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Mestrado em Biologia Marinha

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Declaração de autoria de trabalho

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

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Title

Assessment of source populations for stranded adult loggerhead turtles (*Caretta caretta*) in the north-east Atlantic

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Abstract

a. English

Maritime activities impact the natural migratory routes of marine organisms such as marine mammals and turtles. The coastal waters of Portugal are in the pathway of such routes for loggerhead turtles (*Caretta caretta*). While the local upwelling provides foraging grounds, there are no nesting beaches adjacent to the Iberian coast, offering an excellent opportunity to study the migration of the species. This study is the first report of the natal origins of loggerheads in the North-East Atlantic Ocean. We attempted to investigate the genetic diversity of 194 loggerhead turtles found stranded across the Portuguese coast and to assess the origin of individuals through many-to-one mixed-stock analysis. We successfully produced 16 haplotypes of 776 bp of mitochondrial DNA control region for 127 samples and detected a previously unknown haplotype. Comparison with baseline data from distinct nesting populations of the Mediterranean Sea, Cape Verde Islands and the North-West Atlantic Ocean revealed that most of the loggerheads originated in the North-West Atlantic and to a smaller degree in Cape Verde and the Mediterranean. In particular, the Gulf of Mexico in the North-West Atlantic yielded the largest part of the samples and specifically the Florida rookeries: Canaveral National Seashore, Melbourne Beach, Juno beach and Ft Lauderdale. Furthermore, this study highlights the important role of currents in the long journeys of turtles, since most of the contributing rookeries were along such currents. In conclusion, our results signify the need for conservation measurements like turtle excluder devices, tag tracking and fishing management in Portugal, where migratory species, like the loggerhead turtles, are vulnerable.

b. Portuguese

As actividades marítimas têm um impacto significativo nas rotas migratórias de organismos marinhos, como cetáceos e tartarugas. As rotas da tartaruga-boba (*Caretta caretta*) cruzam as águas costeiras de Portugal cujo tráfego marítimo é muito elevado. A tartaruga-boba é uma das sete espécies de tartarugas marinhas do planeta e uma das cinco que podem ser encontradas no oceano Atlântico. Os adultos podem crescer até mais de 2 metros e pesar 500 kg, constituindo, assim, a segunda maior espécie de tartaruga. *Caretta caretta* assemelha-se à tartaruga verde (*Chelonia mydas*), mas está geneticamente relacionada com a

tartaruga-de-Kemp (*Lepidochelys kempii*) e com a tartaruga-de-Oliva (*Lepidochelys olivacea*). As tartarugas têm pulmões semelhantes aos mamíferos e por isso precisam de vir periodicamente à tona de água para respirar. Estima-se que vivam mais de 50 anos, mas podem atingir a maturidade sexual já na segunda década das suas vidas.

A distribuição geográfica da tartaruga-boba é muito ampla em águas temperadas de todo o mundo, pelo que desempenha um papel importante em vários ecossistemas com a sua dieta diversificada. Estas tartarugas migram constantemente ao longo das suas vidas. Inicialmente, passam quase uma década no mar aberto depois de nascerem. Depois, mudam-se para as zonas costeiras para se alimentarem e à medida que crescem a sua dieta torna-se mais variada. Por último, regressam aos locais de nidificação quando estão prontas para depositar os ovos nos ninhos por ela escavados. A sua ampla dispersão torna-as susceptíveis a numerosas ameaças, sobretudo de origem antropogénica, que se sobrepõem às dos predadores naturais. A principal ameaça a *Caretta caretta* é a captura acessória na pesca artesanal. Além desse factor, as atividades costeiras, como o desenvolvimento industrial, destroem gradualmente os seus locais de nidificação. Também as alterações climáticas representam uma ameaça para esta espécie, uma vez que os seus ovos são incubados numa faixa de temperaturas muito específica (25 a 35 °C). Curiosamente, a temperatura da incubação determina o sexo do embrião e tem-se observado que nascem muito mais fêmeas devido ao aquecimento global. Por tudo isto, esta espécie é considerada uma espécie com o estatuto de ameaçada. Os esforços de conservação incluem (1) o rastreio com transmissores de GPS por satélite, (2) dispositivos que diminuem a captura de tartarugas, (3) a preservação dos seus ninhos por conservacionistas voluntários, e (4) a análise genética para estudar a conectividade entre as populações. Em Portugal, o afloramento local proporciona zonas dealimentação, embora não existam praias de nidificação adjacentes na costa ibérica. Assim, é importante avaliar as origens das tartarugas-boba, numa tentativa de perceber quais as unidades de gestão internacionais da espécie que serão mais afectadas pela actividade na costa portuguesa.

Todos os anos arrojam na costa portuguesa um número significativo tartarugas-boba. É frequente cidadãos particulares e autoridades recorrerem à Rede Nacional de Arrojamentos que é coordenada nacionalmente pelo Instituto da Conservação da Natureza e Florestas (ICNF) e regionalmente pela RAAlg que integra uma equipa técnica de biólogos do CCMAR e Universidade do Algarve, disponível 24 horas durante sete dias por semana.

Este estudo é o primeiro relato sobre as possíveis origens das tartarugas-boba no Nordeste do Oceano Atlântico cujas amostragem resultam da actividade da RAAlg. Foram cedidas amostras de 194 tartaruga-boba arrojadas na costa portuguesa entre 2008 e 2017. Em estudos previamente publicados foi seleccionada a região-controlo do ADN mitocondrial para sequência tartarugas-boba ao longo da sua distribuição. No presente estudo optámos pelo mesmo fragmento do ADN mitocondrial para caracterizar a proveniência das tartarugas encontradas na costa portuguesa. Do total das 194 amostras obtivémos sequências com 776 pares de bases para 127 indivíduos (66%). Uma versão mais curta desta região-controlo com 380 pares de bases foi adicionalmente obtida para comparação com dados de estudos anteriores. As sequências resultaram num total de 16 haplótipos divididos em dois grandes grupos, um grupo a que pertencem indivíduos exclusivamente oriundos do Atlântico e outro grupo de indivíduos do Atlântico e do Mar Mediterrâneo. Além disso, foi detectado um haplótipo novo de origem Atlântica. Os valores elevados das diversidades nucleotídicas e haplotipícas implicam que as tartarugas arrojadas em Portugal representam um stock diversificado de indivíduos. Recorrendo a dados publicados anteriormente, construímos uma matriz de frequências absolutas dos vários haplótipos encontrados no Atlântico (Cabo Verde, Golfo do México, Flórida, Bahamas e unidades de gestão da costa brasileira) e no Mediterrâneo Oriental. Esta matriz constituiu os dados de entrada para a análise bayesiana de “mixed-stock”. Esta análise revelou que as tartarugas arrojadas em Portugal têm várias origens, embora maioritariamente sejam originárias do Atlântico Noroeste. A desconstrução dos dados em matrizes parciais de cada zona geográfica analisada, permitiu-nos detalhar a origem geográfica, e apontar o Golfo do México como sendo a zona que mais contribuiu para os indivíduos arrojados na costa de Portugal. Na zona do Golfo do México, a Flórida, que inclui Canaveral National Seashore, Melbourne Beach, Juno Beach e Fort Lauderdale, foi a região que teve uma contribuição mais elevada. Curiosamente, Cabo Verde foi a segunda área com maior contribuição estimada. No Mediterrâneo, o Golfo da Manfredonia foi a zona que mais contribuiu. Este estudo destaca o importante papel das correntes oceanográficas nas longas viagens das tartarugas, uma vez que as zonas de nidificação que mais contribuíram para a presença de indivíduos na costa portuguesa têm acesso directo à corrente do Golfo que transporta os indivíduos para o outro lado do Oceano Atlântico. No entanto, a contribuição de Cabo Verde e do Mediterrâneo indica algumas migrações individuais contra-corrente.

A baixa frequência de indivíduos adultos na amostragem pode ser explicada pela tendência das fêmeas pós-oviposição ficarem nas proximidades dos seus locais de postura, ou eventualmente pelo facto de indivíduos mais experientes poderem ter aprendido a evitar ameaças como redes e linhas de pesca. Não há outros dados que nos permitam concluir sobre

esta observação e se há outros factores que impeçam os indivíduos mais velhos de frequentar a costa ibérica.

Em conclusão, os nossos resultados reforçam a conectividade entre regiões geográficas, e o que afecta as tartarugas numa região tem implicações noutras, mesmo que a kilometros de distância. A conservação global depende das gestões regionais.

Keywords

Loggerhead turtle, *Caretta caretta*, mix stock, mitochondrial DNA, Atlantic Ocean

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Chapter 1

General introduction

1. The species

1.1. Phylogeography, taxonomy, morphology and identification

The loggerhead turtle *Caretta caretta* is one of the five marine turtle species in the EU and seven in the world (Linnaeus, 1758). It is the largest in size member of the ancient family *Cheloniidae*, presumed to appear around 40 million years ago (Spotila, 2004) and the second largest after the leatherback of the Dermochelidae family (Dodd, 1988; Ernst & Lovich, 2009) (Figure 1). *Cheloniidae* includes the green, olive ridley, hawksbill, flatback and Kemp's ridley sea turtles (Witherington, 2006). Even though morphologically the loggerhead resembles the green turtle, it is closer genetically to the Kemp's ridley sea turtle, olive ridley sea turtle and the hawksbill turtle (Spotila, 2004). It was first classified as *Testudo caretta* by Linnaeus (1758) in the Caribbean sea. The species gets its name from the relatively narrow and large head with strong jaws, and it has a characteristic orange to brown hard carapace, a pale yellow plastron, and pale yellow skin with brown spot-like patterns (Dodd, 1988).

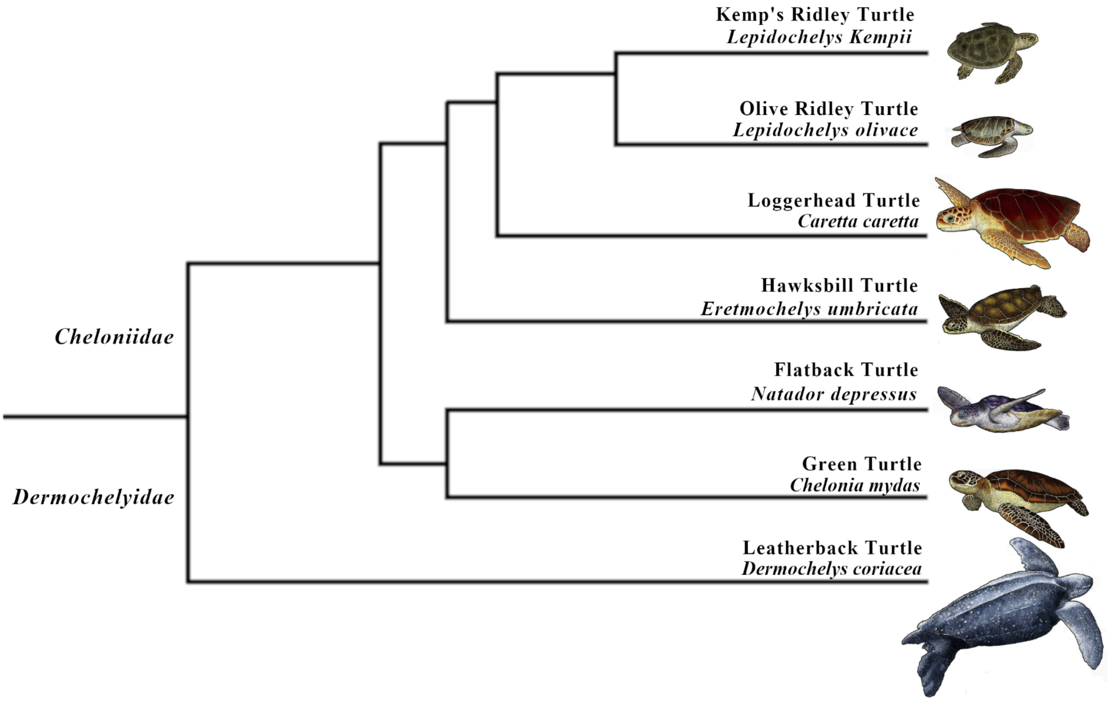

Fig 1.1. Sea turtle phylogeography. The evolutionary relations of sea turtles of today.

1.2. Growth, sexual maturity, nesting and site fidelity

Adult size usually ranges between 70 to 95 cm with an average weight of 135 kg, though they can go up to over 2 meters and 500 kilograms (Ernst & Lovich, 2009). In respect to size, individuals fall under one of four categories, hatchling, juvenile, subadult and adult (Dodd, 1988). Slight differences in coloration between size groups have been observed, along with other minor morphological differences. Hatchlings appear relatively darker than adults, measure about 4,5 cm and weigh about 20 g (Bolten et al., 2003). The carapace scutes start in hatchlings wider than longer, and they elongate as the individuals grow (Dodd, 1988) (Figure 2).

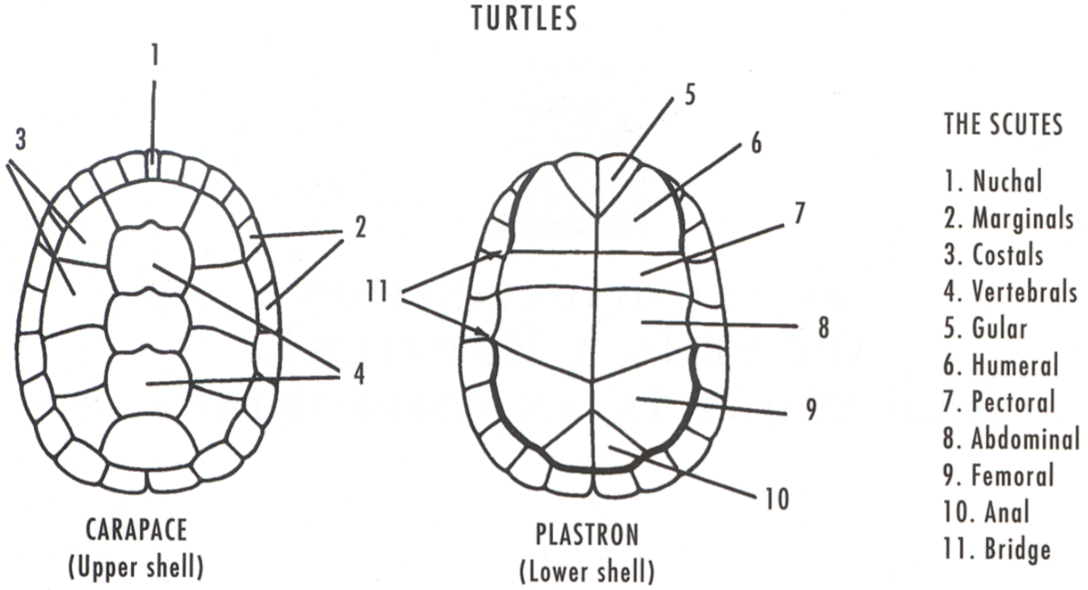

Fig 1.2. Carapace and plastron’s location and names (from Conant and Collins, 1998).

Their internal organs include a pair of multi-chambered lungs that lead to a central intrapulmonary bronchus similar to mammals (Fleetwood & Munnell 1996). Thus they need to breathe air periodically, every few minutes when they are active and travelling, but can stay underwater up to four hours, when they rest and conserve their energy (Ernst & Lovich, 2009). Multiple orientation cues, such as a geomagnetic sensing and visual observation, navigate them throughout the ocean from the beginning of their lives (Lohmann, 1991; Salmon & Wyneken, 1987; Salmon & Lohmann, 1989; Light et al., 1993; Lohmann & Lohmann, 1996; Avens & Lohmann, 2003)

Loggerheads are estimated to live more than 50 years, but they reach sexual maturity between 17 to 35 years of age (Dodd, 1988). Mating takes place every two or three years for each individual, usually in March to early June and the females lay their eggs throughout the summer, but can happen all year long (Dodd, 1988). This species has courtship rituals that include hectic movements, nuzzling and biting. Females rely on this courtship for their ovulation (Manire et al., 2008) and can hold the sperm of multiple partners in their ovulacts, allowing for multiple paternity (Harry & Briscoe, 1988; Bolmer et al., 1999; Zbinden et al., 2007; Tedeschi et al., 2015). Cross-breeding with other species of their family has also been observed, with successful hybridization (Lara-Ruiz et al., 2006; Arantes et al., 2020). Males fight for the females' heart, with preference to the larger individuals, which are shown to produce more and bigger clutches of eggs (Frazer & Richardson, 1986), and eventually the winner attaches to the top of the female and begins the very intense mating ritual that can often result in the female's harm (Miller et al., 2003). Courtship and mating takes place during their migrations between foraging and nesting grounds (Miller et al., 2003). Loggerhead turtles are a highly migratory species but they return to their birthplace to lay their eggs in sandy beaches (Avens et al., 2003; Tucker, 2010). They can lay three to five nests per season, with tenths of eggs in clutches, that hatch after approximately two months (Dodd, 1988). Environmental temperature plays a crucial role in successful incubation, as the eggs need a narrow range of temperature to hatch (between 25 and 35 °C)(Blanck & Sawyer, 1981; Matsuzawa et al., 2002; Reid et al., 2009; Fischer et al., 2014). In that range, the specific temperature and chemical conditions of the sand can affect growth, morphology and even the gender of the embryos (Yntema & Mrosovsky, 1980; Caillouet & Duronslet, 1985; Carthy, 1996; Reece et al., 2002). After they hatch they are ready to use the coastal currents to reach the open ocean, along with some high paced movement, known as swimming frenzy (Wyneken & Salmon, 1992; Salmon et al., 1995).

1.3. Range, dispersal and distribution

Loggerheads undertake two types of migrations: (1) natal homing, where adults embark on long migrations between foraging neritic grounds and their natal nesting beaches for reproduction, while (2) the hatchling and juveniles spend several years (6-11) in open ocean, before transitioning to neritic foraging areas (Bowen & Karl, 2017). Studies have shown that juvenile loggerheads can disperse, not only towards actively chosen locations but also to random grounds driven by oceanographical events (Monzon-Arguello et al., 2012). Additionally, adult loggerheads need more nutrients and larger food quantities as they grow, including prey non-indigenous to one area only, forcing supplementary extensive movements (Hatase et al., 2002; Frick et al., 2009). Eventually, a loggerhead inhabits three different habitats throughout its life. They are born in a sandy terrestrial environment, afterwards they travel to the oceanic zone where they spend their juvenile lives and as adults they hang around the nearshore ecosystems until the mating which will begin the cycle of journeys again (Dodd, 1988).

Therefore, loggerheads are found worldwide, primarily in the warm water of the Atlantic basin with range as far north as Canada or as far south as Argentina, in the Pacific with a similarly extended range from Alaska to Chile, as well as the Indian Ocean and the Mediterranean sea (Baldwin et al., 2003; Margaritoulis et al., 2003; Spotila, 2004) (Figure 3). The warm water gyres that occasionally appear around South Africa provided a way for the species to spread along the ocean basins without obvious speciation (Bowen & Karl, 2007) The biggest nesting aggregations are reported in Peninsular Florida, United States and Masirah Island, Oman (Mrosovsky & Provancha, 1989; Baldwin et al,. 2003; Turkozan et al., 2008; Witherington et al., 2009).

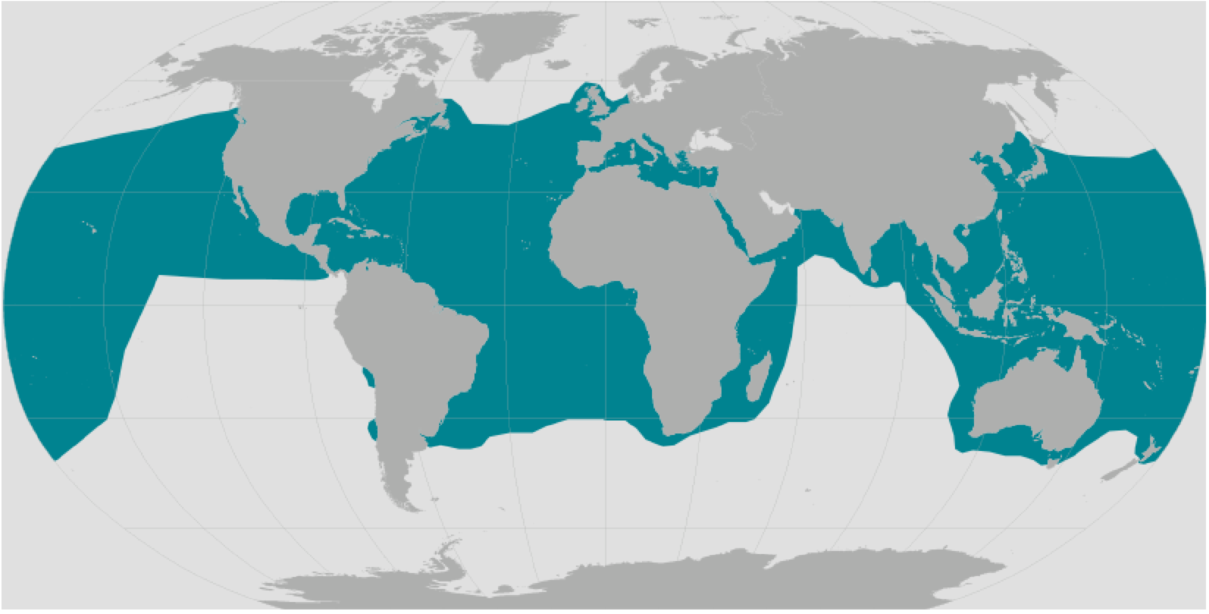

Fig 1.3. Approximate representation of the loggerhead turtle's disperse range in the world (provided by NOAA fisheries)

1.4. Diet and foraging behavior

Loggerhead turtles are omnivorous and their strong jaws permit the feeding on hard shelled animals, like decapods, bivalves, invertebrates and gastropods, but their diet is the most broad amongst sea turtles and can include softer organisms, like jellyfish, sponges and sometimes even seaweed (Ernst & Lovich, 2009). Juvenile loggerheads, after all, spend a lot of time in Sargassum fields, where their prey is abundant, during their oceanic period (Frick et al., 2009). As they grow their need for nutrients leads them to coastal areas (Frick et al., 2009).

Their foraging grounds can be plenty and far apart from the nesting ones, but their movements do not stop there. Around their foraging habitats various cleaning stations can be found, where the turtles let fish and crabs eat residues of damaged tissue, parasitic amphipods and algae in a symbiotic activity (Davenport, 1994; Schofield et al., 2006; Schofield et al., 2017). For harder epibionts, like barnacles, they rub their carapaces and heads in hard rocks found in those stations (Schofield et al., 2017). These activities happen periodically, with a visible increase after mating season (Schofield et al., 2017), in an effort to keep a healthy level of hygiene and a smooth body surface to assist their hydrodynamics (Frick & Pfaller, 2013).

1.5. Threats and conservation

There are many predators of the species, though they change in nature, numbers and risk of mortality, throughout the turtle's life. Hatchlings have it worse, since they are subject to predation from terrestrial animals of all sorts, such as racoons, snakes, various canids, rodents, bears, insects and birds (Ernst & Lovich, 2009; Marco et al., 2015). Eggs can also be found by worms, skunks and wild pigs, along with the above predators (Spotila, 2004). Once they reach the sea, they are also predated by crabs, eels and some fish (Ernst & Lovich, 2009). As they grow, the danger from predation grows thinner, since their carapace and size protects them, yet they can still be chased by sharks, whales and seals (Ernst & Lovich, 2009). Diseases caused by bacteria, fungi or other parasites also seem to decrease in numbers and in risk, as they grow (Ernst & Lovich, 2009).

The only persistent predation of the species would have to be accredited to mankind, since human activities have such a big impact on the turtle's life and go beyond direct taking. Hunting has been banned and is nearly eradicated in most countries, however there are still people who look for their meat and eggs (Aguire et al., 2006; Peckham et al., 2008). Moreover, humanity's intense seawater mobility leads to various collisions with loggerhead turtles. Turtles can be hurt by impact, caught in fishlines and gillnets or get stuck in traps, trawls and dredges (Bolten & Bjorndal, 2003; Peckham et al., 2007; Casale et al., 2007). Additionally, the species' wide diet makes them susceptible to accidental consumption of the marine litter that is dumped consistently in the oceans, often with mortal results (Ryan et al., 2016; Nicolau et al., 2016; Pham et al., 2017).

But the impact of human activity to this species goes deeper. Coastal development, like beach armoring and rising seas from climate change are resulting in the loss of beach habitat which is important for nesting (Witherington et al., 2009; Reese et al., 2013; Butt et al., 2016; Varela et al., 2019). Not only do they lose the sandy habitat, but artificial lighting on and near beaches can deter nesting females from coming ashore to nest and can disorient hatchlings trying to find the sea after hatching (McFarlane, 1963; Salmon & Witherington, 1995; Stewart & Wyneken, 2004; Alava et al., 2006; Pfaller et al., 2009). The sensitivity of their eggs to temperature creates another issue, since global warming leads to the heating of the nests, resulting in more and more females to be born, or to fail incubation altogether (Reid et al., 2009). Furthermore, all these environmental changes directly affect their prey abundance and distribution in their foraging grounds (National Marine Fisheries Service, & U.S. Fish and Wildlife Service, 2021).

Throughout all these threats, the main causes of mortality over the past decades would be the fisheries bycatch of thousands of turtles in the East Atlantic and Pacific ocean (Mast et al., 2005; Peckham et al., 2007; Wallace et al., 2010; Nicolau et al., 2016) and secondary, the marine litter, especially abandoned nets that can trap unsuspected individuals (Nicolau et al., 2016), while the degradation and loss of their nesting habitat has decreased their population numbers significantly around the continent of America and in the Mediterranean coasts, where some of the most popular nesting rookeries exist (Mazaris et al., 2009; Reece et al., 2013).

This is why the loggerhead turtle was first classified as a threatened species in 1978 under the Endangered species act in the U.S.A (U.S. Fish & Wildlife Service) and is now listed as a vulnerable species in the Northeastern Atlantic in the IUCN Red List (Casale & Tucker, 2017) and as a priority species in Annex II of the Habitats Directive (1992).

Efforts to help the species' conservation are made globally, with various approaches. Over the past decades, governments around the world banned the hunting of turtles and by now it is nearly eradicated, however consumption and trade of turtle meat and eggs is still considered legal, imposing an opposing pressure to its conservation (Peckham et al, 2008; Marco et al., 2012). Identified nesting aggregates often draw the attention of various environmental NGOs and local authorities who deploy qualified personnel to protect the nests, the eggs and the hatchlings until they reach the sea (Stancyk et al., 1980; Yerli et al., 1997; Nel et al., 2013; Lei & Booth, 2017).

In order to stop the massive bycatch of turtles by shrimp trawlers, fishermen use trap-like constructions in their nets, that stop turtles and big animals from going deep into the nets and lead them outside instead (Christian & Harrington, 1988; Kendall, 1990). These constructions, called “Turtle Excluder Device” (TED), have been evolving technologically through the years to increase their effectiveness (Mitchell et al., 1995; Shiode & Tokai, 2004; Jenkins, 2012). The efficiency of this device in saving turtles led to the use of TEDs by all major fisheries stakeholders, as it is now required by certain national laws (Yaninek, 1995).

The species' highly migratory nature implores the use of tracking, as a means of avoiding them and their habitats. Simple capture-mark-recapture methods helped keeping track of certain key places, but is not enough to track the individuals when they move (Revelles et al., 2008; Moncada et al., 2010;). Satellite tag tracking has been used in the North Pacific Ocean to locate the turtles in hopes that this knowledge would help fisheries avoid them (Kobayashi et al., 2008). In Hawaii, this approach was taken to the next level with the creation of TurtleWatch, a live tracking map that was shared to fishermen and other managers to help avoid turtle bycatch (Howel et al., 2008).

2. The methods for identifying populations and rookeries and studying their connectivity

2.1. Genetic approach

Nonetheless, loggerhead turtles can migrate thousands of kilometers, indicating a need for combining the direct observation methods with indirect, such as a genetic comparison between nesting stocks of the species (Bolker et al., 2003). The species’ karyotype was first studied by Nakamura (1937), but his methods were obsolete and showed some inaccuracies. Additional studies revealed a diploid genome of 28 pairs of chromosomes with a sex related polymorphism in females (Kamezaki, 1989). With genetic approaches, we can identify the moving populations and link them to nesting rookeries by recognising and comparing genetic markers in them (Queller & Goodnight, 1989). However, the nucleus genome rarely offers as distinctive markers as other cell organoids, throughout a species, due to its frequent recombination (Caetano & Gresshoff, 1997).

Later on, Bowen and his team (1993) managed to identify a multitude of regions on the loggerheads’ mtDNA and realised that distinct nesting populations, or stocks, have major differences in the appearance of such genetic regions. Over the years, with the advancement in technology came the development of molecular techniques that helped surpass more and more limitations in the study of marine turtles through mtDNA markers (Smith et al., 1977; Avise et al., 1992), like the generation of primers that encase bigger fragments of DNA (Shamblin et al., 2012). After all, mtDNA is known to offer a good platform to look for genetic markers, due to the fact that it originates strictly from maternal cells and that it evades recombination, operating on a much lower risk of random mutations and, thus retaining much of its original structure amongst generations (Scheffler, 2011; Tzagoloff, 2012).

Often, combining the study of different markers leads to an even better understanding of the species' life history. In an effort to study parental gene flow, Bowen uncovered with his team (2005), using microsatellites, that paternal gene flow is a lot more frequent amongst closeby nesting populations, than maternal from mtDNA, indicating thus that males can mate with females from more than one rookery. A similar research revealed paternal gene flow amongst rookeries in the Mediterranean, in accordance to the circulation of water masses in the area, linking the gene flow to their movement in the currents (Carreras et al., 2006). Ultimately, the long migrations towards foraging or nesting grounds, the philopatric behavior of females to the nesting sites and the resulting population structure of loggerheads (by reducing gene flow amongst geographically separated nesting beaches) were established using diverse approaches, including (1) capture–mark–recapture studies (Margaritoulis et al., 2003; Revelles et al., 2008; Moncada et al., 2010); (2) satellite telemetry (Cardona et al., 2005; Bentivegna et al., 2007; Revelles et al., 2007; Cardona et al., 2009; Marcovaldi et al., 2010; Casale et al., 2013; Ceriani et al., 2012); (3) stable isotopes (Vander Zanden et al., 2014; Pfaller et al., 2020) and (4) mitochondrial DNA (Carreras et al., 2007; Saied et al., 2012; Garofalo et al., 2013; Clusa et al., 2013; Shamblin et al., 2014; Carreras et al., 2018; Tolve et al., 2018; Splendiani et al., 2017).

2.2. Mixed stock analysis

For most migratory species studying the connectivity between nesting populations is full of obstacles (Webster et al., 2002; Waples & Gaggiotti, 2006). Eventually, markers in mtDNA permitted scientists to assign specific haplotypes to individuals, and thus identify many different rookeries and some of their interactions (Bowen et al., 1995; Encalada et al., 1998; Tinti et al., 2010; Shamblin et al., 2012; Clusa et al., 2014) whilst contributing to research of the species' phylogeography and life history (Bowen & Karl, 2007; Reis et al, 2010; Casale et al., 2012). Now, distinct haplotypes found in *Caretta caretta* are cataloged and can be found in a global database on the Archie Carr Center for Sea Turtle Research (ACCSTR) website at the University of Florida website (https://accstr.ufl.edu/resources/mtdna-sequences/).

Using this database, we can estimate a complete stock composition by comparing the frequencies of such haplotypes (Monzón-Argüello et al., 2010; LeRoux et al., 2012; Jensen et al., 2016). This application is known as mixed-stock analysis and was originally developed to determine the origin of stock composition of Pacific salmon (Grant et al., 1980; Milner et al., 1985). Originally, the criteria for comparison was based on maximum likelihood of haplotype appearance among populations (Pella and Milner, 1987), however, the Bayesian method that relates both stock and mixture observations (Pella and Masuda, 2001) is a better approach for the *Caretta caretta*, who reveal many rare haplotypes (Bass et al., 2004). As such, the sequences of the control region of mtDNA have been effectively used in mixed stock analysis to determine origins of populations of loggerheads in many areas with high activity, such as in the Adriatic Sea (Tolve et al., 2018), the North African coast (Karaa et al., 2016), the French coast (Loisier et al., 2021) and the eastern Turkey coast (Turkozan et al., 2018) in the Mediterranean, as well as in the Atlantic, in the United States (Rankin-Baransky et al., 2001; Stewart et al., 2019), the Caribbean sea (Engstrom et al., 2002) and Canada (LaCasella et al., 2014). Such studies indicate that loggerheads traverse widely around the seas and oceans, throughout their lifetime, most often actively (Bolten et al., 1998; Monzon-Arguello et al., 2009; Clusa et al., 2014).

3. Thesis objectives and hypothesis

The source populations of foraging turtles is a topical subject because the overlap of foraging areas with intensive fishing regions inflicts putative differential mortality rates which have the potential to negatively affect the success of the original rookery (Peckham et al., 2017). Loggerhead turtles do not have any reported nesting sites on the coast of Portugal. However, every year a considerable number of dead loggerhead turtles end up dead, stranded or captured in nets along Portugal's coast (Nicolau et al., 2016). By sequencing the control region of mitochondrial DNA and comparing it with published sequences from individuals from the Western Atlantic, Cape Verde and the Mediterranean by means of mixed-stock analysis, we will be able to identify the source rookeries of the stranded individuals. Muscle tissue samples of stranded loggerhead turtles in Portuguese coast will be sequenced for a fragment of the mtDNA control region with two specific objectives: (1) describe the haplotype composition of the individuals cruising Portuguese feeding grounds in comparison to other geographic areas and (2) determine the natal origin of loggerhead turtles frequenting the Portuguese coast.

Are the turtles following the Northern Atlantic currents to Portugal looking for food, or are they dispersing randomly? These results will contribute crucial information on this species' movements and which source populations are most threatened by human activity in these routes. It will additionally provide important data for developing conservation strategies for this endangered species.

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Chapter 2

Assessment of source populations for stranded adult loggerhead turtles (*Caretta caretta*) in the north-east Atlantic

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Abstract

Maritime activities impact the natural migratory routes of marine organisms such as marine mammals and turtles. The coastal waters of Portugal are in the pathway of such routes for loggerhead turtles (*Caretta caretta*). While the local upwelling provides foraging grounds, there are no nesting beaches adjacent to the Iberian coast, offering an excellent opportunity to study the migration of the species. This study is the first report of the natal origins of loggerheads in the North-East Atlantic Ocean. We attempted to investigate the genetic diversity of 194 loggerhead turtles found stranded across the Portuguese coast and to assess the origin of individuals through many-to-one mixed-stock analysis. We successfully produced 16 haplotypes of 776 bp of mitochondrial DNA control region for 127 samples and detected a previously unknown haplotype. Comparison with baseline data from distinct nesting populations of the Mediterranean Sea, Cape Verde Islands and the North-West Atlantic Ocean revealed that most of the loggerheads originated in the North-West Atlantic and to a smaller degree in Cape Verde and the Mediterranean. In particular, the Gulf of Mexico in the North-West Atlantic yielded the largest part of the samples and specifically the Florida rookeries: Canaveral National Seashore, Melbourne Beach, Juno beach and Ft Lauderdale. Furthermore, this study highlights the important role of currents in the long journeys of turtles, since most of the contributing rookeries were along such currents. In conclusion, our results signify the need for conservation measurements like turtle excluder devices, tag tracking and fishing management in Portugal, where migratory species, like the loggerhead turtles, are vulnerable.

Introduction

There are seven marine turtle species in the world and five of them can be found roaming the Atlantic (Avise and Hamrick, 1996). The leatherback turtle is the largest species, with the loggerhead turtle following in second place (Dodd, 1988). Although they have been an essential part of marine ecosystems for millions of years, all marine turtle species are currently considered critically endangered or vulnerable, due to human activity (Casale & Tucker, 2017). The major threats to the survival of marine turtles in many parts of the world include diverse fisheries impacts (collision, accidental capture by gillnets or longline), direct take, loss of nesting habitats, diseases, and marine pollution (Mast et al., 2005; Nicolau et al., 2016).

The loggerhead turtle is one of the most widespread species. They undertake two major types of migrations in natal homing of adults and in the hatchlings and juveniles that spend close to a decade in open ocean (Bowen & Karl, 2017).The relevance of the species migrational patterns and life cycles is apparent, making the study of their routes and origins a key element to its conservation. Such efforts to help the species are made globally, with various approaches. Satellite tag tracking has been used in the North Pacific Ocean to locate the turtles in hopes that this knowledge would help fisheries avoid them (Kobayashi et al., 2008). However, loggerhead turtles can migrate thousands of kilometers to find spawning grounds, indicating a need for combining the direct observation methods with indirect, such as the analyses of their genetic markers in the comparison between nesting stocks of the species (Bolker et al., 2003).

The Atlantic Iberian coast, and in particular the Algarve region, located in the Portuguese southern coast, is considered a “high-use area,” playing a central role in the feeding ecology of juvenile loggerhead turtles (Nicolau et al., 2016). Over 36-years (1978–2013) loggerheads were the most frequent turtle species (57%)in the area, with the highest stranding density observed on the southern coast during spring and summer (Nicolau et al., 2016). The waters around the Iberian Peninsula are important for this species due to two factors: (1) they harbour areas of upwelling where turtles appear to forage epipelagically (Cameron et al., 2019) and (2) they constitute a migratory route for the adjacent Mediterranean and the remaining North Atlantic regions which have particularly suitable habitats and foraging grounds (Bellido et al., 2010; Carreras et al., 2006). The Portuguese continental waters represent an overlap of the geographic routes of three main source regions of loggerhead populations - the Mediterranean (major nesting areas in Greece, Turkey, Cyprus, and Israel), the North West Atlantic (major nesting areas in the southeastern United States and México) and the North-East Atlantic (major nesting area in Cape Verde) (Wallace et al., 2010). In the past decade bycatch by artisanal fisheries has shown to be the main cause of turtle mortality in the coastal waters of Portugal, especially in demersal long lines and passive gears (set nets-gill or trammel nets) (Nicolau et al., 2016) as well as marine litter (Nicolau et al., 2016). Notably, the lack of suitable nesting beaches in the vicinity of this area precludes a local origin of the loggerheads that forage in the region. The individuals found must have originated in the Mediterranean, NW Atlantic and Cape Verde rookeries. This situation presents an ideal opportunity to implement mixed-stock analysis, to assess the natal origins of individuals in the foraging area.

Differences in the haplotype frequency of the maternally inherited mitochondrial DNA control region between sufficiently differentiated rookeries (baseline data) can be used to assign individuals from feeding grounds composed of mixed-origins (Encalada et al., 1998; Clusa et al., 2014; Karaa et al., 2016) using mixed stock analysis (Jensen et al., 2016; LeRoux et al., 2012; Pfaller et al., 2020). In loggerhead turtles, the sequences of the control region of mitochondrial DNA have been effectively used in mixed stock analysis to determine origins of populations in many areas with high activity (Rankin-Baransky et al., 2001; Engstrom et al., 2002; LaCasella et al., 2014; Tolve et al., 2018; Turkozan et al., 2018; Stewart et al., 2019; Loisier et al., 2021), studies that show that loggerheads can travel to the North Atlantic when they are old enough to swim across the Atlantic and the Gibraltar straits (Clusa et al., 2014; Bolten et al., 1998).

This thesis aims to determine the putative origin of stranded loggerhead turtles (*Caretta caretta*) found along the Portuguese mainland coast. It is a reasonable proposition that demographic parameters, critical for assessing sea turtle population status and trends, may be affected by individual foraging preference areas. If these areas are under particular anthropogenic pressure, individuals frequenting these areas are also endangered. For this purpose, we sequenced a total of 127 samples of stranded loggerhead turtles in the Portuguese coasts, for a fragment of the mtDNA control region with the objectives to describe the haplotype composition of the individuals and to determine the natal origin of these turtles frequenting the Portuguese coastal waters.

Materials & Methods

Sample collection and laboratory procedures

Samples obtained from an agreement between the Portuguese Marine Animal Tissue Bank (MATB) and CCMAR in 2019 (see Appendix : Table 2.1). Over a period of 10 years (2008–2017), 194 loggerhead tissue samples were collected from stranded turtles during the “Rede de Arrojamentos” operation along the Portuguese coast (Figure 2.1). For each individual, information on the date, place of sampling, cause of stranding and tissue condition was recorded. Sex was not determined, but both curved carapace length (CCL) and straight carapace length (SCL) measurements were collected. To divide turtles into stage class, we used the SCL recommendations in the 2009 TEWG report (Turtle Expert Working Group) as a guideline and divided turtles into two main categories: small turtles (≤63 cm), including first years, oceanic juveniles and juveniles that may alternate between oceanic and neritic habitats and large turtles (>63) (including larger juveniles alternating between oceanic and neritic habitat, and adult turtles in both habitats). Skin biopsies were taken from all dead turtles. Tissue samples were preserved in 95% ethanol at 4 °C.

Genomic DNA isolation using Qiagen Blood and Tissue Kit

The ~800 base pairs of the mitochondrial control region (CR) was amplified using primers LCM-15382 (5′ - GCTTAACCCTAAAGCATTGG - 3′) and H950g (5′ - GTCTCGGATTTAGGGGTTTG - 3′) (Abreu-Grobois et al., 2006). PCRs were conducted on Biometra thermocycler, using the following cycling parameters: 3-min denaturation at 94 °C, followed by 30-s denaturation at 94 °C, 30-s annealing at 50 °C, and 1-min extension at 72 °C for 35 cycles and ending with a final extension at 72 °C for 10 minutes. PCR volumes of 25 µl included 1 µl of genomic DNA, 0.2 mM (=0.5 µl) dNTPs (Promega, Madison, WI, USA), 1 U (=0.2 µl) GoTaq polymerase (Promega, Madison, WI, USA), 5 mM de Gotaq Buffer (5x) (=5 µl), 1.5 mM (=1.5 µl) MgCl2 and 10 pmol (=0.5 µl) of forward and reverse primers. Negative controls were run alongside positive samples to identify any contamination present from the extraction step. PCR products were visually verified using 2% agarose gels with GelGreen®.

Samples were precipitated before sequencing, using 10 µl Sodium acetate 3M in a volume of 100 µl, containing 20 µl of our PCR product. After two steps of mixing with Ethanol (100% on the first step and 70% on the second) and high speed centrifugation, we eluded the dry pellet with miliQ H_2O. Only forward strands were cycle sequenced using an ABI ® Big Dye Terminator v3.1 or a v3.1 ABI Prism Terminator Cycle Sequencing Kit and evaluated with

Applied Biosystems ® (models 3130 and 3730) automated genetic analysers (Foster City, CA, USA) at CCMAR.

Data analysis

Raw sequence chromatographs were edited and aligned in Geneious Prime (Biomatters, New Zealand, https://www.geneious.com) using MUSCLE version 3.6 (Edgar, 2004) with default values selected, and visually verified. Sequences were cropped at a standard cropping site of 776 bp (Abreu-Grobois et al., 2006). We also trimmed sequences down to 380 bp to match the short mtDNA control region fragment (Norman et al., 1994) and compared sequence diversity parameters to values reported for *Caretta caretta* in the earlier studies.

Haplotypes were assigned by comparing sequences from the samples to a reference library of long 776-bp haplotypes of published loggerhead sequences from the Atlantic and Mediterranean, using the standardized nomenclature of the Archie Carr Center for Sea Turtle Research (https://accstr.ufl.edu/resources/mtdna-sequences/)

Sequences were collapsed into haplotypes using “haplotype” function of the R-package haplotypes v1.1 (Aktas, 2019) We reconstructed haplotype networks using a statistical parsimony algorithm (Templeton et al., 1992), implemented in TCS v.1.21 (Crandall et al., 2000). The raw output was visualised in the web implementation of tcsBU (Múrias dos Santos et al., 2016).

The standard measures of genetic diversity were computed separately for the main location sites. We used *pegas* R-package (Paradis, 2010) to estimate standard descriptive measures of genetic diversity, including the number of haplotypes and private haplotypes, haplotype diversity (h) (Saitou & Nei, 1987), and nucleotide diversity (π) (Saitou & Nei, 1987) and respective standard deviations.

To estimate the contribution of each origin region to the sampled stock, we used a Bayesian many-to-one mixed-stock approach (Pella & Masuda, 2001) as implemented in the mixstock R-package (Bolker et al., 2007). Applying the Markov Chain Monte Carlo method we used a maximum likelihood (ML) approach that will find the set of parameters with the highest probability of observing the sampled data given a particular set (likelihood). The result is a matrix with the percentage contribution of each origin region (see Appendix : Table 2.2). As a final result we will be showing the percentage of populations of different origins in the stock. We used four different data arrangements: (dataset 1) three major clustered areas: the Mediterranean (EMED), the Western Atlantic (WAT) as a supergroup of the Western rookeries and Cape Verde (CAP); (dataset 2) regions subdivided into Eastern US (EUS), the

Bahamas (BAH), the Gulf of Mexico (GME), the Brazilian coast (BRA), the Cape Verde (CAP) and the Eastern Mediterranean which holds all Mediterranean rookeries (EMED); (dataset 3) all the western Atlantic rookeries : Cape Island, South Carolina and Ossabaw Island, Georgia (GEO), Canaveral National Seashore, and Melbourne beach, Florida (FLO1), Juno beach and Ft. Lauderdale, Florida (FLO2), Cay Sal, Bahamas (BAH), Dry Tortugas, Florida (DRT), Isla Cozumel and Quintana Roo mainland, Mexico (MEX), Keewaydin island, Florida (KEY), Casey Key, Florida (CSK), St George island and Cape San Blas, Florida (FLO3) and Sergipe, Bahia, Espirito Santo and Rio de Janeiro, Brazil (BRA); and (dataset 4) all the Mediterranean rookeries: Calabria, Italy (CAL), Zakynthos island, Kyparissia and Lakonikos bay, Greece (GRE), Crete island, Greece (CRT), Dalyan, Turkey (DLY), Dalaman, Turkey (DAL), Western Turkey (TKW), middle and Eastern Turkey (TME), Alagadi and Akamas beach, Cyprus (CYP), El Mansouri, Lebanon and Israel (LIR) and Sirte and Misurata, Libya (LYB).

Results

Samples characteristics and haplotypes

Most loggerhead stranded turtles were juveniles (N= 170), ranging from 22.0 to 63 cm CCL and only 15 were adults with the larger individual reaching 83 cm and the majority (163) were sampled on the Southern coast of Portugal (Figure 2.1). Stranding numbers were uneven throughout 2008-2017, with the highest in 2011 (N = 88) and the lowest in 2008 (N = 1).

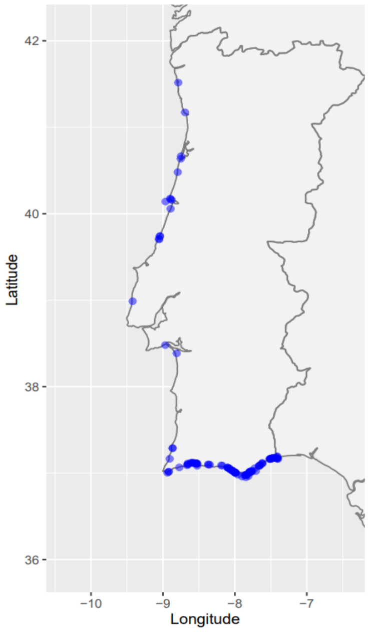

Fig 2.1 Sampling locations along the Portuguese coastline.

Of the 194 samples, we obtained long fragments for 127 individuals (9 adults, 114 juveniles and 4 unknowns). Overall nucleotide diversity was 0.022 (± 0.001) and the haplotype diversity was 0.739 (± 0.027).

We found 16 different haplotypes (Figure 2.2). The most common haplotype was CC-A1.1. (N=53), which is also the most common worldwide haplotype of *Caretta caretta*. It appears mostly in the Eastern US coast and the Gulf of Mexico. The second most abundant haplotype was CC-A2.1 (N=34) which is the most common haplotype in the Mediterranean Sea. Third haplotype found was CC-A1.3 (N=16), which is the most common in Cape Verde rookeries. Haplotypes CC-A3.1 and CC-A14.1 were represented in nine and four individuals respectively, while the remaining haplotypes were singletons. One new haplotype was recorded in one individual, and was submitted to the ACCSTR and the GenBank. The new haplotype differed from CC-A1.1 for one T-to-G transversion at nucleotide site 15,699 of the published *Caretta caretta* mitochondrial genome sequences (ACCN FR694649, KP256531 and NC_016923). The new haplotype was not considered in the MSA as its rookeries of origin have yet to be identified.

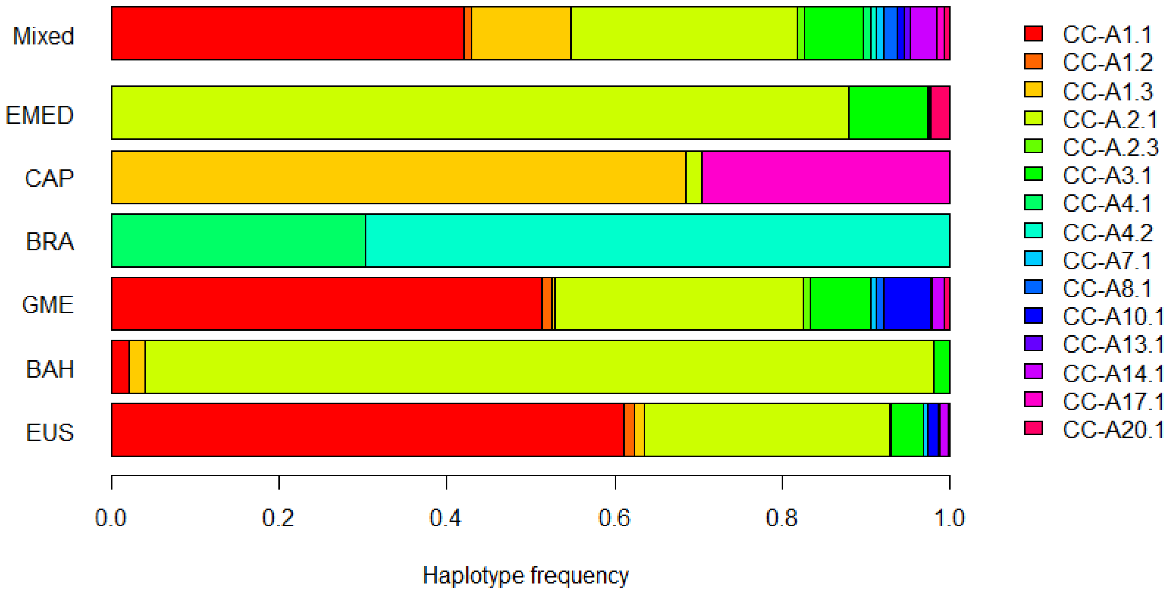

Fig 2.2. Mitochondrial Control Region haplotypes frequency of *Caretta caretta* found in Portuguese waters (MIX) and in six main geographic regions (Eastern USA - EUS; Bahamas - BAH; Gulf of Mexico - GME; Brazil - BRA; Cape Verde - CAP and Eastern Mediterranean - EMED). Horizontal axis shows the color of each haplotype.

Haplotype network

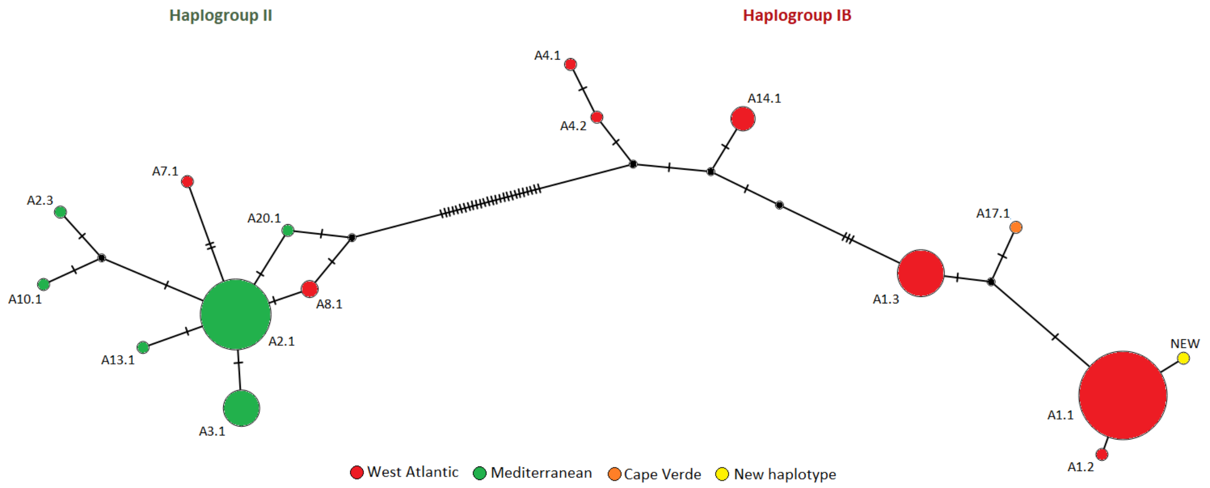

Fig 2.3. Haplotype network obtained for the long fragment of the control region for 127 juveniles and 15 adults of *Caretta caretta*. Substitutions between haplotypes can be transitions, transversions or indels. Grey circles indicate unsampled or extinct haplotypes. Sizes of circles are proportional to sample frequencies.

For the network analysis, 217 sequences previously published (Shamblin et al., 2014; Splendiani et al., 2017) have been added to our dataset, thus representing 57 haplotypes of the long fragment. The parsimony network we built for the control region with minimum spanning distance (Fig. 2.3) indicated two main haplogroups (nomenclature according to Shamblin et al. 2014) that mainly represented geographic groups. Haplogroup IB, clustered around CC-A1.1, included a majority of Atlantic haplotypes and haplogroup II, clustered around CC-A2.1, encompassed haplotypes from both the Atlantic Ocean and the Mediterranean Sea. Haplogroups II and IB are separated by 26 substitutions.

Mixed stock analysis

The MSA of the relative contribution of three main regions (Western Atlantic, Cape Verde and Eastern Mediterranean Sea) to the turtles found in Portuguese waters exhibited a shrink factor = 1 (according to the mixstock manual - http://shorturl.at/pBCOQ, the Gelman-Rubin criteron should be below 1.2 for all parameters in order for the chain to be judged to have converged properly) and narrow confidence intervals. The analysis showed a notable difference between the large (p = 0.82, 95% CI = 5.88E-01 to 0.93) contribution estimates of Western Atlantic rookeries, Cape Verde rookeries (p = 0.12, 95% CI = 5.70E-02 to 0.26) and Eastern Mediterranean rookeries (p = 0.06, 95% CI = 7.96E-06 to 0.25) (Fig. 2.4A).

The following analysis, albeit with very wide confidence intervals, allowed us to pinpoint specific contributions in each major geographic region. According to the MSA with priors weighted by population abundance within the Western Atlantic, the Gulf of Mexico had the highest contribution (p = 0.69, 95% CI = 1.44E–05 to 0.86) (Fig. 2.4B) and within the Gulf of Mexico, Canaveral National Seashore and Melbourne Beach in Florida had the largest contribution (p = 0.49, 95% CI = 5.05E-05 to 0.49) followed by Juno beach and Ft Lauderdale in Florida (p = 0.28, 95% CI = 5.14E-05 to 0.67) (Fig. 2.4C). In the Eastern Mediterranean, the rookery in the Gulf of Manfredonia appeared to contribute the most (p = 0.39, 95% CI = 2.76E-08 to 0.59) followed closely by the middle and Eastern Turkish rookeries (p = 0.35, 95% CI = 2.63E-11 to 0.42) and also with significant probability of contribution from the Dalaman rookery in Turkey (p = 0.21, 95% CI = 2.84E-09 to 0.53) (Fig. 2.4D).

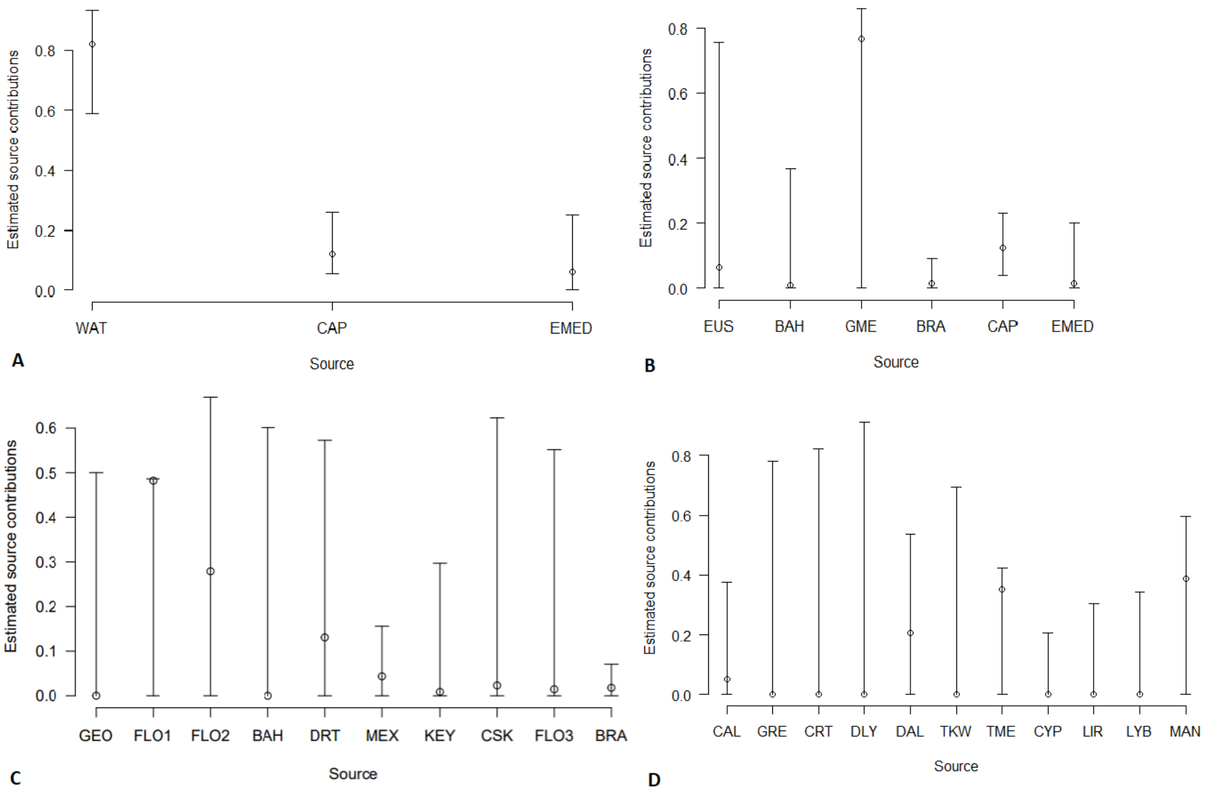

Fig 2.4. Contribution proportions and 95% confidence intervals of the four datasets to the Portuguese stranded loggerhead turtle estimated using an proportional-contribution prior. A - dataset 1, B - dataset 2; C - dataset 3; D - dataset 4 (See definition of data sets in Material and Methods section).

Discussion

This study intends to characterise the population structure and composition of a sampling of loggerhead turtles from stranding and bycatch events reported in the Portuguese coast. This is an important contribution as, thus far, there was no data available in this area of the Northeastern Atlantic Ocean where no rookeries are available and hence only foraging turtles are found. The loggerhead turtles stranded in Portuguese coastal waters have diverse geographic origins. The mixed-stock analysis reveals that the most significant estimated contribution is from the aggregations of the Western Atlantic Ocean (82%), with additional contributions of Cape Verde (12%) and the Eastern Mediterranean basin (6%).

The nucleotide and haplotype diversity of our samples are high in comparison to such values obtained from management units around the world. In fact, the nucleotide diversity (π) is comparable to values obtained in the Florida aggregations (Encalada et al., 1998; Ceriani et al., 2014), where the population size is large and contains a wider range of nucleotides. Likewise to the Mexican aggregations, where we observe a lot of the loggerheads' haplotypes, the haplotype diversity (h) of our samples is also high (Monzón-Argüello et al., 2010). Such results are expected from a mixed stock of many different origins and indicate that we can obtain a lot of information from genetic analysis on this mtDNA marker. Furthermore, the parsimony network reveals two main groups of haplotypes, those that exist specifically in the Atlantic ocean and those that appear in the Mediterranean sea along with the Atlantic. Notably, we found a new haplotype that is one mutation away from CC-A1(Fig 2.3). We can hypothesise that the individual with this haplotype comes from the Western Atlantic, where CC-A1 is most abundant.

Previous studies have revealed that older loggerheads tend to forage within the vicinity of their natal grounds, with only a few individuals known to migrate further away (Pajuelo et al., 2012), while younger loggerheads are more likely to migrate to distant foraging grounds (Hatase et al., 2002; Hawkes et al., 2006; Mansfield et al., 2009). These tendencies fall in line with previous observations where individuals from the Gulf of Mexico and surrounding rookeries follow currents all the way to the Azores and Madeira islands (Bolten et al., 1998) . Studies conducted in the Mediterranean suggest similar turtle migrational behaviour. In the Adriatic sea, loggerheads who frequent the coastal waters are born in Greece, Cyprus and Turkey (Lazar et al., 2004; Splendiani et al., 2017; Tolve et al., 2018) following the Eastern-Southern Adriatic current (Zavatarelli & Pinardi, 2003). Some of those turtles from Greece and Turkey, in fact, go further west to the French coast, following Mediterranean currents in search for upwellings rich in prey (Carreras et al., 2006; Loisier et al., 2021). Similarly, our study detected turtles from the West Atlantic Ocean and to a lesser degree from the Western Mediterranean.

While the species is characterised by philopatry, the Iberian coast does not contain any nursing rookeries, hence all the turtles found in these waters are foraging. The coastal upwelling provides an abundance of prey for marine migratory species, like turtles. The loggerhead turtles stranded in Portuguese waters consist mainly of young and subadults between 22 and 83 cm CCL. The turtles' size is found to be positively correlated with diet volume and variety (Hatase et al., 2002; Frick et al., 2009), which relates their arrival with their need for more prey, after their oceanic stage is over. Nonetheless, it is not clarified at which point the turtles change their diet and it is also speculated that sometimes their change of prey uptake leads to their faster growth (Hatase et al., 2002). The latter can explain why seven small individuals (<32cm) are also found in Portugal. The lack of larger, older adults from the strandings can either be attributed to old individuals heading towards nursery sites, instead of foraging sites, or to the fact that, as they grow, they learn to avoid nets and other threats that caused the strandings.

The observation that many turtles come to the Iberian coast from the West part of the Atlantic is in line with prior expectations for two reasons mainly. First, the Florida and Gulf of Mexico management units are some of the largest in the world, counting thousands of turtles every year, much more than Africa or South America. Secondly, besides the contribution in numbers, the dominant currents of the North Atlantic Drift lead turtles from the American to the European side, facilitating the species dispersal (Monzon-Arguello et al., 2009). In fact, the North Atlantic drift starts to form intensely already in the Gulf of Mexico, where most of the loggerheads we sampled originate (Reverdin et al., 2003). This movement agrees with the hypothesis that the turtles tend to use currents to travel, as a means of treading great distances with minimum energy expansion. Something noteworthy is that the lack of older adults in our samples can be because adult females from the Gulf of Mexico do not travel very far from their natal sites after giving birth (Renaud & Carpenter, 1994; Girard et al., 2009). Unfortunately, our samples were missing data on their gender and reproduction phase, thus hindering an accurate estimation on whether this is the reason older loggerheads (>83 cm) are absent from the strandings.

Marine turtles also have the ability to orient themselves and memorise parts of their journeys (Monzon-Arguello et al., 2012; Bowen and Karl, 2017). This is demonstrated by the fact that we have observed in our stock individuals from the Mediterranean. Loggerheads can travel through the Gibraltar strait, directed towards the Atlantic, whereas the surface currents are stronger towards the Mediterranean (Clusa et al., 2014). Additionally, 50 of the turtles found in Portugese water share haplotypes with turtles from Cape Verde, which is located South of the Portuguese waters. The North Atlantic Current splits in two directions, one going North to waters too cold for the loggerhead turtles and the other going South, towards Cape Verde (Reverdin et al., 2003). This constitutes another example of these turtles moving countercurrent. The haplotype CC-A17.1 which is uniquely found in the Cape Verde rookeries, along with the CC-A1.3 which has frequent appearance in those rookeries, imply that turtles indeed travelled northwards. Cape Verde is, moreover, one of the most important loggerhead turtle rookeries, being recently characterised as a stepping stone for the species' dispersal in the Atlantic (Baltazar-Soares et al., 2020). The contribution of Cape Verde and Mediterranean indicate that the turtles can navigate across or even against currents to whatever direction they choose, matching their hatchlings' orientation and travelling behaviour (Salmon & Wyneken, 1987; Avens & Lohmann, 2003). However, the sample size was insufficient to allow us to draw definite conclusions on how these aggregations can be affected by activities in the European Atlantic coasts.

Considering these findings, the rookeries that are affected the most by threats in the North-East Atlantic, are the ones located in the Gulf of Mexico. Loggerheads have to overcome very similar challenges in both sides of the Atlantic ocean, as the main cause of stranding in the Gulf of Mexico is also bycatch by fisheries, followed by marine litter digestion and the loss of nesting habitat (Lewison et al., 2003; Reece et al., 2013). The implementation of bycatch laws such as the use of turtle excluding devices has helped the conservation of loggerhead turtles in the American continental coast and lead to an increase of their populations (Lewison et al., 2003).

This study suggests that the laws and measurements implemented in the Gulf of Mexico should be implemented in areas like the Portuguese coast, where there might not be turtle nests, but there is an abundant destination for foraging turtles. In addition, data on gender and reproduction stage can help in understanding the absence of older loggerheads from Portuguese waters. Nonetheless, there is a clear presence of big oceanic journeys. Satellite tagging will confirm the species tendency to follow currents and will reveal all the ecosystems they affect. Data collected from stranded individuals may reveal new haplotypes, like the one discovered in our study, and contribute to the global database.

In conclusion, studying marine turtle populations leads to the assessment that proper management and conservation relies on global effort, especially for highly migratory, widely dispersed species like the loggerhead turtle (Wallace et al., 2010; 2011). Of course, regional management is the first shield for conservation, but it is rendered insignificant when organisms travel to regions without any hard measures for their protection (Wallace et al., 2011). If anything, studies on populations and ecosystems consistently point to their inseparable connection.

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Appendix.

#cap	date	region	Location	SCL (cm)	Size class (SCL)	CCL (cm)	Latitude (°N)	Lontitude (°W)	Haplotype
cc001	23-06-2008	S	Faro	43,2	2	47	36.981	7.843	A20.1
cc002	27-08-2010	S	Praia de Faro	42,0	2	46	37.004	7.995	A1.1
cc003	26-05-2010	NC	Aveiro	unknown	2	42,5	40.636	8.752	A1.1
cc004	02-06-2010	S	Praia do Barril	46,0	2	48,7	37.087	7.657	A1.1
cc005	22-06-2010	S	Ilha da Culatra	52,0	3	56,0	36.984	7.833	A1.3
cc006	24-03-2010	S	Ilha da Armona	55,2	3	59,5	37.020	7.768	A2.1
cc007	28-04-2010	S	Alvor	unknown	2	56,2	37.119	8.591	unknown
cc008	28-04-2010	S	Praia da Rocha	40,3	2	44,5	37.113	8.533	A1.1
cc009	22-06-2010	S	Ilha da Armona	unknown	2	50,0	37.014	7.786	A1.1
cc010	25-06-2010	S	Monte Gordo	45,0	2	50,7	37.174	7.436	A2.1
cc011	09-07-2010	S	Ilha do Farol	43,0	2	48,8	36.977	7.848	A1.3
cc012	31-07-2010	S	Marina VRSA	40,0	2	41,0	37.195	7.412	A1.3
cc013	04-08-2010	S	Vila Real Santo António	47,0	2	49,5	37.164	7.402	A1.1
cc014	04-08-2010	S	Vila Real Santo António	37,0	2	39,0	37.172	7.419	A2.1
cc015	19-08-2010	CS	Praia do Amado	unknown	2	43,0	37.166	8.906	A1.1
cc016	02-09-2010	S	Manta Rota	49,0	2	50,5	37.164	7.512	unknown
cc017	21-09-2010	S	Ilha da Armona	35,0	2	38	37.009	7.791	A14.1
cc018	06-12-2010	S	Praia de Quarteira	39,0	2	43,0	37.065	8.102	A1.1
cc019	unknown	unknown	unknown	unknown	unknown	unknown	unknown	unknown	unknown
cc020	12-02-2011	S	Armação do atum - Tunipex	50,0	2	53,0	37.024	7.708	A1.1
cc021	12-04-2011	S	Largo de Lagos	52,0	3	57,5	37.091	8.663	A1.1
cc022	14-05-2011	S	Praia do Trafal	46,0	2	48,0	37.055	8.081	A1.1
cc023	23-05-2011	NC	Figueira da Foz	43,0	2	46	40.175	8.900	A2.1
cc024	27-05-2011	S	Alvor	62,0	3	67,8	37.121	8.610	NEW
cc025	27-05-2011	S	Praia de Faro	47,0	2	51,2	37.008	8.002	A1.1
cc026	02-06-2011	S	Praia da Marinha	31,0	1	35,0	37.089	8.413	unknown
cc027	03-06-2011	S	Praia de Quarteira	32,0	1	35,5	37.064	8.099	A1.1
cc028	05-06-2011	S	Altura	49,0	2	52,2	37.167	7.504	unknown
cc029	16-06-2011	NC	Figueira da Foz	unknown	2	51,0	40.157	8.877	A1.1
cc030	21-06-2011	S	Ilha Deserta	52,0	3	54,0	36.960	7.885	unknown
cc031	02-07-2011	S	Praia de Faro	47,0	2	51,0	37.000	7.989	unknown
cc032	25-07-2011	S	Praia de Altura	44,0	2	46	37.170	7.490	A3.1

cc033	27-07-2011	S	Monte Gordo	47,0	2	49,5	37.176	7.461	A2.1
cc034	22-08-2011	S	Praia do Burgau	54,0	3	58,6	37.067	8.772	A1.1
cc035	15-09-2011	NC	4 milhas Figueira da Foz	unknown	2	44,5	40.141	8.965	A1.1
cc036	22-10-2011	S	Praia da Mareta	52,0	3	53,5	37.001	8.940	unknown
cc037	29-05-2011	S	Alvor	51,0	2	53,0	37.121	8.603	unknown
cc038	14-06-2011	CS	Ribeira d'ilhas	unknown	2	50,0	38.988	9.421	A1.1
cc039	23-09-2011	S	Largo Olhão	62,0	3	64,2	36.967	7.811	A1.1
cc040	14-05-2011	S	Praia do Cabeço	47,0	2	50,0	37.174	7.470	unknown
cc041	23-05-2011	S	Alvor	53,0	3	55,0	37.119	8.590	A1.3
cc042	27-05-2011	S	Praia do Vau	33,5	2	35,0	37.113	8.560	A2.1
cc043	27-05-2011	S	Praia da Rocha	53,0	3	56,4	37.113	8.535	unknown
cc044	04-06-2011	S	Manta Rota	42,0	2	43,5	37.165	7.509	A1.3
cc045	25-06-2011	S	Vale do Lobo	44,0	2	46,8	37.040	8.064	A1.3
cc046	06-07-2011	S	Praia da Rocha	45,0	2	48,2	37.112	8.533	unknown
cc047	23-10-2011	S	Manta Rota	50,0	2	54,5	37.165	7.511	unknown
cc048	13-02-2011	S	Praia do Ancão	53,0	3	55,2	37.026	8.031	A1.1
cc049	20-04-2011	S	Praia do Cabeço	58,0	3	61,5	37.175	7.468	A14.1
cc050	27-04-2011	S	Alvor	48,0	2	51,5	37.119	8.600	A14.1
cc051	28-04-2011	S	Meia Praia	40,0	2	48,0	37.100	8.656	A1.1
cc052	28-04-2011	S	Praia de Faro	43,0	2	45,0	36.995	7.986	unknown
cc053	08-05-2011	S	Barra Olhão-Faro	unknown	unknown	unknown	36.961	7.868	unknown
cc054	09-05-2011	CS	Praia da Comporta	unknown	2	53,5	38.386	8.808	A3.1
cc055	09-05-2011	S	Praia de Faro - Barrinha	30,6	1	33,2	36.987	7.969	unknown
cc056	09-05-2011	S	Praia de Faro - Barrinha	41,0	2	43,0	36.984	7.966	unknown
cc057	09-05-2011	S	Praia de Faro - Barrinha	60,0	3	65,8	36.985	7.967	unknown
cc058	09-05-2011	S	Manta Rota	50,0	2	52,5	37.161	7.521	unknown
cc059	11-05-2011	S	Armação de Pera	54,3	3	55,6	37.100	8.363	A1.1
cc060	17-05-2011	S	Praia Altura	47,0	2	48,5	37.167	7.502	A1.1
cc061	21-05-2011	S	Praia do Ancão	52,0	3	53,0	37.030	8.037	A1.1
cc062	24-05-2011	S	Meia Praia	31,0	1	33,0	37.105	8.650	A1.1
cc063	29-05-2011	S	Vila Real Santo António	43,0	2	45,0	37.170	7.411	A1.1
cc064	03-06-2011	S	Monte Gordo	42,0	2	44,5	37.175	7.440	A1.1
cc065	04-06-2011	S	Praia dos beijinhos	50,0	2	52,0	37.100	8.375	A2.1
cc066	04-06-2011	S	Ilha Deserta	35,5	2	39,0	36.963	7.875	unknown
cc067	05-06-2011	S	Portimão	56,0	3	58,0	37.114	8.540	A1.1

cc068	05-06-2011	S	Portimão	47,0	2	50,0	37.105	8.523	unknown
cc069	08-06-2011	S	Praia de Quarteira	35,0	2	37,0	37.065	8.102	A1.3
cc070	11-06-2011	S	Praia de Faro	33,5	2	35,5	36.993	7.983	unknown
cc071	13-08-2011	S	Praia Verde	39,0	2	42,0	37.173	7.478	A1.1
cc072	17-08-2011	S	Ilha Deserta	50,0	2	55,0	36.961	7.899	A1.1
cc073	21-08-2011	S	Largo Portimão	35,0	2	38,8	37.085	8.524	A2.1
cc074	04-06-2011	NC	Figueira da Foz	unknown	2	42,0	40.168	8.892	A1.1
cc075	02-10-2011	S	Barra do Lavage	51,0	2	53	37.003	7.801	A3.1
cc076	31-03-2011	CS	Praia da Arrifana	45,5	2	54,0	37.288	8.866	A1.1
cc077	12-04-2011	S	Largo de Olhão	42,0	2	44,0	36.952	7.847	A1.1
cc078	19-04-2011	S	Praia de Alvor	54,5	3	57,0	37.119	8.594	unknown
cc079	21-04-2011	S	Praia do Cabeço	53,0	3	55,0	37.174	7.472	A2.1
cc080	07-05-2011	S	Praia do Trafal	50,0	2	55,5	37.052	8.078	unknown
cc081	08-05-2011	CS	Praia do Creiro	unknown	2	54,5	38.481	8.968	A8.1
cc082	09-05-2011	S	Praia de Faro - Barrinha	48,0	2	52,0	36.987	7.972	unknown
cc083	14-05-2011	S	Manta Rota	44,0	2	47,5	37.163	7.514	unknown
cc084	15-05-2011	S	Rio Guadiana	53,0	3	56,0	37.189	7.411	A1.1
cc085	16-05-2011	S	Praia do Martinhal	46,0	2	48,5	37.017	8.918	A2.1
cc086	17-05-2011	S	Praia do Martinhal	53,0	3	59,0	37.014	8.924	unknown
cc087	17-05-2011	S	Praia do Martinhal	53,0	3	57	37.015	8.923	unknown
cc088	17-05-2011	S	Praia do Martinhal	52,0	3	56,0	37.016	8.921	A1.1
cc089	26-05-2011	NC	Praia da Polveira	unknown	2	54,0	39.710	9.056	A2.1
cc090	26-05-2011	NC	Matosinhos	unknown	2	39,0	41.173	8.693	A1.3
cc091	01-06-2011	S	Praia da Rocha	43,0	2	45,0	37.112	8.532	A2.1
cc092	03-06-2011	S	Alvor	58,0	3	61,0	37.119	8.595	unknown
cc093	05-06-2011	NC	Praia do Pico Norte - Peniche	unknown	2	45,0	39.372	9.393	unknown
cc094	07-06-2011	NC	Leirosa	unknown	2	52,0	40.058	8.893	A14.1
cc095	10-06-2011	NC	Paredes Vitória	unknown	2	48,5	39.703	9.052	A1.1
cc096	16-06-2011	NC	Pedra de Ouro	unknown	2	57,0	39.742	9.043	A1.1
cc097	17-06-2011	NC	Areão sul	unknown	2	56,0	40.546	8.777	unknown
cc098	18-06-2011	NC	Paredes Vitória	unknown	2	45,5	39.700	9.053	unknown
cc099	19-06-2011	CS	Pedras Negras	unknown	2	53,5	38.449	9.024	unknown
cc100	20-06-2011	NC	Papoa - Peniche	unknown	3	63,0	39.373	9.379	unknown
cc101	01-07-2011	S	Praia de Quarteira	45,5	2	48,0	37.065	8.101	unknown
cc102	25-07-2011	S	Terra Estreita	unknown	2	49,0	37.093	7.647	A2.1
cc103	17-09-2011	S	Terra Estreita	48,0	2	52	37.092	7.649	unknown

cc104	03-05-2011	S	Portimão	57,5	3	63,0	37.106	8.528	A2.1
cc105	unknown	unknown	unknown	unknown	unknown	unknown	unknown	unknown	A1.3
cc106	unknown	unknown	unknown	unknown	unknown	unknown	unknown	unknown	A3.1
cc107	unknown	unknown	unknown	unknown	unknown	unknown	unknown	unknown	unknown
cc108	05-09-2012	S	Cabanas Tavira	52,0	3	55,4	37.138	7.581	unknown
cc109	23-05-2012	S	Praia de Faro	26,0	1	28,0	37.002	7.987	unknown
cc110	07-09-2012	S	Praia da Lota	66,0	3	71,5	37.162	7.521	unknown
cc111	31-03-2012	S	Praia do Martinhal	56,0	3	57,5	37.017	8.928	unknown
cc112	02-04-2012	S	Praia do Barranco	52,5	3	55,5	37.042	8.895	unknown
cc113	08-04-2012	S	Ilha da Armona	62,0	3	68,0	37.016	7.798	A2.1
cc114	01-05-2012	S	Monte Gordo	72,5	4	75,5	37.177	7.448	unknown
cc115	01-03-2013	CS	Praia da Arrifana	27,0	1	29,0	37.290	8.864	A1.3
cc116	22-08-2013	S	Cabanas Tavira	54,5	3	57,0	37.128	7.601	unknown
cc117	25-04-2013	S	Praia de Faro	42,0	2	46,0	37.006	7.994	A1.1
cc118	05-07-2013	S	Praia de Faro	69,0	3	75,0	37.004	7.990	A2.1
cc119	23-08-2013	S	Tavira - quatro águas	52,0	3	53,0	37.116	7.620	A2.1
cc120	12-06-2013	S	Praia de Faro - lado direito	48,0	2	50,0	37.009	7.998	unknown
cc121	07-07-2013	S	Praia Olhos d'água	43,0	2	46,0	37.090	8.190	A1.2
cc122	08-07-2013	S	Ilha do Farol	59,0	3	64,0	36.979	7.855	A1.1
cc123	25-08-2013	S	Praia da Lota - Manta Rota	48,0	2	51	37.166	7.510	A1.3
cc124	27-08-2013	S	Manta Rota	37,0	2	38,7	37.163	7.519	A2.1
cc125	28-08-2013	S	Praia Vila Real Santo António	43,0	2	44,0	37.169	7.406	A1.3
cc126	23-02-2013	S	Quinta do Lago	59,0	3	65,0	37.023	8.023	A1.1
cc127	08-03-2013	S	Praia de Faro - Barrinha	23,8	1	25,4	36.982	7.957	A17.1
cc128	25-09-2013	S	Praia do Forte Novo - Quarteira	31,0	1	34,0	37.061	8.088	unknown
cc129	25-09-2013	S	Praia dos três irmãos	53,0	3	57,0	37.120	8.584	unknown
cc130	26-09-2013	S	Alvor	54,0	3	unknown	37.005	8.937	unknown
cc131	31-12-2013	S	Praia da Mareta	54,0	3	58,0	37.005	8.937	A13.1
cc132	27-10-2014	S	Largo de Olhão	25,9	1	27,7	37.014	7.778	A2.1
cc133	16-06-2014	S	Entre Praia Verde e Praia da Lota	57,0	3	59,7	37.171	7.489	A3.1
cc134	26-10-2014	S	Barra do Lavage	60,0	3	64,5	37.011	7.800	unknown
cc135	13-03-2014	S	Praia de Faro	30,0	1	33,3	37.003	7.989	A1.1
cc136	16-05-2014	S	Praia dos Pescadores	41,0	2	44,3	37.086	8.251	unknown

cc137	23-05-2014	S	Praia Grande - Armação Pera	23,0	1	28,0	37.098	8.349	A1.3
cc138	22-06-2014	S	Ilha da Armona	50,0	2	52,7	37.019	7.785	unknown
cc139	30-06-2014	S	Vale do Lobo	43,0	2	47,9	37.050	8.068	unknown
cc140	14-07-2014	S	Quatro águas	39,0	2	43,0	37.117	7.620	A1.1
cc141	17-07-2014	S	Ilha de Tavira	55,0	3	58,4	37.105	7.628	unknown
cc142	01-08-2014	S	Praia Verde	44,5	2	49,3	37.174	7.478	unknown
cc143	06-06-2015	S	Molhe de Ferragudo	41,0	2	44,6	37.109	8.523	A2.3
cc144	04-07-2015	S	Praia do Ancão	46,0	2	49,0	37.034	8.041	A1.1
cc145	09-06-2015	S	Meia Praia	50,0	2	52,0	37.112	8.656	A1.1
cc146	11-06-2015	S	Ilha do Farol	56,0	3	58,0	36.976	7.861	A4.2
cc147	21-05-2015	NC	Poço da cruz	54,1	3	60,1	40.481	8.794	A3.1
cc148	03-03-2015		Oceanário	70,4	3	unknown	unknown	unknown	A2.1
cc149	03-04-2015	S	Praia Adão e Eva	41,0	2	43,0	37.176	7.468	unknown
cc150	09-04-2015	S	Praia do Alemão	46,0	2	47,5	37.119	8.564	A1.3
cc151	06-06-2015	S	Praia do Trafal	36,0	2	38,4	37.054	8.076	A2.1
cc152	05-07-2015	NC	Praia de Ofir	79,0	2	83	41.517	8.787	A3.1
cc153	06-07-2015	S	Ilha de Tavira	32,0	1	35,2	37.110	7.620	A1.1
cc154	06-06-2015	S	Praia do Barril	54,0	3	57,8	37.086	7.661	A4.1
cc155	07-07-2015	S	Ilha da Armona	51,5	2	55,8	37.019	7.786	A1.1
cc156	08-07-2015	S	Praia Homem Nú - Barril	49,5	2	53,5	37.081	7.673	unknown
cc157	22-07-2015	S	Praia de Faro	52,5	3	53,4	37.009	7.998	A3.1
cc158	05-11-2015	S	Praia Maria Luísa	63,0	3	66,7	37.089	8.201	unknown
cc159	unknown	unknown	unknown	unknown	unknown	unknown	unknown	unknown	A1.1
cc160	28-02-2016	NC	Praia de São Jacinto	20,0	1	22,0	40.666	8.749	A7.1
cc161	28-05-2016	S	Praia da Rocha	43,0	2	46,3	37.114	8.533	A2.1
cc162	15-06-2016	S	Ilha da Culatra - 600m nascente	47,0	2	52,5	36.986	7.837	A3.1
cc163	06-06-2016	S	Largo de Olhão	44,0	2	49,0	37.023	7.759	A2.1
cc164	28-06-2016	S	Ilha armona - poente	51,0	2	53,5	37.011	7.796	A2.1
cc165	29-06-2016	S	Praia Verde	38,0	2	42,0	37.172	7.483	A1.3
cc166	17-01-2016	NC	Praia Água de Madeiros	25,9	1	29,0	39.740	9.040	A1.1
cc167	25-05-2016	S	Ilha do Farol	36,0	2	36,5	36.976	7.860	unknown
cc168	26-06-2016	S	Praia do Barril	44,0	2	47,0	37.083	7.667	A2.1
cc169	27-06-2016	S	Ilha da Armona	62,0	3	67,0	37.028	7.769	A1.1
cc170	29-06-2016	S	Altura	34,5	2	35,8	37.169	7.492	unknown

cc171	29-06-2016	S	Cabanas Tavira	46,5	2	47,0	37.136	7.583	unknown
cc172	29-06-2016	S	Praia da Lota - Manta Rota	46,0	2	48,5	37.165	7.510	A1.1
cc173	04-07-2016	S	Barra do Lavage	45,0	2	47,5	37.010	7.797	A1.1
cc174	21-06-2016	S	Arroteia - ria	54,0	3	57,2	37.058	7.723	A2.1
cc175	13-06-2017	S	Ilha da Culatra	53,0	3	58	unknown	unknown	unknown
cc176	17-07-2017	S	Praia de Faro	52,0	3	58,0	unknown	unknown	A1.1
cc177	01-05-2017	S	Quinta do Lago - 500m Wdo bar Gigi	49,0	2	51,5	37.028	8.030	unknown
cc178	21-06-2017	S	Monte Gordo	32,0	1	35,0	unknown	unknown	unknown
cc179	21-06-2017	S	Praia Loulé Velho	67,0	3	unknown	unknown	unknown	unknown
cc180	22-07-2017	S	Praia de faro - nascente	60,0	3	64,0	unknown	unknown	unknown
cc181	24-05-2017	S	Praia Alvor poente	62,0	3	66,5	unknown	unknown	A2.1
cc182	03-07-2017	S	Praia do Vau	34,0	2	36,0	unknown	unknown	A2.1
cc183	20-04-2017	S	Manta Rota	44,0	2	47,0	unknown	unknown	A10.1
cc184	07-05-2017	S	Praia do Barranco da Belharuca	51,0	2	54,0	37.088	8.174	A2.1
cc185	10-05-2017	S	Praia da Oura	35,0	2	37,0	37.086	8.224	unknown
cc186	20-05-2017	S	Praia de Faro	48,0	2	50,4	37.010	7.999	A8.1
cc187	21-05-2017	S	Praia Três Irmãos	39,0	2	41,0	37.120	8.584	A2.1
cc188	29-05-2017	S	Praia Loulé Velho	38,0	2	40	unknown	unknown	A1.1
cc189	29-05-2017	S	Praia Rocha Baixinha	52,0	3	57,0	unknown	unknown	A1.1
cc190	20-06-2017	S	Praia de Faro - frente ao paquete	49,0	2	51,0	unknown	unknown	unknown
cc191	21-06-2017	S	Praia Altura - frente ao hotel	39,0	2	43,0	unknown	unknown	A2.1
cc192	21-06-2017	S	Vilamoura	unknown	2	35,0	unknown	unknown	A2.1
cc193	17-07-2017	S	Praia de Faro	40,0	2	45,5	unknown	unknown	A2.1
cc194	18-08-2017	S	Cabanas Tavira	49,0	2	52	unknown	unknown	A2.1

Table 2.1 : Turtle Strandings found in the Portuguese coast.

Dataset 1	WAT	CAP	EMED								
Contribution %	81.97	11.89	6.14								
CI: 97.5%	5.87E-01 to 0.93	5.70E-02 to 0.26	7.96E-06 to 0.25								
Dataset 2	EUS	BAH	GME	BRA	CAP	EMED					
Contribution %	15.18	<0.01	69.20	1.60	12.40	1.61					
CI: 97.5%	1.00E-	3.24E-	1.44E-	4.69E-	3.42E-	3.31E-					

	04 to 0.76	05 to 0.43	05 to 0.86	06 to 0.08	03 to 0.22	06 to 0.18					
Dataset 3	GEO	FLO1	FLO2	BAH	DRT	MEX	KEY	CSK	FLO3	BRA	
Contribution %	6.86	39.68	47.18	<0.01	2.60	1.38	0.16	1.00	0.14	0.99	
CI: 97.5%	1.00E-04 to 0.50	5.05E-05 to 0.49	5.15E-05 to 0.67	2.13E-06 to 0.60	5.89E-09 to 0.57	4.48E-09 to 0.16	3.13E-09 to 0.30	2.36E-09 to 0.62	1.90E-11 to 0.55	2.97E-09 to 0.07	
Dataset 4	CAL	GRE	CRT	DLY	DAL	TKW	TME	CYP	LIR	LYB	MAN
Contribution %	5.29	<0.01	<0.01	<0.01	20.69	<0.01	35.10	<0.01	<0.01	<0.01	38.86
CI: 97.5%	1.00E-04 to 0.37	6.54E-05 to 0.78	1.66E-06 to 0.82	7.98E-06 to 0.91	2.85E-09 to 0.54	7.72E-11 to 0.69	2.64E-11 to 0.42	1.34E-11 to 0.20	1.22E-11 to 0.30	1.22E-11 to 0.34	2.76E-08 to 0.59

Table 2.2 : Percentage contributions of major rookery regions.