Bernat Martí Alsina

Using image analysis to study structure, population size and connectivity patterns of the whale shark *Rhincodon typus* in the Azores archipelago

UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia

2021

Bernat Martí Alsina

Using image analysis to study structure, population size and connectivity patterns of the whale shark *Rhincodon typus* in the Azores archipelago

Mestrado em Biologia Marinha

Supervisors:

Dr. Jorge Fontes

Co-supervisor

Dr. Karim Erzini

UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia

2021

Statement of authorship and copyright

Title*: Using image analysis to study structure, population size and connectivity patterns of the whale shark Rhincodon typus in the Azores archipelago*

Declaro ser o(a) autor(a) deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

_____

Bernat Marti Alsina

_____

Bernat Marti Alsina

English Abstract

In the last recent years, whale sharks (*Rhincodon typus*) have been found to aggregate in the waters of the Azores archipelago, during the summer season. In this study we used photo-identification data collected during years 2008 through 2011, 2013, 2017, 2019 and 2020 to produce the first estimates of population structure, site fidelity, abundance, and movements of these aggregations. A total of 3,705 photographs were collected, of which 545 were suitable for comparison using the semi-automated matching software and photographic identification database Wildbook for Whale Sharks. A total of 186 individuals were uniquely identified through the combination of spot and stripe patterns behind the last gill slit and forward of the dorsal fin (left and right lateral views). Among the 115 sexed individuals, 31% were males and 69% were females, confirming a female-biased aggregation. A total of 38 individuals (20%) presented scars during the different years of the study. The majority of whale shark encounters occurred in the island of Santa Maria (82%), especially during the months of August and September (81%). Of the 29 individuals resighted during the study, 28 were resighted in different occasions during the same year, and 4 individuals were resighted in different years (ranging from 1 to 3 years). Inter-island connectivity was also observed for one whale shark. We estimated the population of whale sharks that visit the Azores region to consist of approximately 283 – 496 individuals (95% confidence interval) based on Cormack-Jolly-Seber open-population models. This study provides the first population abundance estimates and characterization of the whale shark population of Azores, as well as assembling value information regarding their ecology and connectivity patterns.

Portuguese Abstract

A gestão eficiente e as estratégias de conservação de populações de fauna selvagem necessitam de conhecimento sólido da distribuição espacial, conectividade e demografia das mesmas. No entanto, para a megafauna marinha, obter tais informações pode ser desafiante e dispendioso, devido ao comportamento migratório e ao estilo de vida em mar aberto de muitos destes organismos (cetáceos, tubarões, raias, espadins e espadartes), tornando a amostragem extremamente trabalhosa, e demorada. O tubarão-baleia (*Rhincodon typus*, Smith 1828) é um exemplo claro dos desafios que envolvem a avaliação demográfica, distribuição e habitat e padrões de conectividade da megafauna marinha.

Rhincodon typus pertence à família monotípica Rhincodontidae, pertencente à Ordem Orectolobiformes, e é o único representante da ordem que apresenta estilo de vida pelágico. Pensa-se que os tubarões-baleia são cosmopolitas, ocorrendo em todas as águas tropicais e temperadas quentes, com preferência por águas com temperaturas de superfície de 21 - 25ºC. Os tubarões-baleia são uma espécie icónica, com um elevado valor ecológico e económico. No entanto, em muitos aspetos, e apesar da investigação fundamental desenvolvida nas últimas décadas, existem ainda grandes lacunas no que diz respeito à ecologia dos tubarões-baleia, tamanhos das populações regionais, e aspetos chave da sua biologia, tais como taxas de crescimento específicas da idade, taxas reprodutivas, e habitats de reprodução.

A população global de tubarões-baleia sofreu uma redução superior a 50% nos últimos 75 anos, como resultado da ação antropogénica, sob a forma de pesca direta, perturbação do habitat e navegação. Como consequência, *Rhincodon typus* está agora classificado como "Ameaçado" na Lista Vermelha de Espécies Ameaçadas da IUCN.

Apesar de serem largamente oceânicos, passando a maior parte das suas vidas em mar aberto, os tubarões-baleia formam agregações sazonais em águas costeiras de regiões temperadas e tropicais quentes, levando os tubarões-baleia a ter um estilo de vida migratório, realizando grandes migrações transfronteiriças de alimentação. Estas agregações sazonais previsíveis - principalmente de machos juvenis e subadultos parecem ser altamente influenciadas por alterações interanuais na temperatura da superfície do mar (SST) e mudanças climáticas. Estes locais de agregação de tubarões-baleia oferecem aos cientistas uma oportunidade de recolher informação acerca destes grandes animais, e proporcionam oportunidades lucrativas para a indústria do turismo. Por esta razão, são necessários esforços

de investigação das agregações de populações de tubarões-baleia e das suas rotas migratórias, possibilitando a implementação de estratégias adequadas de proteção a nível regional, nacional e internacional, de modo a assegurar a conservação desta espécie a longo prazo.

Observou-se que as agregações de tubarões-baleia ocorrem sazonalmente em várias ilhas, bem como em alguns locais costeiros, tais como 1. Golfo da Baja California, 2. ilhas Galápagos, 3. norte do México, 4. Belize, 5. Santa Helena, 6. Arábia Saudita, 7. Djibuti, 8. Moçambique, 9. Madagáscar, 10. África do Sul, 11. as Seicheles, 12. as Maldivas, 13. a Índia, 14. o Qatar, 15. Filipinas, 16. Ilha Christmas, e 17. Austrália Ocidental. No entanto, observaram-se também aglomerados intermitentes de tubarão-baleia em regiões de latitudes mais elevadas, como o arquipélago dos Açores, representando o limite norte da sua distribuição geográfica no Oceano Atlântico.

Sabe-se que os tubarões-baleia ocorrem na região dos Açores há já algum tempo, embora os primeiros registos oficiais datem de meados dos anos noventa. Historicamente, os avistamentos de tubarões-baleia nos Açores tinham sido tipicamente baixos ou nulos, até ao acentuado aumento observado em 2008, atribuído a um aumento da temperatura média da água. Os avistamentos de tubarões-baleia nos Açores ocorreram principalmente em torno das ilhas orientais - e principalmente em torno da ilha de Santa Maria (Afonso et al., 2014). As séries temporais interanuais de SST média e clorofila (chl-a) na região dos Açores mostraram que os avistamentos ocorreram principalmente durante os meses de Verão, quando a SST estava no seu ponto mais alto, e a chl-a estava a começar a diminuir após o pico da Primavera.

As populações de tubarões-baleia têm sido investigadas utilizando uma variedade de diferentes métodos e técnicas de investigação, tais como estudos genéticos baseados em ADN microsatélite e mitocondrial, ou através da utilização de satélite e telemetria acústica. Contudo, a técnica mais difundida utilizada durante as agregações de tubarões-baleia é a utilização de identificação fotográfica (foto-ID) baseada em métodos de captura-marcação-recaptura (CMR). Os tubarões-baleia nascem com um padrão corporal único de manchas e riscas brancas que é retido ao longo da sua vida. Os padrões de manchas podem ser comparados entre outros tubarões-baleia, utilizando programas de foto-recaptura. Ao procurar correspondências em grandes bibliotecas de foto-IDs, tais como a biblioteca Wildbook for Whale Sharks, podemos então investigar padrões de movimento entre escalas regionais e maiores, padrões de residência, bem como desenvolver estimativas de abundância

populacional utilizando modelos de captura-recaptura e software convencional de modelação CMR.

Neste estudo utilizámos dados de foto-identificação recolhidos durante os anos 2008 a 2011, 2013, 2017, 2019 e 2020 para produzir as primeiras estimativas da estrutura populacional, residência, abundância e movimentos desta espécie em águas dos Açores. Foram recolhidas um total de 3.705 fotografias, das quais 545 foram validadas para comparação utilizando o software de correspondência semi-automatizada e a base de dados de identificação fotográfica Wildbook for Whale Sharks. Um total de 186 indivíduos foram identificados através da combinação de padrões de manchas e riscas atrás da última fenda das branquial e à frente da barbatana dorsal (vista lateral). Entre os 115 indivíduos em foi possível determinar o sexo, 31% eram machos e 69% eram fêmeas, confirmando ser uma agregação dominada por fêmeas. No total, 38 tubarões-baleia (20%) apresentaram algum tipo de cicatrização. A maioria dos encontros de tubarões-baleia ocorreram na ilha de Santa Maria (82%), especialmente durante os meses de Agosto e Setembro (81%). Dos 29 indivíduos avistados durante o estudo, 28 foram avistados em diferentes ocasiões no mesmo ano, e 4 indivíduos foram avistados em anos diferentes (variando de 1 a 3 anos). A conectividade inter-ilhas foi também observada para um indivíduo. Estimamos a população de tubarões-baleia que visitam a região dos Açores em aproximadamente 283 a 496 indivíduos (intervalo de confiança de 95%) com base nos modelos de população aberta de Cormack-Jolly-Seber. Este estudo fornece as primeiras estimativas de abundância populacional e caracterização da população de tubarões-baleia nos Açores, bem como a recolha de informação de fundamental relativa à sua ecologia e padrões de conectividade e residência. Este estudo sublinha também o papel importante que a foto-identificação desempenha no estudo das populações de tubarões-baleia, mas ao mesmo tempo, demonstra a necessidade de utilizar métodos complementares, tais como a marcação por satélite ou estudos genéticos para melhor compreender a estrutura populacional, bem como os padrões de movimento e conectividade das populações.

STATE OF THE ART

Effective approaches for the management and conservation of wildlife populations require a solid knowledge of spatial extent, connectivity and population demographics (Caughley & Gunn, 1996; Andrzejaczek et al., 2016). However, for marine megafauna, obtaining such information can be challenging and expensive, as numerous organisms (cetaceans, sharks, rays, billfishes) are migratory or spend most of their lifetime in the open ocean (Richardson & Poloczanska, 2008; Block et al., 2011; Sequeira et al., 2014; Andrzejaczek et al., 2016), making sampling extremely laborious, and time-consuming (Richardson & Poloczanska, 2008).

Whale sharks (*Rhincodon typus*, Smith 1828) represent a good example of the challenges of ecological studies involving the assessment of the population demographics, spatial extent and connectivity patterns of marine megafauna (Andrzejaczek et al., 2016). The whale shark is the largest living fish in the world, capable of reaching a maximum size of 18.8 m in length (McClain et al., 2015) and presents a circumglobal distribution, inhabiting all tropical and warm waters (Compagno, 2001; Rowat & Brooks, 2012). Although the whale shark has become an iconic shark in many aspects and in spite of substantial research in the last decades, there are still major gaps regarding whale shark ecology, regional population sizes, and key aspects of whale shark biology, such as age-specific growth rates, reproductive rates, and breeding habitats (Dove & Pierce, 2021).

Taxonomy and General Description of Whale Sharks

The species was first named and described by Dr Andrew Smith in 1828 from a specimen harpooned in Table Bay, South Africa (Smith, 1828). *Rhincodon typus* belongs to the monotypic family Rhincodontidae, within the Order Orectolobiformes, which also includes leopard sharks (Stegostomatidae), nurse sharks (Ginglymostomatidae) and wobbegongs (Orectolobidae). The association between these families is based on several morphological and anatomical similarities, including skeletal anatomy, tooth and dermal denticle morphology, fin placement and barbel morphology (Compagno, 1973). However, despite these similarities, *R. typus* is the only orectoloboid that is pelagic in habit (Rowat & Brooks, 2012).

R. typus has a fusiform body and is characterized by its large size, three conspicuous longitudinal ridges (carina) along its dorsal flanks, a large first dorsal fin, and a semi-lunate caudal fin (Figure 1). It has a broad, flattened head with a large terminal mouth and the dorsal part of the body presents a characteristic pattern of light spots and stripes over a dark background, with a light ventral surface (Compagno, 2001; Rowat & Brooks, 2012). The characteristic body markings are a combination of two forms of camouflage which are probably used in a defensive manner and are especially important during their early years to hide from predators; the spots and stripes represent a disruptive coloration, while the lighter ventral surface is termed counter-shading (Wilson & Martin, 2001).

Figure 1: Whale shark in Azores. Photo courtesy of Jordi Chias.

Biology and Ecology

Distribution and habitat

Rhincodon typus is thought to be a cosmopolitan species, occurring in all tropical and warm temperate waters, including sightings in the Mediterranean Sea (Wolfson, 1986; Jaffa & Taher, 2007). It is found in a band around the equator between 30ºN and 35ºS, in both coastal and oceanic waters (Compagno, 1984), although it has occasionally been sighted at latitudes as high as 41ºN and 36.5ºS (Wolfson, 1986). Whale sharks are known to inhabit both deep and shallow coastal waters, as well as lagoons of coral reefs and atolls (Demetrios, 1979), and although they are known to use the entire water column, from surface down to

over 2000 m (Brunnschweiler et al. 2009; Tyminski et al., 2015), they are thought to spend most of their time in the top part of the epipelagic zone, within the top 10-15 meters (Wilson et al., 2006; Rowat & Gore, 2007; Tyminski et al., 2015; Green, 2021). *R. typus* can be found in surface waters of temperatures between 18ºC and 30ºC, although they prefer surface temperatures of 21 – 25ºC (Iwasaki, 1970). Whale sharks occur throughout the Indian and Atlantic Ocean and have been reported in the Gulf of California, Gulf of Mexico, Philippines, Maldives, Seychelles, Comores Islands, and Christmas Island, Santa Helena, Galapagos Islands, Azores and along the coastlines of Honduras, Belize, Madagascar, South Africa, Mozambique, Tanzania, Kenya, Pakistan, India, Sri Lanka, Thailand, Malaysia, Indonesia, and Australia (Colman, 1997; Arnbom & Papastavrou, 1988; Heyman et al., 2001; Hoffmayer et al., 2005; Graham & Roberts, 2007; Hobbs et al., 2009; Ramírez-Macías et al., 2012; Afonso et al., 2014; Clingham et al., 2016; Araujo et al., 2017).

Growth and reproduction

Information about growth, longevity, reproduction and development of whale sharks is very limited (Stevens et al., 2000; Perry et al., 2018; Ong et al., 2020). Generally, growth curves for sharks have been derived from age estimates based upon growth zones (bands or rings) in calcified structures such as vertebral centra (Colman, 1997). However, until the study of Ong et al. (2020) it was still uncertain as to whether vertebrae growth bands were formed annually or biannually, a crucial information in determining the age, growth, and longevity of whale sharks. The growth of whale sharks has been studied opportunistically from animals held in captivity in the Okinawa Expo Aquarium in Japan (Uchida et al., 1990). Preliminary results indicated that in captivity, one pair of growth zones was deposited per year. How this relates to growth in the wild can be determined only by examination of growth zones in vertebral centra samples collected from dead specimens (Colman, 1997). In addition, the results showed that neonatal pups grew faster than larger juveniles, and that juveniles > 3.5 m of total length showed variables rates with the females growing faster than the males (Chang et al., 1997; Leu et al., 1997; Kitafuji & Yamamoto, 1998; Uchida et al., 2000). Nevertheless, Meekan et al. (2020) suggested that in the wild, males grow faster than females in the earlier stages of life, although they ultimately reach a smaller maximum size.

The largest whale shark specimen reported to date was an individual of approximately 20m and 34t in mass reported by the Taiwanese fishery in 1987 (Chen et al., 1997). Measuring large sharks, both in and out of the water, has proven difficult, which helps explain why there is almost no information about growth rates or ageing in whale sharks (Colman, 1997; Rowat & Brooks, 2012). Nowadays, new technology based on digital photography is being used to measure *R. typus* in the water with much greater accuracy and precision, such as the use of laser photogrammetry (Rohner et al., 2011; Rowat & Brooks, 2012).

Historically, it was unclear whether whale shark were oviparous (egg cases expelled from the female's body and hatching on the sea floor) or ovo-viviparous (egg cases hatching in utero, with the female giving birth to live young) until 1995, when a pregnant *R. typus* was landed at a Chen-Kung fish market, Taiwan (Joung et al., 1996; Colman, 1997). The pregnant female had 304 embryos combined from the two uteri and presented a large number of embryos that were not contained in egg cases and were lacking a yolk sac, proving that whale sharks are ovo-viviparous (Rowat & Brooks, 2012).

Information about size at sexual maturity and longevity is sparse. Taylor (1994) speculated that sexual maturity in both sexes may not occur until the sharks are over 9 m in length or over 30 years of age, and that their lifespan is over 100 years. However, pregnant females and pups, ranging from 55 to 93 cm in length are very rarely encountered (Colman, 1997), with gravid females regularly reported only in the Gulf of California (Eckert & Stewart, 2001; Ramírez-Macías, et al., 2012). Apparently pregnant females have been also recorded in Galapagos Islands (Hearn et al., 2013; Acuña-Marrero et al., 2014) and the Philippines (Rowat & Brooks, 2012), although no clear evidence has been confirmed. There are no confirmed records of whale shark between 93 cm and 3 m, whereas juveniles over 3 m in length are encountered worldwide. Most specimens reported in the literature are between 4 and 10 m, but maximum total length is uncertain (Colman, 1997).

Population structure

Movement patterns and seasonal aggregations

Despite being largely oceanic, spending most of their lives in the open ocean, whale sharks form seasonal aggregations in coastal waters of warm temperate and tropical regions (Sequeira et al., 2013), leading whale sharks to have evolved a migratory lifestyle, performing large trans-boundary feeding migrations (Rowat & Gore, 2007; Afonso et al.,

2014). These predictable seasonal aggregations – of mostly juvenile and sub-adult males (Sequeira et al., 2013), have shown to be highly influenced by interannual changes in sea surface temperature (SST), and climatic forcing (Wilson et al., 2001; Cárdenas-Palomo et al., 2010; Sleeman et al., 2010; Afonso et al., 2014). Coastal aggregations of whale sharks offer scientists an opportunity to collect demographic data from these large animals, and a significant economic value to tourism industries (Gallagher & Hammerschlag, 2011; Sequeira et al., 2016). However, anthropogenic impacts on whale sharks such as direct fisheries, habitat disturbance and shipping has led to important declines in global populations. As a consequence, *Rhincodon typus* is now classified as "Endangered" in the conservation status of the IUCN Red List of Threatened Species (Pierce & Norman, 2016).

The temporal and spatial extent of whale shark distribution has been intensively studied, proving that some populations include site-faithful and migratory individuals that form seasonal aggregations (Rowat & Gore, 2007; Rowat et al., 2009). However, the individual movements once they depart from these seasonal aggregation sites, and the connectivity patterns between oceanic habitats are still largely unknown (McKinney et al., 2017). Nevertheless, the large scale seasonal movements of the migratory individuals have been observed to be fundamentally longitudinal between different tropical or subtropical regions (Rowat & Brooks, 2012).

Aggregations of whale sharks have been observed to occur seasonally around several islands as well as in some coastal sites, such as 1. Gulf of Baja California (Ramírez-Macías, et al., 2012), 2. Galapagos Islands (Acuña-Marrero et al., 2014), 3. northern Mexico (Eckert & Stewart, 2001), 4. Belize (Heyman et al., 2001), 5. St. Helena (Clingham et al., 2016), 6. Saudi Arabia (Berumen et al., 2014), 7. Djibouti (Rowat et al., 2007), 8. Mozambique (Wolfson, 1986), 9. Madagascar (Diamant et al., 2016), 10. South Africa (Gifford et al., 2007), 11. the Seychelles (Rowat et al., 2009), 12. the Maldives (Riley et al., 2010), 13. India (Pravin, 2000), 14. Qatar (Robinson et al., 2013), 15. Philippines (Araujo et al., 2019), 16. Christmas Island (Hobbs et al., 2009), and 17. Western Australia (Meekan et al., 2006) (Figure 2). However, whale sharks have been also observed to intermittently aggregate in regions at higher latitudes such as the Azores archipelago, representing the northern limit of its geographic Atlantic distribution (Afonso et al., 2014; Fontes et al., 2020).

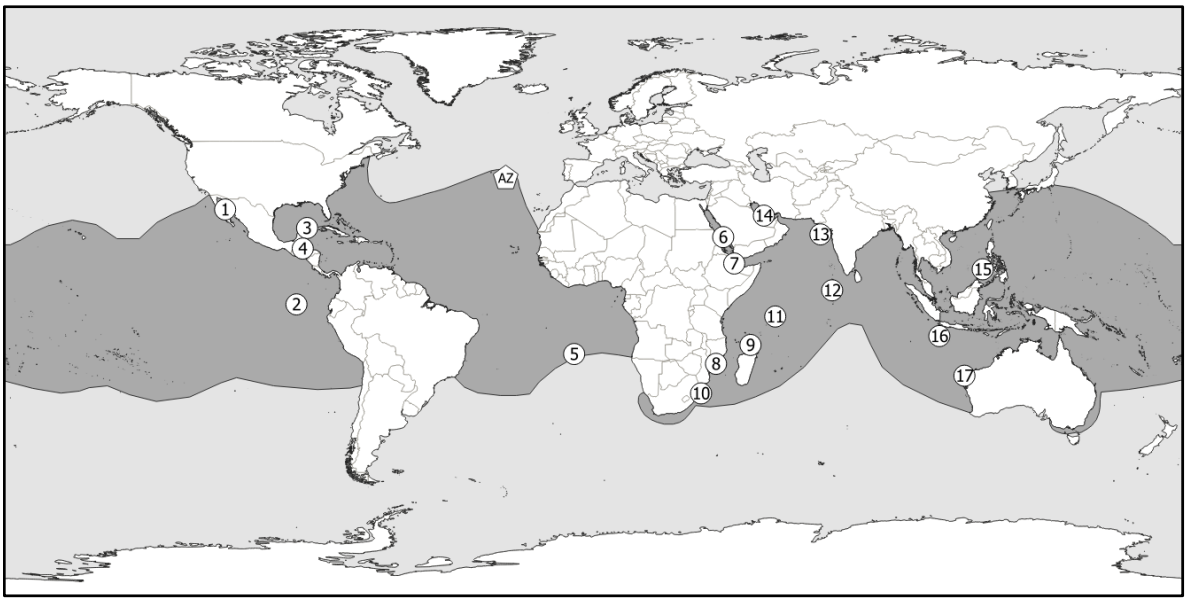

Figure 2: Global whale shark distribution (dark grey) and study site (AZ = Azores) and known whale shark aggregations to date.

Whale sharks in Azores

The Azores archipelago comprises 9 islands of volcanic origin and represents the most isolated archipelago in the North Atlantic Ocean, located about 1500 km from Europe (Silva & Smith, 2004; Afonso et al., 2020). Maximum altitude of the islands ranges from 4402 to 2531 m, and surrounding oceanic depths regularly exceed 1500 m (Santos et al., 1995; Silva & Smith, 2004). The climate of the archipelago is temperate oceanic with a mean annual temperature of 17ºC at sea level (Silva & Smith, 2004). Its temperate geographic location is modulated by subtropical gyres (Afonso et al., 2020), providing the particular conditions to attract the highest cetacean biodiversity in the world, turtles (Saavedra et al., 2018), large bony fishes and aggregations of pelagic sharks and rays, such as whale sharks (Borges et al., 2010; Das & Afonso, 2017).

Azores is characterized by low marine productivity (Paiva et al., 2010). However, due to the influence of equatorial and tropical waters transported by the Gulf Stream, and colder northern waters, localized patches of enhanced productivity around the coastal waters of the islands are produced (Gould, 1985; Santos et al., 1995; Pingree et al., 1999), attracting large marine megafauna. An interesting ecological interaction in the Azores archipelago is the whale shark-tuna associations, which local fleet opportunistically use to locate and fish

tunas (Fontes et al., 2020). It is speculated that the association with tunas may facilitate access to large quantities of small, highly mobile fish for whale sharks, representing a benefit during the summer months, when whale sharks aggregate in the Azores and primary production is at its lowest (Woods & Barkmann, 1995; Fontes et al., 2020).

Whale sharks have been known to occur in the Azores region for some time, although the first official records date from the mid-nineties (Santos et al., 1995, 1997). Historically, whale shark sightings in the Azores across the season had been typically low or null, until the sharp increase observed in 2008, attributed to an increase in the average water temperature (Afonso et al., 2014). A study carried out by Afonso et al. (2014) analyzed a 16-year observer dataset from the pole-and-line tuna fishery across the Azores, to investigate spatial and temporal patterns of whale shark occurrence in relation to oceanographic features. Between 1998 and 2013, there were a total of 17339 tuna fishing events, of which 1443 had an associated whale shark sighting. The period with the highest number of whale shark sightings was in 2008, with 302 monthly sightings in that year alone (Afonso et al., 2014).

Sightings of whale sharks in the Azores have mainly occurred around the eastern groups of islands – and mostly around the island of Santa Maria – with the exception of 2012, that presented the greatest deviation in the spatial pattern of whale shark sightings, mainly concentrating around the large Princess Alice bank located to the SW island of Faial (Afonso et al., 2014). Interannual time series of average SST and chlorophyll (chl-*a*) in the Azores region showed that sightings mostly took place during the summer months when SST was at its highest, and chl-*a* was starting to decrease after the spring bloom (Afonso et al., 2014). Whale shark aggregations in the Azores and its interannual variation could be explained by several environmental drivers using a binomial generalized model (GAM). An increase in SST, exceed chl-*a* threshold, closer distance to seamounts and steeper bottom slopes are the main predictor variables that would help explain larger numbers of whale shark detections in the Azores island (Afonso et al., 2014).

Photo-identification: A pattern recognition approach

Whale shark populations have been investigated using a variety of different research methods and techniques (Perry et al., 2020). Genetic studies based on microsatellite and mitochondrial DNA have suggested that whale shark populations are distinct between the Atlantic and Indo-Pacific, representing two functionally separated populations (Vignaud et

al., 2014; Pierce & Norman, 2016). Satellite and acoustic telemetry are two other techniques that have been used to study the movement ecology of different populations of whale sharks (Eckert & Stewart, 2001; Gifford et al., 2007; Cagua et al., 2015). These techniques, which involve attaching a satellite and/or acoustic transmitter to the shark, have provided information on horizontal movements, vertical diving behavior and residency patterns (Perry et al., 2020). In fact, deployment of satellite tags has provided good evidence for connectivity at regional scales (Eckert et al., 2002; Hearn et al., 2013), but limited evidence for broad-scale, cross-ocean movement (Andrzejaczek et al., 2016).

However, the most wide-spread technique used during whale shark aggregations – and the one used in this study – is the use of photographic identification (photo-ID) based on capture-mark-recapture methods (CMR) (Brooks et al., 2010; Marshall & Pierce, 2012). The general principle of CMR methods is to mark a sample of individuals in a first capture session and then record the proportion of marked individuals in subsequent recapture sessions, providing a set of capture histories per observed individual informative on survival, recruitment and population size (Pradel, 1996; Petit & Valiere, 2006). Individual recognition can be either achieved by applying artificial markings or by using the natural markings of an animal (Mann et al., 2000).

Applying artificial marks to wildlife can alter natural behavior, reduce individual performance, and may affect growth, health, and survival of the organism (Gauthier-Clerc et al., 2004; Thorsteinsson, 2002). The process of marking and tagging may be difficult to apply, and tag losses over time can also compromise the estimation of demographic parameters (Bateson, 1977; Schwarz & Seber, 1999; Bradshaw et al., 2000; Thorsteinsson, 2002; Ogutu et al., 2006). Additionally, ethical and welfare issues often arise with the application of permanent or temporary marks (Thorsteinsson, 2002). Therefore, natural markings have become a powerful alternative to artificial markings.

Whale sharks are born with a unique body pattern of white spots and stripes that is retained throughout their lives (Norman, 2004). This unique and natural pattern shows to be stable over the years, not showing evidence of significant change, enabling individual whale sharks to be uniquely identified (Norman, 1999; Brooks et al., 2010; Andrzejaczek et al., 2016). The area of interest to compare spot patterns among whale sharks is the lateral surface including the upper and lower fifth gill and the inner trailing edge of the pectoral fin, as these

flank areas contain enough large distinctive spots and suitable reference points for comparison between images (Arzoumanian et al., 2005; M. Meekan et al., 2006; Speed et al., 2007).

To visually match images of the same individual "by eye" is potentially feasible with a low number of images, but is impractical with large databases (Kelly, 2001; Matthé et al., 2017). In the recent years, different photo-matching algorithms have been developed and successfully applied to match images of unique individuals in large databases (Bolger et al., 2012). However, these methods are typically not fully automated as the user is required to inspect and manually select potential matches from a number of top ranked matches based on a similarity score (*match score)* calculated by the algorithm (Morrison et al., 2011) (Figure 3). Amongst the different photo-matching software for whale sharks, Wildbook for Whale Sharks and I^3S are the most used in the scientific community (Araujo et al., 2017).

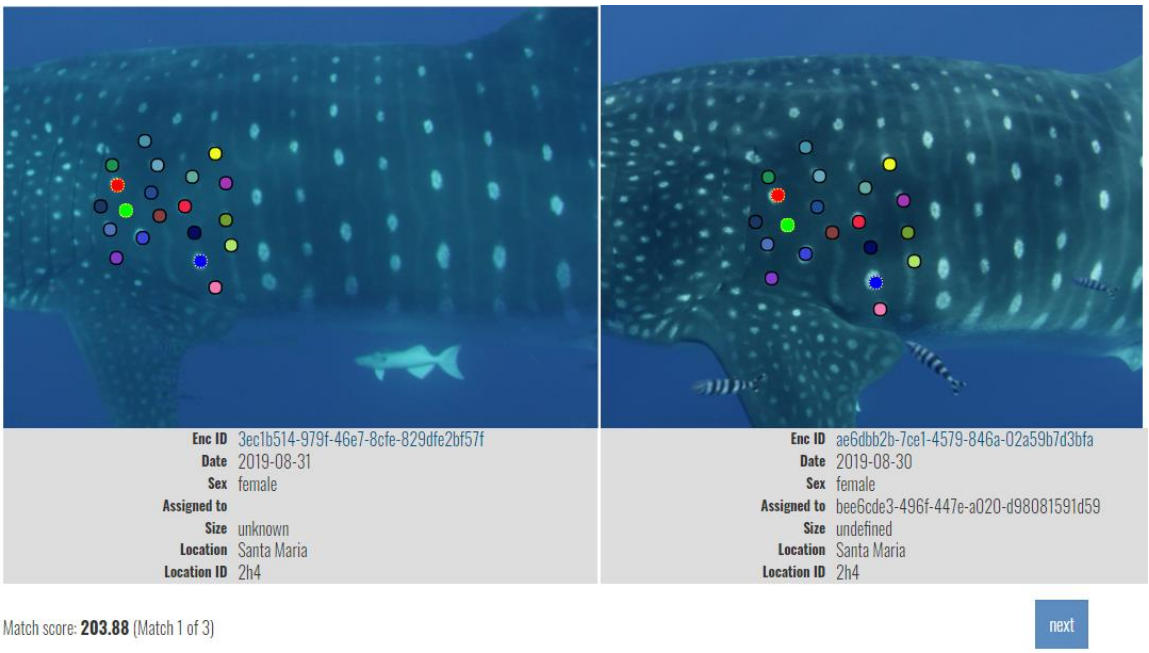

Figure 3: Result of a spot mapping match, and match score calculated by the algorithm.

Wildbook for Whale Sharks photographic identification database (http://www.whaleshark.org) was developed to automate the matching of whale shark identification images based on the Groth algorithm for matching astronomical data (Groth, 1986). Using the Growth algorithm, a 92% success rate of matches was achieved when using 27 pairs of manually, pre-matched images, and 86% success rate when using 111 pairs of un-matched images. The second program developed was a free-ware computer program

(Interactive Individual Identification System, I^3S) (www.reijns.com/i3s), which was originally developed to assist in matching spot patterns on ragged-tooth sharks *Carcharias taurus* (Van Tienhoven et al., 2007) and was shown to also be effective in matching *R. typus* images with an 83% success rate against 58 pre-matched images (Rowat & Brooks, 2012).

In order to identify spots, the area of interest ('the measurement region') is selected, which is located directly behind the 5th gill slit on both the right and left sides of the body (Figure 4). The region is delimited (i) anteriorly by the fifth gill slit, (ii) ventrally by the insertion plane of the pectoral fin, (iii) posteriorly by a line drawn vertically from the insertion point of the trailing edge of the pectoral fin and (iv) dorsally by the most ventral of the three longitudinal ridges (Arzoumanian et al., 2005). Three reference points are selected: 1) top of 5th gill slit, 2) posterior-most point of the pectoral fin and 3) the bottom of the 5th gill slit, allowing the program to re-scale and rotate images to ensure standardized comparisons regardless of the orientation or distance from the shark from which the photograph was taken, and the dots of the whale shark within the reference points are selected for the spot mapping (Speed et al., 2007; Brooks et al., 2010).

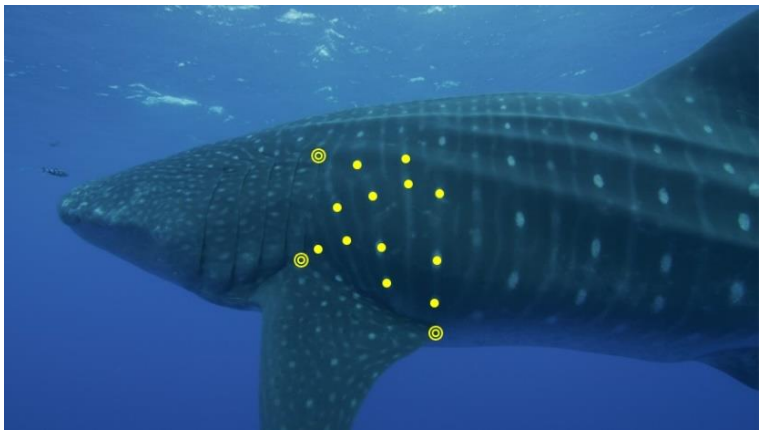

Figure 4: Reference area in whale sharks used for photo-identification defined as the area encompassed by the reference points 1) top of 5th gill slit, 2) posterior-most point of pectoral fin and 3) bottom of 5th gill slit, and points selected for spot mapping.

By searching for matches in large libraries of photo-IDs, such as the Wildbook for Whale Sharks library, we can then investigate patterns of movement among regional and larger scales, residency patterns, as well as develop population abundance estimates using capture-recapture models and conventional CMR modelling software (Schwarz & Arnason, 1996; Meekan et al., 2006; Brooks et al., 2010; Riley et al., 2010; Marshall & Pierce, 2012).

Classical capture-mark-recapture models use repeated observations of identifiable individuals and derive information about the detection probability, *p*, from the detection and non-detection pattern of each marked individual (Kery & Schaub, 2012). The basic theory behind CMR models resides in that the proportion of marked individuals within the second sample should be proportional to the number of marked individuals in the whole population (Parsons, 2004). Mark-recapture models have several assumptions that need to be met, including 1) marks must be recognizable and must not be lost, 2) all animals are equally likely to be caught in each sample, 3) recapture probability is not affected by first capture, 3) death or migration of an individual is not affected by marking 4) all individuals have the same probability of dying or migrating (Parsons, 2004; Navarro Alberto et al., 2014).

Capture-recapture models can be divided into closed-population models and open-population models. These two type of models differ in that in closed-population models no population losses (emigration or deaths) or gains (birth or immigration) occur during the sampling period, whereas in open-population models, the population is open to losses and gains (Kery & Schaub, 2012; Pérez-Jorge et al., 2016).

Closed-population models are conducted over short periods of time, and include a series of eight different models defined by Otis et al. (1978) to cope with variations in the catchability of animals (Evans et al., 1994). M_0 is the most simple model for inference about the size of a population (Kery & Schaub, 2012), and assumes a single common probability of capture. The other models of Otis et al. are denoted as M_t, M_b, M_h, M_{bh}, M_{tb}, M_{th}, and M_{tbh}. The subscripts *t*, *b*, and *h* correspond to temporal variation in common probability of capture, behavioral response to common probability of capture, and individual response to capture, respectively. Multiple subscripts indicate a combination of capture responses (Evans et al., 1994). On the other hand, open-population models are used in samplings with a larger period of time (Speakman et al., 2010). The original Jolly-Seber (JS) model is primarily interested in estimating abundance. Since then, the focus of many mark-recapture changed to estimate survival rates by using the Cormack-Jolly-Seber (CJS) models (Schwarz & Arnason, 2009).

The goal of this study is to shed a light on the unique identification of whale sharks migrating to Azores using a photo-ID approach, allowing to develop the first population structure characterization, and first population abundance estimates through CMR methods, as well as assembling valuable information regarding the basic ecology and connectivity

patterns of whale sharks in the Atlantic Ocean, essential to support conservation strategies for these animals.

For this study, it was hypothesized that the Azores whale shark aggregation is dominated by juvenile male whale sharks, similarly to the majority of whale shark aggregations sites. We also hypothesized that there will be some degree of connectivity within the islands of the Azores archipelago, and in a smaller level, a certain degree with more distant areas of the Atlantic Ocean.

In order to characterize the population of whale sharks visiting the Azores, as well as developing the first population estimates and obtaining information on their movement patterns, whale shark photographic data obtained from researchers, ecotourism industries and professional underwater photographers from years 2008-2020 was used to obtain information on sex ratios, scarring patterns, spatial and temporal distribution, connectivity patterns, and population size.

References

Acuña-Marrero, D., Jiménez, J., Smith, F., Jr, P. F. D., Hearn, A., Green, J. R., Paredes-Jarrín, J., & Salinas-de-León, P. (2014). Whale shark (*Rhincodon typus*) seasonal presence, residence time and habitat use at Darwin Island, Galapagos Marine Reserve. *PLOS ONE*, *9*(12), e115946.

Afonso, P., Fontes, J., Giacomello, E., Magalhães, M. C., Martins, H. R., Morato, T., Neves, V., Prieto, R., Santos, R. S., Silva, M. A., & Vandeperre, F. (2020). The Azores: A Mid-Atlantic hotspot for marine megafauna research and conservation. *Frontiers in Marine Science*, *6*, 826.

Afonso, P., McGinty, N., & Machete, M. (2014). Dynamics of whale shark occurrence at their fringe oceanic habitat. *PLOS ONE*, *9*(7), e102060.

Amorim, P., Perán, A. D., Pham, C. K., Juliano, M., Cardigos, F., Tempera, F., & Morato, T. (2017). Overview of the ocean climatology and its variability in the Azores region of the North Atlantic including environmental characteristics at the seabed. *Frontiers in Marine Science*, *4*, 56.

Andrzejaczek, S., Meeuwig, J., Rowat, D., Pierce, S., Davies, T., Fisher, R., & Meekan, M. (2016). The ecological connectivity of whale shark aggregations in the Indian Ocean: A photo-identification approach. *Royal Society Open Science*, *3*(11), 160455.

Araujo, G., Agustines, A., Tracey, B., Snow, S., Labaja, J., & Ponzo, A. (2019). Photo-ID and telemetry highlight a global whale shark hotspot in Palawan, Philippines. *Scientific Reports*, *9*(1), 1-12, 17209.

Araujo, G., Snow, S., So, C. L., Labaja, J., Murray, R., Colucci, A., & Ponzo, A. (2017). Population structure, residency patterns and movements of whale sharks in Southern Leyte, Philippines: Results from dedicated photo-ID and citizen science. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *27*(1), 237-252.

Arnbom, T., & Papastavrou, V. (1988). Fish in association with whale sharks *Rhiniodon typus* near the Galapagos Islands. *Noticias de Galapagos, 46*, 13-15.

Arzoumanian, Z., Holmberg, J., & Norman, B. (2005). An astronomical pattern-matching algorithm for computer-aided identification of whale sharks *Rhincodon typus*. *Journal of Applied Ecology*, *42*(6), 999-1011.

Bateson, P. (1977). Testing an observer's ability to identify individual animals. *Animal Behaviour*, *25*, 247-248.

Berg, A., Meyer, R., & Yu, J. (2002). Deviance information criterion as a model comparison criterion for stochastic volatility models. (No. 178). Department of Economics, The University of Auckland.

Berumen, M. L., Braun, C. D., Cochran, J. E. M., Skomal, G. B., & Thorrold, S. R. (2014). Movement patterns of juvenile whale sharks tagged at an aggregation site in the Red Sea. *PLOS ONE*, *9*(7), e103536.

Block, B. A., Jonsen, I. D., Jorgensen, S. J., Winship, A. J., Shaffer, S. A., Bograd, S. J., Hazen, E. L., Foley, D. G., Breed, G. A., Harrison, A.-L., Ganong, J. E., Swithenbank, A., Castleton, M., Dewar, H., Mate, B. R., Shillinger, G. L., Schaefer, K. M., Benson, S. R., Weise, M. J., … Costa, D. P. (2011). Tracking apex marine predator movements in a dynamic ocean. *Nature*, *475*(7354), 86-90.

Bolger, D. T., Morrison, T. A., Vance, B., Lee, D., & Farid, H. (2012). A computer-assisted system for photographic mark–recapture analysis. *Methods in Ecology and Evolution*, *3*(5), 813-822.

Borges, P., Costa, A., Cunha, R., Gabriel, R., Gonçalves, V., Martins, A., Melo, I., Parente, M., Raposeiro, P., Rodrigues, P., Santos, R., Silva, L., Vieira, P., & Vieira, V. (2010). *A list of the terrestrial and marine biota from the Azores*. Princípia, 429p.

Bradshaw, C. J. A., Barker, R. J., & Davis, L. S. (2000). Modeling tag loss in New Zealand fur seal pups. *Journal of Agricultural, Biological, and Environmental Statistics*, *5*(4), 475-485.

Brooks, K., Rowat, D., Pierce, S., Jouannet, D., & Vely, M. (2010). Seeing Spots: Photo-identification as a regional tool for whale shark identification. *Western Indian Ocean Journal of Marine Science*, *9*(2), 185-194.

Brunnschweiler, J. M., Baensch, H., Pierce, S. J., & Sims, D. W. (2009). Deep-diving behaviour of a whale shark *Rhincodon typus* during long-distance movement in the western Indian Ocean. *Journal of Fish Biology*, *74*(3), 706-714.

Cagua, E. F., Cochran, J. E. M., Rohner, C. A., Prebble, C. E. M., Sinclair-Taylor, T. H., Pierce, S. J., & Berumen, M. L. (2015). Acoustic telemetry reveals cryptic residency of whale sharks. *Biology Letters*, *11*(4), 20150092.

Cárdenas-Palomo, N., Herrera-Silveira, J., & Reyes, O. (2010). Spatial and temporal distribution of physicochemical features in the habitat of whale shark *Rhincodon typus* (Orectolobiformes: Rhincodontidae) in the north of Mexican Caribbean. *Revista De Biologia Tropical*, *58*(1), 399-412.

Caughley, G., & Gunn, A. (1996). *Conservation biology in theory and practice*. Cambridge, MA: Blackwell Science, 459p.

Chang, W. B., Leu, M. Y., & Fang, L. S. (1997). Embryos of the whale shark, *Rhincodon typus*: Early growth and size distribution. *Copeia*, *1997*(2), 444-446.

Chen, C. T., Liu, K. M., & Joung, S. J. (1997). Preliminary report on Taiwan's whale shark fishery. *Traffic bulletin-wildlife trade monitoring unit*, *17*, 53-57.

Clingham, E., Brown, J., Henry, L., Beard, A., & Dove, A. D. (2016). *Evidence that St. Helena island is an important multi-use habitat for whale sharks, Rhincodon typus, with the first description of putative mating in this species* (No. e1885v1). PeerJ Preprints.

Cochran, J. E. M., Braun, C. D., Cagua, E. F., Jr, M. F. C., Hardenstine, R. S., Kattan, A., Priest, M. A., Sinclair-Taylor, T. H., Skomal, G. B., Sultan, S., Sun, L., Thorrold, S. R., & Berumen, M. L. (2019). Multi-method assessment of whale shark (*Rhincodon typus*) residency, distribution, and dispersal behavior at an aggregation site in the Red Sea. *PLOS ONE*, *14*(9), e0222285.

Colman, J. (1997). A review of the biology and ecology of the whale shark. *Journal of Fish Biology*, *51*(6), 1219-1234.

Compagno, L. J. V. (1973). Interrelationships of living elasmobranchs. In P. H Greenwood, R. S. Miles & C. Patterson (Eds.). *Interrelationships of Fishes,* (pp. 49-51). New York, NY. Academic Press.

Compagno, L. J. V. (1984). Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Part 1. Hexanchiformes to Lamniformes. *FAO Species Catalogue*. *FAO Fisheries Synopsis.* No. 125, Vol. 4, 209-236.

Compagno, L. J. V. (2001). Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Volume 2. Bullhead, mackerel and carpet sharks (Heterodontiformes, Lamniformes and Orectolobiformes). *FAO Species catalogue for fishery purposes.* No. 1, Vol. 2. Rome, FAO. 269p.

Das, D., & Afonso, P. (2017). Review of the diversity, ecology, and conservation of elasmobranchs in the Azores region, Mid-North Atlantic. *Frontiers in Marine Science*, *4*, 354.

Demetrios, E. (1979). Tie-tie malie. *California Academy of Science California Wild, 32*(1), 24-29.

Diamant, S., Pierce, S., Ramirez-Macias, D., Heithaus, M., d'Echon, A., Guillemin-d'Echon, T., & Kiszka, J. (2016). Preliminary observations on whale sharks in Nosy Be, Madagascar. In *The 4th International Whale Shark Conference* (Vol. 2016, No. 2, p. 15). Hamad bin Khalifa University Press (HBKU Press).

Dove, A. D., & Pierce, S. J. (Eds.). (2021). *Whale Sharks: Biology, Ecology, and Conservation*. CRC Press

Ebert, D. A., Fowler, S. L., & Compagno, L. J. V. (2013). *Sharks of the world: A fully illustrated guide*. Plymouth: Wild Nature Press.

Eckert, S., Dolar, L., Kooyman, G., Perrin, W., & Abdul Rahman, R. (2002). Movements of whale sharks (*Rhincodon typus*) in South-east Asian waters as determined by satellite telemetry. *Journal of Zoology, 257*(1), 111-115.

Eckert, S., & Stewart, B. (2001). Telemetry and satellite tracking of whale sharks, *Rhincodon typus*, in the Sea of Cortez, Mexico, and the north Pacific Ocean. *Environmental Biology of Fishes*, *60*, 299-308.

Evans, M. A., Bonett, D. G., & McDonald, L. L. (1994). A general theory for modeling capture-recapture data from a closed population. *Biometrics*, *50*(2), 396-405.

Fontes, J., McGinty, N., Machete, M., & Afonso, P. (2020). Whale shark-tuna associations, insights from a small pole-and-line fishery from the mid-north Atlantic. *Fisheries Research*, *229*, 105598.

Fox, S., Foisy, I., Venegas, R. D. L. P., Pastoriza, B. E. G., Graham, R. T., Hoffmayer, E. R., Holmberg, J., & Pierce, S. J. (2013). Population structure and residency of whale sharks *Rhincodon typus* at Utila, Bay Islands, Honduras. *Journal of Fish Biology*, *83*(3), 574-587.

Fromentin, J.M., & Powers, J. E. (2005). Atlantic bluefin tuna: Population dynamics, ecology, fisheries and management. *Fish and Fisheries*, *6*(4), 281-306.

Gallagher, A. J., & Hammerschlag, N. (2011). Global shark currency: The distribution, frequency, and economic value of shark ecotourism. *Current Issues in Tourism*, *14*(8), 797-812.

Gauthier-Clerc, M., Gendner, J. P., Ribic, C. A., Fraser, W. R., Woehler, E. J., Descamps, S., Gilly, C., Le Bohec, C., & Le Maho, Y. (2004). Long-term effects of flipper bands on penguins. *Proceedings of the Royal Society of London. Biological Sciences*, *271* (Suppl. 6), S423-S426.

Gifford, A., Compagno, L. J. V., Levine, M., & Antoniou, A. (2007). Satellite tracking of whale sharks using tethered tags. *Fisheries Research*, *84*(1), 17-24.

Gould, W. J. (1985). Physical oceanography of the Azores front. *Progress in Oceanography*, *14*, 167-190.

Graham, R., & Roberts, C. (2007). Assessing the size, growth rate and structure of a seasonal population of whale sharks (*Rhincodon typus* Smith 1828) using conventional tagging and photo identification. *Fisheries Research*, *84*(1), 71-80.

Green, S. M., (2021). *The diving behaviour of the whale shark Rhincodon typus related to geographical and environmental conditions in the Galapagos Marine Reserve and the Eastern Tropical Pacific* (Masters Thesis, Universidade do Algarve).

Groth, E. J. (1986). A pattern-matching algorithm for two-dimensional coordinate lists. *The Astronomical Journal*, *91*, 1244-1248.

Gunn, J. S., Stevens, J. D., Davis, T. L. O., & Norman, B. M. (1999). Observations on the short-term movements and behaviour of whale sharks (*Rhincodon typus*) at Ningaloo Reef, Western Australia. *Marine Biology*, *135*(3), 553-559.

Hearn, A., Green, J., Espinoza, E., Peñaherrera, C., Acuña, D., & Klimley, A. (2013). Simple criteria to determine detachment point of towed satellite tags provide first evidence of return migrations of whale sharks (*Rhincodon typus*) at the Galapagos Islands, Ecuador. *Journal of animal telemetry*, *1*, 11.

Heyman, W., Graham, R., Kjerfve, B., & Johannes, R. E. (2001). Whale sharks *Rhincodon typus* aggregate to feed on fish spawn in Belize. *Marine Ecology-Progress Series*, *215*, 275-282.

Hobbs, J., Frisch, A., Hamanaka, T., McDonald, C., Gilligan, J., & Neilson, J. (2009). Seasonal aggregation of juvenile whale sharks (*Rhincodon typus*) at Christmas Island, Indian Ocean. *Coral Reefs*, *28*(3), 577-577.

Hoffmayer, E., Franks, J., & Shelley, J. (2005). Recent observations of the whale shark (*Rhincodon typus*) in the northcentral Gulf of Mexico. *Gulf and Caribbean Research*, *17*(1), 117-120.

Holmberg, J., Norman, B., & Arzoumanian, Z. (2009). Estimating population size, structure, and residency time for whale sharks *Rhincodon typus* through collaborative photo-identification. *Endangered Species Research*, *7*(1), 39-53.

Iwasaki, Y. (1970). On the distribution and environment of the whale shark, *Rhincodon typus*, in skipjack fishing grounds in the Western Pacific. *Journal of the College of Marine Science and Technology, Tokai University*, *4*, 37-51.

Jaffa, K. S., & Taher, N. A. (2007). Whale sharks in Palestinian waters: A whale shark (*Rhincodon typus*, Smith 1828) rescued near the Tantura Beach, Carmel Coast, North Palestine. The first record from the Palestinian Mediterranean Coast. *Gazelle: The Palestinian Biological Bulletin*, *71*, 22-23.

Joung, S.-J., & Chen, C.-T. (1995). Reproduction in the sandbar shark, *Carcharhinus plumbeus*, in the waters off northeastern Taiwan. *Copeia*, *1995*(3), 659-665.

Joung, S.-J., Chen, C.-T., Clark, E., Uchida, S., & Huang, W. Y. P. (1996). The whale shark, *Rhincodon typus*, is a livebearer: 300 embryos found in one 'megamamma' supreme. *Environmental Biology of Fishes*, *46*(3), 219-223.

Kelly, M. J. (2001). Computer-aided photograph matching in studies using individual identification: An example from Serengeti cheetahs. *Journal of Mammalogy*, *82*(2), 440-449.

Kery, M., & Schaub, M. (2012). *Bayesian population analysis using WinBUGS: A hierarchical perspective*. Academic Press.

Kitafuji, M. & Yamamoto, K. (1998). Rearing of the whale shark, Rhincodon typus, in the Osaka aquarium 'Kaiyukan'. *Journal of the Japanese Association Zoological Aquaria*, 39(2), 47- 54.

Leu, M. Y., Chang, W. B. & Fang, L. S. (1997). The success of keeping a baby whale shark from its fetal stage in Taiwan. In *Fourth International Aquarium Congress Tokyo*, pp. 109–111. Tokyo: International Aquarium Congress Central Office.

Mann, J., Connor, R. C., Tyack, P. L., & Whitehead, H. (2000). *Cetacean societies: Field studies of dolphins and whales*. University of Chicago Press, 448p.

Marshall, A. D., & Pierce, S. J. (2012). The use and abuse of photographic identification in sharks and rays. *Journal of Fish Biology*, *80*(5), 1361-1379.

Matthé, M., Sannolo, M., Winiarski, K., Sluijs, A. S. der, Goedbloed, D., Steinfartz, S., & Stachow, U. (2017). Comparison of photo-matching algorithms commonly used for photographic capture–recapture studies. *Ecology and Evolution*, *7*(15), 5861-5872.

McClain, C. R., Balk, M. A., Benfield, M. C., Branch, T. A., Chen, C., Cosgrove, J., Dove, A. D. M., Gaskins, L. C., Helm, R. R., Hochberg, F. G., Lee, F. B., Marshall, A., McMurray, S. E., Schanche, C., Stone, S. N., & Thaler, A. D. (2015). Sizing ocean giants: Patterns of intraspecific size variation in marine megafauna. *PeerJ*, *3*, e715.

McCoy, E., Burce, R., David, D., Aca, E. Q., Hardy, J., Labaja, J., Snow, S. J., Ponzo, A., & Araujo, G. (2018). Long-term photo-identification reveals the population dynamics and strong site fidelity of adult whale sharks to the coastal waters of Donsol, Philippines. *Frontiers in Marine Science*, *5*, 271.

McKinney, J. A., Hoffmayer, E. R., Holmberg, J., Graham, R. T., Driggers, W. B., de la Parra-Venegas, R., Galván-Pastoriza, B. E., Fox, S., Pierce, S. J., & Dove, A. D. M. (2017). Long-term assessment of whale shark population demography and connectivity using photo-identification in the Western Atlantic Ocean. *PLoS ONE*, *12*(8).

Meekan, M., Bradshaw, C., Press, M., McLean, C., Richards, A., Quasnichka, S., & Taylor, G. (2006). Population size and structure of whale sharks (*Rhincodon typus*) at Ningaloo Reef, Western Australia. *Marine Ecology Progress Series*, *319*, 275-285.

Meekan, M. G., Taylor, B. M., Lester, E., Ferreira, L. C., Sequeira, A. M. M., Dove, A. D. M., Birt, M. J., Aspinall, A., Brooks, K., & Thums, M. (2020). Asymptotic growth of whale sharks suggests sex-specific life-history strategies. *Frontiers in Marine Science*, *7*, 774.

Morrison, T. A., Yoshizaki, J., Nichols, J. D., & Bolger, D. T. (2011). Estimating survival in photographic capture–recapture studies: Overcoming misidentification error. *Methods in Ecology and Evolution*, *2*(5), 454-463.

Navarro Alberto, J., Manly, B., & Barrientos Medina, R. C. (2014). Introduction to mark-recapture sampling and closed-population models. In *Introduction to Ecological Sampling* (Manly, B., Navarro Alberto, J.) pp. 87-99. Chapman and Hall/CRC

Nelson, J., & Eckert, S. (2007). Foraging ecology of whale sharks (*Rhincodon typus*) within Bahía de Los Angeles, Baja California Norte, México. *Fisheries Research*, *84*(1), 47-64.

Norman, B. (1999). *Aspects of the biology and ecotourism industry of the whale shark Rhincodon typus in North-Western Australia* (Doctoral dissertation, Murdock University).

Norman, B. M. (2004). Review of the current conservation concerns for the whale shark (*Rhincodon typus*): A regional perspective. *Coast and Clean Seas Project 2127*.

Norman, B. M., Holmberg, J. A., Arzoumanian, Z., Reynolds, S. D., Wilson, R. P., Rob, D., Pierce, S. J., Gleiss, A. C., de la Parra, R., Galvan, B., Ramirez-Macias, D., Robinson, D., Fox, S., Graham, R., Rowat, D., Potenski, M., Levine, M., Mckinney, J. A., Hoffmayer, E., … Morgan, D. L. (2017). Undersea constellations: The global biology of an endangered marine megavertebrate further informed through citizen science. *BioScience*, *67*(12), 1029-1043.

Norman, B. M., & Stevens, J. D. (2007). Size and maturity status of the whale shark (*Rhincodon typus*) at Ningaloo Reef in Western Australia. *Fisheries Research*, *84*(1), 81-86.

Ogutu, J. O., Piepho, H.-P., Dublin, H. T., Reid, R. S., & Bhola, N. (2006). Application of mark–recapture methods to lions: Satisfying assumptions by using covariates to explain heterogeneity. *Journal of Zoology*, *269*(2), 161-174.

Ong, J. J. L., Meekan, M. G., Hsu, H. H., Fanning, L. P., & Campana, S. E. (2020). Annual bands in vertebrae validated by bomb radiocarbon assays provide estimates of age and growth of whale sharks. *Frontiers in Marine Science*, *7*, 188.

Otis, D. L., Burnham, K. P., White, G. C., & Anderson, D. R. (1978). Statistical inference from capture data on closed animal populations. *Wildlife Monographs*, (*62)*, 3-135.

Paiva, V. H., Geraldes, P., Ramírez, I., Meirinho, A., Garthe, S., & Ramos, J. A. (2010). Foraging plasticity in a pelagic seabird species along a marine productivity gradient. *Marine Ecology Progress Series*, *398*, 259-274.

Parsons, D. K. M. (2004). Procedural Guideline No. 4 – 5 Using photographic identification techniques for assessing bottlenose dolphin (*Tursiops truncatus*) abundance and behaviour. In J. Dacies, M. Bradley, D. Connor, J. Khan, E. Murray, W. Sanderson, C. Turnbull and M. Vincent (Eds.). *Marine Monitoring Handbook*. United Kingdom: Joint Nature Conservation Committee.

Pérez-Jorge, S., Gomes, I., Hayes, K., Corti, G., Louzao, M., Genovart, M., & Oro, D. (2016). Effects of nature-based tourism and environmental drivers on the demography of a small dolphin population. *Biological Conservation*, *197*, 200-208.

Perry, C. T., Clingham, E., Webb, D. H., de la Parra, R., Pierce, S. J., Beard, A., Henry, L., Taylor, B., Andrews, K., Hobbs, R., Araujo, G., & Dove, A. D. M. (2020). St. Helena: An important reproductive habitat for whale sharks (*Rhincodon typus*) in the Central South Atlantic. *Frontiers in Marine Science*, *7*, 899.

Perry, C. T., Figueiredo, J., Vaudo, J. J., Hancock, J., Rees, R., Shivji, M., Perry, C. T., Figueiredo, J., Vaudo, J. J., Hancock, J., Rees, R., & Shivji, M. (2018). Comparing length-measurement methods and estimating growth parameters of free-swimming whale sharks (*Rhincodon typus*) near the South Ari Atoll, Maldives. *Marine and Freshwater Research*, *69*(10), 1487-1495.

Petit, E., & Valiere, N. (2006). Estimating population size with noninvasive capture-mark-recapture data. *Conservation Biology*, *20*(4), 1062-1073.

Pierce, S. P., & Norman, B. (2016). *Rhincodon typus*. *The IUCN Red List of Threatened Species 2016: e.T19488A2365291*. Available at https://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T19488A2365291.en. (Accessed 24 December 2020).

Pingree, R. D., Garcia-Soto, C., & Sinha, B. (1999). Position and structure of the Subtropical/Azores Front region from combined Lagrangian and remote sensing (IR/altimeter/SeaWiFS) measurements. *Journal of the Marine Biological Association of the United Kingdom*, *79*(5), 769-792.

Pradel, R. (1996). Utilization of capture-mark-recapture for the study of recruitment and population growth rate. *Biometrics*, *52*(2), 703-709.

Pravin, P. (2000). Whale shark in the Indian coast – Need for conservation. *Current Science*, *79*(3), 310-315.

Ramírez-Macías, D., Vázquez-Haikin, A., & Vázques-Juárez, R. (2012). Whale shark *Rhincodon typus* populations along the west coast of the Gulf of California and implications for management. *Endangered Species Research*, *18*(2), 115-128.

Richardson, A. J., & Poloczanska, E. S. (2008). Under-resourced, under threat. *Science*, *320*(5881), 1294-1295.

Riley, M., Hale, M., Harman, A., & Rees, R. (2010). Analysis of whale shark *Rhincodon typus* aggregations near South Ari Atoll, Maldives Archipelago. *Aquatic Biology*, *8*(2), 145-150.

Robinson, D. P., Jaidah, M. Y., Jabado, R. W., Lee-Brooks, K., El-Din, N. M. N., Malki, A. A. A., Elmeer, K., McCormick, P. A., Henderson, A. C., Pierce, S. J., & Ormond, R. F. G. (2013). Whale sharks, *Rhincodon typus*, aggregate around offshore platforms in Qatari waters of the Arabian Gulf to feed on fish spawn. *PLOS ONE*, *8*(3), e58255.

Rohner, C. A., Richardson, A. J., Marshall, A. D., Weeks, S. J., & Pierce, S. J. (2011). How large is the world's largest fish? Measuring whale sharks *Rhincodon typus* with laser photogrammetry. *Journal of Fish Biology*, *78*(1), 378-385.

Rowat, D., & Brooks, K. (2012). A review of the biology, fisheries and conservation of the whale shark *Rhincodon typus*. *Journal of Fish Biology*, *80*(5), 1019-1056.

Rowat, D., & Gore, M. (2007). Regional scale horizontal and local scale vertical movements of whale sharks in the Indian Ocean off Seychelles. *Fisheries Research*, *84*(1), 32-40.

Rowat, D., Meekan, M., Engelhardt, U., Pardigon, B., & Vely, M. (2007). Aggregations of juvenile whale sharks (*Rhincodon typus*) in the Gulf of Tadjoura, Djibouti. *Environmental Biology of Fishes*, *80*(4), 465-472.

Rowat, D., Speed, C. W., Meekan, M. G., Gore, M. A., & Bradshaw, C. J. A. (2009). Population abundance and apparent survival of the Vulnerable whale shark *Rhincodon typus* in the Seychelles aggregation. *Oryx*, *43*(4), 591-598.

Saavedra, C., Santos, M. B., Valcarce, P., Freitas, L., Silva, M., & Pipa, T. (2018). *Macaronesian Roof Report*. Technical Report. Macaronesia: Project MISTIC SEAS II; 114. Available at https://misticseas3.com/sites/default/files/materialdivulgativo/macaronesian_roof_report_en.pdf (Accessed 8 January 2021).

Santos, R., Hawkins, S., Monteiro, L., Alves, M., & Isidro, E. (1995). Marine research, resources and conservation in the Azores. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *5*(4), 311-354.

Santos, R., Porteiro, F., & Barreiros, J. (1997). *Marine fishes of the Azores: Annotated checklist and bibliography: a catalogue of the Azorean marine Ichthyodiversity*. Universidade dos Açores.

Schwarz, C. J., & Arnason, A. N. (1996). A general methodology for the analysis of capture-recapture experiments in open populations. *Biometrics*, *52*(3), 860-873.

Schwarz, C. J., & Arnason, A. N. (2009). Jolly-Seber models in MARK. In E. Cooch & G. White (Eds.). *Program MARK: a gentle introduction (8th Edition)*. Available at http://www. phidot. org/software/mark/docs/book. (Accessed 3 August 2021)

Schwarz, C. J., & Seber, G. A. F. (1999). Estimating animal abundance: Review III. *Statistical Science*, *14*(4), 427-456.

Sequeira, A., Mellin, C., Fordham, D. A., Meekan, M. G., & Bradshaw, C. J. A. (2014). Predicting current and future global distributions of whale sharks. *Global Change Biology*, *20*(3), 778-789.

Sequeira, A., Mellin, C., Meekan, M. G., Sims, D. W., & Bradshaw, C. J. A. (2013). Inferred global connectivity of whale shark *Rhincodon typus* populations. *Journal of Fish Biology*, *82*(2), 367-389.

Sequeira, A., Thums, M., Brooks, K., & Meekan, M. G. (2016). Error and bias in size estimates of whale sharks: Implications for understanding demography. *Royal Society Open Science*, *3*(3), 150668.

Silva, L., & Smith, C. W. (2004). A characterization of the non-indigenous flora of the Azores Archipelago. *Biological Invasions*, *6*(2), 193-204.

Sleeman, J. C., Meekan, M., Fitzpatrick, B., Steinberg, C. R., Ancel, R., & Bradshaw, C. (2010). Oceanographic and atmospheric phenomena influence the abundance of whale sharks at Ningaloo Reef, Western Australia. *Journal of Experimental Marine Biology and Ecology*, *382*(2), 77-81.

Smith, A. (1828). Descriptions of new, or imperfectly known objects of the animal kingdom, found in the south of Africa. *South African Commercial Advertiser*, 3(2).

Speakman, T., Lane, S., Schwacke, L., Fair, P., & Zolman, E. (2010). Mark-recapture estimates of seasonal abundance and survivorship for bottlenose dolphins (*Tursiops truncatus*) near Charleston, South Carolina. *Journal of Cetacean Research and Management*, *11*(2), 53-62.

Speed, C., Meekan, M. G., & Bradshaw, C. J. (2007). Spot the match – wildlife photo-identification using information theory. *Frontiers in Zoology*, *4*(1), 2.

Speed, C., Meekan, M., Rowat, D., Pierce, S., Marshall, A., & Bradshaw, C. (2008). Scarring patterns and relative mortality rates of Indian Ocean whale sharks. *Journal of Fish Biology*, *72*(6), 1488-1503.

Stevens, J. D., Bonfil, R., Dulvy, N. K., & Walker, P. A. (2000). The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. *ICES Journal of Marine Science*, *57*(3), 476-494.

Taylor, G. (1994). *Whale sharks: The giants of Ningaloo Reef* (1st Edition.) Angus & Robertson, Sydney, 176p

Thorsteinsson, V. 2002. Tagging methods for stock assessment and research in fisheries. Report of concerted action FAIR CT.96.1394 (CATAG). Reykjavik. *Marine Research Institute Technical Report* (79), pp 179.

Tyminski, J. P., Parra-Venegas, R. de la, Cano, J. G., & Hueter, R. E. (2015). Vertical movements and patterns in diving behavior of whale sharks as revealed by pop-up satellite tags in the eastern Gulf of Mexico. *PLOS ONE*, *10*(11), e0142156.

Uchida, S., Toda, M. & Kamei, Y. (1990). Reproduction of elasmobranchs in captivity. In *Elasmobranchs as living resources: Advances in the biology, ecology, systematics, and the status of the fisheries (Pratt, H.L., Gruber, S.H., Taniuchi, T., eds). NOAA Technical Report NMFS, 90*, 211-237.

Uchida, S., Toda, M., Kamei, Y. & Teruya, H. (2000). The husbandry of 16 whale sharks *Rhincodon typus*, from 1980 to 1998 at the Okinawa expo aquarium. In *Abstracts of the American Elasmobranch Society 16th Annual Meeting. La Paz, Mexico, 14-20 June 2000.*

Van Tienhoven, A., Hartog, J., Reijns, R., & Peddemors, V. (2007). A computer-aided program for pattern-matching of natural marks on the spotted raggedtooth shark *Carcharias taurus*. *Journal of Applied Ecology*, *44*(2), 273-280.

Vignaud, T. M., Maynard, J. A., Leblois, R., Meekan, M. G., Vázquez-Juárez, R., Ramírez-Macías, D., Pierce, S. J., Rowat, D., Berumen, M. L., Beeravolu, C., Baksay, S., & Planes, S. (2014). Genetic structure of populations of whale sharks among ocean basins and evidence for their historic rise and recent decline. *Molecular Ecology*, *23*(10), 2590-2601.

Wilson, S. G., Polovina, J. J., Stewart, B. S., & Meekan, M. G. (2006). Movements of whale sharks (*Rhincodon typus*) tagged at Ningaloo Reef, Western Australia. *Marine Biology*, *148*(5), 1157-1166.

Wilson, S. G., Taylor, J. G., & Pearce, A. F. (2001). The seasonal aggregation of whale sharks at Ningaloo Reef, Western Australia: Currents, migrations and the El Niño/Southern Oscillation. *Environmental Biology of Fishes*, *61*(1), 1-11.

Wilson, S., & Martin, R. (2001). Body markings of the whale shark: Vestigial or functional? *Western Australian naturalist*, *24*(2), 115-117.

Wolfson, F. H. (1986). Occurrences of whale shark *Rhincodon typus*, Smith. In *T. Uyeno, R. Arai, T. Taniuchi & K. Matsuura (Eds.). Proceedings of the* 2*nd International Conference on Indo Pacific fishes* (pp. 208–226). Tokyo: Icthyological Society of Japan.

Woods, J. D., & Barkmann, W. (1995). Modelling oligotrophic zooplankton production: Seasonal oligotrophy off the Azores. *ICES Journal of Marine Science*, *52*(3-4), 723-734.

Using image analysis to study structure, population size population size and connectivity patterns of the whale shark *Rhincodon typus* in the Azores archipelago

Bernat Marti Alsina[a,*], Bruno Macena [b,c] , Pedro Afonso[b,c], Karim Erzini[d], Jorge Fontes[b,c]

[a] *Faculty of Sciences and Technology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal*

[b] *OKEANOS-UAc University of the Azores, Horta, Azores, Dept. of Oceanography and Fisheries, 9900-862 Horta, Portugal*

[c] *IMAR-Azores, Institute of Marine Research at the University of the Azores, Dept. of Oceanography and Fisheries, 9900-862 Horta, Portugal*

[d] *Center of Marine Sciences (CCMAR), University of the Algarve, Campus de Gambelas, 8005-139 Faro, Portugal*

***Corresponding author:** bernat18m@gmail.com

Keywords

Elasmobranch · Photo-identification · capture-mark-recapture · Cormack-Jolly-Seber · Citizen science

Abstract

In the last recent years, whale sharks (*Rhincodon typus*) have been found to aggregate in the waters of the Azores archipelago, during the summer season. In this study we used photo-identification data collected during years 2008 through 2011, 2013, 2017, 2019 and 2020 to produce the first estimates of population structure, site fidelity, abundance, and movements of these aggregations. A total of 3,705 photographs were collected, of which 545 were suitable for comparison using the semi-automated matching software and photographic identification database Wildbook for Whale Sharks. A total of 186 individuals were uniquely identified through the combination of spot and stripe patterns behind the last gill slit and forward of the dorsal fin (left and right lateral views). Among the 115 sexed individuals, 31% were males and 69% were females, confirming a female-biased aggregation. A total of 38 individuals (20%) presented scars during the different years of the study. The majority of whale shark encounters occurred in the island of Santa Maria (82%), especially during the months of August and September (81%). Of the 29 individuals resighted during the study, 28 were resighted in different occasions during the same year, and 4 individuals were resighted in different years (ranging from 1 to 3 years). Inter-island connectivity was also observed for one whale shark. We estimated the population of whale sharks that visit the Azores region to consist of approximately 283 – 496 individuals (95% confidence interval) based on Cormack-Jolly-Seber open-population models. This study provides the first population abundance estimates and characterization of the whale shark population of Azores, as well as assembling value information regarding their ecology and connectivity patterns.

Introduction

Knowledge of population demographics, spatial ecology, and connectivity patterns of populations is essential in order to design effective management and conservation strategies for any species (Caughley & Gunn, 1996; Andrzejaczek et al., 2016). However, obtaining such information can be challenging, time-consuming and expensive, particularly for species that are migratory or that reside in the open ocean (Richardson & Poloczanska, 2008; Block et al., 2011; Sequeira et al., 2014b; Andrzejaczek et al., 2016).

Whale sharks provide a good example of the challenges involved with the assessment of population demographics and connectivity patterns of oceanic marine megafauna. The whale shark *Rhincodon typus* (Smith 1828) is an epipelagic, filter feeding organism and the largest living fish in the world (Holmberg et al., 2009; Ebert et al., 2013). *R. typus* has a widespread global distribution occurring in all tropical and warm temperature waters (Colman, 1997; Cochran et al., 2019), with a preference of water temperatures of 21 – 25ºC (Iwasaki, 1970). The species has suffered a larger than 50% decline in global population over the last 75 years, mainly due to targeted and accidental fisheries, as well as habitat loss and degradation and shipping collisions, leading to a conservation status of "Endangered" on the ICUN Red List of Threatened Species and included in the Appendix II of the Convention on International Trade in Endangered Species (CITES) (Pravin, 2000; Pierce & Norman, 2016). Despite these protection plans, a more targeted conservation strategy requires a better knowledge of the distribution, population demographics and movement patterns (Fromentin & Powers, 2005).

While the whale shark's geographical range is well known, information on the global population structure and connectivity patterns remains limited (McKinney et al., 2017). Whale sharks have been found to form predictable seasonal aggregations in several hotspots around the world, which seem to be primarily linked to high productivity areas (Eckert et al., 2002; Graham & Roberts, 2007; Nelson & Eckert, 2007; Rowat & Brooks, 2012; Fox et al., 2013). Predictable seasonal aggregations of whale sharks offer scientists an opportunity to collect data from these large animals, and a significant economic value to tourism industries and local communities through ecotourism practices (Meekan et al., 2006; Gallagher & Holmberg et al., 2009; Jones et al., 2009; Hammerschlag, 2011; Sequeira et al., 2016).

Despite the discovery of high density, predictable aggregations which has sparked a rapid expansion in research and international interest in the conservation of this species, much of its life history, including age-specific growth rates, its reproductive ecology, and migration patterns, as well as regional population sizes remain largely unknown (McKinney et al., 2017; Perry et al., 2020). However, cooperation among research groups, ecotourism tours, and citizen science across known aggregations sites has produced an extensive record of *R. typus* encounters, much of which has been collected in a single online database (www.whaleshark.org) (Cochran et al., 2019).

Whale shark ecology and biology can be investigated using a variety of techniques, including satellite and acoustic telemetry, genetic studies and photo-identification (photo-ID) (Gunn et al., 1999; Rowat & Gore, 2007; Ramírez-Macías et al., 2012b; Perry et al., 2020). Photographic identification is a non-invasive method that uses permanent natural markings to identify individuals within a population, allowing to monitor population demographics and structure, and estimate population sizes through Catch-Mark-Recapture (CMR) modelling techniques (Brooks et al., 2010; Norman et al., 2017; Perry et al., 2020). Whale sharks can be uniquely identified by the distinctive patterns of lines and spots on their flanks that are unique to each individual and serve as an effective natural marker that can be distinguished by eye or by using available computer algorithms (Arzoumanian et al., 2005; Van Tienhoven et al., 2007; Holmberg et al., 2009). Furthermore, photographic identification of whale sharks can be used to investigate seasonal aggregations, residency patterns, as well as regional-scale migrations and potentially enabling to elucidate connectivity between different locations (Brooks et al., 2010; Fox et al., 2013; Acuña-Marrero et al., 2014; McCoy et al., 2018).

The demography and population connectivity of whale sharks at both local and regional (oceanic) scales is a priority for management and conservation strategies (Brooks et al., 2010; Ramírez-Macías, Meekan, et al., 2012). In this study we examined the population structure, connectivity patterns and population size by characterizing the whale sharks summer aggregations in the Azores over more than a decade, using photo-identification (photo-ID). By searching for matches in global libraries of photo-IDs (Wildbook for Whale Sharks) collected at different sites across all ocean basins we were able to investigate movement patterns, connectivity and residency across different time scales, while providing the first insight into the demography of whale sharks that aggregate in the Azores.

MATERIAL AND METHODS

Study Area

Azores

The Azores archipelago is located along the northern mid-Atlantic ridge (latitude 36º - 43º N, longitude 25º - 31º W) and comprises 9 islands of volcanic origin that are grouped into the western, central and eastern island groups (Afonso et al., 2014; Fontes et al., 2020), representing the most isolated archipelago in the North Atlantic Ocean, situated approximately 1500 km from Europe (Silva & Smith, 2004) (Figure 1). The Azores region is characterized by a temperate oceanographic regime resulting from the interaction of the North Atlantic Current (in the North) and the Azores Current (in the south) with many unstable eddies, meanders, and localized patches of enhanced productivity (Santos et al., 1995; Alves & de Verdière, 1999; Johnson & Stevens, 2000; Bashmachnikov et al., 2009). In general, the Azores region experiences large scale spatial and seasonal variations of oceanographic conditions, with minimum sea surface temperatures (SST) in winter (~ 15ºC) and maximum SST in summer (27ºC) (Lafon et al., 2004; Martins et al., 2007). Maximum chlorophyll-a concentrations have been observed during periods of lower SST, usually occurring during winter and spring, whereas minimum chlorophyll-a concentrations take place during the summer, when SST is typically higher (Santos et al., 2013; Amorim et al., 2017). Although these are the general patterns, high inter-annual variability has been observed in the region (Martins et al., 2009; Amorim et al., 2017).

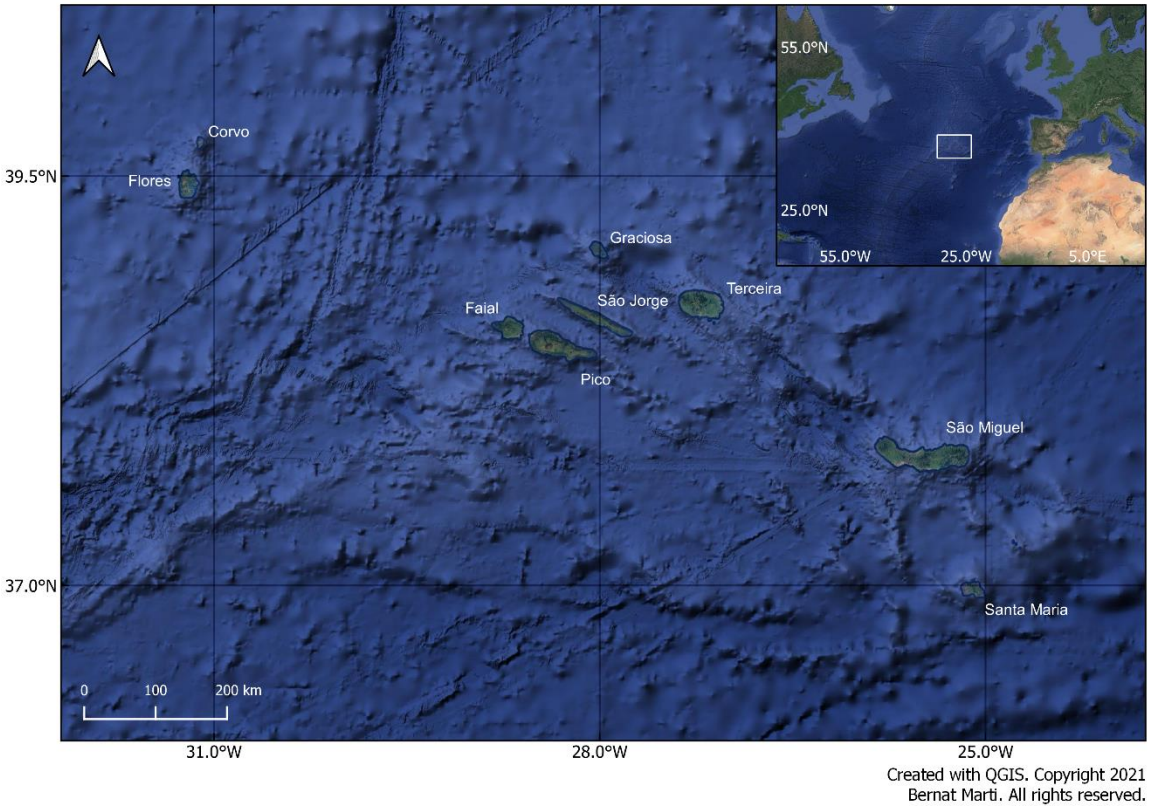

Figure 1: Map of Azores, the study site. Insert: location of Azores archipelago in the North Atlantic.

Data collection

Photographs and occurrence data of whale sharks were collected from over 35 individual contributors, including the authors, researchers, tourists, professional underwater photographers, and members of the ecotourism industry during the 2008-2020 period. Photographic data was only available for years 2008 through 2011, 2013, 2017, 2019 and 2020. Remaining years had no sightings, or occurrence data was not possible to be obtained.

Information on each encounter was recorded, including underwater photographs and videos, date, location, and sex of the shark whenever possible. Sex was determined by the presence (in males) or absence (in females) of claspers on the pelvic fins (Meekan et al., 2006). Claspers are short, soft, and smooth in sexually immature males, but quickly grow and calcify during maturation (Norman & Stevens, 2007), and they often appear rough and abraded in adult sharks (Joung & Chen, 1995). Sex was classified as indeterminate in some cases due to poor visibility, or because the shark swam away before pelvic fins could be examined or photographed. Prominent scars, abrasions, or amputations on the animals were also recorded. Size of maturity was established according to Norman & Stevens (2007).

Sampling effort

Sampling effort were not available for sightings and records provided by tourist neither for the science team records previous to 2019. Sampling effort was only available from dedicated surveys of whale sharks off Pico and Santa Maria Islands in the years 2019 and 2020. Surveys were conducted from RIB research vessels, and survey duration was limited to ten hours per day. Survey days were limited by weather conditions. These were primarily conducted between the months of August and September.

Based on the logged data, a total of 137 whale shark encounters were recorded during a period of 250 hours of surveys, resulting in an average of 5.48 ± 3.76 whale shark encounters per day.

Photographic identification

The unique spot-and-stripe pattern present on the flank of the whale shark in the area forward of the dorsal fin and behind the last gill slit was photographed and used for the individual identification of the whale sharks (Arzoumanian et al., 2005). When possible, both the left and right sides were photographed. To date, these natural markings show no evidence of significant change throughout their lives (Taylor, 1994; Arzoumanian et al., 2005). Identification of individual sharks was performed using the techniques and computer-assisted matching tools described in Arzoumanian et al. (2005). All collected data used in this study was uploaded to the online whale shark database "Wildbook for Whale Sharks" at www.whaleshark.org for photo-identification analysis, and meta-data was recorded on a digital data base containing information of the individual ID, sighting location, sighting date, sex, and presence of injuries.

For each encountered shark, information on the number, type and position of the scars was also recorded. Following Speed et al. (2008) scars and wounds were subdivided into seven categories (abrasions, lacerations, amputations, natural deformations, bites, nicks, and others) (Figure 2), and a probable origin was also attributed (anthropogenic or natural). Anthropogenic scars appeared to be mainly caused by boat strikes, leaving rows of parallel scars along the dorsal surface or the body flanks, open wounds, or amputations. On the other hand, bites, natural deformations, and nicks were classified as natural source. Scars and wounds were also divided into two categories according to severity. Wounds that were considered life-threatening for the animal were defined as 'major scars' and included

complete or near-complete amputations of the caudal fin, first dorsal fin or pectoral fins, deep lacerations, natural deformations around the head or gills, and large bites (> 300 mm in length) (Speed et al., 2008). On the other hand, superficial scars were defined as 'minor scars' and included abrasions, small bites, and nicks (Speed et al., 2008). Finally, injuries were also categorized as recent or fully old.

Figure 2: Scarring categories: **a** abrasion, **b** amputation, **c** bite, **d** natural deformations, **e** laceration, **f** nicks.

Data analysis

Pearson's chi-squared test was applied to test if sex ratio different from 1.0:1.0 female to male ratio. Statistical significance was tested at $P = 0.05$. Percentages of injured sharks were calculated for the different years of the study. ANOVA was used to test if number of recent scars/numbers of animals documented were significantly different among years. Location of each sighting was recorded and transferred to QGIS 3.8.12 to build a distribution map.

Population size

The photographic identifications and re-identifications were treated as captures and recaptures, respectively. With this information, a matrix was created using a binary code, of 1 and 0, for all capture histories in the different sighting dates. The values 1 or 0 indicate the presence or absence of the individual during the sighting occasion. Each series of 1's and 0's corresponds to the encounter history of a specific individual.

Capture-mark-recapture models assume that the samples are representative of the population and that the probability of mortality, emigration, immigration, and recapture is the same for all individuals (Cormack, 1964; Jolly, 1965; Seber, 1965, Rowat et al., 2009). Capture-mark-recapture models can be divided into two groups, models that assume demographic closure (no net immigration or emigration) and open population models (assume that animals are capable of immigration and emigration) (Link & Barker, 2010). Considering that whale sharks are a highly mobile and migratory species (Ramírez-Macías et al, 2012; Fox et al., 2013), photo-identification datasets for years 2019 and 2020 were used to model population size using variants of the open-population Cormack-Jolly-Seber model (Schwarz & Arnason, 1966) in the POPAN option, as described in other studies (Meekan et al., 2006; Rowat et al., 2009; Brooks et al., 2010; Riley et al., 2010; Ramírez-Macías et al., 2012). The model provides estimates of apparent survival (ϕ), capture probability (p) given the animal is alive and available for capture, estimates of probability of entry into the population (β) and population size (N), following the constant equation $\{\Phi(.)\ p(.)\beta(.)N(.)\}$, where (.) indicates that apparent survival (ϕ), capture probability (p), probability of entry into the population (β) and population size (N), remain constant over the whole study period. To test assumption of closed population, program CloseTest (Stanley & Richards, 2011) was used. All models were run in the program R version 1.4.1106 (R Core Team, 2021), using the package RMark, an (Laake 2013) interface of MARK software (White & Burnham, 1999).

Model results were evaluated using the normalized weights and values of the Akaike's Information Criterion (AIC_c) (Akaike, 1973), which is used to measure the likelihood of the goodness-of-fit to the data (Ribarič, 2018). The model with the smallest AIC_c was considered to be the best description (Berg et al., 2002) of whale shark population estimates in the Azores.

RESULTS

Photographic identification and image analysis

From 3,705 images and videos collected from different sources for the present study, 545 (15 %) were suitable for the photo-matching process. A total of 186 individual sharks were identified using photo ID from 2008-2020. Of these, 36 (19%) were male, 79 (43%) female and 71 undetermined sex (38%). Overall, the number of females was statistically greater than males $\chi^2 = 16.078$, df = 1, $P < 0.05$, with a male:female ratio of 1.0:2.2. Although the study was not characterized to describe size distribution within the population, all animals were estimated to be larger than 8 m, and therefore, mature individuals.

A total of 38 whale sharks (20%) encountered during the whole study had different types of scarring. In 2008, the percentage of wounded sharks was 80% (n = 4), in 2019 15% (*n* = 11), while in 2020 was 18% (*n* = 25). No wounded whale shark was recorded in the remaining years (Table 1). Considering only major scars, the general percentage of scarred sharks was 8%. Overall, nicks were the most abundant scar category (34%), followed by abrasions (29%) and amputations (13%) respectively (Table 2). The most affected areas of the whale shark's body were the dorsal fin (34%) followed by the caudal fin (29%). Of the all the scars present in the individuals, 52% were considered to potentially have a natural cause, and 48% potentially an anthropogenic source. Of the total of wounded sharks, 12 individuals (32%) presented recent scars, whereas 26 individuals (68%) presented old scars. Recent scars/number of animals documented was not significantly different among years (ANOVA; $P > 0.05$).

Table 1: Number of identified sharks, and percentage of wounded sharks from the in Azores aggregation in 2008-2020 study period.

Year	Identified sharks	Wounded sharks %
2008	*5*	80
2009	2	0
2010	2	0
2011	4	0
2013	1	0
2017	6	0
2019	71	15
2020	136	18

Table 2: Percentages of injured whale sharks, according to scar type and body area in Azores, for the years where injured whale sharks were observed (2008, 2019, and 2020). Sample size (N) indicates the total number of whale sharks encountered and individually identified on that specific year.

Scars and wounds	2008 (%)	2019 (%)	2020 (%)	Mean ± SD
Type of scar				
Abrasion	0	37	26	21 ± 19
Amputation	0	11	14	8 ± 7
Bites	0	11	0	4 ± 6
Blunt Trauma	0	5	11	6 ± 6
Laceration	0	0	6	2 ± 3
Nicks	100	32	31	54 ± 40
Others	0	5	11	6 ± 6
Body area				
Caudal fin	0	37	28	22 ± 19
Pectoral fins	0	11	6	5 ± 5
Dorsal fin	100	26	31	52 ± 41
Dorsal area	0	0	6	2 ± 3
Head	0	5	6	4 ± 3
Flank	0	21	22	14 ± 13
Ventral area	0	0	3	1 ± 2
Sample size (*N*)	5	71	136	

Population size

After discarding photo-identities of poor information (i.e., no exact date available), the database revealed that a total of 177 sharks were photographed between 2008 and 2020. Due to inconsistent temporal series of photographs available for all years, capture histories were constructed to perform CMR models to estimate population size for years 2019-2020 only. Photo-identities of both the left and right side were not available for all individuals and so left-side identities were used because these were more common (and also the side used in the Wildbook for Whale Sharks database) than those for the right side (160 cf 143), thus avoiding double-counting, and giving a total number of 117 known individuals.

A total of two years of data (2019 and 2020) with 117 individuals photographed (160 separate sightings) over the study period, generated a series of population estimates under

the different models tested. Assumption of closure was violated (Z = -7.554; P<0.001), confirming that whale shark population from the Azores is an open population with gains and losses. Therefore, an open-population approach was selected. POPAN open-population Cormack-Jolly-Seber models, provided an estimated population size of 367 (SE = 53.5; range: 283 – 496) individuals (Table 3) based on the constant model {Φ(.)p(.)β(.)N(.)} which resulted to be the best model to fit the data (Table 4). The constant model indicated that apparent survival, capture probability, probability of entry into the population, and population size remained constant (.) over the whole study period. Resulting estimates of annual abundance from the best-fit POPAN models are listed in Table 5.

Table 3: Population estimates and parameters derived from Cormack-Jolly–Seber open population model (POPAN option) for the combined photo-identification data for 2019 – 2020, providing estimates of apparent survival (Φ), capture probability (p), probability of entry into the population (β), and population size (N). Confidence interval = 95%.

Real Function Parameters of {Φ(.)p(.)β(.)N(.)}

Parameter	Estimate	Standard error (SE)	95% Confidence interval	
			Lower	Upper
Φ	0.838	0.026	0.781	0.882
p	0.076	0.016	0.050	0.113
β	0.016	< 0.001	0.015	0.018
N	367.564	53.578	282.551	496.231

Table 4: Model rankings comparing variants of the Cormack-Jolly-Seber (CJS) model in the option POPAN to estimate whale shark population size in Azores.

Model	Parameters	AICc	weight	Deviance
Φ(~1)p(~1)β(~1)N(~1)	4	635.981	1.00E+00	-288.060
Φ(~1)p(time)β(~1)N(~1)	59	693.638	3.02E-13	-390.944
Φ(~1)p(~1)β(time)N(~1)	58	741.726	0.00E+00	-337.819
Φ(~time)p(~1)β(~1)N(~1)	58	768.329	0.00E+00	-311.217
Φ(~1)p(~time)β(~time)N(~1)	113	1262.931	0.00E+00	-418.938
Φ(~time)p(~1)β(~time)N(~1)	112	1268.386	0.00E+00	-389.950
Φ(~time)p(~time)β(~1)N(~1)	113	1268.474	0.00E+00	-413.396
Φ(~time)p(~time)β(~time)N(~1)	167	6228.297	0.00E+00	-444.047

As bootstrap goodness-of-fit is not available for the POPAN model, estimates of apparent survival and capture probability were calculated by modeling the data with the recaptures-only (CJS) analysis. The constant recaptures-only model {Φ(.) p(.)} was ranked the highest with an AIC_c of 383.683, providing estimates of apparent survival (Φ) = 0.835 ± 0.025 and capture probability (p) = 0.077 ± 0.016.

Table 5: Annual abundance of whale sharks visiting the Azores study area, as estimated in the best-fit Cormack-Jolly-Seber model in the POPAN option for years 2019 and 2020. 95% CI are provided in parenthesis.

Year	Abundance
2019	190 (143 - 270)
2020	201 (111 - 424)

Spatial and temporal patterns

Whale sharks were encountered in four different islands of the archipelago (Flores, Pico, São Miguel, and Santa Maria) as well as in Princess Alice Bank (Figure 3). However, 82% (n = 156) of whale sharks encounters occurred in the vicinity of the island of Santa Maria, the most eastern island of the archipelago.

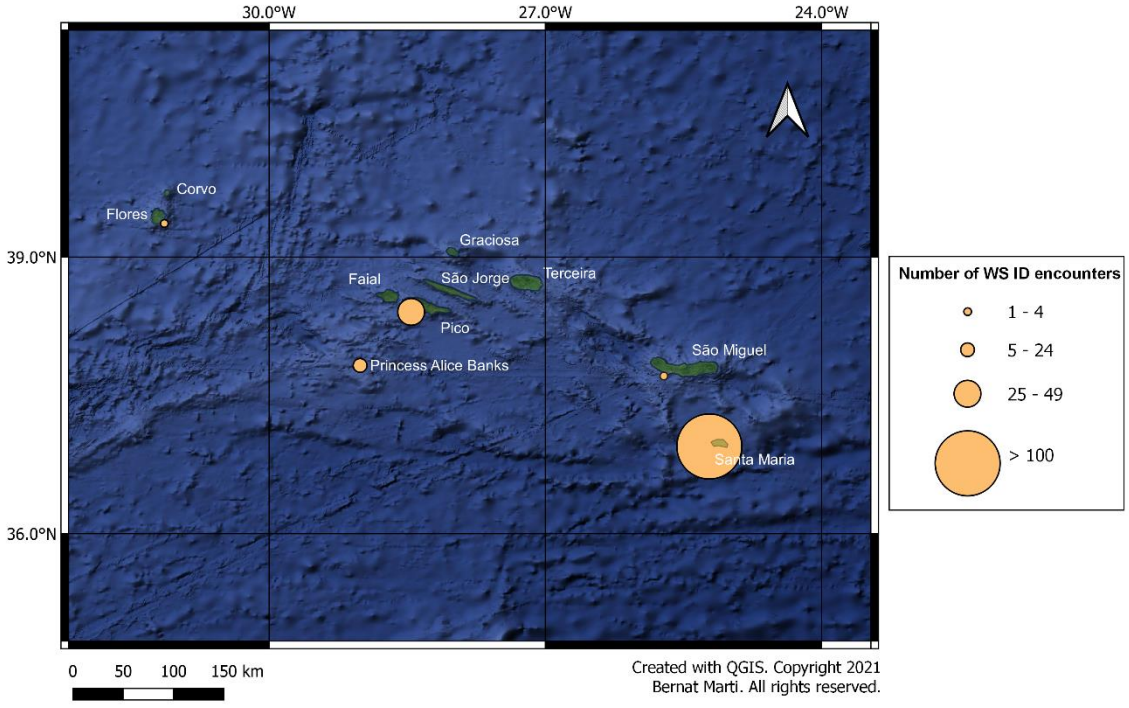

Figure 3: Distribution of the whale shark identification encounters across the archipelago.

Whale shark encounters spanned from July to November, although there was a distinct trend with 81% of all encounters being documented in the months of August and September. More precisely, most of the whale shark encounters occurred during the 2nd week of August until 2nd week of September (Figure 4A). In total, 71 and 136 whale shark individuals were encountered during the 2019 and 2020 season respectively, representing a 31% and 60% of the total whale shark encounters (Figure 4B, Table 1). Remaining encounters occurred in 2008 (2%), 2009 (1%), 2010 (1%), 2011 (2%), 2013 (<1%) and 2017 (3%).

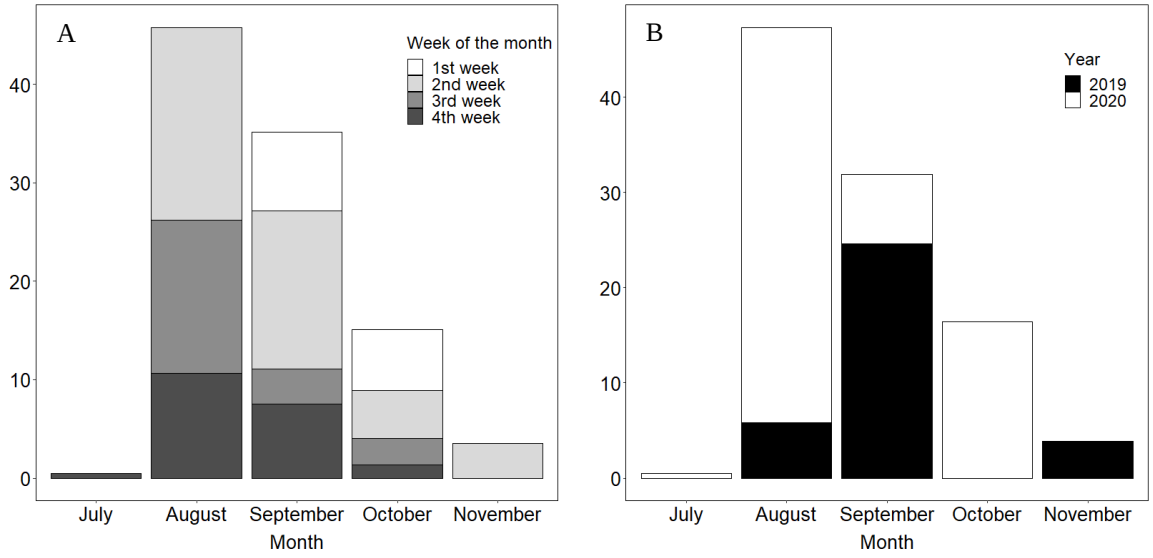

Figure 4: Whale shark encounters across all years aggregated per month (A), per weeks (B), and comparison of whale shark encounters between years 2019 and 2020 (C).

Residency and connectivity

Although the majority (84%) of individuals were encountered only once across the study period, 29 whale sharks were resighted in the study area multiple times (Table 6). The minimum interval between encounters with the same shark that was recorded as a re-sighting for the purpose of data analysis was 2 h. Nine individuals (31%) were resighted on more than three different days. Individual residence time was calculated as the mean of the day difference from first to last encounter in the Azores area. The period between first to last sighting ranged from one to twenty days, with an average of 6.1 ± 5.3 (mean ± SE) days. Of the 29 re-sighted animals, 14% (n = 4) were re-sighted in different years. Of these, three sharks were re-sighted in consecutive seasons, whereas individual AZ-003 was seen with a three-year difference.

Table 6: Summary of re-sighted sharks with information on sex, date of first and last encounter, interval between first and last sighting (days) (within season and between seasons), and location of first and last encounters. * Indicates whale shark individual was also spotted in different dates between first and last encounter. Whale sharks with interannual sightings and interisland sightings are highlighted in bold.

Wildbook ID	Sex	First Encounter	Last Encounter	Day Difference	Next Year Encounter	Interannual Difference	Site nº 1	Site nº 2
AZ-003	NA	29/8/2008			14/8/2011	1080	Santa Maria	Santa Maria
AZ-004	Male	20/8/2020	22/8/2020	2			Santa Maria	Santa Maria
AZ-006 *	Female	14/8/2020	25/8/2020	11			Santa Maria	Santa Maria
AZ-013 *	Female	3/10/2020	15/10/2020	12			Santa Maria	Pico
AZ-014	Male	5/10/2020	20/10/2020	15			Pico	Pico
AZ-018	NA	10/10/2020	18/10/2020	8			Pico	Pico
AZ-035 *	Female	3/10/2020	11/10/2020	8			Pico	Pico
AZ-037	NA	10/10/2020	17/10/2020	7			Pico	Pico
AZ-041	Male	17/10/2020	23/10/2020	6			Pico	Pico
AZ-044	Male	11/10/2020	17/10/2020	6			Pico	Pico
AZ-051	Female	30/8/2019	31/8/2019	1			Santa Maria	Santa Maria
AZ-054	Female	1/9/2019	3/9/2019	2			Santa Maria	Santa Maria
AZ-056	Female	2/9/2019	12/9/2019	10			Santa Maria	Santa Maria
AZ-060 *	Female	3/9/2019	14/9/2019	11			Santa Maria	Santa Maria
AZ-061	Female	10/9/2019	12/9/2019	2			Santa Maria	Santa Maria
AZ-062	Female	10/9/2019	14/8/2020	1	15/8/2020	340	Santa Maria	Santa Maria
AZ-072	Female	14/9/2019	15/9/2019	1	1/10/2020	382	Santa Maria	Santa Maria
AZ-073	Female	15/9/2019	16/9/2019	1			Santa Maria	Santa Maria
AZ-088	Female	14/8/2020	16/8/2020	2			Santa Maria	Santa Maria
AZ-107	Female	11/9/2019	14/9/2019	3			Santa Maria	Santa Maria
AZ-119 *	Female	11/8/2020	23/8/2020	12			Santa Maria	Santa Maria
AZ-121	Male	20/8/2020	23/8/2020	3			Santa Maria	Santa Maria
AZ-122	Male	20/8/2020	21/8/2020	1			Santa Maria	Santa Maria
AZ-126 *	Female	22/8/2020	3/9/2020	12			Santa Maria	Santa Maria
AZ-127	NA	10/8/2020	12/8/2020	2			Santa Maria	Santa Maria
AZ-128 *	Female	9/11/2019	22/8/2020		12/8/2020	277	Santa Maria	Santa Maria
AZ-135	Female	26/9/2019	27/9/2019	1			Santa Maria	Santa Maria
AZ-147	Female	14/8/2020	15/8/2020	1			Santa Maria	Santa Maria
AZ-159 *	Female	14/8/2020	3/9/2020	20			Santa Maria	Santa Maria

Re-sightings of individuals only occurred in the islands of Pico and Santa Maria. Re-sightings of individuals always occurred at the same island where the first sighting occurred, except for AZ-013 that was sighted first in Santa Maria and then in Pico (Table 6).

Whale shark sightings in Azores were contrasted with all available records, including previous records registered by other researchers, and ecotourism companies. While a total of 29 individuals were re-sighted in the region of Azores during the study period, no matches were found with other known whale shark populations.

DISCUSSION

This study aimed to develop the first characterization of population structure and abundance of the whale shark population visiting the Azores archipelago, as well as providing valuable information regarding the basic ecology and connectivity patterns of whale sharks in the Atlantic Ocean. Image analysis of scar patterns and photo-identification methods allowed us to identify individual whale sharks, investigate population structure, residency patterns and connectivity across multiple scales within and between years and between islands and other known aggregations in the Atlantic.

Population structure

Whale shark presence and abundance patterns in the Azores are quite irregular and their abundance has varied significantly since 1998, when the systematic record of whale sharks associated with the local tuna fisheries started. Thus, since 1998, two periods of high abundance were recorded, the first from 2008 to 2013 and more recently, from 2019 to 2020. We were able to successfully identify 186 individuals from eight different years during the period 2008-2020, when whale sharks were present, i.e., 2008, 2009, 2010, 2011, 2013, 2017, 2019 and 2020. Images from 2012 are not available since whale sharks were mainly sighted off shore, south of the Princess Alice seamount in the central group of islands, far from diving operations and tourists Overall, the whale shark population that visits Azores is mostly dominated by females, with a male:female sex ratio of 1.0:2.2. This sex ratio is quite unique as most information regarding *R. typus* population structure comes from coastal aggregations in the Caribbean Sea, Indian Ocean, and South Atlantic and these aggregations tend to be dominated by immature males (Heyman et al., 2001; Meekan et al., 2006; Rowat et al., 2011; Ramírez-Macías et al., 2012; Ketchum et al., 2013; Norman et al., 2017; Perry et al., 2020), with male:female ratios ranging from 3:1 to 10:1 or more (Norman et al., 2017). Female biased populations of whale sharks have been recorded in the Galápagos (Acuña-Marrero et al., 2014) and the Gulf of California (Eckert & Stewart, 2001; Ramírez-Macías, et al., 2012b), both presenting a great majority of adult females, showing signs of a potential pregnancy (Ramírez-Macías et al., 2012b; Acuña-Marrero et al., 2014). Interestingly all aggregation in coastal regions of the Atlantic Ocean are dominated by males, with males typically 2.6 times more abundant than females (Western Atlantic; McKinney et al., 2017). In other remote oceanic locations in the Atlantic, male:female sex ratio of the population are 1.1:1.0 (Central

South Atlantic; Perry et al., 2020) and 1.0:3.7 (Archipelago of São Pedro and São Paulo; Macena & Hazin, 2016), suggesting that large females are mostly encountered in oceanic regions. Although female individuals encountered in the Azores did not present signs of potential pregnancy (i.e, distended abdomen), the female dominance among the aggregations in the archipelago, as well as the presence of large sexually mature males, suggests that the Azores could play a role in the reproductive ecology of whale sharks in the Atlantic. However, copulation has never been documented and only a small fraction of males occasionally presented some sign of abrasion on claspers (Fontes pers.com).

Sexual or size segregation or biased sex ratios appear to be a general characteristic of shark populations (Springer, 1967; Klimley, 1987; Robbins, 2007; Ketchum et al., 2013; Vandeperre et al., 2014), including whale sharks (Rowat & Brooks, 2012) and also occurring in other large planktivorous sharks, such as the basking shark (*Cetorhinus maximus*) (Wilson, 2004). In most coastal and oceanic whale shark aggregations, sexual or size segregation occurs. Most coastal aggregations sites are composed predominantly by immature males, as occurs in Western Australia (Meekan et al., 2006; Norman et al., 2007), Djibouti (Rowat et al., 2007b), Seychelles (Rowat et al., 2007), Maldives (Riley et al., 2010), Belize (Graham & Roberts, 2007), Honduras (Fox et al., 2013) and Mexico (Ramírez-Macías et al., 2012, 2012b), whereas large adult whale sharks are more common at oceanic sites such as Galapagos Islands (Acuña-Marrero et al., 2014), St. Helena (Perry et al., 2020), Archipelago of São Pedro and São Paulo (Macena & Hazin, 2016), and the Azores (present study). Segregation or biased sex ratios are thought to be related to sex differences in body size, reproductive cycle, predation risk, forage selection, competition for food or mates, mating behavior, and social factors (Sims et al., 2001; Wearmouth & Sims, 2008; Kock et al., 2013). In coastal aggregations biased sex ratios may also result from different migration patterns (Pratt, 1979), or different mortality rates within the population (Heithaus, 2001). In the Azores, the presence of only adult whale sharks could be linked to the reproductive cycle or mating behavior, as also observed by the adult aggregation of whale sharks in the oceanic islands of St. Helena and Galápagos (Acuña-Marrero et al., 2014; Perry et al., 2020). Another potential explanation could be that individuals are linked to a suboptimal thermal threshold, which only adults could tolerate. Further research will be needed in order to confirm these hypotheses.

Despite the lack of direct observation of predatory attacks or collisions with ships experienced by whale sharks, some evidence of these events may be left in the form of scars or injuries on the body (Speed et al., 2008). Similar to populations in the Indian Ocean (Speed et al., 2008), several *R. typus* individuals from the Azores archipelago bare evidence of natural and anthropogenic scars. Most lacerations, abrasions and amputations could have been caused by boat collisions. However, the percentage of scarred individuals was lower than the percentage reported at Ningaloo Reef (27%), Mozambique (37.2%), the Seychelles (67%) and Mexico (69%) possibly because the population of whale sharks in Azores mainly comprises large adults that are known to be essentially oceanic, thus reducing the exposure to highly populated coastal areas where most boat traffic is concentrated. While it is possible that the scars were caused by collisions with boats at the Azores archipelago, we cannot discard that the damage occurred in some other area of their habitat range, given the tendency of whale sharks to migrate long distances (Eckert & Stewart, 2001; Rowat & Gore, 2007), as well as the high percentage of old scars (68%) compared to recent scars (32%) that individuals presented.

Population size

The photo-identification data was used to provide the first estimates of population abundance for the Azores. However, due to the small number of resighted individuals and the short study period, abundance estimates should be treated with caution. Based on open population models, the population of whale sharks utilizing the Azores archipelago as an aggregation site consisted of approximately 283 – 496 individuals, with an estimated annual abundance of 190 individuals in 2019, and 201 in 2020. Population presented a high survival rate (~80%), since the population is comprised mostly by adults, and a low recapture probability (~7%), possibly due to the low sampling size/effort in collecting the photographs.

By comparison, similar size or larger whale shark populations have been found to occur at the Ningaloo Reef (320 – 440 individuals) (Meekan et al., 2006), Seychelles (469 – 557 individuals) (Brooks et al., 2010), or Mexico (521 – 809 individuals) (Ramírez-Macías et al., 2012). In 2017, photo-identification data was also used to provide the first Western Central Atlantic Ocean (WCA) regional population estimates from multiple known aggregation sites within Honduras, Belize, Mexico, United States of America, and the greater Caribbean region (McKinney et al., 2017). Using maximum likelihood models, the

population size of whale sharks in the WCA was estimated to be 2.167 (± 345) individuals, including several mete populations with high connectivity potential.

The increase of the photo-identification database should allow more accurate estimates of population size in the future and this first estimate should be reviewed in the future. Similar to other studies (Rowat et al., 2009; Brooks et al., 2010), the test for closure of the population was violated and the rate of entry into the population could not be estimated, consistent with the preliminary satellite tracking data from the region (J. Fontes, pers. comm.).

Spatial and temporal patterns

Whale sharks at the Azores archipelago appeared to concentrate mainly around the island of Santa Maria, which is consistent with the encounter pattern reported by the tuna fisheries observers (Afonso et al 2014, Fontes et al. 2020), although our fisheries independent data recently revealed a minor aggregation at the southern part of Pico Island (Figure 3). Afonso et al. (2014), Fontes et al. (2020) also reported a large aggregation, south of the central group, close to the largest seamount in the region, in the year 2012. In our data set, most sightings tend to occur in August and September. Sparce sightings were also reported in July, although they were quite rare. These patterns may be related to variability in the mean annual summer sea surface temperature (SST) as well as chlorophyll (chl-*a*) concentrations patterns. According to Afonso et al. (2014), higher percentage of whale shark sightings occurred during the summer months when SST was at its highest and chl-*a* started to decrease after the spring bloom. Whale shark sightings tended to be associated to an overall SST range between 22 and 24ºC and in areas of enhanced chl-*a* biomass. Spatially, whale sharks were found to be closely associated with seamounts and in areas with large bathymetry slopes (Afonso et al., 2014). In addition, the location of the 22ºC isotherm helped predict the presence of whale sharks in Azores. Previous to 2008, the average position of the 22ºC isotherm during the SST peak season (July-September) was south of the islands following an east-west orientation (Afonso et al., 2014). In 2008, when whale shark sightings dramatically increase, the isotherm was found much further north (past Santa Maria), followed by a return to a more southerly position during 2009 and 2010. Whale shark sightings have generally followed this pattern (Afonso et al., 2014).

Therefore, based on the unique dataset of Afonso et al. (2014), which spanned 16

years (1998-2013), it was demonstrated that changes in the mean annual summer SST is correlated with the abundance of whale sharks in the Azores, which represents the thermal boundary for this species. Thus it is possible that the increase in whale shark sightings in the Azorean waters in recent years may be related to the large-scale climate changes, while the reasons for the spatial variability within the region remain unknown (Afonso et al., 2014).

However, we should also take into consideration that a higher presence of whale shark sightings around the island of Santa Maria during the summer months could also be influenced by a much higher general sampling effort during this period. It is important to note that most photographs used for the study were obtained from the ecotourism industry, and professional underwater photographers. Santa Maria is a well-known hotspot of marine life, accommodating several ecotourism industries (diving companies, island tours, dolphin tours, sailing etc.). Both, ecotourism industry and underwater photographers are more active during the summer months, when climatic and water conditions are more favorable, thus, providing a higher record of whale shark encounters and photographs during this period of the year. Therefore, it is important to acknowledge that our data set is not ideal to characterize spatial and temporal abundance as the sampling effort distribution is quite dependent on multiples factors including but not limited to human behavior, availability of infrastructure or availability of researchers and ecotourism industries.

Other oceanic migratory elasmobranchs have been observed to present similar seasonal patterns in the Azores archipelago (Filipa Sobral, 2013). *Mobula tarapacana* is a species of large migratory mobulid that aggregates in the waters of Azores. The spatial ecology and demography of this species is poorly understood, especially in areas as the Azores which constitute their fringe oceanic habitat (Filipa Sobral, 2013). The study carried out by Filipa Sobral (2013) observed that *M. tarapacana* was more abundant between June and October and had a preference for shallow bank habitats (seamounts), as they are thought to increase the vertical flux of nutrients and to retain water masses promoting a higher local productivity (Morato et al., 2008; White et al., 2008), as well as having distinctive magnetic signatures that might be used as specific cues for migratory species (Garrigue et al., 2015). Although a higher presence of mobulids in the seamounts of Azores was linked to summer months, when SST was higher, it could not be concluded that SST was the only driver influencing abundance of *M. tarapacana* in the Azores region (Filipa Sobral, 2013).

Residency and connectivity

Individual identification allowed to investigate the residency of whale sharks in the Azores both within a season and across years. While we found that some individuals remained in the Azores for at least a few weeks within a season, interannual resighting indicate that some animals returned in successive years. A total of 29 individuals (16%) were resighted in the study area multiple times, mostly within a season. One individual was resighted on six occasions (AZ-006), within an interval of 11 days from first to last sighting. Of these 29 resighted individuals, 28 were resighted in different occasions within the same season, and four individuals were resighted in consecutive years, suggesting they returned to Azores in the following year after their first sightings (2019-2020), except for individual AZ-003 which was re-sighted three years after the first sighting (2008-2011). In addition, one individual (AZ-013) was sighted first in Santa Maria and later in Pico in 2020 (Table 6).

These results are the first evidence to confirm that at least some animals return to the Azores region with annual periodicity. Also, although the area around Santa Maria Island is the hotspot for whale shark aggregation in the Azores, individual whale sharks were observed to disperse from this hotspot to other areas within the region, consistent with the preliminary satellite tracking data (J. Fontes, pers. comm.). Although the motivation for this regional movement is unclear, the exceptional occurrence of a small aggregation in south shore of Pico Island, was coincident with the decrease in tuna and bait fish abundance in Santa Maria and the increase in tuna and bait fish in the Pico Island area (J. Fontes, pers. comm.), suggesting that some individuals may respond to changes in tuna and bait fish distribution. Fontes et al. 2020, investigated the association between whale sharks, tunas and the local tuna fisheries and found that tuna distribution patterns across the Azores differed between years where whale sharks were present and periods when whale sharks were not reported. While it's unclear if tuna and whale sharks distribution follow the same environmental cues or if they actively associate for mutual benefit, we often find the whale sharks in the Azores closely associated with tunas and whale sharks have frequently been observed feeding simultaneously with tuna on bait fish and swimming with hundreds to thousands of tunas (J. Fontes, pers. comm.), suggesting that whale shark tuna associations may be mutually beneficial (Fontes et al, 2020).

In addition, the different re-sightings within the short study period suggests that the

population may consist of a few number of site-faithful individuals along with a large number transient individuals that might be harder to detect with low sampling effort and large and variable space use patterns, making hard to detect them. This hypothesis may be tested as the data set continuous to grow and sampling effort increases by the addition of more years of photo-identification data. Our data shows that inter-annual site fidelity in Azores Islands (2.1%) is lower than most other populations, such as the population at the Seychelles (15 to 23%), Bahía de La Paz (16 to 35%), Bahía de los Ángeles (2.5 to 50%), Holbox Island (11 to 27%), St. Helena (12%), but higher than the Galápagos (1.2%) (Ramírez-Macías et al., 2012b; Acuña-Marrero et al., 2014). This low percentage of inter-annual re-sightings in Azores can either result from significantly lower sampling effort and short season in the Azores, where whale shark tours are opportunistic, compared to the most popular tropical whale shark aggregations, or may also result from the wither offshore oceanic habitat use patterns in the Azores, making sightings comparatively more unlikely and unpredictable. The third possibility is that multiple strategies coexist within the population, and while some sharks return in consecutive years, others may adopt a longer returning cycle. It has been hypothesized that some individuals from different populations move following a clockwise pattern in their respective ocean basins, a movement that can take months or in some cases, up to 2 years to complete (Sequeira et al., 2013). In the Western Atlantic Ocean, most whale shark sightings occur in Belize, Honduras, Mexico and the United States, whereas in the Eastern Atlantic Ocean they are mainly sighted off the coast of Gabon, Senegal, and Cape Verde (Sequeira et al., 2014; Mckinney et al., 2017). It has been hypothesized that interannual variation in sightings at the ocean-basin scale could be linked to such cyclic pattern (Sequeira et al., 2014). In the case of the Azores region, *R. typus* individuals might only travel there when the Gulf Stream is strong, with the warmer-than-usual waters near the Azores encouraging more northerly forays (Sa, 2008). Therefore, it is hypothesized that individuals travelling to the Azores might originate from the Gulf of Mexico. However, as waters closer to the Saint Peter and Saint Paul archipelago and West Africa tend to be warmer, movements towards these regions are more typical (Sequeira, et al., 2014).

Although a high level of transience was overall observed, it was estimated that whale sharks appeared to spent a mean of 6.1 days before departing the site, with a maximum residence time observed of 20 days (Table 6). This estimate is higher than that observed in

the Galápagos Islands (2.09 days, Acuña-Marrero et al., 2014), yet lower than that estimated at other parts of the Atlantic, such as in St. Helena (19 days, Perry et al., 2020), or in coastal sites of the Indian Ocean, such as Tanzania (31 days, Prebble et al., 2018), or the Ningaloo Reef (33 days, Holmberg et al., 2009). In the Western Atlantic Ocean, whale shark individuals were observed repeatedly in the same area for several days to months, whereas in other cases, more than 16 years passed between re-sightings. Furthermore, despite the high level of transience observed in the Azores archipelago, no matches with populations from other aggregations sites of the world were obtained, which raises the question of where are these whale sharks coming from, and where are they travelling to. However, having both site-faithful and transient individuals poses challenges for management and for this reason, it is essential that migration pathways of whale sharks are established (Meekan et al., 2006; Rowat et al., 2009).

Number of re-sightings has been found to be negatively correlated with population size, where aggregations with larger population sizes present lower yearly resighting rates (Andrzejaczek et al., 2016). Whale shark individuals from populations of the Indian Ocean experienced a rate of migration lower than 5% between the different localities (Andrzejaczek et al., 2016). However, it is important to note that differences in sampling effort can pose a significant influence in the variation of resighting rates. For this reason, describing the sampling effort is fundamental, in order to be able to make comparisons with other studies and sites. In the Western Central Atlantic, newly-identified whale sharks were encountered each year of the study of McKinney et al. (2017), without exhibiting an asymptote. However, percentage of resighted individuals remained constant from 2011-2015 at 75.0% (McKinney et al., 2017). Whale sharks observed during the same year among different regions of the Mesoamerican Reef presented a lag time of 26 ± 4.7 days between Belize and Honduras, and 4.4 ± 0.3 months between Belize/Honduras and Mexico (McKinney et al., 2017).

Socio-economic value

Coastal communities near aggregation sites have the potential to provide incentive-based conservation for this species, while benefiting immensely (socially and economically) with the presence of this species through the ecotourism practices (Cisneros-Montemayor et al., 2013; Bentz et al., 2014; Anna & Saputra, 2017). While the aggregation of whale sharks in Azorean waters can provide additional interest and income on the marine tourism industry

of the region, it can also have negative impacts due to its intermittency and unpredictability (J. Fontes, pers. comm.). Information on the economic value of whale shark tourism is very important for management purposes, and value of whale sharks had been quantified individual, locally and even nationally in whale shark aggregations sites (Catlin & Jones, 2010; Cagua et al., 2014; Anna & Saputra, 2017). Different studies concluded that shark ecotourism can generate more than $314 million annually worldwide, with an input of whale shark tourism ranging $1.8 – $13.3 million dollars annually (Cisneros-Montemayor et al., 2013; Cagua et al., 2014). This economic profit contrasted with the price for which a whale shark can be sold in the market (*ca.* $20,000-$30,000) (Chen and Phipps, 2002; Lee, 2014), evidences the need to protect this species from direct and accidental fisheries and work towards more sustainable practices

However, community-based ecotourism do not always promote biodiversity conservation and economic and sociocultural sustainability for the host communities (Neves, 2004; Silva, 2015). Furthermore, to ensure the conservation of the whale shark and habitats involved, as well as the long-term sustainability of the whale shark tourism industry, several steps should be taken into consideration in the management of marine wildlife tourism: 1) Increase of research on the biology and ecology of whale sharks; 2) Structured enforcement of existing policies and enhancement of ecological awareness of visitors through active education; 3) Application of a management framework to continuously improve the codes of conduct employed; 4) Involvement of different stakeholders and local communities in the development and improvement of the marine wildlife tourism activity (Trave et al., 2017).

Limitations and future research

Our approach, while providing valuable and novel insight, has some limitations. First, although size of individuals was qualitatively described, it could not be quantitatively determined, thus excluding an important parameter for population characterization. Although the aggregation of whale sharks in Azores is known since 2008, a larger quantity of encounter data has been available in the recent years, and therefore, several procedures still need to be set. Thus, ecotourism industries and professional underwater photographers are still not trained to provide estimates of total size of the individuals, although they all agree that individuals are at least 8 m. However, new methods are now being implemented, such as

laser photogrammetry as well stereo video photogrammetry to measure the body size of whale sharks in the Azores. Last, but not least, although photographic data (images and videos) were obtained from a large number of collaborators, more robust results could be obtain with a larger quantity of photographic data. Therefore, this first characterization of the whale shark population in Azores, and first population size estimation should be revised in the future, with the addition of new encounter data.

Conclusions

Images and videos from whale sharks visiting the Azores Islands were collected for years 2008-2020, allowing to uniquely identify 186 individuals using a photo-identification approach. Scars and wounds were recorded and categorized according to the type, severity of the injure and source, and in some cases, it helped to properly identify the individuals. A higher abundance of whale sharks during the months of August and September and around the island of Santa Maria was observed. Of the total individuals identified, four individuals showed site fidelity, returning to Azores on the following years. First estimates of population abundance using capture-mark-recapture models concluded that the population of whale sharks utilizing the Azores archipelago might consist of approximately 283 – 496 individuals, mostly dominated by female individuals, indicating that the Azores whale shark population structure is quite unique in the context of the known aggregations in the Atlantic.

In conclusion, photo-identification can play a useful role in answering some of the questions of the population of female whale shark that aggregate in Azores. However, photo-identification cannot be used alone and other monitoring methods, such as satellite tagging, or genetic studies should be used simultaneously. The effective conservation and management of this endangered species depends on understanding its population status as well as its migratory and movement patterns. The results shown here points out the significance that the Azores archipelago has on whale sharks and the urge of concentrated research efforts, as well as a properly implementation of regional, national and international protection strategies in order to ensure the long-term conservation of the whale shark, an ecologically and economically important species in risk of extinction.

Acknowledgements

Throughout the writing of this Master Thesis, I have received a great deal of support and assistance, which I am very thankful for.

I would first like to thank my supervisor Dr. Jorge Fontes and my co-supervisor Dr. Karim Erzini for their support, help and guidance throughout the thesis process, and whose expertise was invaluable in formulating the research questions and methodology to follow. I also want to thank Dr. Bruno Macena for all the hours he spent helping me, giving advice, and sharing all his knowledge, and for teaching me and learning together how to work with capture-mark-recapture models in R. I would also like to acknowledge Jason Holmberg and Simon J. Pierce for introducing me to Wildbook for Whale Sharks and for your immediate help when problems with the software arose. I am profoundly grateful to Sofia Green, for being there since the beginning of the thesis, for your continuous help and suggestions to improve my work, and for sharing with me all your knowledge, passion and love for whale sharks. I hope to never stop learning from you.

Also, I would like to thank my parents and my brother for believing in me all this time, for being there during the last tough months, and for supporting and encouraging me to chase my dreams.

To my friends and my second family in Faro, who we stayed united and motivated together during times of struggle. Thanks for your incredible support and for all the amazing moments we lived together during this Master.

Last but not least, I would like to thank all the collaborators that participated in the collection of whale shark photography in Azores. My research work would have not been possible without your collaboration. Therefore I want to thank Alba Iglesias, Ana Besugo, BestSpot Azores, Bruno Macena, CW Azores, Daniel Machado, Diana Serrano, Eurico Roseiro, Fadia AlAbar, Filipe Lopes da Silva, Francisco Pires, Gonçalo Graça, Henrique Ramos, Jonas Karlsson, Jordi Chias, Jorge Botelho, Jorge Fontes, Leire Lopetegui, Luís Quinta, Mantamaria, Manuel Anselmo Assunção, Martijn Schouten, Nuno Sa, Nuno Vasco, Pedro Afonso, StudioFreek, Tiago Castro, and Wahoo Diving for sharing with me your whale shark images, videos and encounter data.

References

Acuña-Marrero, D., Jiménez, J., Smith, F., Jr, P. F. D., Hearn, A., Green, J. R., Paredes-Jarrín, J., & Salinas-de-León, P. (2014). Whale shark (*Rhincodon typus*) seasonal presence, residence time and habitat use at Darwin Island, Galapagos Marine Reserve. *PLOS ONE*, *9*(12), e115946.

Afonso, P., McGinty, N., & Machete, M. (2014). Dynamics of whale shark occurrence at their fringe oceanic habitat. *PLOS ONE*, *9*(7), e102060.

Akaike, H. (1973). Information theory as an extension of the maximum likelihood principle. In: Petrov, B. and Csaki, F (eds). Proceedings of the Second International Symposium on Information Theory. Budapest, Hungary, pp. 276-281.

Alves, M. L., & Colin de Verdière, A. (1999). Instability dynamics of a subtropical jet and applications to the Azores Front Current System: eddy-driven mean flow. *Journal of Physical Oceanography*, *29*(5), 837-864.

Amorim, P., Perán, A. D., Pham, C. K., Juliano, M., Cardigos, F., Tempera, F., & Morato, T. (2017). Overview of the ocean climatology and its variability in the Azores region of the North Atlantic including environmental characteristics at the seabed. *Frontiers in Marine Science*, *4*, 56.

Andrzejaczek, S., Meeuwig, J., Rowat, D., Pierce, S., Davies, T., Fisher, R., & Meekan, M. (2016). The ecological connectivity of whale shark aggregations in the Indian Ocean: A photo-identification approach. *Royal Society Open Science*, *3*(11), 160455.

Anna, Z., & Saputra, D. S. (2017). Economic valuation of whale shark tourism in Cenderawasih Bay National Park, Papua, Indonesia. *Biodiversitas Journal of Biological Diversity*, *18*(3), 1026-1034.

Arzoumanian, Z., Holmberg, J., & Norman, B. (2005). An astronomical pattern-matching algorithm for computer-aided identification of whale sharks *Rhincodon typus*. *Journal of Applied Ecology*, *42*(6), 999-1011.

Bashmachnikov, I., Machín, F., Mendonça, A., & Martins, A. (2009). In situ and remote sensing signature of meddies east of the mid-Atlantic ridge. *Journal of Geophysical Research: Oceans*, *114*(C5).

Bentz, J., Dearden, P., Ritter, E., & Calado, H. (2014). Shark diving in the Azores: Challenge and opportunity. *Tourism in Marine Environments*, *10*(1-2), 71-83.

Berg, A., Meyer, R., & Yu, J. (2002). *Deviance information criterion as a model comparison criterion for stochastic volatility models*. (No. 178). Department of Economics, The University of Auckland. Available at https://ideas.repec.org/p/auc/wpaper/178.html (Accessed 20 July 2021).

Block, B. A., Jonsen, I. D., Jorgensen, S. J., Winship, A. J., Shaffer, S. A., Bograd, S. J., Hazen, E. L., Foley, D. G., Breed, G. A., Harrison, A.-L., Ganong, J. E., Swithenbank, A., Castleton, M., Dewar, H., Mate, B. R., Shillinger, G. L., Schaefer, K. M., Benson, S. R., Weise, M. J., … Costa, D. P. (2011). Tracking apex marine predator movements in a dynamic ocean. *Nature*, *475*(7354), 86-90.

Brooks, K., Rowat, D., Pierce, S., Jouannet, D., & Vely, M. (2010). Seeing Spots: Photo-identification as a regional tool for whale shark identification. *Western Indian Ocean Journal of Marine Science*, *9*(2), 185-194.

Cagua, E. F., Collins, N., Hancock, J., & Rees, R. (2014). Whale shark economics: A valuation of wildlife tourism in South Ari Atoll, Maldives. *PeerJ*, *2*, e515.

Catlin, J., & Jones, R. (2010). Whale shark tourism at Ningaloo Marine Park: A longitudinal study of wildlife tourism. *Tourism Management*, *31*(3), 386-394.

Caughley, G., & Gunn, A. (1996). *Conservation biology in theory and practice*. Cambridge, MA: Blackwell Science, 459p.

Chen, V. Y., and M. J. Phipps. 2002. Management and Trade of Whale Sharks in Taiwan. Taipei: TRAFFIC-East Asia Report.

Cisneros-Montemayor, A. M., Barnes-Mauthe, M., Al-Abdulrazzak, D., Navarro-Holm, E., & Sumaila, U. R. (2013). Global economic value of shark ecotourism: Implications for conservation. *Oryx*, *47*(3), 381-388.

Cochran, J. E. M., Braun, C. D., Cagua, E. F., Jr, M. F. C., Hardenstine, R. S., Kattan, A., Priest, M. A., Sinclair-Taylor, T. H., Skomal, G. B., Sultan, S., Sun, L., Thorrold, S. R., & Berumen, M. L. (2019). Multi-method assessment of whale shark (*Rhincodon typus*) residency, distribution, and dispersal behavior at an aggregation site in the Red Sea. *PLOS ONE*, *14*(9), e0222285.

Colman, J. (1997). A review of the biology and ecology of the whale shark. *Journal of Fish Biology*, *51*(6), 1219-1234.

Cormack, R. M. (1964). Estimates of survival from the sighting of marked animals. *Biometrika*, *51*(3/4), 429-438.

Ebert, D. A., Fowler, S. L., & Compagno, L. J. V. (2013). *Sharks of the world: A fully illustrated guide*. Plymouth: Wild Nature Press.

Eckert, S., Dolar, L., Kooyman, G., Perrin, W., & Abdul Rahman, R. (2002). Movements of whale sharks (*Rhincodon typus*) in South-east Asian waters as determined by satellite telemetry. *Journal of Zoology*, *257*(1), 111-115.

Eckert, S., & Stewart, B. (2001). Telemetry and satellite tracking of whale sharks, *Rhincodon typus*, in the Sea of Cortez, Mexico, and the north Pacific Ocean. *Environmental Biology of Fishes*, *60*, 299-308.

Filipa Sobral, A. (2013). *Biology, ecology and conservation of Mobulid rays in the Azores* (Master Thesis, Universidade dos Açores).

Fontes, J., McGinty, N., Machete, M., & Afonso, P. (2020). Whale shark-tuna associations, insights from a small pole-and-line fishery from the mid-north Atlantic. *Fisheries Research*, *229*, 105598.

Fox, S., Foisy, I., Venegas, R. D. L. P., Pastoriza, B. E. G., Graham, R. T., Hoffmayer, E. R., Holmberg, J., & Pierce, S. J. (2013). Population structure and residency of whale sharks *Rhincodon typus* at Utila, Bay Islands, Honduras. *Journal of Fish Biology*, *83*(3), 574-587.

Fromentin, J.M., & Powers, J. E. (2005). Atlantic bluefin tuna: Population dynamics, ecology, fisheries and management. *Fish and Fisheries*, *6*(4), 281-306.

Gallagher, A. J., & Hammerschlag, N. (2011). Global shark currency: The distribution, frequency, and economic value of shark ecotourism. *Current Issues in Tourism*, *14*(8), 797-812.

Garrigue, C., Clapham, P. J., Geyer, Y., Kennedy, A. S., & Zerbini, A. N. (2015). Satellite tracking reveals novel migratory patterns and the importance of seamounts for endangered South Pacific humpback whales. *Royal Society Open Science*, *2*(11), 150489.

Graham, R., & Roberts, C. (2007). Assessing the size, growth rate and structure of a seasonal population of whale sharks (*Rhincodon typus* Smith 1828) using conventional tagging and photo identification. *Fisheries Research*, *84*(1), 71-80.

Gunn, J. S., Stevens, J. D., Davis, T. L. O., & Norman, B. M. (1999). Observations on the short-term movements and behaviour of whale sharks (*Rhincodon typus*) at Ningaloo Reef, Western Australia. *Marine Biology*, *135*(3), 553-559.

Heithaus, M. R. (2001). The biology of tiger sharks, *Galeocerdo Cuvier*, in Shark Bay, Western Australia: Sex ratio, size distribution, diet, and seasonal changes in catch rates. *Environmental Biology of Fishes*, *61*(1), 25-36.

Heyman, W., Graham, R., Kjerfve, B., & Johannes, R. E. (2001). Whale sharks *Rhincodon typus* aggregate to feed on fish spawn in Belize. *Marine Ecology-Progress Series*, *215*, 275-282.

Holmberg, J., Norman, B., & Arzoumanian, Z. (2009). Estimating population size, structure, and residency time for whale sharks *Rhincodon typus* through collaborative photo-identification. *Endangered Species Research*, *7*(1), 39-53.

Iwasaki, Y. (1970). On the distribution and environment of the whale shark, *Rhincodon typus*, in skipjack fishing grounds in the Western Pacific. *Journal of the College of Marine Science and Technology, Tokai University*, *4*, 37-51.

Johnson, J., & Stevens, I. (2000). A fine resolution model of the eastern North Atlantic between the Azores, the Canary Islands and the Gibraltar Strait. *Deep Sea Research Part I: Oceanographic Research Papers*, *47*(5), 875-899.

Jolly, G. M. (1965). Explicit estimates from capture-recapture data with both death and immigration-stochastic model. *Biometrika*, *52*(1/2), 225-247.

Jones, T., Wood, D., Catlin, J., & Norman, B. (2009). Expenditure and ecotourism: Predictors of expenditure for whale shark tour participants. *Journal of Ecotourism*, *8*(1), 32-50.

Joung, S.-J., & Chen, C.-T. (1995). Reproduction in the sandbar shark, *Carcharhinus plumbeus*, in the waters off northeastern Taiwan. *Copeia*, *1995*(3), 659-665.

Ketchum, J., Galván-Magaña, F., & Klimley, A. (2013). Segregation and foraging ecology of whale sharks, *Rhincodon typus*, in the southwestern Gulf of California. *Environmental Biology of Fishes*, *96*(6), 779-795.

Klimley, A. P. (1987). The determinants of sexual segregation in the scalloped hammerhead shark, *Sphyrna lewini*. *Environmental Biology of Fishes*, *18*(1), 27-40.

Kock, A., O'Riain, M. J., Mauff, K., Meÿer, M., Kotze, D., & Griffiths, C. (2013). Residency, habitat use and sexual segregation of white sharks, *Carcharodon carcharias* in False Bay, South Africa. *PLOS ONE*, *8*(1), e55048.

Laake J (2013). "RMark: An R interface for analysis of capture-recapture data with MARK." AFSC Processed Rep. 2013-01, Alaska Fish. Sci. Cent., NOAA, Natl. Mar. Fish. Serv., Seattle, WA.

Lafon, V. M., Martins, A. M., Bashmachnikov, I. L., Jose, F., Melo-Rodrigues, M., Figueiredo, M. P., Mendoca, M., & Macedo, L. M. (2004, November). SST variability in the Azores region using AVHRR imagery: regional to local scale study. In *Remote Sensing of the Ocean and Sea Ice 2004* (Vol. 5569, pp. 130-139). International Society for Optics and Photonics.

Lee, J. L. 2014. Slaughterhouse said to process "horrifying" number of whale sharks annually. National Geographic, January 30, 2014. Available at https://www.nationalgeographic.com/animals/article/140129-whale-shark-endangered-cites-ocean-animals-conservation (Accessed 12 September 2021).

Link, W. A., & Barker, R. J. (2010). Chapter 11: Open population models. In W. A. Link & R. J. Barker (Ed.). *Bayesian Inference* (p. 239-270). Academic Press.

Macena, B. C., & Hazin, F. H. (2016). Whale shark (Rhincodon typus) seasonal occurrence, abundance and demographic structure in the mid-equatorial Atlantic Ocean. *PLoS One*, *11*(10), e0164440.

Martins, A. M., Amorim, A. S. B., Figueiredo, M. P., Souza, R. J., Mendonça, A. P., Bashmachnikov, I. L., & Carvalho, D. S. (2007, October). Sea surface temperature (AVHRR, MODIS) and ocean colour (MODIS) seasonal and interannual variability in the Macaronesian islands of Azores, Madeira, and Canaries. In *Remote Sensing of the Ocean, Sea Ice, and Large Water Regions 2007* (Vol. 6743, p. 67430A). International Society for Optics and Photonics.

Martins, A., Mendonça, A., Figueiredo, M., & Baschmachnikov, I. (2009, April). Historical Ocean Colour and SST inter-annual variability for the Azores region from 2002 to 2008-comparison with in situ data. In *EGU General Assembly Conference Abstracts* (p. 12519).

McCoy, E., Burce, R., David, D., Aca, E. Q., Hardy, J., Labaja, J., Snow, S. J., Ponzo, A., & Araujo, G. (2018). Long-term photo-identification reveals the population dynamics and strong site fidelity of adult whale sharks to the coastal waters of Donsol, Philippines. *Frontiers in Marine Science*, *5*, 271.

McKinney, J. A., Hoffmayer, E. R., Holmberg, J., Graham, R. T., Driggers, W. B., de la Parra-Venegas, R., Galván-Pastoriza, B. E., Fox, S., Pierce, S. J., & Dove, A. D. M. (2017). Long-term assessment of whale shark population demography and connectivity using photo-identification in the Western Atlantic Ocean. *PLoS ONE*, *12*(8).

Meekan, M., Bradshaw, C., Press, M., McLean, C., Richards, A., Quasnichka, S., & Taylor, G. (2006). Population size and structure of whale sharks (*Rhincodon typus*) at Ningaloo Reef, Western Australia. *Marine Ecology Progress Series*, *319*, 275-285.

Morato, T., Varkey, D., Damaso, C., Machete, M., Santos, M., Prieto, R., Santos, R., & Pitcher, T. (2008). Evidence of a seamount effect on aggregating visitors. *Marine Ecology Progress Series*, *357*, 23-32.

Nelson, J., & Eckert, S. (2007). Foraging ecology of whale sharks (*Rhincodon typus*) within Bahía de Los Angeles, Baja California Norte, México. *Fisheries Research*, *84*(1), 47-64.

Neves, K. (2004). Revisiting the tragedy of the commons: Ecological dilemmas of whale watching in the Azores. *Human Organization*, *63*(3), 289-300.

Norman, B. M., Holmberg, J. A., Arzoumanian, Z., Reynolds, S. D., Wilson, R. P., Rob, D., Pierce, S. J., Gleiss, A. C., de la Parra, R., Galvan, B., Ramirez-Macias, D., Robinson,

D., Fox, S., Graham, R., Rowat, D., Potenski, M., Levine, M., Mckinney, J. A., Hoffmayer, E., … Morgan, D. L. (2017). Undersea constellations: The global biology of an endangered marine megavertebrate further informed through citizen science. *BioScience*, *67*(12), 1029-1043.

Norman, B. M., & Stevens, J. D. (2007). Size and maturity status of the whale shark (*Rhincodon typus*) at Ningaloo Reef in Western Australia. *Fisheries Research*, *84*(1), 81-86.

Perry, C. T., Clingham, E., Webb, D. H., de la Parra, R., Pierce, S. J., Beard, A., Henry, L., Taylor, B., Andrews, K., Hobbs, R., Araujo, G., & Dove, A. D. M. (2020). St. Helena: An important reproductive habitat for whale sharks (*Rhincodon typus*) in the Central South Atlantic. *Frontiers in Marine Science*, *7*, 899.

Pierce, S. P., & Norman, B. (2016). *Rhincodon typus*. *The IUCN Red List of Threatened Species 2016: e.T19488A2365291*. Available at https://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T19488A2365291.en. Downloaded (Accessed 24 December 2020).

Pratt, H. (1979). Reproduction in the blue shark, *Prionace glauca*. *Fishery Bulletin*, *77*(2), 445-470.

Pravin, P. (2000). Whale shark in the Indian coast – Need for conservation. *Current Science*, *79*(3), 310-315.

Prebble, C., Rohner, C., Pierce, S., Robinson, D., Jaidah, M., Bach, S., & Trueman, C. (2018). Limited latitudinal ranging of juvenile whale sharks in the Western Indian Ocean suggests the existence of regional management units. *Marine Ecology Progress Series*, *601*, 167-183.

Ramírez-Macías, D., Meekan, M., de la Parra, R., Remolina-Suárez, F., Trigo-Mendoza, M., & Vázques-Juárez, R. (2012). Patterns in composition, abundance and scarring of whale sharks Rhincodon typus near Holbox Island, Mexico. *Journal of fish biology*, *80*(5), 1401-1416.

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ramírez-Macías, D., Vázquez-Haikin, A., & Vázques-Juárez, R. (2012b). Whale shark *Rhincodon typus* populations along the west coast of the Gulf of California and implications for management. *Endangered Species Research*, *18*(2), 115-128.

Ribarič, D. (2018). First report on abundance and distribution of common bottlenose dolphins (*Tursiops truncatus*) in the NATURA 2000 area, Istria, North-eastern Adriatic Sea. *Journal of the Marine Biological Association of the United Kingdom*, *98*(5), 1039-1053.

Richardson, A. J., & Poloczanska, E. S. (2008). Under-resourced, under threat. *Science*, *320*(5881), 1294-1295.

Riley, M., Hale, M., Harman, A., & Rees, R. (2010). Analysis of whale shark *Rhincodon typus* aggregations near South Ari Atoll, Maldives Archipelago. *Aquatic Biology*, *8*(2), 145-150.

Robbins, R. L. (2007). Environmental variables affecting the sexual segregation of great white sharks *Carcharodon carcharias* at the Neptune Islands South Australia. *Journal of Fish Biology*, *70*(5), 1350-1364.

Rowat, D., & Brooks, K. (2012). A review of the biology, fisheries and conservation of the whale shark *Rhincodon typus*. *Journal of Fish Biology*, *80*(5), 1019-1056.

Rowat, D., Brooks, K., March, A., McCarten, C., Jouannet, D., Riley, L., Jeffreys, G., Perri, M., Vely, M., & Pardigon, B. (2011). Long-term membership of whale sharks (*Rhincodon typus*) in coastal aggregations in Seychelles and Djibouti. *Marine and Freshwater Research*, *62*(6), 621.

Rowat, D., & Gore, M. (2007). Regional scale horizontal and local scale vertical movements of whale sharks in the Indian Ocean off Seychelles. *Fisheries Research, 84*(1), 32-40.

Rowat, D., Meekan, M., Engelhardt, U., Pardigon, B., & Vely, M. (2007). Aggregations of juvenile whale sharks (*Rhincodon typus*) in the Gulf of Tadjoura, Djibouti. *Environmental Biology of Fishes*, *80*(4), 465-472.

Rowat, D., Speed, C. W., Meekan, M. G., Gore, M. A., & Bradshaw, C. J. A. (2009). Population abundance and apparent survival of the Vulnerable whale shark *Rhincodon typus* in the Seychelles aggregation. *Oryx*, *43*(4), 591-598.

Sa, N. (2008). Gigantes na ilha de Santa Maria. *National Geographic*. Available at http://www.nationalgeographic.pt/sumario.jsp?id=1744887/ (Accessed 13 October 2021).

Santos, R., Hawkins, S., Monteiro, L., Alves, M., & Isidro, E. (1995). Marine research, resources and conservation in the Azores. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *5*(4), 311-354.

Santos, M., Moita, M. T., Bashmachnikov, I., Menezes, G. M., Carmo, V., Loureiro, C. M., Mendonça, A., Silva, A. F., & Martins, A. (2013). Phytoplankton variability and oceanographic conditions at Condor seamount, Azores (NE Atlantic). *Deep Sea Research Part II: Topical Studies in Oceanography*, *98*, 52-62.

Seber, G. A. F. (1982). The estimation of animal abundance and related parameters.

Schwarz, C. J., Arnason, A. N. (1966). A general methodology for the analysis of capture-recapture experiments in open populations. *Biometrics, 52*, 860-873

Sequeira, A. M., Mellin, C., Floch, L., Williams, P. G., & Bradshaw, C. J. (2014). Inter-ocean asynchrony in whale shark occurrence patterns. *Journal of experimental marine biology and ecology*, *450*, 21-29.

Sequeira, A., Mellin, C., Fordham, D. A., Meekan, M. G., & Bradshaw, C. J. A. (2014b). Predicting current and future global distributions of whale sharks. *Global Change Biology*, *20*(3), 778-789.

Sequeira, A. M., Mellin, C., Meekan, M. G., Sims, D. W., & Bradshaw, C. J. (2013). Inferred global connectivity of whale shark Rhincodon typus populations. *Journal of Fish Biology*, *82*(2), 367-389

Sequeira, A., Thums, M., Brooks, K., & Meekan, M. G. (2016). Error and bias in size estimates of whale sharks: Implications for understanding demography. *Royal Society Open Science*, *3*(3), 150668.

Silva, L. (2015). How ecotourism works at the community-level: The case of whale-watching in the Azores. *Current Issues in Tourism*, *18*(3), 196-211.

Silva, L., & Smith, C. W. (2004). A characterization of the non-indigenous flora of the Azores Archipelago. *Biological Invasions*, *6*(2), 193-204.

Sims, D., Nash, J., & Morritt, D. (2001). Movements and activity of male and female dogfish in a tidal sea lough: Alternative behavioural strategies and apparent sexual segregation. *Marine Biology*, *139*(6), 1165-1175.

Speed, C., Meekan, M., Rowat, D., Pierce, S., Marshall, A., & Bradshaw, C. (2008). Scarring patterns and relative mortality rates of Indian Ocean whale sharks. *Journal of Fish Biology*, *72*(6), 1488-1503.

Springer S (1967) Social organization of shark populations. In P. W. Gilbert, R. W. Mathewson & D. P. Rall (Eds.). *Sharks, skates and rays* (pp. 149-174). Baltimore, MD. John Hopkins Press.

Stanley, T.R., & Richards, J. D. 2011. CloseTest. USGS Fort Collins Science Center.

Taylor, G. (1994). *Whale sharks: The giants of Ningaloo Reef* (1st ed.) Angus & Robertson, Sydney, 176p.

Trave, C., Brunnschweiler, J., Sheaves, M., Diedrich, A., & Barnett, A. (2017). Are we killing them with kindness? Evaluation of sustainable marine wildlife tourism. *Biological Conservation*, *209*, 211-222.

Vandeperre, F., Aires-da-Silva, A., Fontes, J., Santos, M., Santos, R. S., & Afonso, P. (2014). Movements of blue sharks (*Prionace glauca*) across their life history. *PLOS ONE*, *9*(8), e103538.

Van Tienhoven, A., Hartog, J., Reijns, R., & Peddemors, V. (2007). A computer-aided program for pattern-matching of natural marks on the spotted raggedtooth shark *Carcharias taurus*. *Journal of Applied Ecology*, *44*(2), 273-280.

Wearmouth, V. J., & Sims, D. W. (2008). Sexual segregation in marine fish, reptiles, birds and mammals behaviour patterns, mechanisms and conservation implications. *Advances in Marine Biology*, *54*, 107-170.

White, M., Bashmachnikov, I., Aristegui, J., & Martins, A. (2008). Physical processes and seamount productivity. In *Seamounts: Ecology, Fisheries and Conservation* (p. 62-84).

White, G. C., Burnham, K. P. (1999). Program MARK: survival estimation from populations of marked animals. *Bird Study*, *46*, S120-S139.

Wilson, S. (2004). Basking sharks (*Cetorhinus maximus*) schooling in the southern Gulf of Maine. *Fisheries Oceanography*, *13*(4), 283-286.