Population Structure, Habitat Use and Conservation of Short-Finned Pilot Whales *Globicephala macrorhynchus* in the Archipelago of Madeira

DOCTORAL THESIS

Filipe Marco Andrade Alves

DOCTORATE IN BIOLOGICAL SCIENCES

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ORIENTAÇÃO

Manfred Josef Kaufmann

CO-ORIENTAÇÃO

Caterina Maria Fortuna

Tânia (10 anos; desenho) e Vítor (7 anos; pintura)

“…the water becometh as red as blood, …so that the pilot whales cannot see to run away; it is a strange thing, to see that these strong creatures make no resistance, …till death cometh upon them”

Lucas Debes 1676

“Pilot whales are beautiful animals, and even if they are no longer ‘good to eat’ because of pollution, contamination and the dumping of waste in our oceans, from a whale health perspective they, just like the rest of the biosphere, deserve a good, healthy life in a clean environment of their own.”

in Pilot Whaling in the Faroe Islands, by Jóan Pauli Joensen (2009)

Dedicado à minha família,

a minha verdadeira 'piloto' que me guiou…

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This thesis is the product of the contributions of many people, which somehow participated in the data collection, shared scientific advises, influenced my state of mind or helped me moving forward during this journey over the last years. I will try not to forget any, but sorry if I did.

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NOTE FROM THE AUTHOR

The data used in the present thesis was analyzed between 2010 and 2013 while as PhD student, and was collected throughout several scientific projects that have provided the co-financial support for the development of the field and laboratory work. These projects were co-funded by the Machico Town Hall and by EU and national programs. The acronym, name, co-funding program and period covered by each project are listed below (ordered chronologically):

- CETACEOSMADEIRA "Projecto para a Conservação dos Cetáceos no arquipélago da Madeira (*Project for the Conservation of Cetaceans in the archipelago of Madeira*)" - LIFE 99NAT/P/6432 (2000 to 2004)

- MACETUS "Estudo da estrutura populacional, distribuição, movimentos e utilização do habitat do *Physeter macrocephalus*, *Globicephala macrorhynchus*, *Tursiops truncatus* e *Stenella frontalis* na Macaronésia (*Population structure, distribution, movements and habitat use of four species around the archipelagos of the Azores, Canary and Madeira*)" - FEDER/INTERREG IIIB MAC/4.2/M10 (2004 to 2005)

- GOLFINICHO "Utilização diferencial do nicho espacial e trófico entre o golfinho-comum (*Delphinus delphis*) e o golfinho-pintado (*Stenella frontalis*) nos Açores e Madeira (*Ecological niche partitioning between two species of dolphins around the Azores and Madeira*)" - FCT POCI/BIA-BDE/61009 (2005 to 2006)

- EMECETUS "Estudo, Monitorização e Educação para a Conservação dos cetáceos na Macaronésia (*Study, Monitoring and Education for the Conservation of cetaceans in the Macaronesia*)" - FEDER/INTERREG IIIB 05/MAC/4.2/M10 (2006 to 2008)

- CETACEOSMADEIRA II "Identifying critical marine areas for bottlenose dolphin and surveillance of the cetaceans' conservation status in Madeira archipelago" - LIFE+07 NAT/P/000646 (2009 to 2013)

The projects were developed by the Madeira Whale Museum, which I joined in February 2004. Hereby, I declare to have actively participated in the data collection since then, including taking photographs, collecting biopsy samples, developing and deploying the tags, and participating in all types of surveys. However, I want to highlight that collecting such amount of data during over a decade was only possible as team work (see ‘Acknowledgments’). Data collected prior to my arrival to the Madeira Whale Museum was only used in Chapter V. I also declare to have created and maintained, all by myself, the photo-identification catalogue used in this study. And I assume responsibility for all the statistical analysis and writing presented here, although I had a major contribution from the geneticist Sophie Quérouil that run the genetic analyses and helped interpreting the results presented in Chapter II, and from Adalberto Carvalho, technician on Geographic Information System (GIS), that converted the surveyed track-lines into quantified data per grid cells.

Finally, I would like to state that the experiments comply with the current Portuguese laws. The Biopsy samples were obtained under credentials Of. 668/04 DAC/DSCN from the Instituto de Conservação da Natureza. The remotely-sensed data used in this effort were acquired as part of the activities of NASA's Science Mission Directorate, and are archived and distributed by the GES DISC.

STRUCTURE OF THE THESIS

The outline of the thesis covers one introductory chapter, four data chapters and a final discussion chapter. The general introduction contextualizes the goals presented here, and presents a description of the aspects of the biology and ecology of the target species relevant for this study as well as a characterization of the study area. Each data chapter is a separate study and corresponds to a research manuscript, either published or submitted to peer-reviewed journals (see 'List of publications'). Finally, a general discussion pulls together the major findings, reviews the implications for the conservation of the species and looks ahead.

LIST OF PUBLICATIONS

In peer-reviewed journals, corresponding to Chapters II-V, respectively:

- Alves F, Quérouil S, Dinis A, Nicolau C, Ribeiro C, Freitas L, Kaufmann M, Fortuna C (2013). Population structure of short-finned pilot whales in the oceanic archipelago of Madeira based on photo-identification and genetic analyses: implications for conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems* doi: 10.1002/aqc.2332

- Alves F, Dinis A, Nicolau C, Ribeiro C, Kaufmann M, Fortuna C, Freitas L (submitted). Survival and abundance of short-finned pilot whales in the archipelago of Madeira, NE Atlantic. *Marine Mammal Science*

- Alves F, Dinis A, Ribeiro C, Nicolau C, Kaufmann M, Fortuna CM, Freitas L (in press). Daytime dive characteristics from six short-finned pilot whales *Globicephala macrorhynchus* off Madeira Island. *Arquipelago - Life and Marine Sciences*

- Alves F, Freitas L, Carvalho A, Ribeiro C, Dinis A, Nicolau C, Fortuna C, Kaufmann M (submitted). Distribution and habitat preferences of short-finned pilot whales *Globicephala macrorhynchus* in Madeira, Portugal. *Wildlife Biology*

In Conferences:

- Alves F, Quérouil S, Dinis A, Nicolau C, Ribeiro C, Kaufmann M, Fortuna C, Freitas L (2013). Population structure and parameters of short-finned pilot whales in an oceanic archipelago (Madeira, NE Atlantic): implications for conservation. Presented as oral communication, In *27th Conference of the European Cetacean Society*, April, Setúbal.

- Alves F, Dinis A, Nicolau C, Ribeiro C, Carvalho A, Quérouil S, Fortuna C, Kaufmann M, Cañadas A, Freitas L (2013). Ecologia populacional da baleia-piloto e do roaz no arquipélago da Madeira: metodologias utilizadas, resultados e perspectivas futuras. Presented as oral communication, In *Simpósio do Mar - Horizon 2020*, June, Madeira.

CONTENTS

ABSTRACT

This thesis provides information on the grouping structure, survival, abundance, dive characteristics and habitat preferences of short-finned pilot whales occurring in the oceanic archipelago of Madeira (Portugal, NE Atlantic), based on data collected between 2001-2011, and contributes for its conservation. Photo-identification methods and genetic analyses demonstrated that there is a large degree of variability in site fidelity, including resident, regular visitor and transient whales, and that they may not be genetically isolated. It is proposed that the pilot whales encountered in Madeira belong to a single population encompassing several clans, possibly three clans of island-associated (i.e. resident and regular visitor) whales and others of transients, each containing two to three matrilineal pods. Mark-recapture methods estimated that the island-associated community is composed of less than 150 individuals and that their survival rate is within the range of other long-lived cetacean species, and that around 300 whales of different residency patterns uses the southern area of the island of Madeira from mid-summer to mid-autumn. No significant trend was observed between years. Time-depth recorders deployed in adult whales during daytime revealed that they spend over ¾ of their time at the surface, that they have a low diving rate, and that transient whales also forage during their passage. The analyses of visual data collected from nautical and aerial line-transect surveys indicate a core/preferred habitat area in the south-east of the island of Madeira. That area is used for resting, socializing, foraging, breeding, calving and birthing. Thus, that area should be considered as an important habitat for this species, at least seasonally (during autumn) when the species is more abundant, and included in conservation plans. No direct threat needing urgent measures was identified, although the impact of some activities like whale-watching or marine traffic should be assessed.

Key-words: population ecology, photo-identification, genetics, nautical/aerial surveys, cetacean, Macaronesia.

RESUMO

A estrutura populacional, a taxa de sobrevivência, a abundância, as características de mergulho e as preferências do habitat da baleia-piloto tropical foram estudadas no arquipélago da Madeira. Utilizaram-se dados recolhidos entre 2001-2011, permitindo obter informações robustas para a sua conservação. Através de métodos de foto-identificação e de análises genéticas, demonstrou-se que as baleias apresentam diferentes padrões de residência na área de estudo, incluindo residentes, visitantes regulares e transeuntes. Constatou-se ainda que existe troca de fluxo genético entre as baleias de diferentes padrões de residência. Sugere-se que estas baleias pertencem a uma população única que comporta vários clãs, possivelmente com três associados ao arquipélago (incluindo baleias residentes e visitantes regulares) e outros transeuntes, e que cada clã contém dois a três grupos matrilineares. Através de métodos de captura-recaptura, estimou-se que existem menos de 150 indivíduos associados ao arquipélago, os quais com uma elevada taxa de sobrevivência, e que cerca de 300 indivíduos utilizam a área sul da ilha da Madeira entre o meio do verão e o do outono. Através da colocação de marcas, analisou-se o perfil de mergulho diurno de baleias adultas. Observou-se que passam mais de ¾ do tempo à superfície, que apresentam uma reduzida taxa de mergulho, e que as transeuntes também efetuam mergulhos profundos para alimentação aquando da sua passagem. Através da análise de censos visuais náuticos e aéreos, identificou-se que as águas do sudeste da ilha da Madeira constituem um habitat importante para a baleia-piloto dado que, pelo menos no outono quando a espécie é mais abundante, é utilizada para repouso, socialização, alimentação, reprodução e criação. Esta área deve ser tida em especial atenção na definição de planos de conservação. Não foram identificadas ameaças que necessitem de medidas urgentes, contudo algumas atividades como a observação comercial de cetáceos ou o tráfego marítimo devem ser avaliadas.

Palavras-chave: ecologia populacional, foto-identificação, genética, censos náuticos/aéreos, cetáceo, Macaronésia.

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CHAPTER I

. General Introduction

Chapter cover:

Pilot whales travelling in an east direction off the south coast of Madeira Island. Photograph taken from the research inflatable boat *Roaz* in November 2010.

Photo credits: Nuno Sá

Cetacean Ecology and Conservation

Cetaceans (Chordata, Cetacea) include 87 species of whales, dolphins and porpoises (Hoyt 2011). This diverse group ranges in adult size from less than 1.5 m to 33 m in length, inhabits all major ocean basins and adjoining seas as well as certain lakes and river systems, and exhibits a diverse array of life history strategies (Perrin 1991, Nowak 2003, Rojas-Bracho *et al.* 2009). Despite their diversity of form, behavior and habitat, these aquatic mammals share some key features, such as being air-breathing and live-bearing homeotherms, like their terrestrial ancestors (Ballance 2009). However, studies of the biology of cetaceans tend to differ from any other research due to the particular challenges presented by the aquatic lifestyle. Ecologists and conservation biologists have to deal with the fact that cetaceans are often difficult to observe due to spending most of their time underwater, due to fast moving, and occupying a vast environment both in terms of the geographical range of the species and their use of the water column. In many cases, their large body size, endangered status, or high level of public attention can also contribute for making ecological studies so challenging (Boyd *et al.* 2010).

Balance (2009) has defined cetacean ecology as the study that describes the relationships between cetaceans and their physical environment, including their interactions with prey, predators, competitors and commensals. The human interest and fascination by marine mammals, the depletion of some stocks due to over-exploitation (e.g. by whaling), and the technological developments in recent decades, have increased the scientific research upon these animals and have greatly facilitated our understanding of their ecology. Cetaceans have been described as intelligent, highly mobile, and adaptive mammals that exploit a diversity of habitats (Reeves & Stewart 2003). Their ecological roles can vary by species, geographic area, season, age class or stage, and even sex. However, there are two ways in which cetaceans and their ecosystems can interact. Not only are they affected by the ecosystem but also it is likely that they affect it, since, for example, as apex predators, they are known to take tons of prey biomass from the ecosystem (Estes 2009). In fact, their food and the habits of their prey have been described as the main factors driving their morphologic, physiologic, and behavioral adaptations to the environment. This allows dividing their ecological adaptations in habitat types such as ‘open ocean’, ‘semipelagic’, or ‘coastal’ (Würsig 2009).

Information about the structure, the life-history strategies and the habitat use of a population are at the core of both cetacean ecology and conservation biology. Analysis of the population structure has been revealed to be of great importance in identifying conservation units, either using photo-identification methods and/or molecular genetics (e.g. Hoelzel *et al.* 2002, Aschettino *et al.* 2011). On the other hand, the life-history strategies have been defined by parameters that describe how individuals allocate vital resources such as survival, providing knowledge for understanding the population’s demography. Yet, such parameters are known in detail for relatively few species, and most of the biological data have been collected as a result of cross-sectional studies from animals taken during whaling operations, fisheries bycatch or strandings (Chivers 2009). Longitudinal studies, which can provide the required data for addressing the population’s demographic parameters, are known for only three species (e.g. Olesiuk *et al.* 1990, Wells & Scott 1990, Clapham 1996). Finally, studies on the habitat use can provide information on the cetaceans’ distribution, either on the vertical or on the horizontal scale. While the former allows to analyze their foraging behavior mainly throughout the use of archival tags (revised in Hooker & Baird 2001, Ropert-Coudert *et al.* 2009), the latter examines the relative importance of various environmental components in determining their distribution and abundance at different spatial and temporal scales from the analysis of survey data (e.g. Cañadas 2006, Redfern *et al.* 2006).

Conservation can be regarded from different viewpoints. One view is considering it as the effort made by people to maintain the autonomous regeneration of natural populations under natural conditions (Reeves & Reijnders 2002). However, this is not so simple in practice, as 'natural conditions' may no longer exist (pristine ecosystems are extremely rare) and many of today’s conservation problems to many cetacean populations are more complex than they were a few decades ago. These involve multifaceted and poorly understood threats such as toxic contaminants, noise from shipping and industrial activities, or military operations, when compared to other clear threats such as excessive harvest, mortality in fisheries or boat strikes (Reeves & Stewart 2003, Read 2010). Other aspect is that biological diversity exists at various levels (e.g. species, population, community, individuals) and some threats may affect only a lower level. Therefore, the scientific community is focusing more in conservation efforts at those lower levels by ensuring the survival of local or geographical populations (Reeves 2009, Taylor *et al.* 2010).

Efforts to conserve cetaceans have been made at the international, national, regional, and local levels. To know the legislation and frameworks in force is a necessary step regarding the research and conservation of cetaceans. There is a vast list of established treaties and conventions protecting species of cetaceans, carried out by intergovernmental as well as by non-governmental organizations (see Reeves 2009). Some of the most important, and with relevance for the present study, are the International Whaling Commission (IWC), the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), the International Union for the Conservation of Nature (IUCN), the Convention on the Conservation of Migratory Species of Wild Animals (Bonn Convention), the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention), and the Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area (ACCOBAMS). The Red List, maintained by the IUCN, and despite some criticism (Mrosovsky 1997), remains the primary international standard for assessing conservation status (Rodrigues *et al.* 2006). Finally, the designation of especially marine protected areas is a potential and rising tool to achieve conservation goals. Many times these areas are created purposely for cetacean species due to their charismatic features, although they bring greater protection to all of the adjoining marine life (Hoyt 2011).

Target Species

The short-finned pilot whale *Globicephala macrorhynchus* Gray (1846) is among the most familiar cetacean species because of its circumglobal distribution throughout tropical and warm temperate waters, its general abundance and its proclivity for mass stranding (Reeves *et al.* 2002). Despite being known as whales, this species is in fact part of the large species delphinid group (Family Delphinidae). Several specific names have been used in literature in the past, although all those are actually considered synonyms of *G. macrorhynchus* (revised in Fraser 1950, van Bree 1971, Klinowska 1991, Rice 1998).

The external appearance is very similar of its cool-water relative, the long-finned pilot whale *G. melas*. Adults reach an average size of approximately 6 m, are black or dark grey, with a robust body and a bulbous melon with no beak. The dorsal fin is wide, broad-based and falcate, and a distinct post-dorsal grey saddle patch as well as a post-orbital blaze may be visible in most individuals (Olson 2009). Miyashita *et al.* (1990), Shane & McSweeney (1990) and Auger-Méthé & Whitehead (2007) have analyzed the feasibility of using these natural markings to photographically identify pilot whales, although marks in the dorsal fin have been the most successful and widely used in photo-identification studies (e.g. Heimlich-Boran 1993, Ottensmeyer & Whitehead 2003, de Stephanis *et al.* 2008). Pilot whales exhibit sexual dimorphism in size, with males being bigger than females (Olson 2009). However, recent findings discouraged distinguishing the gender in long-finned pilot whales based on visual observations or on photographs (Augusto *et al.* 2013). If the same should be applied to short-finned pilot whales remains unknown. The morphological characteristic distinguishing the two species at sea are subtle, requiring a visual inspection of the pectoral fins or of the ventral color pattern, and preferentially in adults. The pectoral fins tend to be proportionally shorter and the kedge-shaped throat mark with a streak extending to the inguinal area tends to be much darker and less conspicuous in the short-finned pilot whale (Sergeant 1962, van Bree 1971, Bloch *et al.* 1993). Moreover, numerous characters, such as size or pigmentation, have been found to vary at the inter-individual as well as at the inter-population level in the short-finned pilot whale

(Yonekura *et al.* 1980, Kasuya *et al.* 1988, Heimlich-Boran 1993). The latter case is highly evident between two discrete forms (or populations), the 'southern' and the 'northern', described in short-finned pilot whales caught off Japan. Apart from morphological differences (Kasuya *et al.* 1988), significant differences in gene frequencies between those two forms were also identified (Wada 1988).

Apart from the study of Wada (1988), the genetic differentiation of short-finned pilot whales has been analyzed only (to my best knowledge) in the studies of Chivers *et al.* (2003) and Oremus *et al.* (2009). While the former provide preliminary evidence for population structure within the eastern North Pacific Ocean, the latter analyzed worldwide population structure and, apart from corroborating the two genetically-distinct forms off Japan, found strong mtDNA differentiation between oceanic basins and low haplotype and nucleotide diversity compared to other abundant widespread cetaceans. On the other hand, probably because entire groups of long-finned pilot whales are still taken regularly in traditional drive fisheries in the Faroe Islands (Zachariassen 1993, Joensen 2009, Fielding 2010), there is a considerably higher number of works that have used genetic analyses to evaluate population structure in this species (e.g. Amos *et al.* 1991a, 1991b, 1993a, 1993b, Anderson 1993, Fullard 2000). As so, and due to being related to the target species, some of the output from those works about the long-finned pilot whale is also described in this paragraph. Such works indicate that groups in the Faroe Islands are composed of maternally related males and females that rarely inter-breed (Amos *et al.* 1991b, 1993b), and that substructure does exist between different regions of the North Atlantic (Fullard *et al.* 2000). The molecular ecology of the mass strandings of long-finned pilot whales around New Zealand and Australia was later investigated to test the hypothesis that individuals stranding together are part of an extended matrilineal group, and results demonstrated that groups are sometimes composed of unrelated maternal lineages (Oremus 2008, Oremus *et al.* 2013). Still for the long-finned pilot whale, and based on photo-identification, Ottensmeyer & Whitehead (2003) suggested short-term associations of individuals over hours to days and long-term associations with a subset of those individuals over years, and de Stephanis *et al.* (2008) showed a non-random social structure made by constant companions. In the short-finned pilot whale, Heimlich-Boran (1993) found support to the hypothesis that the species has a matrilineal hierarchical system, and Mahaffy (2012) showed a non-random social structure with gradual dissociation over time. The resulting picture is that social and population structure in pilot whales are not yet fully understood and suggests that, in order to clarify it, molecular genetics and photo-identification should be used in combination.

The short-finned pilot whale is generally nomadic, but several resident populations have been documented based on photo-identification (Olson 2009). These included locations such as the waters off California (Shane & McSweeney 1990), Japan (Miyashita *et al.* 1990), Hawaii (Shane & McSweeney 1990, Mahaffy 2012) and Canary Islands (Heimlich-Boran 1993). However, while some were based in only few recaptures, Heimlich-Boran (1993) and Mahaffy (2012) have analyzed large datasets over multiple years. Following Bigg *et al.* (1990) that defined longitudinally stable groups in their study of killer whales *Orcinus orca* in the coastal eastern North Pacific, the terms ‘pods’ and ‘units’ have been used to define that type of groups in the resident populations described by Heimlich-Boran and by Mahaffy, respectively. Moreover, a group of short-finned pilot whales generally contains 15 to 50 animals of mixed sex and age, with an approximate sex ratio of one mature male to eight mature females (Reeves *et al.* 2002). Finally, quantitatively derived estimates of abundance in this species are limited to line transect methods and mainly for near shore populations off Japan, Hawaii, eastern tropical Pacific, West coast of US and US Gulf of Mexico (reviewed in Olson 2009, including estimates for each area and references).

The short-finned pilot whale is found mainly over deep waters and its preferential habitat includes areas of high topographic relief, island slopes and continental shelf-breaks (Hui 1985, Heimlich-Boran 1993, Montero & Arechavaleta 1996, Andrews *et al.* 2011, Wells *et al.* 2013). Off California, seasonal inshore/offshore movements and abundance of short-finned pilot whales appears to be related to the distribution and spawning of squid (Hui 1985, Shane 1995). The species is mainly teuthophagous, although fish is also consumed. Most of this information has been obtained through the examination of stomach contents from carcasses of stranded or animals caught as bycatch in California (Seagars & Henderson 1985, Sinclair 1992), Canary Islands (Hernández-García & Martín 1994, Fernández *et al.* 2009), Japan (Kubodera & Miyazaki 1993), New Caledonia (Bustamante *et al.* 2003) and North Carolina (Mintzer *et al.* 2008). Although results from the different regions agree that their diet consist primarily of squid, with lesser amounts of fish, differences in the prey type and habitat were observed. For example, oceanic preys were described as the most important in the stomach contents of whales in North Carolina, which differs from the Pacific coast where neritic species dominate the diet. Mintzer *et al.* (2008) also suggest that the short-finned pilot whale feeds on deep-water species. Foraging dives in the short-finned pilot whale to catch deep-water prey have been corroborated by the use of acoustic DTAGs (logging sound, depth and orientation) attached to whales in the Canary Islands (Hindell 2008, Soto *et al.* 2008, Jensen *et al.* 2011). It is also known that the foraging dives seem to adapt to circadian rhythms, with deeper dives and longer time periods of shallow diving or surface resting occurring during the day (Baird *et al.* 2003, Soto *et al.* 2008, Andrews *et al.* 2011).

The life history parameters of the short-finned pilot whale have been recognized primarily from the analysis of groups that were stranded or hunted off Japan. They have been analyzed for the 'southern' and the 'northern' form, which showed similarity in the age at maturation, breeding life span, age dependent decline in reproductive activity and longevity (Kasuya & Tai 1993). The species has a low estimated annual pregnancy rate (13%), declining rapidly with increasing age until, before age 40 years, reaching zero percent, even though they may live up to 63 years. In contrast, males have a maximum longevity of only 46 years. Gestation is estimated to be between 15 and 16 months. Lactation lasts for a minimum of two years, but associations between mothers and calves can last until around the age of sexual maturity in males (15 to 22 years) and perhaps after maturity in females (7 to 12 years). Females produce an average of four to five calves during their life. The old, low- and post-reproductive females comprise a significant part of a group and probably contribute towards its maintenance and the survival of the younger individuals. The total mortality rate is lowest in the post-pubertal stage, it increases with age, and is unbalanced in favor to males at all ages. The juvenile total mortality rate is probably higher than that of post-pubertal animals (Kasuya & Marsh 1984, Kasuya & Matsui 1984, Marsh & Kasuya 1984, 1986, Kasuya & Tai 1993, Kasuya *et al.* 1993, Johnstone & Cant 2010).

Concerning its status and conservation, the species is considered to remain relatively abundant throughout its range even after centuries of exploitation. Hunting is known to have occurred in populations off Japan, Indonesia, Sri Lanka and in the eastern Caribbean (Reeves *et al.* 2002). Additionally, incidental bycatch in a variety of fishing gears have been recorded off California, Hawaii, and in the northeast US waters, although the impact of the removal of individuals by the local populations is unknown (Olson 2009). As a result, its conservation status is described as 'Data Deficient' on a global scale, and its population trend is unknown (IUCN 2012). The species is listed in the Annex II of Bern Convention, Annex IV of Habitats Directive and Appendix II of CITES. At a regional scale, the species is legally protected in the Exclusive Economic Zone (EEZ) of the archipelago of Madeira since 1986 by the Dec.-Leg. Regional 6/86/M (Cabral *et al.* 2005).

The status of the short-finned pilot whale in the archipelago of Madeira is defined as 'Least Concern' (Cabral *et al.* 2005). However, scientific knowledge for this species in Madeira is scarce. Ecological studies towards this species started only during the last decade, and the results are presented in this thesis. Prior to the present study, literature describing this species in Madeiran waters is resumed to the identification of two individuals (Maul & Sergeant 1977) and to anecdotal sightings (Freitas *et al.* 1998). The identification of the two individuals is resumed to a first identification by Sarmento (1936, 1948) and Nobre (1938) as long-finned pilot whales (*G. melas*), and later by a correction by Fraser (1950) and a corroboration by Maul and Sergeant (1977) as being short-finned pilot whales (*G. macrorhynchus*).

Study Area

This study was conducted in the archipelago of Madeira, Portugal. The archipelago is located in the warm-temperate waters of the northeast Atlantic Ocean (~33° N, 017° W), at approximately 1000 km of the European continent and 500 km of the West African coast. It is one of the most isolated archipelagos of the North Atlantic Ocean, with an intermediate geographical position between the archipelago of the Azores (at 900 km distance) and the Canaries (at 400 km). The archipelago, of volcanic origin from the Miocene (Schmincke 1973, Klügel *et al.* 2009), comprises the main island of Madeira and the smaller Desertas and Porto Santo, with distances of 20 to 40 km between them, and the Selvage Islands at about 300 km to the South. Madeira is the largest island of the group with an area of 741 km^2 (57 per 22 km), a coastline of 157 km and a mountain ridge reaching 1862 meters altitude. The Madeira EEZ has approximately 446000 km^2 and features mainly deep (abyssal) oceanic waters (Figure I.1).

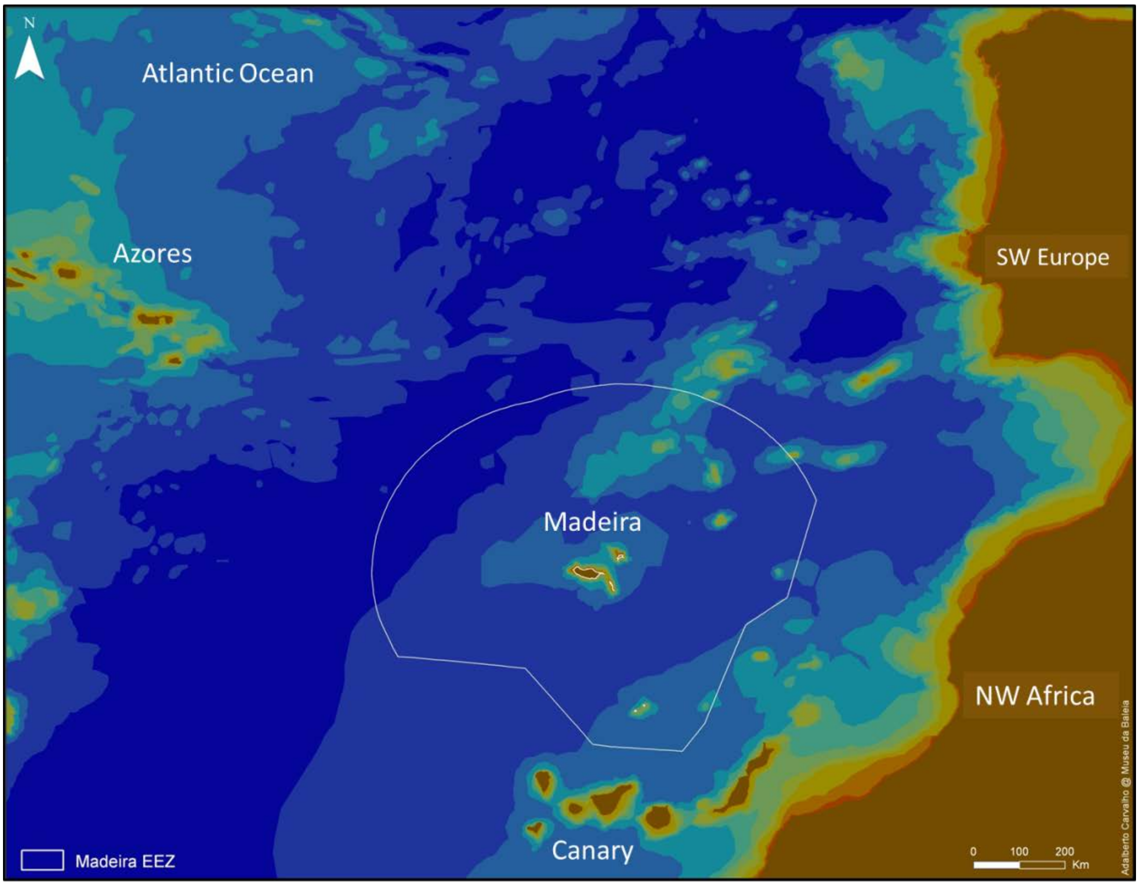

Figure I.1. Map of the northeast Atlantic showing the location of the archipelago of Madeira and its Exclusive Economic Zone (EEZ).

This archipelago is characterized by the presence of the Portuguese Current, the Azores Current, and the Canary Current, all part of the eastern anticyclonic North Atlantic subtropical gyre (Sala *et al.* 2013). The eastward branch of the Azores Current that joins the Canary Current north and around the archipelago of Madeira (New *et al.* 2001) are responsible for most of its seasonal and inter-annual complex oceanographic patterns, resulting in high salinity, high temperature and low-nutrient regime waters (Johnson & Stevens 2000). Although not yet considered, similarly to what occurs at the Canaries (Aristegui *et al.* 1997), African filaments are known to travel offshore, and occasionally, they might also reach Madeira (Caldeira & Sangrà 2012). Nevertheless, Caldeira *et al.* (2002) have shown that the island mass effect phenomena also affect Madeira regional oceanographic setting. The obstruction by the islands mountain range to the dominant northeast trade winds (Tomczak & Godfrey 1994) and the underwater ridges resulting from collapsed crater rising abruptly from the deep ocean (e.g. 1000 to 200 m) near the coast, results in the formation of leeward productive eddies and fronts in the flanks of the islands. Caldeira *et al.* (2002) have also shown that northern waters were consistently colder than the warm and salty southern waters, and that localized cold water with high chlorophyll concentrations was also observed around the coast of the island of Madeira. Finally, these authors have shown that winter and fall geostrophic currents reach the island mostly from the north, whereas during the summer, there is a strong south inflow and during spring a strong western incoming flow. More recently, Caldeira & Sangrà (2012) have shown that the complexity of the archipelago wake is influenced by the small neighboring island of Desertas and Porto Santo, leading to destabilization of the shear layers of the main island, and by the archipelago shelf, contributing to asymmetric vertical migration of oceanic vorticity. Although complex regional patterns of ocean circulation is known to affect the distribution of marine organisms (Santos *et al.* 1995), only few studies (e.g. Caldeira *et al.* 2001) have tried to characterize it in Madeira.

I have performed surveys in the offshore waters of the archipelago (>40 km of the coast) using platforms of opportunity, yet, these were only addressed briefly to help discussing some of the findings of this study. Therefore, the main explored area of this study was the waters encompassing the islands of Madeira, Desertas and Porto Santo (Figure I.2), up to a maximum of 40 km off the coast. The area covers 8633 km^2, and is characterized by a narrow continental shelf, with steep submarine canyons and deep waters (ca. 1500 m) (Geldmacher *et al.* 2000), thus providing a truly pelagic/oceanic habitat for cetaceans at short distances from the coast.

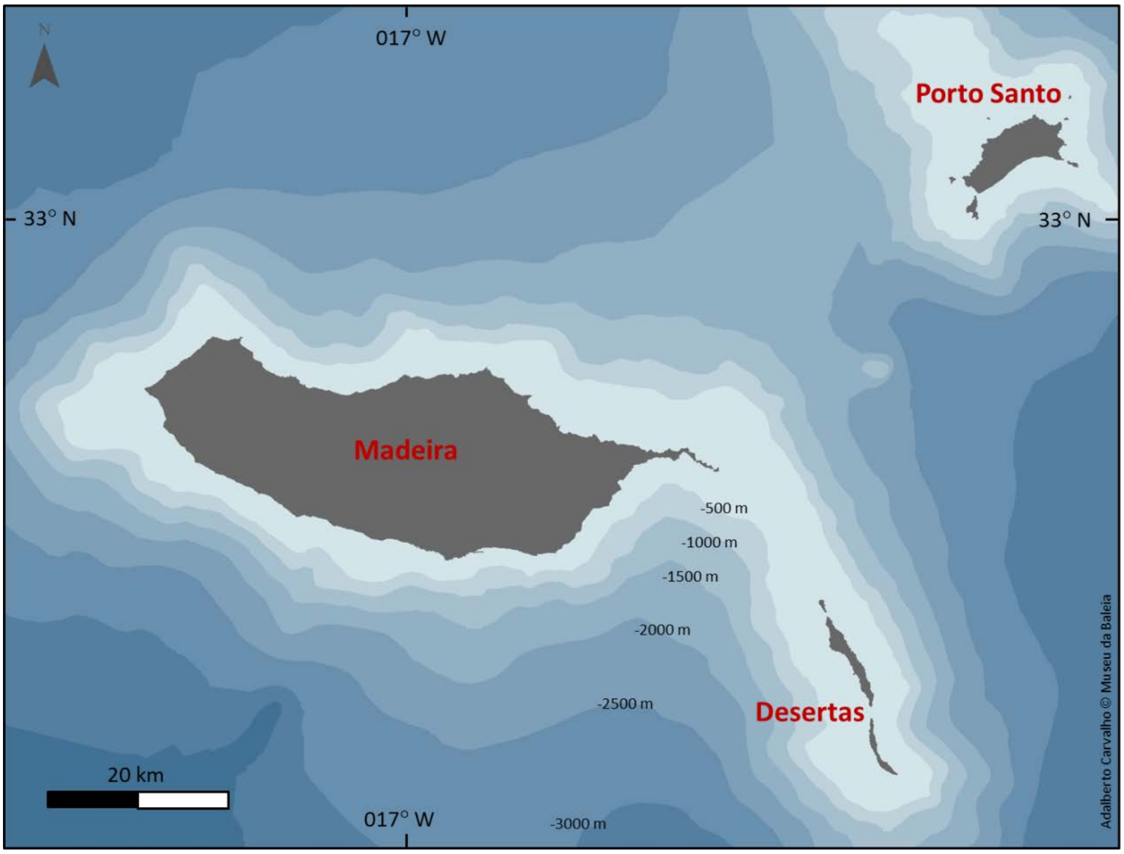

Figure I.2. Bathymetric map of the study area.

Madeira relies on tourism as its main source of economic income, and development of marine touristic activities had been growing during the last decade (Vera 2012). Indeed, a study assessing the anthropogenic threats with effects on the cetaceans in this archipelago have shown that from a total of eight threats listed with identified impacts and nine listed with potential impacts, only whale-watching and recreation vessels needed immediate monitoring and management measures (Dinis *et al.* 2006). A study in 2007 estimated that the whale-watching industry involved 1.5 million euros per year in Madeira (Ferreira 2007). In that year, the fleet was composed of 10 vessels operating year-round mainly along the south of the island of Madeira (between the south-west of Madeira and Desertas), and short-term effects on delphinids were recorded, with changes in speed during and after the encounter with boats (Ferreira 2007). By the end of the present study, in May 2013, the whale-watching activity became legally regulated by the Dec.-Leg. Regional 15/2013/M.

In respect to the occurrence of cetaceans in Madeira, a similar scenario to that of its neighboring archipelagos is found, hosting at least (based on confirmed records) 25 species (Reiner *et al.* 1993, Ritter 2001, Freitas *et al.* 2012). While several species uses the waters of the archipelago mainly during their migratory courses such as the fin whale *Balaenoptera physalus* or the minke whale *B. acutorostrata* (Gordon *et al.* 1995, Alves *et al.* 2005), others are present seasonally such as the Bryde's whale *B. brydei*, the common dolphin *Delphinus delphis* or the Atlantic spotted dolphin *Stenella frontalis* (Alves *et al.* 2010, Quérouil *et al.* 2010, 2013), and others are even present throughout the year such as the bottlenose dolphin *Tursiops truncatus*, the short-finned pilot whale or the sperm whale *Physter macrocephalus* (Alves *et al.* 2007, Freitas *et al.* 2004, 2006). Information on the feeding habits and diet of cetaceans in Madeira is restricted to the common dolphin and the Atlantic spotted dolphin (Cordeiro *et al.* 2007, Dinis *et al.* 2008, Quérouil *et al.* 2013). Finally, genetic analyses from biopsies taken from free-ranging bottlenose dolphins (Quérouil *et al.* 2007), as well as from common dolphins and Atlantic spotted dolphins (Quérouil *et al.* 2010), have revealed low genetic differentiation when compared with samples taken from populations of the neighboring archipelago of the Azores.

Objectives

Most of the research on the short-finned pilot whale has been conducted along continental coasts, mainly due to the operational constraints normally associated with the survey of offshore/oceanic habitats. The reduced (almost absent) continental shelf in the archipelago of Madeira offers a privileged location for the study of the ecology of cetaceans in a pelagic environment. Additionally, this northeast Atlantic oceanic archipelago relies in a region where information on the pilot whales' life history and ecology is scarce, being mainly restricted to studies on social and diving behavior in the Canary Islands (e.g. Heimlich-Boran 1993, Soto *et al.* 2008). Even looking at it on a wider scale, the Atlantic Ocean, population estimates are mainly confined to the US

Gulf of Mexico (Waring *et al.* 2007). Overall, scientific knowledge on the population structure, demographic parameters, trend, movements, threats and critical habitats of this species, as well as on the trophic processes and topographic factors influencing its habitat use is scarce. And such knowledge constitutes the base-line to establish an adequate conservation status and to apply proper management measures.

As mentioned above, the regional status for this species in the archipelago of Madeira is defined as 'Least Concern' (Cabral *et al.* 2005), but scientific information, especially at the population level, lacks to support the definition of such criteria. Moreover, most of the knowledge about this species in the archipelago of Madeira has been obtained from its coastal waters, with no valid information from the remaining EEZ. At the beginning of this work, two potential threats were suspected of causing disturbance to the short-finned pilot whale in this archipelago. First, the tuna fishermen association communicated to the Madeira Whale Museum that these whales are interfering with the fishery. Second, being among the most common species and occurring year-round (Freitas *et al.* 2004) makes it vulnerable for the fast growing and only very recently regulated whale-watching industry in Madeira. Additionally, a preliminary analysis using photo-identification methods on the short-finned pilot whale in Madeira (using a subset of the data used in this study) suggests that these waters are visited by resident (regularly present) and transient pods (Alves *et al.* 2007, Servidio *et al.* 2007). However, knowledge on patterns of site fidelity and on gene flow among whales with different residency status remains unknown. Similarly, information on their numbers, survivorship, preferential areas and site-specific activities is not available. Therefore, this work aims to provide information about the ecology of the population of short-finned pilot whales occurring in the oceanic habitat of the archipelago of Madeira, and to contribute for its conservation.

The aim of Chapter II (the first data chapter) is to infer the population structure of short-finned pilot whales in the archipelago of Madeira and to determine whether individuals associate with kin. For this purpose, and for the first time in pilot whales (according to an extensive literature search), a combination of photo-identification and molecular genetics was used. The grouping structure is studied using nine years of individually recognizable whales in order to assess site fidelity, residency and association patterns. Additionally, mtDNA sequences and microsatellite markers are used to estimate inter-individual relatedness within and between groups of whales encountered in two years. Two hypotheses are tested: (1) there is at least one pelagic and one or more island-associated communities, and (2) groups consist of related individuals, with a matrilineal social structure. The chapter ends with a discussion about the implications of these findings for the establishment of effective conservation measures in Madeira.

Chapter III extends to the next logical step in the study of population ecology, the population demographic parameters. These are crucial to define conservation strategies for these whales, especially when in the presence of island-associated communities. The aim of the chapter is thus to estimate survivorship and abundance of these whales using mark-recapture methodology from photo-identification data collected over seven years. The heterogeneity in capture probability between individuals is addressed, and different population models are tested. Despite some limitations, the chapter reports the first estimates of population parameters for short-finned pilot whales using mark-recapture methodology.

Chapter IV is part of one of the major goals in this thesis, the study of the habitat use. While its complementary chapter (Chapter V, the main chapter focused on the habitat use) explores the habitat use on a horizontal scale, Chapter IV evaluates the whales' vertical movements. The aim of the chapter is to provide data on the proportion of time spent at the surface and at different dive phases (descent, bottom and ascent), dive depths and dive rates. Six biologgers (time-depth recorders) attached with suction-cups are deployed in resident and transient adult whales in their suggested preferred habitat area to evaluate their daytime diving characteristics.

The aim of Chapter V (the last data chapter) is to investigate this species relative density and spatio-temporal distribution in relation to dynamic and physiographic variables, and to assess its biological factors such as behavior, proportion of calves and inter-specific relationships. Here, I analyze 11 years of effort-related sightings collected during dedicated aerial and nautical line-transect surveys, conducted year-round up to

40 km off the coasts of the islands of Madeira, Desertas and Porto Santo. Finally, the relevance of the results is discussed towards the identification of important habitats for the conservation of the species.

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CHAPTER II

. Population structure of short-finned pilot whales in the oceanic archipelago of Madeira based on photo-identification and genetic analyses: implications for conservation

Chapter cover:

Resident pod R4 off the south-west coast of Madeira Island. This was the most captured pod during this long-term study. Old ‘friends’, Gma089 (foreground) and Gma002 (background) were photographed together most of the times. Gma002 is known to use this area for at least for 14 years. Photograph taken from the whale-watching vessel *Ribeira Brava* in August 2009.

Photo credits: Claudia Gomes, Lobosonda

Abstract

Pilot whales *Globicephala* spp. are known to display a hierarchical social pattern, but longitudinal data to infer population structure of short-finned pilot whales *G. macrorhynchus* are rare. Using data collected between 2003-2011 in the oceanic archipelago of Madeira, the grouping structure of short-finned pilot whales was studied using photo-identification methods and mtDNA sequences and microsatellite markers to test the hypotheses that (1) there is at least one pelagic and one or more island-associated communities, and (2) groups are made of related individuals, with a matrilineal social structure. Pilot whales demonstrated a large degree of variability in site fidelity, including residents (up to 14-year interval), regular visitors and transients. The social and temporal analyses revealed a well-differentiated society with long-lasting relationships (of years). The genetic analyses suggested that individuals of the three residency patterns may not be genetically isolated, and that small groups are made up of related individuals, suggesting some degree of social philopatry, while large groups are probably temporary associations of smaller groups. It is proposed that the pilot whales encountered in Madeira belong to a single population encompassing several clans, possibly three clans of island-associated whales and others of transients, each containing two to three matrilineal pods, each with a mean of 15 individuals (SD=9, range: 4-29). It is suggested that the clans interact for mating purpose when they meet. For management decisions, it is considered that the island-associated whales should not be regarded as demographically independent populations, but instead as stable social entities to be included in governmental management plans and requiring periodic evaluation of their status. The high proportion of marked individuals and low rate of mark change encourages further research in this species.

Introduction

Attempts to understand the behavioral ecology and social structure of wild populations require analysis of the social networks of associations and relationships at the inter-individual level. Such analyses can result in the definition of social entities and influence the definition of management policies. Photo-identification (photo-id), which is based on the repeated identification of individuals, has become an essential technique in providing information on group stability and association patterns in longitudinal studies (Würsig & Würsig 1977, Würsig & Jefferson 1990). Examples include the intensively studied resident killer whales *Orcinus orca* where natal pod philopatry has been evidenced in both sexes (Bigg *et al.* 1990), or the long-finned pilot whales *Globicephala melas* with non-random social systems based on constant companions (Ottensmeyer & Whitehead 2003, de Stephanis *et al.* 2008).

In the case of the short-finned pilot whales *Globicephala macrorhynchus*, photo-id was described as a feasible technique for studying their social organization (Miyashita *et al.* 1990, Shane & McSweeney 1990), and Heimlich-Boran (1993) found support to the hypothesis that this species has a matrilineal hierarchical system. Even though, up to date, and despite this species being distributed worldwide from tropical to warm-temperate waters and considered abundant throughout its range (Olson 2009), behavioral longitudinal studies on this species using photo-id are scarce; only Heimlich-Boran (1993) and Mahaffy (2012) have analyzed large datasets (of hundreds of individuals) over multiple years.

Another technique that has become widely used for the analysis of population structure in free-ranging cetacean species is molecular genetics. It has been revealed to be of great importance in identifying conservation units and estimating gene flow between demes (cf. Hoelzel *et al.* 2002). Molecular analyses have also been used to obtain information on social and geographic philopatry in several delphinid species, such as bottlenose dolphins *Tursiops aduncus* (Wiszniewski *et al.* 2010), killer whales (Pilot *et al.* 2010) or striped dolphins *Stenella coeruleoalba* (Gaspari *et al.* 2007). In most toothed whale species, females form stable matrilineal lineages as a result of social philopatry, while males either disperse (e.g. sperm whales *Physeter macrocephalus*) or display geographic philopatry (e.g. bottlenose dolphins) or even social philopatry (e.g. long-finned pilot whales and resident populations of killer whales, Amos *et al.* 1991a, 1991b, 1993a, 1993b, Connor 2000).

To our best knowledge, inference on the genetic differentiation of short-finned pilot whales is confined to the studies of Wada (1988) and Oremus *et al.* (2009). While the former found two genetically isolated forms off the Pacific coast of Japan, the latter analyzed worldwide population structure, finding strong mtDNA differentiation between oceanic basins and low haplotype and nucleotide diversity compared to other abundant widespread cetaceans. Nevertheless, and contrarily to its related species (see Amos *et al.* 1991a, 1993a, Fullard *et al.* 2000), very little information is available on the population structure of short-finned pilot whales either for the Atlantic or for offshore archipelagoes.

The pelagic waters of the archipelago of Madeira are amongst the most isolated oceanic habitats of the north Atlantic. Field surveys conducted over the last decade within the frame of several research projects showed that pilot whales are among the four most encountered odontocete species (unpublished data from the Madeira Whale Museum). However, scientific knowledge on these whales' ecology and population structure in Madeiran waters is lacking. One study using photographically-identified individuals suggested seasonal site fidelity for Bryde's whales *Balaenoptera brydei* (Alves *et al.* 2010). Knowing if short-finned pilot whales in Madeiran waters belong to a single large oceanic population and thus be regarded as a single conservation unit, or if they are divided into multiple populations, could greatly improve future management decisions. One could expect an absence of population structure and genetic isolation given the wide spread oceanic habitat surrounding the archipelago and the pelagic nature of this species, which is known to undertake large-scale movements (Bernard & Reilly 1999). Nevertheless, the presence of island-associated whales in Madeira should be regarded as a possibility, based on a preliminary photo-id analysis (Alves *et al.* 2007) and on the occurrence of resident pods of this species (Heimlich-Boran 1993, Mahaffy 2012).

The present study is the first one to use a combination of photo-id and molecular genetics to determine whether individuals associate with kin and to infer the population structure of pilot whales. In the archipelago of Madeira, the grouping structure of short-finned pilot whales was studied using individually recognizable whales, in order to assess site fidelity, residency and association patterns between 2003-2011. Additionally, mtDNA sequences and microsatellite markers were used to estimate inter-individual relatedness within and between groups of whales encountered during 2004 and 2005. It was hypothesized that (1) there is at least one pelagic and one or more island-associated communities, and (2) groups are made of related individuals, with a matrilineal social structure. Finally, it was expected to contribute to the establishment of effective conservation measures as part of a long-term governmental management plan.

Material and Methods

Study site

The study was conducted around the warm-temperate islands of Madeira, Desertas and Porto Santo, archipelago of Madeira (Portugal). These oceanic volcanic islands lie at 670 km from Morocco, and steep submarine canyons and deep waters (ca. 1500 m) are common around them due to a reduced continental shelf (Geldmacher *et al.* 2000). The study area covered a total of 4818 km^2 up to 20 km offshore around the three islands. However, since data were obtained through different sources, a more intensely surveyed core area of ≈900 km^2 was defined along the south and east of Madeira Island, up to 15 km offshore (Figure II.1).

Field procedures

Individual identification photographs were obtained through year-round systematic and non-systematic surveys, as well as from other sources. One hundred and six systematic surveys (with a mean duration of 11 h 30 min) were carried out in the whole study area following predefined line-transects using the 18 m research-yacht *Ziphius* (12 km h^{-1}) during 2004 and 2007-2011. Here, time constraints have limited full photographic coverage of some encounters. One hundred and seventy non-systematic surveys (with a mean duration of 6 h 16 min) were carried out mainly in the core area using the 6.5 m Rigid Inflatable Boat *Roaz* (15-25 km h^{-1}) between 2004-2011. All groups of short-finned pilot whales seen were approached and attempts to photograph the left- and right-side of all individuals were made, independently of age class and of how distinctive a dorsal fin was.

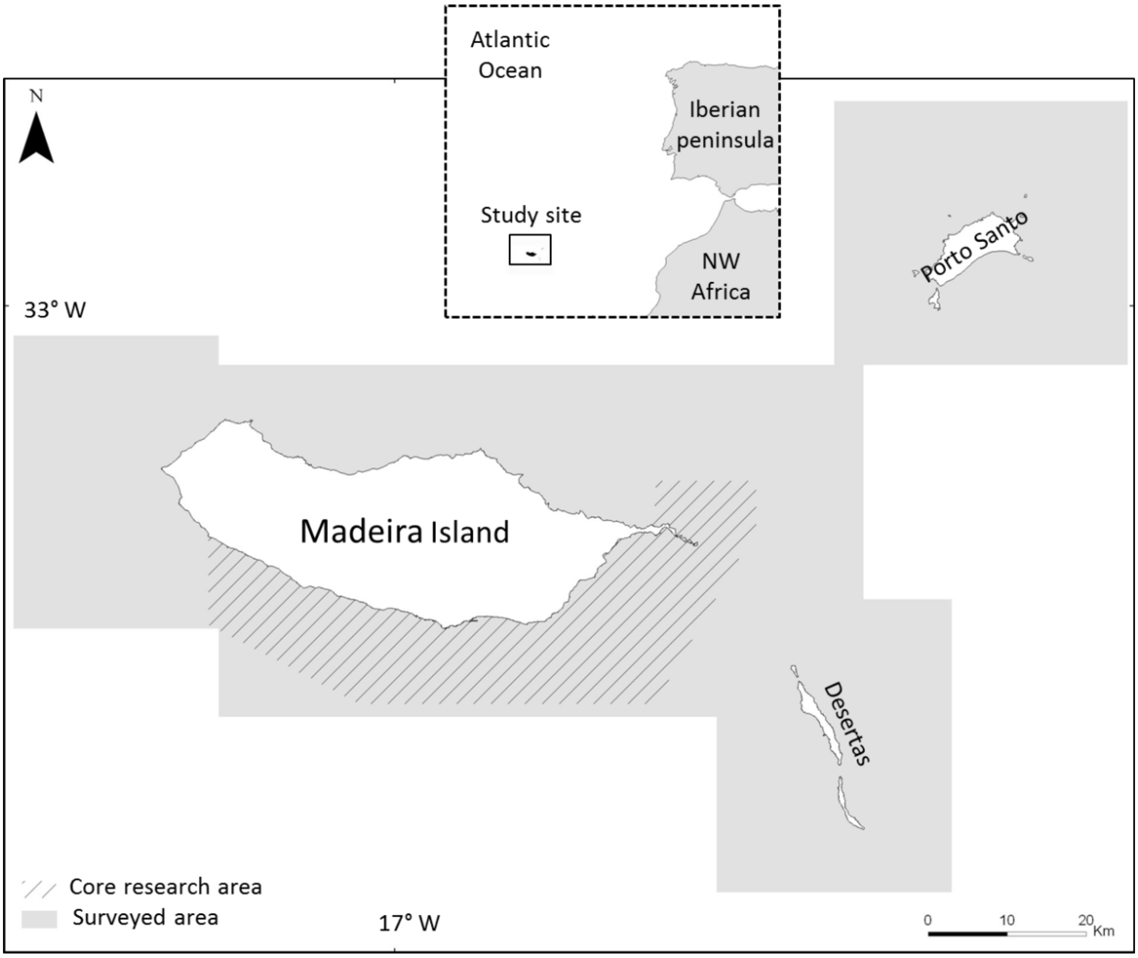


Figure II.1. Location of the islands of Madeira, Desertas and Porto Santo, showing the surveyed and the core research areas.

A mean of three observers searched the area in Beaufort sea state ≤3, and track courses were registered using a GPS. Weather, effort and sighting information (location, initial time, best estimate of group size and composition) were recorded. A group was defined as all individuals within a 250 m radius of each other and exhibiting similar behavior (Heimlich-Boran 1993). An encounter was defined as a sampling event that provided at least one photographic capture.

Photo-id

Members of the research team took photographs using digital cameras with lenses ranging between 70-400 mm. Additionally, digital photographs from other photographers taken between 2003-2011 onboard whale-watching boats that operated year-round in the core area were also analyzed, and film photographs taken by our team during opportunistic surveys in 1997 and 2001 at south Madeira underwent individual matching examination to help determining long-term site fidelity. A photo-id catalogue was compiled and a dataset of capture histories per encounter was created using the individual identification photographs (Würsig & Jefferson 1990). A capture was defined as an individual identification within an encounter.

Photo processing consisted in cropping the dorsal fin and the surrounding body area, and adjusting contrast with Adobe Photoshop Elements 3.0. The result was a processed image that highlighted the trailing edge, thus facilitating sorting and matching (Figure II.2). Photo matching consisted in comparing processed images based primarily on the number of unique notches on the dorsal fin, which allowed matching left- and right-side photographs. A notch was considered as an indentation in the leading or trailing edge of the dorsal fin, identifiable from both sides (Auger-Méthé & Whitehead 2007). Other secondary features such as fin shape or scars (Auger-Méthé & Whitehead 2007) were only used to sort individuals without unique notches, as well as to confirm matches. At the end, all matches were double-checked and all individuals compared in order to avoid false-positives and false-negatives. The photo-id was done by the same person (Filipe Alves), and the long-term matches were confirmed by a second researcher experienced in photo-id (Ana Dinis).

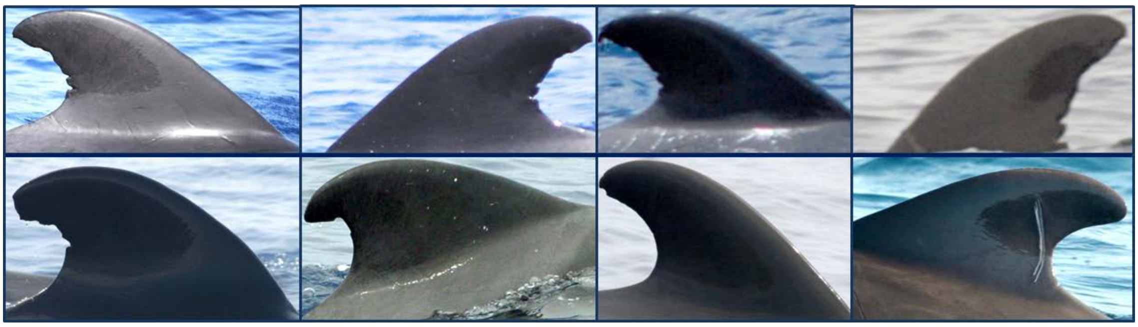


Figure II.2. Top (left to right): examples of excellent, good, fair and poor quality images of the very distinctive short-finned pilot whale number 112. Bottom (left to right): examples of very distinctive, distinctive, slightly distinctive and non-distinctive individuals.

A photo quality to the best image of each capture and a distinctiveness category to each catalogued whale were assigned an independent 1-4 rating following Baird *et al.* (2009); consisting of poor, fair, good or excellent quality, and as non-distinctive, slightly distinctive, distinctive or very distinctive, respectively (Figure II.2). The photo quality rating was based on focus, clarity and contrast of the image, and on the angle and size of the dorsal fin relative to the frame (Friday *et al.* 2000). Captures featuring photo quality 3-4 were defined as high-quality (HQ) images. Non-distinctive individuals were based only on scars or on atypical fin shapes, slightly distinctive individuals had few small notches along the trailing edge, distinctive individuals had multiple notches or at least a big one in the dorsal fin, and very distinctive individuals had similar types of markings but to a greater extent. Individuals with distinctiveness 3-4 were defined as well-marked (WM). Unless otherwise stated, this study used only WM-individuals and HQ-images, thus increasing the certainty of matches. The coverage of the proportion of captures per encounter, calculated as the number of individuals captured divided by the number of individuals in the group, was graded as: low-coverage if this proportion was <0.3, medium-coverage if ≥0.3 and <0.8, high-coverage if ≥0.8 and <1, and full-coverage if =1.

Whales were classified in age categories based on the comparison of the relative size of individuals using photographs featuring multiple individuals as well as using field notes, according to descriptions by Yonekura *et al.* (1980) and Kasuya & Marsh (1984).

This study considered immature whales as (calves or juveniles) individuals that had a lighter (grey) color without a pronounced melon, and that were <⅔ the length of the adult individual that it was in close association with. Sub-adults were individuals slightly smaller than adults. Due to the difficulty to distinguish between sub-adult males and adult females, the latter were considered only when observed constantly in the presence of an immature.

Photographic analysis

Three residency patterns were considered based on the individual capture histories. Individuals that exhibited multi-year and year-round site fidelity (captured ≥5 times in at least 3 years and 3 seasons, i.e. spring, summer, autumn and winter) were termed residents; individuals captured only once were termed transients; and individuals that fell between these thresholds were considered temporary emigrants/immigrants or regular visitors and thus termed visitors. Exceptions were made to few cases when individuals were captured only twice within ≤8 days interval, even if in two seasons. These animals were also considered transients since they (1) were probably only passing through, and (2) presented a clear distinct residency pattern from the visitors, that were commonly captured (more than twice) yearly and/or within one or two seasons. Immatures captured only once were assigned the same residency pattern as the respective adults that accompanied them.

The proportion of WM-individuals within groups was estimated using only data from encounters with full-coverage obtained during surveys. A one-way ANOVA and a post-hoc Tukey test were used to check if significant differences existed in the proportion of WM-individuals between groups composed exclusively of residents, of visitors or of transients. Similarly, the proportion of at least slightly distinctive individuals was also estimated to allow comparison with other studies. Estimations included encounters with small (3) and large (33) number of individuals, thus not biasing results to sampling only small or large groups, and reducing variance due to not sampling exclusively small groups. To estimate the rate of mark change due to the acquisition of new notches in the dorsal fin over time, the time between the first and last capture for all recaptured individuals was summed and divided by the minimum and maximum number of mark changes that occurred (Aschettino *et al.* 2011).

Group size was determined at sea using only encounters from surveys, ensuring less biased counts. While the analysis of group size per month used data from encounters of all coverage, the analysis of group size per residency pattern was restricted to encounters with high and full-coverage. Additionally, a group featuring individuals of different residency patterns was termed mixed group, and in these cases, encounters of all coverage were also used (since a group with a minimum of two individuals of different residency patterns was already considered a mixed group). A two-way ANOVA test was used to check if significant differences in group size existed between months and type of groups (according to residency pattern). To test for a preferential period of occurrence of mixed groups, a binomial two-sample proportions test was used to assess if the proportion of mixed groups (compared to other types of groups, i.e. groups dominated by residents, by visitors or by transients) was significantly different between two predefined seasons, i.e. January-June vs. July-December. The Shapiro-Wilk and the Levene's tests were used for the ANOVA assumptions (α=0.05). All the preceding analyses were carried out using the R 2.14.2 statistical package (R Development Core Team 2012).

Associations

A social network diagram was created using NetDraw 2.119 (Borgatti 2002) to illustrate individual association patterns. All encounters were considered, but the ones resulting in a single individual were excluded due to not providing any linking information. Residency pattern was included as individual attribute.

The simple ratio (Ginsberg & Young 1992) and the half-weight (Cairns & Schwäger 1987) association indices were used to represent the strength of the behavioral relationships between dyads, where 0 means two individuals were never recorded associated, and 1 that they were always associated. Considering that the inferences drawn were similar and that the half-weight index is less biased when not all associates are identified (Whitehead, 2008), as it is in the case of this dataset, the results presented here concern the half-weight index.

Hierarchical cluster analysis was used to classify and illustrate relationships between the whales that were captured more often. Only whales captured on ≥4 encounters with high- or full-coverage were used to ensure a more accurate representation. Sampling period as well as associations was defined as individuals grouped within an encounter. Associations were calculated using the average-linkage method due to presenting the highest cophenetic correlation coefficient (CCC) when compared to single-, complete- and Ward's-linkage (not shown), and also due to being less affected by sampling error (Milligan & Cooper 1987). Knowing that dendrograms impose a hierarchical structured society for a valid interpretation (Whitehead 2008), the CCC was considered (where a value between 0.8 and 1 is a good fit; Bridge 1993) and the preferred/avoided associations were tested using permutation tests (Bejder *et al.* 1998; Whitehead 1999). The null hypothesis was that individuals associate with the same probability with all other individuals, given their availability. Observed SD and CV of the pairwise association indices that were significantly larger than those from permuted datasets were taken as evidence that individuals preferred or avoided companions (Whitehead 1999). To ensure that *P*-values were stable, it were generated 1000, 10000 and finally 20000 permutations, and performed three runs using the simple ratio plus three using the half-weight association index. Modularity-G (Newman, 2004) and knot-diagram (Wittemyer *et al.* 2005, Whitehead 2008) methods were used for population division. The resulting social entities were defined as 'pods' (Bigg *et al.* 1990, Heimlich-Boran 1993), and an alphanumeric ID was assigned.

Standardized lagged association rates (SLAR) were used to address temporal patterning in social relationships (Whitehead 1995). To interpret SLAR the null rates were also considered, which were the values animals would have if associated randomly (see Whitehead 1995 for equations). Moving average was chosen as that which best adjust between precision and smoothing. SLAR were plotted on a log x-axis time lag scale since time differences between sampling periods ranged over an order of magnitude. Data from encounters with high- and full-coverage were used, independently of the number of captures. Sampling period was defined as day, and associations as individuals grouped within an encounter. The precision (SE) was estimated using the temporal jackknife method on each sampling period (Whitehead 2008). Exponential models representing simulated social structures (Whitehead 1995) were fit to the SLAR (Table II.1). According to Whitehead (2008), the best fit model was chosen as that which minimized the Quasi Akaike Information Criterion (QAIC). All association analyses were performed with the compiled version of SOCPROG 2.4 (Whitehead 2009).

Table II.1. Fit of social system models to the standardized lagged association rate (SLAR) for all individuals. τ is time lag in days. CA – casual acquaintances, CC – constant companions. Models ranked by the Quasi Akaike Information Criterion (QAIC); the lowest indicates the best-fitting model. CA is just one interpretation of the SLAR pattern (according to Whitehead 1995) and not appropriate in this case where there are long-term relationships falling off only after about three years.

Description of model	Model formula	Number of parameters	QAIC
CA	$0.092309\, e^{(-0.0003214\,.\,\tau)}$	2	3973.4
Two levels of CA	$4.2545\, e^{(-3.8546\,.\,\tau)} + 0.091674\, e^{(-0.0003165\,.\,\tau)}$	4	3975.7
CC + CA	$0.069697 + 0.19069\, e^{(-0.24657\,.\,\tau)}$	3	4001.9
CC	0.071094	1	4006.6

DNA analyses

Thirty-two skin samples were collected in September-November 2004 and in February, September and October 2005 using a biopsy darting system (cf. Mathews *et al.* 1988). Samples were collected from 14 groups, most were from adult individuals, and the number of samples per group did not exceed 33.3% of the group size. Details on sampled groups are given in Table II.1SM (see 'Supplementary Material'). Samples were stored in 90% ethanol and processed at INETI, Lisbon, Portugal. DNA extractions were performed using the DNeasy tissue isolation kit (Qiagen) following the manufacturer's protocol. About 1-2 mm^3 of skin were minced and rinsed in dd-water. Digestion by recombinant proteinase K was extended overnight at 56 °C.

Part of the tRNA-Thr, the tRNA-Pro and the most variable part of the mitochondrial control region were amplified using the primers Dloop-16^L (Hoelzel *et al.* 1991; *n*=24 short sequences) and H00034 (Rosel *et al.* 1994) or Dloop-19^H (Hoelzel *et al.* 1991; *n*=5 long sequences). PCR reactions were carried out in a 25 µl volume using 0.75 units of *Taq* DNA polymerase (MBI Fermentas) in KCl buffer with 2 mM $MgCl_2$ and 0.3 µM of each primer. The number of cycles was set to 35 and the annealing temperature to 52 °C. PCR products were purified with the GFX PCR DNA purification kit (Amersham Biosciences). Sequencing was done on an ABI-prism capillary sequencer (Applied Biosystems) by Macrogen, Korea. All samples were sequenced with the 16^L primer, using an annealing temperature of 55 °C. Five randomly selected samples were also sequenced with the reverse primer, and amplification and sequencing were repeated for two randomly chosen samples, and no mismatches were found. All sequences were carefully checked for errors by visual inspection of peak heights with Chromas Lite 2.01 (freeware available on-line at http://www.technelysium.com.au/chromas.html). Dubious nucleotides were systematically verified. Alignment was performed visually and no gap was found. Short sequences were of 609 base pairs (bp) and long sequences of 999 bp.

Eight polymorphic dinucleotide microsatellite loci were analysed. PCR reactions were performed in multiplex, using a touch-down protocol with a temperature decrease of 0.1 °C per cycle over 25 cycles. The initial annealing temperature was set at 52 °C for Sw10 and Sw19 (Richard *et al.* 1996), and 56 °C for EV37 (Valsecchi & Amos 1996), FCB1 and FCB17 (Buchanan *et al.* 1996), as well as for Mk6 (Krützen *et al.* 2001) and d22 (Shinohara *et al.* 1997). Locus 468/9 (Amos *et al.* 1993a) was amplified separately, with a fixed annealing temperature of 45°C. Fragments were scanned on an ABI 310 capillary sequencer using the size marker ROX350 (Applied Biosystems). Molecular sexing was performed by co-amplification of a short fragment of the male-specific SRY gene (CSY, 157 bp; Abe *et al.* 2001) and a tetranucleotide microsatellite used as a PCR control for positive identification of females (GATA028; Palsbøll *et al.* 1997), using a touch-down protocol with an initial annealing temperature of 52 °C. Unsuccessful PCR reactions were repeated up to three times. DNA extraction and genotyping were repeated whenever a sample was found not to amplify at three or more loci. Samples that failed this second attempt (*n*=2) were removed from the data set. Three randomly chosen samples were genotyped twice to verify that results were reproducible.

Identification of replicated samples

Genotypes were checked for potential errors and replicated individuals using Microsatellite Tools (MsTools; Park 2001). Allowing for a maximum of two mismatches between samples, one pair of potential replicates with identical genotypes was identified. Extraction and genotyping was repeated for these two samples. Genotype identity was confirmed and one of the two samples was removed from the dataset prior to analyses. There were also two pairs of individuals differing by three mismatches. The mismatching loci were re-analysed and the initial genotypes were confirmed.

Genetic statistical analyses

As the mtDNA analyses yielded a single haplotype, no statistical analyses were performed on the mtDNA sequences. As for microsatellites, it was verified that loci were not in linkage disequilibrium and each locus was tested for departure from Hardy-Weinberg frequencies using the exact test implemented in Genepop on the internet (http://genepop.curtin.edu.au/index.html; Genepop 3.3 by Raymond and Rousset 1995), with default settings. A sequential Bonferroni correction was used to compensate for multiple tests (cf. Rice 1989). Probability of genotype identity between unrelated and first-order related individuals, allelic diversity, observed (H_O) and expected (H_E) heterozygosity were calculated with GenAlEx v.6 (Peakall & Smouse 2006). Despite the small number of samples, an attempt was made to estimate genetic differentiation between resident and transient individuals by means of F_{ST} and R_{ST}

calculated with Arlequin 3.1 (Excoffier *et al.* 2005). Significance was tested by 10000 permutations. Pairwise relatedness coefficients between individuals were calculated using SPAGeDi 1.2d (Hardy & Vekemans 2002). The coefficient $r_{Q\&G}$ (Queller & Goodnight 1989) was selected for being widely used and readily interpretable. For each residency pattern, the mean degree of relatedness and its variance were calculated following Ritland (2000); significant deviation from zero was tested by 10000 permutations, using SPAGeDi 1.2d (Hardy & Vekemans 2002).

The distribution of pairwise relatedness coefficients within and between groups were plotted to search for potential first order relatives ($r_{Q\&G}>0.5$). Whether relatedness was higher within than between groups was tested using a permutation procedure, which allowed accounting for the fact that pairwise relatedness coefficients were not independent from one another. For that, Genetix 4.03 (Belkhir *et al.* 2001) was used to perform a Mantel test as suggested by Sokal & Rohlf (1995). It was searched for a correlation between a matrix of pairwise genetic relatedness ($r_{Q\&G}$) and a matrix of group membership encoded as 1 for pairs of individuals sampled in the same group and 0 for pairs of individuals not sampled in the same group. The number of permutations was set to 10000. Whether there was a correlation between mean intra-group relatedness and group size was also tested by means of a Spearman's rank test performed with Statistica 6.1. Finally, using all biopsied individuals that had been photo-identified, a correlation between pairwise relatedness and their half-weight index was searched by means of a Mantel test with 10000 permutations performed with Genetix 4.03 (Belkhir *et al.* 2001). Half-weight indexes were calculated for all biopsied individuals that had been photo-identified, independently of the number of times they had been encountered. Only HQ-images and groups with high- or full-coverage were included.

Results

Photo-id

Over 45000 photographs taken from 405 encounters resulted in 1114 captures and 364 WM-individuals based on HQ-images (Table II.2). The mean duration of each encounter, calculated as the time between the first and last photograph, was 27 min (SD=23, *n*=105) during surveys, and 14 min (SD=12, *n*=300) during whale-watching trips; totaling 7014 min of photo-id. The capture frequency histogram shows different capture probabilities; 71.7% of the individuals were captured only once and a subset of 10 individuals presented 25 to 33 captures (Figure II.3). The discovery curve slightly stabilized throughout the years but not completely by the end of the study, indicating that not all individuals using the area have been captured (Figure II.4). Encounters with 100% of recapture started being common after 2006 (≈350 captures). The sampling effort (number of captures) was higher in 2010 and 2011 (Figure II.4).

Table II.2. Total number of captures and individuals (in bold) and percentages of their respective categories for high-quality images and well-marked individuals.

Captures (total number)		**1114**
Photo quality	good	44.5%
	excellent	55.5%
Individuals (total number)		**364**
Distinctiveness	distinctive	52.2%
	very distinctive	47.8%
Age	adults	95.3%
	sub-adults	4.1%
	immatures	0.6%

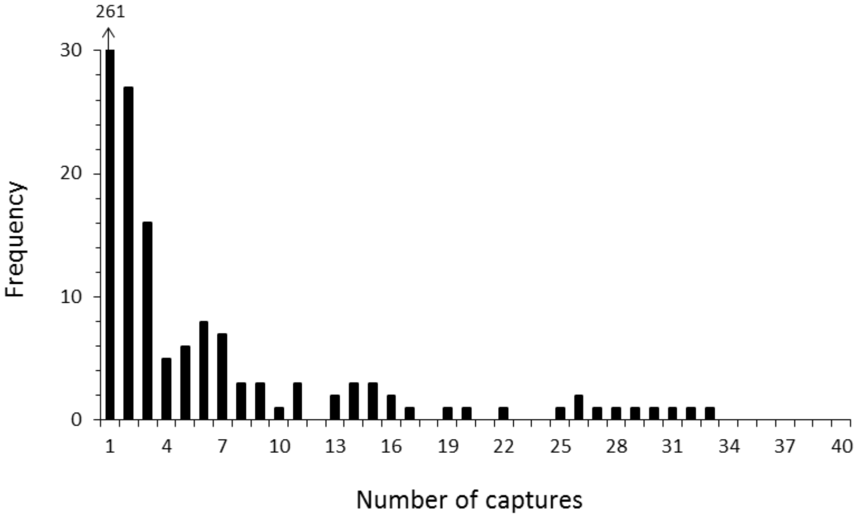


Figure II.3. Capture frequency histogram for well-marked individuals based on high-quality images. The y-axis was cut to facilitate visualization of the smaller frequencies.

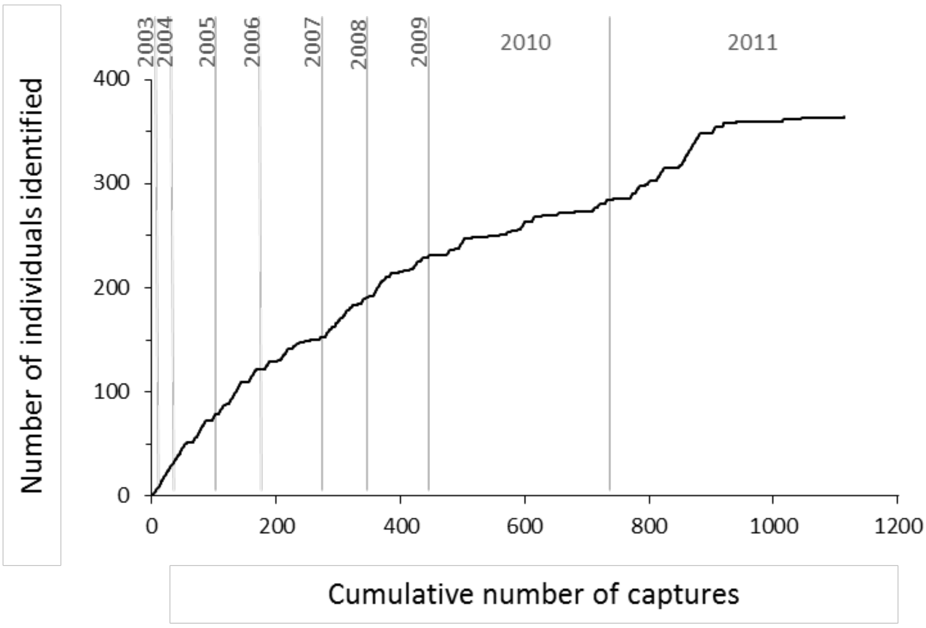


Figure II.4. Discovery curve for well-marked individuals based on high-quality images between 2003-2011 (years separated by vertical bars).

Of the 364 individuals, 28.3% (103 individuals) were recaptured. Of these, 82.5% (85) were captured in different years. The median inter-annual interval between the first and the last capture for these 85 individuals was 4 years (mean=3.9, SD=2.2, range: 1-8) for the study period (2003-2011). For the 103 recaptured whales, individuals captured in 2 years were the most frequent, >10 individuals were captured in 3 and 4 years, <10 individuals were captured in 5, 6 and 7 years, and one individual was captured in 9 years; indicating site fidelity for a subset of individuals (Figure II.5). The matching examination of 19 individuals from opportunistic surveys revealed that (1) one individual captured in October 1997 was an individual that had been captured yearly between 2004-2011, thus presenting a 14-year recapture interval, and (2) one individual captured in January and another one captured in May 2001 were recaptured in January 2011 and June 2010, presenting a 10- and a 9-year recapture interval, respectively.

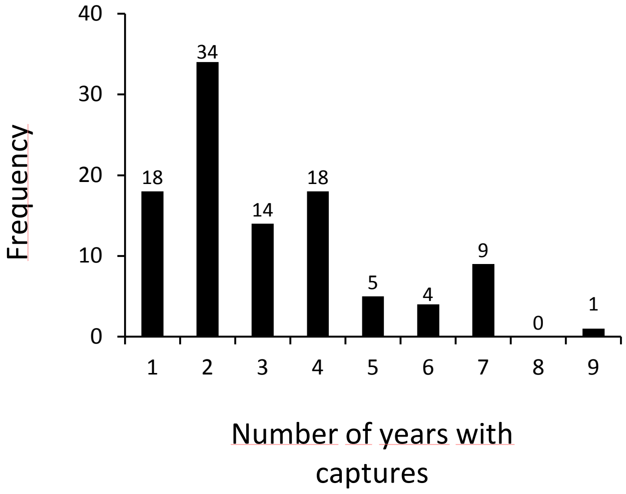


Figure II.5. Frequency of the number of years with captures for the 103 well-marked individuals recaptured between 2003-2011. Note that the maximum number of years an individual could be captured was 9.

Photographic analysis

Based on 32 groups, a mean proportion of 0.51 individuals (SD=0.24, range: 0.17-1) were WM and 0.78 (SD=0.16, range: 0.42-1) were at least slightly distinctive.

Proportions of marked individuals within groups composed exclusively of residents, visitors or transients, differed between groups (Table II.3). The inference tests showed that the proportions of marked individuals in groups composed exclusively of transients were significantly higher than those from groups composed exclusively of residents, when either considering WM or slightly distinctive individuals. Twelve groups including individuals of different residency patterns (mixed groups) were excluded (Table II.3).

Of the 103 WM-individuals with recaptures, 29 (28.2%) incurred new notches in the dorsal fin. Of these, 17 underwent multiple mark changes. Mark changes were recorded on 43 occasions, for a total of 52 new notches. Considering that multiple mark changes between recaptures could have occurred on either a single or multiple occasions, the actual number of mark change events was somewhere between 42 and 53. The cumulative time between the first and last capture for all individuals captured more than once was 125737 days (343.5 years), thus a new mark event was calculated to occur every 6.6-8.0 years (mean=7.3 years).

Table II.3. Mean proportions (±SD, range) of well-marked and of at least slightly distinctive individuals from groups composed exclusively of residents (R), of visitors (V), and of transients (V); and *P*-values for the one-way ANOVA and post-hoc Tukey tests (* 0.01<*P*<0.05). *n* - number of samples.

	Well-marked individuals	At least slightly distinctive individuals	*n*
Residents	0.35 (±0.13, 0.22-0.50)	0.68 (±0.13, 0.56-0.83)	5
Visitors	0.49 (±0.16, 0.33-0.67)	0.79 (±0.11, 0.67-1)	7
Transients	0.66 (±0.22, 0.25-1)	0.89 (±0.11, 0.71-1)	8
all groups	0.51 (±0.24, 0.16-1)	0.78 (±0.16, 0.42-1)	32
one-way Anova	*P* =0.0259*	*P* =0.0159*	
post-hoc Tukey			
R - T	*P* =0.0222*	*P* =0.0125*	
R - V	*P* =0.3983	*P* =0.2601	
V - T	*P* =0.2096	*P* =0.2229	

Pilot whales were captured in every calendar month. The mean group size was 18 individuals (median=15, SD=12, range: 2-60, *n*=105). Larger groups were recorded between May-October and were composed of individuals of different residency patterns (Figure II.6A-B), however, the two-way ANOVA test revealed no significant differences in group size between months (*P*=0.58), between type of groups (*P*=0.09), or due to the interaction of both factors (*P*=0.84). Groups dominated by resident individuals were naturally recorded year-round. Groups dominated by transients were also recorded year-round (although masked in the second semester by being recorded in mixed groups). Finally, groups dominated by visitors, as well as mixed groups, were proportionally more frequent between August-December (Figure II.7). The two-sample proportions test showed that the proportion of mixed groups (compared to other types of groups) was significantly higher (*P*<0.001) for the season July-December than for January-June. Despite the non-inclusion of the effort analysis in Figure II.7, the probability of encountering mixed groups or another type of groups was the same within each month. The different number of encounters used in each month does not reflect seasonal abundances. A spatio-temporal analysis taking effort in consideration is in preparation and will be published elsewhere. A closer examination of capture histories revealed that mixed groups included all types of possible associations between individuals of different residency patterns.

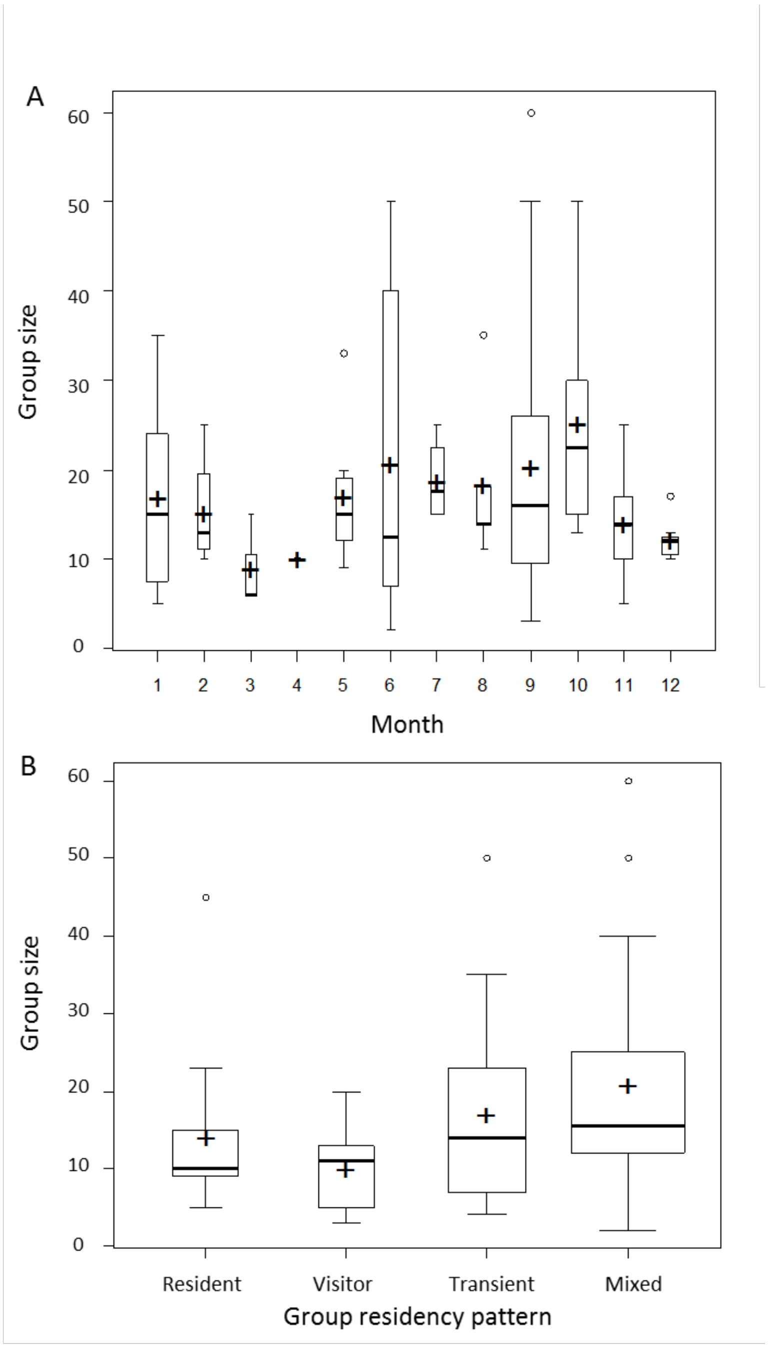


Figure II.6. A: boxplots of group size per month, using encounters from surveys (*n*=105). B: boxplots of group size according to group residency pattern (see definitions in 'Material and Methods'), using encounters where it could be applied a category to all (or almost all) individuals of the group (*n*=86). Middle bold lines indicate median values, + shows the mean, upper and lower lines of boxes encompass the spread of data from the first to the third quartile, and upper and lower horizontal bars show minimum and maximum group sizes unless outliers are present (○), in which case the horizontal bar is defined as the third quartile plus 1.5. The width of the bars is proportional to sample size: *n*=3 in March and *n*=35 in September; *n*=10 for visitors and *n*=42 for mixed. No significant differences were found for any factor (see text).

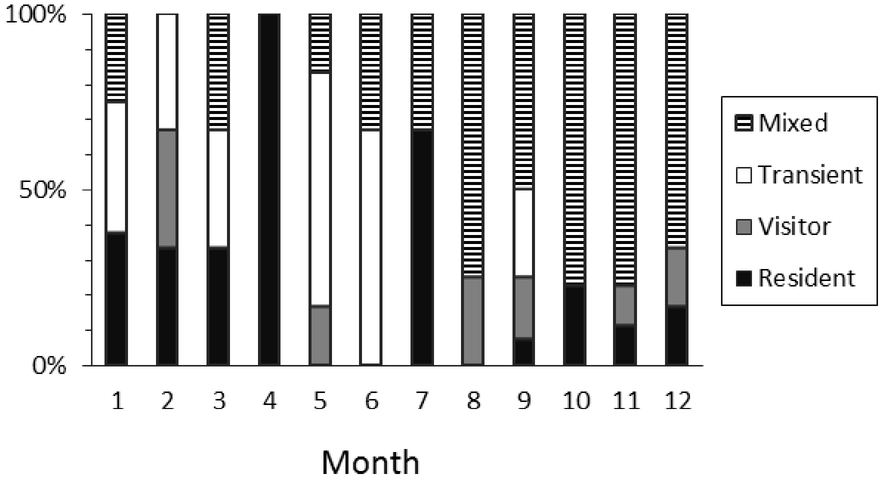


Figure II.7. Monthly relative percentage according to group residency pattern (see definitions in 'Material and Methods'). The number of encounters used was 8, 3, 3, 1, 6, 6, 3, 4, 28, 9, 9 and 6, respectively for January-December. Despite the unequal monthly effort, the probability of capture of each of these groups was equal within each month. The proportion of mixed groups (compared to other types of groups) was significantly higher for the season July-December than for January-June (binomial two-sample proportions test, *P*<0.001).

Associations

The social network diagram, based on 344 individuals from 246 encounters, shows that 124 individuals (36%) form a core social network and the remaining 220 individuals (64%) form satellite clusters (Figure II.8). The diagram comprises 39 (11.4%) residents, 72 (20.9%) visitors and 233 (67.7%) transients. While the main component includes all the resident individuals, but also visitors and transients, the 46 satellite clusters do not include any resident and were dominated by clusters of transients only (84.8%). Additionally, the satellite clusters include clusters of visitors only (6.5%) as well as clusters with both transients and visitors (8.7%).

The SD and CV of the observed pairwise association indices were significantly higher than those from permuted data sets, when using either the simple ratio or the half-weight index (*P*<0.001), thus rejecting the null hypothesis that individuals associated randomly. The cluster dendrogram (Figure II.9, CCC=0.96) shows that most of the whales captured on ≥4 encounters were captured with preferred companions, and one pair was always captured together (ID numbers 310 and 493 from pod V3, Table II.4). The mean of the half-weight association index per dyad was 0.77. The estimate of social differentiation was 1.87 (SE=0.16). A total of 44 individuals from 66 encounters with high- or full-coverage between 2005-2011 were used for this analysis, 33 (75.0%) residents and 11 (25.0%) visitors.

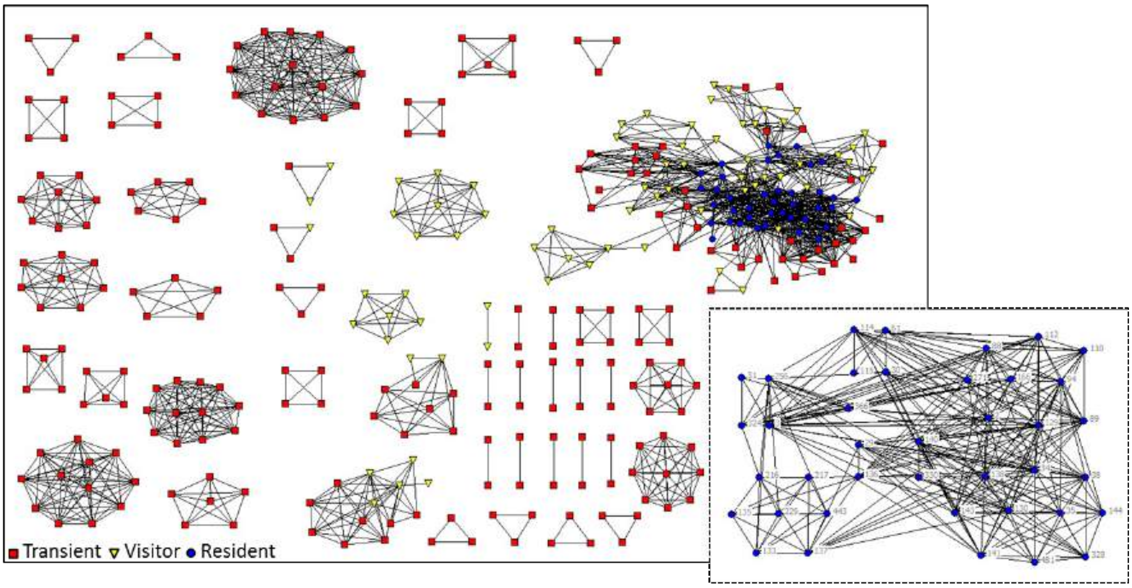


Figure II.8. Social network diagram showing associations of 344 well-marked pilot whales around Madeira Islands, using only high-quality images. Nodes correspond to individuals, while lines between nodes represent presence within a group. Symbol shapes and colors indicate individuals' residency pattern (see legend inside figure). One hundred and twenty four individuals (36%) are linked to a main cluster, which includes all the residents but also visitors and transients, and the remaining individuals form satellite clusters (mainly composed by transients). Bottom right: detailed view of the resident whales.

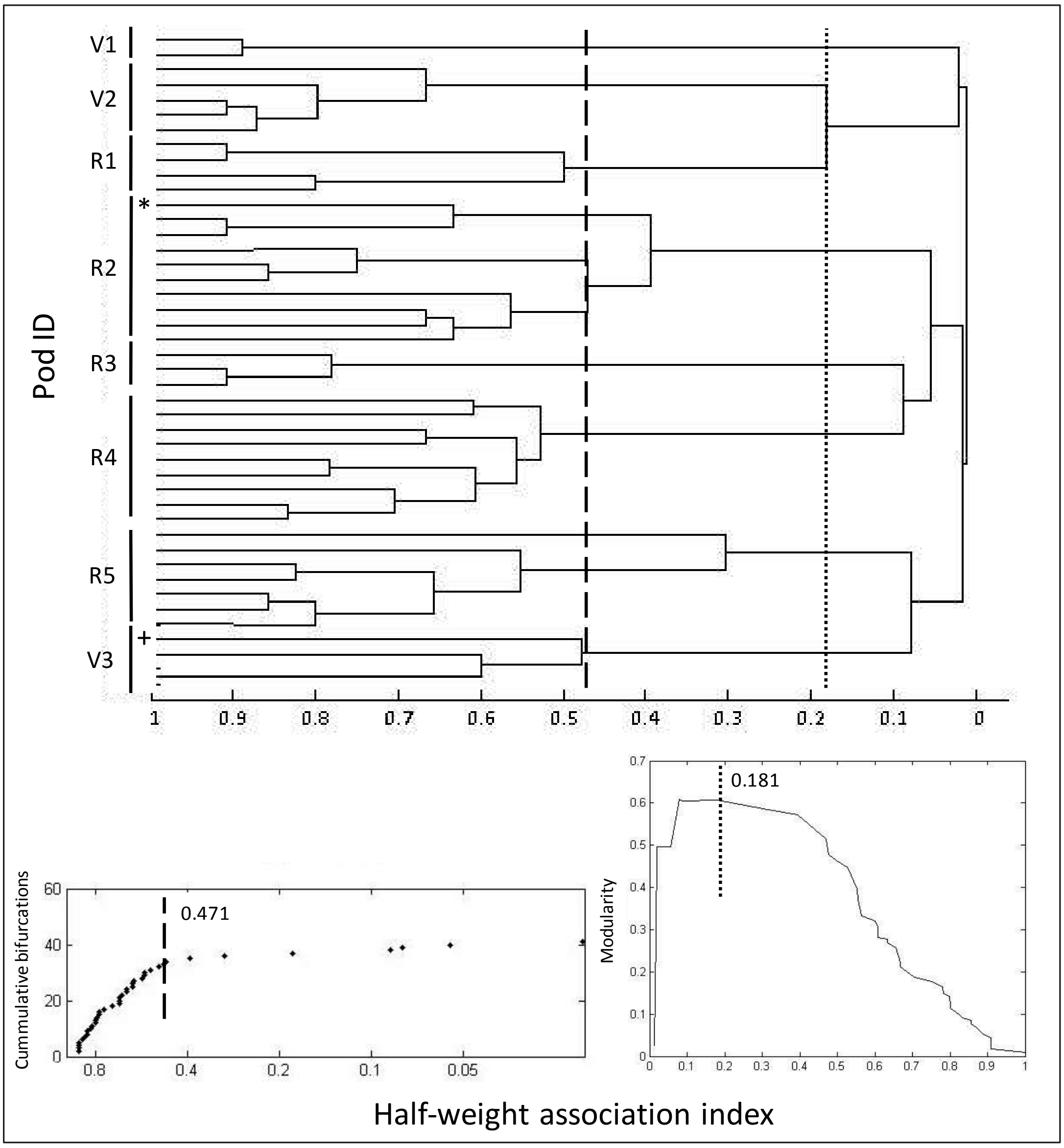
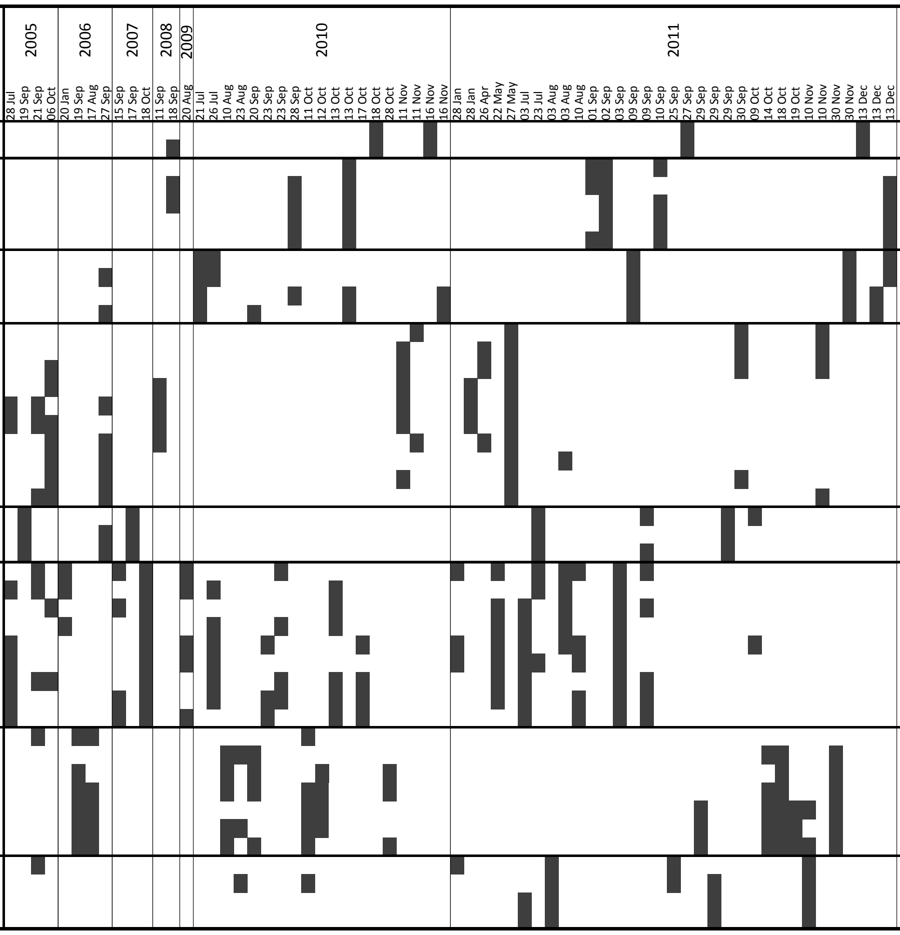


Figure II.9. Top: dendrogram produced using average-linkage hierarchical cluster analysis (CCC=0.96) for 44 well-marked pilot whales based on high-quality images captured on ≥4 encounters with high- or full-coverage. Sampling period as well as associations was defined as individuals grouped within an encounter. Eight pods composed either by residents (R) or by visitors (V) were identified, with exceptions in pods R2 and V3 where one visitor (*) and one resident (+) individuals (as defined in Material and Methods) were included, respectively (see individuals' attributes in Table II.4). Bottom left: the knot-diagram suggests a knot at an association index of 0.471 (dashed line). Bottom right: the modularity-G suggests that the best division into pods is with an association index of 0.181 (dotted line).

Table II.4. Attributes and capture histories (black cells mean presence and blank cells mean absence) of the individuals composing each resident (R) and visitor (V) pod, according to Figure II.9. a - adult, sa - sub-adult, f - female, m - male, # - number. Sex was determined by genetic analysis. * and + concerns to exceptions in residency pattern (see Figure II.9). Pods size was estimated using the correction factor calculated in this study; 0.49 for visitor pods and 0.35 for resident pods (see Table II.3).



Pod ID	Individual ID	Age class	Sex	# captures	Estimated pod size
V1	325	a		4	4
	321	a		5	
V2	473	sa		4	10
	433	a		6	
	380	a		6	
	379	a		5	
	308	a		6	
R1	324	a		5	11
	003	a		6	
	031	a		7	
	256	a		8	
R2	538*	a		4	29
	481	a		5	
	144	a		6	
	035	sa		5	
	034	a		7	
	138	a		7	
	038	a	m	6	
	143	a		4	
	141	a		5	
	130	a	f	5	
R3	061	a	m	6	9
	030	a		5	
	114	a	m	6	
R4	089	a		13	26
	002	a		10	
	094	a		9	
	166	a		9	
	088	a		13	
	271	sa		10	
	129	a		12	
	112	a		13	
	110	a		11	
R5	137	a		4	20
	229	a		6	
	443	a		7	
	216	a		10	
	217	a		10	
	135	a	f	11	
	133	a		12	
V3	136+	a		5	8
	383	a		6	
	493	a		4	
	310	a		4	

When assessing population division into social entities, the modularity-G suggests that the best division is with an association index of 0.181, and the knot-diagram suggests a knot at an association index of 0.471 (Figure II.9). The modularity-G peak (0.608) is high, showing that with this division, there is much more total association within pods than would be expected for randomly determined pods. The modularity-G was the selected method for pod division due to its high maximum value and due to the subjective interpretative nature of the knot-diagram. Therefore, a total of eight pods of variable size and association strength were identified, five being composed by resident individuals (R1-5) and three by visitors (V1-3). Exceptions occurred in pod R2 where one visitor individual is included, and in pod V3 where one resident individual is included (Figure II.9, Table II.4). A mean pod size of 15 individuals (SD=9, range: 4-29) was estimated including marked and non-marked individuals by applying the previously estimated correction factors of the proportion of well-marked individuals to resident and visitor pods (Table II.4). A one-way ANOVA revealed no significant differences (*P*=0.38) between the pod and group mean sizes.

A total of 90 individuals comprising residents, visitors and transients captured during 66 encounters (corresponding to 53 days between 2005-2011) were used to address temporal pattern in social relationships between all individuals in the study area. The SLAR line never reached the null association rate, indicating the absence of random associations for the time scale of ≈2000 days. The line started to decline only after about three years (Figure II.10). The best fit model also indicates a social system with long-lasting relationships. Therefore, the fitted model described as ‘casual acquaintances’ is not appropriate in this case (Table II.1). To address temporal pattern in social relationships between individuals of a resident pod the analysis was restricted to the nine individuals from pod R4 (the pod with more captures). The social system model that best fit the curve is described by the model including the constant companion hypothesis over this period (Figure II.1SM).

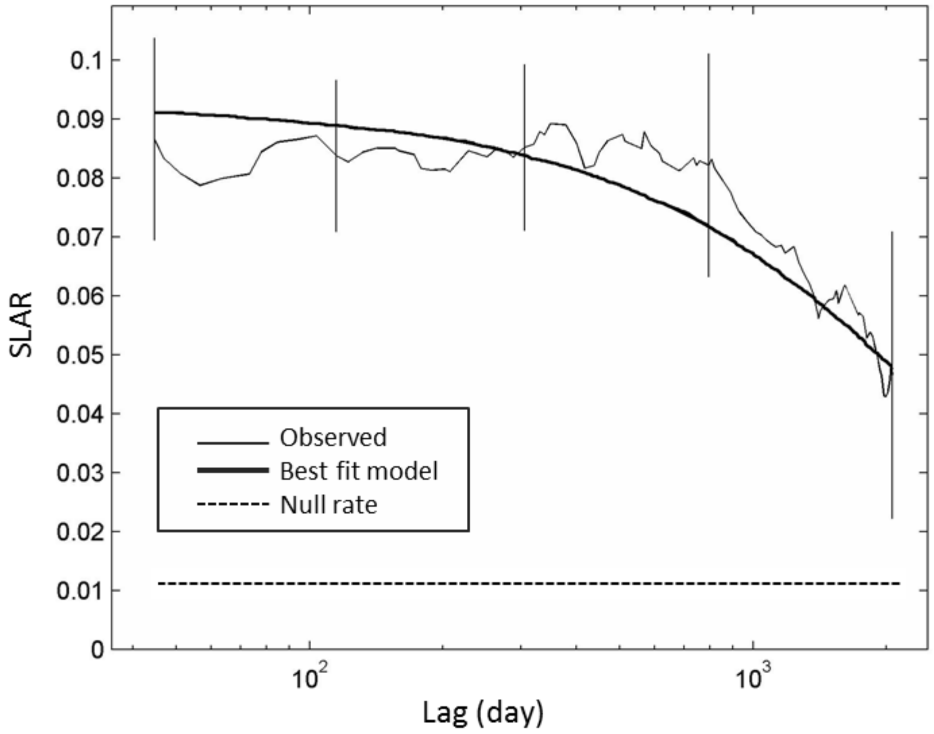


Figure II.10. Standardized lagged association rate (SLAR) for 90 well-marked pilot whales captured between 2005-2011. It was used only high-quality images from encounters with high- or full-coverage, and it was chose a moving average of 8000 associations. Sampling period was defined as day, and associations as individuals grouped within an encounter. Vertical bars indicate SE calculated using the temporal jackknife method on each sampling period. The best fit model indicates a social system with long-lasting relationships. The null association rate represents the theoretical SLAR if individuals associated randomly.

Genetic analysis

Among the 29 distinct samples successfully analyzed, 12 were females and 17 were males. Females and males of all three residency patterns were recorded (Table II.1SM). All short and all long sequences were identical. The haplotype corresponding to the short sequences perfectly matched the haplotype corresponding to the long sequences (GenBank accession numbers JX144881 and JX144880, respectively). Thus, haplotype diversity was null. Microsatellite allelic diversity ranged between 4 and 11 (Table II.5). Average expected heterozygosity ranged between 0.423 and 0.831. No loci departed from HWE. Loci were not in linkage disequilibrium, except for FCB1 and FCB17

(*P*<0.001). The probability of genotype identity was 3.8 e^{10-7}, and 2.6 e^{10-3} for first order relatives.

Table II.5. Locus-specific microsatellite information: allelic diversity (K), observed (H_O) and expected (H_E) heterozygosity, and probability of departure from Hardy-Weinberg Equilibrium (HWE, exact test *P*-value).

Locus	K	H_O	H_E	HWE (*P*)
D22	5	0.414	0.423	0.305
EV37	7	0.552	0.687	0.077
FCB1	11	0.828	0.831	0.445
FCB17	8	0.793	0.725	0.567
Mk6	4	0.655	0.565	0.691
Sw10	4	0.552	0.458	0.237
Sw19	8	0.621	0.698	0.330
468/9	5	0.679	0.620	0.641
Mean	6.5	0.636	0.626	
SD	2.4	0.135	0.138	

Genetic differentiation between individuals identified as resident (*n*=13) and transient (*n*=6) was not significant (F_{ST}=0.015, *P*=0.181; R_{ST}=-0.020; *P*=0.611). As for relatedness, mean $r_{Q\&G}$ was 0.004 (SE=0.238) among individuals identified as resident, -0.054 (SE=0.307) among those identified as transient and -0.037 (SE=0.246) in the entire data set. Related individuals ($r_{Q\&G}$>0.1) tended to be over-represented within groups (Figure II.11). Noticeably, individuals with $r_{Q\&G}$>0.4 represented 12.1% of intra-group pairwise comparisons, while between groups their proportion was only 1.9%. In the entire dataset, there were three pairs of individuals with $r_{Q\&G}$>0.5, which could be considered as potential first order relatives. A resident adult female (ID 116) and a resident subadult male that were sampled in the same group had $r_{Q\&G}$=0.745. They shared one allele in common for each microsatellite locus, indicating that they could be mother and offspring. There were also a resident female and a transient male with $r_{Q\&G}$=0.500 and a resident male and a male of unknown residency pattern with $r_{Q\&G}$=0.574, which had not been sampled in the same groups. These individuals could not be parent and offspring as they did not share one allele in common at every loci.

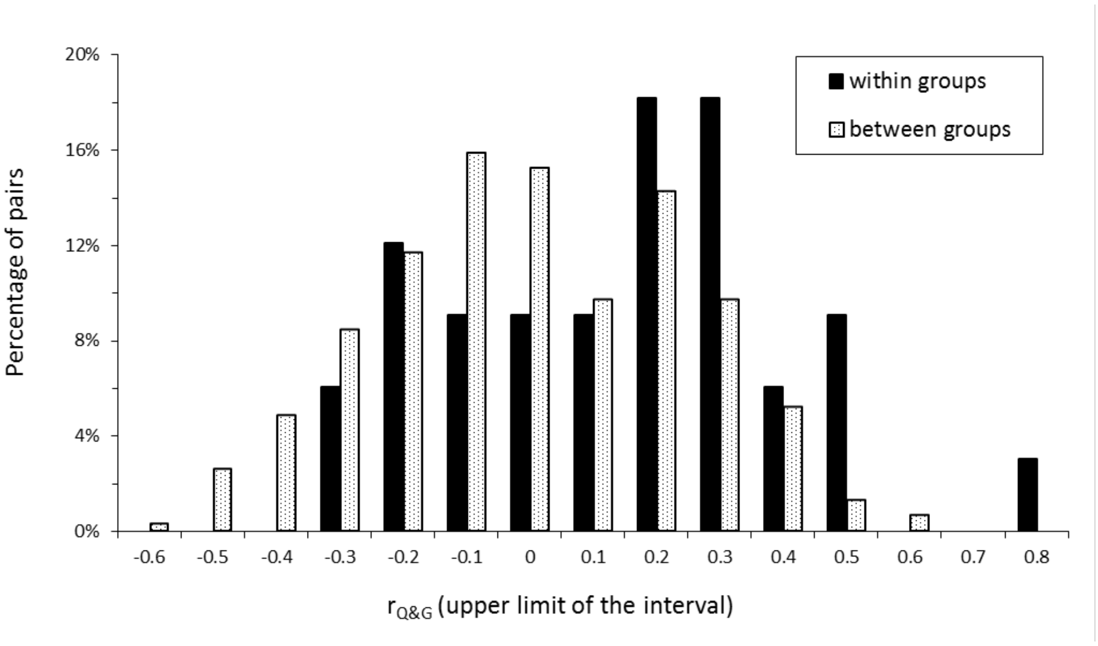


Figure II.11. Distribution of pairwise relatedness coefficients within groups (*n*=33) and between groups (*n*=308).

Mean $r_{Q\&G}$ within groups (mean=0.097, SE=0.265) was higher than between groups (mean=-0.016, SE=0.237). The Mantel test revealed a significant correlation between genetic relatedness and group membership (Pearson *r*=0.161, *P*=0.003). Mean intra-group relatedness was negatively correlated to group size (Spearman's rank coefficient, *n*=8, *r*=-0.807, *P*<0.05; Figure II.12). Mean relatedness was high (around 0.3) in groups of up to 30 individuals, and low (below 0) in groups of 45-50 individuals. In a group of 50 resident individuals where six samples were obtained, average relatedness was low, but two subgroups with a mean relatedness of 0.148 (first two samples) and 0.068 (last four samples) could be defined. In a group of 45 resident individuals where three samples were obtained, the first sample that was obtained was unrelated to the last two, while the latter two presented a mean relatedness of 0.251. Finally, there was a significant correlation between pairwise relatedness coefficients and half-weight indexes for the 12 individuals that had been biopsied and photo-identified (Mantel test: Pearson *r*=0.380, *P*=0.002).

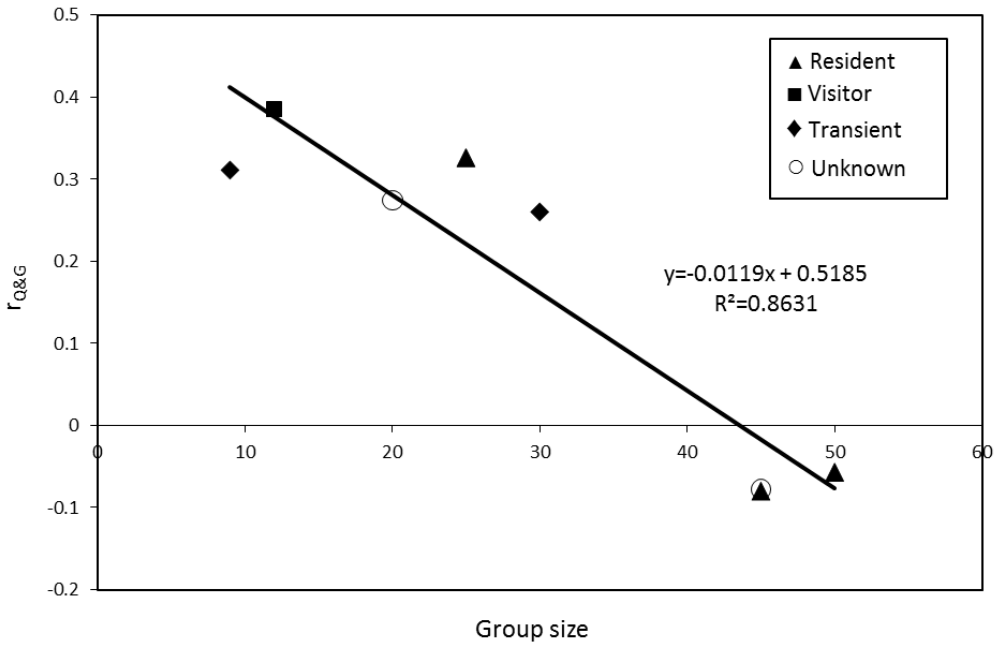


Figure II.12. Correlation between mean relatedness within group and group size. Symbols represent group residency pattern.

Discussion

This study shows that pilot whales demonstrate a large degree of variability in site fidelity, including residents, regular visitors and transients. These differences in patterns of occurrence were not due to a sampling bias since whales from the three types of residency pattern were recorded over the entire course of the study and all were highly distinctive, thus having similar probabilities of being recaptured. The fact that some resident individuals have >30 recaptures, and that one resident individual had a 14-year recapture interval, suggests that some short-finned pilot whales exhibit long-term site fidelity in Madeiran waters. Nevertheless, the range of their distribution is unknown, and findings outlined here do not imply that they were always present in the study area. The fact that the visitors regularly visit (and not just pass by) Madeira suggests that these animals come to these waters for either mating and/or feeding. Transients, on the other hand, were captured throughout the entire course of the study and year-round and this suggests that they belong to an open pelagic population that is far from being fully catalogued, and they use the Madeiran waters as a 'passage'. The analysis of the social network diagram shows that these whales associate with visitors and residents during their passage. Independently of the importance of the Madeiran waters for these whales, they constituted about ⅔ of our catalogue. Heimlich-Boran (1993) also reported whales captured only once in the neighboring archipelago of the Canary Islands. The extent of this 'meta-population' is unknown, and it is likely that some of these whales visit both archipelagoes. As suggested by Heimlich-Boran, perhaps they simply return at intervals greater than the length of the study. In fact, in the present study, two whales catalogued as transients had a 10- and a 9-year recapture interval; which may comply with the previous suggestion. Varying patterns of residency have been also commonly documented among cetacean populations (Silva *et al.* 2008, Baird *et al.* 2009), however, for pilot whales, only Mahaffy (2012) had described a pattern of habitat usage similar to ours.

The results from mark rate support that we are facing different communities. When comparing the proportion of marked individuals between groups composed exclusively of residents, visitors or transients, it was found that groups of transients have more distinctive individuals than groups of residents ($P<0.05$). Not discarding bias potentially associated with the analysis, individuals of all ages and both sexes had the same capture probability between each residency pattern, thus natural differences can in fact occur. The reason why transients may be more likely to bear dorsal fin notches is beyond the scope of this study. To our best knowledge, this is the first study testing the distinctiveness of individuals according to residency pattern, and this question should be addressed in future photo-id studies where groups of different residency patterns co-occur.

Our results suggest that, despite the existence of three residency patterns in pilot whales around Madeira, resident whales are not isolated from visitors and transients.

Mixed groups of individuals with different residency status were frequently observed between July and December, and the social network diagram shows that whales of different residency pattern were photographed together. The genetic analyses did not reveal any differentiation between resident and transient whales, neither for mitochondrial-DNA nor for nuclear-DNA. These analyses are preliminary given the low number of biopsied whales with known residency pattern. Lack of genetic differentiation will have to be confirmed by the analysis of a higher number of individuals of the three residency patterns. It is noteworthy that mitochondrial-DNA haplotype diversity was null. Although short-finned pilot whales are known to have very low mtDNA diversity, which is likely to result from their matrilineal social structure (Whitehead 1998), this complete lack of diversity was unexpected. It suggests a common ancestry for all biopsied whales, independently of their residency pattern.

The results from group size also support that we are facing one community in the islands and others that move and breed when they meet. As observed in various studies performed year-round in other areas, large groups tend to occur at a specific season of the year (e.g. Felleman *et al.* 1991, Cañadas & Sagarminaga 2000, de Stephanis *et al.* 2008). Heimlich-Boran (1993) reported that larger groups of short-finned pilot whales occurred during the summer months in the Canary Islands. His findings are in accordance with ours suggesting that, in the warm-temperate waters of the north-east Atlantic, larger groups of this species occur in the warmer months of the year. The reason for such aggregations could be linked to foraging behavior, as evidenced for this species and Risso's dolphins *Grampus griseus* off California (Shane 1995). However, there is no sufficient information about prey availability to infer a similar association for pilot whales in Madeira, nor for the Canary Islands (Heimlich-Boran 1993). An alternative and non-exclusive hypothesis is that whale aggregations are linked to breeding behavior. The season of larger group size corresponds to the likely mating period observed in the Canary Islands (Heimlich-Boran 1993). Similarly, in Madeira, the observation of larger groups coincides with an increase of visitors and a statistically significant higher proportion of mixed groups during the warmer months, making it possible for individuals of different residency patterns to breed.

Furthermore, in a matrilineal species, increasing group sizes is believed to occur mainly for reproduction, as suggested for aggregations of long-finned pilot whales (de Stephanis 2007). Recently, satellite tagging of different clans of pilot whales in the Mediterranean Sea also supported the hypothesis that clans interact for mating purpose when they find each other (Verborgh *et al.* 2012). Finally, the estimated mean pod size (15, SD=9, range: 4-29, *n*=8) was lower (but not statistically significant) than the mean group size (18, SD=12, range: 2-60, *n*=105). While the low number of pods analyzed limits our interpretation, this discrepancy could arise from temporary associations between pods. The means of pod and group sizes obtained in this study are in agreement with observations made in other pilot whale populations (Heimlich-Boran 1993, Ottensmeyer & Whitehead 2003, de Stephanis *et al.* 2008, Mahaffy 2012).

This study shows that pilot whales in Madeira exhibit a well-differentiated society with long-lasting relationships. The results of the social analysis rejected the null hypothesis that individuals associated randomly and the dendrogram revealed that most of the whales captured on ≥4 encounters were with preferred companions. The temporal analysis also indicated an absence of random associations for the time scale of ≈2000 days (six years). Additionally, despite the SLAR decline, a detailed examination of the capture histories reveal that some resident individuals have been frequently captured together during the six years period considered for the temporal analysis, as corroborated by the SLAR of the pod R4 that fit a constant companion model, with no disassociations over that period. The SLAR decline after a stabilization of approximately two-three years indicates some gradual disassociations over that period, which may indicate a demographic event where individuals are dying or leaving the identified population (Whitehead 2008). Another explanation is that, when a newborn arrives, the relationship between a mother and a previous calf decreases with time. Considering that the weaning period in this species ranges between 2.5 and 5.5 years (Kasuya & Marsh 1984), it could have contributed to the decline in the SLAR. The positive correlation between association indexes and genetic relatedness coefficients suggested that individuals who associated more tended to be genetically related. This preliminary result would be worth investigating by further analyses.

It was found that genetic relatedness was higher within groups than between groups and that highly related individuals tended to be over-represented within groups as compared to between groups. Mean intra-group relatedness was negatively correlated to group size. Although mean relatedness is naturally expected to decline with group size due to a dilution effect, the existence of a correlation implies that small groups are made up of related individuals. Interestingly, genetically related subgroups could be defined within two of the largest groups, which suggest that large groups may be temporary associations of two or more unrelated groups. Similarly, a genetic study on striped dolphins in the Ligurian Sea showed that average relatedness significantly decreased with group size, and concluded that large groups were aggregations of smaller social units (Gaspari *et al.* 2007). Also in that study, mean relatedness within groups was higher than between groups, especially in females where $r_{Q\&G}$ reached a mean value of 0.221.

Our results indicate that the pods outlined here represent stable social entities and suggest that short-finned pilot whales may exhibit natal group philopatry, a type of stable matrilineal social structure implying relatedness between members of both sexes within the natal group, as described in killer whales in the coastal eastern North Pacific (Bigg *et al.* 1990, Barrett-Lennard 2000). The number of samples available for the genetic analyses was too small to compare relatedness between genders. Mean relatedness coefficients obtained for resident as well as for transient individuals were low, and much lower than in populations of killer whales exhibiting philopatry in both sexes, where mean $r_{Q\&G}$ ranged between 0.102 in transient and 0.305 in resident populations (Pilot *et al.* 2010). Nonetheless, this study tends to corroborate the hypothesis described by Heimlich-Boran (1993), that the short-finned pilot whale has a matrilineal hierarchical system. The temporal analysis, fitting a constant companion model to a resident pod, also supports the hypothesis that we are facing a matrilineal system. These findings, together with the evidence that long-finned pilot whales also exhibit a matrilineal structure (Amos *et al.* 1991a, 1991b, 1993a, 1993b, Fullard 2000) support that *Globicephala* spp. follow this social pattern.

The final goal of this study is to discuss the implication of our findings towards the long-term conservation of this species and the possible management measures that could be implemented. If several populations had found, or at least one of island-associated/residents and other(s) of oceanic/transients, those would require a high level of concern, with intensive monitoring and possible severe conservation measures, given that population differentiation would prevent gene flow and not enable the island-associated/residents to recover from dramatic events. Instead, the results suggest potential gene flow between different types of communities, which would prevent genetic divergence of island-associated communities. Therefore, there might be a single population in the warm-temperate oceanic waters of the north-east Atlantic. From a conservation standpoint, this ‘meta-population’ can be regarded as a single ‘management unit’, whose preservation can be of extreme importance as it may act as a pool for resident communities. Similar studies should be conducted in the neighboring archipelagoes of the Canary and Azores to determine whether there is a need for concerted conservation policies at the scale of the North Atlantic. On the other hand, the presence of island-associated whales, whether residents or regular visitors, imply a high risk of exposure to local threats, which can also result in implications for the whole population. Even if it is considered that different communities should not be regarded as demographically independent populations, these island-associated whales should be considered as stable social entities in governmental management plans. Therefore, an assessment of the potential threats should be carried out, as well as a periodic evaluation of the status of these social entities. Due to the growing whale-watching industry, proper legislation and/or educative workshops with the operators are also recommended, given that it could minimize the impact over those entities.

To conclude, it would be interesting to raise an often debated but still unsolved question; what is a social entity, or a conservation unit? The social entities observed in the present study were defined as ‘pods’. However, the biological significance of pods has been questioned. The term ‘pod’ that has been used by Bigg *et al.* (1990) to describe groups of closely related matrilines of killer whales that travel, forage, socialize and rest with each other ≥50% of the time, and are stable over many generations, has been later redefined by Ford & Ellis (2002) as transitional groupings that reflect the relatedness of recently diverged matrilines. The clusters interpreted here and by Heimlich-Boran (1993) as meaningful pods are likely the closest equivalent (since it satisfies the criterion of being associated ≥50% of their time) to 'units' in other studies characterizing stable social entities of pilot whales (Ottensmeyer & Whitehead 2003, de Stephanis *et al.* 2008, Mahaffy 2012). Following the suggestion that the social structure of pilot whales is based on clans (i.e. pods which associate regularly; de Stephanis 2007, de Stephanis *et al.* 2008), the results from the hierarchical cluster analysis suggest possibly three clans of island-associated whales, one made by pods V1, V2 and R1, another by R2, R3 and R4, and a possible third one by R5 and V3.

It is then proposed that the pilot whales encountered in Madeira belong to a single population encompassing several clans, possibly three clans of island-associated whales and others of transients, each containing two to three matrilineal pods, each with a mean of 15 individuals (SD=9, range: 4-29). Given the difficultly in identifying these pods at sea, it is suggested that all clans, whether composed of island-associated or of transient whales, should be treated equally as a precautionary approach. Finally, further research in this species is encouraged due to the high proportion of marked individuals and low rate of mark change obtained in this study.

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Supplementary Material

Table II.1SM. Information on sampled groups: sampling date, group size (best estimate), group type (T: transient, V: visitor, R: resident), reference of photo-id individuals (ID), number of samples collected (M: male, F: female, All: total), and mean relatedness ($r_{Q\&G}$). Calves were observed in all sampled groups, except the group of 13 individuals of the 22/11/2004 for which the information is missing.

Date	Group size	Group type	Individual (photo) ID	M	F	All	Mean $r_{Q\&G}$
06-09-2004	20	T		1	0	1	
08-09-2004	9	T	049	1	2	3	0.311
08-09-2004	30	T		2	0	2	0.258
25-10-2004	20	?		1	2	3	0.274
03-11-2004	12	?		1	0	1	
22-11-2004	30	?		1	0	1	
22-11-2004	13	?		1	0	1	
23-11-2004	45	?		2	0	2	-0.078
22-02-2005	12	V	082, 085	1	1	2	0.385
19-09-2005	25	R	061, 114, 116, 182	3	1	4	0.326
21-09-2005	50	R	118, 130, 134, 135	2	4	6	-0.057
06-10-2005	45	R	038	1	2	3	-0.078

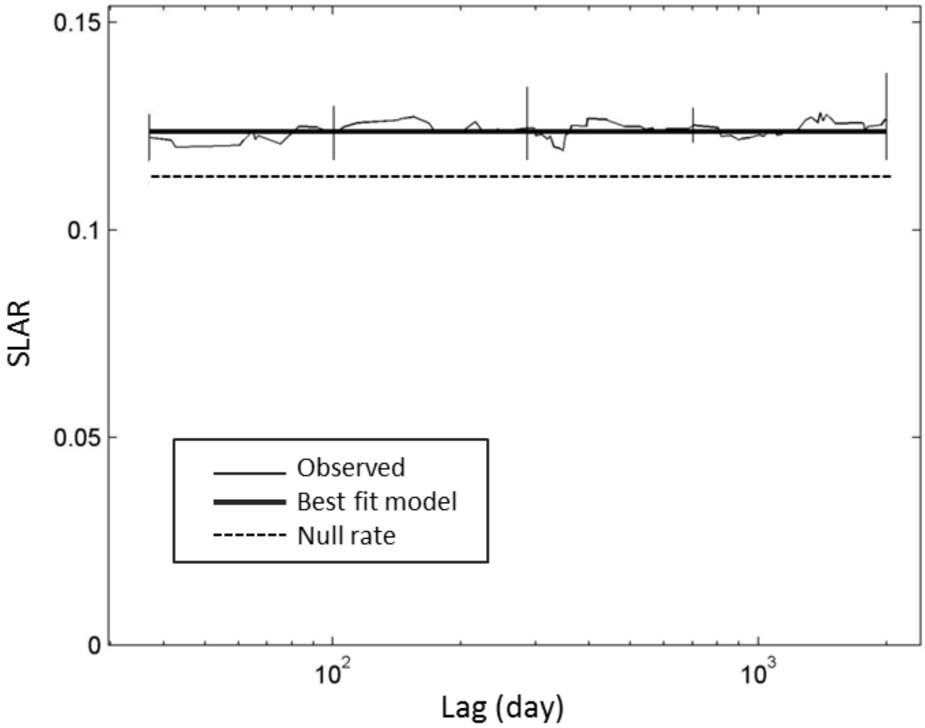

Figure II.1SM. Standardized lagged association rate (SLAR) for the nine resident (see definition in 'Material and Methods') well-marked pilot whales from pod R4 (the pod with more captures; see Table II.4 and Figure II.9), captured on 19 days between 2005-2011. It was used only high-quality images from encounters with high- or full-coverage, and it was chose a moving average of 1600 associations. Sampling period was defined as day, and associations as individuals grouped within an encounter. Vertical bars indicate SE calculated using the temporal jackknife method on each sampling period. The best fit model (based on the lowest QAIC) represents associations with constant companions [$g(\tau)$=0.12354]. The null association rate represents the theoretical SLAR expected if individuals associated randomly.

CHAPTER III

. Survival and abundance of short-finned pilot whales in the archipelago of Madeira, NE Atlantic

Chapter cover:

Underwater ‘shadows’ in the crystal waters of Madeira. Photograph taken from the whale-watching vessel *Ventura do Mar* in August 2011.

Photo credits: Raquel Marques

Abstract

Estimates of population parameters for the short-finned pilot whale *Globicephala macrorhynchus* are scarce in literature; contributing to an IUCN (International Union for Conservation of Nature) status of 'Data Deficient'. Here, photo-identification data collected over seven years off Madeira was used to estimate survivorship, capture probability and abundance for the first time in this species using mark-recapture methodology. The Cormack-Jolly-Seber estimated that the adult island-associated (i.e. resident and regular visitor) whales had a constant survival rate of 0.960 (95% CI: 0.853 to 0.990) and an annual capture probability varying between 0.372 (CI: 0.178 to 0.619) and 0.843 (CI: 0.619 to 0.947). A parameterization of the Jolly-Seber model estimated that 140 island-associated whales (CI: 131 to 151) used the area throughout the course of the study. Based on a closed population model, the less biased (lower CV) annual estimate of the total number of pilot whales using the southern and eastern waters of Madeira (≈900 km^2) in a three month period covering summer/autumn is 334 animals (CI: 260 to 437). No trend was observed. Despite including biases, the approach used in this study provided plausible estimates of population parameters. These are expected to contribute to the regional conservation strategies.

Introduction

Population parameters are essential for health assessments and management decisions on a stock. Little is known about the population parameters of the short-finned pilot whale *Globicephala macrorhynchus*, which has a wide distribution from tropical to warm temperate waters (Olson 2009). The small amount of literature available describes estimates of mortality, growth and reproductive rates from stranded and hunted whales in Japan (Kasuya & Marsh 1984), and reports estimates of abundance from line-transects (reviewed by Olson 2009). On a global scale, the conservation status of the short-finned pilot whale is described as 'Data Deficient' and its population trend is unknown (IUCN 2012).

This is the first study presenting estimates of survival and abundance for short-finned pilot whales using a non-invasive mark-recapture methodology based on photo-identification of naturally marked animals (Seber 1982, Hammond *et al.* 1990). Mark-recapture models have been applied to estimate population parameters in several taxa (e.g. Péron *et al.* 2013) including cetaceans (e.g. Speakman *et al.* 2010, Cantor *et al.* 2012). The reliability of the obtained estimates depends on the underlying assumptions of the model used, and these are not always easy to fulfill (Hammond 1986). To minimize bias in the present study, the model assumptions were carefully addressed according to each estimated parameter.

A photo-identification study of short-finned pilot whales in the archipelago of Madeira (Portugal) suggests varying patterns of occurrence with resident (up to 14 years), transient and temporary emigrant whales occupying the study area (Alves *et al.* 2013). In that study, several entire pods (72% of the 364 catalogued individuals) were captured only once during the study period (2003-2011), while other individuals were recaptured up to 33 times in multiple years. The transience and the emigration and re-immigration into the study area indicate a wide ranging movement of these whales, causing unequal capture probabilities and consequently introducing problems for the analysis. It further demonstrates that the population under study uses a more extensive area than that surveyed.

Genetic analyses suggest that individuals of the different residency patterns encountered in Madeira may not be genetically isolated and that they may interact for mating purposes upon meeting (Alves *et al.* 2013). Additionally, the genetic information suggests that small groups are made up of related individuals with long-lasting relationships and that large groups are probably temporary associations of smaller groups. No other information related to stock boundaries or movements of short-finned pilot whales, from satellite tags or acoustics, is known to be available for the sampled population.

Estimating abundances of the island-associated whales, defined as residents or regular visitors (i.e. individuals with >1 capture, according to Alves *et al.* 2013) could be more relevant for addressing the impacts of localized disturbances (Conn *et al.* 2011).

However, estimating the total number of animals that uses the area annually is also important for conservation management. Therefore, the aim of this study is to estimate survivorship and abundance of the island-associated and the total number of these poorly known whales.

Material and Methods

Data collection

Individual identification photographs were obtained through systematic boat surveys conducted around the islands of Madeira, Desertas and Porto Santo, and through non-systematic and opportunistic surveys from a smaller area (≈900 km^2) (Figure III.1). Details on systematic and non-systematic surveys are described in Alves *et al.* (2013). The opportunistic surveys were carried out in touristic boats that operate bi-daily trips to search for cetaceans, and not pilot whales in particular, in the whole smaller selected area (Figure III.1). These allowed observers of our research team, as well as experienced whale-watching operators, to collect additional digital photographs. All types of surveys were performed year-round from 2003 to 2011, although only a subset was used in the analysis (see ‘Dataset and analysis’). The survey effort varied according to the type of survey and among years, with a general increase in the number of surveys during the two latest years (Table III.1).

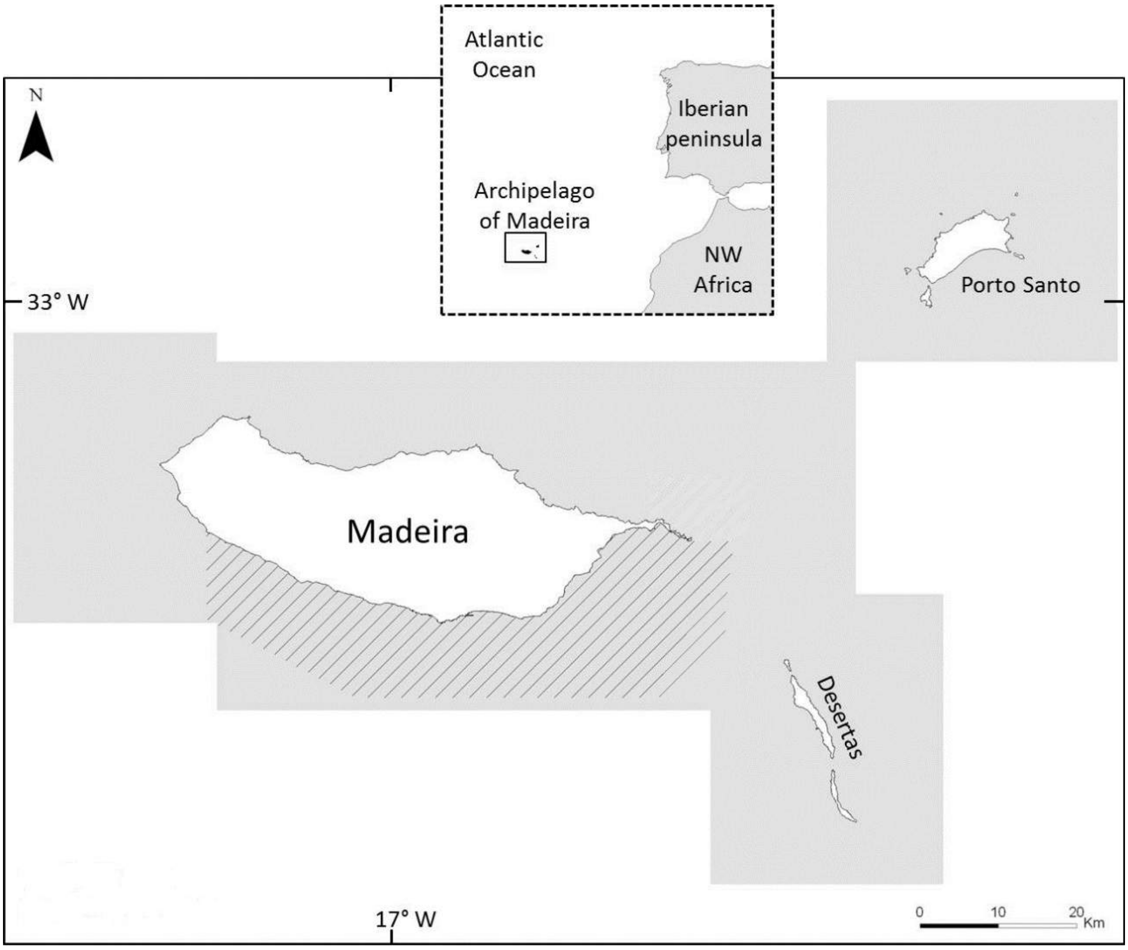

Figure III.1. Surveyed area (in grey on the larger picture) in the archipelago of Madeira (Portugal), showing the smaller selected area used for the analysis of survival and abundances (in diagonal stripes).

Table III.1. Sampling effort and photo-identification (of well-marked whales based on high-quality images) per period (start-end) used for the data analysis in this study. See text for details on each type of survey.

		Number of surveys / number of captures			
Year	Period	Systematic	Non-syst.	Opportunistic	Total
2005	(28 Jul-22 Oct)	0	11/52	7/8	18/60
2006	(19 Jul-27 Sep)	0	7/36	6/1	13/37
2007	(25 Jul-19 Oct)	2/9	7/27	26/40	35/76
2008	(11 Sep-21 Oct)	1/12	0	10/15	11/27
2009	(22 Jul-24 Oct)	0	0	11/46	11/46
2010	(26 Jul-28 Oct)	3/14	8/35	67/153	78/202
2011	(17 Jul-19 Oct)	2/8	11/21	81/131	94/160
Total		8/43	44/171	208/394	260/608

Attempts to photograph all short-finned pilot whales encountered were made from a close distance, both on the left- and right-side, independently of age/sex class and dorsal fin distinctiveness. During all types of surveys, sighting data included location, time of encounter, best estimate of group size and composition. While cameras Nikon D2H and lenses were used during the entire study period, cameras Nikon D700 were used only during 2010-2011. Time constraints have limited full photographic coverage of some encounters during systematic and opportunistic surveys.

Photo-identification

A photo-identification catalogue was compiled (Würsig & Jefferson 1990) and photo processing and matching is described in Alves *et al.* (2013). Individuals were identified based only on unique notches on the trailing edge (Auger-Méthé & Whitehead 2007). A four-level photo quality was assigned to each photograph according to Friday *et al.* (2000) and a four-level independent distinctiveness category was given to each individual based on the number of notches in the dorsal fin. This study used only high-quality (HQ) images and well-marked (WM) whales. Photographs of HQ-images and WM-whales, as well as the definition of sub-adults and immatures are shown/described in Alves *et al.* (2013).

Dataset and analysis

Only data collected in the smaller selected area (Figure III.1) was used in the analysis due to being more intensively researched and given the whales' geographical preferences for that area (Filipe Alves, unpublished data from the Madeira Whale Museum). Moreover, only sighting histories generated from data collected in three month periods were used in the analysis in order to minimize violation of model assumptions (see 'Discussion'). Data was restricted to the periods with more captures (summers/autumns) during 2005-2011 (Table III.1). Analysis limited to data from single type of surveys was less robust, with lower number of captures and higher CV and overdispersion (not shown), than when using data from the three types of surveys. Therefore, all the analysis in this study used the combined data from all types. Different approaches had to be used to estimate survival of adult whales (ϕ_{ad}), abundance of island-associated whales of all ages (N_{asso}) and abundance of all whales using the area per period in each year (N_{tot}). A summary of the datasets, models and programs used is described in Table III.2.

Table III.2. Summary of the models used in this study. See 'Material and Methods' for Model/Estimator specifications.

ID	To estimate	Target whales	Period	Population model	Model[a]/ Estimator	Software
ϕ_{ad}	Survival	Island-associated adults	All years	Open	CJS	MARK
N_{asso}	Abundance	Island-associated all-age	All years	Open	JS	POPAN
N_{tot}	Abundance	All	Each year	Closed	Chao	CAPTURE

The Cormack-Jolly-Seber (CJS) models (Cormack 1964, Jolly 1965, Seber 1965), which are based on the sighting histories of known individuals, were used for estimating survival (ϕ) between years and capture probability (p) for each year (Lebreton *et al.* 1992). Since mortality in mammals can vary according to age class (Reilly 1984, Gabriele *et al.* 2001, Silva *et al.* 2009), survival estimate was restricted to adults due to being the most representative category. Since recent findings discouraged distinguishing the gender in pilot whales based on photographs (Augusto *et al.* 2013), the survival was not estimated for sexed animals. Moreover, survivorship was restricted to island-associated whales since the inclusion of individuals with different capture probabilities, as in the case of this study (Alves *et al.* 2013), would violate the assumption of equal probability of capture (Cubaynes *et al.* 2010, Madon *et al.* 2012), resulting in a lower survival rate estimate. An ad hoc method presented by Pradel *et al.* (1997) was chosen to overcome the transience problem, excluding the first capture of each individual (e.g. Ramp *et al.* 2006). This left truncation of the dataset suppresses the influence of the lower survival estimate of individuals with null recapture probability, which corresponded to individuals captured only once, i.e. permanent emigration (Pradel *et al.* 1997). Therefore, the survival was estimated for island-associated adult whales. For this analysis, captures of individual whales made during the same year were pooled and each year was treated as a sampling occasion (Table III.3). The left truncation and the exclusion of non-adults reduced the dataset to 60 whales.

Table III.3. Summary of the pooled data per period (see Table III.1) in each year, with number of photo-identified well-marked whales.

Year	Marked	New	In catalog
2005	47	47	47
2006	29	20	67
2007	46	25	92
2008	24	7	99
2009	34	16	115
2010	84	39	154
2011	58	17	171

A parameterization of the Jolly-Seber model (Schwarz & Arnason 1996) was used to obtain an estimate of the number of island-associated whales that used the area throughout the course of the study. Here, whales of all ages could be estimated since the total abundance was based on the proportion of marked to unmarked animals (see 'Total abundance'). As in the previous open population model, the dataset to estimate N_{asso} was left truncated (e.g. Conn *et al.* 2011), captures of individual whales made during the same year were pooled and each year was treated as a sampling occasion (Table III.3). The left truncation reduced the dataset to 64 whales of all ages and sexes. Although the model used to estimate N_{asso} could provide a survival estimate this was not considered due to including non-adult whales.

Given that unequal capture probabilities between individuals are difficult to model in the open population framework, closed population models were chosen to estimate abundance of all whales using the area in each year since these can deal with such type of heterogeneity (Chao 1989). To model each year independently in program CAPTURE, each three month period contained multiple sampling occasions (between three and nine, Table III.4), each comprising pooled data from close surveys (median=1 day-apart, range: 0 to 5) and separated by large intervals (median=11 days-apart, range: 5 to 52); following Conn *et al.* (2011).

Table III.4. Number of sampling occasions, captures and individuals marked per period (see Table III.1) in each year, used to estimate abundances in program CAPTURE (see Table III.2).

Year	Sampling occasions	Captures	Individuals
2005	4	57	47
2006	4	36	28
2007	6	66	40
2008	3	21	21
2009	5	37	32
2010	9	151	78
2011	8	105	53

An additional procedure to estimate abundance of all whales was used in order to assess consistency. The Chapman's modification of the Petersen estimator (Hammond 2009) estimated abundance between every two years using pooled data of individuals captured per three month period (Table III.3).

Modelling ϕ_{ad}

A goodness-of-fit (GOF) test was used to check if the chosen general model fitted the data adequately, using the program U-CARE (Choquet *et al.* 2009). GOF tests compare observed vs. expected frequencies, and provided an overall test statistics and four different tests, each testing different aspects of the data to evaluate potential violations of assumptions (Burnham *et al.* 1987, Choquet *et al.* 2009). The clearest tests are the TEST 2.CT that is known to examine heterogeneity in capture probability and the TEST 3.SR that is known to examine if there is a transience effect (Choquet *et al.* 2005). The GOF test failed to reject the general model (*P*=0.22) with only one of the components being slightly significant (TEST 3.SR, *P*=0.04) (Table III.1SM, see 'Supplementary Material'). Additionally, overdispersion is an important aspect to consider in mark–recapture data (Anderson *et al.* 1994). An estimation of the overdispersion factor (Lebreton *et al.* 1992), also called variance inflation factor ($\hat{c}$), was obtained by dividing the χ^2 statistics of GOF tests by the number of degrees of freedom (Choquet *et al.* 2009). Whereas $1<\hat{c}<3$ is indicative of overdispersion in the data, a $\hat{c}>3$ may represent more fundamental problems (Lebreton *et al.* 1992). Given that the GOF test showed that model structures were correct and that the $\hat{c}$ was 1.298 we proceeded with the analysis.

Two candidate models were used to test the effects on survival and capture probabilities. One included a more parameterized general model considering the full time-variation (t), and the other considered constant (.) survival rates. The estimated $\hat{c}$ was incorporated in the model, allowing the overdispersion to be included in the computation of the standard error (SE) and confidence interval (CI, 95%) of the parameter estimates ϕ and p. For model selection, instead of the Akaike Information Criterion (AIC_c), it was used the Quasi-likelihood AIC_c ($QAIC_c$) given that models were adjusted for overdispersion (Anderson *et al.* 1994). It was based on the lowest $QAIC_c$ value, and the normalized $QAIC_c$ weights were used to measure the strength of evidence of a specific model.

Modelling N_{asso}

Given that the dataset used to estimate N_{asso} had only four more (non-adult) whales than the dataset used to estimate ϕ_{ad}, and that only a fraction (the proportion of marked animals) of the total estimate of N_{asso} would be tested, GOF tests were not applied. Two candidate models were used, one considering full time-variation (t) and another considering constant (.) survival rates. The link functions were specified according to Cooch & White (2008). Model selection was based on the lowest AIC_c value. The 'Deviance' could not be directly computed (Cooch & White 2008).

Modelling N_{tot}

For the closed population models, model selection procedure for each year was performed from the 'Appropriate' option that uses maximum value criteria. Yet, behavioral response to 'capture' was not considered given that it is generally agreed that photo-identification of cetaceans should not lead to changes in capture probabilities (Wilson *et al.* 1999, Fortuna 2006). Therefore, heterogeneity in capture probabilities was taken into account in models considering the temporal variability (t) and the individual heterogeneity (h), as well as their combination (th) and the null model (o). Moreover, estimates were restricted to the Chao estimator (Chao 1989) given it is suggested to be less biased than others (Brittain & Böhning 2009) and is likely more appropriate when sampling effort is not equal on each sampling occasion (as in this case) or when probabilities of capture are small (Fortuna 2006). Given that there is no GOF test available for closed population models, these were not adjusted for overdispersion.

Heterogeneity was also modelled with mixture models (Pledger 2000) using 2 mixtures of capture probabilities but these were discarded due to causing over-parameterization (not shown). Additionally, an attempted to address emigration with the Pollock's robust design was made (Pollock 1982; Kendall *et al.* 1995, 1997) but with no success due to requiring a larger number of captures (not shown). All mark-recapture modelling procedures were run in MARK (White & Burnham 1999). Trends were assessed through linear regressions using the R 2.14.2 statistical package (R Development Core Team 2012).

Total abundance

Given estimates of abundance ($\hat{N}$) from models do not pertain to the whole population, total pilot whales abundance ($\hat{N}_{tot}$) occurring in the study area was calculated by dividing $\hat{N}$ by the mean proportion of WM-individuals ($\hat{\theta}$). This proportion was estimated as the number of WM-individuals divided by the number of all photographed individuals, using HQ-images from 32 groups of various sizes (3 to 33 individuals) where all individuals were captured (total = 348 individuals). It was estimated for each year since a binomial GLM of the proportion of WM-individuals per year showed that most years were significant ($P<0.05$). The variance of $\hat{N}_{tot}$ was estimated using the delta method as $\hat{N}_{tot}^2$ x {(var $\hat{N}$ / $\hat{N}^2$) + (var $\hat{\theta}$ / $\hat{\theta}^2$)} (Wilson *et al.* 1999). Lower and upper log-normal CIs for $\hat{N}_{tot}$ were calculated as MNA + {($\hat{N}_{tot}$ - MNA) / $C_{ʃ}$} and MNA + {($\hat{N}_{tot}$ - MNA) x $C_{ʃ}$}, respectively, where MNA is the minimum number of animals known to be alive in the population (i.e. the number of animals that were captured) and $C_{ʃ} = \exp \{1.96\sqrt{} \ln (1 + \text{var } \hat{N}_{tot} / [\hat{N}_{tot} – \text{MNA}]^2)\}$ (Thompson *et al.* 1998).

Model assumptions

Assumptions of open population models include: (1) marks are not lost or missed, (2) individuals are immediately released after being sampled, and the duration of each sampling event is small in relation to the total duration of the sampling period, (3) all marked individuals in the population that are alive on a given sampling occasion have the same probability of surviving to the next sampling occasion, (4) all animals have the same capture probability, (5) captures are assumed independent of one another, and (6) all emigration is permanent (Hammond 1986, Hammond *et al.* 1990).

Closed models imply other assumptions such as (7) marking not affecting the catchability, (8) the population is closed (Hammond 1986, Kendall *et al.* 1995), and (9) the animals can mix between sampling occasions (Hammond 2009). Potential violations of models assumptions are addressed in the ‘Discussion’.

Results

Estimates of ϕ_{ad}

The full time-variation model fitted the data poorly, and the model with constant survival represented the best model (Table III.2SM). After applying the ĉ, the estimated constant survival rate was 0.960 (SE=0.028, 95% CI: 0.853 to 0.990).

Capture probabilities from the best model fluctuated between 0.372 (SE=0.120, CI: 0.178 to 0.619) in 2008 and 0.843 (SE=0.081, CI: 0.619 to 0.947) in 2010 (Figure III.2). A linear regression fitted to the capture probabilities showed no significant trend (adjusted r^2=-0.04, P=0.43).

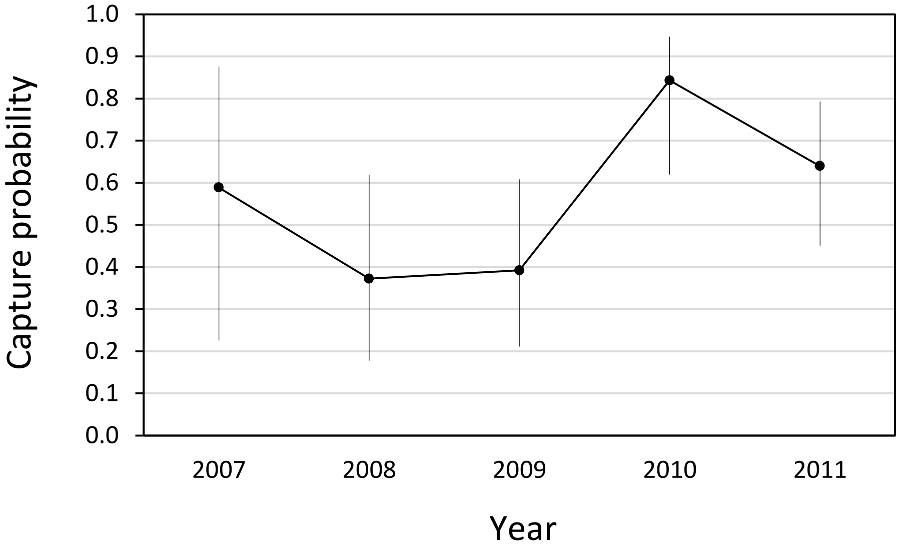

Figure III.2. Estimates of capture probability for island-associated adult whales with 95% CI (vertical bars).

Estimates of N_{asso}

The model with constant survival represented the best model (Table III.3SM). The estimated number of marked island-associated whales using the selected area throughout the course of the study was 69 animals (CV=0.05, CI: 66 to 80). Correcting for the unmarked proportion of the population (51%) the total N_{asso} was estimated at 140 animals (CI: 131 to 151).

Estimates of N_{tot}

Total abundances estimates varied from a low of 105 whales (CI: 83 to 136) in 2006 according to M(o) to a high of 334 (CI: 260 to 437) in 2010 according to M(t) (Table III.5). Parameters for 2008 could not be estimated. A linear regression fitted to the abundances showed no significant trend (adjusted r^2=0.33, P=0.14).

Table III.5. Estimates of the proportion of well-marked individuals (θ) and abundance (N) from the closed population models that were used to estimate total abundance (N_{tot}) of whales using the selected area (see Figure III.1) per period (see Table III.1) in each year. Appropriate model probably given in CAPTURE was used for model selection, and Chao was used as estimator (see 'Material and Methods'). See text for definition of M(t), M(h), M(th) and M(o). The number of whales (n) from which θ was estimated was zero for 2006, thus the overall mean was used for that year (†).

				Model			Total	
Year	θ	SE	*n*	Selected	N	CV	N_{tot}	95% CI
2005	0.80	0.07	30	M(t)	115	0.31	144	109 - 198
2006	0.49†	0.10	0	M(o)	52	0.26	105	83 - 136
2007	0.52	0.08	27	M(th)	93	0.26	179	134 - 247
2008	0.57	0.09	44		-			
2009	0.63	0.08	27	M(o)	98	0.38	156	109 - 231
2010	0.37	0.04	35	M(t)	124	0.16	334	260 - 437
2011	0.43	0.03	185	M(h)	91	0.20	213	174 - 265

Estimates from the Chapman's modification are shown only for the pair of years 2010-2011 because it was considered the most reliable and representative one. This was due to presenting the lowest CV and having used the largest number of captures, as a result of the higher sampling effort in those periods. The estimated number of marked whales was 142 animals (CV=0.08). Correcting for the unmarked proportion of the population for those years (58%) the total number of all whales using the selected area in 2010 and 2011 was 340 animals (CI: 306 to 381).

Discussion

Model assumptions

Given that only WM-individuals and HQ-images were used, that those whales have a low rate of mark change in the dorsal fin (every 6.6-8.0 years, Alves *et al.* 2013), and that captures were collected from short-term photo-identification events, greatly contributed to avoid violating the two first assumptions (Wilson *et al.* 1999, Stevick *et al.* 2001).

The third and fourth assumptions can be easily violated when using heterogeneous data as in this case. The fact that a high percentage of new individuals were identified each year, including the last year of the study (see Table III.3), and that about ⅔ of the individuals were captured only once (see Figure III.1SM), strongly suggests temporary immigration and transience into the study area. The high number of recaptures observed for a subset of the catalogued individuals and the low rate of mark change in the dorsal fin (Alves *et al.* 2013) corroborates that the continuous addition of new individuals cannot be explained by the acquisition of new marks. Therefore, it is assumed to be an open population, leading to heterogeneous data. The first capture of each individual was censored to reduce transience, and this was addressed through GOF tests. Despite still indicating transience, the tests show that model structures are correct. Moreover, the ĉ-values indicate that the excess variation was within acceptable limits. Nonetheless, the assumption that all animals have the same capture probability can rarely be met in a real study (Evans & Hammond 2004).

The assumption that captures are assumed independent of one another is likely to be violated in most studies estimating population parameters in cetaceans due to their social structure, and a gregarious species such as the short-finned pilot whale (Heimlich-Boran 1993) is no exception. Yet, to the best of our knowledge this issue has not been properly addressed in cetaceans' studies (Choquet *et al.* 2013). Although the population used in this study is socially structured, not all pairs of individuals are expected to have been seen closely associated the entire time (Alves *et al.* 2013). Moreover, by having grouped different days into one session, which joined data of many different social groups, greatly contributed for minimizing the violation of this assumption. In all cases, this may have caused only an underestimation of the model-conditioned standard errors and not lead to biased estimates (Williams *et al.* 2002, Conn *et al.* 2011).

While the assumption of marking not affecting the catchability of an animal is assumed to be met through the use of photo-identification in cetaceans (Hammond *et al.* 1990, Wilson *et al.* 1999), possible violations to closure should not be discarded. Although, by having analyzed a dataset restricted to a three month period likely contributed to ensure, or at least minimize the violation of, closure. Finally, the fact that a group of pilot whales traveled ≈600 km between Madeira and Canary Islands in 20 days (Servidio *et al.* 2007) suggests that mixing is likely to have occurred between the sampling periods in the present study, especially if we consider that these were 11 days-apart (range: 5 to 52) and the relatively small size of the study area.

Survival

Excluding the first capture of each individual appears to be a useful approach to address the heterogeneity arising from transience. The same procedure was used in Ramp *et al.* (2006) to deal with transience in estimating blue whales *Balaenoptera musculus* survival rates. Yet, even after truncation, there was still heterogeneity in the dataset. Since permanent emigration was no longer a problem it is assumed that this heterogeneity was caused by the presence of temporary emigrant whales (Alves *et al.* 2013), which unfortunately could not be addressed with the Pollock's robust design. Additionally, incorporating a variance inflation factor increased the SE and the CI of the estimates but reflected the extra amount of variance in the data.

Silva *et al.* (2009) used CJS and robust design models to estimate survivorship, but only the former provided reliable results when dealing with transience, and Ramp *et al.* (2006) estimated survivorship using only open CJS models, as in the case of the present study. The best estimate presented here considers only the island-associated adult pilot whales. Nevertheless, the survival rate should not vary greatly among residency-type due to being a long-lived mammal species.

The analysis excluded non-adult individuals, which eliminated possible heterogeneity arising from differences in age class, but included whales of both sexes. Kasuya & Marsh (1984) described that in Japan male short-finned pilot whales have a higher mortality rate than females at any given age. As so, further photo-identification studies on this population should combine genetic sampling for sex determination in order to address this possible source of heterogeneity.

The high survival rate of 0.960 presented here is in accordance with estimates for other long-lived cetaceans (Barlow & Clapham 1997, Currey *et al.* 2008), and agrees with the mortality rates of 4.5% for females and 8.3% for males of this species (Kasuya & Marsh 1984; noting they considered all age classes). Nonetheless, the survival rate described here is lower than the estimate reported for adult long-finned pilot whales *G. melas* in the Strait of Gibraltar (0.982, CI: 0.955 to 0.993; Verborgh *et al.* 2009).

Capture probability

Capture probabilities showed no trend but varied during the study period, suggesting time-dependency, as expected in the majority of mark-recapture studies in cetaceans (Hammond 2009). Although any annual fluctuations in whale abundance or environmental factors such as distribution and availability of prey might have contributed to variations in capture probabilities, most variations could likely be explained by differences in sampling effort. This could have included differences in the survey effort, type of surveys, type of vessels, or even type of cameras.

Abundance

This study estimated that 140 island-associated whales (CI: 131 to 151) used the southern and eastern waters of Madeira during the three month periods covering summers/autumns of 2005-2011. Based on individuals captured on ≥4 encounters with high coverage during all year, Alves *et al.* (2013) described at least eight pods of resident and regular visitor pilot whales in Madeira, each with an estimated mean of 15 individuals (SD=9, range: 4 to 29). The number of island-associated whales based on pods is likely to include others than those ≈120 animals if accounted for the individuals captured on <4 occasions. Therefore, this corroborates that the estimated number of island-associated whales found in this study should not be too biased and may be representative of the animals using the area throughout the year.

On the other hand, total abundances are difficult to model with open population models due to the presence of transients that induces unequal capture probabilities (Hammond 1986). Instead, closed models were used, which allowed accounting for heterogeneity since robust estimators have been developed to obtain less biased population estimates (Otis *et al.* 1978, Chao 1989). In general, abundance estimates varied between years, although with no significant trend. The less biased estimate (based on the lower CV, e.g. Silva *et al.* 2009) corresponds to 2010, the year with greater sampling effort and higher estimate of abundance. Also, the number of individuals used to estimate the proportion of WM-individuals was much higher in that year, which correspond to a higher precision. Thus, the less biased estimate of short-finned pilot whales using the southern and eastern waters of Madeira in a three month period covering summer/autumn is 334 animals (CI: 260 to 437). Although, it should be noted that the annual estimates can be overestimated since do not incorporate temporary emigration (i.e. some animals may be available in some years but not in others). The Chapman's modification estimated that the total number of all whales using the same area and periods of 2010 and 2011 was 340 animals (CI: 306 to 381). This estimate, that may include biases associated with the violation of closure between periods, gives consistency that the estimates obtained from Chao models may be overestimated.

The approximately 213 long-finned pilot whales reported every summer in the central part of the Strait of Gibraltar (Verborgh *et al.* 2009) are within the range of our estimates. Even though, these studies covered different methodological approaches, species and areas. No other estimates of abundance using mark-recapture data on pilot whales are known to have been reported to date.

Conclusions

Analyzing free-ranging animals in an open pelagic environment induced limitations in the dataset that consequently challenged the analysis. Yet, the approach used in this study provided plausible estimates for survival of adult whales (within the range of other long-lived cetacean species) as well as for abundance of island-associated whales and of individuals using the area seasonally. The estimates presented here should be taken in consideration by managers as a base-line for conservation strategies. The area used for the analyses coincides with the main area of marine traffic in the island of Madeira and with the main area of operation of the whale-watching vessels. Similarly, the period of year used in the analyses covers the main season of that industry. Given that the whale-watching industry is increasing in Madeira, it can become a potential pressure to the island-associated pilot whales. Therefore, we stress the need for long-term monitoring, especially of the animals that use the area regularly, since it could help to assess the health of the population. As future consideration, a larger dataset covering a higher number of years would likely improve the issue of temporary emigration since it would increase the chances of recapturing those whales visiting the area sporadically.

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Supplementary Material

Table III.1SM. Results from goodness-of-fit tests run in U-CARE for the dataset used to estimate survival in the Cormack-Jolly-Seber program.

	TEST 2.CT	TEST 2.CL	TEST 3.SR	TEST 3.SM	Global test
df	3	2	3	3	11
χ^2	1.5	0.6	8.4	3.8	14.3
P -value	0.67	0.76	0.04	0.28	0.22

Table III.2SM. Model selection for the survival (using Cormack-Jolly-Seber) candidate models of probabilities of survival (ϕ) and capture (p), considering constant (.) and time-variation (t); ΔQAICc reflects the QAICc difference from Model 1.

#	Model	QAICc	ΔQAICc	QAICc weight	Likelihood	No. para.	Deviance
1	ϕ(.) p(t)	158.38	0	0.947	1	6	42.8
2	ϕ(t) p(t)	164.15	5.78	0.053	0.056	9	41.5

Table III.3SM. Model selection for the abundance of island-associated whales (using Jolly-Seber) candidate models of probabilities of survival (ϕ), capture (p) and probability of entry (pent), considering constant (.) and time-variation (t); ΔAICc reflects the AICc difference from Model 1.

#	Model	AICc	ΔAICc	AICc weight	Likelihood	No. para.	Deviance
1	ϕ(.) p(t) pent(t)	252.56	0	0.693	1	12	0
2	ϕ(t) p(t) pent(t)	254.18	1.62	0.307	0.444	13	0

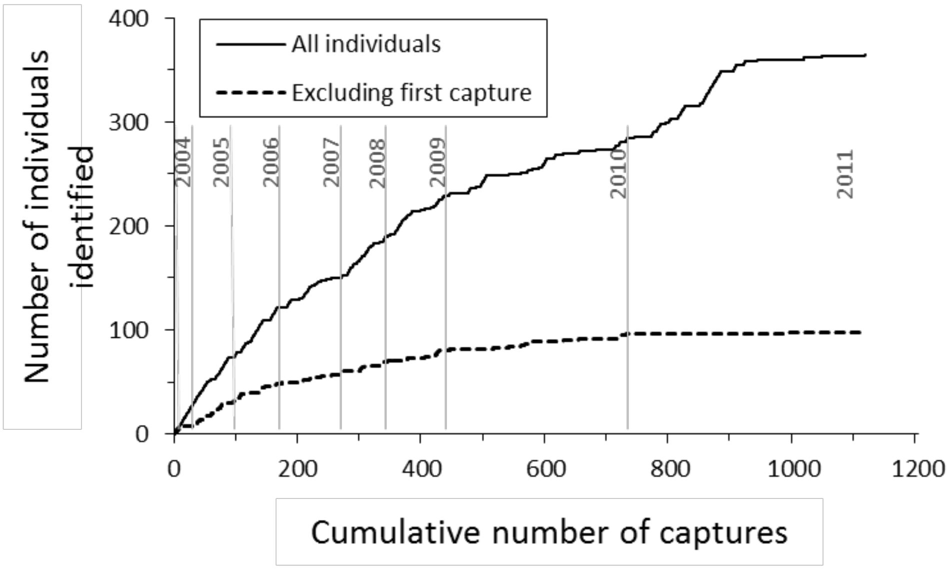

Figure III.1SM. Discovery curve for all and for island-associated (all excluding first capture) pilot whales, considering only well-marked individuals based on high-quality images for all studied years (separated by vertical bars) and surveyed area.

CHAPTER IV

. Daytime dive characteristics from six short-finned pilot whales *Globicephala macrorhynchus* off Madeira Island

Chapter cover:

The author deploying a time-depth-recorder in a short-finned pilot whale. Photograph taken from the research rigid inflatable boat *Roaz* in November 2010.

Photo credits: Nuno Sá

Abstract

Six time-depth recorders attached with suction-cups were deployed in resident and transient adult short-finned pilot whales to evaluate their daytime diving characteristics in their preferred habitat area off Madeira Island. Here, data on the proportion of time spent at the surface and at different dive phases (descent, bottom and ascent), dive depths and dive rates are presented. With a mean attachment durations of 2 h 31 min (SD=2 h), the whales spent a considerable amount of time at the surface (mean=76.3%, SD=18.6) and presented a low diving rate (mean=6.8 dives h^{-1}, SD=6.1). The maximum dive depth recorded in this study was 988 m, and dives deeper than 500 m, which were recorded from resident and transient whales, suggest foraging activity along their preferred habitat area. The analysis of dives deeper than 100 m shows that the percentage of time spent on descent, bottom or ascent varied between dives, with means of ≈40, 30 and 30%, respectively.

Introduction

Obtaining information on the diving behavior of marine mammals is of major interest for management purposes (Hooker & Baird 2001). When combined with knowledge on group behavior, the percentage of time a species spends at the surface can help converting the at-surface abundance to total estimates of abundance, given that adjusts for whales that are not detected at the surface during visual surveys (Laake & Borchers 2004). On the other hand, information on the subsurface dive profile is important in the study of feeding ecology (Panigada *et al.* 1999) or in assessment of depth-specific (e.g. acoustic) impacts (Hooker & Baird 2001).

Short-finned pilot whales *Globicephala macrorhynchus* are top predators inhabiting circum-tropical and warm temperate waters. Several aspects of this species diving behavior have been documented, such that foraging dives seem to adapt to circadian rhythms, with deeper dives and longer periods of time shallow diving or surface resting occurring during the day (Baird *et al.* 2003; Soto *et al.* 2008). Yet, time-per-depth data for this species has only been addressed by Wells *et al.* (2013), based on post-release monitoring via satellite-linked telemetry of two adult males released directly from a mass stranding. Moreover, the proportion of each dive spent at the bottom has only been described for the related long-finned pilot whale *G. melas* (Baird *et al.* 2002, Heide-Jørgensen *et al.* 2002).

In Madeira Archipelago (NE Atlantic) short-finned pilot whales demonstrate a large degree of variability in site fidelity (Alves *et al.* 2013). It is suggested that transient and resident whales interact for mating purposes when they meet (Alves *et al.* 2013), and that there is a preference for these whales to use the southern and eastern waters of Madeira Island (Filipe Alves, unpublished data from the Madeira Whale Museum). In this study, and for the first time in Madeira, biologgers were used in short-finned pilot whales to evaluate their daytime diving characteristics. Six time-depth recorders (TDRs) attached with suction-cups were deployed in resident and transient adult whales in their preferred habitat area. Here, data on the proportion of time spent at the surface and at different dive phases (descent, bottom and ascent), dive depths and dive rates are presented.

Material and Methods

Fieldwork was undertaken from the 18 m research yacht *Ziphius* or from the 6.5 m inflatable boat *Roaz*, from 2005 to 2011, along the south and east coast of Madeira Island (Figure IV.1). Tags technical specificities, deployment procedures, and data download are described in Alves *et al.* (2010). TDRs were set to sample depth once per second, to a maximum of 2000 m. At each tag deployment, the reaction of the animal was recorded, as well as the presence of calves, group size, animal's behavior, GPS position and travel direction. To minimize disturbance and record more natural behaviors, the boat left the group approximately 10 min after each deployment. During tag deployment, each tagged whale was digitally photographed for dorsal fin comparison to 683 individuals recorded between 2001 and 2011 and catalogued in a photo-identification database held at the Madeira Whale Museum. This allowed assignment of a residency status and a pod affiliation (in the case of resident whales) according to Alves *et al.* (2013). The photographs, together with field observations, allowed to categorise all tagged whales as adults, and classifying their sex according to descriptions by Yonekura *et al.* (1980) and Kasuya & Marsh (1984).

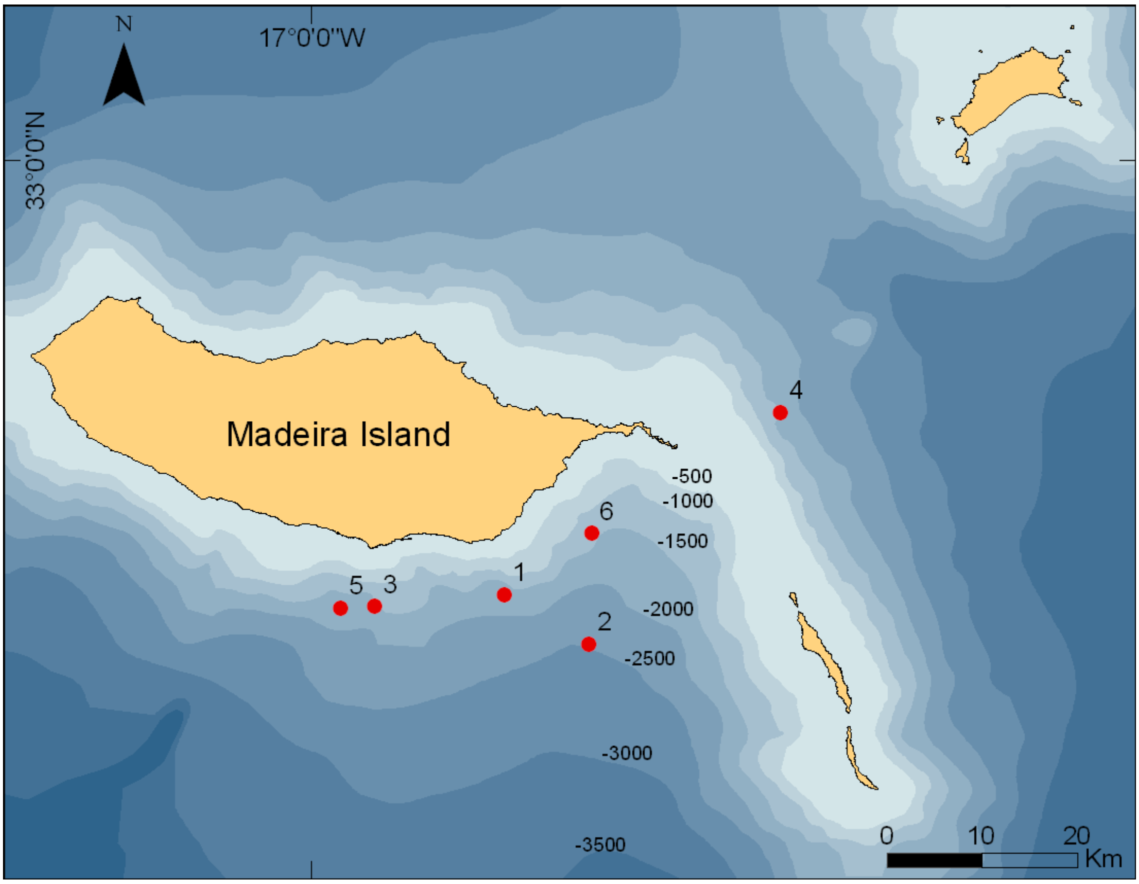

Figure IV.1. Bathymetric map showing the location of the six deployments used in this study, archipelago of Madeira, Portugal. Numbers 1-6 correspond to whales ID. Depths in meters.

A dive was defined as submergence deeper than 10 m (about two body lengths) to exclude brief shallow submersions between respirations. The proportion of time spent at the top 10 m layer and the diving rate (number of dives h^{-1}) were determined for each whale. The mean proportion of time spent at the top 10 m and the mean diving rate were calculated per component (sex, residency pattern and resident pod);

however the limited number of samples did not allow a robust analysis. Additionally, the total proportion of time spent and at different depth bins was determined.

To characterise the proportion of time spent at each phase in dives deeper than 100 m, the following definitions were used: ‘descent’ - the time between the beginning of the dive, from the surface, to the bottom; ‘bottom’ - the time between the point characterised by an inversion of the vertical direction in the last ⅔ of maximum depth and/or followed by a stabilisation of depth, and the point when ascent begins at constant rate; and ‘ascent’ - the time between the end of bottom and surface.

A variety of depths (e.g. >100, >300, >500 m) are described in literature for establishing deep foraging dives in short-finned pilot whales (Baird et al. 2003, Soto et al. 2008, Jensen et al. 2011). In this study this was done by searching for a bimodal distribution on a plot of the duration and maximum depth of dives (following Hooker & Baird 2001). Two sets of dives longer and deeper than the majority of dives were found: one between 100 and 200 m, and other deeper than 500 m (Figure IV.2). Therefore, instead of establishing one depth defining deep dives, the dive statistics were analysed for these categories.

Results

Six tag deployments (out of 18 attempts) yielded 15 h 06 min of data, and attachment durations ranged from 22 min to 5 h 08 min, with a mean of 2 h 31 min (SD=2 h) (Table IV.1). Tag recordings occurred between 11:44 and 18:49 local time, i.e. before sunset independently of the sampling season. Tags were applied to four residents (three females from the same pod and one male from a different pod) and two male transients (Table IV.1). The water depth during deployments ranged from 1000 to 2500 m (Figure IV.1). Tagging caused only short-term (few seconds) reactions to tagged animals, such as sudden shallow dive or tail slapping. These animals then rejoined the group and regained their natural behavior. A visual inspection of the dive profiles showed no discrepancies during the first minutes (Figure IV.3 and IV.4), thus these were not excluded from the data analysis.

The dive profiles in relation to time of day showed periods of surfacing and shallow diving (shallower than 100 m) intercalated with periods of dives between 100 and 200 m (whales 1, 2, 5 and 6; Figure IV.3), and with periods of dives between 500 and 1000 m (whales 3 and 4; Figure IV.4). With the exception of one animal (whale 3), the percentage of time each tagged whale spent in the top 10 m was >73%, with an overall mean of 76.3% (SD=18.6) (Table IV.1). Similar values were obtained between all components (sex, residency pattern and pod affiliation; Table IV.2).

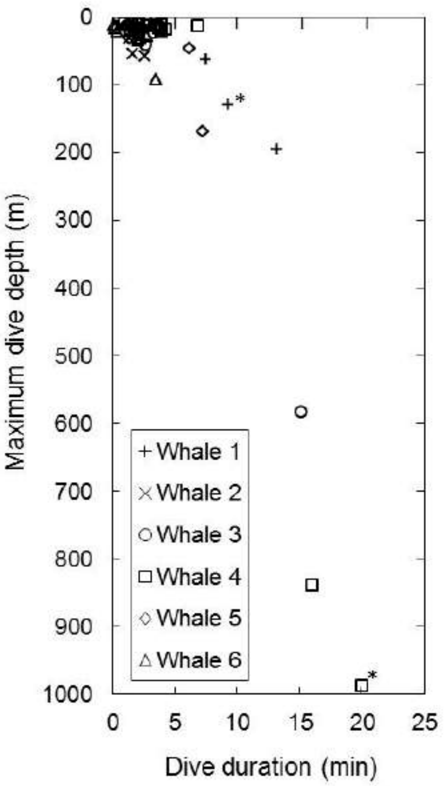

Figure IV.2. Dive duration and maximum dive depth for 59 dives deeper than 10 m recorded from six adult short-finned pilot whales. * - estimated values since tag detached during beginning of ascent; using the mean ascent rate of the previous (similar) dive of the same whale. Two dives were not considered since tag detached during descent. Three dives between 100 and 200 m and three deeper than 500 m differentiate from the majority of dives, which were shallower than 100 m and shorter than 5 min.

Whales performed a total of 61 dives deeper than 10 m, of which three were between 100 and 200 m, and three were deeper than 500 m, presenting mean dive rates of 6.8 (SD=6.1), 0.6 (SD=0.6) and 0.3 (SD=0.6) dives h^{-1}, respectively (Table IV.1). Transient males presented a considerably higher number of dives (Table IV.2). The analysis of the total proportion of time from the six tagged whales per depth bin shows that 94.1% of the time was spent in the 100 m bin, of which most was spent in the shallower bins (84.3% in the top 10 m, Table IV.3). The whales then spent 1.89% of their time in the 200 m bin (i.e. between 101 and 200 m), followed by the 600 m bin (0.80%) and the 800 m bin (0.63%) (Table IV.3).

Table IV.1. Summary of the tags deployed on adult short-finned pilot whales, including diving behavior. MWM - Madeira Whale Museum. *With calf. †Sex confirmed by genetic analysis from a biopsy collected in another encounter (Alves *et al.* 2013). Residency pattern and pod ID according to Alves *et al*. (2013).

Whale				Deployment			Diving behaviour			
ID	# MWM photo-id catalogue	Sex	Residency pattern	Date	Time to sunset when detached	Duration	% Time top 10 m	Dive rate (dives h^{-1}) deeper than 10 m	100 m	500 m
1	089	♂	Resident - Pod R4	13-06-2005	>2 h	2 h 57 min	78.0	5.8	0.7	0
2	149	♂	Transient	20-10-2005	>2 h	22 min	73.7	18.8	0	0
3	137	♀	Resident - Pod R5	19-07-2006	>6 h	37 min	41.2	6.5	1.6	1.6
4	373	♂	Transient	06-05-2008	4 h	5 h 08 min	84.3	2.5	0.4	0.4
5	133	♀*	Resident - Pod R5	18-10-2011	3 h	1h 33 min	85.3	3.9	0.6	0
6	135	♀[†]	Resident - Pod R5	30-11-2011	12 min	4 h 29 min	95.0	3.1	0	0
					Mean *(SD)*	2 h 31 min (2 h)	76.3 (18.6)	6.8 (6.1)	0.6 (0.6)	0.3 (0.6)
					Total	15 h 06 min				

Table IV.2. Mean of the percentage of time spent in the top 10 m and of the dive rate deeper than 10 m per sex, per residency pattern and per resident pod.

Component	% Time top 10 m	Dive rate (dives h^{-1})
Male	78.7 (SD=5.3, *n* =3)	9.0 (SD=8.3, *n* =3)
Female	73.8 (SD=28.7, *n* =3)	4.5 (SD=1.8, *n* =3)
Resident	74.9 (SD=23.5, *n* =4)	4.8 (SD=1.6, *n* =4)
Transient	79.0 (SD=7.5, *n* =2)	10.7 (SD=11.5, *n* =2)
Male-Resident	78.0 (*n* =1)	5.8 (*n* =1)
Male-Transient	79.0 (SD=7.5, *n* =2)	10.7 (SD=11.5, *n* =2)
Female-Resident	73.8 (SD=28.7, *n* =3)	4.5 (SD=1.8, *n* =3)
Pod R4	78.0 (*n* =1)	5.8 (*n* =1)
Pod R5	73.8 (SD=28.7, *n* =3)	4.5 (SD=1.8, *n* =3)

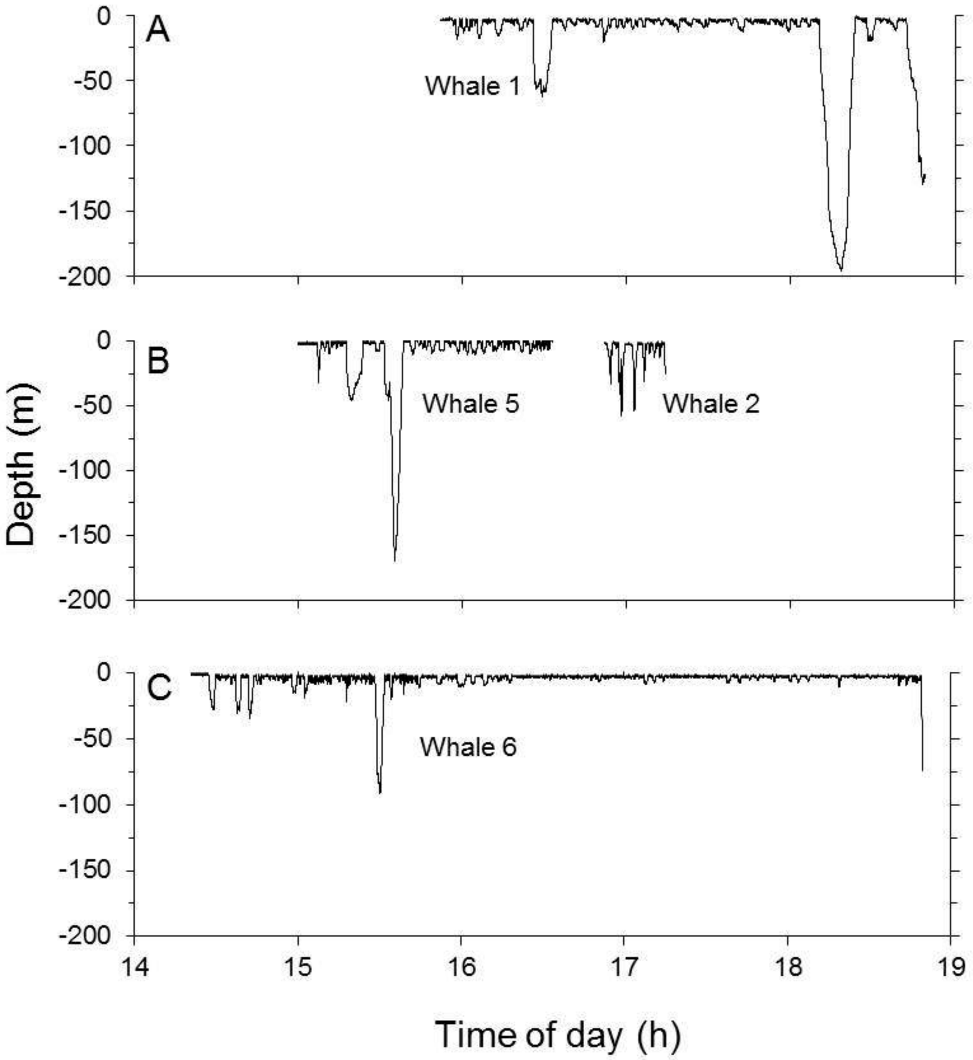

Figure IV.3. Dive profiles in relation to local time of day for whales 1 (A), 2, 5 (B) and 6 (C). The last dive of whales 1, 2 and 6 is incomplete due to tag detachment at depth. Dive profiles show periods of surfacing and shallow diving intercalated with dives to almost 200 m.

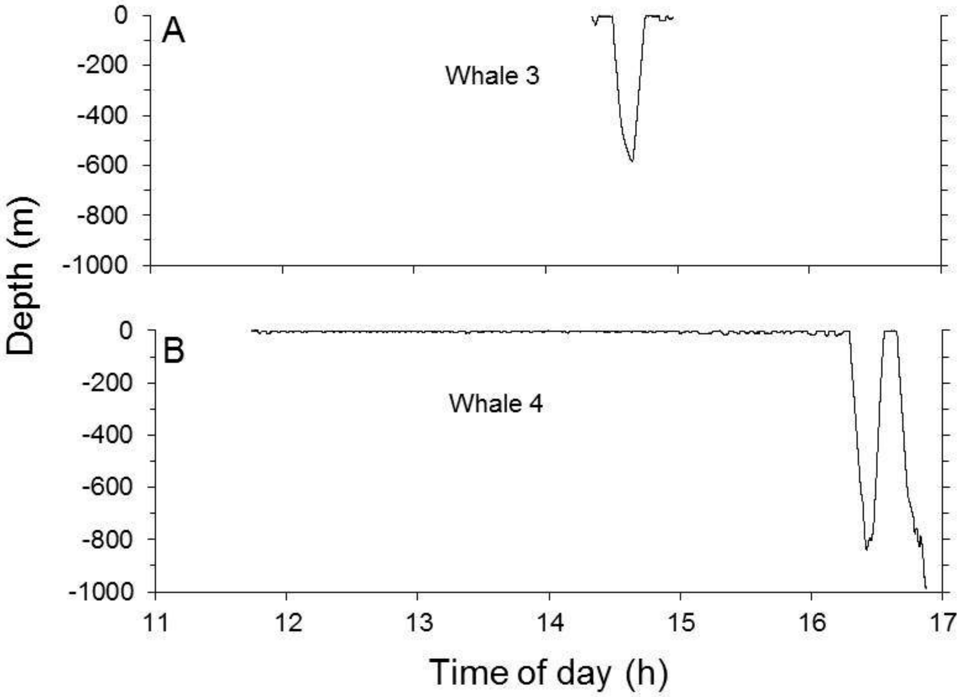

Figure IV.4. Dive profiles in relation to local time of day for whales 3 (A) and 4 (B). The last dive of whale 4 is incomplete due tag detachment at depth. Dive profiles show periods of surfacing and shallow diving intercalated with dives to almost 1000 m.

Table IV.3. Total percentage of time spent by six tagged whales at each depth bin. The depth bin '10' covers the top 10 m, the bin '20' covers depths between 11 and 20 m, etc. The depth bin '200' covers depths between 101 and 200 m, etc. The whales spent 94.1% of time in the 100 m depth bin.

10 m depth bin	% Time	100 m depth bin	% Time
10	84.32		
20	5.26	200	1.89
30	1.31	300	0.46
40	0.94	400	0.46
50	0.72	500	0.55
60	0.76	600	0.80
70	0.23	700	0.49
80	0.23	800	0.63
90	0.22	900	0.47
100	0.15	1000	0.12
Sum			100

Apart from three dives, all dives shallower than 100 m lasted less than 5 min and, as a general trend, deeper dives tended to be longer (Figure IV.2). The maximum dive depth recorded was 988 m, based on an incomplete dive (Table IV.4). The maximum dive depth and duration recorded from a complete dive were 839 m and 16.08 min (Table IV.4). The analysis of dives deeper than 100 m shows that the percentage of time spent on descent, bottom or ascent varied between dives, with means of ≈40, 30 and 30%, respectively (Table IV.4). Higher mean rates of descent and ascent were recorded in the dives deeper than 500 m (Table IV.4).

Table IV.4. Dive statistics of daytime dives deeper than 100 m. Dives ranked by maximum depth. [a]Minimum value since tag detached during beginning of ascent, but the whale could have descended again. [b]Estimated value (due incomplete dive) using the mean ascent rate of the previous (similar type and depth) dive of that whale. [c]Approximate value due incomplete dive. [d]V-shaped dive with no defined bottom. [e]See definition in 'Material and Methods'.

Whale ID	Maximum depth (m)	Duration (min)	% Time at descent	bottom[e]	ascent	Mean rate ($m\ s^{-1}$) of descent	ascent
1	130[a]	9.32[b]	50[c]	25[c]	-	0.4	-
5	170	7.22	56.9	-[d]	43.1	1.2	1.0
1	196	13.20	30.2	42.5	27.3	0.7	0.8
3	584	15.15	24.2	41.3	34.6	1.9	1.8
4	839	16.08	49.4	18.7	32.0	1.8	2.4
4	988[a]	20.09[b]	43[c]	27[c]	-	1.6	-
Mean (SD) dives deeper than 100 m	485 (373)	13.51 (4.69)	42.3 (12.6)	30.9 (10.5)	34.2 (6.6)	1.3 (0.6)	1.5 (0.7)
dives deeper than 500 m	804 (204)	17.11 (2.62)	38.8 (13.1)	29 (11.4)	33.3 (1.9)	1.8 (0.2)	2.1 (0.4)

Discussion

This study shows that six adult pilot whales tagged during daytime in Madeira spent a considerable amount of time at the surface and presented a low diving rate. Even not being directly comparable due to using different depth bins, Wells *et al.* (2013) have shown that two whales tagged in the West Atlantic also spent most of the time at the surface and at shallower depths. Similar findings have also been described for the long-finned pilot whale (Heide-Jørgensen *et al.* 2002). However, and despite the consistency in the results found among whales of different components in this study, a higher number of deployments or of hours of data is required to address the many different parameters that might influence diving behavior.

Information on the time the animals spent at surface can be very helpful in the analysis of visual line-transect surveys assuming the contribution to convert the at-surface abundance to total estimates of abundance (Laake & Borchers 2004). However, individual dive behavior can differ from group diving behavior, so that information is biased if not combined with information on dive synchrony. While in the study of Shane (1995) and in this one (unpublished data) the authors have observed entire pods of short-finned pilot whales submerging during several minutes, acoustic studies have shown an apparent lack of dive synchrony (Jensen *et al.* 2011, Soto 2006, Soto *et al.* 2008). Therefore, further information on dive synchrony is needed in order to determine with accuracy the proportion of time whales are 'detectable', thus accounting more adequately for the 'availability bias' (Laake & Borchers 2004). That information can be acquired with visual behavioral studies, acoustic studies or possibly through tagging of all individuals in a small (3-5 individuals) pod.

The dives recorded in this study are likely to represent maximum diving performance for the species given that dive capability is known to generally increase with body size (Schreer & Kovacs 1997) and all the tagged whales were identified as adults, and given that dives were not depth limited. The depths reported here are in agreement with depth ranges described for daytime deep dives of short-finned pilot whales off Tenerife (Soto *et al.* 2008). Dives deeper than 500 m recorded in this study suggest foraging activity, especially as they are comparable to results in Baird *et al.* (2002) and

Soto *et al.* (2008). Such deep dives have a high energetic cost and likely enable the animals to get a high-gain in return (Thompson & Fedak 2001), for example feeding on high-caloric prey such as squid which have been described as the primary diet of pilot whales (Desportes & Mouritsen 1993, Mintzer *et al.* 2008). Therefore, at least three dives, one from a resident (whale 3) in the south and two from a transient (whale 4) in the east of Madeira Island, are assumed to be foraging dives.

The duration of dives in this study is in accordance with values obtained for this species in the West Atlantic (Wells *et al.* 2013) and long-finned pilot whales (Baird *et al.* 2002, Heide-Jørgensen *et al.* 2002). The mean rate of descent and ascent recorded for dives deeper than 500 m are comparable to the values obtained for similar dives of short-finned pilot whales off Tenerife (Soto *et al.* 2008). Concerning the percentage of time at bottom, the ≈30% obtained during five dives deeper than 100 m in this study are within the range of the means presented for its related species (Baird *et al.* 2002).

The limited number of successful TDR tag deployments during this study may have been caused by difficulties in approaching animals and by a limited number of animals available for tagging. This limited amount of data per disturbance discourages further use of these tags, in substitution of the technologically improved satellite-linked TDRs. Yet, attachment durations in this study did not differ much from those described in Baird *et al.* (2002) and Soto *et al.* (2008).

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CHAPTER V

. Distribution and habitat preferences of short-finned pilot whales *Globicephala macrorhynchus* in Madeira, Portugal

Chapter cover:

A group of pilot whales resting in their preferred habitat area, west of Desertas Islands. Photograph taken from the research rigid inflatable boat *Roaz* in October 2005.

Photo credits: Filipe Alves © Museu da Baleia da Madeira

Abstract

In the waters of the oceanic archipelago of Madeira the short-finned pilot whale *Globicephala macrorhynchus* occurs year-round, is among the most common cetacean species, and transient groups are known to interact with island-associated ones. Yet, one of the needs for the conservation of this species is information on the habitat use, which remains widely unknown. Here, the aim is to investigate this species' relative density and spatio-temporal distribution and to assess its biological factors (behavior, proportion of calves and inter-specific relationships). Additionally, remotely-sensed (Sea Surface Temperature, Chlorophyll-*a* and Colored Dissolved Organic Matter) and physiographic variables (depth, slope, and distance from the coast) are used to help explaining any temporal or spatial distribution pattern, respectively. Effort-related sightings were collected in dedicated nautical and aerial line-transect surveys conducted year-round between 2001-2011 off the coasts of the islands of Madeira, Desertas and Porto Santo. Significant higher encounter rates (ER) were observed during autumn, but only in the area of highest use. Clear spatial distribution patterns of short-finned pilot whales indicate a core/preferred habitat area in the south-east of the island of Madeira. It is suggested that these waters are used for breeding, foraging, resting, socializing, calving and birthing, and thus should be considered an important habitat for this species, at least seasonally. No significant correlation was found between monthly ER and any of the dynamic variables and the whales showed higher ER above deep bathymetries, on low to moderate slopes and in offshore habitats. The information provided here should be considered in governmental conservation plans, and the exposure of the core area to marine traffic and to whale-watching activities should be assessed. The approach used in this study reduced potential biases associated with the analysis of ER per grid cell, providing representative estimates of occurrence to be used by managers.

Introduction

The short-finned pilot whale *Globicephala macrorhynchus* is among the most common cetacean species occurring in the oceanic archipelago of Madeira (Freitas *et al.* 2004). Individuals of different residency patterns have been described, and it is suggested that they belong to a single population encompassing several clans of island-associated whales and others of transients, that interact for mating purpose when they meet (Alves *et al.* 2013). However, and despite the clear importance that the waters of Madeira play for short-finned pilot whales, there is still no information on seasonal fluctuations of abundance, on preferential habitats or on site-specific activities.

Given the increasing number of anthropogenic activities in Madeira with potential impact to cetaceans, with special reference to the whale-watching industry (Ferreira 2007, Vera 2012), appropriate habitat use and distribution studies, to describe the whales' habitat preferences and indicate possible critical habitats, are therefore needed to support conservation measures. Especially, when dealing with a species that on a global scale is described as 'Data Deficient' (IUCN 2012) and in a study area where the existent marine protected sites covers only the coastal waters (up to 100 m depth) (Menezes *et al.* 2011). A key-information for managers is therefore the identification of potential hotspots across time (Hoyt 2011), preferably when based on long-term data sets as in the case of this study.

On the other hand, cetaceans' distribution and habitat preferences have been described by linking their presence to different habitat variables, such as physiographic (e.g. Cañadas *et al.* 2002, Panigada *et al.* 2005, Robinson *et al.* 2009), oceanographic (e.g. Cañadas *et al.* 2005, Panigada *et al.* 2008), or biological (e.g. Littaye *et al.* 2004, Gilles *et al.* 2011). These variables can be used as proxies for prey abundance (de Stephanis *et al.* 2008), that are rarely available at the required spatial resolution to be used for habitat use analysis (as in this case), and is known to most often determine the cetacean distribution patterns as a predator response (Redfern *et al.* 2006). Yet, information on the distribution of cetaceans in relation to physiographic and biological ocean properties in Madeira remains widely unknown.

The present study analyzes sighting data of short-finned pilot whales collected between 2001-2011 in the archipelago of Madeira from nautical and aerial line-transect surveys. Here, using the species' encounter rate (ER), the aim is to investigate its distribution through: 1) time (intra- and inter-annually) and how it is related to remotely-sensed variables (Sea Surface Temperature SST, Chlorophyll-*a* Chl-*a*, and Colored Dissolved Organic Matter CDOM), and 2) space (at a high resolution) and how it is related to physiographic variables (depth, slope, and distance from the coast). Taking the biological factors into account, such as behavior, aggregation size, proportion of calves and inter-specific relationships, the relevance of the results is discussed towards the identification of important areas for the conservation of the species.

Material and Methods

Study area

The study area encompasses the waters around the islands of Madeira, Desertas and Porto Santo in the archipelago of Madeira (Figure V.1). The area covers 8633 km^2, up to 40 km off the coast. It is characterized by a narrow continental shelf, steep submarine canyons and deep waters (ca. 1500m) (Geldmacher *et al.* 2000). The oceanographic patterns are complex due to being influenced by the Portuguese, the Azores and the Canary Currents, as well as by African filaments and by the regional dynamics (Caldeira *et al.* 2002, Caldeira & Sangrà 2012, Sala *et al.* 2013). These oceanographic conditions lead to productive cyclonic eddies and fronts being formed in the flanks of the islands (Caldeira *et al.* 2002).

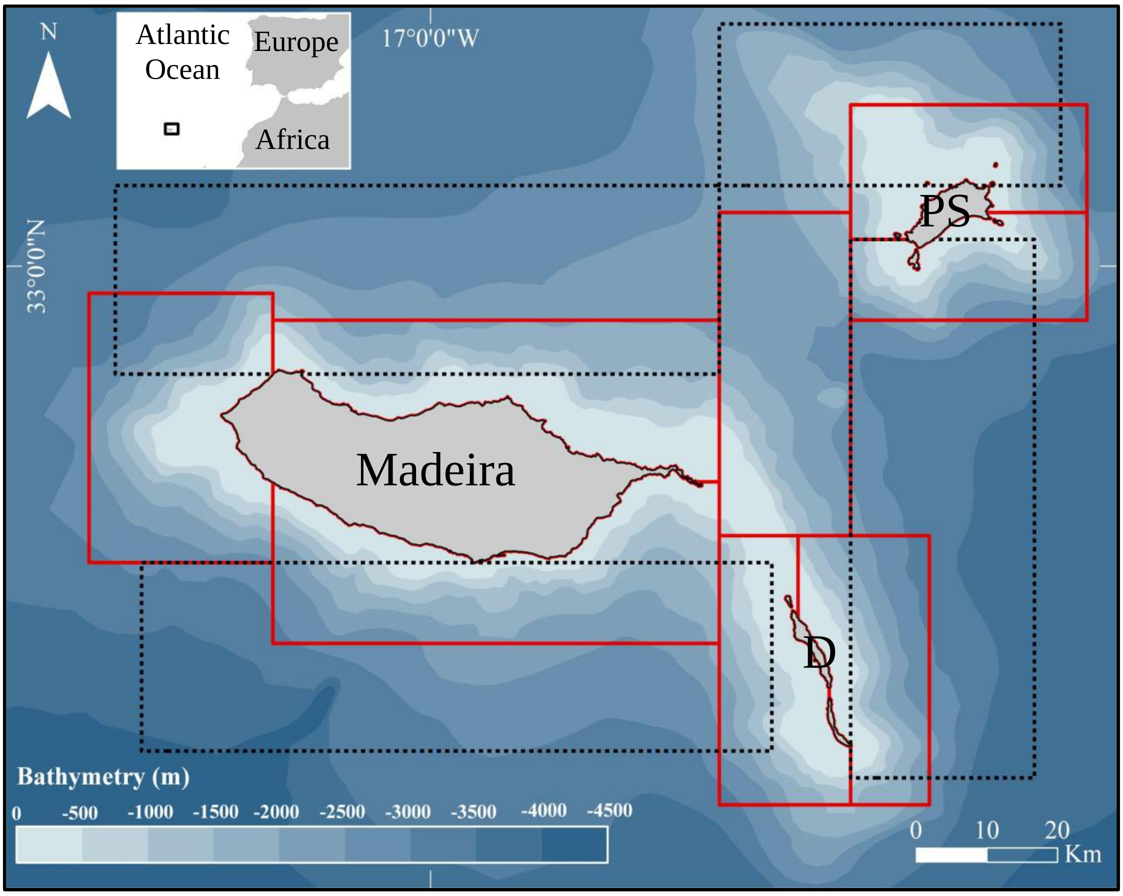


Figure V.1. Localization of the study area in the NE Atlantic and bathymetry of the archipelago of Madeira. The eight sectors from nautical surveys are indicated by solid red lines, and the four sectors from aerial surveys are indicated by dotted black lines. PS: island of Porto Santo, D: island of Desertas.

Data collection

Sighting data on short-finned pilot whales was collected year-round between January 2001 and December 2011 using non-systematic and systematic nautical surveys, and systematic aerial surveys (Table V.1). Non-systematic surveys were carried out mainly from the 6.5 m research rigid inflatable boat *Roaz* (at speeds of ≈25 km h^{-1}), but also from the 18 m research yacht *Ziphius* (12 km h^{-1}). These were conducted more intensively along the south and east of the island of Madeira (≈900 km^2), and an attempt was made to cover all distances and depths within that area. A mean of three observers (min. two, max. four) scanned the area by naked eye, at an eye-height of ≈2 m above the sea level (when using *Roaz*). Track line was registered using a Global

Positioning System (GPS), and Beaufort sea state, effort and sighting data were recorded on printed data forms.

Systematic nautical surveys were carried out from *Ziphius* (2004-2011), but also from the 12 m vessel *Calcamar* (2001-2002; 11 km h^{-1}), over eight established sectors that covered the waters around the islands of Madeira, Desertas and Porto Santo (4637 km^2; see Figure V.1). Three observers searched the area up to the horizon, assisted with 7 x 50 binoculars, at an eye-height of ≈5 or ≈3 m above the sea level when using *Ziphius* or *Calcamar*, respectively. Systematic aerial surveys were carried out from a helicopter Ecureiul AS350B over four established sectors that covered a more offshore area (6312 km^2; intersecting 2316 km^2 of the area of nautical surveys; see Figure V.1). Three observers searched the area from the bow to 90° on each side, at 185 km h^{-1} and 153 m altitude. Both nautical and aerial systematic surveys followed predefined equal spaced zig-zag line-transects generated by Distance 4.0 (Thomas *et al.* 2010) to ensure, as much as possible, equal geographic coverage, and data and track courses were recorded on a laptop using the Data Logging Software Logger 2000 (developed by the International Fund for Animal Welfare) connected to a GPS.

Table V.1. Number of km surveyed under favorable conditions and number of groups of short-finned pilot whales sighted per year, per month and per type of survey.

	Non-systematic nautical surveys		Systematic nautical surveys		Systematic aerial surveys		Total	
Year	km	Groups	km	Groups	km	Groups	km	Groups
2001			2577	5			2577	5
2002			1943	2	1479	1	3422	3
2003					2017	0	2017	0
2004	454	2	652	2	1781	1	2888	5
2005	1858	9			2516	3	4374	12
2006	2005	7			3704	2	5709	9
2007	649	2	919	2	3772	5	5340	9
2008			2274	9	2060	1	4334	10
2009			500	2			500	2
2010	952	10	1551	4			2503	14
2011	3270	25	2510	9			5779	34
Month								
1	308	6	1003	4	1515	0	2826	10
2	528	3	793	0	1483	0	2804	3
3	307	1	942.8	2	1382	4	2632	7
4	567	0	1371	1	2116	1	4054	2
5	1046	2	1312	5	1011	1	3369	8
6	1575	3	1158	3	1016	0	3750	6
7	1244	2	690	1	1755	0	3689	3
8	485	2	974	2	2154	1	3613	5
9	1710	16	2052	9	1172	2	4933	27
10	650	10	1337	2	1166	2	3153	14
11	629	8	787	0	1741	1	3157	9
12	139	2	505	6	820	1	1464	9
Total	9188	55	12926	35	17331	13	39444	103

Beaufort sea state and effort were recorded every hour or every time changes occurred. All sighted groups of short-finned pilot whales were approached for photo-identification purposes (only during nautical surveys; Alves *et al.* 2013), and for confirmation of group size and of inter-specific relationships. The sighting data included coordinates (the closest to the group), initial time, initial behavior pattern (only during nautical surveys), best estimate of group size, and best estimate of number of calves (only during nautical surveys).

Behavior patterns were ranked in three categories: (1) travelling (moving animals, normally on steady course, independent if slowly, moderately or fast); (2) resting (stationary in one place, almost without movement); (3) socializing (clear and constant interaction between the animals in the group, normally stationary in the area, with non-synchronized movements and very active); adapted from Cañadas & Hammond (2006). Calves were defined according to descriptions in Yonekura *et al.* (1980) and Kasuya & Marsh (1984) and could include animals up to few years of age. A group was defined as all individuals within 250 m distance to each other and exhibiting similar behavior (Heimlich-Boran 1993). Two or more species were considered associated when observed in close association and exhibiting a similar behavior during the entire duration of the encounter.

Search effort was measured as the number of kilometers travelled on effort mode under favorable conditions (i.e. Beaufort sea state ≤3). Sightings made off effort and sightings from radio calls were only considered for the analyses of group size, proportion of calves, and inter-specific relationships. Effort and sighting data were transferred into ArcView 9.3.1, which was used for data preparation for the analysis. The study area was divided into a 2’ x 2’ grid, and search effort, sightings, and static variables were associated with each cell using Geographic Information System (GIS) tools.

The static variables used in the analyses were mean depth, mean slope and minimum distance from the coast, and were calculated for each cell using GIS tools. The selected dynamic variables were SST, Chl-*a* and CDOM, and were obtained through remote sensing from the Goddard Earth Sciences (GES) Data and Information Services Center (DISC) for at approximately 5 km south of the island of Madeira (32° 37’ N, 017° 01’ W). While SST data was obtained for the whole study period as monthly averages with a resolution of 9 km, Chl-*a* and CDOM was available only since July 2002 as monthly averages with a resolution of 4 km.

Temporal distribution

The monthly ER (ER_m) was used to assess the temporal distribution of short-finned pilot whales. It was calculated as the number of groups sighted per 100 km of search effort within each sampled month. Data analysis was carried out independently for each type of survey due to using different platforms (with different detection probabilities) and to covering different (or only partial) areas. We used the Pearson's correlation test to assess if the response variable (ER_m) was correlated with each dynamic variable, matching each surveyed month with corresponding data for each variable. Three two-way ANOVA tests and post-hoc Tukey tests, one per type of survey, were used to check if the ER was significantly different between seasons and years. Seasons were defined as winter (January-March), spring (April-June), etc. We used the Shapiro-Wilk and the Levene's tests for the ANOVA assumptions ($\alpha=0.05$). Analyses were carried out using the R 2.14.2 statistical package (R Development Core Team 2012).

Spatial distribution

The ER was calculated for each cell (ER_c) using the number of groups sighted per 100 km of search effort, pooled over the years of the study since no significant fluctuations were found between years. Data analysis was carried out independently for each type of survey, and cells with <5 km of search effort were excluded from the analysis to avoid small sample biases (Panigada *et al.* 2005, de Stephanis *et al.* 2008). The 5 km was selected based on the diagonal of the cell (Fortuna 2006), calculated using ArcGIS.

To investigate if short-finned pilot whales showed fine-scale habitat preference, the observed sighting frequencies in each cell were compared with the expected sighting frequencies, calculated after taking into account the survey effort. The expected sighting frequency of cell i (E_i) was calculated as $E_i = n \times l_i / L$, where n = total number of sightings of short-finned pilot whales, l_i = survey effort in cell i, and L = total survey effort (Silva *et al.* 2012). To investigate if there were annual changes in the pattern of habitat use, the observed and expected sighting frequencies of each cell were calculated separately for each year. The mean ratio of observed to expected sighting frequencies and the standard error (SE) of this ratio were used to assess intensity and persistence of use of each cell. A mean ratio >1 suggests that whales used the cell more often than predicted by the survey effort. Sightings included the behavioral pattern as an attribute to assess site-specific activities.

For the analysis of the ER_c in relation to the static variables, the depth, slope and distance from the coast were ranked into bins. Slope values were expressed in degrees, where 0 represents horizontal and 90 represents vertical.

Group size, proportion of calves and inter-specific relationships

Sightings recorded in search and off effort during all type of surveys, as well as sightings collected opportunistically during 2012 (in the study area from *Roaz* or *Ziphius*), were considered in order to provide more robust analyses. Group size, number of calves and presence of other species were recorded at sea. A one-way ANOVA test was used to check if significant differences in group size existed between months. A binomial Generalized Linear Model (GLM) with logit link function was used to test if the proportion of groups with calves differed significantly between months and years (as factors), and a two-way ANOVA test was used to assess if the percentage of calves in groups with calves differed significantly between months and years. Data from aerial surveys were excluded from the analysis of the proportion of calves due to the difficulty in recording the presence and numbers of calves during flights. Finally, the percentage of encounters that displayed inter-specific relationships was calculated.

Results

Temporal distribution

A total of 103 sightings were recorded during 39444 km (see Table V.1 for yearly and monthly values according each type of survey). These were recorded in every calendar month, and higher mean ER_m are shown from non-systematic nautical surveys in almost every month (Figure V.2). No significant correlation was found between the ER_m and any of the variables, for each type of survey (*P*-values are n.s., Figure V.2). The two-way ANOVA tests revealed no significant differences in the ER between seasons, between years, or due to the interaction of both factors (*P*-values are n.s.), for each type of survey, exception made for seasons on non-systematic nautical surveys where autumn showed significant higher ER when compared with the other seasons ($P<0.01$).

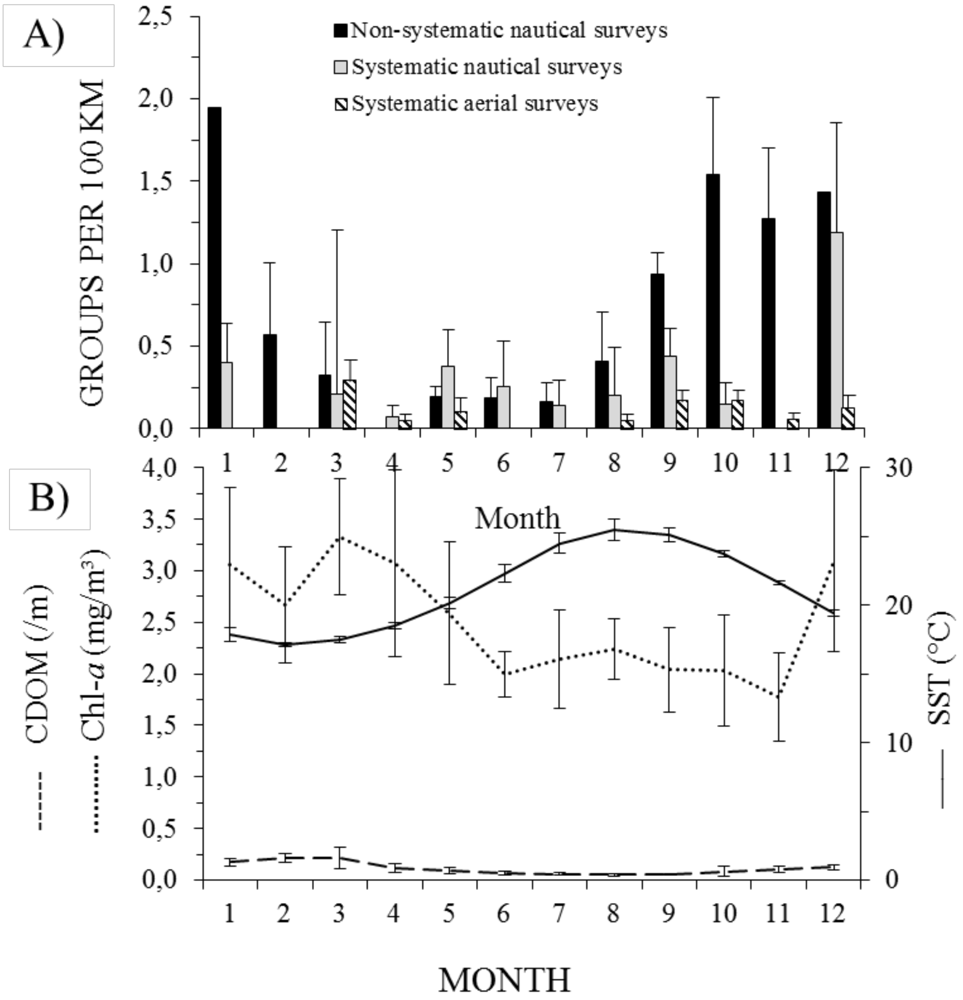


Figure V.2. A) Mean monthly encounter rate per type of survey, calculated separately for each year (±SE); B) mean monthly values of SST, Chl-*a* and CDOM from a single location (see 'Material and Methods'), calculated separately for each year (±SD). No significant correlation was found between monthly encounter rates and any of the variables, for each type of survey (Pearson's correlation, *P*-values are n.s.).

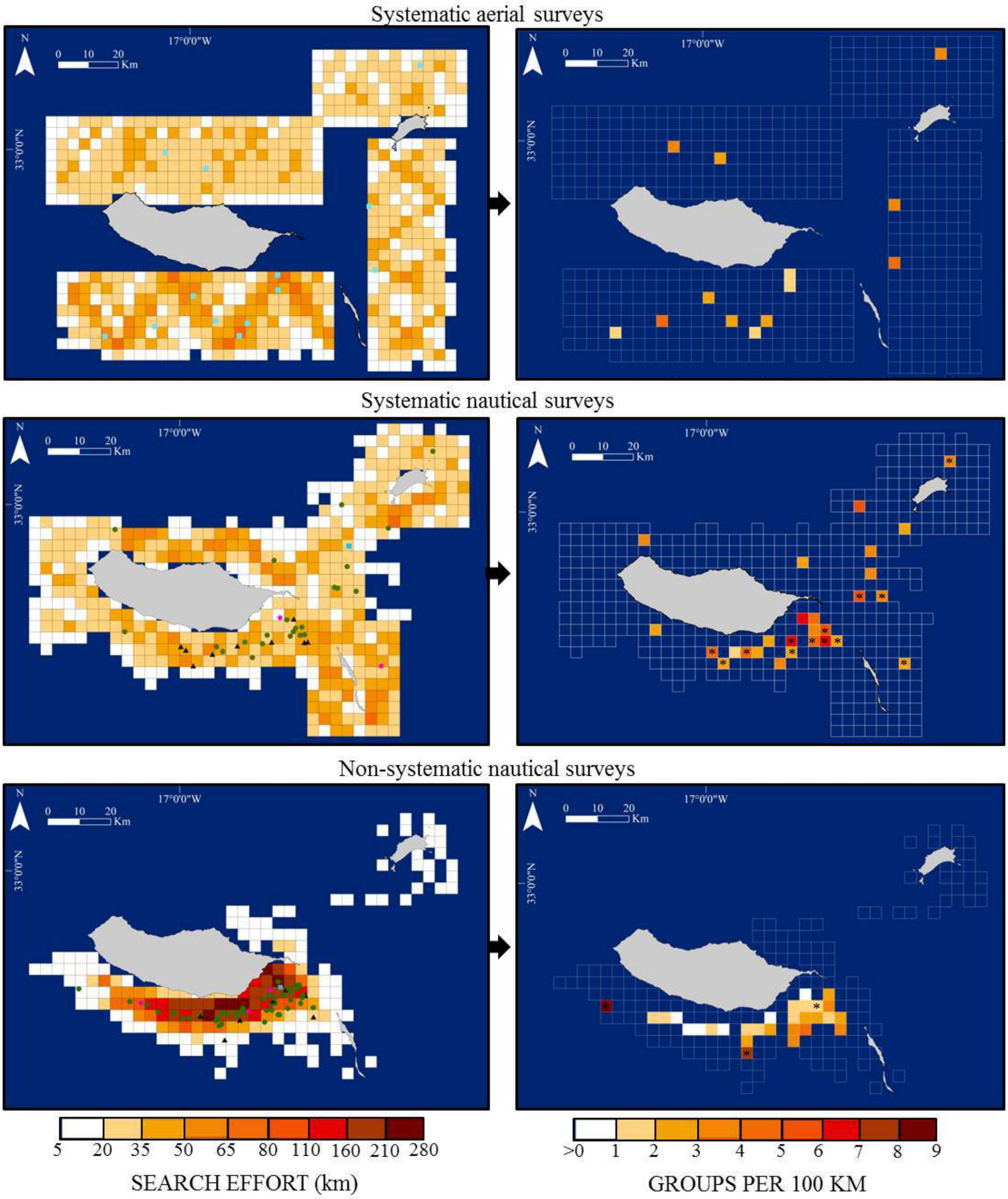
Spatial distribution

Limiting the analysis to cells with ≥5 km of search effort resulted in the exclusion of 252 km of transect line and one sighting from non-systematic surveys, of 134 km from systematic nautical surveys, and of 128 km from systematic aerial surveys. The search effort used in the analysis covered 185, 461 and 644 cells, respectively (Figure V.3). The 54 sightings from non-systematic surveys were recorded in 29 cells (16% of the cells surveyed), the 35 from systematic nautical surveys were recorded in 27 cells (6%), and the 13 from aerial surveys were recorded in 13 cells (2%) (Figure V.3).

In cells with sightings the ER_c varied from 0.37 to 8.71 during non-systematic surveys, from 1.94 to 6.93 during systematic nautical surveys, and from 1.53 to 4.62 during aerial surveys. The systematic surveys, which covered the three islands, indicated a higher number of cells with ER_c>0 in the south and south-east of the island of Madeira. The non-systematic surveys, which presented greater search effort in that area, indicated that all the cells with ER_c>0 were concentrated in the south and south-east of Madeira, with one exception in the south-west of that island. The cells with the two highest ER, located in the south-west (ER_c=8.71) and in the south of Madeira (ER_c=7.51), corresponded to cells with low search effort (11 and 13 km, respectively) (Figure V.3).

Data from systematic aerial surveys were excluded from the calculation of the mean ratio of observed to expected sighting frequencies. Sixteen cells, 3 from the analysis of the non-systematic and 13 from the systematic nautical surveys, presented a mean ratio >1 (Figure V.3 and V.4). Eleven (69%) of those cells are located in the south and east of the island of Madeira (indicated with an asterisk in Figure V.3).

Behavioral patterns were attributed to 87 groups, 53 during non-systematic and 34 during systematic nautical surveys (Figure V.3). Of these, 66 groups (76%) were travelling, 16 (18%) were resting and 5 (6%) were socializing. While travelling groups were recorded all over the study area, resting groups were restricted to the south and south-east of the island of Madeira. Socializing groups were recorded along the south of Madeira (four occasions) and in the east of Desertas (one occasion) (Figure V.3).



Systematic aerial surveys

Systematic nautical surveys

Non-systematic nautical surveys

Figure V.3. Distribution of the search effort and sightings of short-finned pilot whales per behavior pattern (left column), and of the encounter rate (right column) by cell over the study area according to each type of survey. Cells with <5 km of search effort were not considered. Behavior patterns: ● travelling, ▲ resting, ⬟ socializing, ■ undetermined. Cells with a mean ratio of observed to expected sighting frequencies >1 are indicated with an asterisk (*) according to Figure V.4.

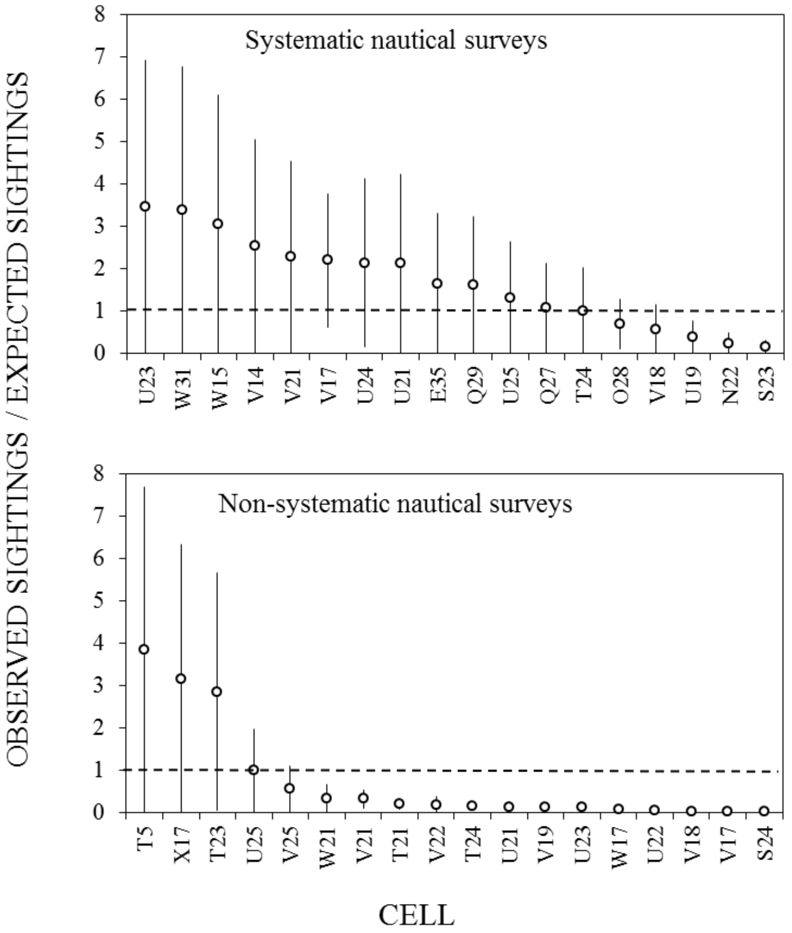


Figure V.4. Mean ratio of observed to expected sighting frequencies in each cell, per type of survey. Ratios were calculated separately for each year, considering only cells with ≥5 km of search effort. Vertical bars represent SE. Cells with mean ratio <0.03 are omitted.

The distribution of the ER with respect to the effort in each bin of the static variable showed that higher ERs were recorded over bathymetries ranging between 1000 and 2500 m, on slopes ranging between 5 and 15°, and in distances from the coast ranging between 5 and 20 km (Figure V.5).

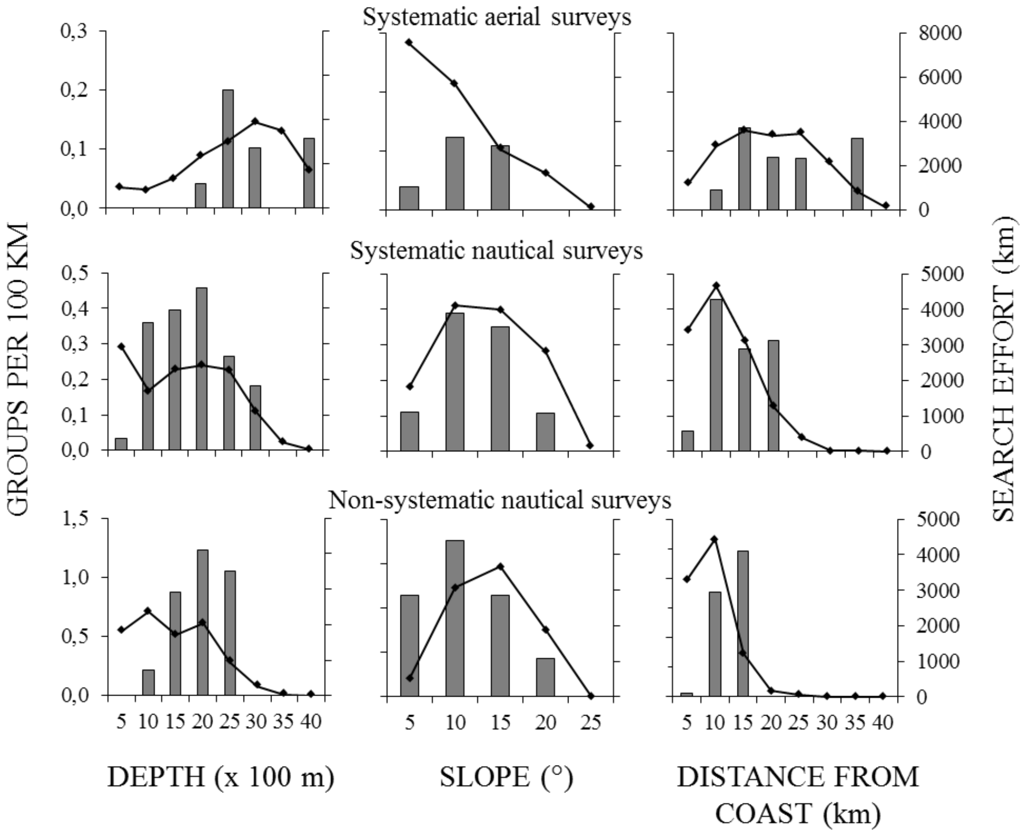


Figure V.5. Distribution of encounter rate (bars) and search effort in favorable conditions (♦) in relation to depth, slope and distance from the coast, per type of survey. The bin 5 covers the interval 0-5, the bin 10 covers the interval 5-10, etc.

Group size, proportion of calves and inter-specific relationships

The mean group size was based on 167 sightings, being 102, 47, and 18 during non-systematic nautical surveys, systematic nautical surveys, and systematic aerial surveys, respectively. The mean group size was 17 individuals (median=13, SD=13, range: 1-80). The one-way ANOVA test revealed no significant differences in group size between months (*P*=0.55).

Based on 149 sightings, 110 groups (73%) were recorded with calves. The binomial GLM showed no significant differences (*P*-values are n.s.) in the proportion of groups with calves per month and per year (Figure V.6). The mean percentage of calves in those 110 groups was 16% (SD=10%). The two-way ANOVA test revealed no significant differences in the percentage of calves between months, between years, or due to the interaction of both factors (*P*-values are n.s., Figure V.6).

Based on 167 sightings, 54 groups (33.5%) were associated with other species. Of those, 91.1% of the times were with bottlenose dolphins *Tursiops truncatus*, 5.4% with non-identified small delphinids, 3.6% with sperm whales *Physeter macrocephalus*, and 1.8% with Atlantic spotted dolphins *Stenella frontalis*.

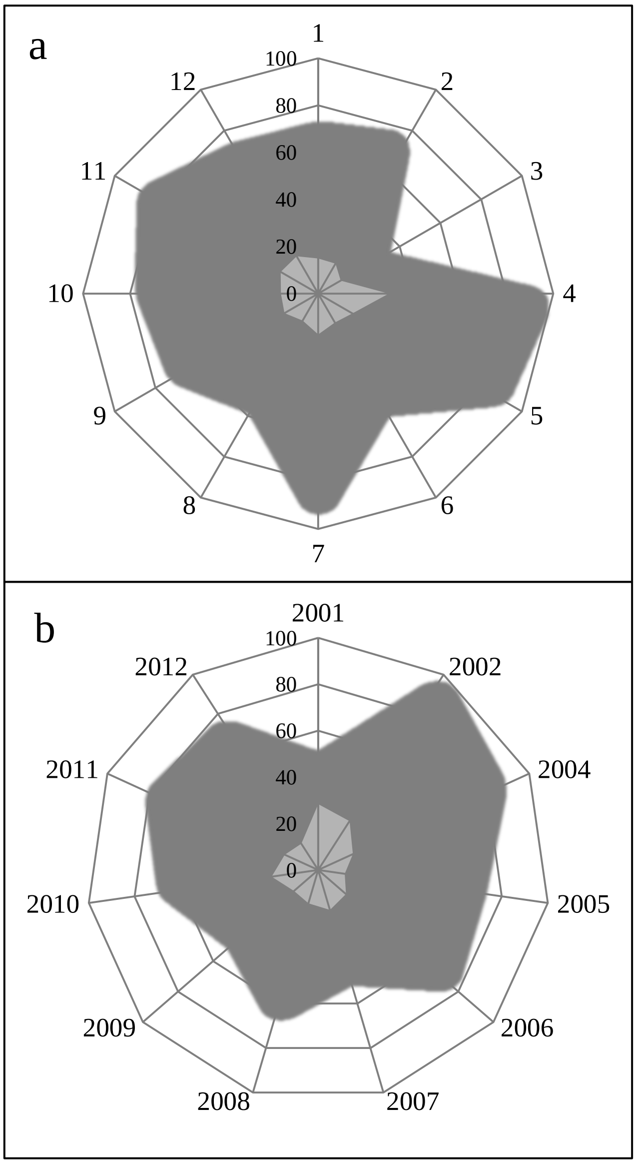


Figure V.6. Proportion of groups with calves (dark grey outside area, *n*=149) and mean percentage of calves in those groups (light grey inside area, *n*=110) per month (a) and year (b).

Discussion

This study presents 11 years of effort-related sightings based on nautical and aerial surveys, providing new information on the spatio-temporal distribution patterns of short-finned pilot whales in the archipelago of Madeira. One of the main findings is that this species shows preferential usage during a specific season and over a confined geographical space. Significant higher ERs were observed in autumn, but only when using data from non-systematic nautical surveys. This is likely because the majority of its effort coincided with the species' preferred area of occurrence, contrarily to the other types that survey that area only partially. Higher ERs of short-finned pilot whales have been also described over this same season in the neighboring archipelago of the Canaries (Heimlich-Boran 1993, Carrilo *et al.* 2010), which support the hypothesis that transient clans move to inshore waters of these warm-temperate archipelagos of the north-east Atlantic to meet and breed with island-associated whales (Alves *et al.* 2013).

A core area, in the south-east of the island of Madeira, is indicative of the whales' habitat preferences. Interestingly, this area was described as a particularly dynamic area and a region of interest with localized upwellings (Caldeira *et al.* 2002). Unfortunately, there is a lack of information on how it affects local prey resources, as well as on the diet of this species in the study area. Yet, given the suggested deep foraging dives by short-finned pilot whales around this area (Alves *et al.* in press) and the fact that a local fishery directed to squid, known as their main diet (Desportes & Mouritsen 1993, Mintzer *et al.* 2008), operates almost every year during July/August in this area (own observations), may indicate that it constitutes an important feeding habitat for this population. Adding the information on behavior patterns shows that all the groups observed resting and ⅘ of the groups observed socializing were also in, or its surrounding, area, which reinforce its importance.

Several biases, such as variations in detection probability due to weather conditions or platforms, are known to potentially affect the type of analysis used in this study (Matthiopoulos & Aarts 2010). However, our approach addressed several factors that reduced seasonal and geographical biases, thus contributing for obtaining representative estimates of occurrence. These included: a) analyzing year-round data, b) pooling the data over the years, c) providing coverage throughout the whole study area, d) estimating variability in the data, e) excluding cells with low search effort, f) limiting the search effort to favorable conditions, g) using the closest sighting coordinates to the groups, and h) analyzing each type of survey independently. Although the maps do not reflect seasonal relative densities of short-finned pilot whales, these are likely to represent the preferential spatial distribution patterns since no other hotspot has been detected. Moreover, the absence of significant differences in group size between months indicates that the detection probability of a group in a month is independent of the size of the group. Finally, as methodological consideration it should be highlighted that, although having covered a different (or only partial) area, the much lower values obtained from aerial surveys suggest potential associated bias, which should be addressed in studies using such platforms.

On the other hand, this study also tried to assess how dynamic and physiographic variables affect the distribution of short-finned pilot whales. Yet, only the latter provided important information, with the whales being distributed over deep bathymetries, in agreement with Baird *et al.* (2006) and Carrillo *et al.* (2010), on low to moderate slopes, and in offshore habitats. The Chl-*a* values presented here are higher than *in situ* data (as also observed by Caldeira *et al.* [2002] that compared remote sensing and *in situ* data in Madeira), and thus should not be considered as absolute values.

Being aware that management and conservation issues are addressed by providing a preliminary identification of areas of particular importance for a species (Panigada *et al.* 2008), the findings described here should be taken into consideration by managers. It is thus suggested that the south-east of the island of Madeira constitutes an important area for this species, either for resting, socializing, foraging or breeding between pods. While breeding is suggested to be mainly associated with the increase in the relative density during autumn, the area plays an important role for the species throughout the year. A complete understanding of the oceanographic and ecological processes guiding the Madeiran waters is still missing (Caldeira & Sangrà 2012). Improving it and understanding how it can affect cetaceans' occurrence may allow to more accurately describe areas of particular importance. As precautionary approach it is recommended that managers should consider a large area to accommodate uncertainty in the definition of critical or important habitat of a wide-ranging species such as this (Hoyt 2011).

Finally, the large proportions of groups with calves (73%) and of calves in those groups (16%) recorded throughout the year in this study suggest that these waters are used for calving. The observation of four dead neonates in the core area (Cátia Nicolau, unpublished data from the Madeira Whale Museum) suggests that the area may be also used for birthing. Moreover, ⅓ of the groups were observed in inter-specific relationships, especially with a species listed in Annex II of the Habitats Directive, which is actually the target species for a proposal of a Site of Community Importance to be delivered by the Madeira Whale Museum to the Regional Government. In light of all this, it is proposed that this region, with special emphasis for the south-east of the island of Madeira and particularly during autumn, constitutes an important habitat for this species. Additionally, the exposure of the south-east area to marine traffic and to whale-watching activities should be assessed and considered in governmental conservation plans.

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CHAPTER VI

. General Discussion

Chapter cover:

The author using a directional antenna and a radio telemetry receiver to search for a signal from a tag deployed in a short-finned pilot whale. Photograph taken in the top of Deserta Grande (Desertas Islands) in November 2010.

Photo credits: Nuno Sá

Synthesis

The assessment of the conservation status of a species and the definition of management measures by biologists and managers must be based on a fair knowledge of the species ecology and of the environment in which they live. The basic support of such knowledge should rely on rigorous science, which in the case of cetaceans usually involves large data-series that are logistically challenging and expensive (Harwood 2001, Evans & Hammond 2004). Science can provide, for example, information on the structure and size of the populations that are to be conserved, its distribution patterns, how the animals use their habitat and the impact of the threats over the populations. It can, and must, contribute also to the identification of the most effective management actions (Cañadas 2006). In the present work, the study of all factors constituting the environment in which pilot whales live (either on their socio-economic role or on the identification of anthropogenic threats) and the evaluation of the impact that threats may have over their population fell out of its main scope, being addressed only lightly. On the other hand, the present work, based on data collected over an 11 years' period, contributed substantially to a better understanding of the ecology of the short-finned pilot whale, and indirectly to its conservation in the archipelago of Madeira.

The analysis of the grouping structure in Chapter II provided one of the major findings of this work, with short-finned pilot whales demonstrating a large degree of variability in site fidelity. It revealed the presence of at least one island-associated community, including resident and regular visitor whales, and several pelagic communities, based on transient whales. Resident whales were observed year-round and exhibited long-term site fidelity to the area. The social and temporal analyses revealed a well-differentiated society with long-lasting relationships (at least of the duration of the study). An important finding from a conservation standpoint is that individuals with different residency status do mix. These have been photographed together, especially between July and December, and the genetic analyses support it. The latter suggest that individuals of the three residency patterns may not be genetically isolated, and that small groups are made up of related individuals, implying some degree of social philopatry, while large groups are probably temporary associations of smaller groups. It is then proposed that the pilot whales encountered in Madeira belong to a single population encompassing several clans that interact for mating purpose when they meet. The several clans include possibly three clans of island-associated whales and others of transients, each containing two to three matrilineal pods, each with about 15 individuals.

This study, the first one using a combination of photo-id and genetics in pilot whales, tends to corroborate the hypothesis described by Heimlich-Boran (1993), that the short-finned pilot whale has a matrilineal hierarchical system, which, being similar to that of the long-finned pilot whale (Amos *et al.* 1991a, 1991b, 1993a, 1993b, Fullard 2000), support that *Globicephala* spp. adopt this social pattern.

The analysis of the same photo-identification dataset used in Chapter II, based only on high-quality and well-marked individuals, have provided estimates of some vital demographic parameters for this population in Chapter III using mark-recapture methods. The analysis corroborated the presence of island-associated whales and the presence of a 'meta-population' using the area with high levels of permanent emigration. Despite transience was expected as a consequence of analyzing free-ranging animals in an open pelagic environment, the limitations that it induced in the dataset subsequently challenged the analysis. Additionally, because this constitutes the first estimates of population parameters for short-finned pilot whales using mark-recapture methods, and especially because the literature using similar procedures in gregarious cetacean species (like in this case) is rare (e.g. Verborgh *et al.* 2009), the analytical challenges were even more difficult to overcome. The approach used in this work used different population models, and even including some limitations, it provided plausible estimates for survival of adult whales (0.96, which is within the range of other long-lived cetacean species) as well as for abundance of island-associated whales (less than 150 individuals) and of individuals using the area seasonally (around 300 individuals) in the south of the island of Madeira. Although studies analyzing trends in abundance usually require a larger dataset than the one used in the present work (Chaloupka *et al.* 1999, Wilson *et al.* 1999, Cañadas 2006), the seven years used here showed no trend. Nevertheless, these results provide a useful baseline when considering conservation actions.

The analysis of the data collected from the biologgers deployed in six adult short-finned pilot whales during daytime is presented in Chapter IV. It revealed that the whales spent over ¾ of their daytime at the surface and presented a low diving rate. Notwithstanding the low number of tagged whales, one interesting feature is that an *a posteriori* analysis as an outcome of Chapter V indicated that all whales were tagged in their preferred habitat area. The deep dives, which are usually associated to foraging behavior (Baird *et al.* 2002, Soto *et al.* 2008), could suggest that their preferred area is also used to forage. Despite the consistency in the results found in this study among whales of different gender and residency pattern, and the similarity of these results with those of other telemetry studies in pilot whales (Baird *et al.* 2002, Heide-Jørgensen *et al.* 2002, Soto *et al.* 2008, Wells *et al.* 2013), the low number of deployments prevented a more robust analysis. Nevertheless, that suggestion should not be ignored when considering conservation actions. Information that could corroborate the indication that short-finned pilot whales' habitat preference (see below) in Madeira is driven by their diet (e.g. following the distribution of prey or of the squid fishery) is lacking.

Finally, in Chapter V, the analysis of approximately 40000 km of effort-related sightings collected over a decade provided consistent information on the spatio-temporal distribution of short-finned pilot whales up to the 40 km off the islands of Madeira, Desertas and Porto Santo. The whales showed higher encounter rates in offshore habitats, above deep bathymetries and on low to moderate slopes, and a core area was identified in the south-east of the island of Madeira. Significant higher encounter rates were observed during autumn, but only in the area of highest use. The approach used in this chapter analyzed year-round data with no significant inter-annual fluctuations, provided coverage throughout the whole study area, estimated variability and accounted for reducing geographical and seasonal biases in the data, thus ensuring representative estimates of occurrence. Additionally, besides from the suggestion that the core area could represent a feeding habitat for this species

(Chapter IV) and that transient/pelagic pilot whales move to inshore waters of this archipelago to meet and breed with island-associated whales (Chapter II), in Chapter V it has been shown that all the groups observed resting and ⅘ of the groups observed socializing were in the south and south-east of the island of Madeira. It also shows that about ¾ of the groups have calves and that approximately ⅓ of the groups were observed in inter-specific relationships. Therefore, it is suggested that the core area could constitute an important habitat for this species, and this should be considered in conservation actions.

Towards Conservation

One of the objectives of the present thesis was to contribute to the conservation of the short-finned pilot whale in the archipelago of Madeira. Conservation-related issues have been addressed in the 'Discussion' of the majority of the data chapters, especially in Chapters II and V. The suggested potential gene flow between the different types of communities can prevent genetic divergence of island-associated communities (Chapter II), in which a single population in the warm-temperate oceanic waters of the north-east Atlantic (the 'meta-population', Chapters II and III) could be regarded as a single 'management unit'. However, the presence of island-associated whales implies a high risk of exposure to local threats. Those island-associated whales should not be regarded as demographically independent populations but instead as stable social entities to be included in governmental management plans and requiring a periodic evaluation of their status. This could include the estimation of the populations' demographic parameters, as done in Chapter III, from data collected during surveys established by a systematic monitoring plan. Such a plan towards the conservation status of cetaceans in the archipelago of Madeira (Freitas & Alves 2004) has been proposed to the Madeira Regional Government as a result of the LIFE project CETACEOSMADEIRA (see 'Note from the author') and should be continued.

The suggestion of an important area in the south-east of the island of Madeira for the target species (Chapter V) should be taken seriously by the competent authorities when considering conservation plans for cetaceans in Madeira. It has been shown that it represents a seasonally important area (at least in autumn), for resting, socializing, foraging or breeding between pods (Chapters II, IV and V). To a wider extent, the study area is also important for calving and birthing (Chapter V). These activities are even in agreement with the criteria for determining the critical habitat of cetaceans according to Hoyt (2009, 2011), which reinforces the importance of that area. Those two studies also mention the importance of a critical area to cetacean prey, to upwelling conditions or ocean fronts, and to the presence of topographic structures favorable for enhancing foraging opportunities for cetaceans. These three criteria seem to apply to the case presented here and have been also addressed in Chapter V. The possibility of the south-east of the island of Madeira representing a critical area for the target species should be further investigated.

After the first step of identifying an important habitat for a cetacean species, protecting it becomes also a difficult task. Marine Protected Areas (MPAs) have been proven one of the best ways to protect the habitat of wide-ranging species such as cetaceans, offering the possibility of a far-reaching impact on conservation (Hoyt 2011). As mentioned in Chapter V, there are no MPA’s for cetaceans in Madeira. However, a proposal of a Site of Community Importance (pSCI) is to be delivered to the Regional Government of Madeira as a result of the LIFE+ project CETACEOSMADEIRA II (see ‘Note from the author’). The SCI is known to target only species listed in the Annex II of the Habitats Directive, and in Madeira, the bottlenose dolphin figures as the only species with such a status. Nevertheless, an SCI can have an ‘umbrella’ effect, which means that defending one species automatically implies that many other species get protected too (Zacharias & Roff 2001, Cañadas 2006, Fortuna 2006). In the scenario of the creation of the SCI, the short-finned pilot whale could certainly be one of those species, especially if the identified important habitat (the south-east of the island of Madeira) will be included in the area of the pSCI. Being listed in the Annex IV of the Habitats Directive, the output provided here on the short-finned pilot whale should be included in the pSCI as additional information. The fact that approximately ⅓ of the groups of short-finned pilot whales had been observed with bottlenose dolphins (Chapter V) may reinforce the importance of including this species.

Yet, in order to be effective, MPA's should have an ecosystem-based management approach. This is a management regime that recognizes that maintaining the structure and function of ecosystems is vital, and that human uses and ecosystem health are interdependent (UNESCO 2009, Hoyt 2011), i.e. management priorities should start from the ecosystem rather than from the target species (Pikitch *et al.* 2004, Harwood 2010). An impact assessment of the main threats to the short-finned pilot whale, and to cetaceans in general, should be carried out in Madeira. On the other hand, all stakeholders (Madeira Regional Government, schools, general public, etc.) should take advantage of these charismatic species, using them as a 'flagship' to the protection of the entire ecosystem, either throughout educational events or specific legislation.

Even not being one of the main objectives of the present work, the knowledge acquired during the thousands of hours of fieldwork and the knowledge of the local realities, allowed me to comment on potential threats to the target species as mentioned in the 'Objectives' (Chapter I). Firstly, as mentioned in the 'Study Area' (Chapter I), me and other three observers had the opportunity to embark in platforms of opportunity (tuna fishing vessels) in the waters of the Madeira EEZ for the project CETACEOSMADEIRA II. This allowed monitoring 295 tuna fishing events between 2010-2012, covering approximately 18% of the fishing events in that period. During those events the short-fined pilot whale was never recorded (Nicolau *et al.* 2013). Moreover, only three sightings of short-fined pilot whale were recorded from 5220 km of survey effort in the offshore waters (>40 km from the coast) of the Madeira EEZ (Cátia Nicolau, unpublished data from the Madeira Whale Museum). Considering the low probability of interaction with the fisheries, this preliminary assessment indicates that there is little potential disturbance to these whales by the fishermen (mentioned in the 'Objectives' as a potential threat). Although, the level of disturbance is unknown when in interaction, and that should be assessed.

Secondly, I believe that the short-finned pilot whale is among the most vulnerable cetacean species to an unregulated whale-watching industry. This is because the species is present year-round, has less than 150 island-associated whales, lives in matrilineal cohesive pods, and the area identified as important habitat partially coincides with the main area used by this industry. The fact that very recently the whale-watching activity had become legally regulated should, or is expected to, minimize some of the potential negative effects (see for example Bejder *et al.* 2006a, 2006b, Lusseau & Bejder 2007) on this population.

The area identified as important habitat coincides with one of the highest areas exposed to marine traffic in Madeira (Inês Cunha, unpublished data from the Madeira Whale Museum), being between the two main harbors of the island. Although the local marine traffic is not comparable to the high levels of other areas of the world, such as the Strait of Gibraltar that also host a resident population of pilot whales (de Stephanis 2007), this pressure and the assessment of its potential impact should not be ignored when considering conservation plans for this species in Madeira.

Finally, based on the scientific findings of this work, especially on the gene flow between island-associated and transient whales, and on the lack of any major identified threats with direct impact on these whales for the near future, I foresee no apparent reason to change the conservation status of the short-finned pilot whale for the archipelago of Madeira, defined in Cabral *et al.* (2005) as 'Least Concern'. However, the monitoring plan of the status of cetaceans in the archipelago of Madeira should continue, with special attention to the proposed important habitat.

Future Work

New questions typically arise at the end of most ecological studies, and this thesis constitutes no exception. Here, I suggest future studies in the line of the present work.

1. To study the movements of the resident, regular visitor and transient short-finned pilot whales through satellite tagging or comparison of photo-identification catalogues with neighboring regions. This would allow determining their range, with implications for conservation on a wider scale.

2. To continue to study the population demographic parameters in order to assess trends, either with estimates from photo-identification (mark-recapture) and/or line-transect methods.
3. To determine if the observed strong inter-specific relationship with bottlenose dolphins concerns always the same individuals (of both species). This could be done by photo-identification.
4. To analyze the diet of the short-finned pilot whale and the distribution of their prey, and control the landings of the fishery of their possible prey in Madeira, which are fundamental parameters for better understanding the distribution of these whales and to assess the level of human exploitation on their main prey. The stomach contents of all stranded whales should be analyzed, and biochemical analysis of blubber fatty acids and/or stable isotope analyses from skin samples collected from wild animals through biopsies could be used.
5. To compare the vocal repertoires between clans in Madeira, and with clans of neighboring regions, which could help assigning ID's to clans (see Scheer 2013).
6. To assess the impact of some of the main potential threats outlined in this work. This includes the assessment of: i) the level of disturbance caused by the tuna fishermen when in the presence of short-finned pilot whales during fishing events, ii) how the whale-watching legislation improved the way operators approach the whales, and iii) the acoustic impact of marine traffic.
7. To perform oceanographic studies in the area of the underwater ridge that connects the island of Madeira and Desertas, which is a particular dynamic area that coincides with the proposed important habitat. This would allow a greater understanding of the oceanographic and ecological processes underlying that area of high importance for this species.
8. Finally, high levels of heavy metals have been detected in several fish species in Madeira (e.g. Afonso *et al.* 2007), and the short-finned pilot whale is an apex predator. It would be interesting to study the level of those pollutants in these whales to assess their health.

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