Ecology and conservation of green turtles in Guinea-Bissau

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Introduction

Green turtle, a great traveller

Green turtles (*Chelonia mydas*) are a highly migratory species, establishing connectivity between distant areas (Scott et al. 2014a). They venture into their first great migration as soon as they emerge from their nests at sandy beaches and crawl into the sea, where they associate with prevalent currents to disperse into the open ocean (Putman et al. 2010; Scott et al. 2014b). During this oceanic period, they can travel several thousand kilometres, living an epipelagic life-style, which can last 3-5 years (Reich et al. 2007). After this stage, juvenile turtles recruit to shallow coastal foraging areas, where they may remain resident until adulthood, or travel between nearby feeding grounds (Bolten et al. 2003).

During the dispersal stage of green turtles, in the first years of life, animals from various populations mix with the help of ocean currents (Patrício et al. 2017). As a result, in coastal feeding areas, it is common to have aggregations composed of animals from several nesting beaches (mixed stocks; Bolker et al. 2007). It is essential to know the origin of these animals, to understand the threats to which they are subjected throughout their life cycle, and to establish collaborations between countries that share this resource. Through genetic characterisation of individual turtles, it is possible to compare mixed-stock foraging aggregations with nesting populations, to estimate their rookery origin.

Finally, as adults, they perform cyclic breeding migrations, every two to five years on average (Seminoff et al. 2015), between nesting beaches and neritic foraging grounds, covering hundreds to thousands of kilometres each time (Scott et al. 2014a). For such a vagrant animal as the green turtle, the

effective conservation of populations depends highly on understanding the links established between the different areas occupied throughout their life cycle, so that the full-range of threats can be identified and addressed.

Conservation challenges

After centuries of overexploitation for the consumption of meat, eggs, oil and soup (Rieser 2012), the green turtle has been recovering in most of its distribution range, thanks to decades of conservation efforts (Mazaris et al. 2017). However, this species is now facing several different anthropogenic-induced threats, with bycatch (Wallace et al. 2010), plastic pollution (Wilcox et al. 2018; Duncan et al. 2019) and coastal development (Biddiscombe et al. 2020) having the greatest impact. Green turtles are also highly vulnerable to upcoming climate change (Varela et al. 2018; Patrício et al. 2021) and these pressures can act synergistically, further enhancing negative impacts.

The conservation challenges are ever greater, as the aforementioned threats are ubiquitous in our seas and coastal habitats, and even more so because the source/s of some threats are impossible to pinpoint (e.g. plastic pollution or climate change). Ensuring the protection of a range of suitable habitats to allow populations to thrive and adapt is, thus, a priority for population continuity. However, to define which areas are key to protect we must first understand the spatial distribution of populations and the connectivity they establish, through dispersal and migration, between breeding and foraging sites. This is particularly urgent for areas that are poorly known, due to limited research in the past, such as West Africa.

Major population of green turtles in West Africa

West Africa is a region of global importance for green turtles, hosting one of the largest populations globally (SWOT 2011; Patrício et al. 2019). The core breeding rookery for this population is located at Poilão Island, in the southeast limit of the Bijagós Archipelago, Guinea-Bissau (Catry et al. 2009; Barbosa et al. 2018), where an average of 27,251 clutches are laid annually (2013-2017; Broderick & Patrício 2019). Lower numbers of nesting occur on several other islands of the archipelago and on the northern continental coast of Guinea-Bissau (Catry et al. 2002). There is also nesting in other countries of the region, but in much lower numbers (~10 to ~100 nests/year; Agyekumhene et al. 2017).

Foraging aggregations are also known to occur across the Bijagós (Barbosa *pers. comm.*), with an important developmental area around the islands of Unhocomo and Unhocomozinho (Catry et al. 2010), which has yet to be genetically characterized. Important green turtle foraging areas are also known at Cabo Verde (Marco et al. 2011; Monzón-Argüello et al. 2010), at the National Park of the Banc d'Arguin (PNBA), in Mauritania (Cardona

et al. 2009; Godley et al. 2010), around the Bijol Islands, in The Gambia (Hawkes et al. 2008) and in Guinea (Fretey et al. 2008). Additionally, bycatch data support the presence of foraging green turtles in other countries of the region (e.g. Ghana, Togo and Benin; Agyekumhene et al. 2017).

Potentially, several of the foraging aggregations in West Africa are linked to the major rookery of the Bijagós, yet there is a paucity of tracking and of genetic data to confirm this hypothesis. The exception is the connectivity with the PNBA, which was confirmed through satellite tracking of post-breeding females from Poilão Island (Godley et al. 2010). However, the sample size for this study was limited (n = 4) and a more recent work suggests the existence of plasticity in foraging strategies for this population (Patrício et al. 2019), possibly associated with multiple post-breeding destinations.

Study aims

Considering the challenges intrinsic in the conservation of migratory species, and the knowledge gaps in the study region, we set out to understand the spatial distribution of green turtles nesting at Poilão Island, and the connectivity between juvenile green turtles from the Bijagós and Atlantic rookeries.

Specifically, we use 1) genetic analyses to assess the origin of the juvenile green turtles foraging in the Bijagós archipelago, and 2) satellite telemetry to investigate the inter-nesting spatial distribution, the post-breeding migrations and the foraging habitat use by green turtles that nest at Poilão Island. Ultimately, our results will provide the scientific basis to inform marine spatial planning and other conservation measures in the region, aimed at protecting this resource.

Study site

The Bijagós is a deltaic archipelago west of the mainland coast of Guinea-Bissau. It comprises 88 islands and islets and covers an area of 10,000km^2. Only 21 of the islands are permanently inhabited, with a human population of *ca.* 25,000, mostly from the Bijagó ethnic group (Campredon & Catry 2016). Some of the uninhabited islands are considered sacred and only accessed during religious and social ceremonies. These traditional restrictions have contributed to the protection of the archipelago's remarkable biodiversity, which includes several other charismatic species besides the green turtle, notably the West African manatee (*Trichechus senegalensis*), the Atlantic humpback dolphin (*Sousa teuszii*) and the 'marine' hippopotamus (*Hippopotamus amphibious*). A large part of the islands is surrounded by mangroves and extensive mudflats, which provide shelter and developmental areas for many species of fish, molluscs, and crustaceans, and feeding grounds for wintering shorebirds. In addition to the green turtle, three other species of sea turtle nest on the beaches of the archipelago: the olive ridley (*Lepidochelys olivacea*), leatherback

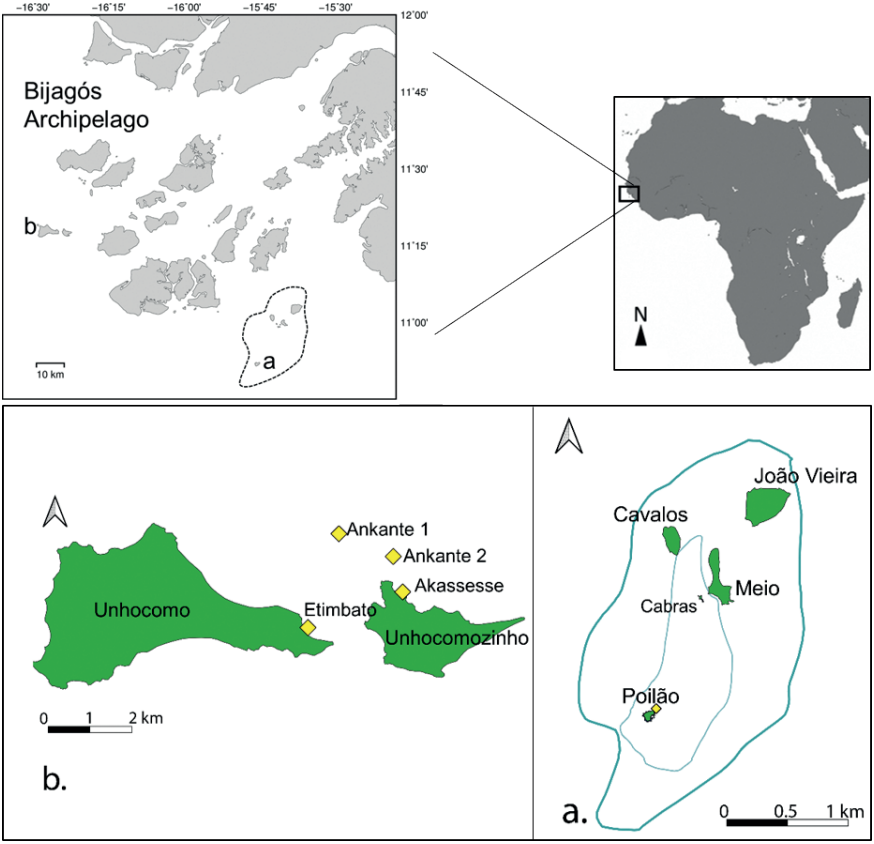

Fig. 1. Upper, right to left: location of the Bijagós Archipelago in West Africa and zoomed map of the Bijagós Archipelago showing the location of the study sites; a. Poilão Island and b. Unhocomo and Unhocomozinho Islands (U&U). Dashed line shows limits of the João-Vieira Poilão National Marine Park (PNMJVP). Lower, right to left: a. Outer limits and delineation of the no-take zone (central zone) of the PNMJVP, and islands within the MPA, including Poilão (yellow diamond); b. Unhocomo and Unhocomozinho Islands (U&U) and in-water capture locations (yellow diamonds).

(*Dermochelys coriacea*), and hawksbill (*Eretmochelys imbricata*). Loggerhead turtles (*Caretta caretta*) are present in the surrounding waters, but do not nest in Guinea-Bissau. This rich biodiversity led to the establishment of three marine protected areas (MPAs) within the archipelago, and to the designation of the Bijagós-Bolama Biosphere Reserve by UNESCO in 1996.

It is within one of the archipelago's MPAs, the João Vieira-Poilão Marine National Park (PNMJVP in its Portuguese acronym), in the southeast limit of the Bijagós, at Poilão Island, that the main green turtle rookery is located (Fig. 1a). Poilão (N 10.87°, W 15.72°) is a low-lying small island, with an area

of 43ha, covered by undisturbed tropical forest, and surrounded by a rocky subtidal zone. It has a tropical climate, with the rainy season between May and November, peaking in August, coinciding with the peak of the nesting season.

Besides Poilão, this MPA has three other islands – João Vieira, Cavalos and Meio – and three islets – Cabras, Águias and Baixo de Gaivotas. After Poilão, most nesting occurs on Cavalos (2,507 nests in 2016), followed by Meio (2,063 nests in 2016) and João Vieira (596 nests in 2011; Barbosa et al. 2018). Although there is marked interannual variability in nesting numbers, the relationship of nesting abundance (absolute number of nests) among these islands has been consistent over the years. Nesting density (nests per square metere) is very high at Cabras Islet, but nesting abundance has yet to be quantified there. The other two islets are submersed during high tide and thus not suitable for nesting.

The main green turtle feeding and developmental grounds in the Bijagós are located at the westernmost limit of the archipelago, in the shallow waters surrounding the islands of Unhocomo and Unhocomozinho (N 11.31°, W 16.40°; Fig. 1b). These feeding grounds are characterized by areas of rocky seabed covered with algae (*Caulerpa* sp., *Sargassum* sp. and *Dictyota* sp.), adjacent to mangroves (*Rhizophora* sp.) and sandy areas, with low-density seagrass patches (*Halodule* sp.) and sparse rocks covered in algae. The sea surface temperature average is 27.3°C (ranging from 25.1°C to 29.5°C).

To assess the connectivity of green turtles from the Bijagós, we collected biopsy samples from juvenile green turtles foraging around Unhocomo and Unhocomozinho and deployed satellite tags on females found nesting at Poilão Island. We also continued the standard monitoring of nesting activities and report the nesting number estimates for the last 15 years.

Methods

Monitoring of nesting activities

Since 2007, IBAP has used a standard protocol to monitor the nesting activities of green turtles at Poilão. Because the island is, for its most part, surrounded by intertidal rocks which are exposed at low tide (Fig. 2), the turtles must wait for high tide to access the beach to nest. It is therefore around the peak of the night high tide that IBAP patrols the 2km beach to assess the number of nesting females. In the early morning, there is an additional survey to count tracks from the previous night, and to count the number of turtles that are stranded on the intertidal rocks. These turtles usually rest in intertidal pools, where they can keep their body temperatures within tolerable limits before returning to the sea with the high tide.

Fig. 2. Left: Poilão Island, in the Bijagós Archipelago, Guinea-Bissau, with intertidal rocks exposed due to low tide. Right: green turtles waiting for high tide to return to the sea. Photos by IBAP (left) and Rita Patricio (right).

Monitoring is conducted annually from August to November, to encompass the nesting distribution. Because nesting density at Poilão is too high to allow counting all the nests, we estimate the number of clutches laid per season by multiplying the number of nesting female emergences by 0.813, to adjust for nesting success in Poilão (Catry et al. 2009).

Genetic assessment

We collected biopsy samples from the epidermis of juvenile green turtles found foraging at Unhocomo and Unhocomozinho for genetic analysis. The number of biopsy samples collected was minimal without jeopardising statistically valid results, and the sampling techniques chosen have been refined over the years of practice to minimise impact on animals (e.g. reduced handling time, reduced size of samples). All procedures used are widely applied in the field of sea turtle research and were carried out by trained personnel following recommended guidelines (NMFS-SFC, 2008) in order to reduce stress to the animals and ensure their welfare.

To capture turtles, we used an entanglement net (800m long, 20cm mesh size) which was deployed from a pirogue operated by Bijagós fishers. Water depth varied from 4-1m. Each net set lasted one hour, and we swam the length of the net throughout this period to look for entangled turtles (i.e. three people started at equidistant points along the net and kept swimming to ensure that turtles were released from the net as fast as possible). Most turtles did not get entangled; instead, they would swim along the net trying to find a way out, and were captured by hand. Once captured, we brought the turtles to a logistic vessel anchored next to the net.

Once at the logistic vessel, we measured the curved carapace length (CCL) using a flexible metric tape to the closest millimetre for each individual and tagged both front flippers with uniquely numbered, self-piercing Inconel tags (©National Band & Co). We used sterile biopsy punches (6mm diameter) to

collect skin samples from the right shoulder, after disinfecting the area with a diluted povidone-iodine solution. We used single-use surgical gloves and biopsy punches for each new sample. Samples were stored in 96% ethanol, inside 2ml Eppendorf tubes uniquely labelled.

We will extract the DNA of samples and amplify specific regions of the mitochondrial DNA (i.e. ~860bp fragment from control region and short tandem repeats; Shamblin et al. 2012; Tikochinski et al. 2018), used in sea turtle population genetic assessments and shown to improve resolution of connectivity studies (Shamblin et al. 2012; Tikochinski et al. 2018). We will then compare the haplotypes found in the Bijagós with haplotypes throughout Atlantic green turtle rookeries, to estimate the contribution of each putative source to this foraging aggregation.

Satellite tracking

During the 2018 to 2020 nesting seasons at Poilão Island, we deployed 45 satellite tags on nesting females (Fig. 3) to study their movements. We also applied flipper tags and passive integrated transponders (PIT) tags to each turtle. To deploy the satellite transmitters, we waited for the turtles to start laying their eggs and then executed the attachment procedure within 20 minutes, while the turtle continued to lay. First, we sanded the second vertebral scute where the tag was to be attached and cleaned it with acetone. Then we applied a base of fibreglass and fast-dry epoxy (®Devcon-5min Epoxy) and allowed it to dry for five minutes. A 'cushion' for the tag was then made with magic metal (®Loctite) before we pressed the tag gently on top of the fibreglass base and allowed ten minutes for it to dry further. For the last step, we applied again fibreglass with epoxy around the tag to secure it, and allowed five minutes for the second fibreglass and epoxy application to dry. All turtles continued with their nesting activity throughout the tag application, successfully laid their eggs, and covered and camouflaged the nest; therefore, we are confident that the procedure did not disturb them.

Preliminary results

This population shows high inter-annual variability in nesting numbers (Fig. 4), and the year 2020 was a record year, with over 60,000 clutches estimated at Poilão Island alone (Fig. 4). This variability in nesting numbers across years is common among sea turtle populations, particularly among green turtles, and it is potentially linked to the availability of food resources at foraging areas, in addition to the natural reproductive cycle of breeding females (Broderick et al. 2001). The very high number of clutches in 2020 reinforces the importance of this population for the maintenance of foraging aggregations across the West Africa region, and potentially, the Atlantic.

Fig. 3. Green turtle with ®Wildlife Computers Spot tag, at Poilão Island, Guinea-Bissau. Photo by Rita Patricio.

At Unhocomo and Unhocomozinho we captured a total of 103 green turtles, of which 99 were immatures, ranging from recruit (i.e. a turtle that recently moved from oceanic to neritic waters) to subadult sizes (CCL: 35-79cm; Fig. 5), indicating that this is mainly a developmental site. We also caught four adults, two females and two males (CCL: 87.5-97.0cm). The genetic assessment at Unhocomo and Unhocomozinho will allow us to estimate the origin of this foraging aggregation, and estimate the connectivity between Atlantic nesting beaches and this juvenile developmental area, further highlighting the importance of the Bijagós Archipelago for Atlantic green turtles.

Thanks to satellite tracking technology, we have obtained daily movements of green turtles from this large population and have some very interesting findings. The spatial distribution during the inter-nesting intervals will be key to understanding whether the current limits of the PNMJVP ensure adequate protection to turtles during the nesting season. Tracking data from their post-nesting migrations will allow us to understand the connectivity established between Poilão and foraging grounds along West Africa. We have found that this population feeds in coastal areas in Guinea-Bissau, Senegal, The Gambia and Mauritania. Knowledge of the spatial distribution of this

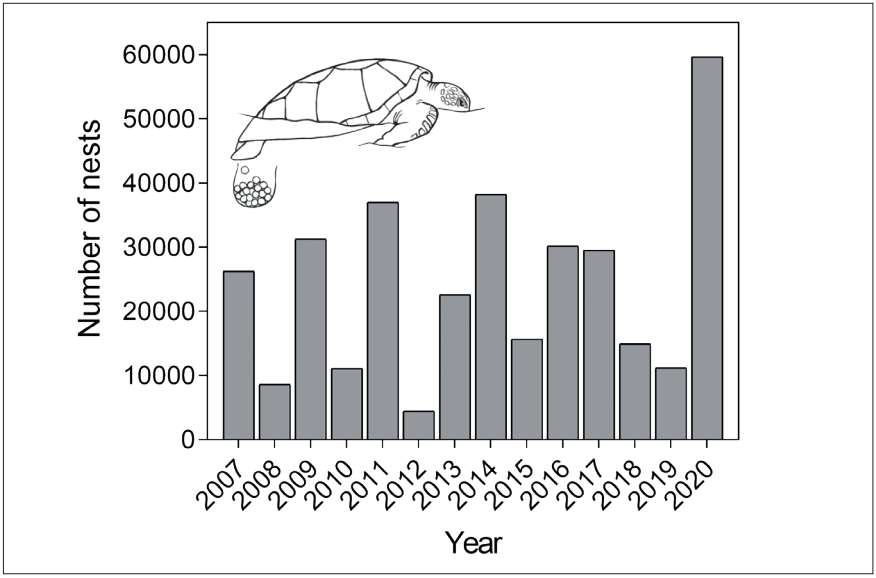

Fig. 4. Estimated number of green turtle clutches at Poilão Island, in the Bijagós Archipelago, Guinea-Bissau.

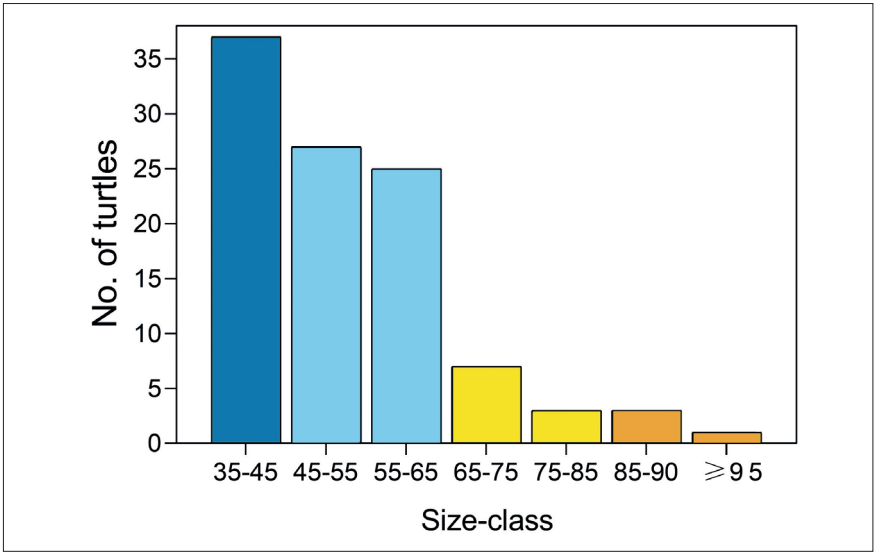

Fig. 5. Size-classes of green turtles found foraging at the water surrounding the islands of Unhocomo and Unhocomozinho, in the Bijagós Archipelago, Guinea-Bissau. Dark blue: recruits (i.e., turtles that have recently moved from oceanic to neritic waters); light blue: resident juveniles; yellow: subadults; orange: adults.

population outside the breeding period will allow us to understand the range of threats that this population may be exposed to, including identifying areas of conflict with fishing activities. Ultimately, these results should help establish collaborations among sea turtle conservation projects within the region, and potentially help define areas in need of protection.

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