Ecology of the green sea turtle (*Chelonia mydas* L) in a changing world

Submitted by

Ana Rita Caldas Patrício

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Signed:

Ana Rita Patrício

Ana R. Patrício

Green turtle hatchling moving towards the ocean at Poilão, Guinea-Bissau

Abstract

Climate change is threatening biodiversity, causing populations and species to adapt, or otherwise, become extinct. Sea turtles have survived dramatic climate changes in the past, however, due to a history of intense human exploitation, and the current anthropogenic threats, their current resilience may be jeopardized. The main pursuits of this thesis were to i) evaluate the resistance of green turtles to predicted climate change impacts, using a globally significant rookery, in Poilão, Guinea-Bissau, as a case study; and ii) assess key population parameters to inform the conservation management of this resource. As the work developed I additionally had the opportunity to study the dynamics of an emerging disease in a juvenile foraging aggregation from Puerto Rico, which contributed to a broader understanding of resilience in this species. Specifically, I investigate the nest site selection behaviour of green turtles, their nesting environment, and the outcomes for their offspring, at Poilão, and apply this information to infer on the resilience of this population under future scenarios of climate change. I explore the connectivity established by the dispersal of post-hatchlings from Poilão, followed by their recruitment to foraging grounds, to set the geographical context of this major population. Lastly, I model the dynamics of Fibropapillomatosis, which affects juvenile green turtles globally, and examine the potential for disease recovery. The green turtle rookery in Poilão shows some resilience to expected climate change impacts. This significant population likely contributes to all juvenile foraging aggregations along the west coast of Africa, and to some extent to those in South America. Currently, green turtles are capable of recovery from Fibropapillomatosis, however, the incidence of disease may be enhanced by climate change.

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Author’s declaration

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Chapter 1: Balanced primary sex ratios and resilience to climate change in a major sea turtle population

Ana R. Patrício, Ana Marques, Castro Barbosa, Annette C. Broderick, Brendan J. Godley, Lucy A. Hawkes, Rui Rebelo, Aissa Regalla and Paulo Catry

ARP, PC, BJG, ACB and LAH planned fieldwork methods. ARP, AM, CB, and PC conducted fieldwork. CB and AR coordinated fieldwork logistics and research permits. AM and RR conducted histological examination of hatchlings gonads. ARP assembled and analysed data, produced all figures and tables, and was lead author on the manuscript. BJG, PC, RR, ACB and LAH provided guidance on data analysis and writing, and all co-authors provided useful comments on the manuscript.

Chapter 2: Nest site selection repeatability and success of green turtle *Chelonia mydas* clutches

Ana R. Patrício, Miguel R. Varela, Castro Barbosa, Annette C. Broderick, Brendan J. Godley, Maria B. Ferreira Airaud, Aissa Regalla, Dominic Tilley, Paulo Catry

ARP, PC, BJG, and ACB planned fieldwork methods. ARP, MRV, CB, MBFA, DT, and PC conducted fieldwork. CB and AR coordinated fieldwork logistics and research permits. MRV conducted drone aerial surveys, photogrammetry analysis and created the orthophotos and digital elevation models (DEMs) of the nesting beach. ARP assembled and analysed data, produced all figures and tables, and was lead author on the manuscript. PC, BJG and ACB provided guidance on data analysis and writing, and all co-authors provided useful comments on the manuscript.

Chapter 3: Climate change resilience of a globally important green turtle population

Ana R. Patrício, Miguel R. Varela, Annette C. Broderick, Paulo Catry, Lucy A. Hawkes, Aissa Regalla, Brendan J. Godley

ARP, MRV, and PC conducted fieldwork. AR coordinated fieldwork logistics. MRV conducted drone aerial surveys, photogrammetry analysis and created the orthophotos and digital elevation models (DEMs) of the nesting beach. ARP assembled and analysed data, produced all figures and tables, and was lead author on the manuscript. BJG, LAH, PC, and ACB provided guidance on data analysis and writing, and all co-authors provided useful comments on the manuscript.

Chapter 4: Dispersal of green turtles from Africa's largest rookery assessed through genetic markers

Ana R. Patrício, Angela Formia, Castro Barbosa, Annette C. Broderick, Mike Bruford, Carlos Carreras, Paulo Catry, Claudio Ciofi, Aissa Regalla, Brendan J. Godley

ARP, CB and PC conducted fieldwork. CB and AR coordinated fieldwork logistics and research permits. AF contributed partially to the molecular analyses, with the collaboration of MB and CCiofi. CCarreras supervised molecular analyses conducted by ARP. ARP assembled and analysed data, produced all figures and tables, and was lead author on the manuscript. BJG, CCarreras, PC, and ACB provided guidance on data analysis and writing, and all co-authors provided useful comments on the manuscript.

Chapter 5: Novel insights into the dynamics of green turtle fibropapillomatosis

Ana R. Patrício, Carlos E. Diez, Robert P. van Dam and Brendan J. Godley

ARP, CED and RPD conducted fieldwork. ARP assembled and analysed data, produced all figures and tables, and was lead author on the manuscript. BJG, provided guidance on data analysis and writing, and all co-authors provided useful comments on the manuscript.

List of notations and abbreviations

Notations:

h – Haplotype diversity
π – Nucleotide diversity
ΦST – Genetic distances
K – Fulton’s body condition index
R - Repeatability

Abbreviations:

AR5 – Fifth assessment report
BCI – Body condition index
BLAST - Basic Local Alignment Search Tool
bp – Base pairs
CCL – Curved-carapace-length
ChHV5 – Chelonid herpesvirus-5
CMA – *Chelonia mydas* Atlantic
CMR – Capture-mark-recapture
CSI – Cumulative Impact Score
DEM – Digital elevation model
DPS – Distinct population segment
ESD – Environmental-dependent sex determination
F – ‘Forest’
FB – ‘Forest border’
FDR – False discovery rate
FP - Fibropapillomatosis
GAM – Generalized additive model
GCP – Ground control point
GLM – Generalized linear model
GSD – Genotypic sex determination
HST – Highest spring tide
IP – incubation period to hatching

$\mathrm{IP}_{\mathrm{mid}}$ – middle third of the incubation period

IPCC – Intergovernmental Panel on Climate Change

m2m MSA – Many to many mixed stock analysis

MPA – marine protected area

MSA – Mixed stock analysis

mtDNA – Mitochondrial DNA

OS – ‘Open sand’

OSR – Operational sex ratio

PCoA – Principal components analysis

PIT – Passive integrated transponder (tag)

PNMJVP – National Marine Park of João Vieira and Poilão

RCP - Representative concentration pathways

SCL – Straight-carapace-length

SLR – Sea level rise

STR – Short tandem repeats

TRT – Transitional range of temperatures

TS – Tumour score

TSD – Temperature-dependent sex determination

TSP – Thermosensitive period

General introduction

Marine turtles have been swimming in the world's oceans and nesting on its fringing beaches for over 200 million years, surviving the mass extinction which saw the loss of dinosaurs. Since prehistory these resilient, long-living, marine ectotherms have been part of the human culture, playing important roles in mythology around the world (Stookey 2004), and used in religious ceremonies (Allen 2007, Catry et al. 2009), as well as representing an important protein source for coastal populations (Frazier 2003, Allen 2007). More recently, extensive trading of their meat, eggs, cartilage, oil, carapaces, and body parts, used as talismans, jewellery or other luxury items, led to the over-exploitation of sea turtles globally, and depletion of local populations (Bjorndal & Jackon 2002).

Among the seven extant sea turtle species, the green turtle *Chelonia mydas* L, is probably the most charismatic (Rieser 2012), and historically the most widely exploited for human consumption (Aiken et al. 2001, Rieser 2012). Conservation efforts for the past decades, leading to laws protecting sea turtles and their habitats and increased awareness, have contributed to the recovery of several of the major green turtle populations worldwide (Broderick et al. 2006, Chaloupka et al. 2008). However, the list of threats to these animals remains considerable, most notably bycatch from industrial and artisanal fisheries, illegal harvesting, habitat degradation, plastic ingestion, and climate change (Hamann et al. 2010). Sea turtles have endured pronounced climate changes in the past (Poloczanska et al. 2009), yet, it is uncertain whether they will be able to adapt to the current rapid changes, particularly as they face other human-induced threats that may act synergistically with climate change impacts (Brook et al. 2008).

In the present thesis, **'Ecology of the green sea turtle (*Chelonia mydas* L) in a changing world'**, throughout five chapters, written as independent units of study, I explore the impacts of climate change, and potential for adaptation, on a globally important green turtle population in Guinea-Bissau; I look into the connectivity of this population for a more complete understanding of its significance at a regional level; and I use the case study of a green turtle juvenile aggregation in Puerto Rico to assess the current impacts of an emerging disease, which may be enhanced by climate change (Harvell et al. 2002).

In the first chapter, **‘Balanced primary sex ratios and resilience to climate change in a major sea turtle population’**, we model the population-specific sex determination response to incubation temperatures at Poilão, Guinea-Bissau, and apply the fitted model to estimate the primary sex ratio across the nesting season and nesting habitats. Our results are surprisingly different from the most common reports of highly-female biased primary sex ratios, and we found that the native vegetation was crucial for the production of male hatchlings. Additionally, we highlight the importance of using population-specific parameters and of estimating the transitional range of temperatures, to understand the response of populations to climate change.

In the second chapter, **‘Nest site selection repeatability and success of green turtle *Chelonia mydas* clutches’**, we monitor the nest site selection behaviour of adult females in the same rookery, and the consequences for their offspring. We conduct the first repeatability analysis of nest site choice in green turtles, and found that individuals were both highly repeatable on their nesting habitat, and highly philopatric at a very fine-scale. Nest site selection involved tradeoffs in hatchling phenotype, but overall it enhanced clutch survival, suggesting it is an adaptive behaviour, while the high repeatabilities indicate potential for heritability of this trait. We explore here the potential of this behaviour for mitigation of predicted climate change impacts.

In the third chapter, **‘Climate change resilience of a globally important green turtle population’**, we apply a vulnerability framework to conduct a comprehensive assessment of climate change resistance, using the most uptodate climate models by the IPCC, together with empirical data. We estimate the impacts of global warming on the primary sex ratio and on female hatchling output, and of sea level rise on the current nesting habitat. We further explore the availability of spatial and temporal microrefugia, and, based on the knowledge obtained from this and the two previous chapters, discuss the potential for adaptation/mitigation of expected impacts. We found this rookery to be resistant to climate change with potential for resilience to expected impacts. The methodology used is transferrable to other rookeries, allowing comparisons between populations, and region-wide assessments.

Due to their migratory behaviour, marine turtles establish important links between distant geographic areas, encountering a range of threats throughout their movements. It was therefore important for us to unravel the connectivity of this major green turtle rookery. So, in the fourth chapter, **‘Dispersal of green turtles from Africa’s largest rookery assessed through genetic markers’**, we analyse the genetic composition of the rookery at Poilão, and conduct a regional mixed stock analysis, incorporating all data available from Atlantic green turtle nesting populations and juvenile foraging aggregations. We identified a haplotype previously only detected among green turtle juveniles, in African and South American aggregations. We estimated that the majority of the post-hatchlings disperse along the west coast of Africa, recruiting to African foraging grounds, but a meaningful proportion accomplishes a transatlantic migration, lilkely recruiting to South American juvenile aggregations.

In the fifth and final chapter, **‘Novel insights into the dynamics of green turtle fibropapillomatosis’**, we model the dynamics of Fibropapillomatosis (FP), an infectious neoplastic disease of marine turtles, using a long-term dataset from a juvenile aggregation in Puerto Rico. Although in this last chapter we study green turtles belonging to a different regional management unit, the work is relevant to the population in Guinea-Bissau, and globally, as insight gained should be applicable to other foraging aggregations affected by this disease. This is in fact the case of some West African aggregations, namely in Príncipe Island and Corisco Bay, to which the rookery of Poilão contributes, as revealed in chapter 4. We found that FP does not currently seem to be a major threat to green turtle populations, however, there is a paucity of data on disease prevalence in many regions, which needs to be addressed, particularly as human-induced stressors, in particular increased sea surface temperatures due to climate change, can lead to deviations in host−pathogen relationships and enhance disease virulence.

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Chapter 1: Balanced primary sex ratios and resilience to climate change in a major sea turtle population

Ana R. Patrício[1, 2], Ana Marques[3], Castro Barbosa[4], Annette C. Broderick[1], Brendan J. Godley[1], Lucy A. Hawkes[1], Rui Rebelo[3], Aissa Regalla[4] and Paulo Catry[2]

[1] Centre for Ecology and Conservation, University of Exeter, TR10 9FE, Penryn, UK
[2] MARE – Marine and Environmental Sciences Centre, ISPA – Instituto Universitário, 1140-041, Lisbon, Portugal
[3] Centre for Ecology, Evolution and Environmental Changes (CE3C), Faculdade de Ciências, Universidade de Lisboa, Bloco C2, Campo Grande, 1749-016 Lisboa, Portugal
[4] Institute of Biodiversity and Protected Areas of Guinea-Bissau (IBAP), CP – 70, Bissau, Guinea Bissau

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Abstract

Global climate change is expected to have major impacts on biodiversity. Sea turtles have temperature-dependent sex determination, and many populations produce highly female-biased offspring sex ratios, a skew likely to increase further with global warming. We estimated the primary sex ratio at one of the world's largest green turtle *Chelonia mydas* rookeries in Guinea-Bissau, West Africa, and explored its resilience to climate change. In 2013 and 2014, we deployed dataloggers recording nest (n=101) and sand (n=30) temperatures, and identified hatchling sex by histological examination of gonads. A logistic curve was fitted to the data, to allow predictions of sex ratio across habitats and through the nesting season. The population-specific pivotal temperature was 29.4ºC, with both sexes produced within incubation temperatures from 27.6 to 31.4ºC: the transitional range of temperatures (TRT). Primary sex ratio changed from male- to female-biased across relatively small temporal and spatial scales. Overall it was marginally female biased, but we estimated an exceptionally high male hatchling production of 47.7% (95% CI: 36.7–58.3%) and 44.5% (95% CI: 33.8–55.4%) in 2013 and 2014, respectively. Both the temporal and spatial variation in incubation conditions and the wide range of the TRT suggest resilience and potential for adaptation to climate change, if the present nesting habitat remains unchanged. These findings underline the importance of assessing site-specific parameters to understand the response of populations to climate change, particularly with regard to identifying rookeries with high male hatchling production that may be key for the future conservation of sea turtles, under projected global warming scenarios.

Introduction

Sex ratio is an important parameter to assess population viability and resilience (Melbourne and Hastings 2008, Mitchell et al. 2010). Balanced sex ratios, where males and females are approximately equal in numbers, seem to be the norm among species with genotypic sex determination (GSD) where frequency-dependent selection on the primary sex ratio is strong (Fisher 1930). In species with environmental-dependent sex determination (ESD) however, deviations from this equilibrium are widely observed (Bull 1983). Temperature-dependent sex determination (TSD) is the most common mechanism of ESD, in which offspring sex is determined by the incubation temperatures experienced during the thermosensitive period (TSP), corresponding approximately to the middle third of embryogenesis (Bull 1983). This is the mechanism of sex differentiation among crocodilians (Lang & Andrews, 1994), sphenodontians (Mitchell et al. 2010), some lizards (Viets et al. 1994), and most turtle species (Mrosovsky & Yntema 1980).

Among sea turtles, clutches demonstrate a thermal tolerance of 23 ºC to 35 ºC during incubation (Ackerman 1997, Howard et al. 2015). During the TSP, higher incubation temperatures produce female offspring, and lower incubation temperatures produce males (Mrosovsky & Yntema 1980). Between these extremes, there is a transitional range of temperatures (TRT) at which both sexes can be produced (Mrosovsky & Yntema 1980). The constant temperature resulting in a 1:1 sex ratio is known as the pivotal temperature, and it has been shown under laboratory conditions to be approximately 29 ºC for most sea turtle species (Ackerman 1997, Hawkes et al. 2009; Witt et al. 2010). Under natural conditions incubation temperatures fluctuate, typically associated with rainstorm events (Godfrey et al. 1996, Houghton et al. 2007, Lolavar & Wyneken, 2015, Matsuzawa et al. 2002) or diel temperature variation (Georges 2013), therefore, the equivalent of the pivotal temperature is given as the mean of the temperatures experienced during the middle third of development leading to a balanced sex ratio (Mrosovsky & Pieau 1991, Girondot & Kaska 2014). Relatively few field studies have derived ‘pivotal temperatures’ (but see Broderick et al. 2000, Godley et al. 2002).

Because extreme temperatures could lead to the production of hatchlings of a single sex, sea turtles have been considered vulnerable to rapid climate and habitat change, as these may modify the thermal environment of their nests, skewing primary sex ratios (Hawkes et al. 2009, Mitchell & Janzen 2010, Poloczanska et al. 2009, Witt et al. 2010). Only one study thus far has described male-biased primary sex ratios (Esteban et al. 2016). The majority of studies at sea turtle rookeries have estimated female-biased hatchling sex ratios, likely to worsen with future climate change (Hawkes et al. 2007, Fuentes et al. 2009, Fuentes et al. 2010a, Katselidis et al. 2012, Reneker & Kamel 2016), and beachfront deforestation (Kamel & Mrosovsky 2006a, Kamel 2013) . Feminising temperatures prolonged through generations could potentially lead to adaptive responses; by phenotypic plasticity and/or microevolutionary shifts in threshold temperatures, or otherwise lead to population extinction (Hulin et al. 2009, Mitchell & Janzen 2010). Although sea turtles have endured pronounced past climate variations (Poloczanska et al. 2009), it is uncertain whether they can adapt to the predicted future scenarios of change. Additionally, despite the fact that many major populations are recovering from historical exploitation following conservation efforts (McClenachan et al. 2006, Weber et al. 2014), climate change impacts may act synergistically with other existing threats to arrest population growth (Brook et al. 2008). Populations of sea turtles that nest across a wider range of thermal conditions should produce a broader variation in offspring sex ratio and thus should be more resilient to climate change and have higher chances of adaptation (Fuentes et al. 2013, Abella Perez et al. 2016).

Despite the increase in research on sea turtle primary sex ratios, and on the impacts of climate change in this trait (Rees et al. 2016), there are significant gaps in information at both regional and species levels (Fuller et al. 2013, Hawkes et al. 2009). The majority of research has been focused on loggerhead turtles *Caretta caretta*, followed by green turtles *Chelonia mydas*, with less data on the remaining species (Hawkes et al. 2009). Geographically, most studies have been conducted on Mediterranean (Broderick et al. 2000, Casale et al. 2000, Godley et al. 2001a, Kaska et al. 2006, Zbinden et al. 2007, Katselidis et al. 2012, Fuller et al. 2013, Candan & Kolankaya 2016), West Atlantic (Marcovaldi et al. 1997, Godfrey & Mrosovsky 2006, Hawkes et al. 2007, Houghton et al. 2007, Mrosovsky et al. 2009, LeBlanc et al. 2012, Patino-Martinez et al. 2012, Kamel 2013, Marcovaldi et al. 2014, Braun McNeill et al. 2016, Laloë et al. 2016, Marcovaldi et al., 2016, Reneker & Kamel 2016) and Australian (Booth & Freeman 2006, Fuentes et al. 2009, Fuentes et al. 2010a) turtle populations. Very limited information is yet available for most of the Pacific (King et al. 2013, Kobayashi et al. 2017), the Indian (Esteban et al. 2016), and the Eastern Atlantic Oceans (Abella Perez et al. 2016).

Poilão Island, in Guinea-Bissau, West Africa, hosts one of world’s largest green turtle nesting populations (Catry et al. 2002, Catry et al. 2009), and is the main nesting site within the green turtle Southern Atlantic distinct population segment (DPS, Seminoff et al. 2015). A study using dead hatchlings to predict primary sex ratios estimated 45% and 15% of male offspring for early and late-season clutches respectively, with these differences likely being explained by rainfall (Rebelo et al. 2012). Although Rebelo et al. (2012) importantly detected a temporal variation in male production at Poilão, their study did not encompass the duration of the nesting season, nor the diversity of nesting habitats. We aimed to contribute to the regional knowledge on green turtle primary sex ratios, and set out to (1) estimate population-specific pivotal temperature and TRT, (2) determine the range of temporal and spatial incubation conditions available throughout the nesting season, and (3) predict the current primary sex ratio at Poilão Island.

Materials and methods

Study site

In Guinea-Bissau, green turtles nest throughout the Bijagós Archipelago, with the vast majority of the clutches laid at Poilão (10°52’N, 15°43’W, Catry et al. 2002, Catry et al. 2009), the smallest and southernmost island within the João Vieira and Poilão Marine National Park (JVPMNP, Fig. 1a). An estimate of 29,000 clutches are laid annually here (Catry et al. 2009). Poilão has a total area of 43 ha, is covered by undisturbed tropical forest, and sandy beaches extend for 2km of the ca. 4km coastline (Fig. 1b). The nesting season (mid-June to mid-December, peaking in August and September; Catry et al. 2002), largely coincides with the rainy season (May to November), although sporadic nesting occurs year-round (C. Barbosa, *pers. obs.*).

Temporal nesting distribution

To assess the number of adult female emergences we conducted systematic track counts from 7 August to 21 November 2013 (106 d), and from 10 August to 28 November 2014 (111 d). Weather conditions prevented us from surveying the beach on seven (6.6% of the period covered) and three (2.7% of the period covered) days, in 2013 and 2014, respectively. We used linear interpolation to account for missing data (Godley et al. 2001b). Our surveys did not cover the beginning and end of the nesting season, so previous surveys (2000 and 2007; Catry et al. 2009) were used to reconstruct mean nesting frequency distribution at Poilão, at the start and end of the season. Following Metcalfe et al. (2015), we pooled daily counts into half-month bins, and divided each half-month value by the maximum half-month value (i.e. bin with the highest track count) to obtain a distribution of the mean proportion of the season’s maximum. We did not divide each bin by the total sum of the track counts because (as mentioned above) not all of each season’s emergences were recorded. We further reconstructed one half-bin at the beginning of the season, starting in 15 June, by attributing a value of 50% of the subsequent half-month bin, to cover the whole nesting season (Metcalfe et al. 2015).

Spatial nesting distribution

The nesting area was divided in four beach sections, from West to East (1-4, Fig.1b). A smaller beach in the east (5; Fig.1b) was not monitored due to difficult access; nests there represented <5% of the overall numbers (C. Barbosa, *pers. obs.*). Within each section we classified the distribution of nests according to three habitats: 'forest', 'forest border' and 'open sand'. The forest habitat encompassed the nesting area surrounded by vegetation and was shaded, the forest border comprised a band within 0-1m of the vegetation and experienced partial shade, and the open sand corresponded to the area from >1m of the vegetation to the high tide line, which was exposed to the sun throughout all or most of the day (see Fig. S1).

Due to the exceptionally high nesting density at Poilão, females typically disturb each other's nests (Catry et al. 2009), making it impractical to locate these, even on the subsequent morning. Thus, to determine nest distribution across habitats we monitored turtle nesting activity at night, for three nights in 2013 (n=407 nests identified) and six nights in 2014 (n=1,152 nests identified), during the peak of the nesting season, and determined the habitat and beach section for all 1,559 nests. During these focused assessments we surveyed all four beach sections (2km), at high tide (see Catry et al. 2002), and as quickly as possible (typically <1 hour), to ensure that most females were detected. Only females that were laying, covering or camouflaging nests were counted, as otherwise turtles could still change their location or abandon nesting activity. To avoid counting the same female twice, this survey was conducted by one person only, and only in one direction (i.e. on return no turtles were counted), additionally, in wider beach sections with higher density, temporary marks were drawn in the sand to identify a counted animal. We used chi-square statistics to test if the distribution of nests among beach sections, and among habitats within each beach section, was independent of survey date, within and between years.

Nest and sand temperatures

From September to November 2013, and August to October 2014, encompassing the peak of the nesting seasons, we recorded hourly nest temperatures with Tinytag-TGP-4017 dataloggers (Gemini Data Loggers, ± 0.3°C accuracy, 0.1°C resolution). We placed dataloggers in the centre of each clutch (n=101 nests; 46 and 55 in 2013 and 2014, respectively), after *ca.* 50 eggs were laid, and we encircled each nest with three wooden poles, to help prevent destruction by other nesting females. The dataloggers had a long red string attached, extended to the subsurface, so it was easier to find them upon nest excavation, additionally, we surveyed these nests daily, to detect any perturbation. For a subset of nests (n=30; 16 and 14 for 2013 and 2014 respectively), control dataloggers were deployed 1m from the clutch, at a mean mid-clutch depth of ~70cm (local unplubl. data), to estimate the difference in sand temperature associated with metabolic heat produced by the eggs (Broderick et al. 2001a). Nest and control loggers were distributed across the four beach sections (section 1: n=19 nests; 5 control sites, section 2: n=25; 7, section 3: n=26; 8, section 4: n=31; 10), and the three habitats identified ('open sand': n=64 nests; 11 control sites, 'forest border': n=21; 9, 'forest': n=16; 10). All dataloggers were calibrated before and after each field season in a constant temperature room (24h at 28ºC) and used only if accuracy was ≤0.3ºC. Data were used to calculate mean temperatures during the middle third of incubation (IP_{mid}), with the incubation period (IP) ending at hatching (identified as a peak in temperature followed by a decrease until emergence; Matsuzawa et al. 2002). We discarded the initial four hours of temperature records, to enable data loggers to equilibrate with the surrounding sand (Broderick et al. 2001a).

For each nest we recorded beach section and habitat. At nest excavation we further recorded: nest chamber depth (after all nests contents were removed), clutch size (from a count of hatched and unhatched eggs), hatching success (H%=n hatched egg shells/clutch size), and emergence success (E%=(n egg shells – n dead and live hatchlings found inside nest chamber)/clutch size).

A 'reference' datalogger was left to measure sand temperature from March 2013 to March 2015, to encompass both nesting seasons, and to enable comparisons with local air temperature. Due to the risk of dataloggers being removed outside of the monitoring campaign (by turtles or people), the reference datalogger was secured to a fixed structure, within the 'forest border' habitat, minimizing chances of loss. We assessed the relationships between sand temperatures at the 'open sand' and the 'forest' habitats against the 'forest border' habitat, where we had the reference datalogger, and used the later as reference to extend sand temperature estimations at each habitat through the entirety of the nesting seasons. We estimated IP_{mid} mean incubation temperatures for nests laid from 15 June to 15 December (2013 and 2014) by calculating an 18-day moving average of sand temperature at each habitat, 18 days corresponding to the mean duration of IP_{mid} (this study), and added mean metabolic heating (0.5 ± 0.4 ºC, mean value for this study). Sand temperature was regressed against air temperature, obtained from the National Climatic Data Centre (http://cdo.ncdc.noaa.gov/CDO/cdo, Bolama station, 50km distance), to reconstruct sand temperatures for periods of missing data (i.e. when no dataloggers recorded sand temperature).

Sex ratio estimations

In 2013 we deployed wire traps (50cm diameter x 30cm height, wire mesh 1cm^2) above 27 of the monitored nests (i.e. nest with dataloggers) from Day 45 of incubation, checking them daily for emergent hatchlings. A random sample of four to five hatchlings per nest (total 131 hatchlings) were sacrificed, following procedures in Stocker (2005), for sex identification. Straight-carapace-length (SCL) of hatchlings was measured to 0.01cm with a digital caliper. Sampling and handling protocols were approved by the research ethics committee of the University of Exeter, and the government of the Republic of Guinea-Bissau. Kidney-gonad complexes were extracted through dissection and stored in 96% ethanol. In an effort to compensate for this action, across the two field seasons, we saved over 2,000 hatchlings from stranding on the intertidal rocks, where they generally die from exposure to sunshine and avian predators. Histological examination of gonads was conducted at the University of Lisbon. Cross sections of the kidney-gonad complex were kept for 16 hours in a 50:50 mix of resin (Kulzer, Technovit 7100 system) and 96% ethanol, followed by 24 hours in 100% resin, and a further 24 hours in a mix of resin and hardener (Kulzer, Technovit® 7100 hardener, 1ml for each 15ml of resin). The cross sections were then sectioned further into 3um-width slices using a Leica RM 2155 microtome, allowed to dry for 24 hours, stained with toluidine blue for one minute and mounted with NeoMount glue. Photographs of each section were obtained with a Leica DFC 290, using software Irfanview v.4.27 (Skiljan 2012). Identification of gonad structures and paramesonephric ducts followed criteria described in Miller & Limpus (2003). Sex assignment was independently conducted by two researchers (AM and RR). Consistency in sex identification was 95% (compared for 131 hatchlings); for mismatched assignments (n=7) observers conferred until reaching agreement.

Data analysis

Generalised Linear Models (GLM) with Gaussian error structure and identity link function were used to test for the effects of beach, habitat, nest depth and clutch size (independent variables) on i) IP_{mid} mean incubation temperature (response variable); and ii) hatching and emergence successes (response variables).

Most studies consider the IP_{mid} as the TSP, however, as gonad differentiation depends on embryonic development rather than incubation duration, the TSP in nests with fluctuating temperatures may differ from the IPmid (Girondot & Kaska, 2014). We thus used R package embryogrowth v.6.4 (see Girondot & Kaska, 2014 for detailed methods), which accounts for the stages of embryonic development in response to temperature, to estimate the beginning, end, and mean incubation temperatures of the TSP, for each nest with sexed hatchlings, using gastrula size for *C. mydas* from Kaska & Downie (1999), mean hatchling size (SCL) from our data, and remaining parameters following Girondot & Kaska (2014). GLMs with binomial errors and logit function were fitted to our data of sex ratio (response variable) against the following independent variables: i) IP_{mid} mean incubation temperature, ii) TSP mean incubation temperature, and iii) IP (to hatching). We assessed goodness-of-fit of GLMs through p-values and deviance. The best-fit logistic response function with 95% confidence intervals (CI) and reconstructed TSP mean incubation temperatures, across habitat and nesting season, were used to estimate primary sex ratios in 2013 and 2014. All statistical tests and models were conducted using R v.3.2.5 (R Development Core Team 2008). Estimates are presented as mean ± SD, unless stated otherwise.

Results

Nesting distribution

During our daily surveys, from early August to late November, we counted 48,696 green turtle tracks in 2013, and 83,304 in 2014, corresponding to 24,348 and 41,652 female emergences, respectively (each emergence corresponding to an ascending and a descending track). Following Catry et al (2009), we multiplied the number of emergences by 1.05, to account for the period of the nesting season that we did not monitor, and by 0.813 to adjust for nesting success (Catry et al. 2009). We estimate that in total 20,785 clutches (95% CI: 18,049 – 22,855) were laid in 2013 and 35,556 clutches (95% CI: 30,877 – 39,099) were laid in 2014. Peak nesting activity in both years was from August to September, coinciding with heavier precipitation (Fig. 2a, b, e, f).

The largest proportion (34.7 ± 1.4%) of tracks were found in section 1, followed by 24.9 ± 0.2% in section 4 and 20.4 ± 0.6%, and 20.0 ± 1.0% in sections 3 and 2 respectively. There was no difference in nesting distribution among beach sections ($\chi^2_{(3)}$=0.14, *P*=0.98) or habitats ('forest', 'forest border', 'open sand'; Table S1) within and between study years. We thus calculated the mean nesting distribution among habitats; within each beach section (Fig.1b), and overall. Most of the clutches were laid in the open sand 64.2 ± 7.9 %, followed by the forest 22.1 ± 7.8%, and forest border 13.7 ± 5.1%.

Incubation temperatures

Clutch size (120.3 ± 30.2, n=98, $F_{1,95}$=0.7, *P*=0.4) and bottom nest depth (0.8 m ± 0.2, n=98, $F_{1,97}$=0.8, *P*=0.4) were poor predictors of IP_{mid} mean incubation temperatures. However, there were significant differences among nesting habitats ($F_{2,89}$=27.1, *P*<0.01), with IP_{mid} mean incubation temperatures increasing from the 'forest' (28.3 ºC ± 0.7; range: 27.5 – 29.0 ºC, n=16), to the 'forest border' (29.7 ºC ± 0.7; range: 28.5 – 30.3 ºC, n=21), and to the 'open sand' (30.6 ºC ± 0.8; range: 29.2 – 32.3 ºC, n=64). Additionally, there were significant differences in IP_{mid} mean incubation temperatures among beach sections ($F_{3,89}$=27.1, *P*<0.01), and within habitats among beach sections (i.e. interaction of beach section and habitat: $F_{6,89}$=27.1, *P*=0.04). A post hoc Tukey HSD test indicated that the IP_{mid} mean incubation temperature at the 'open sand' habitat in eastern beach sections (3 and 4 in Fig.1b) was significantly warmer (31.1 ºC ± 0.6; range: 29.7 – 32.8 ºC, n=38, Fig. S2, Table S2) than in the western sections (1 and 2 in Fig.1b). In addition, IP_{mid} mean incubation temperatures of the open sand nests located in the western sections (29.9 ºC ± 0.6; range: 29.2 – 31.1 ºC, n=25) were not significantly different from the nests located in the 'forest border' (P=0.45). Thus, clutches laid at the open sand in the western beach sections' experienced the same incubation temperatures predicted for the forest border habitat.

To estimate mean incubation temperatures at each habitat throughout both nesting seasons, we added mean daily differences in sand temperature, at the open sand (1.0 ºC; Fig. S3a, b) and at the forest habitat (-1.5 ºC; Fig. S3a, b), to the 18-day moving averages of the reference sand temperatures ('forest border'). Sand temperatures were highly correlated among habitats (open sand vs. forest border r^2=0.96, and forest border vs. forest r^2=0.94; Fig. S3c). We were unable to get sand temperatures for December 2013 and for July 2014, so we reconstructed these with air temperature using the equation $T_{sand}=0.94T_{air} + 3.04$ (T=temperature ºC, $F_{1,37}$=54.53, P<0.0001, r^2=0.60; Fig. S4). Finally, we added 0.5 ºC of mean metabolic heating, estimated for the IP_{mid} (0.5 ºC ± 0.4, range: -0.4 – 1.2 ºC, n=20). There were no significant differences among habitats in metabolic heating ($F_{12, 17}$=1.7, P=0.22). Lower IP_{mid} incubation temperatures were predicted for nests laid in July and August, with higher temperatures expected for clutches laid in September and October (Fig. 2c, d).

Incubation period

We were able to estimate the IP (to hatching) of 88 nests, ranging from 40 to 70 days, with a mean of 53.5 ± 5.0 days. For the remaining 13 nests we estimated the IP by subtracting from the emergence date the mean length of the period between hatching and emergence, which was 5.0 ± 1.4 days. The IP was inversely correlated with mean incubation temperature (IP = -3.4644 * mean incubation temperature + 156.92, r^2=0.87, P<0.0001). Consequently, mean IP decreased from the forest habitat (60.2 ± 5.1 days, n=13), to the forest border (55.5 ± 3.9 days, n=16), and to the open sand (51.3 ± 3.5 days, n=59).

Hatching and emergence successes

Hatching success ranged from 0 to 100%, with a mean of 65.4 ± 33.9%, and we found no significant relationship with either clutch size ($F_{1, 93}$=2.6, P=0.113), nest depth ($F_{1, 92}$= 0.2, P=0.647), beach section ($F_{3, 94}$=1.9, P=0.126), or habitat ($F_{2, 95}$=2.2, P=0.119). The emergence success was also independent of clutch size ($F_{1, 93}$=3.6, P=0.062), nest depth ($F_{1, 92}$=0.3, P=0.592), and beach section ($F_{3, 94}$=3.1, P=0.052), but dependent on nesting habitat ($F_{2, 95}$=3.7, P= 0.028). Emergence success decreased from the open sand (66.1 ± 30.8%, range: 0.0 – 100%, n=62), to the forest border (51.9 ± 38.3 %, range: 0.0 – 98.2%, n=20), to the forest habitat (42.2 ± 41.6%, range: 0.0 – 96.2%, n=16). It should be noted that nests in this study were relatively protected from the destructive action of nesting females, such that these parameters may be slightly overestimated.

Sex ratio estimates and hatchling size

We identified the sex of 131 hatchlings from 27 nests, laid from 1 to 22 of September and distributed across the three habitats and the four beach sections (Table S3), with an average of 4.9 ± 0.4 hatchlings per nest. Male hatchlings were significantly larger (4.95 ± 0.19cm, range: 4.44 – 5.33cm, n=83) than females (4.73 ± 0.18cm, range: 4.26 – 5.11cm, n=48, $t_{(95)}$=-6.542, P<0.0001). The beginning of the TSP was 2.0 ± 0.7 days later than the start of the IP_{mid} (range: 0.8 – 3.2 days), and the end of the TSP was 3.3 ± 1.1 days later than the end of the IP_{mid} (range: 2 – 5 days). Thus, the mean length of the TSP was highly coincident with the mean length of the IP_{mid} (differing only by 1.3 ± 0.6 days), justifying the use of the 18-day average to predict the incubation temperature felt by clutches during the critical period of gonad differentiation. Additionally, the resulting difference in mean incubation temperatures between the TSP and the IP_{mid} was negligible; 0.3 ± 0.1ºC (range: 0.0 – 0.5ºC). All three covariates: i) IP_{mid} mean incubation temperature, ii) TSP mean incubation temperature, and iii) IP (to hatching) were significantly correlated with expected sex ratio; P<0.0001. We used the logistic equation with TSP mean temperatures as the independent variable to estimate sex ratios across habitats and nesting seasons, as this model had smaller residual deviance (null deviance of GLMs = 127.9, residual deviance of GLMs using i) IP_{mid} mean temperatures = 56.8, ii) TSP mean temperatures = 56.0, iii) IP = 62.9). The pivotal temperature was 29.4ºC, and the TRT ranged from 27.6-31.4ºC (Fig. 3a). Some nests behaved atypically, for instance we sampled only males from a nest incubated at feminizing temperatures (>30ºC, Fig. 3a). The IP equivalent to the pivotal temperature was 55.1 days (Fig. 3b). We estimated that 47.7% (95% CI: 36.7 – 58.3%) and 44.5% (95% CI: 33.8 – 55.4%) of hatchlings that were produced in 2013 and 2014, respectively, were male (Fig. 4). These estimates were reduced by 3.5%, when considering the emergence success at each habitat (i.e. 44.2% and 40.9% post-emerged males for 2013 and 2014, respectively). The proportion of male offspring produced was higher in the western beach sections (Fig. 1.b). Both the nesting habitat and clutch date influenced sex ratios. The mean expected proportion of males for both years at the open sand was 29.5% (95% CI: 20.2 - 40.9%), at the forest border was 56.6% (95% CI: 43.5 - 68.3%), and the forest was 90.3% (95% CI: 79.2 - 95.5%). The sex ratio at the forest habitat was always male-biased (Fig. 5), and a higher proportions of males were produced during the month of August (Fig. 4).

Discussion

We report here the first field-based estimates of primary sex ratio, pivotal temperature and transitional range of temperatures (TRT), from one of the major green turtle nesting rookeries worldwide, and the largest in the Southern Atlantic DPS (Seminoff et al. 2015, Fig. 6). We found temporal and spatial heterogeneity in incubation conditions, leading to variation in estimated sex ratios, but an overall balanced primary sex ratio when the entire nesting season was considered. These estimates diverge from the primarily reported female-biased hatchling sex ratios at most rookeries. Our site-specific sex ratio curve enabled us to generate robust population-specific estimates, and can be applied for future monitoring of climate change impacts on the primary sex ratio. Insights gained from this work have broad application for the conservation management of sea turtle nesting habitats, and will specifically inform local decision makers towards an improved management of the marine protected area (MPA) of João Vieira and Poilão. We recommend conservation actions, and highlight a way forward to more fully understand the full scope of population resilience to climate change, and its potential for adaptation.

Population-specific pivotal temperature and TRT

The pivotal temperature estimated here was similar to recent values found for other green turtle populations (Broderick et al. 2000, Godley et al. 2002, Godfrey & Mrosovsky 2006). This parameter alone however, is insufficient to predict primary sex ratios; accounting for the TRT is critical to characterize a population’s response to incubation temperatures (Mrosovsky & Pieau 1991, Hulin et al. 2009). A wider TRT will result in more mixed-sexed clutches, and a wider range of temperatures within which heritability may influence offspring sex ratio (Bull et al. 1982, Hulin et al. 2009). Thus, populations with wider TRT have a lower risk of sex ratio bias under climate change (Hulin et al. 2009). A narrow TRT, on the other hand, leads to mostly single-sex nests, and even a slight change in incubation temperatures can have a dramatic impact on primary sex ratios, if the thermal conditions that allow for differentiation of both sexes ceases to be available (Mrosovsky & Pieau 1991, Hulin et al. 2009). Nevertheless, few studies have estimated population-specific pivotal temperatures, and the TRT is rarely reported (Hulin et al. 2009). Typically, laboratory-derived curves are applied to infer primary sex ratios in the wild. However, because these curves rely on a small number of clutches (2-4 clutches; Mrosovsky 1988, Godfrey et al. 1999, Mrosovsky et al. 2002, Godfrey & Mrosovsky 2006), that are exposed to less variable incubation conditions than those in the nesting beach, they have resulted in steep logistic curves with narrow TRTs, which may not reflect the real population variability and resilience. Here we estimated a TRT of 3.8ºC, suggesting that even with substantial increases in incubation temperatures, as predicted by the Intergovernmental Panel on Climate Change (i.e. 2-3ºC; Stocker et al. 2013) some nests would continue to produce males.

Within-population variability in primary sex ratio response

We found inter-clutch variation on the sex ratio response to mean incubation temperatures and to incubation period, similar to other field studies (Spotila et al. 1987, Godfrey & Mrosovsky 1997, Mrosovsky et al. 1999, Godley et al. 2002, King et al. 2013, Wyneken & Lolavar 2015). Such variation has been attributed to the effect of fluctuating temperatures in embryos’ development (Girondot et al. 2010). However, this should not be the case here, as we accounted for the embryo thermal reaction norm to estimate the beginning and end of the TSP (Girondot & Kaska 2014). Interestingly, these were mostly coincident with the middle third of incubation, which normally is expected under constant temperature environments (Bull 1983), possibly due to the buffering effect against sudden temperature changes facilitated by the depth of the green turtle nests (Kaska et al. 1998). Both the spatial variation in incubation temperatures within clutches (<1ºC, decreasing from the top to the bottom; Kaska et al. 1998, Booth & Astill 2001), and our small sample size (inherent to studies involving lethal sampling of hatchlings), may contribute to some of the variation, but are unlikely to explain more atypical observations (e.g. 100% males under a TSP mean incubation temperature of 30.3ºC). Heritability, on the other hand, could be a more reasonable explanation, as similar within-population divergence is seen under constant incubation conditions (Bull et al. 1982, Mrosovsky 1988). Alternatively, overlooked environmental parameters could be influencing hatchling sex. Recently, moisture was shown to override the effect of temperature on gonad differentiation; such that clutches incubated at female-biased temperatures, but with high humidity, produced more males than expected (Wyneken & Lolavar 2015). Relative humidity is likely an important attribute of nests at Poilão, given the coincidence between the nesting and the rainy seasons. Moreover, the groundwater level after heavy rain episodes or spring tides is sufficiently high, that accumulated water can be seen inside abandoned nest chambers and body pits at areas with low elevation. Interestingly, the atypical nest mentioned above, with 100% males at feminizing incubation temperatures, was very close to the high tide line (~1m). An interaction between the effects of humidity and those of heritability, on the mechanisms of TSD, may be driving the observed variation within the TRT. Most important, both the variability in sex ratio response to incubation temperatures, and the wide TRT, are suggestive of resilience and potential for adaptation to climate change. It should be noted that the observed variation is not expected to bias sex ratio estimations, as the atypical values (i.e. more males than predicted under 'female-biased' temperatures, and vice versa), to some extent, cancelled each other out, because incubation temperatures during the TSP are fairly evenly distributed above and below the pivotal temperature at Poilão (Mrosovsky et al. 1999).

Temporal and spatial refugia: resilience and adaptation to climate change

Male hatchling production varied greatly over relatively small spatial scales; both from the exposed beach area to the dense vegetation (increasing from 30% to 91%), and from the east to the west beach sections (increasing from 35% to 56%); and over short temporal scales. Differences in sand temperature between nearby beaches have been attributed to sand albedo (Godley et al. 2002, Fuller et al. 2013), at Poilão however, there is no marked difference in sand color between west and east sections. Alternatively, this variation may be driven by beach orientation (Booth & Freeman 2006, Fuentes et al. 2010a), for instance the western beach sections may be more exposed to Atlantic winds. or by distance to the high tide line, as the western beach sections are narrower, so that nests are on average closer to the sea experiencing cooler temperatures (Fuentes et al. 2010a). Both the cooling effect of vegetation cover (Janzen 1994, Kamel 2013), and rainfall (Godfrey et al. 1996, Houghton et al. 2007, Lolavar & Wyneken 2015), on incubation temperatures have been previously recognized. This emphasizes the importance of accounting for the spatial and temporal distribution of nesting when estimating population primary sex ratios.

The heterogeneity found here, across space and time, suggests that nesting females at Poilão may very well be capable of adaptation through phenotypic plasticity, if air temperatures and/or changes in precipitation lead to unfavorable incubation conditions. For example, in the future, females may adjust the start of the nesting season, to have peak activity coinciding with the colder months (December and January). This would enhance male hatchling production, and clutch survival, under future global warming scenarios, as extremely high incubation temperatures induce hatchling mortality (Godley et al. 2001c, Santidrián Tomillo et al. 2014, Hays et al. 2017). Changes in nesting phenology in response to climate change have been reported, however it remains unclear whether the start of nesting is triggered by the sea surface temperatures at breeding sites (Weishampel et al. 2004), or at foraging grounds (Mazaris et al. 2009). Additionally, other aspects influence sea turtle reproductive phenology, such as availability of food and energy allocated for reproduction (Broderick et al. 2001b), making predictions of phenological adaptations to climate change a challenge. Another possible way for females to adapt would be through nest-site selection, as some TSD species seem to adjust their nesting site to achieve optimal thermal conditions (Doody et al. 2006, Mitchell et al. 2013), although others have displayed behaviors that increased, rather than minimize, their vulnerability to warmer temperatures (Telemeco et al. 2017). Interestingly, individual inter-annual consistence in nest-site selection has been observed in sea turtles (Kamel & Mrosovsky 2006b). This provides scope for natural selection to occur, as females choosing to nest at cooler sites will probably have enhanced fitness under future global warming scenarios (Hays et al. 2017). There may be a trade-off however, between improved thermal conditions and reduced emergence success, as we found the latter to be significantly lower at the vegetated area, likely a consequence of the presence of roots entangling hatchlings, as is frequently observed upon nest excavations.

Primary sex ratio and implications for breeding sex ratio

Overall we estimated a balanced seasonal primary sex ratio. This may imply a male-biased operational (breeding) sex ratio (OSR) for the green turtle population at Poilão, as several populations with female-biased primary sex ratios have been found to have balanced to male-biased OSRs (Wright et al. 2012a, Rees et al. 2013, Stewart & Dutton 2014). These discrepancies, to some extent, may result from males breeding more frequently than females (James et al. 2005, Hays et al. 2014, but see Wright et al. 2012b), compensating partially for female-biased effective population sex ratios. Additionally, balanced juvenile sex ratios, when female-biased were expected, have also been reported (Casale et al. 2006), leading to the hypothesis of differential survival between female and male post-hatchlings (Wright et al. 2012b). Male-biased incubation temperatures typically generate larger hatchlings with superior locomotor abilities, more likely to evade predators (Booth & Evans 2011, Kobayashi et al. 2017). At our study site males were indeed larger, and ghost crabs have been found to preferentially prey on smaller hatchlings here (Rebelo et al. 2011). Finally, some inconsistencies between predicted hatchling sex ratios and observed juvenile and adult sex ratios may derive from poor primary sex ratio estimations, not accounting for population-specific pivotal temperatures and TRTs. At any rate Poilão likely produces a significant number of adult males, which may contribute to a wider Eastern Atlantic metapopulation (Roberts et al. 2004, James et al. 2005, Wright et al. 2012a), endowing it of global importance for the future of the green turtle in a warming world, particularly given the scale of magnitude of this population (> one million hatchlings produced every year). Considering that some TSD-species populations are expected to produce 100% female offspring under predicted climate change scenarios (Hawkes et al. 2007, Patino-Martinez et al. 2012, Laloë et al. 2016), it is of global importance to identify nesting rookeries with high male hatchling production, as these are likely to become of higher conservation value in the future.

Conclusions

Significant information gaps on sea turtle primary sex ratios exist, both at a species and at a geographic level. Adding Poilão to the regional map of green turtle primary sex ratios will contribute to assessments of the metapopulation. There are now robust estimates of this population parameter from the three main nesting rookeries within the Southern Atlantic DPS, but estimates are still lacking from other significant rookeries (e.g. Aves Island, French Guiana and Trindade Island, Fig. 6).

A key outcome of this study is the evidence supporting the importance of native vegetation for population resilience. Poilão currently enjoys a full protection of its habitat, thanks to national laws and its sacred status among the local communities (Catry et al. 2009). However, on nearby islands where numerous clutches are also laid annually (IBAP unpubl. data), significant deforestation for slash-and-burn agriculture has taken place in recent years. Forest conservation and the enforcement of rules banning the felling of trees inside the MPA are critical actions, and of broad impact, contributing to the conservation of both sea turtles and other species using the coastal forest habitat, notably the globally endangered Timneh parrots *Psittacus timneh* (Lopes 2014).

Our findings indicate that despite current climate changes the population at Poilão seems resilient to warming temperatures, however, other aspects of climate change must be considered. Thermal expansion of the ocean will increase the mean sea level, causing inundation and erosion of coastal areas, worsened further by predicted increased storm intensity. Extensive losses of sea turtle nesting habitat have been predicted under median sea-level-rise (SLR) scenarios (Baker et al. 2006, Fuentes et al. 2010b, Katselidis et al. 2014). It is thus critical to investigate how predicted future SLR will impact the low lying nesting habitat at Poilão and neighbouring islands, to fully understand how resilient this population may be to climate change.

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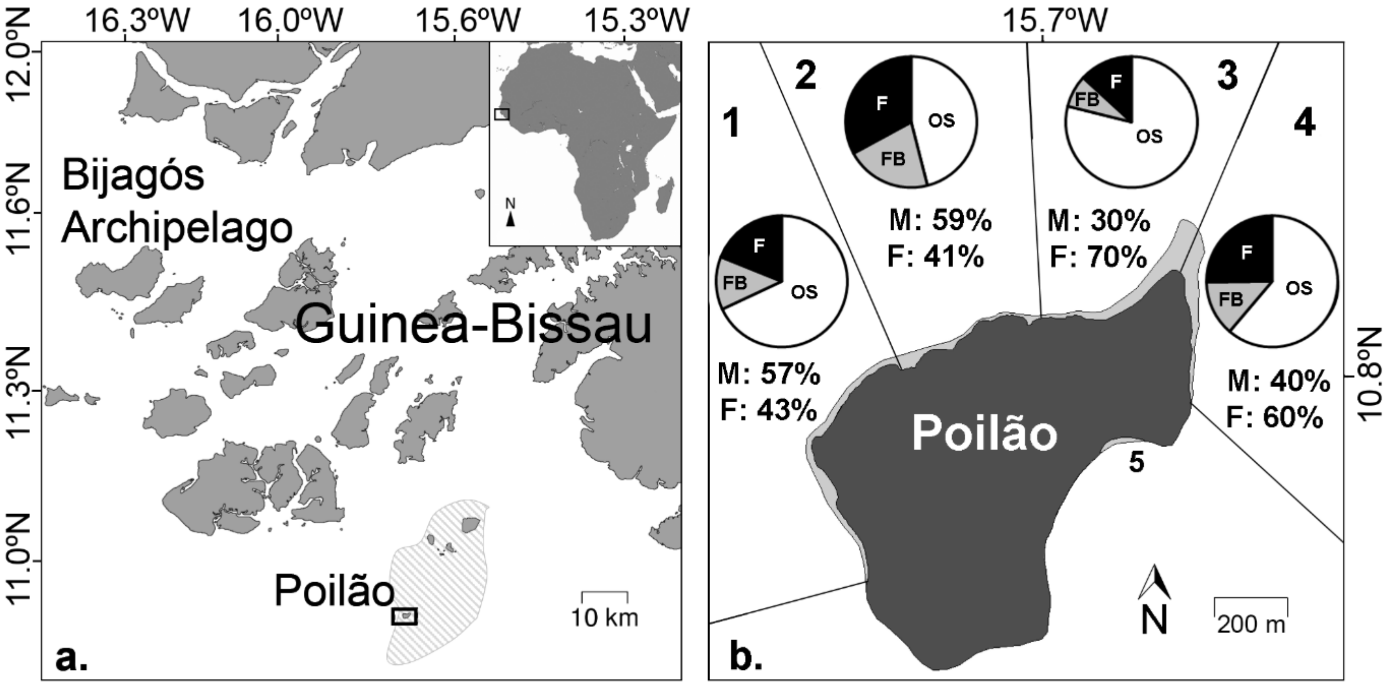

Figure 1a. Map of the Bijagós Archipelago, Guinea-Bissau: the João Vieira and Poilão Marine National Park is represented by the striped area, and the black frame depicts Poilão Island; **b.** Map of Poilão Island showing the four green turtle nesting beach sections monitored in this study (1-Farol, 2-Acampamento Oeste, 3-Acampamento Este, 4-Cabaceira). Pie charts present the mean nesting distribution across three habitats: 'open sand' (OS: white), 'forest border' (FB: grey), and 'forest' (F: black), in each section. Estimated mean proportion of males (M) and females (F) produced in each section are given (average across 2013 and 2014). Section 5-Praia Militar, was not monitored in this study due to difficult access and the small proportion of nesting hosted there (Maps created using www.seaturtle.org/maptool).

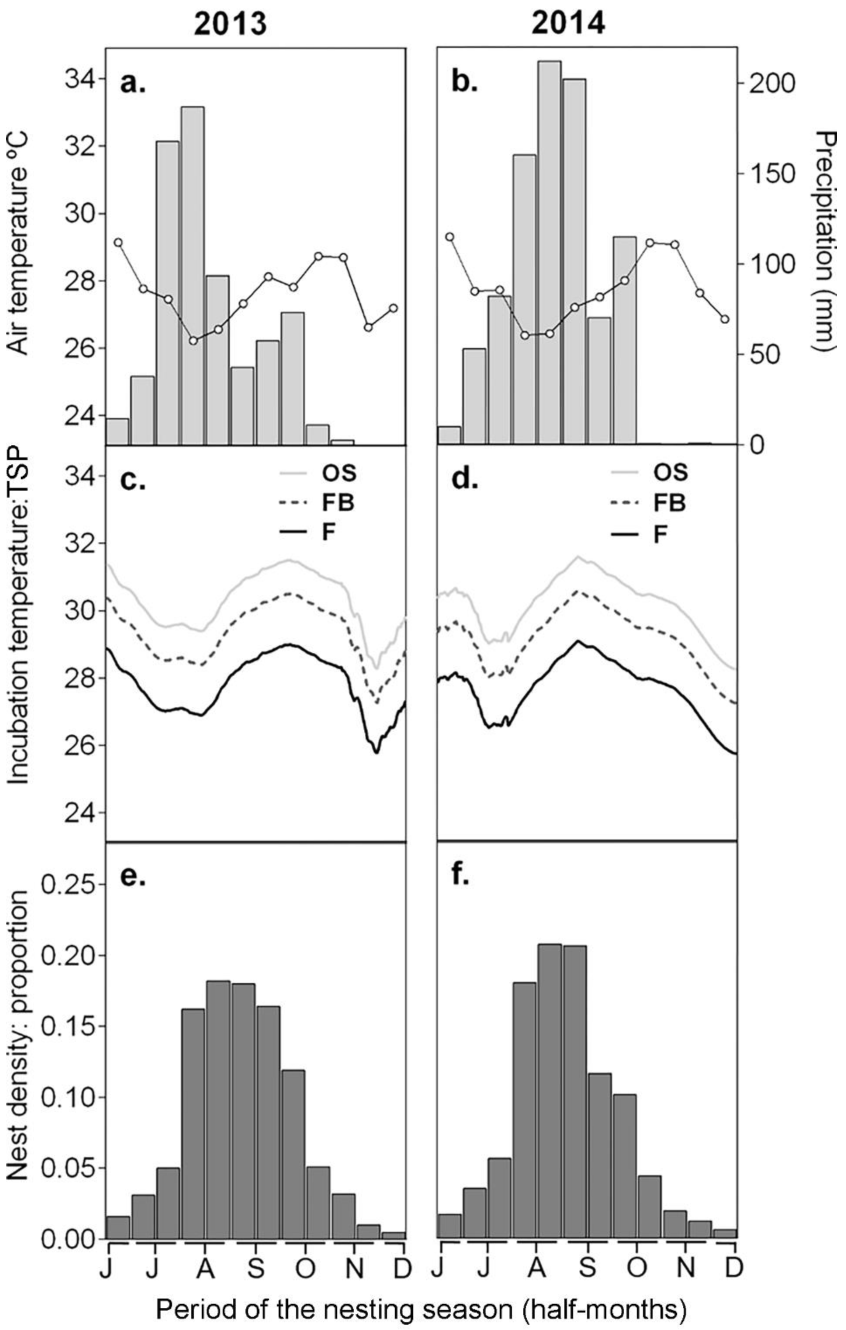

Figure 2a, b. Mean bi-weekly air temperature (open circles) and precipitation (bar) at Bolama Island (http://cdo.ncdc.noaa.gov/CDO/cdo); **c, d**. estimated mean incubation temperature during the thermosensitive period (TSP) experienced by green turtle clutches laid from 15 June to 15 December at Poilão Island, at three habitats (OS-open sand, FB-forest border, F-forest); **e, f.** bi-weekly proportion of green turtle nesting distribution at Poilão.

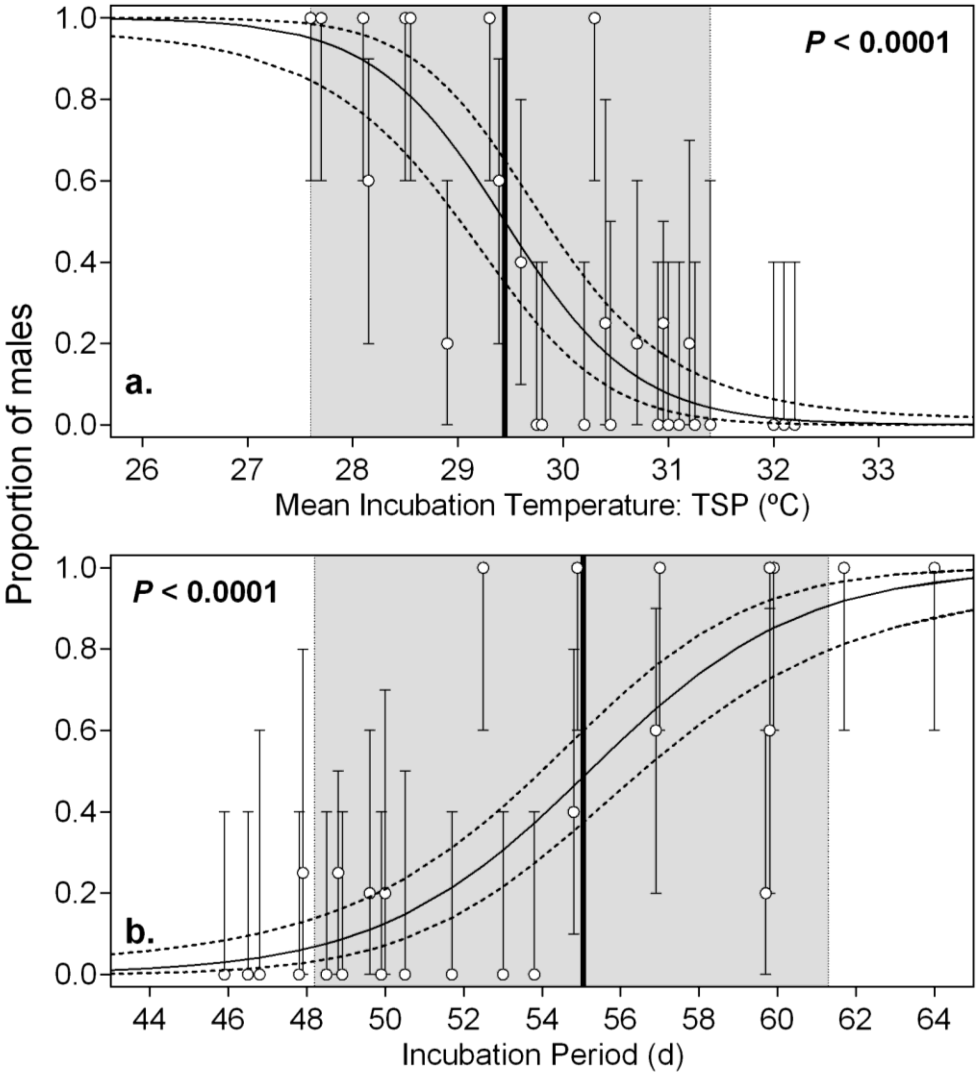

Figure 3. Logistic function (solid curve) and 95% confidence intervals (CI, dashed curves) showing expected proportion of green turtle male hatchlings, as a function of **a.** thermosensitive period (TSP) mean incubation temperatures, and **b.** incubation duration, at Poilão Island, Guinea-Bissau. Open circles and 95% CI error bars show the proportion of males found in natural nests (n = 27), with a mean sample size of 4.9 ± 0.4 SD hatchlings per nest. Shaded areas show: limits of transitional range of temperatures (TRT: 27.6-31.4ºC) in a., and corresponding limits of incubation periods (48.1 – 61.3 days, y=-3.4644x + 156.92, r^2 =0.87) in b. Straight solid line indicates the pivotal temperature (29.4ºC) in a., and incubation length equivalent (55.1 days) in b.

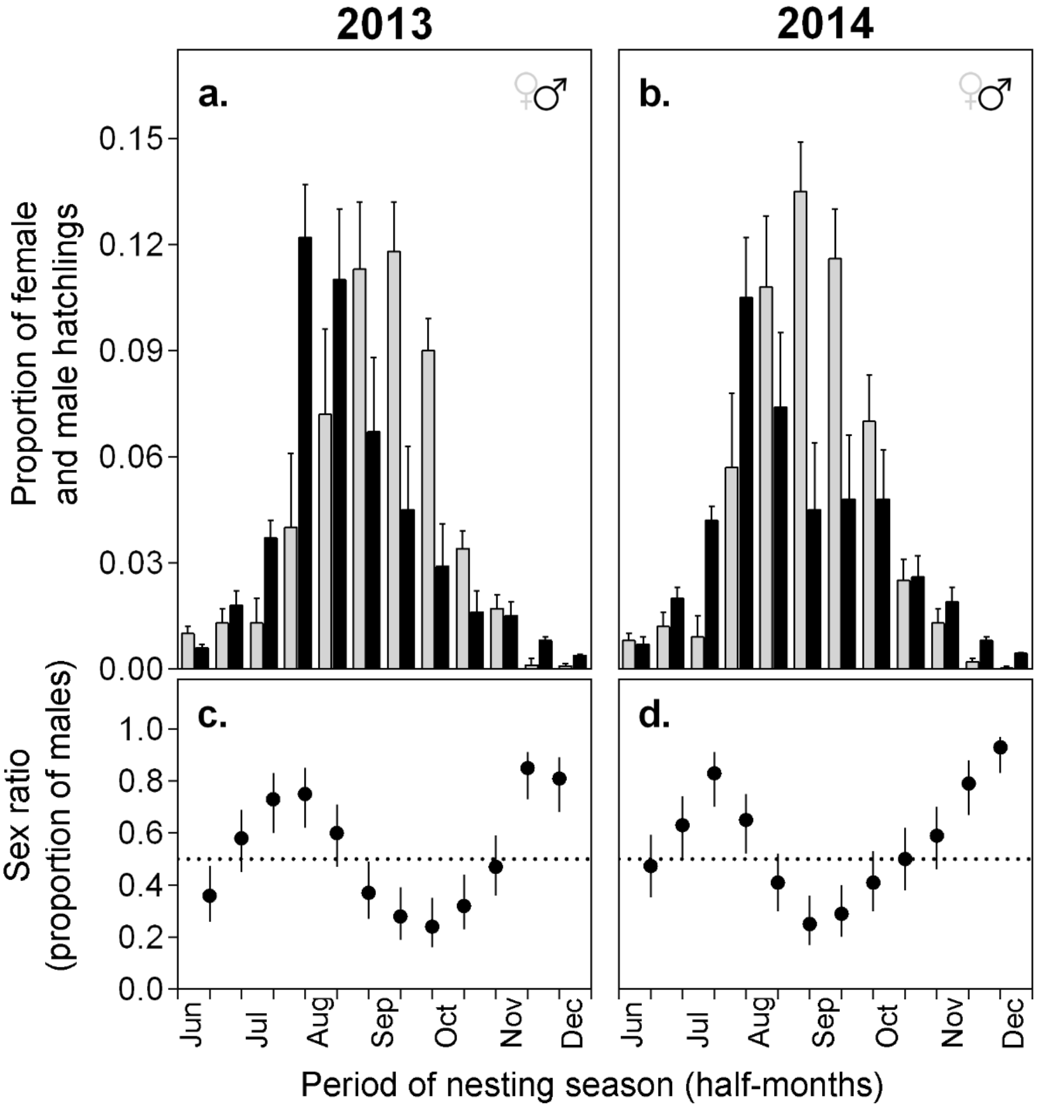

Figure 4a, b. Bi-weekly proportion of female (light grey) and of male (dark grey) green turtle hatchlings predicted to have been produced in Poilão Island, Guinea-Bissau, with error bar showing upper 95% confidence interval (CI); and c.d. estimated mean sex ratio, with 95% CI, along the nesting season, in 2013 and 2014 (average across years).

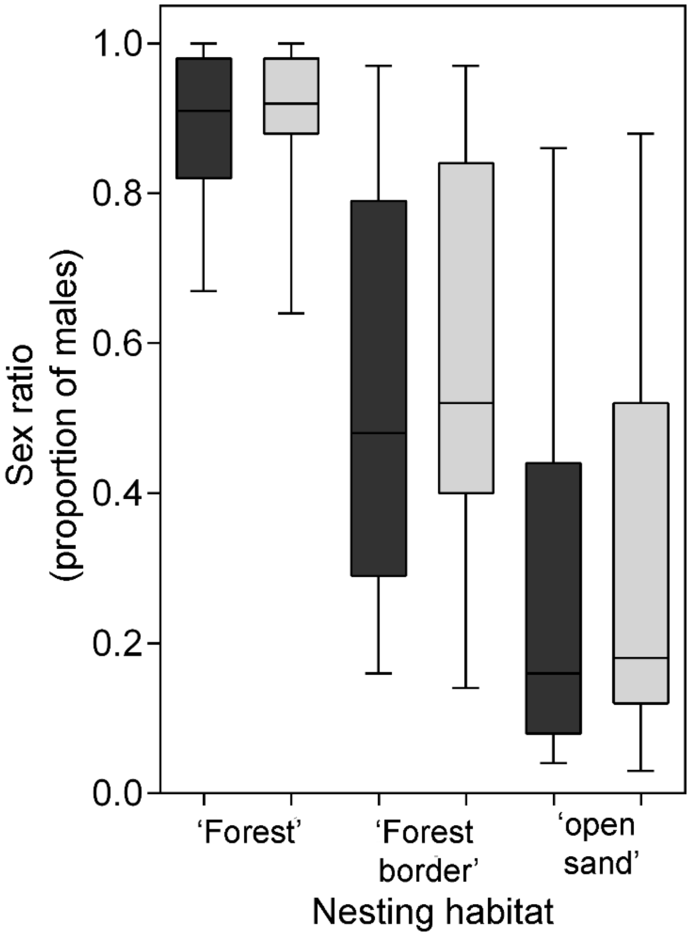

Figure 5. Estimated mean primary sex ratio (proportion of males) of green turtle hatchlings in each of three habitats: 'forest', 'forest border' and 'open sand', at Poilão Island, Guinea-Bissau, for 2013 (dark grey) and 2014 (light grey). Boxes show median, upper and lower quartile, and whiskers show highest and lowest observation.

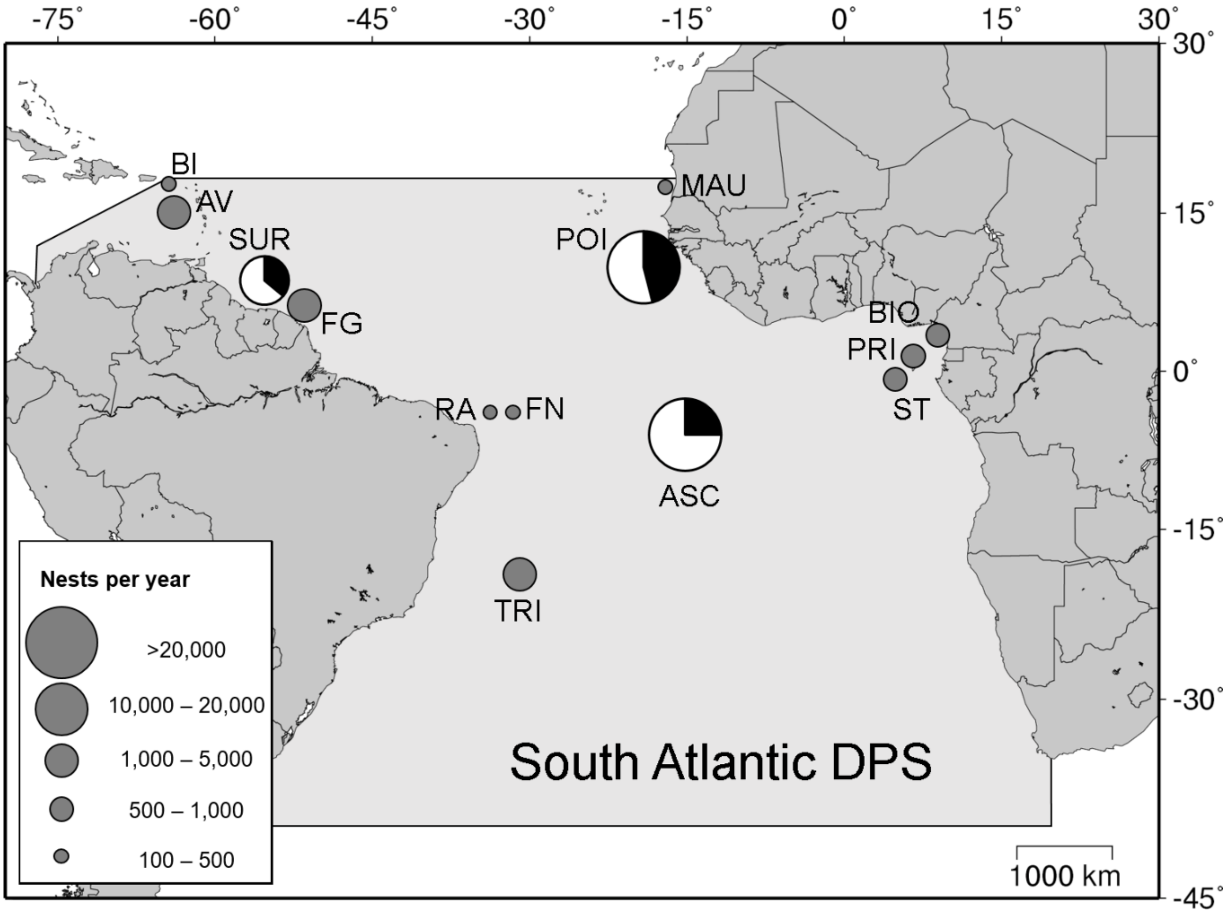

Figure 6. Limits of green turtle South Atlantic distinct population segment (DPS), showing rookeries with 100 or more nests per year. Pie charts indicate primary sex ratio (females: white, males: black), estimated for the three main nesting sites: Suriname (SUR; Godfrey et al. 1996, Seminoff et al. 2015), Ascension Island, UK (ASC; Godley et al. 2002, Weber et al. 2014), and Poilão Island, Guinea-Bissau (POI; this study, Catry et al. 2009). Other rookeries represented by grey circles do not have estimates of primary sex ratios: Buck Island, UK (BI; Seminoff et al. 2015), Aves Island, Venezuela (AV; Garcia Cruz et al. 2015), Yalimapo, French Guiana (FG; Chambault et al. 2016.), Rocas Atol, Brazil (RA; Bellini et al 2013), Fernando de Noronha, Brazil (FN; C. Bellini, Centro Tamar, *pers. comm.*), Trindade Island, Brazil (TRI; Almeida et al. 2011), Mauritania (MAU; J. Fretey *pers. comm.*), Bioko Island, Equatorial Guinea (BIO; Honarvar et al. 2016), and Sao Tome (ST; ATM/MARAPA 2016) and Principe (PRI; Principe Trust Foundation, *pers. comm.*), Sao Tome and Principe (Map created using www.seaturtle.org/maptool).

Chapter 1: supplementary Information

Table S1. Chi-square statistics testing if the distribution of green turtle nests at Poilão Island, Guinea-Bissau, along three habitats: ‘open sand’, ‘forest border’ and ‘forest’, at each beach section, was dependent on sampling occasion, within year (2013 and 2014), and between the two years. n refers to sample occasions.

Beach section (number / name)	**2013 (n = 3)**			**2014 (n = 6)**			**2013 vs. 2014 (n=2)**		
	chi-square	**df**	***P***	**chi-square**	**df**	***P***	**chi-square**	**df**	***P***
1 / Far	2.78	2	0.25	13.39	10	0.20	1.24	2	0.54
2 / AO	2.33	2	0.38	14.05	10	0.17	0.83	2	0.66
3 / AE	0.68	2	0.83	9.30	10	0.50	0.75	2	0.72
4 / Cab	2.40	2	0.30	7.05	10	0.72	1.53	2	0.49

Table S2. Summary of Tukey HSD test results, looking at differences in mean incubation temperature during the middle third of development at four beach sections (see Fig.1b) and three habitats: ‘open sand’ from ≥1m of vegetation or tree canopy to high tide line, ‘forest border from 0-1m of vegetation or tree canopy,’ and ‘forest’, under vegetation or tree canopy. ‘diff’ is the difference in mean temperatures between beach sections, ‘lwr’ and ‘upr’ are the low and upper 95% confidence intervals, and *P* gives the significant level after adjustment for the multiple comparisons.

Beach section	**Habitat**	**diff**	**lwr**	**Upr**	***P***
1 vs. 2	Open sand	0.32	-0.46	1.10	0.97
1 vs. 3		1.51	0.82	2.20	**<0.001**
1 vs. 4		1.13	0.41	1.86	**<0.001**
2 vs. 3		1.20	0.50	1.89	**<0.001**
2 vs. 4		0.82	0.09	1.55	**<0.01**
3 vs. 4		-0.38	-1.01	0.26	0.69
1 vs. 2	Forest border	0.40	-0.63	1.43	0.98
1 vs. 3		0.88	-0.33	2.09	0.39
1 vs. 4		0.08	-1.31	1.48	1.00
2 vs. 3		0.48	-0.55	1.51	0.92
2 vs. 4		-0.32	-1.56	0.92	1.00
3 vs. 4		-0.80	-2.19	0.60	0.74
1 vs. 2	Forest	-0.12	-2.03	1.79	1.00
1 vs. 3		0.01	-1.90	1.91	1.00
1 vs. 4		0.06	-1.42	1.54	1.00
2 vs. 3		0.13	-1.78	2.03	1.00
2 vs. 4		0.18	-1.30	1.66	1.00
3 vs. 4		0.06	-1.42	1.54	1.00

Table S3. Summary information for 27 green turtle clutches, incubated under natural conditions at Poilão Island, Guinea-Bissau, and respective number and proportions of male hatchlings sexed from each clutch. IP: incubation period to hatching; IP_{mid}: middle third of IP; TSP: thermo-sensitive period; Δ: difference in days between start and end of TSP (estimated using 'embryogrowth' v.6.4 R package, Girondot and Kaska 2014) and IPmid (TSP – IP_{mid}); CI: confidence interval. Habitat definitions can be found in the 'Materials and methods' section in the main article. For beach section definitions see Fig.1b.

Nest ID	**Lay date**	**Habitat**	**Beach section**	**IP**	**Mean temperature °C**		**Δ TSP and IP_{mid} (days)**		**Sexed hatchlings**		**Proportion of males**		
					IP_{mid}	**TSP**	**Start**	**End**	**total**	**males**	**mean**	**low 95%CI**	**up 95%CI**
N54	12-Sep	forest	3	61.7	27.6	27.6	1	3	5	5	1.0	0.6	1.0
N66	16-Sep	forest	3	61.4	27.5	27.7	2	4	5	5	1.0	0.6	1.0
N78	18-Sep	forest	4	59.8	27.8	28.1	2	4	5	3	0.6	0.2	0.9
N77	18-Sep	forest	2	59.8	27.8	28.1	2	4	5	5	1.0	0.6	1.0
N53	11-Sep	forest	1	59.7	28.3	28.5	2	3	5	5	1.0	0.6	1.0
N70	17-Sep	forest	4	56.9	28.1	28.5	2	5	5	5	1.0	0.6	1.0
N51	10-Sep	forest	4	59.7	28.8	28.9	1	2	5	1	0.2	0.0	0.6
N79	18-Sep	forest	4	54.8	28.9	29.3	3	4	5	5	1.0	0.6	1.0
N40	04-Sep	forest border	4	56.9	29.4	29.4	1	2	5	3	0.6	0.2	0.9
N39	03-Sep	forest border	2	54.8	29.4	29.6	2	4	5	2	0.4	0.1	0.8
N76	18-Sep	forest border	2	53.8	29.7	29.8	1	2	5	0	0.0	0.0	0.4
N81	20-Sep	forest border	1	51.7	29.3	29.8	3	5	5	0	0.0	0.0	0.4
N73	17-Sep	forest border	1	48.9	29.7	30.2	3	5	5	0	0.0	0.0	0.4
N62	15-Sep	open sand	1	52.5	30.1	30.3	1	2	5	5	1.0	0.6	1.0
N63	15-Sep	open sand	1	50.5	30.1	30.4	2	4	4	0	0.0	0.0	0.5
N84	22-Sep	forest border	3	47.8	30.0	30.4	3	5	4	1	0.3	0.0	0.8
N57	13-Sep	open sand	2	49.6	30.5	30.7	2	3	5	1	0.2	0.0	0.6
N44	08-Sep	open sand	1	48.8	30.6	30.9	3	5	4	1	0.3	0.0	0.5
N72	17-Sep	open sand	2	49.9	30.8	30.9	1	2	5	0	0.0	0.0	0.4
N71	17-Sep	open sand	4	48.8	30.8	31.0	1	2	5	0	0.0	0.0	0.4
N32	01-Sep	open sand	4	53.0	30.9	31.1	2	2	5	0	0.0	0.0	0.4
N60	15-Sep	open sand	4	46.5	30.8	31.2	2	4	5	0	0.0	0.0	0.4
N37	03-Sep	open sand	3	50.0	30.8	31.2	3	4	5	1	0.2	0.0	0.7
N82	21-Sep	open sand	2	46.8	30.9	31.4	2	3	4	0	0.0	0.0	0.6
N68	16-Sep	open sand	2	45.9	31.8	32.0	2	2	5	0	0.0	0.0	0.4
N34	01-Sep	open sand	2	48.5	31.6	32.1	3	3	5	0	0.0	0.0	0.4
N47	09-Sep	open sand	2	47.8	32.2	32.2	1	2	5	0	0.0	0.0	0.4

Figure S1. Nesting habitats utilized by green turtles at Poilão Island, Guinea-Bissau, according to vegetation cover: a. ‘open sand’ habitat, from >1m of the vegetation to high tide line, completely exposed to the sun; b. ‘forest border’, comprised between 0 – 1m of the vegetation line, with partial shade; c. ‘forest’, nesting area completely surrounded by trees or tall bushes, shaded throughout most or all of the day. Wooden poles surround clutches.

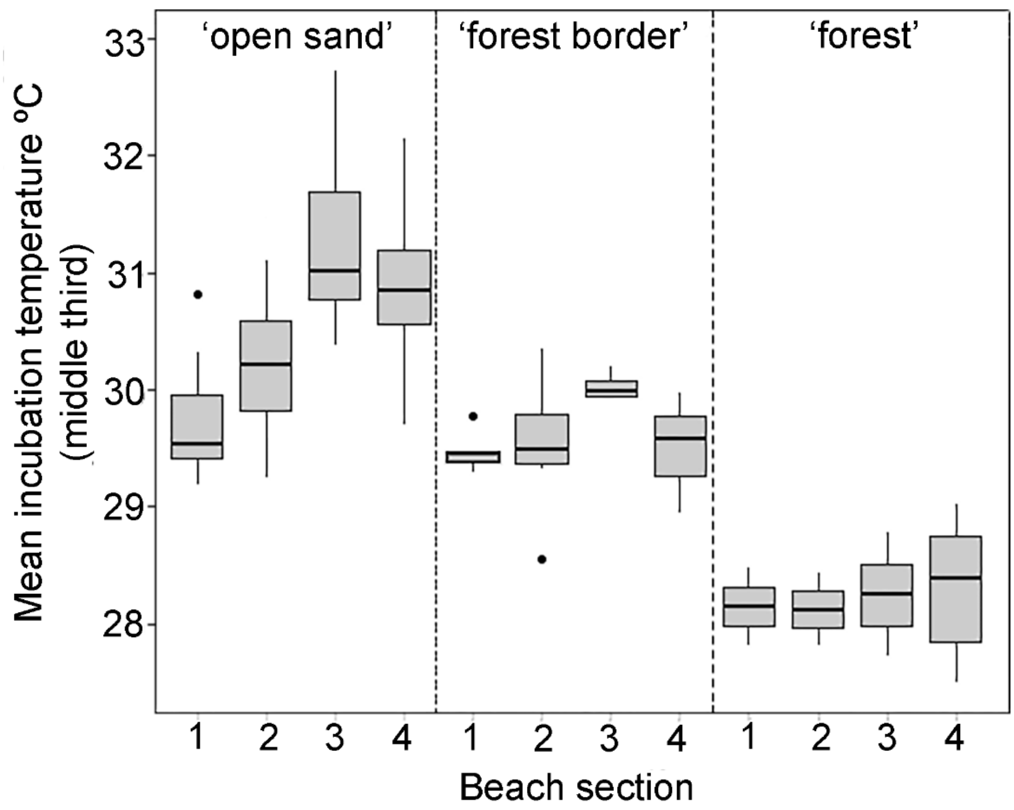

Figure S2. Mean incubation temperature during the thermosensitive period (middle third of development) of green turtle nests in three different habitats and four beach sections, at Poilão Island, Guinea-Bissau. For beach sections see Fig.1b. Habitat definitions can be found in the methods section and Fig. S1.

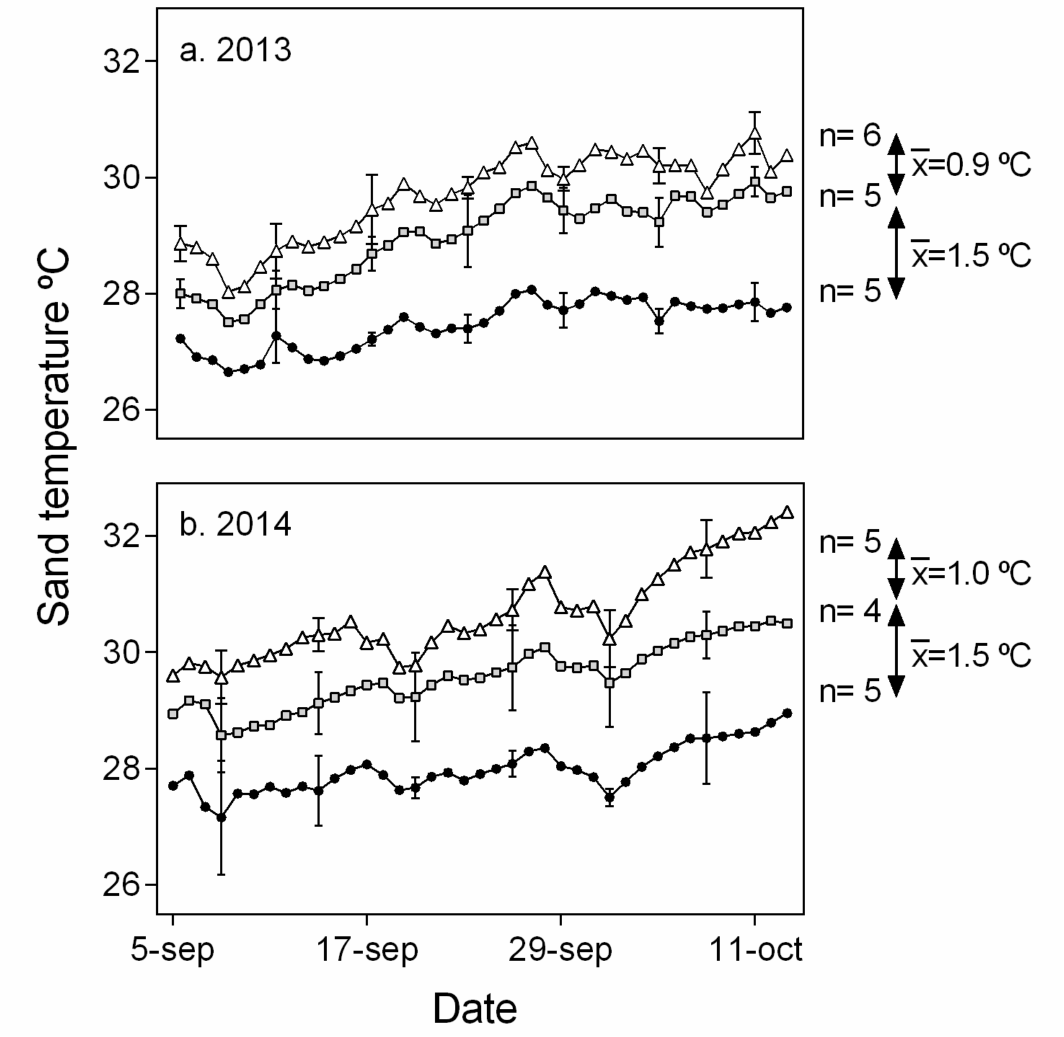

Figure S3. Sand temperature in three nesting habitats for green turtles, at Poilão Island, Guinea-Bissau: ‘open sand’ (open triangles), ‘forest border’ (grey squares), and ‘forest’ (black circles), for 2013 (a) and 2014 (b). ‘n’ is the number of data loggers recording temperature at each habitat (0.3 ºC resolution), and x̄ denotes mean difference between habitats. Habitat definitions can be found in the methods section and Fig. S1.

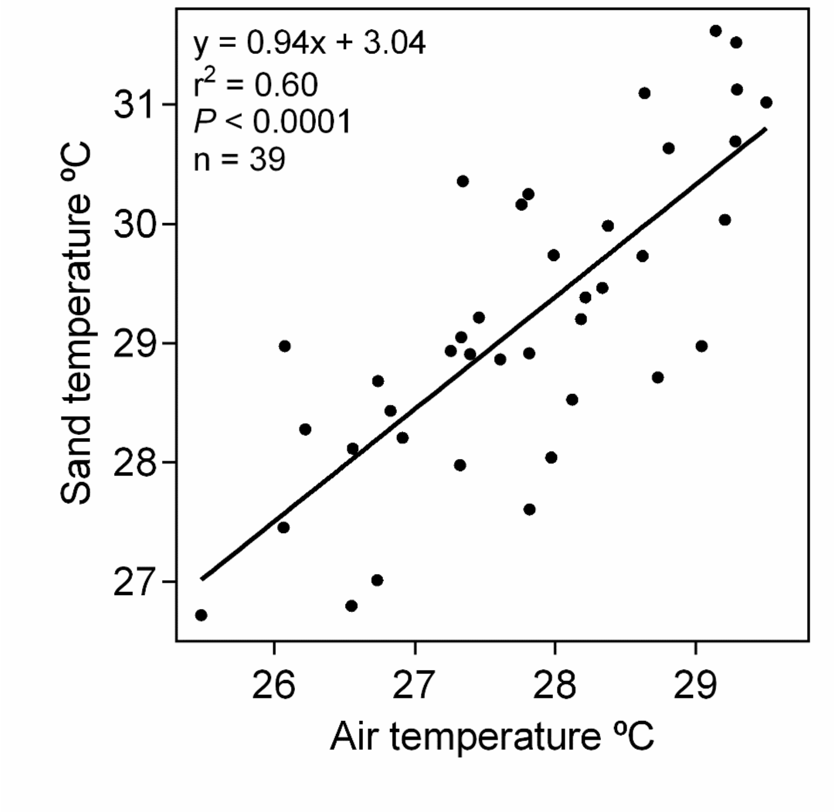

Figure S4. Linear regression between mean bi-weekly sand temperature at Poilão and air temperature in Bolama (http://cdo.ncdc.noaa.gov/CDO/cdo, 50km distant).

Chapter 2: Nest site selection repeatability and success of green turtle *Chelonia mydas* clutches

Ana R. Patrício[1, 2], Miguel R. Varela[1], Castro Barbosa[3], Annette C. Broderick[1], Brendan J. Godley[1], Maria Betânia Ferreira Airaud[2], Aissa Regalla[3], Dominic Tilley[1], Paulo Catry[2]

[1] Centre for Ecology and Conservation, University of Exeter, UK
[2] MARE – Marine and Environmental Sciences Centre, ISPA – Instituto Universitário, Lisbon, Portugal
[3] Institute of Biodiversity and Protected Areas of Guinea-Bissau

Abstract

Nest site selection is a critical behaviour, particularly in species with no parental care, as it can greatly impact offspring survival. Marine turtles depend on sandy beaches to nest, where they select from a range of microhabitats that may differently affect hatchling survival and phenotype. Here we describe the degree of nest site selection at one of the largest green turtle rookeries globally, in Guinea-Bissau, West Africa, and how this impacts offspring. In 2013 and 2014 we recorded the spatial distribution of 1,559 nests, and monitored 657 females during oviposition, to assess population and individual preferences on nesting site. Overall, females tended to nest close to the vegetation, at a preferred elevation interval of 4.8–5.0m, which was above the highest spring tide (4.7m), enhancing clutch survival. Individuals displayed high repeatability in nesting microhabitat type (open sand, forest border, and forest), distance along the beach, distance to the vegetation, and elevation, which may result from this behaviour having a genetic basis, or from fine-scale nest site philopatry. Hatchlings from cooler nests were larger, potentially dispersing faster and more able to evade predators, while smaller hatchlings, from warmer nests, retained more energetic reserves (residual yolk), which may also be advantageous for initial dispersal, particularly if food is scarce. Thus, individual preferences in nest site selection led to trade-offs in offspring fitness, but overall, most nesting females elected sites that enhanced offspring survival, suggesting that nest site selection is an adaptive trait that has been under selection. As under future climate change scenarios females nesting at upper shaded areas should have enhanced fitness, individual consistency in nesting microhabitat provides opportunity for natural selection to occur.

Introduction

Nest site selection is a key behaviour, because the surrounding environment can greatly impact offspring survival and phenotype (Spencer 2002). This is particularly true in species without parental care, for which nest site selection is essentially the last step in parental investment. Marine turtles are an example of such species, as females lay multiple clutches each breeding season, typically every two to four years, and show no parental care (Ehrhart 1982). Reproductive females usually exhibit natal philopatry, returning to their beach of origin to nest (Meylan et al. 1990). Upon emergence at the beach, however, nest site selection may be influenced by microhabitat conditions, most significantly beach morphology, dune vegetation, and sediment attributes (e.g. sand temperature, moisture, grain size; Kelly et al. 2017). Preferences can differ among species and populations (Kelly et al. 2017), yet a range of microhabitats is often used, each differently affecting clutch success (Kamel & Mrosovsky 2004, Pfaller et al. 2009).

Clutches laid closer to the sea will be more vulnerable to tidal inundation and erosion, while those near the vegetation may have roots piercing through the eggs or entangling hatchlings (Kamel & Mrosovsky 2004). Also, a higher risk of misorientation is predicted for hatchlings emerging at forested areas on the back of the beach (Godfrey et al. 1996, Kamel & Mrosovsky 2004). On the other hand, shaded areas promote cooler incubation temperatures leading to larger hatchlings with superior locomotion abilities (Booth & Evans 2011, Kobayashi et al. 2017). Additionally, as sea turtles have temperature-dependent sex determination (TSD, Mrosovsky & Yntema 1980), the nesting site will further determine the sex of hatchlings. Thus, nest site selection may involve trade-offs in hatchling fitness, which can shift under changing environmental conditions. Overall, population-level preferences on nesting site, observed in different species, seem to benefit offspring survival (Spencer & Thompson 2003, Turkozan et al. 2011, Zare et al. 2012), suggesting that nest site selection is an adaptive trait.

Individual fidelity in nest site selection has also been observed in some turtle species, using repeatability analysis (Spencer & Thompson 2003, Kamel &

Mrosovsky 2004, 2005, 2006). Such behaviour, under spatially variable threats, could accelerate natural selection, if only a fraction of the females consistently elect conditions that enhance the fitness of their offspring. However, knowledge on individual nest site selection among sea turtles, its consequences for fitness and its evolutionary potential is still very limited. The evolution of a behaviour, or of any trait, is a result of both selection on phenotypic variation and inheritance of the variants (Fisher 1958). In the context of selection, repeatability is directly useful, as it measures the proportion of total variation that is due to differences among individuals (Falconer & Mackay 1996), therefore revealing the within-individual consistency (Boake 1989). With regard to inheritance, a high heritability in a behavioural trait should correspond to high repeatability in this trait, and a statistically significant repeatability suggests potential for a genetic basis (Dohm 2002).

Poilão Island, Guinea-Bissau, hosts one of the largest green turtle rookeries in the Atlantic, and worldwide, with an estimate of ca. 29,000 clutches laid annually (Catry et al. 2009). The nesting microhabitat characteristics here vary across beach width and length (e.g. elevation, vegetation cover/shading, and sand temperature), likely affecting offspring fitness differently. We looked into the nest distribution in this population, and explored three questions: (1) do females choose their nesting site randomly or based on specific microhabitat characteristics?; (2) are females repeatable in their nesting site conditions, and if so, is this a consequence of fine scale philopatry or of habitat selection?, and (3) how does nesting site affect offspring survival and phenotype. The potential of this behaviour for selection and heritability is discussed.

Materials and methods

Study site

Poilão Island (10.8º N, 15.7º W, Fig. 1), is part of the João Vieira and Poilão Marine National Park, in the Bijagós Archipelago, Guinea-Bissau, and it hosts one of the major green turtle nesting populations worldwide (Catry et al. 2002, 2009). Poilão has a total area of 43ha, is covered by undisturbed tropical forest, and sandy beaches extend for 2km of the ca. 4km of coastline. The island is surrounded by intertidal rocks (Fig. 1), which are exposed during low tide, blocking the access of nesting females to the beach, and/or preventing them from returning to the sea, at the risk of getting stranded and dying of hyperthermia or desiccation. Thus, the temporal pattern of nesting activity at Poilão is centred on the peak of high tide, lasting approximately two to three hours each night. The nesting season extends from mid-June to mid-December, peaking in August and September (Catry et al. 2002), largely coinciding with the rainy season (May to November; Catry et al. 2002). For the purpose of this study we monitored green turtle nesting activity during the 2013 and 2014 nesting seasons.

Nesting distribution at the population level

The nesting area was divided in four beach sections, from west to east (1-4, Fig. 1). Within each section we classified the distribution of nests according to three habitats: ‘forest’, ‘forest border’ and ‘open sand’. The ‘forest’ habitat encompassed the nesting area under the vegetation and was shaded, the ‘forest border’ comprised a band up to 1m of the vegetation and experienced partial shade, and the ‘open sand’ characterized the area from >1 m of the vegetation to the high tide line (see Fig. S1 in Patrício et al. 2017). Due to the magnitude of nesting at Poilão, females mask each other’s activities, precluding the identification of nests even in the following morning. Thus, to determine the nest distribution at the population level, we surveyed all females found laying in each of three nights in 2013 (407 nests), and six nights in 2014 (1,152 nests), during the peak of the nesting season, following the protocol described in Patrício et al. (2017). As a result of these focused surveys, we recorded the GPS location of 1,559 nests, using a hand held GPS (Garmin GPSmap 62), assigning one of the three habitats to each of them. A chi-test revealed that there was no significant differences in the nesting distribution across beach sections and microhabitats between the two years (Patrício et al. 2017), and these represent independent samples, as females nesting in 2013 did not return to nest in 2014, therefore we pooled the data to describe the overall nesting distribution at Poilão.

Characterization of the nesting habitat at the population level

Because of the extent of nests assessed for the population-level assessment, together with survey time constrains, we used remote sensing to measure nest distance to the vegetation line, and nest surface elevation. From 11 to 12 November 2016 we flew a drone (35m altitude), coupled with a digital compact camera, and took aerial photos of the nesting beach, with a minimum of 80% overlap, to create an orthophoto (i.e. orthorectified image with uniform scale), and a digital elevation model (DEM), using Agisoft Photoscan Professional v1.3.1 (© Agisoft, supplementary methods). This work could not be conducted earlier as the technology was still under development. However, given the protection provided by the intertidal rocks, the overall beach morphology at Poilão remained relatively stable over the sampling period. To georeferenced the DEM/orthophoto, and enhance DEM accuracy, we applied in the model the coordinates of 20 ground control points (GCPs: square tiles 25 x 25cm), evenly distributed along the beach (every 100m), obtained using a Piksi GPS (www.swiftnav.com/piksi-multi, accuracy: horizontal=4.1cm, vertical=5.2cm; Fazeli et al. 2016). The DEM and the orthophoto were then exported as rasters to ArcGIS 10.3 (ESRI), together with the GPS locations of the 1,559 nests surveyed, for spatial analysis. We used the 3D Analyst Tools to estimate the surface elevation of the nests, with the DEM as the input surface (i.e. surface with information on elevation). We used the orthophoto to calculate the area (km^2) of the open sand, forest border and forest habitats, within each beach section. To define the extent of the forest habitat (i.e. shaded area used for nesting), we previously measured, in the field, the distance from the vegetation line to the last nest inwards, every 50m, along the beach extension (1800m). Thus, the forest habitat extended 3m into the vegetation for most of the beach, except for the last 150m at the end of section 4 (Fig. 1), where it was set to 8m inwards, as vegetation here consist of tall trees with large open spaces underneath, and turtles penetrate deeper. The Euclidean distances of each of the 1,559 nests to the vegetation line were calculated using the ‘near’ function in Analysis Tools. Finally, we estimated the kernel nest density (2m output cell size, 30m radius search), using Spatial Analyst Tools.

Nest site selection at the individual level

Every night from August to November, throughout the 2013 and 2014 nesting seasons, we monitored the nesting activity of green turtles. Given the large number of females nesting simultaneously, and the relatively short time frame to conduct the monitoring (approx. two hours around the peak of the high tide), we could not assess all females. Thus, each night a team surveyed sections 1 and 2, and another team surveyed sections 3 and 4, targeting the first turtles seen about to lay a clutch. Monitored turtles were tagged on both front flippers with Monel tags, each identified with a unique reference, and the following information was recorded: female id (flipper tags), female curved-carapace-length (CCL, using soft tape measure, to the nearest 0.1cm), GPS of clutch location, distance of clutch to the vegetation line and along the beach (using a 50m surveyors tape measure), habitat (open sand, forest border, or forest), and nest surface elevation (measured the following day). Nest elevation was estimated by measuring the elevation from the nest surface to the high tide line, using an Abney level, and adding the elevation of the tide for the survey day, using the tidal table for João Vieira (17km distance). After all measurements were collected, the teams would carry on to find the next turtles about to lay a clutch, monitoring an average of four turtles per night ($\bar{X}_{2013}$=3 ± 2 SD, $\bar{X}_{2014}$= 5 ± 3 SD). Meanwhile, one member of each team inspected all turtles met along the survey for flipper tags, and when a previously tagged female was found nesting, the same measurements as above were recorded. Females were tagged and measured after laying the eggs, to minimize disturbance. In 2015 a team looked for nesting females tagged in 2013 and 2014, and recorded the clutch habitat and beach section.

Hatchling survival and phenotype

During the nesting season, the disturbance caused by the numerous turtles each night can lead to the destruction of previous clutches, and loss of nest markings. Thus, to secure the follow up of clutch success, we protected a subset of nests (n_{2013}=48, n_{2014}=72, total 120), surrounding them with three wooden poles, and daily monitored these, until emergence date or loss. After 45 days of incubation, we placed wire cages on top of nests (see Patrício et al. 2017), to trap emerged hatchlings in order to measure. After emergence, we evaluated nest contents and calculated hatching success: H%=(n hatched eggs / clutch size) x 100, and emergence success: E%=((n hatched eggs – n dead and live hatchlings inside egg chamber) / clutch size) x 100, and measured the depth to the bottom of the nest (i.e. after all nest contents were removed). Hatchlings found inside traps were taken to our working station, where each nest was processed in under 30min. We measured hatchling straight-carapace-length (SCL) with callipers to the nearest 0.1cm, weighed them with a spring scale to the nearest 0.1g, and calculated a condition index, Fulton's index: $K=W/SCL^3$, to infer the relative amount of energy reserves, in this case residual yolk. The use of K here is appropriate as all individuals are hatchlings, so no error is introduced by growth rates (Peig & Green 2010). After processing, hatchlings were kept in the shade, inside buckets with moist sand, and released near the water after the sunset. All sampling and handling protocols were approved by the research ethics committee of the University of Exeter, and the government of the Republic of Guinea-Bissau.

Statistical analyses

To evaluate if the distribution of nests across beach sections, and across habitats at each beach section, was random, i.e., if turtles were using all of the available nesting area, we used the chi-square test.

To assess if there was within individual preferences on nest site selection we used the measure of repeatability (Nakagawa & Schielzeth 2010). Repeatability analysis for Gaussian data, i.e. distance along the beach, distance to the vegetation, and nest surface elevation, was performed using R package rtpR, method LMM.REML (Nakagawa & Schielzeth 2010). Repeatability analysis for multinomial data, i.e. habitat (open sand, forest border, forest), was conducted using a generalized multinomial model, with the habitat as dependent variable and with multinomial distribution (three levels corresponding to the different habitats), and cumlogit link function (see Appendix A in Dean et al. 2011 for details). Additionally, to explore if observed repeatabilities were linked to habitat selection, or a consequence of nesting site philopatry at a very fine-scale, we fitted generalized linear models (GLMs) to our data, with: i. nest elevation, and

ii. distance to the vegetation as response (dependent) variables; beach section and nesting habitat as factor predictors (independent variables); and two control variables (covariates), elevation or distance to the vegetation (accordingly) of the previous nest from the same female. We compared models with different factor predictors to infer on their significance to the response variables.

To assess which nesting site features predicted clutch survival we fitted generalized additive modelling (GAM), with binomial error structure and logistic function, using r package mgcv (Wood & Wood 2015), with i. hatching, and ii. emergence successes as response variables, and four spatial predictors: nest elevation, habitat, distance along the beach, and distance to the vegetation line. The models also included three maternal covariates: female CCL, clutch size, and nest depth; and one temporal covariate: year. In the GAM with emergence success as a response variable we further included hatching success as a control variable, to disentangle the effect of hatching. We opted for GAMs as some predictors are not expected to have monotonic relationships with the response variables (e.g. distance along the beach).

Hatchling phenotype (SCL, locomotion, sex), can be affected by incubation temperature (Booth & Astill 2001, Godfrey & Mrosovsky 2006, Ischer et al. 2009), which at Poilão is linked to nesting habitat; increasing from the forest, to the forest border, and to the open sand (Patrício et al. 2017). Thus, we fitted GLMs with Gaussian error structure and identity function to test if the nesting habitat had a significant impact on hatchling i. SCL, ii. weight, and iii. Condition index (K), using female CCL and clutch size as control variables (the effect of habitat on hatchling sex is treated in Patrício et al. 2017). All statistical tests and models were conducted using R v.3.2.5 (R Development Core Team 2008). Estimates are presented as mean ± SD, unless stated otherwise.

Results

Nest site preferences at the population level

Despite the presence of intertidal rocks, limiting access to nesting areas at low tide, the clutches were widely distributed along the full extension of the beach (Fig. 2, and Fig.S1 for nest density per nesting season), with 30% laid at section 1, 28% at section 3, 22% at section 4, and 20% at section 2. Nests however, were not distributed randomly across beach sections (Table 1), with more clutches than expected at sections 1 and 2, where beach width is very narrow. Female green turtles did not nest randomly across the nesting habitats either (Table 1), tending to nest disproportionally close to the vegetation line, within the forest border habitat (see also Table S1 for nest distribution across habitats, by section). Further, at the open sand habitat, most clutches were laid within the two quarters closer to the vegetation (Fig. 3a). Mean nest elevation was significantly different among beach sections ($F_{3,1555} = 62.53$, $P<0.0001$), lower at section 1 (Fig. 3b). Consequently, there was a lower proportion of nests above the highest spring tide (HST=4.7m) at section 1 (Fig. 3b). Overall nest elevation was 4.8±0.5m, with 57% of the nests located above the HST.

Nest site preferences at the individual level

A total of 657 females were tagged during this study (n_{2013}=201, n_{2014}=456). From these, 29% and 36% were re-sighted again, in 2013 and 2014, respectively, with a mean re-sighting success of 0.33 for both years. All re-sights were within years, i.e., no turtle tagged in 2013 was seen in 2014. For the repeatability analysis we used only the observations for which we could determine the clutch location (excluding from the dataset the encounters where the turtles were crawling or still preparing the nest). Thus, for the measure of repeatability on nesting habitat (multinomial variable) we gathered information from 179 females (n_{2013}=59, n_{2014}=120), seen nesting on four (n=6), three (n=34) or two occasions (n=139), for a total of 404 separate nesting events. Of these, 269 were in the open sand (67%), 73 were in the forest border (18%) and 62 were in the forest (15%). We found high repeatability within individuals on nesting habitat: R=0.67, SE=0.003, 95% CI: 0.49-0.79. More detailed information on clutch location, i.e. distance along the beach, distance to the forest line and nest elevation, was available for 110 unique turtles (n_{2013}=29,

n_{2014}=81), observed nesting on four (n=2), three (n=21) or two occasions (n=87), for a total of 245 nests. These nests were widely distributed along the beach (1046 ± 503m, range: 30-1790m, Fig. S2a), across the distance to the vegetation (6.7 ± 11.7m, range: -10-50m, Fig. S2b), and along the elevation gradient (4.7 ± 0.4m, 3.5-6.3m, Fig. S2c), indicating between individual variance in nest-site choice. We found significant repeatability within individuals on nest location in relation to i) distance along the beach: R=0.65, 95% CI: 0.54 – 0.74, *P*<0.0001 (Fig.4a); ii) distance to the vegetation line: R=0.44, 95% CI: 0.29 – 0.57, *P*<0.0001, (Fig. 4b); and iii) elevation: R=0.25, 95% CI: 0.09 – 0.39, *P*=0.006 (Fig. 4c). When considering only these 110 turtles, the repeatability within individuals on nesting habitat was very high: R=0.77, 95% CI: 0.59-0.91). Nest elevation was significantly affected by beach section, but not by nesting habitat (Table 2), whereas, on the contrary, the distance to the vegetation was significantly affected by nesting habitat, but not by the beach section (Table 2). Additionally, 16 turtles first tagged in 2013 were re-sighted nesting in 2015. Of these, all returned to the same habitat, and only one changed beach section, supporting inter-season maintenance of nest site selection.

Impacts of nest location on hatchling survival

We managed to follow 108 nests to completion (i.e. emergence or clutch failure, n_{2013}=45, n_{2014}=63). We lost the location of the remaining 12 nests (10%) due to disturbance by other nesting females. The GAM with hatching success as a response variable was a good fit, with 74.3% of the deviance explained. Hatching success was significantly higher in the open sand (68.4 ± 30.5%, Table 3), compared to the forest border (62.9 ± 39.5%), and the forest habitats (61.1 ± 34.9%), and it increased significantly with nest elevation (Fig. 5, Table 3), with a mean of 81.6 ± 17.4% for nests placed at or above the highest observed spring tide (HST=4.7; Fig. 5), compared to 34.4 ± 16.2% for the nests under the HST. It should be noted, however, that mean bottom-clutch depth at our study site is 0.8m (Patrício et al. 2017), such that a nest surface elevation of ≥4.7m corresponds to a mid-clutch elevation of ≥3.9m. Thus, these clutches may still be partially subjected to degrees of flooding, particularly during spring tides, which supports the observed variation in hatching success among nests from higher beach zones. All clutches with hatching success <10% (n=15) were at some point of development flooded. There was also a significant effect of the distance along the beach (Table 3), however we found no obvious pattern (Fig. S2), and this could potentially be a sampling effect. The distance to the vegetation and the control variables (maternal and temporal) had no significant effect on hatching success (Table 3, Fig. S2). The GAM with emergence success as response variable was, as expected, a good fit (79.3% of the deviance explained), since hatching success was included as a control variable and these parameters are intrinsically related. Most important, the effect of forest habitat was significant (Table 3), with lower emergence success here (39.0 ± 37.2ºC), compared to the forest border (59.9 ± 38.8ºC), and the open sand (63.9 ± 31.3ºC). It should be noted that, as nests in this study were relatively protected from the destructive action of nesting females, it is possible that these parameters may be slightly overestimated.

Impacts of nest location on hatchling phenotype

We gathered measurements of straight-carapace-length (SCL) and weight of 10 hatchlings from each of 62 nests (n_{2013}=30, n_{2014}=32), for a total of 620 hatchlings. The mean straight-carapace-length (SCL) of hatchlings was 4.8 ± 0.2cm in 2013 and 4.8 ± 0.1cm in 2014, with no significant difference between the two years. We found no significant effect of either female curved-carapace-length (CCL) or clutch size on hatchling SCL, but nesting habitat had a significant effect (Table 4), with hatchlings from the forest habitat being significantly larger (4.9 ± 0.1cm) than hatchlings incubated in the warmer forest border (4.8 ± 0.1cm) and open sand habitats (4.7 ± 0.1cm, Fig. 6a). The hatchlings were significantly heavier in 2014 (19.1 ± 2.0g), compared to 2013 (17.2 ± 1.4g), and we did not find any significant effect of either nesting habitat, or maternal covariates on hatchling weight, although female CCL was marginally significant (Table 4). Similarly to the SCL, nesting habitat also had a significant effect on the condition index (K; Table 4), but in the opposite direction, with significantly higher K at the open sand (0.177 ± 0.02, Fig. 6b), compared to the forest border (0.162 ± 0.02) and the forest (0.146 ± 0.01). Interestingly, although we did not detect an effect of female CCL on hatchling SCL, females nesting in the forest were significantly larger (103.2 ± 4.1cm, $F_{2,105}$=3.05, P=0.05), compared to those who nested at the forest border (100.5 ± 5.6cm), and the open sand (99.6 ± 5.8cm).

Discussion

Nest-site selection in species with no parental care will essentially determine the fate of offspring and population fitness. This behaviour is nevertheless not well understood for sea turtles, in particular with regards to individual choices of nesting site. Here, we explore the nesting distribution and related consequences for hatchling survival and phenotype, in a major green turtle rookery, at Poilão Island, Guinea-Bissau. This is the first study on the repeatability of nest site selection in green turtles, and we found among the highest repeatabilities reported for this trait in the literature (see also Kamel & Mrosovsky 2005, 2006), which may reflect an underlying genetic basis for this behaviour (Boake 1989).

Population nest site preferences and adaptive value

There was a trend for females to nest close to the vegetation line, and at more elevated areas, similar to that observed among other green turtle (Horrocks & Scott 1991, Wang & Cheng 1999, Turkozan et al. 2011, Santos et al. 2015), hawksbill (Horrocks & Scott 1991, Zare et al. 2012), and loggerhead populations (Garmestani et al. 2000, Wood & Bjorndal 2000). Recurrent evidence on the importance of elevation and proximity to the vegetation for nesting suggests that these are elemental cues for nest site selection, at least in some marine turtle species. Additionally, we frequently observed abandoned nests with water filled chambers or with strong plant roots in the bottom, at the lower beach and under the vegetation, respectively. Thus, nest site selection may be guided by both positive responses to environmental cues (i.e. elevation, distance to the vegetation), and negative responses to environmental deterrents (e.g. water and roots found while digging). However, due to the high number of females disturbing the sand and masking previous activities, we did not systematically assess the distribution of failed nesting attempts.

Nest location had impacts on both hatching and emergence success at Poilão. Hatching success was higher at the open sand habitat, and it increased with nest elevation because nests laid in the lower beach were frequently flooded during spring tides. The emergence success, however, decreased under the supralitoral vegetation, likely a consequence of the presence of roots entangling hatchlings, as frequently observed upon nest excavation. The fact that at

Poilão, most clutches are laid at the open sand, near the vegetation, and at a preferential elevation above the highest spring tide (HST), may be an indication that nest site selection is an adaptive trait that has been under selection. In the western section however, most nests were placed below the HST, more prone to inundation. Interestingly, the surrounding intertidal rocks, where both hatchlings and nesting females often get stranded, facing high risk of depredation (hatchlings only), and desiccation, are not a major obstacle for this population, as no preferential nesting at the section free of rocks was observed. One caveat of this study is that partial protection of clutches with wooden poles could potentially have created a spatial bias, for example enhancing hatching success in high nesting density areas, where the probability of clutch destruction by another nesting female is typically higher. A future study should investigate the impact of nesting density on hatching survival.

Individual consistency in nest site selection and evolutionary potential

We found within-individual consistency in nest site selection, with the highest repeatability in habitat and position along the beach, concurring with Kamel & Mrosovsky (2005) findings for hawksbill turtles. One possible explanation for these consistencies would be that, once a nesting female successfully lays a clutch, it then returns to the same location for subsequent nesting, leading to very fine-scale philopatry, and consequently selecting consistent microhabitat features. For instance, nest elevation was significantly dependent of beach section (indicator of philopatry), but not of nesting habitat. This could be advantageous in relatively morphologically stable beaches like Poilão, assuring that females reach a known successful nesting spot (Eckert 1987). Given the particular physical structure of Poilão, there could be an additional benefit of such strategy, as both arriving and leaving the beach involves a difficult crossing over intertidal rocks around the peak of the high tide, and familiarity with the path could reduce the risk of stranding. Supporting this hypothesis, of 16 green turtles first tagged in 2013 and re-sighted in 2015, all but one went back to the same beach section, evidencing that fine-scale philopatry is kept across nesting seasons. Distances to the vegetation, however, depended on the nesting habitat selected, but not on the beach section, suggesting that, regardless of fine-scale philopatry, turtles consistently choose specific conditions to nest. Indeed, all turtles recaptured in 2015 were seen nesting in the same habitat as in 2013. Another hypothesis for our observations would be that the variation among females has a genetic basis, and nest site choice is a heritable trait, which is plausible, given the high repeatabilities observed (Boake 1989). An interesting finding in our study was that females nesting at the forest habitat were larger (but note that female size did not significantly affect hatchling size). Larger females could potentially be more able to clear the vegetation and break strong roots found while digging, thus being more successful nesting in the forest, but to our knowledge, there is no evidence of this. On the other hand, in freshwater turtles and other reptile species, higher incubation temperatures lead to faster growth rates in post-hatchlings (Booth 2006). If this trait is similar in sea turtles, and is maintained through juvenile phases, smaller hatchlings from warmer nests are expected to mature at smaller sizes, and vice-versa (Atkinson 1994, Van der Have & Jong 1996). This would occur because cell differentiation is faster than body growth (Van der Have & de Jong 1996). Hence, the fact that we see larger females nesting at habitats which generate larger hatchlings is compelling for heritability in nest site selection, meriting further research.

Nest site selection trade-offs for hatchling survival and phenotype

Hatchlings from clutches incubated at cooler (i.e. shaded) sites were larger, compared to hatchlings incubated at warmer temperatures in the open beach (Patrício et al. 2017), agreeing with previous studies (Hewavisenthi & Parmenter 2001, Glen et al. 2003, Ischer et al. 2009, Read et al. 2013). As there was no effect of nesting habitat on hatchling weight, smaller hatchlings had higher condition index (K), indicative of a larger yolk reserve, which has not been converted into body tissue (Hewavisenthi & Parmenter 2001, Booth & Evans 2011). There are potential advantages for different phenotypes under certain conditions. Being larger increases chances of escaping gape-limited predators (Booth et al. 2004), and predators in general due to enhanced locomotion (Ischer et al. 2009, Kobayashi et al. 2017). However, larger hatchlings are mostly generated under the vegetation at the back of the beach, thus crawling longer distances to reach the ocean, increasing the exposure to land predators (e.g. palm nut vultures; Carneiro et al. 2017), and risking misorientation (Kamel & Mrosovsky 2005, 2004). Being small may increase vulnerability to predators, as is the case of Poilão, where the ghost crab

Ocypode cursor preferentially preys on smaller hatchlings (Rebelo et al. 2011). Yet, these hatchlings typically originate in areas clear of vegetation closer to the water, facilitating sea finding, and have more energy reserves (i.e. residual yolk) for their initial dispersal. Additionally, nesting habitat also influences hatchling sex, with males being mainly produced at the forest habitat and females in the open sand (Patrício et al. 2017). Thus, sea turtle nest site selection involves trade-offs in offspring survival and phenotype, which can shift under changing environmental conditions.

Potential for adaptation to a rapidly changing world

Future global warming is expected to enhance the production of female hatchlings, the predominant sex at higher incubation temperatures (Ackerman 1997), and eventually increase clutch mortality, as temperatures rise to more extreme values (Godley et al. 2001, Santidrián Tomillo et al. 2014, Hays et al. 2017). Simultaneously, it will cause the mean sea levels to rise, with greater risk of inundation (revised in Hawkes et al. 2009). Climate change will thus create spatially variable threats, with nests exposed to higher temperatures (in the open sand), and at lower elevations being more threatened. Females may potentially adapt their nesting site in response to changing environmental cues, mitigating the predicted impacts. Indeed, nest site selection was proposed to mitigate potential climate change impacts on the primary sex ratio among TSD species (Janzen & Morjan 2001, Doody et al. 2006, but see Telemeco et al. 2009, 2017). However, it is uncertain whether marine turtles will be capable of adaptation to the current rapid changes. Individual consistency in nest site selection, along with inter-individual variation, observed here, nevertheless, provides opportunity for natural selection to occur.

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Table 1. Estimated area and proportion of each of three habitats, and each of four beach sections, used by green turtles nesting at Poilão Island, Guinea-Bissau, with the distribution of expected and observed nests at each habitat/beach section, and respective chi-square test results for random distribution hypothesis. For habitat and beach sections definitions see methods.

Term	Area (m^2)	Proportion of nesting area	Expected number of nests	Observed number of nests	Chi-square	df	*P*
Nesting habitat							
Open sand	24858	0.72	1115	998	86.692	2.00	< 0.0001
Forest border	2890	0.08	130	218			
Forest	7044	0.20	316	343			
Total	34751	1.00	1559	1559			
Beach section							
Section 1	8981	0.26	403	470	22.74	3	< 0.0001
Section 2	6232	0.18	280	306			
Section 3	10554	0.30	473	433			
Section 4	8984	0.26	403	350			
Total	34751	1.00	1559	1559			

Table 2. Summary of model comparison, to determine which environmental factors, beach section (beach), and nesting habitat (habitat: 'forest', 'forest border' or 'open sand') predict i. nest elevation (elev), and ii. clutch distance to the vegetation (dveg), using as control variables "same female previous nest elevation (elev_p)" and "same female previous distance to the vegetation (dveg_p)", accordingly. df: degrees of freedom, Dev: deviance explained by model. Bold indicates significant values (*P*<0.05).

Generalized linear models	**df**	**Dev**	**test**	**F-test**	***P***
i. response variable: nest elevation					
1. elev ~ elev_p + beach + habitat	8	0.22	-	-	-
2. elev ~ elev_p + habitat	5	0.08	1 vs. 2	7.38	**0.0001**
3. elev ~ elev_p + beach	6	0.19	1 vs. 3	2.23	0.112
i. response variable: distance to the vegetation					
1. dveg ~ dveg_p + beach + habitat	8	0.37	-	-	-
2. dveg ~ dveg_p + habitat	5	0.34	1 vs. 2	2.40	0.071
3. dveg ~ dveg_p + beach	6	0.23	1 vs. 3	14.61	**<0.0001**

Table 3. Summary of generalized addditive models (GAMs) looking at effects of nesting site (spatial predictors) on green turtle clutch survival at Poilão Island, Guinea-Bissau, with maternal and temporal variables as covariates. SE: standard error, df: estimated degrees of freedom of smooth term (1 = linear), NA: not applicable.

Term	**Hatching success %**				**Emergence success %**			
	Estimate	**SE**	**t**	***P***	**Estimate**	**SE**	**t**	***P***
Parametric								
Habitat: OS	0.70	0.23	3.09	**0.003**	0.12	0.24	0.51	0.609
Habitat: FB	1.00	0.30	3.37	0.351	0.55	0.33	1.69	0.095
Habitat: F	0.50	0.33	1.50	0.138	-1.16	0.36	-2.87	**0.005**
Year	0.14	0.24	0.59	0.555	0.34	0.23	1.47	0.145
		df	**F**	***P***		**df**	**F**	***P***
Non-parametric								
Nest elevation		2.76	30.57	**<0.0001**		1	0.39	0.534
Distance: beach		5.06	4.12	**0.001**		2.70	1.65	0.195
Distance: vegetation		1.00	1.52	0.221		1.68	0.50	0.604
Female CCL		1.39	1.89	0.102		1	0.44	0.510
Clutch size		1.32	0.84	0.543		1	0.44	0.507
Nest depth		5.00	1.45	0.210		1	1.95	0.166
Hatching success		NA	NA	NA		3.69	15.15	**<0.0001**

Table 4. Summary of generalized linear models (GLMs) looking at the effect of nesting habitat (‘open sand’ – OS, ‘forest border’ – FB, ‘forest’ – F) on green turtle hatchlings straight-carapace-length (SCL, cm), weight (g) and condition index (K=weight/SCL^3), at Poilão Island, Guinea-Bissau, with maternal and temporal variables as covariates.

Term	**SCL**				**Weight**				**K**			
	Estimate	**SE**	**t**	***P***	**Estimate**	**SE**	**t**	***P***	**Estimate**	**SE**	**t**	***P***
Habitat: OS	4.41	0.38	11.63	**< 0001**	9.64	5.43	1.77	0.082	0.12	0.06	2.14	**0.037**
Habitat: FB	4.46	0.39	11.42	**< 0001**	8.86	5.57	1.59	0.118	0.11	0.06	1.88	0.065
Habitat: F	4.61	0.39	11.60	**< 0001**	9.56	5.66	1.69	0.097	0.10	0.06	1.65	0.104
Clutch size	0.00	0.00	-1.36	0.180	-0.02	0.01	-1.44	0.155	0.00	0.00	0.11	0.914
Female CCL	0.00	0.00	1.02	0.311	0.10	0.06	1.71	0.092	0.00	0.00	0.78	0.440
Year	0.04	0.04	0.92	0.361	1.66	0.55	3.01	**0.004**	0.01	0.01	1.89	0.065

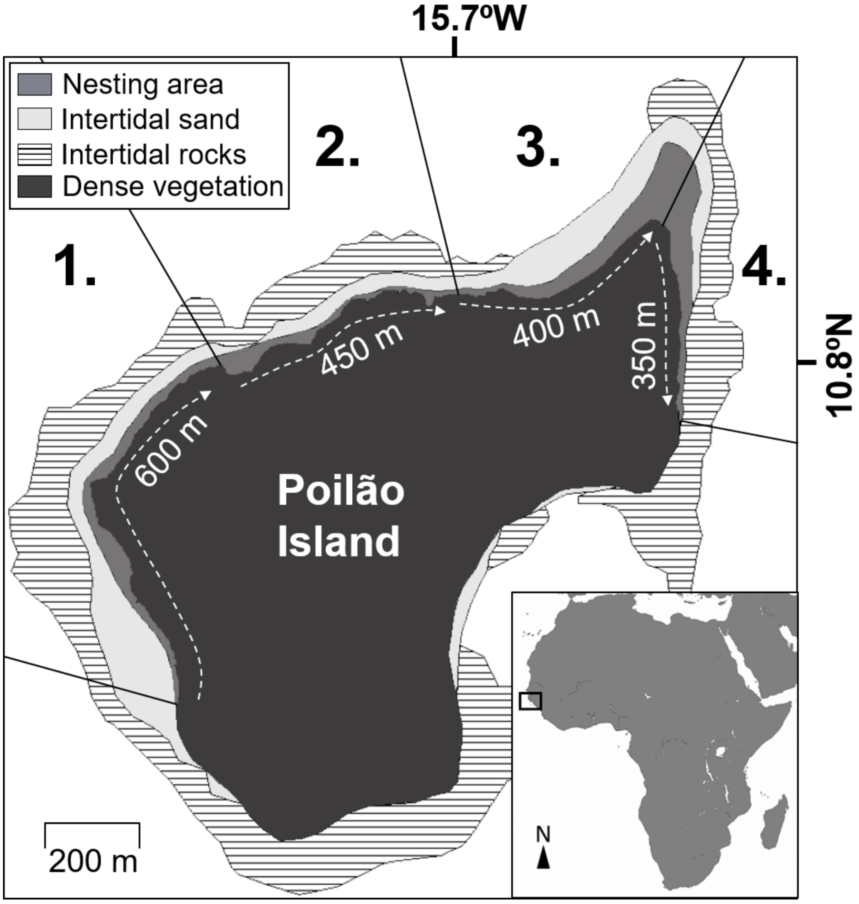

Figure 1. Map of study site: green turtle rookery at Poilão Island, Guinea-Bissau. The nesting beach is divided in four beach sections; 1: Farol, 2: Acampamento Oeste, 3: Acampamento Este, and 4: Cabaceira. The island is surrounded by intertidal rocks, except at beach section 3.

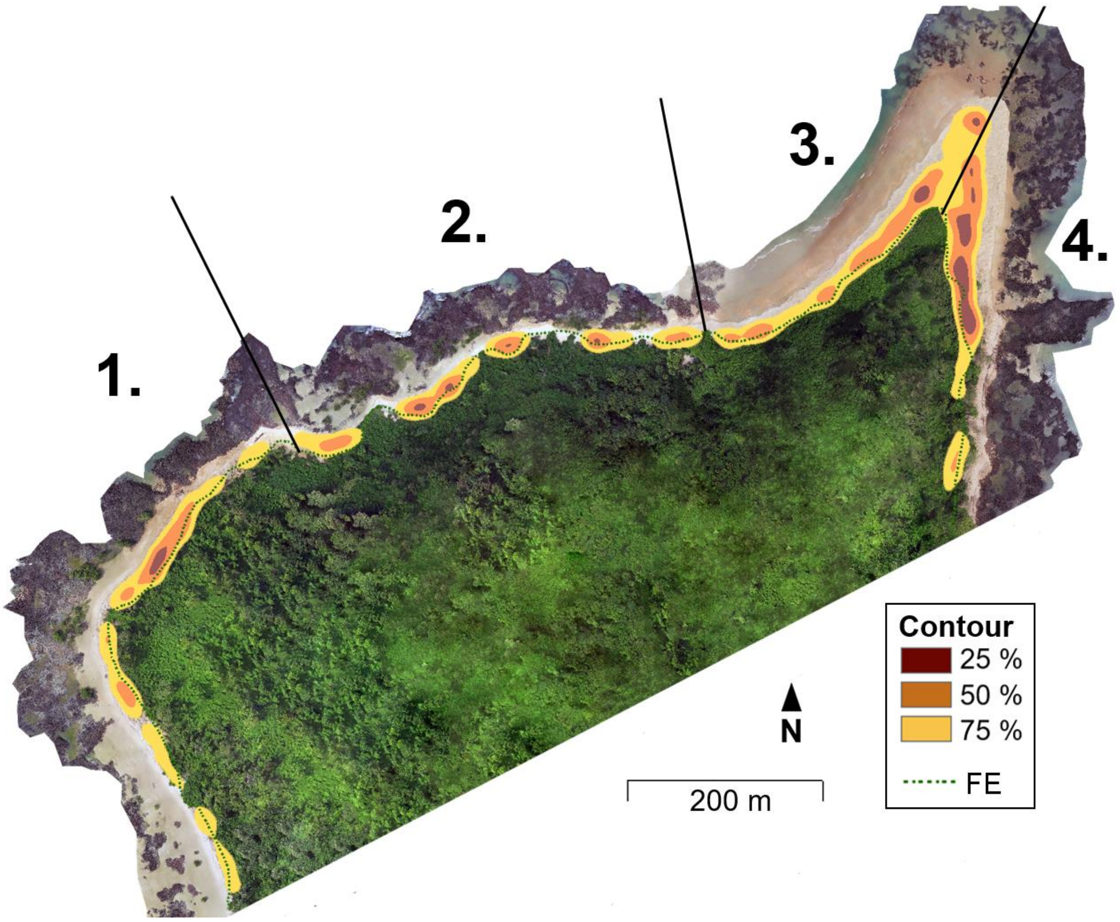

Figure 2. Orthophoto of green turtle nesting beach at Poilão Island, Guinea-Bissau, with kernel nesting density along four beach sections, based on 1,559 nest locations. FE: forest edge. Coloured contours indicate the smallest region containing each probability number of nests (25%, 50%, 75%).

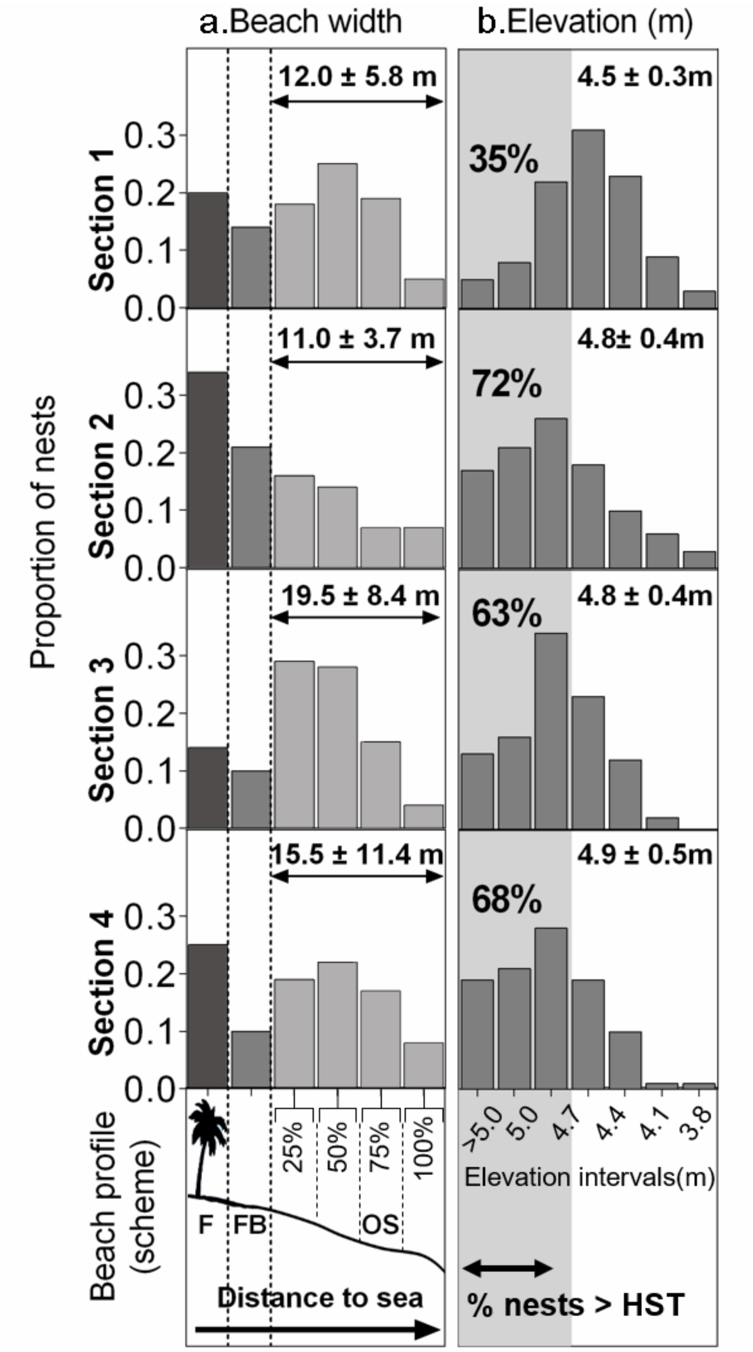

Figure 3. Distribution of green turtle nests (N=1,559) at four beach sections (1: 470; 2: 306; 3: 433; 4: 350), at Poilão Island, Guinea-Bissau: **a)** across beach width, at three habitats: F - forest (dark grey), FB – forest border, and OS – open sand (light grey): each bar at the open sand represents a fourth of the habitat's extension from the forest border to the sea. Mean beach width ± SD is given for each beach section; **b)** along elevation: the shaded area highlights the nests that are above the highest spring tide (HST=4.7m, João Vieira Island tidal table, 17km distant). The mean nest elevation ± SD is given for each section.

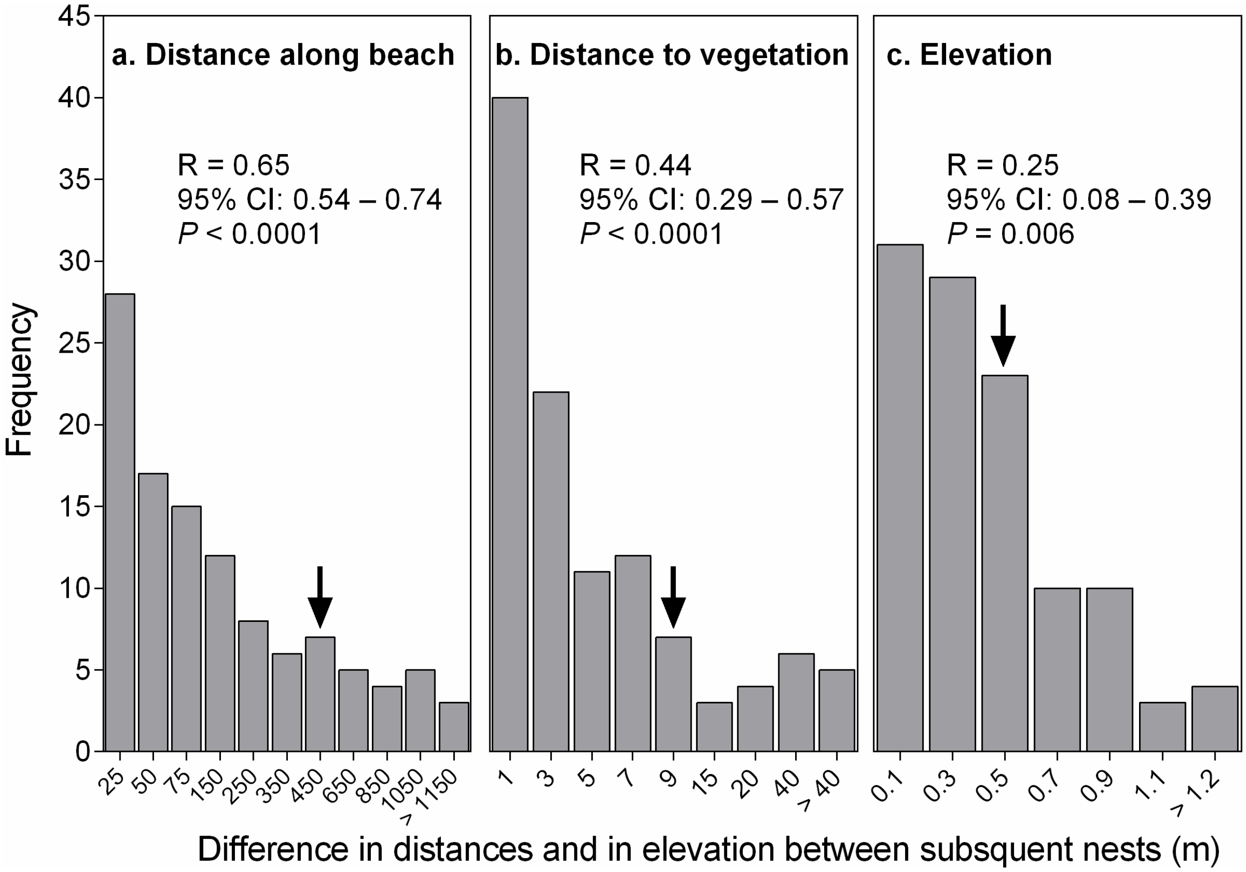

Figure 4. Frequency distribution of differences between two consecutive nests of green turtle females (n=220 nests, from 110 females), at Poilão island, Guinea-Bissau in: **a**. distance along the beach, **b.** distance to the vegetation, and **c.** elevation, with respective measure of repeatability (R), along with 95% confidence intervals (CI) and significant values. Arrows indicate the mean difference between any two random nests after 10,000 iterations, for each of the variables observed. Only two nests from each female were considered to avoid introducing bias by pseudoreplication (i.e. if females with three or more clutches are highly consistent or vive-versa).

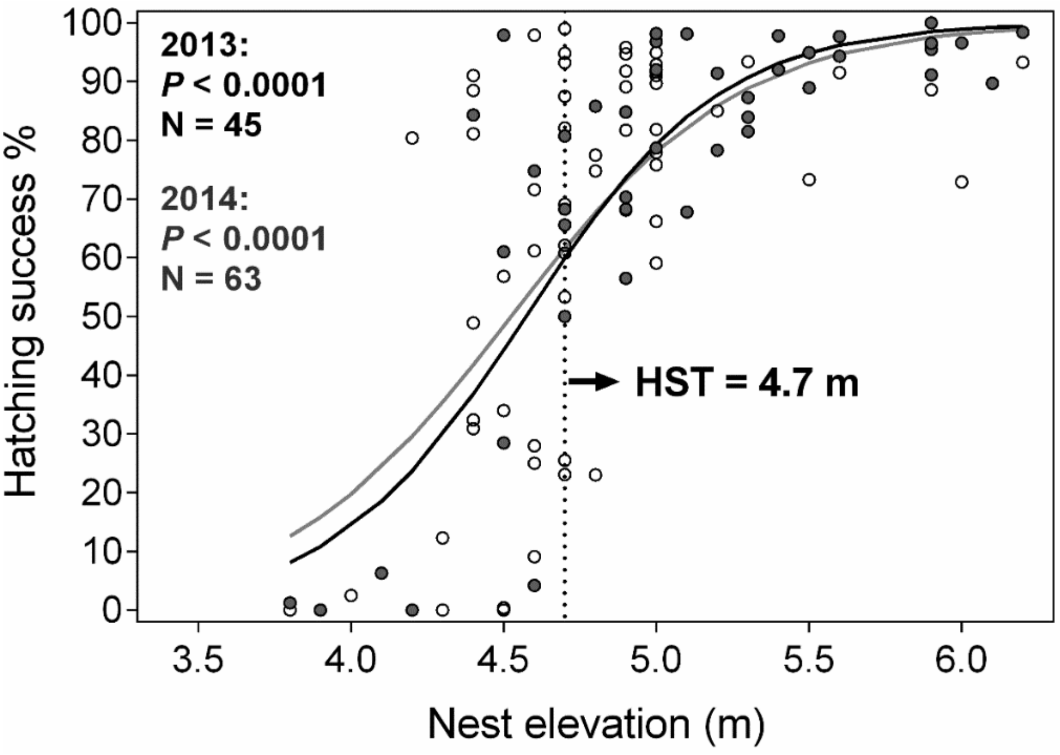

Figure 5. Hatching success of green turtle nests against nest elevation, at Poilão, Guinea-Bissau: circles represent raw values (2013: grey, 2014: open), curves show fitted logistic regression (2013: black, 2014: light grey). Significance of fit and sample size is shown for each year. The dotted vertical line indicates the elevation of the highest spring tide (HST) observed during the study years.

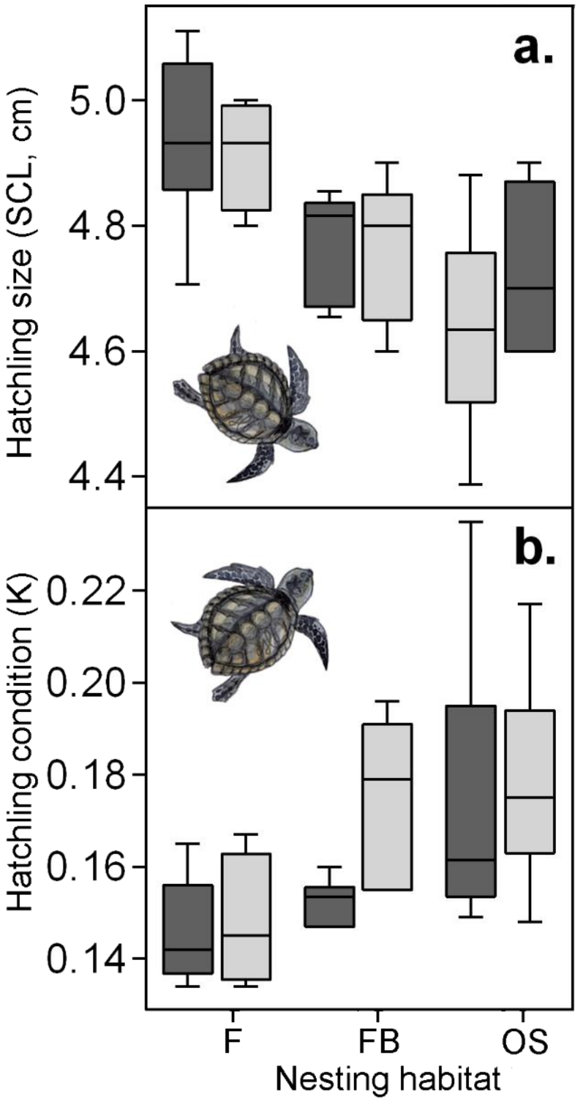

Figure 6. Effect of nesting habitat on green turtle hatchling phenotype, at Poilão Island, Guinea-Bissau: **a.** straight-carapace-length (SCL), and **b.** condition index (K = weight / SCL^3), in 2013 (dark grey), and 2014 (light grey). F: forest; FB: forest border; OS: open sand.

Chapter 2: supplementary information

Table S1. Distribution of expected and observed nests at three nesting habitats for green turtles, at Poilão Island, Guinea-Bissau, and respective chi-square test results for random distribution hypothesis, for each of four beach sections, and for the total extension of the beach.

Beach section	Nesting habitat	Area (m^2)	Proportion of nesting area	Expected number of nests	Observed number of nests	Chi-square	df	*P*
Section 1	Open sand	6060	0.67	317	323	19.282	2.00	< 0.0001
	Forest border	731	0.08	38	60			
	Forest	2190	0.24	115	87			
	Total	8981	1.00	470	470			
Section 2	Open sand	3580	0.57	176	139	34.804	2.00	< 0.0001
	Forest border	663	0.11	33	64			
	Forest	1989	0.32	98	103			
	Total	6232	1.00	306	306			
Section 3	Open sand	9118	0.86	374	344	21.388	2.00	< 0.0001
	Forest border	400	0.04	17	34			
	Forest	1077	0.10	44	55			
	Total	10554	1.0	433	433			
Section 4	Open sand	6100	0.68	238	216	6.548	2.00	< 0.05
	Forest border	1096	0.12	43	48			
	Forest	1788	0.20	70	86			
	Total	8984	1.00	350	350			
Total nesting area	Open sand	24858	0.72	1115	998	86.692	2.00	< 0.0001
	Forest border	2890	0.08	130	218			
	Forest	7044	0.20	316	343			
	Total	34751	1.00	1559	1559			

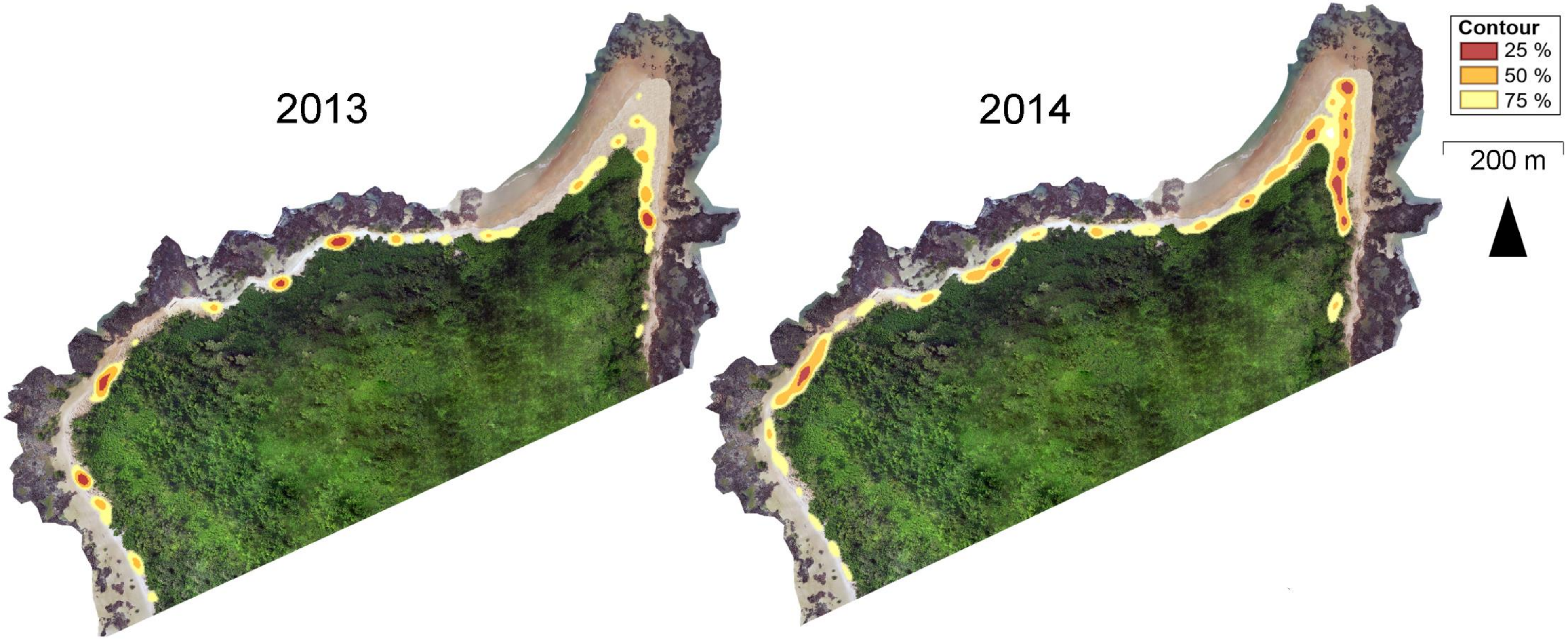

Figure S1. Orthophoto of Poilão Island, Guinea-Bissau, showing green turtle kernel nest density, in 2013 and 2014. Nest distribution was assessed through surveying all females found nesting in each of three nights in 2013 (n=407), and six nights in 2014 (n=1,152), during the peak of the nesting seasons. Coloured contours indicate the smallest region containing each probability number of nests (25%, 50%, 75%).

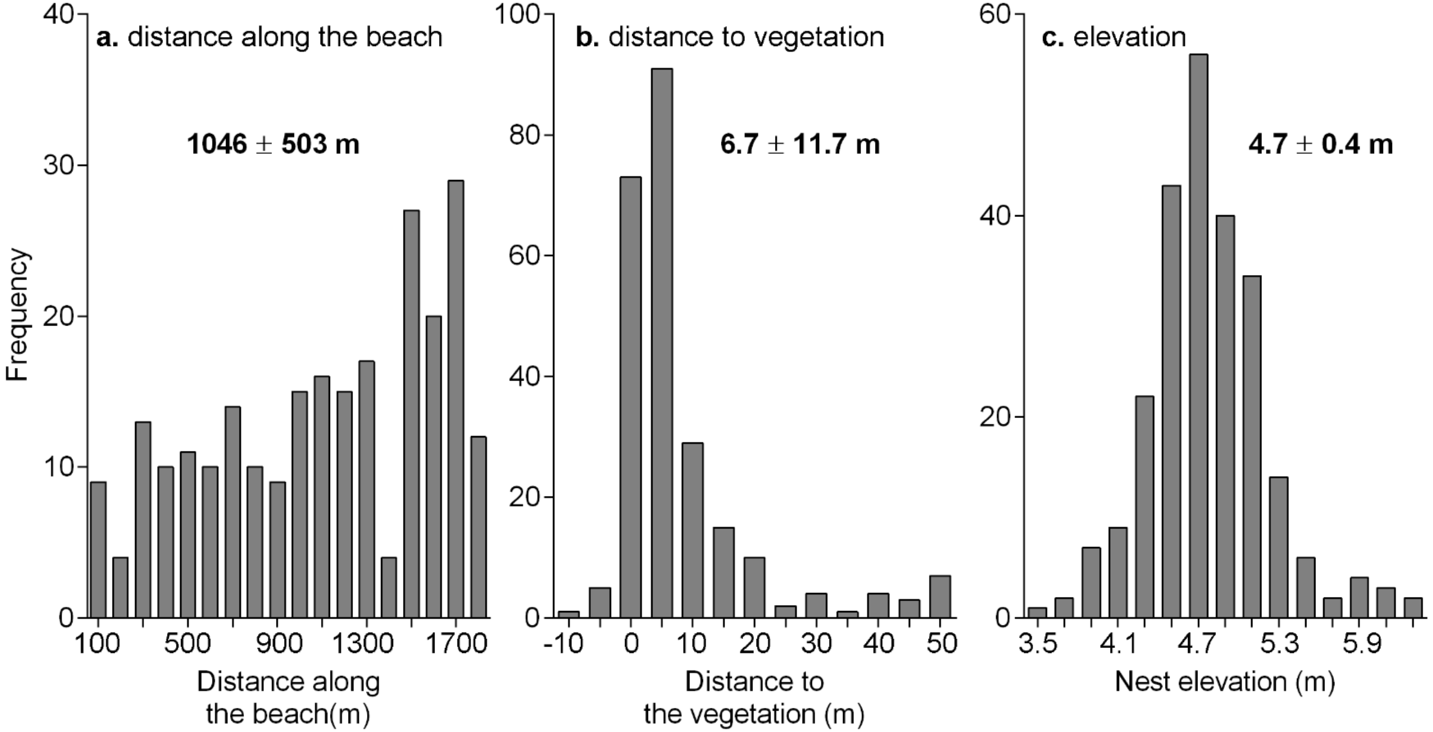

Figure S2. Distribution of nests from 110 green turtles, at Poilão Island, Guinea-Bissau: **a.** along the beach, **b.** in relation to the distance to the vegetation (negative numbers indicate nests under the vegetation), and **c.** across elevation. These are not meant to represent the population distribution, but to show that there was sufficient between-individual variation on nest site selection, such that the measure of repeatability would reflect within-individual variability.

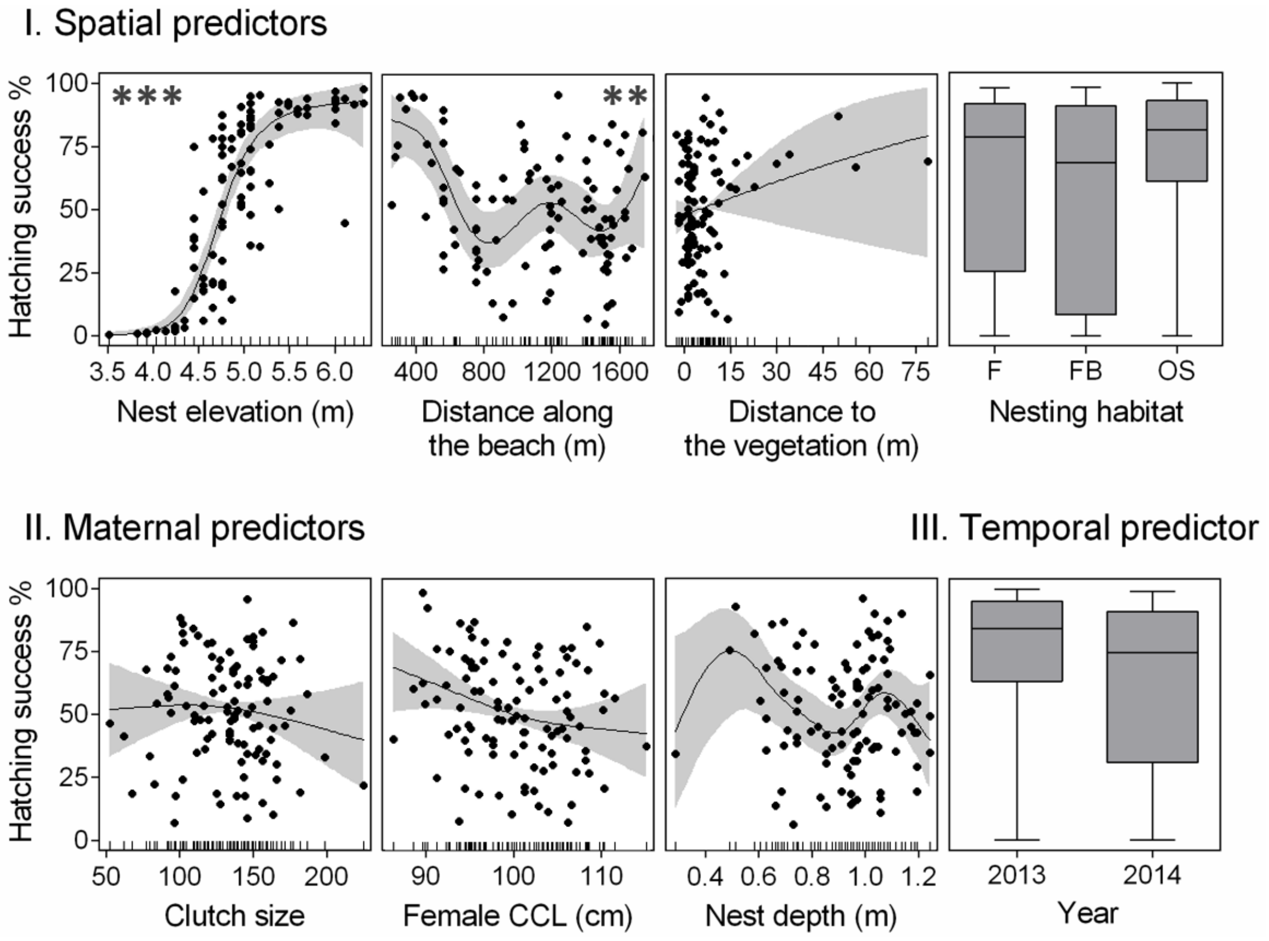

Figure S3. Summary of generalized additive model (GAM), looking at the relationship between hatching success of green turtle clutches laid at Poilão Island, Guinea-Bissau, and: i.four spatial predictors: nest elevation, distance along the beach, distance to the vegetation line, nesting habitat (‘forest’, ‘forest border’, ‘open sand’); ii. three maternal covariates: clutch size, female curved-carapace-length (CCL), and nest depth; and iii. one temporal covariate, year.

Supplementary methods:

Creation of Digital Elevation Model (DEM) and Orthophoto

Data Collection

We used a quadcopter custom made drone, based on the Tarot 650 carbon fibre frame (www.tarot-rc.com), equipped with a Canon S100 compact digital camera, to collect aerial photos of the nesting beach. The drone was controlled with a Pixhawk flight controller from PX4 open-hardware (https://pixhawk.org), and flown in automated mode, assuring a consistent overlap between the aerial images ≥80%, required for accurate DEM/orthophoto (Haala et al. 2013). We used the open source APM Mission Planner (http://plane.ardupilot.com) to setup the following flight parameters: overlap between images, flight time, altitude, and area covered. The drone flew at 35m altitude, at a velocity of 4m/s, allowing for 80% of photo overlapping, and 60% sidelap. The camera focus was fixed to auto, aperture at f4.5, shutter speed 1/1200, and ISO 400. We used the Canon Hack Development Kit (http://chdk.wikia.com/wiki/CHDK) installed on the SD card, to set the camera to take a photo every two seconds, and tilted the camera obliquely, at approximately 30 degrees, to strengthen the network geometry and minimise systematic DEM deformation (James & Robson 2014).

To improve the accuracy of the final model, following Tonkin et al. (2014), we distributed 20 ground control points (GCPs, 25 x 25cm tiles) evenly along the nesting beach, and recorded their coordinates with a Piksi GPS (www.swiftnav.com/piksi-multi). The Piksi GPS is a novel, low cost alternative carrier phase RTK GPS, with an announced centimetre level relative positioning accuracy in real time, in 10Hz position/velocity/time update rate. Two field studies assessed the accuracy of the Piksi, finding horizontal and vertical accuracies of 4.1-8.2cm and 1.1-5.2cm, respectively (Fazeli et al. 2016, Zollo & Gohalwar 2016). We also compared the Piksi against a Leica total station (accuracy ≤1cm) previous to this study, having found a mean horizontal error of 5.0cm and a mean vertical error of 5.5cm. The Piksi consists of two modules: the rover, used to survey the GCPs, and the base station, kept stationary in a GCP placed on the high tide mark. Each GCP was surveyed with the rover placed directly on top of it in a static position for approximately 1min.

Photogrammetry workflow

After manually removing all photos from take-off, landing and blurred ones, the selected photos were imported to Agisoft Photoscan Professional v1.3.1 (© Agisoft). We then went through the steps of the photogrammetry workflow, which have been previously described in detail (Westoby et al. 2012, Gonçalves & Henriques 2015). The parameters used are shown in Table 1. The coordinates of the GCPs were applied to refine camera calibration parameters, georeference the model, and optimize the geometry of the output point cloud in Agisoft Photoscan. The final result was a georeferenced orthophoto and a DEM of the nesting beach. An orthophoto is an image that is free of distortion (i.e. it has been orthorectified) such that the scale is uniform, allowing measurements as if it were a standard map. A DEM is a specialized database that represents the surface between points of known elevation, using interpolation with elevation data. To check if the orthophoto/DEM were correctly georeferenced, we exported a KMZ file of the model into Google Earth, and confirmed that it matched the satellite image.

Table 1. Photogrammetry workflow in Agisoft Photoscan (© Agisoft)

Workflow	Parameters
Align photos	Accuracy: High Generic Preselection Key Point Limit: 60,000 Tie Point Limit: 10,000 Adaptive Camera Model Fitting
Build Dense Cloud	Quality: High Depth Filtering: Moderate
Build Mesh	Surface Type: Height Field Source Data: Dense Cloud Face Count: High Interpolation: Disabled
Build DEM	Geographic Source Data: Dense Cloud Interpolation: Enabled Resolution: 0.0206277
Build Orthophoto	Geographic surface: DEM Blending Mode: Mosaic Enable hole filling Resolution in Metres: 0.0103139 Resolution: 0.03570

References of supplementary material

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Zollo D, Gohalwar R (2016) Piksi TM for UAV Aerial Surveying RTK Direct Georeferencing with Swift Navigation’s Piksi GPS Receiver. Report, pp.1–15

Chapter 3: Climate change resilience of a globally important green turtle population

Ana R. Patrício[1,2], Miguel R. Varela[1], Annette C. Broderick[1], Paulo Catry[2], Lucy A. Hawkes[1], Aissa Regalla[3], Brendan J. Godley[1]

[1]Centre for Ecology and Conservation, University of Exeter, UK
[2]MARE – Marine and Environmental Sciences Centre, ISPA – Instituto Universitário, Lisbon, Portugal
[3]Institute of Biodiversity and Protected Areas of Guinea-Bissau

Abstract

Few studies have attempted estimate the resilience to climate change of populations of wild animals. We use a model higher vertebrate, the sea turtle, as its life history and demography are fundamentally affected by climatic conditions. We use empirical data from a globally important nesting population in West Africa describing multiple aspects of its ecology to assess vulnerability to climate change in a quantitative framework. We project 200 years of primary sex ratios, and create a digital elevation model of the nesting beach to project sea level rise (SLR). As higher temperatures enhance female production, we assess future nesting female recruitment. Primary sex ratio is currently almost balanced, with 52% of hatchlings produced being female. Under IPCC models we predict an increase in the proportion of females by 2100 to 74–94%. Cooler temperatures, both at the end of the nesting season and in shaded areas, will guarantee male hatchling production. Under IPCC scenarios, SLR will lead to loss of 33.4–43.0% of the current nesting area, although, under semi-empirical models of SLR this estimate rises to 86.2%. Climate change will, however, contribute to population growth through population feminization, with 32-64% more nesting females expected by 2120. As incubation temperatures approach lethal levels, population growth will halt and start to decline, but the long-term survival of this population does not seem to be at risk. Overall, this population should resist climate change, and the availability of spatial and temporal microrefugia indicate potential for resilience to climatic change impacts, through the evolution of nest site selection behaviour or changes in nesting phenology. This is the single most comprehensive assessment to date of climate change resilience of a marine reptile, using the most up-to-date IPCC models, including the impacts of temperature and SLR, integrated with population size and trajectory.

Introduction

Anthropogenically-induced climate change is re-shaping the world’s ecosystems at an unprecedented rate, with major impacts on biodiversity (Hoegh-Guldberg & Bruno 2010, Diffenbaugh & Field 2013, Batllori et al. 2017). Many species are already responding by changing their phenology and distribution range (Root et al. 2003, Sunday et al. 2012, Jenouvrier 2013), among other adaptations (Walther et al. 2002), while others seem unlikely to be able to adapt sufficiently (Thomas et al. 2004, Maclean & Wilson 2011). To define priority conservation targets in a changing world, it is thus critical to understand how organisms can resist change, i.e. their capacity to withstand perturbation, and their potential for resilience, i.e. their ability to return to a pre-disturbance state (Connell & Sousa 1983, O’Leary et al. 2017). Few studies have ever attempted to make quantitative estimates of the potential resistance of a population of wild animals to climate change (Williams et al. 2008).

Species with temperature-dependent sex determination (TSD) have been considered among the most vulnerable to climate change, because increasing incubation temperatures may favour the production of one sex in detriment of the other (Mitchell & Janzen 2010). This fundamental life history trait can have deep demographic effects in extreme conditions, as highly skewed sex ratios may lower fecundity and threaten population viability (Mitchell et al. 2010, Santidrián Tomillo et al. 2015). Environmental temperatures are associated with anticipation of breeding seasons in many species, with birds, butterflies and amphibians among the most conspicuous (Walther et al. 2002), and with latitudinal range shifts, marine species adjusting more strictly to their limits of thermal tolerance (Sunday et al. 2012). Simultaneously, ocean thermal expansion is leading to global mean sea level rise (SLR), causing saline intrusion into the water table, flooding of coastal areas, and heightened coastal erosion, further enhanced by increasing storminess, affecting mostly species which rely on coastal habitats (Fish et al. 2005, Hoegh-Guldberg & Bruno 2010). Sea turtles are an excellent example of a vertebrate with distinct sensitivity to climatic conditions throughout incubation and development (Wibbels 2003, Girondot & Kaska 2014), and into adult life stages (Hawkes et al. 2007, Anderson et al. 2013, Dudley et al. 2016). They have TSD, with high incubation temperatures (above approximately 29 ºC; Hawkes et al. 2010) yielding more females and low temperatures more males, and depend on low-lying sandy beaches for reproduction. Together, these threats make sea turtles potentially highly susceptible to climate change impacts (Hawkes et al. 2007, 2009, Poloczanska et al. 2009, Hamann et al. 2010).

Most marine turtle populations studied to date have female-biased primary sex ratios which are expected to skew further with climate warming (Hawkes et al. 2007, Fuentes et al. 2009, Katselidis et al. 2012, Reneker & Kamel 2016). Incubation temperatures above a certain threshold (32.7ºC; Laloë et al. 2017) are expected to reduce clutch survival (Godley et al. 2001a, Santidrián Tomillo et al. 2014, Hays et al. 2017), and hatchling locomotor ability (Fuentes et al. 2010, Booth & Evans 2011). Significant losses of 8-65% of nesting habitat are predicted for several sea turtle rookeries, under climate change scenarios of median severity (Fish et al. 2005, 2008, Baker et al. 2006, Fuentes et al. 2010, Katselidis et al. 2014). Additionally, temporary inundation of beaches, associated with the increasing prevalence and intensity of storms, is expected to lower hatching success (Van Houtan & Bass 2007, Pike et al. 2015). Although it is yet uncertain if sea turtles will be able to adapt to the current rapid changes, they have certainly endured climate change in the past (Poloczanska et al. 2009).

As higher temperatures enhance female hatchling production, it has been argued that climate change may boost the numbers of reproductive females, and consequently nest numbers, promoting population growth (Boyle et al. 2014, Hays et al. 2017). This is dependent, however, on the existence of both sufficient males to fertilize clutches, and incubation temperatures within the thermal tolerance of populations (Santidrián Tomillo et al. 2015, Hays et al. 2017). Additionally, behavioural polymorphism acting on nest-site choice (Kamel & Mrosovsky 2006), and phenological changes of nesting season (Weishampel et al. 2004, Mazaris et al. 2013) have been observed in sea turtle populations, with implications for hatchling sex ratio and survival, suggesting potential for adaptation to climate change impacts.

Integrated assessments of climate change resilience, considering a broad range of impacts and adaptive potential, will enable managers to prioritize conservation efforts, and use realistic measures to mitigate threats. Too often, climate change-induced threats are considered independently (Hawkes et al. 2007, Patino-Martinez et al. 2012, Laloë et al. 2014, Santidrián Tomillo et al. 2015; but see Fuentes et al. 2013, Abella Perez et al. 2016, Butt et al. 2016). Here we apply and extend a vulnerability framework originally posited by Abella Perez et al. (2016), to make a comprehensive assessment of climate change resistance in a globally important green turtle population, and infer on the resilience capacity of this population. This represents the first such attempt to make an empirically based assessment of resistance to climate change in marine turtles and could form an excellent blueprint for comparative studies within and among taxa.

Materials and Methods

Vulnerability framework

For an overview of population resistance to climate change, and adapting the qualitative framework proposed in Abella-Perez et al. (2016) we scored nine criteria, on a five-point scale from 0 (worst) to 100 (best), under three different climate models by the Intergovernmental Panel for Climate Change (IPCC; RCP4.5, RCP6, RCP8; Collins et al. 2013): 1. primary sex ratio; 2. hatchling emergence success; 3. spatial microrefugia; 4. temporal microrefugia; 5. sea-level-rise impact; 6. foraging plasticity; 7. other threats; 8. population trend; and 9. population size. Criteria 8 and 9 are an addition to the original framework. For each climate model we summed the scores of all criteria and divided by n=9, resulting in an overall score of 0 – 100, being 0 the most vulnerable to climate change and 100 the less vulnerable (i.e. more resistant). For scoring system see Table S1.

Climate models

We use projections from three of the four Representative Concentration Pathways (RCPs), in the IPCC fifth report (Collins et al. 2013, Table 1), to provide estimates for each criterion by 2100. We opted to use two intermediate (RCP4.5, RCP6), and one high emissions scenario (RCP8.5). For the trajectories of annual mean incubation temperatures and primary sex ratio, however, we use the Special Report on Emission Scenarios (SRES, Nakicenovic et al. 2000), as annual mean temperature anomalies for the region, enabling trajectory reconstruction, are only available for SRES. Additionally, as several studies indicate that the IPCC process-based projections of SLR are very conservative (Horton et al. 2014, Dutton et al. 2015), and semi-empirical approaches result in more extreme scenarios (Rahmstorf, 2006, Vermeer & Rahmstorf 2009, Grinsted et al. 2010), for SLR impacts we consider the RCPs (Collins et al. 2013) and the most recent estimate based on semi-empirical models (1.2m SLR by 2100; Horton et al. 2014).

Primary sex ratio

a. Historical and projected air temperature trajectory

This research was conducted at Poilão Island (10.8º N, 15.7º W), within the João Vieira and Poilão Marine National Park, at the Bijagós Archipelago, Guinea-Bissau. The green turtle population of the Bijagós is the largest in Africa, among the top six populations worldwide (Catry et al. 2002, 2009, SWOT 2011), with most of the nesting concentrated at Poilão (>90%, C. Barbosa *pers. comm.*). The nesting season extends from mid-June to mid-December, peaking in August and September (Catry et al. 2002, Patrício et al. 2017a). This work encompasses four nesting seasons, from 2013-2016. We used mean monthly historical air temperature data for Bissau (ca. 75km distant, closest station with historical data), ranging from 1901 to 2016, obtained from the Climatic Research Unit of the University of East Anglia (https://crudata.uea.ac.uk/cru/data/temperature/), to reconstruct historical mean air temperatures during the nesting season. To project the trajectory of mean air temperatures to 2100 we added to a historical reference (1970-1999) mean annual temperature anomalies for the region, obtained from the United Nations Development Program (http://www.geog.ox.ac.uk/research/climate/projects/undp-cp/). We used the SRES A1B scenario, which predicts a mean increase in air temperature of 3.13ºC by 2100 (most similar to RCP8.5, Table 1).

b. Sand and incubation temperatures

Sand temperature was recorded at mean clutch depth (0.7m, Patrício et al., 2017a) with Tinytag-TGP-4017 dataloggers (Gemini Data Loggers, Chichester, UK, ± 0.3°C accuracy, 0.1°C resolution), in 2013 (n=16), and 2014 (n=14) nesting seasons. The sand temperature at Poilão varies in relation to the amount of shading, and we defined three microhabitats: 'open sand', 'forest border', and 'forest', as per Patrício et al., (2017a). Thus, temperature dataloggers were distributed along the nesting beach, at the open sand (n=11), forest border (n=9), and forest (n=10). Air temperature has been shown to be a good predictor of sand temperature (Laloë et al. 2014, 2016, Abella-Perez et al. 2016). At our study site air and sand temperatures are also strongly correlated ($T_{sand}=0.94T_{air}+3.04$, $r2=0.60$, $P<0.0001$, $n=39$, T=temperature, Patrício et al. 2017a). The slope of this relationship is not 1 due to a lag of sand temperatures in relation to air temperatures, during our study period, a consequence of the sand's heat capacity, however, when comparing temperatures across multiple years this lag effect should disappear and the slope becomes 1. Given that our slope is nevertheless very close to 1 we applied this equation to obtain historical and projected annual sand temperatures. We then added mean metabolic heating during the thermosensitive period (TSP; the period during middle third of development, when sex is irreversibly defined) to estimate annual mean incubation temperature during the TSP (Godley et al. 2002). Metabolic heating during the TSP at Poilão is 0.5 ± 0.4ºC (Patrício et al. 2017a). Sand temperature at the open sand was on average 1.0ºC above that of the forest border, and 2.5ºC above that at the forest (Patricio et al. 2017a).

c. Sex ratio estimates

In a previous study we modelled the population-specific sex determination response to TSP incubation temperatures (Patrício et al. 2017a) with a logistic function. Here we applied this function to estimate the proportion (P) of female and male hatchlings within each microhabitat (i.e. open sand, forest border, and forest), from 1901 to 2100, as follows:

$$P_{(females)} = 1 / (1 + e^{(-44.856 - 1.527 * \text{TSP temperature})})$$

and

$$P_{(males)} = 1 – P_{(females)}$$

We then accounted for temperature-induced hatchling mortality, using the logistic equation described in Laloë et al. (2017), which models the relationship between emergence success (E) and incubation temperature (T):

$$E_{(T)}=A / 1+e^{-\beta(T-T0)}$$

where the upper asymptote A=86%, the growth rate constant β=-1.7ºC, and the inflection point T0=32.7°C (Laloë et al. 2017). To reconstruct a 200-year trajectory of primary sex ratios by nesting microhabitat, we divided the proportion of female hatchlings produced by the proportion of hatchlings that survived (emergence success).

Spatial and temporal microrefugia

We conducted daily surveys during the nesting season, from August to December, across four years, 2013-2016, and counted all green turtle tracks to assess the temporal distribution of nesting. We followed methodology detailed in Patrício et al. (2017a) to reconstruct mean nesting frequency distribution at the start and end of the season. Daily climate data, available from the National Climatic Data Centre (http://cdo.ncdc.noaa.gov/CDO/cdo, Bolama, 50km distant), were used to compare half-month mean air temperatures, and total precipitation, with mean half-month nesting distribution, across the four years (2013-2016). Note that mean monthly air temperatures at Bissau (used for the historical reconstruction of annual air temperatures) are compatible with those at Bolama, with a mean difference of 0.4 ± 0.3ºC, during the study period). To explore the availability of temporal microrefugia, we calculated the mean annual air temperature for the four years, and classified each half-month as 'cold' if mean air temperature fell below the overall mean, and 'hot' if it was above the mean, and estimated the percentage of nesting occurring in cold and hot months. To assess the presence of spatial microrefugia we examined the current nesting distribution across 'thermal' habitats according to Patrício et al. (2017a; warm, open sand in beaches 3 and 4 =31% of all nests laid; medium, forest border and open sand in beaches 1 and 2 =47%; and cool, forest =22%, Patrício et al. 2017a), and enumerated the proportion laid in the coolest part of the island. For an integrated assessment of spatial and temporal microrefugia, we estimated the primary sex ratio by 2100, for each half-month, from beginning to end of the nesting season (15 June to 15 December), at each of the three microhabitats, under three climate models (RCPs 4.5, 6 and 8, Table 1), considering the emergence success per microhabitat (open sand=66.1 ± 30.8% eggs producing live hatchlings, forest border=51.9 ± 38.3 %, and forest=42.2 ± 41.6%, Patrício et al., 2017a).

Vulnerability to sea level rise (SLR)

We assessed the percentage of nests that would be flooded with increments of 0.1m of SLR if no changes occur in beach morphology, and used this as a proxy for nest area loss. This approach is more meaningful than estimating the available nesting area that would be flooded, as it considers the population's nest site preferences (Katselidis et al. 2014). The distribution of 1,559 nests, surveyed during the peak of the 2013 (n=407) and 2014 (n=1,152) nesting seasons were used to represent the overall nesting distribution (see Patrício et al. 2017a). We created a digital elevation model (DEM) of the beach in Agisoft Photoscan Professional v1.3.1 (© Agisoft), using aerial photos (80% overlap, 35m altitude) taken from a drone (Varela et al. submitted). During the study period, high tide at Poilão ranged from 3.2m (neap tide) to 4.8m (spring tide), with mean high tide (MHT)=4.0m ± 0.3 SD (Bubaque Island tide tables, 40km distant, source: Hydrographic Institute of Lisbon). In the DEM we set the MHT to 0m, to measure nest elevation above it, following previous studies (Fish et al. 2005, Fuentes et al. 2010). We then exported the DEM to ArcGIS 10.3 (ESRI), together with the GPS locations of the 1,559 nests surveyed, and used 3D Analyst Tools to attribute surface elevation to each nest, with the DEM as the input surface. Because mean clutch depth is 0.7m (Patrício et al. 2017a), a nest with a surface elevation >MHT may still be subjected to varying degrees of flooding. Based on a previous study (Patrício et al. submitted) however, nests with a surface elevation below the MHT have a hatching success (H%) ≈ 0%, thereon increasing with elevation, indicating that this is a good reference for complete loss due to inundation.

Foraging plasticity

Population-level foraging plasticity would be advantageous under climate change, if future climatic conditions affect trophic chains and prey availability (Abella Perez et al. 2016). Limited information is available on the foraging behaviour of green turtles from Poilão. We sampled 186 nesting green turtles in 2013 (n=78), 2014 (n=71), and 2016 (n=37), and inferred the dietary range of this population using Nitrogen stable isotope ratios (δ15N: δ14N) from nesting females (see supplementary methods; Godley et al. 1998, Bearhop et al. 2004, Lemons et al. 2011), and on a study by Godley et al. (2010), which recorded the trajectories of eight post-nesting females using satellite transmitters. Nesting females were sampled throughout the season in 2013 and 2014, and in November 2016.

Other threats

Following Abella-Perez et al. (2016), we considered the presence of any known threats to the study population, such as directed harvesting, intentional and incidental captures in fisheries, shipping strikes, ocean and beach pollution, coastal development, invasive species, and ocean acidification, using the Cumulative Impact Score (CIS; a non-linear metric from Halpern et al. 2015), which quantifies 19 anthropogenic threats across the global oceans into one ‘score’.

Population size and trend

a. Female recruitment

Higher temperatures are expected to increase the number of females in populations of sea turtles (Hays et al. 2017). To model a ‘recruitment index’ trajectory for the study population, under SRES A1B, we divided annual estimates of female hatchling production from 2017 to 2100 (i.e. proportion of females emerged from nests) by the current estimates of female hatchling production over the four study years (2013-2016). This gives us a relative index of the number of female hatchlings being produced in relation to the present (Laloë et al. 2014). We then considered 20 years as the minimum age at sexual maturity for Atlantic green turtles in tropical regions (Bell et al. 2005, Patrício et al. 2014), for a ‘recruitment index’ of females to the adult population, assuming that other demographic patterns remain unchanged (Laloë et al. 2014).

b. Nest numbers

Nesting density at Poilão is sufficiently large to preclude complete counting of nests laid (Catry et al. 2009, Patrício et al. 2017a). We therefore estimated the number of nests laid per season from 2013-2016, by multiplying the number of nesting female emergences (each corresponding to an ascending and a descending track) by 1.05, to account for the period of the nesting season not monitored, and by 0.813, to adjust for nesting success (Catry et al. 2009). Then, for a prediction of the number of nests in the future, under the different RCPs (Table 1), we multiplied the mean nest number across the four seasons by i. the nesting female ‘recruitment index’ (above), and ii. 1 – proportion of nests loss to SLR.

Results

Primary sex ratio and emergence success

Historical mean annual air temperatures have increased since the mid-1970s to the present, with a consequent average increase of ca. 1.0ºC in modelled incubation temperatures (Fig. 1a), and an estimated average increase in the proportion of female hatchlings by 20% (Fig. 1b). Increase in female production will be particularly marked in the open sand (ca. 40% increase, Fig. 1b), whereas incubation temperatures in the forest will promote high to moderate male hatchling production throughout the 21st century. Considering both the effects of microhabitat and increased temperatures on hatching success, mean emergence success could drop as low as 40.9% by 2100 (RCP 8.5, Table 1), with 98% of the hatchlings expected to be female (RCP 8.5, Table 1). The relatively wide range of the TRT for this population, 27.6 – 31.4 ºC (Patrício et al. 2017a), however, would allow for male production even under the most extreme RCP.

Spatial and temporal microrefugia

Currently the nesting season largely coincides with both the rainy season and relatively low air temperatures (Fig. 2). We estimated that 46% of the clutches laid at present have the TSP during cold periods (Table 1). Most male hatchlings are produced from clutches laid in late November to early December, and in forest (Fig. 3). Primary sex ratio here remained male-biased under RCP4.5 (42% female hatchlings by 2100), and almost balanced under RCP6 (53%), only becoming female-biased under the most extreme projection, RCP8.5 (82%), but still producing males, particularly towards the end of the season (Fig. 3). The percentage of female hatchlings being produced in the open sand by 2100 is expected to increase from current 61% to 99%, with RCP8.5 (Table 1). Under the same climate scenario, at the forest border, primary sex ratio will increase from 39% to 97% female (Table 1).

Vulnerability to SLR

At present, most clutches are laid 0.8 to 1.0m above MHT (range: -0.6 m to 2.3 m). Because the expected mean SLR according to RCP4.5 and RCP6 are very similar (0.47 vs. 0.48m; Collins et al., 2013), and our DEM has a vertical accuracy of 5cm, we considered these climate models together for projections of SLR impacts. We estimated that by 2100, 33.4% of the current nesting area will be lost under RCP4.5 and RCP6, while 43.0% will be lost under RCP8.5 (Fig. 4, Table 1). Considering semi-empirical models of SLR, however, as much as 86.2% of current nesting habitat could become completely flooded by 2100 (Fig. 4).

Foraging plasticity

Nitrogen isotope ratios (δ15N) varied from 6‰ to 16‰, with a mean of 11.8‰ ± 2.3 SD (Fig. 5), indicating that individual green turtles from Poilão are likely foraging at multiple trophic levels (herbivory and carnivory). An ANOVA test indicated significant differences between years; F1,183 = 5.83, P = 0.003, with the mean δ15N in 2016 significantly higher than that of 2013 (P = 0.03), and in 2014 (P=0.002), but with no difference between the years 2013 and 2014 (P = 0.51), as revealed by a Tukey HSD test. Additionally, Godley et al. (2100) found that some post-nesting females migrated North reaching Mauritania, while others remained local. Thus, foraging plasticity seems to be present at least at the population level, with turtles foraging at different trophic levels, and different feeding grounds (Godley et al. 2010), likely consuming five or more species of prey.

Other threats

In Guinea-Bissau, although marine turtles are fully protected by the national fisheries law, illegal take for local consumption continues to occur (Catry et al. 2009). Poilão and the surrounding waters, however, are virtually free from illegal harvesting, as they benefit from the Bijagós traditional ‘law’, restricting access to the island to very rare ceremonies (Catry et al. 2009). Considering other anthropogenic threats, the CIS for Guinea-Bissau was 3.94, (119th of 238 Exclusive Economic Zones evaluated; Halpern et al. 2015) but we removed the impact score for SLR (0.38), which was already considered separately above, and assumed the nesting beach threats equal to zero. Thus, the total score for ‘other threats’ is 3.57 (Table 1).

Population size and trend

We predicted an increase in nesting female recruitment by 2100 of 58%, 64%, or 32% relative to present, under RCP4.5, RCP6, and RCP8.5, respectively (Table 1). Due to temperature-linked hatchling mortality, however, female recruitment reaches a plateau around 2085, and starts to decrease after 2110 (Fig. 6). Neglecting this important factor would leave scenarios forecasting indefinite increase in female recruitment (Fig. 6). An estimated 20,785 clutches (95% CI: 18,049-22,855) were laid in 2013; 35,556 (95% CI: 30,877-39,099) in 2014; 16,054 (95% CI: 13,941-16,653) in 2015; and 29,348 (95% CI: 25,486-32,272) in 2016. The mean number of nests per year from 2013-2016 was 25,436 (95% CI: 22,088-27,970). Using this value as reference, and accounting for both nesting female recruitment and SLR impacts, we predicted that an average of 26,753 clutches could be laid and survive complete flooding by 2120 under RCP4.5, 27,707 with RCP6, and 19,145 with RCP8.5. These estimates are conservative, as they assume no changes in either beach morphology, spatial distribution of nesting, and mortality patterns at sea.

Vulnerability framework

The corresponding estimate for each criterion of the quantitative vulnerability framework, under each of the three RCPs considered in this study, can be seen in Table 1, together with the scoring for each criterion, and the overall score in climate change resistance for each RCP. The population of green turtles from the Bijagós, Guinea-Bissau, scored 72 (in a scale of 0-100, with 100 being most resistant) under RCPs 4.5 and 6, and 64 with RCP8.5 (Table 1), showing overall high to medium resistance to climate change.

Discussion

Ongoing climate change is driving simultaneously the adaptation and the extinction of populations, species and entire ecosystems (Maclean & Wilson 2011, Xu et al. 2016). Using empirical data and a quantitative framework we conducted a holistic assessment of climate change resistance of a globally significant green turtle nesting population. We document the surprising finding that this population appears to have medium to high resistance under future expected climate change. We highlight the importance of integrated assessments of climate change impacts, instead of considering threats individually, the use of population-specific parameters, and the applicability of this approach to make comparisons with other populations.

Sex ratio

The primary sex ratio at Poilão is among the most balanced reported for green turtle populations, comparable to estimates found in Suriname (54% females; Mrosovsky 1994), Turkey (55.7% females; Candan & Kolankaya 2016), and in one beach of Ascension Island (53.4% females; Broderick et al. 2001), with, to our knowledge, only one study reporting male-biased primary sex ratios (63% males; Esteban et al. 2016). Although the proportion of male hatchlings produced at Poilão may decrease in the future, our results suggest that the complete feminisation of the hatchlings is unlikely. However, the threshold proportion of male hatchlings at which population viability can be jeopardized is yet unknown for marine turtles (Hawkes et al. 2009). Interestingly, recent studies have found that several populations with female-skewed primary sex ratios have approximate numbers of females and males breeding annually (i.e. ‘operational sex ratio’; Wright et al. 2012a, Rees et al. 2013, Stewart & Dutton 2014). These discrepancies between primary and operational sex ratios can result from one or a combination of mechanisms, such as differential survival between female and male post-hatchlings (Wright et al. 2012b), different breeding periodicities (Hays et al. 2014), and males mating with several females from different populations (Roberts et al. 2004, Wright et al. 2012a). Given that the population at Poilão is the largest in Africa, and the sixth largest in the world (Catry et al. 2009, SWOT 2011), more males are likely produced there than in all green turtle African rookeries combined. It is therefore possible that these males contribute significantly to the wider Eastern Atlantic metapopulation, (supported by evidences of male-mediated gene flow across populations, and tracking data, Roberts et al. 2004, Wright et al. 2012a), and may become more important in the future, when sex ratios elsewhere become increasingly female biased.

Vulnerability to sea level rise and storminess

Under the most extreme IPCC projection of future SLR, over half of the current nesting habitat will remain suitable by 2100. Recent studies, however, indicate that IPCC projections are underestimated, and predict higher SLR (Grinsted et al. 2010, Horton et al. 2014, Dutton et al. 2015), under which the proportion of nesting habitat loss at Poilão would increase significantly (ca. 86%). In addition to SLR, future increases in the prevalence and intensity of storms, with heavier precipitation and higher swells, may lead to more frequent temporary inundation of the nesting area (Pike et al. 2015). Large uncertainty of current models precluded us from quantifying these impacts, however, as there is no physical barrier (e.g. cliff, human construction) restricting the nesting beach at Poilão, a likely response to SLR and increased storminess will be coastal realignment. Thus the beach at Poilão may itself be resilient to some degree of climate change. There will be, nonetheless, a limitation to coastal retreat, because Poilão has a very small area (43ha; Catry et al. 2002).

Spatial and temporal microrefugia

In this study, we assessed climate change impacts under the assumption that the spatial and temporal distribution of nests remained unchanged. However, this may not be the case. Poilão is covered by undisturbed tropical forest (Catry et al. 2002), which provides cool incubation conditions, yet currently, under a quarter of the clutches are laid here. There is thus potential for nesting females to use the forest as refuge, mitigating the temperature-linked impacts on the sex ratio and the hatching success, while simultaneously preventing clutch flooding due to SLR and storm events, as the forest is at higher elevations.

Adjusting the start of the nesting season could further reduce feminisation of the population. Beginning to nest two months later, for instance, would synchronize the peak of the TSP with the colder period of the year. Such displacement could have other associated impacts, however, as it would move nesting to the dry season, and moisture provided by rainfall may be important for nest construction (Mortimer & Carr 1987), and male hatchling production (Wyneken & Lolavar 2015). Yet, there is already nesting occurring during this period at Poilão (>100 clutches/year, C. Barbosa pers. comm.), and successful populations nest under dry conditions elsewhere (Godley et al. 2001b, Marco et al. 2012). Notably, if females started to nest slightly earlier instead, it would also decrease TSP incubation temperatures, compared to the present. However, predictions on phenological responses to climate change among sea turtles remain elusive, as it is not clear if the onset of nesting is triggered by sea surface temperatures at breeding (Weishampel et al. 2004) or foraging areas (Mazaris et al. 2009), and whether the response to higher temperatures is anticipation (Weishampel et al. 2004, Mazaris et al. 2009), or delaying of nesting (Neeman et al. 2015). In any case, there is scope for adaptation, and remarkably, flexibility in phenological response.

Population growth

Female production appears to have been rising since the mid-1970s, potentially contributing to current population expansion, as the number of nests in Poilão has increased by 258% in the past ten years (unpublished data, IBAP-Guinea-Bissau). We predicted that this tendency will continue throughout the century, thus climate change will contribute to population growth. As incubation temperatures approach lethal levels, towards the end of the century, growth is expected to reach a plateau, and eventually start to decline. This is in agreement with previous studies, indicating that resilience of TSD species to climate change will eventually be overcome, due to unviable high temperatures (Santidrián Tomillo et al. 2015, Laloë et al. 2017). However, as mentioned earlier, the existence of thermal microrefugia can potentially allow for continued population growth. Additionally, TSD species could, theoretically, cancel (or reduce) the expected temperature-linked hatchling mortality, by experiencing microevolutionary shifts in threshold temperatures, i.e. transitional range of temperatures (TRT: incubation temperatures at which both male and female hatchlings are produced), and pivotal temperature (the incubation temperature resulting in a 1:1 primary sex ratio). This is more likely in populations with wider TRTs (Hulin et al. 2009), as is the case in Poilão (TRT: 27.6-31.4ºC; Patrício et al. 2017a).

Foraging plasticity and external threats

Despite not having samples from prey items to fully understand the diet of the green turtles from Poilão, the values reported here fall well within an omnivorous diet, typically observed among the more generalists loggerhead turtles (Wallace et al., 2009, McClellan et al. 2010), but previously reported for green turtles (Lemons et al. 2011). Having a wide variety of food items is preferable for population persistence, thus, the foraging plasticity evident in this population should be advantageous in the future. A proportion of the nesting females from Poilão migrate northward after the breeding season, to forage at the Banc d'Arguin, in Mauritania (>1000km; Godley et al. 2010), potentially encountering a range of threats along the way. The juvenile turtles originating at Poilão recruit mainly to foraging grounds along the west coast of Africa, in Cape Verde, Liberia, Benin, Equatorial Guinea, and Sao Tome and Principe, with a smaller proportion recruiting to Southwest Atlantic aggregations, in Brasil, and Argentina (Patrício et al. 2017b). Aside from the Equatorial Guinea and Argentina, all other countries have a higher (i.e. worse) CIS, compared to Guinea-Bissau, with Cape Verde and Mauritania scoring the worst, being 60th and 44th, respectively, in a list of 238 Exclusive Economic Zones, mostly due to the presence of extensive artisanal and industrial fisheries, with high rates of bycatch (Zeeberg et al. 2006, Wallace et al. 2010, Halpern et al. 2015). This highlights that population resistance may be compromised by external threats, justifying the ongoing collaborations for the conservation of these species across-boarders. Future work should include satellite tracking of more individuals, in tandem with stable isotope analysis of both turtles and potential food sources, to further unveil their foraging behaviour.

Potential for climate change resilience

In a previous study we found that the green turtles at Poilão currently nest at a preferred elevation, above the high spring tide, enhancing hatching success (Chapter 2), suggesting that nest site choice is an adaptive behaviour that has been under selection. Additionally, nesting turtles displayed high fidelity to nesting microhabitat characteristics (i.e. habitat type, distance to the vegetation, location along the beach and elevation; Chapter 2), also seen among hawksbill turtles (Kamel & Mrosovsky, 2006, 2005), suggesting a possible genetic basis for nest site selection. This provides opportunity for natural selection to act, as females deciding to lay their clutches at higher elevations (safer from flooding) and under cooler conditions (in the forest, but also later in the season) may have enhanced fitness under climate change scenarios, as they should produce more offspring, and notably, almost all of the male hatchlings in the population, which could father the majority of the clutches in the future. Thus, the availability of spatial and temporal microrefugia, together with fidelity to nesting site, suggest potential for mitigation of climate change impacts, through the evolution of nest site selection behaviour. This could lead to the maintenance, or return, to pre-disturbance conditions of the primary sex ratio and of unflooded nests. Overall, we estimate that this population has medium to high resistance to climate change impacts, and can potentially be resilient to changes. This is the single most comprehensive assessment to date of climate change resistance of a marine reptile, using the most updated IPCC models, including the impacts of temperature and SLR, and the population size and trajectory. The approach used here is highly transferable to other marine turtle rookeries, enabling comparisons among populations and species, potentially contributing to regional assessments.

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Table 1. Mean projections of representative concentration pathways (RCPs) from the IPCC fifth assessment report (Collins et al. 2013), and mean estimated values for each of nine criterion used to assess the resistance to climate change of the major green turtle rookery in Africa, at the Bijagós Archipelago, Guinea Bissau, with respective resistance score in parenthesis, following the scoring system in Table S1 (adapted from Abella-Perez et al. 2016). AT: air temperature; SLR: sea level rise

Criterion	**Unit**	**Climate change scenario**					
		RCP 4.5		**RCP 6**		**RCP 8.5**	
Peak Greenhouse Gas emissions	Year	2040		2080		continue to rise	
Mean AT anomaly	2081-2100 (ΔT ºC)*	1.6 ± 0.4		2.0 ± 0.4		3.3 ± 0.6	
Mean SLR	2081-2100 (m)	0.47		0.48		0.63	
1. Primary sex ratio	% female hatchlings	84.0%	(50)	89.0%	(50)	98.0%	(25)
2. Emergence success	% emerged hatchlings	57.0%	(75)	55.7%	(75)	40.9%	(50)
3. Spatial refugia	% nests in warmest habitat	64.2%	(75)	64.2%	(75)	64.2%	(75)
4. Temporal refugia	% nests warmest periods	54.0%	(50)	54.0%	(50)	54.0%	(50)
5. Sea level rise	% nests flooded	33.4%	(75)	33.4%	(75)	43.0%	(50)
6. Foraging plasticity	putative no. prey species	5-10	(50)	5-10	(50)	5-10	(50)
7. Other threats	regional and local threats	3.57	(75)	3.57	(75)	3.57	(75)
8. Population trend	% female recruitment	58.0%	(100)	64.0%	(100)	32.0%	(100)
9. Population size	no. nests**	26,753	(100)	27,707	(100)	19,145	(100)
Resistance score (Σcriteria/ncriteria)		**72**		**72**		**64**	

*****Tropical regions

** Nests in 2120, considering 20 years as minimum age at maturity (Bell et al., 2005; Patrício et al., 2014)

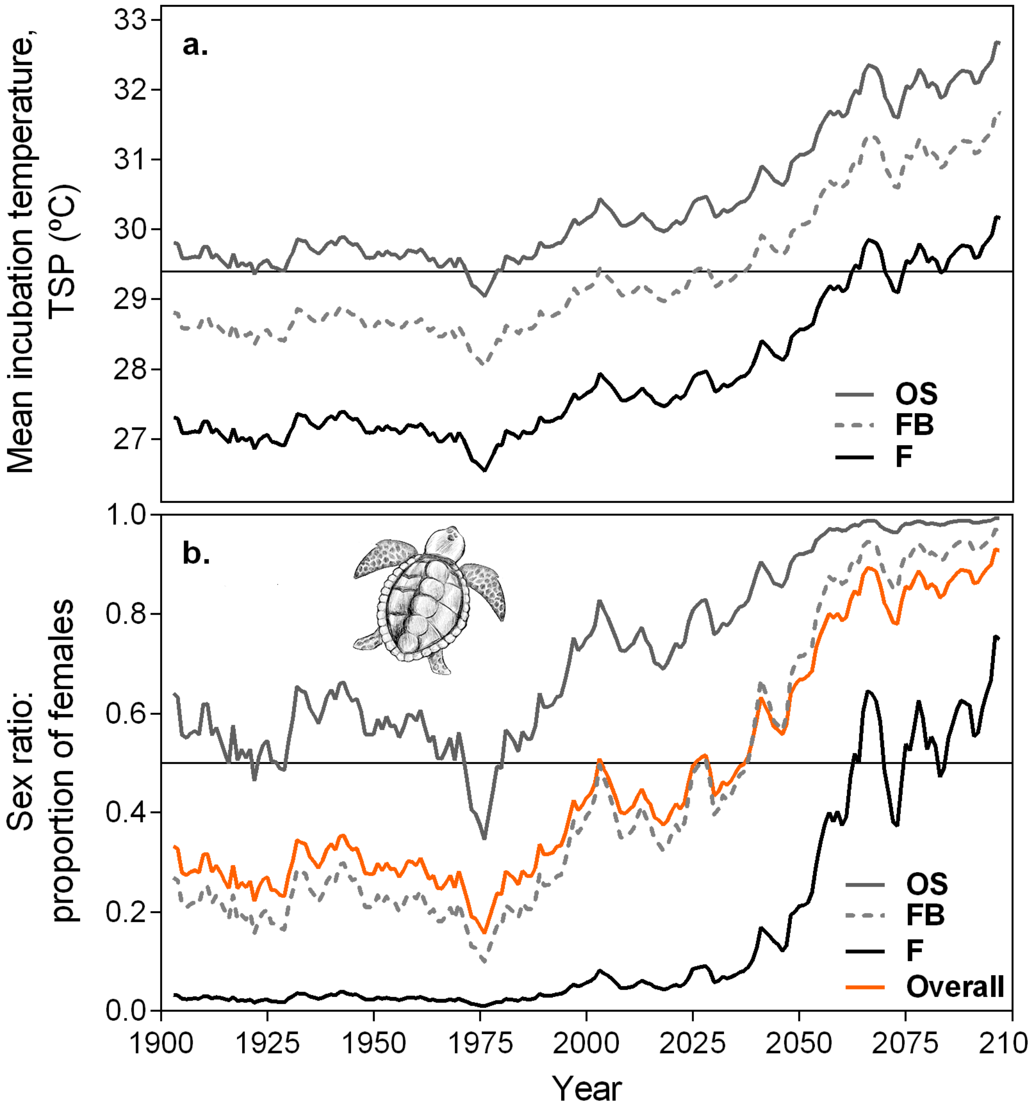

Figure 1. Historical and projected **a.** incubation temperatures, and **b.** proportion of hatchlings expected to be female, in three nesting microhabitats for green turtles, at Poilão Island, Guinea-Bissau. OS – 'open sand', FB – 'forest border', F – 'forest'. Orange curve (overall) shows projection of primary sex ratio accounting for the current nesting distribution across microhabitats, and for the emergence success at each microhabitat. Solid horizontal line indicates **a.** pivotal temperature for this population (29.4 ºC, Patrício et al. 2014), and **b.** 1:1 sex ratio.

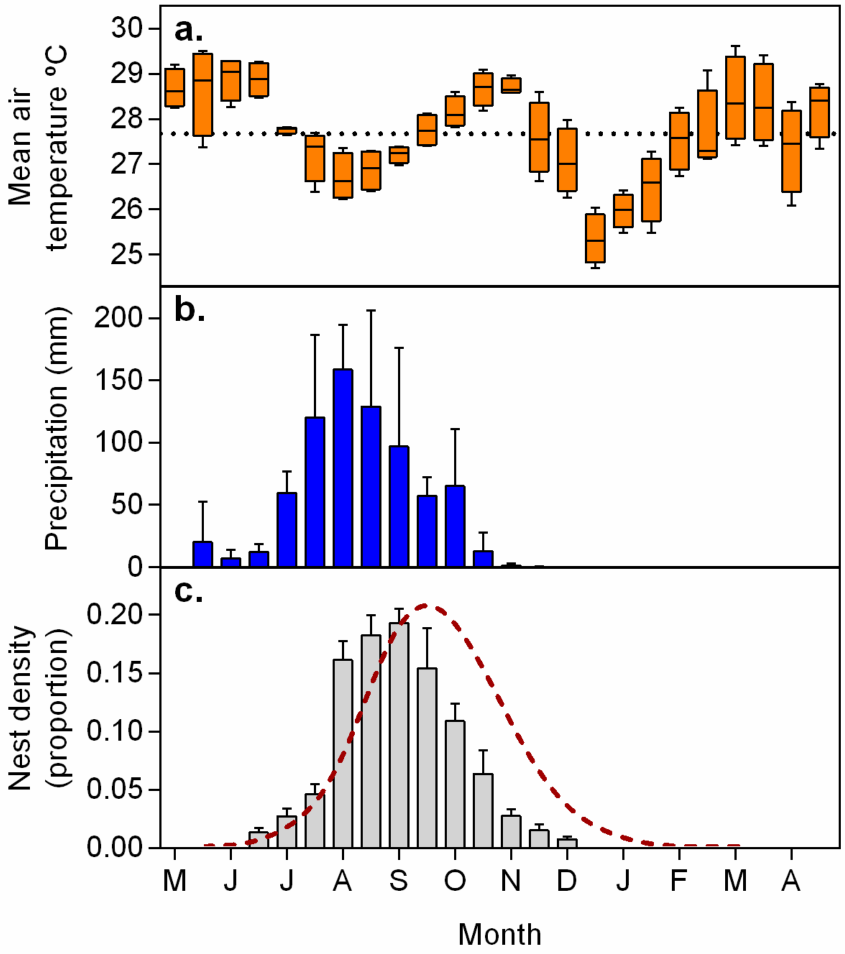

Figure 2. a. Mean bi-weekly air temperature, **b.** precipitation and **c.** green turtle nesting distribution with density curve of thermosensitive period distribution (dashed red line), at Poilão Island, Guinea-Bissau, averaged across four years: 2013-2016. Climate data obtained from the National Climatic Data Centre (http://cdo.ncdc.noaa.gov/CDO/cdo, closest meteorological station Bolama Island, 50km distant).

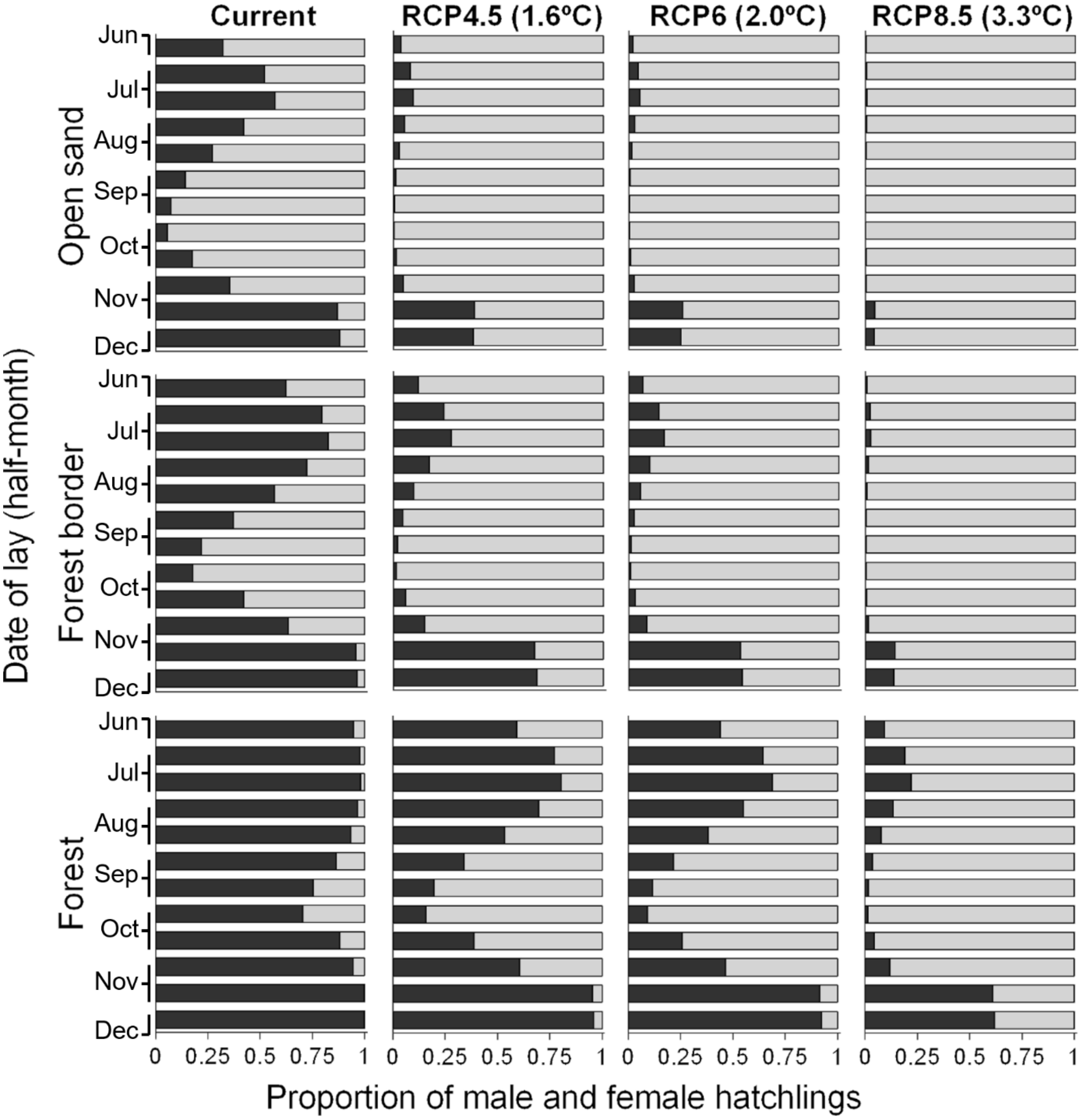

Figure 3. Proportions of male (black) and female (grey) green turtle hatchlings (x-axes), in three nesting microhabitats, across the nesting season, at Poilão Island, Guinea-Bissau: current estimates and projections for 2100, under three climate models, RCP4.5, RCP6 and RCP8.5 (Collins et al. 2013). See Table 1 for climate model details, see methods for habitat definitions.

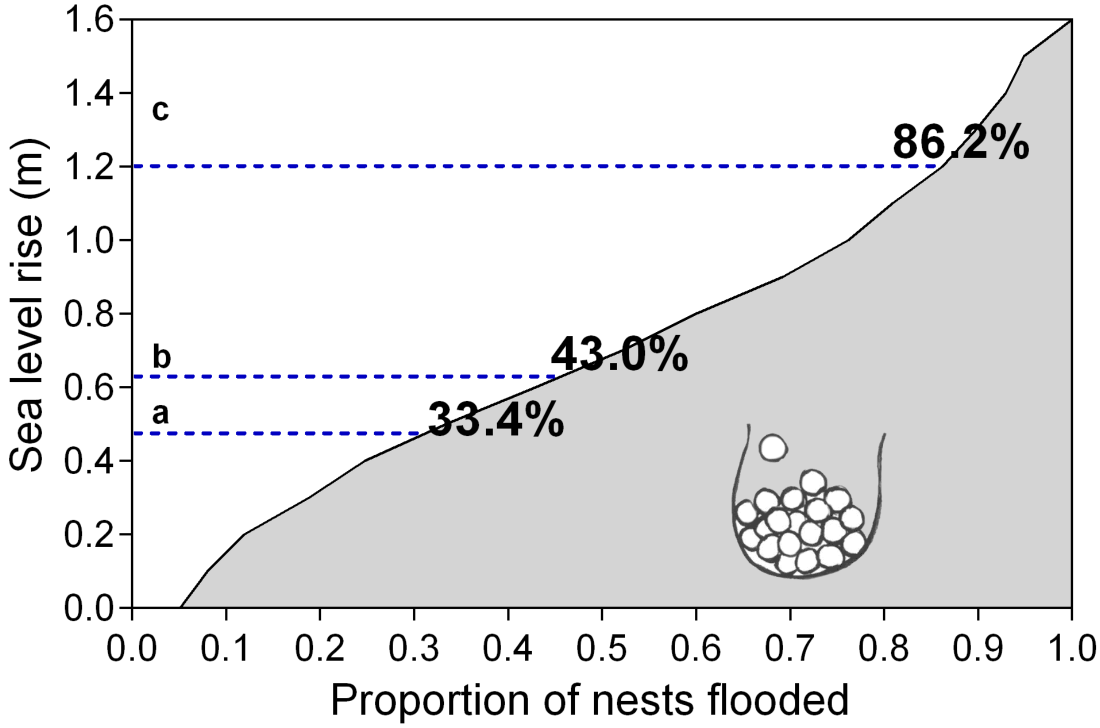

Figure 4. Expected sea level rise (SLR) impact on the current nesting habitat: proportion of green turtle nests at Poilão Island, Guinea-Bissau, that would be flooded with increments of 0.1m of SLR. Dashed lines indicate future scenarios of SLR: a. RCP4.5-0.47m, and RCP6-0.48m; b. RCP8.5-0.63m (from IPCC AR5; Collins et al. 2013), and c. projection derived from semi-empirical models: 1.2m (Horton et al. 2014).

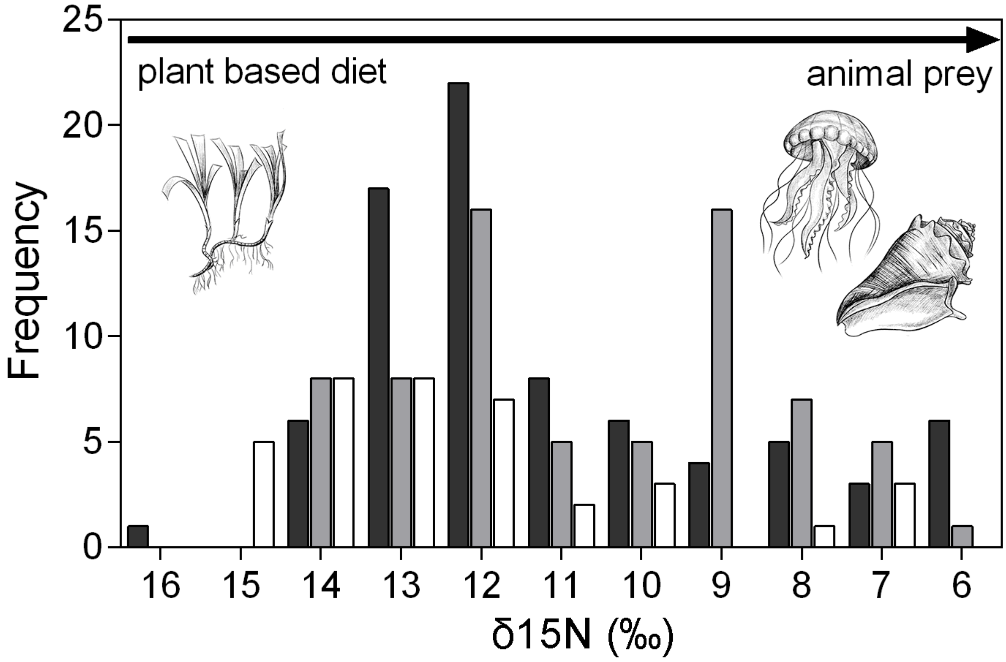

Figure 5. Frequency distributions of nitrogen stable isotopic signature (δ15N) for nesting green turtles from Poilão Island, Guinea-Bissau, in 2013 (11.6 ‰ ± 2.4 SD, n=78, black), 2014 (11.2 ‰ ± 2.2 SD, n=71, grey), and 2016 (11.8 ‰ ± 2.3 SD, n=37, white).

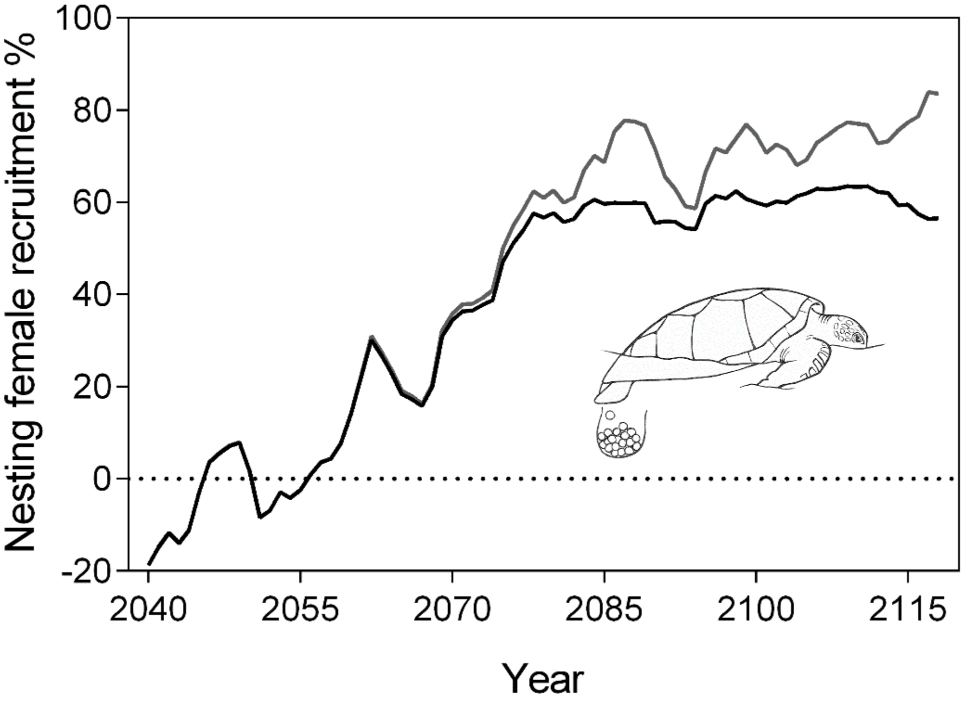

Figure 6. Nesting female recruitment to the green turtle rookery in Poilão Island, Guinea-Bissau, in relation to the present (i.e. 2013-2016), considering a minimum age at maturity of 20 years (Bell et al. 2005, Patrício et al. 2014). In the y-axis, a 0 (dashed line) indicates no change in the number of nesting females, and a recruitment of 100% indicates a doubling. The black curve accounts for the temperature-linked hatchling mortality effect, absent in the grey curve.

Chapter 3: supplementary information

Table S1. Climate change resistance scoring for sea turtles, adapted from Abella-Perez et al. (2016), defined as: 1. Primary sex ratio: % of female hatchlings; 2. emergence success: % of hatchlings emerging from nests; 3. availability of spatial microrefugia: % of clutches laid in the warmest microhabitat (see methods section for definition of microhabitats); 4. availability of temporal microrefugia: % of clutches laid during the warmest periods (above the mean annual temperature); 5. sea level rise: % of current nesting habitat expected to become completed flooded; 6. foraging plasticity: putative number of prey species consumed, from highly specialized to generalist diets; 7. other threats: combination of presence of direct harvest at breeding site and a cumulative anthropogenic impact from Halpern et al. (2015); 8. population trend: % of adult females recruiting to the rookery; and 9. population size: expected number of nests. An option per row is selected and corresponding scores (0, 25, 50, 75, 100) for each column summed and averaged, for a final resistance score between 0 and 100.

Criterion		**Unit**	**Worst**		**Average**		**Best**
			0	**25**	**50**	**75**	**100**
1. Primary sex ratio		% female hatchlings	≥ 99	91 - 98	81 - 90	61 - 80	≤ 60
2. Emergence success		% emerged hatchlings	≤ 10	11 - 30	31 - 50	51 - 75	> 75
3. Spatial microrefugia		% nests in warmest habitat	≤20% of nests in coolest habitat	>20% of nesting in warmest habitat	>40% of nesting in warmest habitat	>60% of nesting in warmest habitat	>80% nests in warmest habitat
4. Temporal microrefugia		% nests warmest periods	≤20%of nests in coolest period	>20% of nesting in warmest period	>40% of nesting in warmest period	>60% of nesting in warmest period	>80% of nesting in warmer period
5. Sea level rise		% nests flooded	>80% of nesting area below SL	>60% of nesting area below SL	>40% of nesting area below SL	>20% of nesting area below SL	≤20% of nesting area below SL
6. Foraging plasticity		putative no. prey species	1-2 (specialist)	2 - 5	5 - 10	10 - 20	> 20
7. Other threats:	direct take	frequency of ocurrence	common	frequent	infrequent	rare	absent
	others	cumulative impact score	6.32 - 8.23	4.16 - 6.31	3.76 - 4.16	2.58 - 3.75	0 - 2.57
8. Population trend		% female recruitment	< 0	0 - 5	5 - 10	10	> 10
9. Population size		no. nests	≤ 100	101 - 500	501 - 1000	1001 - 5000	> 5000

Supplementary methods:

Stable isotope analysis of nitrogen

Skin samples were collected from the shoulder area of nesting green turtles, after all eggs were laid, using a disposable biopsy punch (4-6 mm diameter, Acuderm®), and preserved in 96% ethanol at room temperature. All turtles were individually marked with two Monel flipper tags (front flippers), each identified with a unique reference. Skin samples were rinsed with distilled water, and the epidermis (stratum corneum) was separated from the underlying tissue (stratum germinativum), and finely diced using a scalpel blade. Epidermal samples were then dried at 60°C for 48 hours, following standard protocol described in Ceriani et al. (2014). After completely dry, 0.7 ± 0.1mg of each sample was weighed, and loaded into a sterilized tin capsule, for nitrogen stable isotope analysis (SIA). Isotope analysis was conducted at the Stable Isotope Facility of the Environment and Sustainability Institute (ESI; University of Exeter, Penryn Campus), using a continuous flow isotope ratio mass spectrometer (CF-IRMS), and a Sercon Integra2 stable isotope analyser. Stable isotope ratios are expressed using a conventional notation as δ values defined as parts per thousand or permil (‰) according to the following equation as per Bond & Hobson (2012):

$$\Delta 15N = [(Rsample / Rstandard)-1] \times 10^3$$

Where Rsample and Rstandard are the corresponding ratios of heavy to light isotopes (15N/14N) in the sample and standard (Lemons et al. 2011). Atmospheric nitrogen was used as the nitrogen isotope standard. The standard deviation of the laboratory reference material among runs for δ15N was: 0.18 ‰ for IAEA N1 (δ15N = +0.4 ‰) and, 0.25 ‰ for IAEA N2 (δ15N = +0.25 ‰).

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Chapter 4: Dispersal of green turtles from Africa's largest rookery assessed through genetic markers

Ana R. Patrício[1,2], Angela Formia[3,4], Castro Barbosa[5], Annette C. Broderick[1], Mike Bruford[6], Carlos Carreras[7,8], Paulo Catry[2], Claudio Ciofi[4], Aissa Regalla[5], Brendan J. Godley[1]

[1]Centre for Ecology and Conservation, University of Exeter, TR10 9EZ, Penryn, UK
[2]MARE – Marine and Environmental Sciences Centre, ISPA – Instituto Universitário, 1140-041, Lisbon, Portugal
[3]Wildlife Conservation Society, Marine Program, BP 7847, Libreville, Gabon
[4]Department of Biology, University of Florence, Sesto Fiorentino, 50019, Fl, Italy
[5]Institute of Biodiversity and Protected Areas of Guinea-Bissau (IBAP), CP – 70, Bissau, Guinea Bissau
[6]School of Biosciences, Cardiff University, CF10 3AX, Cardiff, UK
[7]University of Barcelona, Department of Genetics, Microbiology and Statistics, 08028, Barcelona, Spain
[8]Institute of Biodiversity Research of Barcelona, IRBio, 08028, Barcelona, Spain

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Abstract

Marine turtles are highly migratory species that establish multiple connections among distant areas, through oceanic migration corridors. To improve the knowledge on the connectivity of Atlantic green turtles, we analysed the genetic composition and contribution to juvenile aggregations of one of the world's largest rookeries at Poilão Island, Guinea-Bissau. We amplified 856bp mitochondrial DNA (mtDNA) control region sequences of this population (n=171) containing the ~490bp haplotypes used in previous studies. Haplotype CM-A8 was dominant (99.4%) but it divided in two variants when the whole 856bp was considered: CM-A8.1 (98.8%) and CM-A8.3 (0.6%). We further identified the haplotype CM-A42.1 (0.6%), found previously only in juvenile foraging grounds at Argentina, Brazil and Equatorial Guinea. The Poilão breeding population was genetically different from all others in the Atlantic (F_{ST} range: 0.016-0.961, $P < 0.001$). An extensive 'Many-to-many' mixed-stock analysis (MSA) including 14 nesting populations (1,815 samples) and 17 foraging grounds (1,686 samples) supported a strong contribution of Poilão to West Africa (51%) but also to Southwest Atlantic (36%). These findings, in particular the strong connectivity within West Africa, where illegal harvesting is still common, should motivate conservation partnerships, so that population protection can be effectively extended through all life-stages. Our study expands the knowledge on migration patterns and connectivity of green turtles in the Atlantic, evidences the importance of larger sample sizes and emphasises the need to include more finely resolved markers in MSAs and more genetic sampling from West African foraging grounds to further resolve the connectivity puzzle for this species.

Introduction

Many marine species undertake migratory movements among distant geographic areas and across distinct habitats, for feeding, reproduction or development. As a result they may be subject to a diverse range of threats during their extensive movements. Sea birds (Catry et al. 2011), marine mammals (Rasmussen et al. 2007), large fish (Bonfil et al. 2005, Rooker et al. 2014) and sea turtles (Hays & Scott 2013) undertake such movements and are known to play important ecological roles. Understanding their dispersal patterns and the links they establish among different areas is critical to contextualize threats and inform effective management strategies (Rees et al. 2016).

Marine turtles are long-lived organisms and their life histories are marked by ontogenic habitat shifts and large-scale migrations (Bowen & Karl 2007). Green turtles (*Chelonia mydas* L) associate with oceanic currents after hatching and undergo an oceanic pelagic stage, which is thought to last 3-5 years (Reich et al. 2007). After this period, often referred to as 'the lost years', as the whereabouts of the turtles at this phase are poorly known, they generally recruit to coastal habitats, which may change seasonally (Fukuoka et al. 2015), and shift into benthic foraging at a straight-carapace-length of 25-35cm (Bolten 2003). These neritic zones are used as developmental habitats and turtles may spend several years foraging in the same area until reaching a size or maturity stage that triggers them to migrate to additional foraging areas (Patrício et al. 2011, Patrício et al. 2014, Shimada et al. 2015). Upon reaching maturity, adults make periodic migrations between their neritic foraging areas and natal rookeries (Bowen & Karl 2007). This complex migratory behaviour creates multiple connections among distant coastal areas through oceanic migration corridors (Velez-Zuazo et al. 2008). Genetic studies have been critical in enlightening such connectivity (Encalada et al. 1996, Naro-Maciel et al. 2007, Prosdocimi et al. 2012).

Most studies have used sequences of the control region of the mitochondrial DNA (mtDNA), a maternally inherited genetic marker (Bowen & Karl 2007). This marker shows generally high levels of genetic structuring among marine turtle nesting populations worldwide, supporting the natal homing hypothesis, in which the females of marine turtles return to the beaches were they were born to reproduce, as a consequence of philopatry (Meylan et al. 1990). In contrast, foraging aggregations are usually mixed stocks composed of individuals from different rookeries (Bowen & Karl 2007). The high genetic structuring of nesting populations allows the use of mixed stock analysis (MSA; Millar 1987), to estimate contributions of rookeries (stocks) to mixed foraging grounds (mixed stocks). A Bayesian MSA (Pella & Masuda 2001) has been widely applied, allowing the incorporation of informative priors, such as rookery size or geographic distance. Bolker et al. (2007) subsequently developed a 'many-to-many' mixed stock analysis (m2m MSA), aiming to simultaneously answer the questions: 1) where do the individuals from a given source population go? and 2) where do individuals from a given mixed foraging ground originate? Limitations of MSAs have been pointed out however, in particular the assumption that all source populations and mixed aggregations have been adequately sampled (Proietti et al. 2012). The existence of orphan haplotypes at juvenile foraging grounds indicates that some stocks still lack genetic assessment or have not yet been adequately sampled; hence estimates should be interpreted cautiously and along with meaningful ecological data.

One controversial result of recent MSAs of the Atlantic green turtles is the suggested potential connectivity between Guinea-Bissau, West Africa, and the Southwest Atlantic. Although MSAs have supported this migration (Bolker et al. 2007, Monzón-Argüello et al. 2010, Naro-Maciel et al. 2012), the fact that the population at Poilão, Guinea-Bissau, was found to be fixed for the common South Atlantic haplotype (CMA-8; Encalada et al. 1996, Formia et al. 2006, Godley et al. 2010) has limited the interpretations of these results. Notably, the discovery of exclusive haplotypes at low frequency is highly dependent on sample size. This putative migration seems to involve movements greater than expected, according to the 'closest to home' hypothesis where immature turtles tend to move to and settle in foraging grounds closest to their natal beach after recruiting to neritic habitats (Bolker et al. 2007). Additionally, studies using particle dispersal modelling with major oceanic currents did not support this connectivity (Godley et al. 2010, Putman & Naro-Maciel 2013). However, when Putman & Naro-Maciel (2013) estimated the origins of the green turtle Atlantic mixed stocks, tracking particles back through time, this crossing seemed feasible, albeit at low incidence. Lagrangian drifter data have further shown this route to be possible with particle drift (Monzón-Argüello et al. 2010, Proietti et al. 2012). Finally, a similarly large-scale migration of post-hatchling green turtles from Suriname to Cape Verde was supported using mtDNA (Monzón-Argüello et al. 2010).

With this is mind we investigate two questions: 1) where do the post-hatchlings from Poilão disperse to?, and 2) do some of the juveniles found at Southwest Atlantic foraging grounds originate in Poilão? To answer these questions we greatly increased the available sample to characterize the genetic composition of Poilão's nesting population, in an attempt to detect rare haplotypes. We then sought to improve our understanding of the migration patterns and connectivity among Atlantic green turtle populations by comparing our results with molecular data (n=3,501 sequences) from 14 nesting populations and 17 foraging grounds, resulting in the most extensive analysis thus far for this species in the Atlantic.

Materials and methods

Study site and sampling

Poilão Island (N10º52’, W15º43’) is part of the João Vieira and Poilão Marine National Park (PNMJVP), in the Bijagós Archipelago, Guinea-Bissau. It hosts one of the major green turtle nesting populations worldwide (Catry et al. 2002, 2009).This population has been monitored yearly around the peak of the nesting season (August -September) since 2004. In 2013 and 2014 we collected skin samples from 171 nesting females. Samples were taken from the shoulder area using a 6mm sterile biopsy punch as the females laid their eggs and stored in 96% ethanol at room temperature. All sampled individuals were identified with unique tags on both front flippers to avoid sample duplication. Furthermore, the loss of a metal tag leaves scar marks easily recognized within, so we were certain that no previously tagged individual was mistakenly identified as ‘new’. Sampling protocols were approved by the research ethics committee of the University of Exeter and the government of the Republic of Guinea-Bissau.

Sequencing and haplotype assignment

We extracted DNA using the QIAGEN® DNeasy blood & tissue kit, according to the manufacturer’s instructions. A fragment of ~860bp of the mtDNA control region was amplified in a polymerase chain reaction (PCR) with the primers LCM15382 (5’-GCTTAACCCTAAAGCATTGG-3’) and H950 (5’-TCTCGGATTTAGGGGTTT-3’) (Abreu-Grobois et al. 2006) which includes the short region (~486bp) traditionally surveyed for green turtle genetic studies (Encalada et al. 1996, Lahanas et al. 1998, Bjorndal et al. 2006, Formia et al. 2007). Amplifications were performed in a total volume of 25µl, containing 2.5µl of Taq buffer, 3µl of dNTPs, 1µl of $MgCl_2$, 0.5µM of each primer at 10µM, and 0.2µl of Taq DNA polymerase. Cycling conditions were 94ºC for 5min, followed 35 cycles at 94ºC for 1min, 55ºC for 1min and 72ºC for 1min with a final extension step at 72ºC for 10min. Desired PCR products were purified with a combined Exonuclease I and Shrimp Alkaline Phosphatase solution (ExoSAP®). The reaction was incubated for 15min at 37°C, followed by 15min incubation at 80°C to inactivate the two enzymes. Sequences of forward and reverse DNA strands were performed at Macrogen Inc. (Netherlands).

Sequences were assembled and aligned manually using BioEdit 7.2.5 (Hall 1999). Unique haplotypes were identified using the Basic Local Alignment Search Tool (BLAST) from the National Centre for Biotechnology information (http://www.ncbi.nlm.nih.gov/), following the nomenclature of the Archie Carr Center for Sea Turtle Research (ACCSTR; https://accstr.ufl.edu/resources/mtdna-sequences/).

Population structure

To assess the genetic diversity of the nesting population at Poilão compared with the other Atlantic nesting populations, we truncated the mtDNA fragments to 490bp length, the fragment historically explored and for which most genetic information of other locations is currently available. We used Arlequin 3.5.1.3 (Excoffier & Lischer 2010) to estimate the haplotype (*h*) and nucleotide (π) diversity of nesting populations, to estimate the genetic distances among population pairs (Φst) and to test the significance of differentiations with exact tests based on haplotype frequencies. A false discovery rate (FDR) correction (Narum 2006) was applied to calculate the most fitting threshold for the *P*-value significance considering the number of comparisons involved in the analysis and under an expected original threshold of $P<0.05$. To contextualize our sampling location within the Atlantic region, the genetic distances were used to perform a principal coordinate analysis (PCoA) using the package GenAlEX 6.5.0.1 (Peakall & Smouse 2012). We tested the significance of the PCoA grouping with an AMOVA, using Arlequin 3.5.1.3 (Excoffier & Lischer 2010).

‘Many-to-many’ mixed-stock analysis

We generated a dataset of 14 nesting populations (n=1,815) and 17 foraging grounds (n=1,686) when including our new mtDNA data for Poilão to the previously existing data for Atlantic nesting populations and foraging grounds (see Fig. 1 and Table 1 for sites included in this study and literature sources). We used only sequences generated by this study to characterize the genetic composition of Poilão in order to avoid potential pseudoreplication with datasets obtained in previous years. Relative contributions to foraging areas from nesting populations (mixed stock-centric approach), and probable use of foraging grounds from nesting populations (source-centric approach) were estimated with m2m MSA, using the R package mixstock (Bolker et al. 2007) and

WinBUGS (Lunn et al. 2000). We conducted the MSA including the number of nesting females in each population (Seminoff et al. 2015) as a weighting factor (Prosdocimi et al. 2012). We used the Gelman-Rubin diagnostic to assess convergence of the chains to the posterior distribution, assuming that there was no evidence of non-convergence at values <1.2 (Pella & Masuda 2001). As it is reasonable to assume that other African juvenile aggregations remain to be identified, we simulated a juvenile foraging ground fixed for haplotype CM-A8 (similar to Naro-Maciel et al. 2012), with a sample size equal to the mean of the foraging grounds sample sizes (n=99), and added this sample to the dataset to conduct another m2m MSA, as described above.

Results

Genetic composition of Poilão

Genetic variability of the Poilão nesting population was the lowest of all Atlantic populations (h ± SD=0.012 ± 0.011, π ± SD=0.0001 ± 0.0003, Table 2). The haplotype CM-A8 was dominant as suggested by previous studies (Formia et al. 2006). However the use of longer sequences (856bp sequences) distinguished two variants of this haplotype: CM-A8.1 (98.8%) and CM-A8.3 (0.6%). We also identified the haplotype CM-A42.1 (0.6%), a previously orphan haplotype found to date only in juveniles from West Africa and South American foraging aggregations (see Table S1 for haplotype frequencies of nesting populations). Because this is a rare haplotype and not previously detected in the population we performed two independent PCRs, and sequenced the amplified fragment in two independent occasions, to confirm that this result was not a product of genotyping error.

Population Structure

The nesting population at Poilão was significantly different from all other Atlantic green turtle rookeries (Table S2). All other nesting populations were distinct from each other except when comparing Ascension Island with Bioko Island, Aves with Suriname, and Aves with Buck Island. The comparisons between Suriname and Buck Island, and between Sao Tome and Principe and Bioko became non-significant after FDR correction. Populations pairs where genetic differentiation was not detected were kept as discrete sources for the m2m MSA, based on their divergence in population size and geographic position (Monzón-Argüello et al. 2010, Putman & Naro-maciel 2013). The PCoA separated rookeries by region and evidenced three major groups: South Atlantic, Southeast Caribbean and Northwest Caribbean (Fig. 2), each group defined by a major haplotype(s): CM-A8, CM-A5 and CM-A3/A1, respectively. An accumulated 85.5% of the genetic variability was explained by the two principal coordinates of the PCoA. Although located in the North Atlantic, Poilão clustered within the South Atlantic group. Using this *a-priori* grouping in the AMOVA, highly significant structure was observed among the three groups (Φ_{ST}=0.691, P<0.001), with 55.9% of the variation found among groups.

‘Many-to-many’ mixed-stock analysis

The source-centric m2m MSA indicated that most of Poilão’s hatchlings recruit to African foraging grounds (51.4%), but 36.2% would reach juvenile aggregations in the Southwest Atlantic and 8.6% reached North Atlantic aggregations (Fig. 3). A small proportion of the Poilão rookery was attributed to an ‘unknown’ foraging area (3.7%). The foraging ground-centric m2m MSA estimated that at Sao Tome, Corisco Bay and ‘West Africa’ (Liberia to Benin) foraging grounds, over 60% of the juveniles originate at Poilão, as do 31% of the green turtles foraging at Cape Verde (Fig. 4). Notably, at the Southwest Atlantic foraging aggregations proportions ranging from 16 – 41% were attributed to Poilão (Fig. 4). Adding the simulated West African foraging ground did not change contributions at a regional scale, but the relative contributions to the Gulf of Guinea were significantly lower (8 to 14 % lower, Fig. S1), to accommodate a large contribution to this putative aggregation. Because CM-A42 is a rare haplotype, and therefore difficult to detect when sampling a population, we decided to run two additional MSAs using simulated datasets, each of these including haplotype CM-A42 in one of the other two major green turtle rookeries in the Atlantic (i.e. Costa Rica and Ascension Island), and observed no significant changes (Fig. S1).

Discussion

One of the principal techniques that can offer insight into the migratory connectivity of species with complex life cycles is genetics. The robustness of subsequent inferences, however, are highly dependent on the amount of information available, including the number of populations and foraging grounds analysed, and the strength of the signal, including sample sizes at each site and length of the genetic sequence and number of genetic markers analysed. Here we substantially increased the sampling effort at one of the largest Atlantic green turtle rookeries, in Poilão, Guinea-Bissau, in order to resolve the uncertainties surrounding the connectivity between this nesting population and distant juvenile aggregations. We successfully found the origin of a previously orphan haplotype, present in West Africa but also in South American foraging grounds, giving strength to the hypothesis of east-to-west connectivity.

Post-hatchling dispersal to east and west

The contributions estimated by the m2m MSA confirm a strong connectivity within West Africa, as previously hypothesized (Godley et al. 2010), particularly with foraging grounds in the Gulf of Guinea (i.e. 'Sao Tome', 'West Africa' and 'Corisco'). This dispersal was also predicted under an ocean circulation model and through passive drifting associated with the Guinea current (Putman & Naro-Maciel 2013). Due to the large size of the nesting population at Poilão, it is likely however that significant proportions of other African juvenile aggregations originate there. In Guinea-Bissau there are at least two known aggregations of immature green turtles; i) at Unhocomo and Unhocomozinho Islands, in the Bijagós Archipelago, ca. 100km NE from Poilão Island, and ii) at Varela beach, ca. 200km NE from Poilão, that have not been genetically described. The same is true for a foraging ground in Mauritania, mentioned in Godley et al. (2010), and in Congo. We have shown that the estimated proportions of post-hatchlings distributed among West African foraging grounds depend on the inclusion of new juvenile aggregations. To fully understand the connectivity of the large nesting population at Poilão it is essential that investigation into identifying and genetically characterizing these aggregations is undertaken. The MSA also suggests the existence of a transatlantic developmental migration for the green turtle, from east to west, potentially associated with the Equatorial currents, and continuing south, reaching foraging grounds in the south of Brazil and in Argentina.

Studies using estimations of passive drift with major oceanic currents to predict the movements of post-hatchlings have suggested that dispersal from Guinea-Bissau to Southwest Atlantic is unlikely (Godley et al. 2010, Putman & Naro-Maciel 2013). However, marine turtle hatchlings are capable of oriented swimming significantly impacting trajectories (Putman et al. 2012a, 2012b, Scott et al. 2012), and able to swim against currents (Booth 2014). Indeed, recent research has shown that drifter tracks can diverge substantially from those of young turtles (Putman & Mansfield 2015), and it is likely that this process is contributing to observed divergence between genetic- and drift-based predictions (Naro-Maciel et al. 2016). Because CM-A42 is a rare haplotype and therefore difficult to detect, we ran additional MSAs using simulated datasets, including this haplotype in each of the two other major green turtle rookeries in the Atlantic (i.e. Costa Rica and Ascension Island), and observed no significant changes (Fig. S1).

Expanded sample size and geographic coverage

Formia et al. (2006) assessed the genetic composition of Poilão nesting females (n=51) and found it was fixed for the South Atlantic dominant mtDNA haplotype CM-A8. By extending this previous sample size, we were able to detect a rare haplotype, CM-A42, which to date had only been reported from juvenile green turtles foraging in South America, and in West Africa. This enabled the differentiation of Poilão from other Atlantic rookeries, agreeing with the high philopatry characteristic of the green turtle, and the fine scale differentiation existent in other places. Increasing sample size has previously been shown to improve statistical power of detection of structure among populations, through the finding of rare haplotypes (Formia et al. 2007).

The existence of non-significant comparisons among certain population pairs could result from i) recent isolation, such that haplotype frequencies did not have time to differentiate, or ii) current gene flow, mediated by incidental deviations from natal homing. Lack of differentiation between Bioko and Ascension Island has been attributed to recent colonization of the former

(Formia et al. 2006). Likewise, Aves and Buck Island may be more recent than the more diverse population in Suriname. Alternatively, the proximity between Aves and Buck Island (<300km), and between Bioko and Sao Tome (<400km), may be more likely to result in occasional migrants preventing substantial differentiation at an evolutionary timescale (Formia et al. 2006).

Our study further expands the geographic coverage of previous MSAs of the green turtle in the Atlantic, incorporating 14 nesting populations and 17 foraging grounds in our dataset. In particular the inclusion of African foraging grounds (i.e. Corisco Bay, Sao Tome and 'West Africa') improved the estimates for the distribution of hatchlings from Poilão, significantly reducing the estimate of the putative "unknown" foraging site (here 3.7%) compared to a recent MSA (14.3%; Putman & Naro-Maciel 2013), as well as substantially reducing the confidence intervals. In a previous m2m MSA a high contribution of Ascension Island to Corisco Bay was estimated (ca. 40%; Bolker et al. 2007). Here that contribution drops to 9.2%, and we predict a much stronger connectivity between Poilão and Corisco. By including more foraging grounds in our analyses, we show that Ascension rookery contributes primarily to juvenile aggregations along the Southwest Atlantic (71.6%), also seen in Putman & Naro-Maciel (2013). Analogously, the foraging ground-centric MSA in Bolker et al. (2007) attributes most of the Corisco Bay foraging ground to Ascension Island (>70%), while we estimate that 60.5% of the aggregation origins at Poilão, and only 27.7% would come from Ascension. Additionally, the contributions of Aves Island and NE Brazil to Corisco Bay estimated before (ca. 15% each; Bolker et al. 2007) were considerably lower in our study (2.7% and 4.8%, respectively), and these populations also seem to contribute more to the Southwest Atlantic. See tables S3 and S4 for m2m MSA summary results.

Limitations of MSA and future directions

Although increasing the available sample size at Poilão and expanding the dataset for Atlantic green turtles has improved MSA estimates, this analysis is based on a single marker and on a short fragment of the mtDNA. To further unveil the green turtle connectivity puzzle in the Atlantic (and elsewhere) the strength of the genetic signal can be enhanced, at a lesser cost than substantially increasing sample sizes. Data from the longer mtDNA sequences should be obtained from existing samples and made available, to be incorporated in MSAs. Additionally, a new marker consisting of four AT short tandem repeats (STRs) in the 3’ end of the mtDNA, the mtSTR, has been shown to add information on the genetic variability within unique mtDNA haplotype classes, and to contribute to improve the knowledge on population connectivity and evolutionary relationships (Tikochinski et al. 2012, Shamblin et al. 2015). Recent research using nuclear markers have found significant structure among sea turtle rookeries, supportive of male phylopatry (Carreras et al. 2011, Naro-Maciel et al. 2012, Roden et al. 2013, Naro-Maciel et al. 2014). Finally, new genomic approaches have the potential to greatly increase the signal resolution and detect fine-scale population structure (Funk et al. 2012, Milano et al. 2014, Benestan et al. 2015). Some of the above information is now becoming available at local scales. Hopefully future collaborations among research groups at wider scales will lead to significant advances in our understanding of the dispersal and distribution of marine turtles.

Adult linkage

Godley et al. (2010) recorded the trajectories of eight post-nesting females from Poilão using satellite transmitters, finding that they foraged either locally, at the Bijagós Archipelago (n=4) , or regionally (n=4), at the Banc d’Arguin National Park, Mauritania (>1000km distant). This aspect of investigation would clearly benefit from enhanced sampling effort, preferably across multiple seasons, at different points of the season and across a range of size classes, to avoid inter-annual (Witt et al. 2011), seasonal (Rees et al. 2010) and phenotypic (Hawkes et al. 2006) biases in dispersal. Future satellite tracking should be conducted in tandem with stable isotope analysis to facilitate the posterior assignment of turtles to these areas, facilitating the analyses of larger sample sizes, more relevant for population studies (Zbinden et al. 2011).

If nesting females from Poilão are limited to the East Atlantic it does not necessarily contradict our suggestion of transatlantic dispersal as post-hatchlings. Post-hatchling turtles forage during their developmental migration (Reich et al. 2007), which allows them to travel much longer distances than adults that typically fast during their reproductive migrations (Hays & Scott 2013, Scott et al. 2014). According to Scott et al. (2014), if the developmental foraging area is so far as to be too costly to be repeatable during the cyclic reproductive migrations, adults may forage locally, as observed at the Bijagós, instead of returning to the sites experienced when younger. This mechanism reduces the consumption of reproductive energy utilized, potentially increasing fecundity, however it is dependent on the availability of foraging areas.

Conservation implications

In this study we show the importance of Poilão rookery for the recruitment of juvenile green turtles in West Africa, and also that the link with the Southwest Atlantic is very likely. In Guinea-Bissau, despite marine turtles being fully protected by the national fisheries law, illegal take continues to occur without much law enforcement effort (Catry et al. 2009), particularly at the Bijagós Archipelago, where turtles are frequently harvested at the nesting beaches, mostly for local consumption (Catry et al. 2009). The nesting population at Poilão is one exception, thanks to the Bijagós traditional 'law' (reinforced by state authorities), restricting access to the island on very rare ceremonies of social and religious significance (Catry et al. 2009). Off Guinea-Bissau and along the coast of West Africa however, vast artisanal fleets and many industrial fishing fleets operate, using trawlers without turtle excluder devices (Zeeberg et al. 2006, Catry et al. 2009), and longlining (Moore et al. 2010). Unfortunately, there is a scarcity of quantitative data in the region, either on bycatch or on targeted harvesting of marine turtles, particularly from artisanal fisheries (Moore et al. 2010). The foraging grounds in the Southwest Atlantic to which Poilão seems to contribute to, on the other hand, are mostly protected from illegal harvesting (Marcovaldi & dei Marcovaldi 1999), although bycatch may be a problem (Wallace et al. 2010). Despite the existing threats, major green turtle populations are recovering globally following decades of conservation efforts (Broderick et al. 2006, Catry el at. 2009, Bourjea et al. 2015). It may be that the long term enhanced protection in South America and the efforts in Poilão itself are the principle factors involved in the recovery of this population.

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Table 1. Nesting populations (n=14) and foraging grounds (n=17) for Atlantic green turtles *Chelonia mydas* included in a many-to-many mixed-stock analysis, using the control region of mtDNA as a marker (490bp).

Site name	Abbreviation	Reference
Nesting Populations:		
East central Florida	EcFL	Shamblin et al. (2014)
South Florida	SFL	Shamblin et al. (2014)
Southwest Cuba	CUB	Ruiz-Urquiola et al. (2010)
Quintana Roo, Mexico	MEX	Encalada et al. (1996)
Tortuguero, Costa Rica	CR	Bjorndal et al. (2005), Encalada et al. (1996)
Matapica/Galibi, Suriname	SUR	Encalada et al. (1996), Shamblin et al. (2012)
Buck Island	BUC	Shamblin et al. (2012)
Aves Island	AV	Lahanas et al. (1998, 1994), Shamblin et al. (2012)
Rocas/Fernando Noronha	RC/FN	Bjorndal et al. (2006), Encalada et al. (1996)
Trindade Island	TRI	Bjorndal et al. (2006)
Ascension Island	ASC	Encalada et al. (1996), Formia et al. (2007)
Poilão, Guinea-Bissau	POI	This study
Bioko Island, Eq. Guinea	BIO	Formia et al. (2006)
Sao Tome and Principe	STP	Formia et al. (2006)
Foraging grounds:		
North Carolina, USA	NC	Bass et al. (2006)
East central Florida, USA	EcFL	Bagley (2003), Bass & Witzell (2000)
Bahamas	BHM	Lahanas et al. (1998)
Barbados	BRB	Luke et al. (2004)
Almofala, Brazil	ALF	Naro-Maciel et al. (2007)
Rocas Atoll, Brazil	RC	Naro-Maciel et al. (2012)
Fernando de Noronha, Brazil	FN	Naro-Maciel et al. (2012)
Bahia, Brazil	BA	Naro-Maciel et al. (2012)
Espirito Santo, Brazil	ES	Naro-Maciel et al. (2012)
Ubatuba, Brazil	UB	Naro-Maciel et al. (2007)
Arvoredo Island, Brazil	AI	Proietti et al. (2012)
Cassino Beach, Brazil	CB	Proietti et al. (2012)
Buenos Aires, Argentina	BuA	Prosdocimi et al. (2012)
Cape Verde	CV	Monzón-Argüello et al. (2010)
Corisco Bay, Equatorial Guinea	COR	Formia et al. (2006)
‘West Africa’: Liberia to Benin	WA	Formia et al. (2006)
Sao Tome, Sao Tome and Principe	ST	Formia et al. (2006)

Table 2. Haplotype and nucleotide diversity (means ± SD) of Atlantic green turtle *Chelonia mydas* nesting populations (n=14) included in a 'many-to-many' mixed-stock analysis, using the control region of mtDNA as a marker (490bp). Number of females refers to total number of reproductive females in each population (Seminoff et al., 2015). The present study population is in **bold**. Site abbreviations as in Table 1.

Nesting Population	**Sample size**	**No. of females**	**No. of haplotypes**	**Haplotype diversity (*h*)**	**Nucleotide diversity (π)**
EcFL	311	4490	9	0.512 ± 0.02	0.0016 ± 0.0013
SFL	174	3302	10	0.444 ± 0.043	0.0022 ± 0.0016
CUB	26	2226	7	0.648 ± 0.089	0.0053 ± 0.0033
MEX	20	18257	7	0.816 ± 0.058	0.0051 ± 0.0032
CR	433	131751	5	0.163 ± 0.023	0.0033 ± 0.0022
SUR	46	13067	4	0.132 ± 0.053	0.0013 ± 0.0011
BUC	61	63	2	0.153 ± 0.065	0.0030 ± 0.0020
AV	55	2833	2	0.140 ± 0.055	0.0029 ± 0.0020
RC/FN	69	345	7	0.463 ± 0.071	0.0026 ± 0.0018
TRI	99	2016	7	0.505 ± 0.052	0.0012 ± 0.0011
ASC	245	1417	13	0.303 ± 0.038	0.0008 ± 0.0008
POI	**171**	**29016**	**2**	**0.012 ± 0.011**	**0.0001 ± 0.0003**
BIO	50	850	2	0.184 ± 0.068	0.0004 ± 0.0006
STP	26	376	7	0.569 ± 0.110	0.0026 ± 0.0019

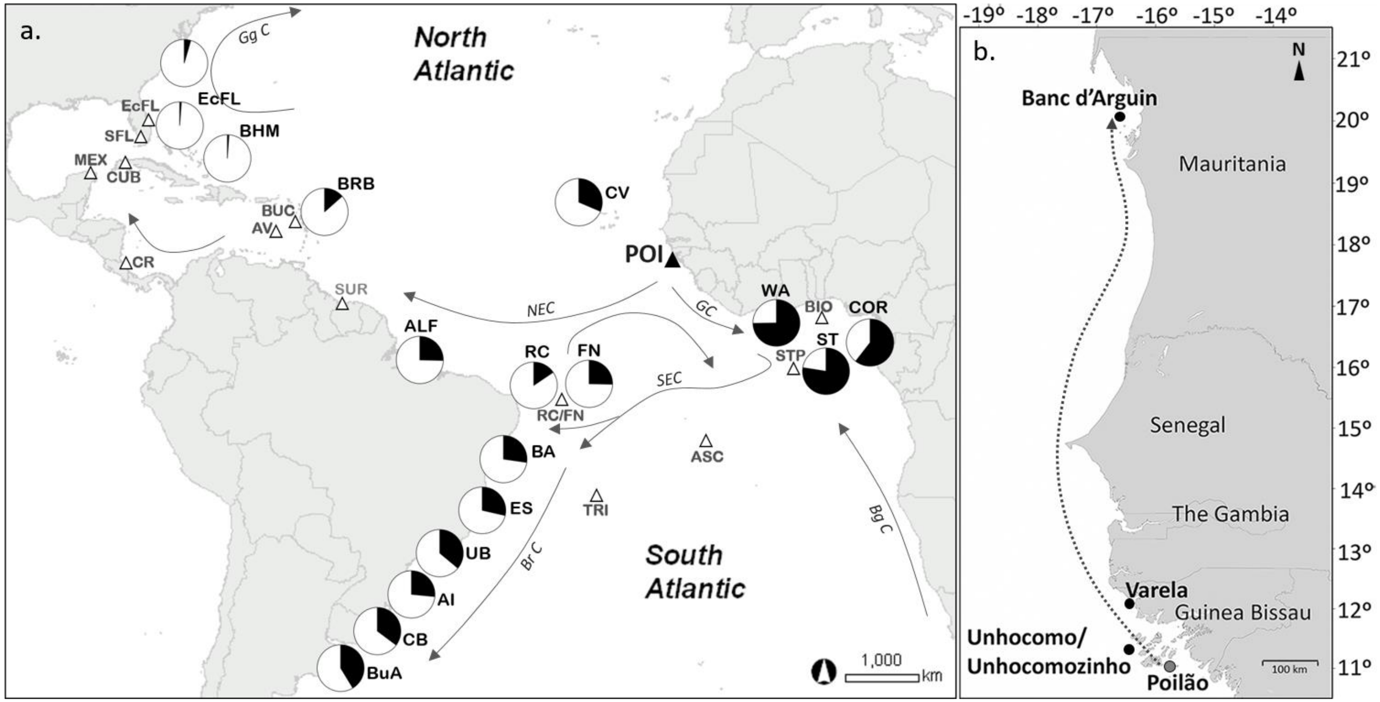

Figure 1. a. Atlantic green turtle *Chelonia mydas* nesting populations (Δ; n=14) and foraging grounds (n=17) used in a 'many-to-many' mixed-stock analysis (MSA), and results of foraging ground-centric MSA (pie charts: in black proportion of each foraging site that originates from the study population in **bold**; see Table 1 for abbreviations and data sources. Arrows indicate general direction of major currents. GfC: Gulf Current, NEC: North Equatorial Current, SEC: South Equatorial Current, BrC: Brazil Current, GC: Guinea Current, BgC: Benguela Current. **b.** Region map with study site, Poilão, and three juvenile foraging grounds likely to partly originate at Poilão, but genetically uncharacterized: Unhocomo/Unhocomozinho and Varela (Guinea-Bissau), and Banc d'Arguin (Mauritania). Dashed arrow illustrates the direction of four adult female green turtles tracked from Poilão to Banc d'Arguin (Godley et al. 2010). (Maps created using www.seaturtle.org/maptool).

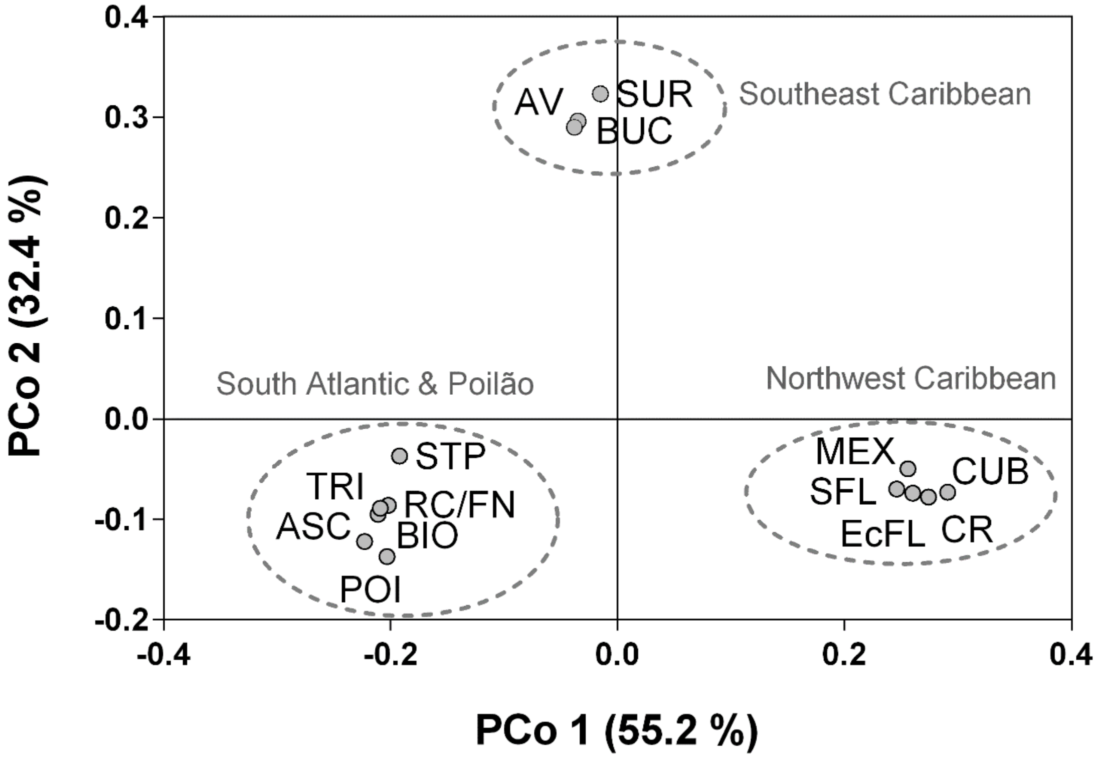

Figure 2. Principal coordinate analysis (PCoA) of 14 Atlantic green turtle *Chelonia mydas* populations using Φ_{ST} distances, and considering the 490bp mtDNA fragment. Rookeries were grouped in three clusters: the South Atlantic & Poilão, the Southeast Caribbean, and the Northwest Caribbean. Percentage of variability explained by each coordinate is shown in brackets. See Table 1 for site abbreviations.

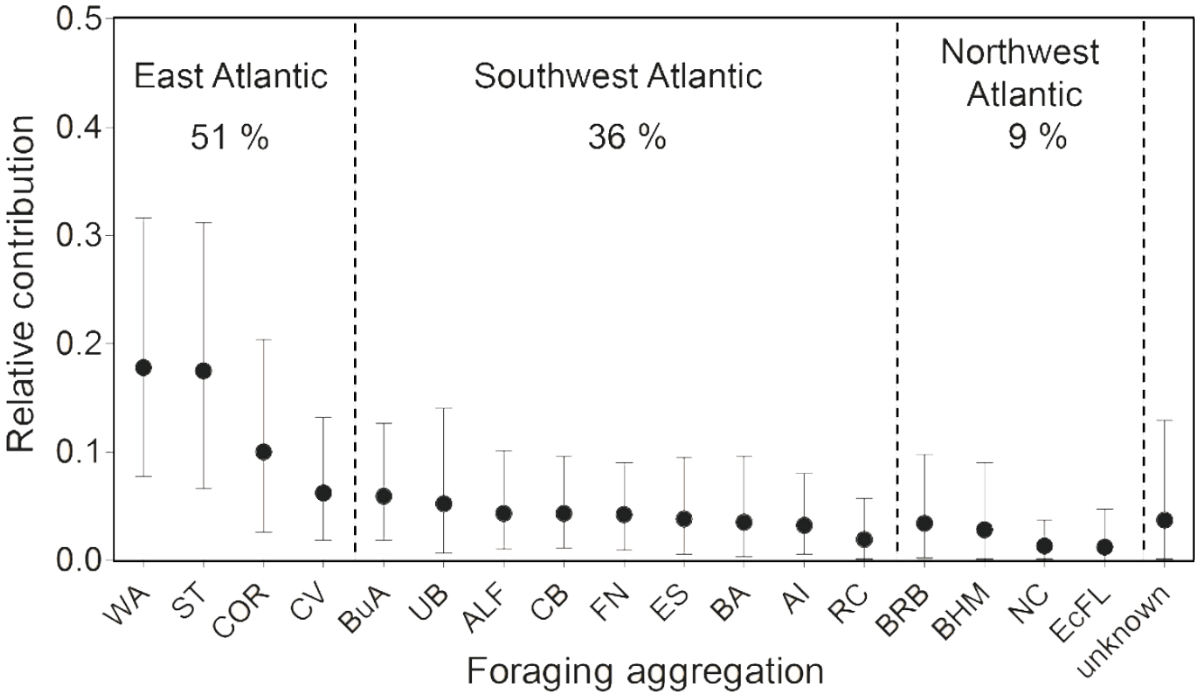

Figure 3. Mean relative contribution of the Poilão nesting population of Atlantic green turtles *Chelonia mydas* to 17 foraging grounds, estimated by a 'many-to-many' mixed-stock analysis. Error bars show 95% confidence intervals. See Table 1 for site abbreviations. Dashed lines separate geographic regions.

Chapter 4: supplementary information

Table S1. mtDNA control region haplotype frequencies (490bp), at 14 Atlantic green turtle nesting populations with total no. of samples per area. See Table 1 for site abbreviations. Long haplotypes (856bp) for study area are shown in the table below.

Haplotype	Nesting Populations													
	EcFL^{a,b}	SFL^{b}	MEX^{a}	CR^{c,d}	CUB^{e}	BUC^{f}	AV^{d,f,g}	SUR^{a,f}	RC/N^{a,h}	TRI^{h}	ASC^{a,i,j}	POI^{k}	BIO^{i}	STP^{i}
CM-A1	197	27	7		3									
CM-A2	7	4												
CM-A3	92	127	5	395	16		5	1						
CM-A4				1		16								
CM-A5	2	4	1	32		45	62	68						1
CM-A6								3			11		5	1
CM-A7								1						
CM-A8	1								50	67	204	170*	45	17
CM-A9									7	19	9			
CM-A10									2		5			
CM-A11									1	1				
CM-A12									5					
CM-A13	7	2												
CM-A15			1											
CM-A16	2	1	1											
CM-A17		2	2											
CM-A18	1	1	3											
CM-A20				2										
CM-A21				3										
CM-A23										6	1			
CM-A24										1	7			

Table S1. Continuation

Haplotype	Nesting Populations													
	EcFL[a,b]	**SFL[b]**	**MEX[a]**	**CR[c,d]**	**CUB[e]**	**BUC[f]**	**AV[d,f,g]**	**SUR[a,f]**	**RC/N[a,h]**	**TRI[h]**	**ASC[a,i,j]**	**POI[k]**	**BIO[i]**	**STP[i]**
CM-A25									3		1			
CM-A27					1									
CM-A28	2	3			1									
CM-A32									1	4	1			
CM-A33										1				
CM-A35														1
CM-A36														3
CM-A37														1
CM-A38														2
CM-A39											1			
CM-A42												1*		
CM-A44											1			
CM-A45											1			
CM-A46											2			
CM-A48					5									
CM-A50											1			
CM-A53		3												
CM-A56					1									
CM-A57					1									
n	311	174	20	433	28	61	67	73	69	99	245	171	50	26

[a]Encalada et al. 1996, [b]Shamblin et al. 2014, [c]Bjorndal et al. 2005, [d]Lahanas et al. 1998, [e]Ruiz-Urquiola et al. 2010, [f]Shamblin et al. 2012, [g]Lahanas et al. 1994, [h]Bjorndal et al. 2006, [i]Formia et al. 2006,[j]Formia et al. 2007,[k]This study

* Long haplotypes (856bp): CMA8.1 (n=169), CMA8.3 (n=1), CMA42.1 (n=1)

Table S2. Pairwise exact test *P*-values (above diagonal) and pairwise Φ_{ST} values (below diagonal), among 14 Atlantic green turtle *Chelonia mydas* nesting populations, based on ~490bp sequences of the control region of the mtDNA. The study site is in grey and in bold, and abbreviations follow those in Table 1. Asterisks indicate statistically significant comparisons (**P*<0.05, ***P*<0.01, ****P*<0.001) i) prior to corrections, in the low diagonal, ii) after false discovery rate (FDR) correction, in the above diagonal. Non-significant values, after FDR (Narum 2006) correction, are marked in bold (for a *P*< 0.05 FDR=0.0098, *P*< 0.01 FDR=0.0020, *P*< 0.001 FDR=0.0002).

	MEX	EcFL	SFL	CR	AV	BUC	CUB	SUR	TRI	RC/FN	ASC	**POI**	BIO	STP
MEX	-	0.009*	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	**0.000*** **	0.000***	0.000***
EcFL	0.082**	-	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	**0.000*** **	0.000***	0.000***
SFL	0.182***	0.197***	-	0.000***	0.000***	0.000***	0.009*	0.000***	0.000***	0.000***	0.000***	**0.000*** **	0.000***	0.000***
CR	0.202***	0.254***	0.033***	-	0.000***	0.000***	0.009*	0.000***	0.000***	0.000***	0.000***	**0.000*** **	0.000***	0.000***
AV	0.796***	0.895***	0.872***	0.820***	-	**0.342**	0.000***	**0.108**	0.000***	0.000***	0.000***	**0.000*** **	0.000***	0.000***
BUC	0.783***	0.897***	0.873***	0.822***	0.000	-	0.000***	**0.045**	0.000***	0.000***	0.000***	**0.000*** **	0.000***	0.000***
CUB	0.104***	0.243***	0.131**	0.154***	0.822***	0.811***	-	0.000***	0.000***	0.000***	0.000***	**0.000*** **	0.000***	0.000***
SUR	0.880***	0.920***	0.905***	0.849***	0.021	0.031*	0.887***	-	0.000***	0.000***	0.000***	**0.000*** **	0.000***	0.000***
TRI	0.860***	0.899***	0.885***	0.820***	0.657***	0.659***	0.873***	0.759***	-	0.009*	0.000***	**0.000*** **	0.000***	0.000***
RC/FN	0.787***	0.886***	0.863***	0.810***	0.567***	0.554***	0.812***	0.666***	0.031**	-	0.000***	**0.000*** **	0.000***	0.009*
ASC	0.913***	0.918***	0.914***	0.852***	0.728***	0.735***	0.922***	0.795***	0.060***	0.037***	-	**0.000*** **	**0.243**	0.000***
POI	**0.953*** **	**0.931*** **	**0.929*** **	**0.855*** **	**0.805*** **	**0.823*** **	**0.950*** **	**0.895*** **	**0.146*** **	**0.070*** **	**0.016*** **	-	**0.000*** **	**0.000*** **
BIO	0.877***	0.909***	0.894***	0.824***	0.640***	0.646***	0.878***	0.789***	0.093***	0.037***	0.003	**0.106*** **	-	**0.036**
STP	0.766***	0.895***	0.870***	0.811***	0.522***	0.505***	0.792***	0.671***	0.083***	0.036*	0.067***	**0.201*** **	0.045*	-

Table S3. Summary of source-centric mixed stock analysis of Atlantic green turtle *Chelonia mydas* nesting populations (n=14) and juvenile foraging grounds (n=17), using ~490bp sequences of the control region of the mtDNA.

Nesting Population	Foraging grounds																	
	NC	EcFL	BHM	BRB	ALF	RC	FN	BA	ES	UB	AI	CB	BuA	CV	COR	ST	WA	X
Poilão, Guinea Bissau																		
Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.2	0.2	0.0
CI: 97.5%	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.3	0.3	0.1
CI: 2.5%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0
Bioko, Eq.Guinea																		
Mean	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
CI: 97.5%	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
CI: 2.5%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sao Tome and Principe																		
Mean	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
CI: 97.5%	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.2	0.2
CI: 2.5%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ascension Island, UK																		
Mean	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0
CI: 97.5%	0.0	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.1
CI: 2.5%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Trindade, Brazil																		
Mean	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0
CI: 97.5%	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.3	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1
CI: 2.5%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rocas/F.Noronha, Brazil																		
Mean	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
CI: 97.5%	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
CI: 2.5%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Suriname																		
Mean	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.2	0.0	0.0	0.0	0.0
low C.I.	0.0	0.1	0.2	0.2	0.2	0.1	0.3	0.2	0.2	0.1	0.1	0.1	0.1	0.3	0.0	0.1	0.1	0.1
upper C.I.	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0

Table 3. Continuation

Nesting Population	Foraging grounds																	
	NC	**EcFL**	**BHM**	**BRB**	**ALF**	**RC**	**FN**	**BA**	**ES**	**UB**	**AI**	**CB**	**BuA**	**CV**	**COR**	**ST**	**WA**	**X**
Aves Island, VNZ																		
Mean	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1
CI: 97.5%	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.1	0.2	0.2	0.2
CI: 2.5%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Buck Island																		
Mean	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
CI: 97.5%	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
CI: 2.5%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tortuguero, CR																		
Mean	0.0	0.2	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3
CI: 97.5%	0.0	0.3	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6
CI: 2.5%	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mexico																		
Mean	0.2	0.4	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
CI: 97.5%	0.4	0.7	0.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5
CI: 2.5%	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Southeast Cuba																		
Mean	0.3	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1
CI: 97.5%	0.5	0.3	0.3	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.0	0.1	0.1	0.4
CI: 2.5%	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
South Florida, USA																		
Mean	0.2	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1
CI: 97.5%	0.5	0.5	0.4	0.4	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.2	0.0	0.1	0.1	0.4
CI: 2.5%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
East central Florida, USA																		
Mean	0.3	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
CI: 97.5%	0.6	0.5	0.4	0.3	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.4
CI: 2.5%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 4. Summary of foraging ground-centric mixed stock analysis of Atlantic green turtle *Chelonia mydas* rookeries (n=14) and foraging grounds (n=17), using ~490bp sequences of the control region of the mtDNA.

Foraging grounds	**Nesting populations**													
	EcFL	**SFL**	**MX**	**CR**	**CUB**	**BUC**	**AV**	**SUR**	**RC/N**	**TRI**	**ASC**	**GB**	**Bio**	**STP**
North Carolina, USA														
Mean	0.19	0.10	0.35	0.16	0.08	0.00	0.01	0.02	0.00	0.01	0.02	0.05	0.01	0.00
CI: 2.5%	0.02	0.00	0.14	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CI: 97.5%	0.41	0.26	0.59	0.36	0.18	0.00	0.05	0.06	0.01	0.03	0.06	0.11	0.02	0.01
East central Florida, USA														
Mean	0.03	0.02	0.26	0.63	0.01	0.00	0.01	0.01	0.00	0.00	0.01	0.01	0.00	0.00
CI: 2.5%	0.00	0.00	0.12	0.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CI: 97.5%	0.11	0.08	0.43	0.78	0.03	0.00	0.02	0.04	0.00	0.01	0.03	0.04	0.01	0.00
Bahamas														
Mean	0.01	0.01	0.03	0.92	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00
CI: 2.5%	0.00	0.00	0.00	0.84	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CI: 97.5%	0.03	0.02	0.08	0.97	0.01	0.00	0.01	0.04	0.00	0.01	0.01	0.03	0.00	0.00
Barbados														
Mean	0.06	0.06	0.15	0.28	0.02	0.00	0.03	0.14	0.00	0.01	0.09	0.13	0.01	0.00
CI: 2.5%	0.00	0.00	0.02	0.08	0.00	0.00	0.00	0.03	0.00	0.00	0.01	0.01	0.00	0.00
CI: 97.5%	0.18	0.19	0.31	0.46	0.09	0.00	0.11	0.25	0.01	0.05	0.22	0.28	0.03	0.01
Almofala, Brazil														
Mean	0.01	0.03	0.02	0.14	0.02	0.00	0.04	0.19	0.00	0.02	0.26	0.25	0.01	0.00
CI: 2.5%	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.08	0.00	0.00	0.10	0.09	0.00	0.00
CI: 97.5%	0.04	0.09	0.07	0.23	0.06	0.00	0.15	0.29	0.02	0.07	0.43	0.41	0.04	0.02
Rocas Atol, Brazil														
Mean	0.02	0.03	0.02	0.05	0.02	0.00	0.06	0.21	0.01	0.05	0.37	0.16	0.02	0.01
CI: 2.5%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.15	0.01	0.00	0.00
CI: 97.5%	0.06	0.09	0.06	0.12	0.07	0.00	0.20	0.33	0.03	0.15	0.57	0.35	0.06	0.02

Table S4. Continuation

Foraging grounds	Nesting populations													
	EcFL	**SFL**	**MX**	**CR**	**CUB**	**BUC**	**AV**	**SUR**	**RC/N**	**TRI**	**ASC**	**GB**	**Bio**	**STP**
Fernando Noronha, Brazil														
Mean	0.02	0.02	0.03	0.02	0.01	0.00	0.04	0.40	0.00	0.02	0.16	0.26	0.01	0.00
CI: 2.5%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.26	0.00	0.00	0.02	0.09	0.00	0.00
CI: 97.5%	0.05	0.06	0.08	0.06	0.05	0.00	0.15	0.50	0.02	0.06	0.33	0.42	0.04	0.02
Bahia, Brazil														
Mean	0.02	0.02	0.02	0.03	0.02	0.00	0.06	0.25	0.01	0.08	0.21	0.27	0.02	0.01
CI: 2.5%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.01	0.02	0.03	0.00	0.00
CI: 97.5%	0.07	0.08	0.08	0.09	0.07	0.00	0.19	0.39	0.02	0.21	0.48	0.51	0.06	0.02
Espirito Santo, Brazil														
Mean	0.01	0.01	0.01	0.01	0.01	0.00	0.05	0.26	0.01	0.04	0.29	0.29	0.02	0.01
CI: 2.5%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.09	0.06	0.00	0.00
CI: 97.5%	0.03	0.03	0.03	0.04	0.03	0.00	0.16	0.36	0.02	0.12	0.52	0.47	0.07	0.02
Ubatuba, Brazil														
Mean	0.01	0.01	0.01	0.02	0.01	0.00	0.04	0.08	0.01	0.03	0.40	0.36	0.01	0.01
CI: 2.5%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.16	0.08	0.00	0.00
CI: 97.5%	0.04	0.04	0.04	0.05	0.04	0.00	0.13	0.17	0.02	0.10	0.68	0.60	0.05	0.02
Arvoredo Island, Brazil														
Mean	0.01	0.01	0.01	0.01	0.01	0.00	0.06	0.16	0.01	0.07	0.37	0.27	0.01	0.01
CI: 2.5%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.17	0.07	0.00	0.00
CI: 97.5%	0.03	0.03	0.04	0.04	0.03	0.00	0.18	0.27	0.02	0.16	0.59	0.47	0.06	0.02
Casino Beach, Brazil														
Mean	0.01	0.01	0.01	0.01	0.01	0.00	0.05	0.15	0.01	0.06	0.31	0.35	0.01	0.01
CI: 2.5%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.12	0.15	0.00	0.00
CI: 97.5%	0.03	0.04	0.04	0.04	0.03	0.00	0.16	0.26	0.03	0.15	0.53	0.55	0.06	0.03

Table S4. Continuation

Foraging grounds	Nesting populations													
	EcFL	**SFL**	**MX**	**CR**	**CUB**	**BUC**	**AV**	**SUR**	**RC/N**	**TRI**	**ASC**	**GB**	**Bio**	**STP**
Buenos Aires, Argentina														
Mean	0.01	0.01	0.01	0.01	0.01	0.00	0.04	0.17	0.00	0.05	0.25	0.41	0.01	0.00
CI: 2.5%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.08	0.20	0.00	0.00
CI: 97.5%	0.04	0.04	0.04	0.04	0.03	0.00	0.16	0.27	0.02	0.14	0.48	0.58	0.05	0.02
Cape Verde														
Mean	0.03	0.03	0.04	0.04	0.02	0.00	0.04	0.40	0.00	0.01	0.04	0.31	0.01	0.00
CI: 2.5%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.14	0.00	0.00
CI: 97.5%	0.10	0.09	0.13	0.14	0.07	0.00	0.16	0.54	0.02	0.05	0.16	0.47	0.04	0.02
Corisco Bay, Eq. Guinea														
Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.04	0.00	0.01	0.28	0.60	0.01	0.01
CI: 2.5%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.37	0.00	0.00
CI: 97.5%	0.02	0.01	0.02	0.02	0.01	0.00	0.05	0.08	0.02	0.04	0.47	0.77	0.07	0.04
Sao Tome, Sao Tome and Principe														
Mean	0.01	0.01	0.01	0.01	0.01	0.00	0.02	0.04	0.00	0.01	0.07	0.77	0.01	0.01
CI: 2.5%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.62	0.00	0.00
CI: 97.5%	0.04	0.04	0.05	0.05	0.04	0.00	0.07	0.10	0.01	0.05	0.19	0.87	0.04	0.03
West Africa: Liberia to Benin														
Mean	0.01	0.01	0.02	0.02	0.01	0.00	0.02	0.06	0.00	0.01	0.08	0.75	0.01	0.00
CI: 2.5%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.58	0.00	0.00
CI: 97.5%	0.05	0.05	0.06	0.07	0.04	0.00	0.07	0.13	0.01	0.04	0.22	0.86	0.03	0.01

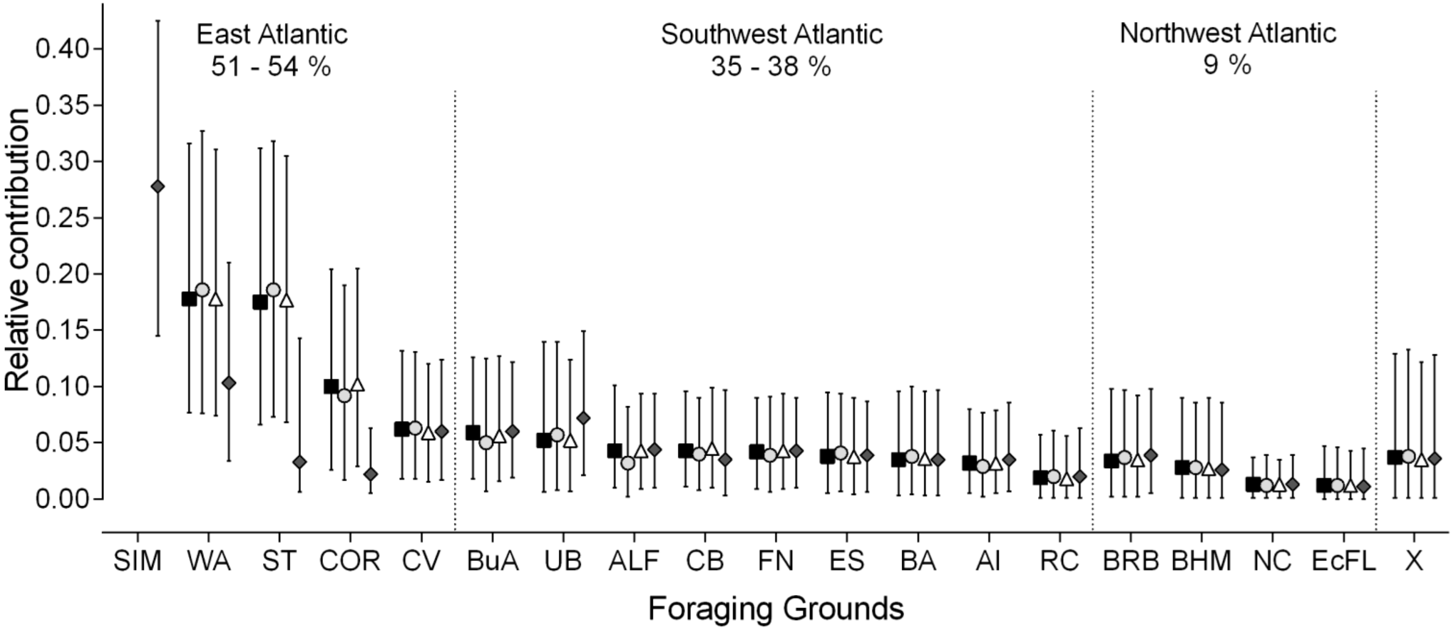

Figure S1. Comparison of mean contributions, and 95% confidence intervals, from Poilão rookery (West Africa) to 17 green turtle Atlantic foraging aggregations, estimated through a 'many-to-many' mixed stock analysis, using different simulated datasets against the actual dataset - black squares. Grey circle – including a rare haplotype (CM-A42) found at Poilão in Ascension Island sample, white triangle – including CM-A42 in Costa Rica sample, and grey diamond – adding a putative foraging ground fixed for haplotype CM-A8 (n=99). SIM: simulated foraging ground, WA: 'Western Africa' – Liberia to Benin, ST: Sao Tome, COR: Corisco Bay, CV: Cape Verde, BuA: Buenos Aires, UB: Ubatuba, ALF: Almofala, CB: Cassino Beach, FN: Fernando de Noronha, ES: Espírito Santo, BA: Bahia, AI: Arvoredo Island, RC: Rocas Atol, BRB: Barbados, BHM: Bahamas, NC: North Carolina, EcFL: East central Florida. Dashed lines separate geographic regions.

Chapter 5: Novel insights into the dynamics of green turtle fibropapillomatosis

Ana R. Patrício[1,2], Carlos E. Diez[3], Robert P. van Dam[4] and Brendan J. Godley[1]

[1] Centre for Ecology & Conservation, College of Life and Environmental Sciences, University of Exeter, Cornwall Campus, Penryn TR10 9EZ, UK
[2] MARE – Marine and Environmental Sciences Centre, ISPA – University Institute, 1149-041, Lisbon, Portugal
[3] Program of Protected Species, Department of Natural and Environmental Resources of Puerto Rico, San Juan, Puerto Rico
[4] Chelonia, San Juan, Puerto Rico

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Abstract

Outbreaks of fibropapillomatosis (FP), a neoplastic infectious disease of marine turtles, have occurred worldwide since the 1980s. Its most likely aetiological agent is a virus, but disease expression depends on external factors, typically associated with altered environments. The scarcity of robust long-term data on disease prevalence has limited interpretations on the impacts of FP on marine turtle populations. Here we model the dynamics of FP at 2 green turtle foraging aggregations in Puerto Rico, through 18 yr of capture-mark-recapture data (1997−2014). We observed spatiotemporal variation in FP prevalence, potentially modulated via individual site-fidelity. FP expression was residency dependent, and FP-free individuals developed tumours after 1.8 ± 0.8 yr (mean ± SD) in the infected area. Recovery from the disease was likely, with complete tumour regression occurring in 2.7 ± 0.7 yr (mean ± SD). FP does not currently seem to be a major threat to marine turtle populations; however, disease prevalence is yet unknown in many areas. Systematic monitoring is highly advisable as human-induced stressors can lead to deviations in host-pathogen relationships, and enhance disease virulence. Finally, data collection should be standardized for a global assessment of FP dynamics and impacts.

Introduction

Emerging diseases in marine ecosystems have increased over the past few decades (Harvell et al. 1999, 2004, Maynard et al. 2011). Climate change and anthropogenic pressure (e.g. habitat degradation, pollution), appear to contribute to marine wildlife disease outbreaks either by depressing host resistance or facilitating pathogen transmission (Harvell et al. 2004). Examples include recent outbreaks of infectious coral diseases worldwide (Maynard et al. 2011), the Caribbean-wide mass mortality of the long-spined sea urchin (Chiappone et al. 2002), mass mortalities of seals due to morbillivirus infection (Jensen et al. 2002), and several infectious neoplastic diseases associated with novel viral pathogens in marine mammals (Bossart 2007).

Fibropapillomatosis (FP) is an infectious neoplastic disease of marine turtles. It was first described in 1938 in a green turtle captured in Florida (Smith & Coates 1938), but since the 1980s, disease outbreaks in the wild have been increasingly reported (Jacobson et al. 1989, Williams et al. 1994, Work et al. 2004, Foley et al. 2005). The tumours can be both external and internal and, though benign, depending on site and size, they can hamper vital activities such as feeding, vision and swimming, and impede organ function (Herbst 1994, Herbst & Klein 1995). Neritic juveniles and subadults are the most susceptible life stages, whereas in adults the disease is rare (Herbst & Klein 1995, Work et al. 2004, Foley et al. 2005). Although more frequent among green turtles (Hirama & Ehrhart 2007), FP has been reported in all species of hardshelled sea turtles (Herbst 1994, D’Amato & Moraes-Neto 2000, Guillen & Villalobos 2000). A novel alphaherpesvirus, the Chelonid herpesvirus-5 (ChHV5), has been consistently detected by PCR analysis in tumour tissue samples from sea turtles (Quackenbush et al. 1998, Herbst et al. 2004, Ene et al. 2005, Patrício et al. 2012), and acknowledged as the most likely aetiological agent of FP (Herbst et al. 2004). However, recently, ChHV5 has been detected in several individuals not expressing visible tumours (Page-Karjian et al. 2012, Alfaro-Núñez et al. 2014).

Anthropogenically altered environments are associated with high FP prevalence (Herbst 1994, Aguirre & Lutz 2004, Van Houtan et al. 2010), implying that factors in these environments promote disease outbreak, e.g. facilitating virus transmissibility, and/or enhancing disease expression (Keller et al. 2014). A strong spatial heterogeneity observed in the distribution of ChHV5 variants in Florida, along with sympatric species of marine turtles sharing virus variants suggests local infection after recruitment to coastal habitats (Ene et al. 2005). Transmission routes remain unclear, but may involve the direct contact between super spreaders and naïve individuals (Work et al. 2014).

The study of stranded turtles has provided insight into the spatiotemporal trends of FP prevalence in eastern USA and in Hawaii (Work et al. 2004, Foley et al. 2005, Chaloupka et al. 2008a); however, this could give biased estimates of FP trends, if turtles with FP have mainly stranded as a consequence of advanced disease, leading to an overrepresentation of severely afflicted animals and potentially missing mild FP states. Alternatively, analyses of capture-mark-recapture (CMR) records can generate reliable estimations of disease incidence (LaPorte et al. 1992). CMR data have been widely applied to assess key population dynamic parameters of sea turtle populations, i.e. survival, abundance and somatic growth (Bjorndal et al. 2000, Chaloupka & Balazs 2005, Patrício et al. 2011, 2014), but rarely used to evaluate disease dynamics (but see Chaloupka et al. 2009). Overall, long-term data on chronic wildlife disease prevalence among live individuals are still scarce (Harvell et al. 2002, Lloyd-Smith et al. 2005, Chaloupka et al. 2009).

At Puerto Rico, reports of FP from occasional stranded turtles date from 1985 (Williams et al. 1994, Ortiz-Rivera et al. 2002). Since 1997, two foraging grounds for immature green turtles, Tortuga Bay and Puerto Manglar, have been monitored annually through CMR. FP was first observed in 2000, and has been present since. Here, we modelled the dynamics of FP disease on these coastal aggregations through the analyses of 18 years (1997 - 2014) of live CMR records. We investigated the effects of body size, year and abundance, on FP risk, and estimated for the first time the periods from recruitment to expressing FP, and from FP expression to complete recovery.

Materials and methods

Study site and sampling

Puerto Manglar (18.30°N, 65.25°W) and Tortuga Bay (18.32°N, 65.23°W) are foraging grounds for immature green turtles, located on the islands of Culebra and Culebrita, respectively, which lie east of the main island of Puerto Rico (see Fig. 1 in Patrício et al. 2011). Puerto Manglar (18.30°N, 65.25°W) is a mangrove-lined bay, bordered by *Rhizophora mangle* (red mangrove), surrounded by wetlands and minor residential development. Maximum depth is 5m and the water has high turbidity (Diez et al. 2010). Tortuga Bay (18.32°N, 65.23°W) is located at the uninhabited island of Culebrita, managed by the US Fish and Wildlife Service as part of the Culebra National Refuge. A sandy beach surrounds the bay, underwater vegetation is sparser than at Puerto Manglar, water transparency is greater and depth goes to 12m (Diez et al. 2010). Turtles were captured with an entanglement net 200m long and 5m deep (nylon twine, 25cm stretch mesh), deployed for ~1h in areas <5m deep using a 7m motor boat. Swimmers snorkelled continually along the net to extract entangled turtles. Turtles were tagged in the front flippers with 2 external tags (inconel and/or plastic tag) plus 1 internal passive integrated transponder (PIT) tag. Multiple tagging (i.e. flipper tags plus PIT tag) plus photo identification (facial profile photographs; Reisser et al. 2008) of each captured turtle assured that throughout our CMR program we were able to correctly identify all unique individuals. Straight-carapace-length (SCL, from the nuchal notch to the posterior-most tip) was measured to the nearest 0.1cm. All individuals were examined for the presence of cutaneous or conjunctival FP (Brooks et al. 1994), and assessed for tumour score (1-3; Work & Balazs 1999). Turtles were kept covered with wet towels and handling time was minimized to 15min per individual, after which they were released near their capture location. Sampling effort ranged from 5 to 16 net sets.y^{-1}, with 5.9 ± 3.5 net sets.y^{-1} (mean ± SD) in Tortuga Bay and 6.6 ± 3.6 net sets.y^{-1} (mean ± SD) in Puerto Manglar.

Data set

From 1997 to 2014 (except 1999) we recorded 764 capture events; 443 at Puerto Manglar, corresponding to 218 unique individuals, and 321 at Tortuga Bay, comprising 143 individual turtles (Table S1). Mean yearly individual captures at both sites corresponded to a proportion of 0.39 ± 0.15 (mean ± SD) of the estimated annual abundance (range: 0.13 – 0.68; Patrício et al. 2014).

Linear mixed effects modelling

Body condition indices have been used to describe the well-being of several wild species (Stevenson & Woods 2006). We calculated body condition index (BCI) for each capture as follows: BCI=weight / SCL^3 (Bjorndal et al. 2000). Tumour score (TS; Work & Balazs 1999) was attributed to each capture of an FP turtle. We analysed the relationship between having FP and BCI, using the data set of all captures (n=764), with linear mixed effects analysis using lme4 (Bates et al. 2015) implemented in R v.3.1.2 (R Development Core Team 2008). FP presence was included in the model as a fixed effect and turtle identity as a random effect. Similarly, within the group of captures corresponding only to turtles with FP we assessed the relationship between TS (fixed effect) and BCI, also using turtle identity as a random effect. *P*-values for fixed effects were obtained by likelihood ratio tests of the models with the effect against models without it. Residual plots were visually inspected to confirm non obvious deviations from homoscedasticity or normality.

Non-linear modelling

We applied generalized additive mixed modelling (GAM), available from package mgcv (Wood & Wood 2015), applied in R v.3.1.2 (R Development Core Team 2008), to assess the relationship between FP presence and three potential explanatory covariates: SCL, year, and abundance. GAMs are a semi-parametric form of generalized linear models that use smooth functions to fit the data, thus allowing for nonlinear relationships between the response and explanatory variables (Hastie & Tibshirani 1995), and perform well with binary responses (Wood & Wood 2015). A range of different models were tested, including different combinations of the potential predictors, until only significant covariates were kept. GAMs had a Binomial error distribution and logit link. Model selection was based on Akaike's information criteria (AIC; Sugiura 1978) and smoothing selection performed with restricted maximum likelihood estimation (REML; Corbeil & Searle 1976). Annual aggregation abundance estimations were extended to 2014 using the methods in Patrício et al. (2014).

Results

Prevalence

FP was first observed in Puerto Manglar in 2000, with FP prevalence peaking in 2003 when 75% of individuals captured presented tumours. Disease prevalence slowly decreased until 2007, and has since remained low (Fig. 1, Table S2). At Tortuga Bay, FP was not observed until 2005, and prevalence peaked in 2009 at 33%. FP has persisted since, albeit with a low prevalence (Fig. 1, Table S2). At Puerto Manglar, 21% of the turtles (45/218) were observed with FP during the sampling period, from which 31% were later observed in a fully recovered state. At Tortuga Bay, only 9 turtles were captured with FP (6%), and none have yet been observed having recovered.

Body Condition Index

There was no effect of FP on BCI ($F_{1,763}$=0.80, *P*=0.37; Fig. S1a), and the effect of individual (i.e. turtle identity) accounted for negligible amounts of variance (see model summary in Table 1). For the 85 captures of turtles with external fibropapillomas (corresponding to 54 unique individuals; 59% with TS1, 36% with TS2, and 5% with TS3), the effect of individual on BCI was also negligible (Table 1), and there was no effect of TS on BCI ($F_{2,82}$=0.81, *P*=0.45; Fig. S1b).

FP Risk

For Puerto Manglar, the minimal adequate GAM showed that both SCL (GAM edf=2.75, Ref.df=3.48, χ^2=26.01, *P*<0.001) and sampling year (GAM edf=5.17, Ref.df=6.20, χ^2=71.25, *P*<0.001) were significant explanatory variables for FP risk, and the model containing these two covariates was a good fit, with R^2=0.42 (deviance explained=40.4%). The size-specific function was nonmonotonic, with the probability of having FP increasing first with SCL, plateauing around 57-59cm SCL then decreasing with carapace length (Fig. 2a). The year-specific function was also nonmonotonic, with FP rapidly increasing to a peak in 2003, from then on decreasing and apparently stabilizing (Fig. 2b. For Tortuga Bay, the best minimal GAM also retained SCL (GAM edf=1.00, Ref.df=1.00, χ^2=7.02, *P*<0.01), and sampling year (GAM edf=2.18, Ref.df=2.74, χ^2=11.43, *P*<0.01). The model, however, had lower fit, R^2=0.18 (deviance explained=28.3%), probably due to a very small sample size of turtles with FP. According to the

GAM, the probability of having FP increased linearly with SCL (Fig. 2c). It also increased with year until 2009, plateauing thereafter (Fig. 2d). There was no significant effect of abundance on the presence of FP, at either site. See Table 2 a GAM summary.

Discussion

This study extends our knowledge on the dynamics of FP in green turtles by monitoring individuals through all stages of expression, i.e. prior to disease, diseased, and recovered, using long-term live CMR records. We observed the outbreak of an FP epidemic at Puerto Manglar in 2000, peaking in 2003, with 75% of the turtles exhibiting tumours. There was no evidence of disease-specific detectability at our study sites (Patrício et al. 2011), indicating no sampling bias or behavioural differences for FP turtles, so these are unbiased prevalence estimates (Jennelle et al. 2011). Located ca. 5km away, Tortuga Bay appeared free of FP until 2005, thereafter FP prevalence remained low. This variability in FP prevalence between the two bays is consistent with the previously recognized individual turtle fidelity to foraging site (Hirama & Ehrhart 2007, Patrício et al. 2011). This attribute of behaviour could be an important factor limiting the spread of FP among foraging grounds, if highly infectious individuals, responsible for disease transmission (super-spreaders; Work et al. 2014) stay resident.

High FP prevalence has been associated with anthropogenic change and habitat degradation (Williams et al. 1994, Van Houtan et al. 2010, Keller et al. 2014), and existing ChHV5 variants were shown to pre-date FP outbreaks (Herbst et al. 2004; Patrício et al. 2012), further implying the role of the environment. Stress has also been posited as a risk factor (Lu et al. 2003). Puerto Manglar, where higher FP prevalence was observed, is potentially more anthropogenically altered, contrasting with Tortuga Bay located at an uninhabited island. An assessment of water quality in 2007, using DNA markers, identified widespread human faecal contamination at Puerto Manglar, while at Tortuga Bay it was only detected next to a boat (Diez et al. 2010). Additionally, nitrogen isotopic values (δ15N) of macroalgae at Manglar suggested an intermediate level of wastewater impact (Diez et al. 2010). Ecological differences could also be involved. Macroalgae and *Thalassia testudinum* dominates at Puerto Manglar, in contrast to the seagrasses *Syringodium filiforme* and *Halodule wrightii* at Tortuga Bay (Diez et al. 2010). Foraging aggregations of green turtles are, however, typically small (such as the ones in the study) and demographic stochasticity alone (i.e. the probabilities of immigration, emigration, death, disease transmission and recovery) could affect FP prevalence (Lloyd-Smith et al. 2005).

Turtles did not appear to be diseased upon arrival at our study sites, supporting the hypothesis of local infection (Ene et al. 2005). Our model indicates that FP prevalence is low among smaller and larger individuals at Puerto Manglar, whereas medium-sized turtles are the most likely to present with signs of the disease. Size distributions of healthy, FP, and recovered individuals at this site evidence the fact that FP appears at intermediate sizes and that only large turtles were seen recovered (Fig. 3). We believe that the size effect on FP expression observed in the GAM, and previously reported (Work et al. 2004, Foley et al. 2005, Patrício et al. 2014), is in reality the reflection of i) residency plus tumour development, and ii) tumour regression. We estimate that it takes 1.8 ± 0.8 years (mean ± SD, range: 1.0 – 3.4 years, Fig. 4a) from recruitment to FP expression at Puerto Manglar, through the records of 12 turtles, which were first captured healthy and later with fibropapillomas. These individuals were never missed for more than one year in our CMRs and were first captured when FP was already present at the foraging ground (i.e. from 2000 onwards).

As FP prevalence at Puerto Manglar was greater earlier in our sampling period, sufficient time has elapsed to be able to observe recovery from the disease; a total of 31% of afflicted turtles were confirmed to have become tumour-free. This is likely a conservative estimate nevertheless, as a previous analysis on the survival probability (ϕ) of turtles in the study aggregations found a much lower apparent survival among subadults ($SCL \geq 65cm$, $\phi=0.529$) compared to juveniles ($SCL<65cm$, $\phi=0.832$), most likely attributed to the permanent emigration of the larger turtles (Patrício et al. 2011). The mean SCL of turtles at first capture after disease recovery was 67.5cm, well within the subadult category. So we believe that FP regression is in reality higher, as larger turtles are both recovering from FP and permanently leaving the foraging ground (Patrício et al. 2011, 2014). If turtles are likely to recover from FP acquiring immunity in the process this could explain the rarity of the disease among adult turtles.

The time from FP expression to complete recovery was 2.7 ± 0.7 years (mean ± SD, range: 1.5-4.0 years, Fig. 4b), estimated for 12 individuals (of 14 confirmed to have recovered) never missed for more than one year. Evidence of high disease recovery at Puerto Manglar suggests that one factor involved in disease fadeout could be herd immunity, as more turtles became resistant to FP, and the number of susceptible individuals decreased (Lloyd-Smith et al. 2005). The annual size-structure of green turtles at Manglar appears to support this hypothesis, as there seems to have been very little recruitment (Fig. 5, size-class<40cm SCL) between the peak years of the FP epidemic and its fadeout, keeping the stock of vulnerable individuals low. If this is the case, the replenishment of susceptibles, by recruitment of new individuals to the forage aggregation could potentiate a new epidemic (Lloyd-Smith et al. 2005). Here we observed from 2008 onwards an increase in the smaller size-class (Fig. 5), indicative of recruitment, and indeed we detected a slight increase in FP prevalence in the last two sampling years at Puerto Manglar, attributed entirely to new individuals (i.e. first tagged in 2013). This could suggest that cyclic epidemics may occur at this site, depending on the immigration rate of individuals naïve to FP.

Previous studies have shown that FP did not affect survival rates or somatic growth at Puerto Manglar and Tortuga Bay foraging grounds (Patrício et al. 2011, Patrício, Diez & van Dam 2014). In Florida, FP was also shown to have no significant effect on somatic growth (Kubis et al. 2009), and in Hawaii, growth rates were only lower in severe cases of the disease (Chaloupka & Balazs 2005). Most FP turtles at our study sites were mildly to moderately affected, and we found no significant differences on mean BCI between healthy and afflicted turtles or among tumour scores, comparable to what was reported in Hawaii (Work et al. 2004). There was evidence for a high rate of disease recovery, as discussed above. Similarly, at the Hawaiian archipelago in a foraging ground in Maui, photo-identification revealed a regression rate of 32% (Bennett et al. 1999), whereas in a different Hawaiian population, at Molokai, 13% to 18% annual recovery probabilities were estimated (Chaloupka et al. 2009). Tumour regression was further observed in Florida (22/24, 88%, Hirama & Ehrhart 2007), Brazil (2/8, 25%, Guimarães et al. 2013), Australia (proportion undetermined, Limpus et al. 2005) and in olive ridley turtles from Costa Rica

(20/42, 48%, Aguirre et al. 1999). Despite the FP epidemic at Puerto Manglar, a positive trend in aggregation size since the beginning of the CMR programme was detected, with a mean annual increase of 10.9% (Patrício et al. 2014). Most remarkable, the once severely depleted Hawaiian green turtle population has recovered notwithstanding major FP outbreaks during the 1980s and 1990s (Chaloupka et al. 2009). Analogously, high FP prevalence in Florida has not halted population recovery (Chaloupka et al. 2008b). These optimistic findings suggest that FP is not a current major threat to marine turtle populations.

Conclusion and monitoring recommendations

Anthropogenic activities, predicted to increase disease occurrence are on the rise (Harvell et al. 2002, 2004). Human-mediated climate change may also increase disease prevalence in the marine environment (Harvell et al. 2002) or lead to deviations in host-pathogen relations and disease virulence. Additionally, recent research has shown that selective harvesting of healthy individuals can increase FP prevalence in a population (Stringell et al. 2015). To better understand the dynamics of wildlife disease and attempt to predict outbreaks, it is essential to gather baseline data, and to develop rapid response capability to identify, monitor, and manage disease outbreaks as they occur (Harvell et al. 2004). FP disease monitoring can be easily integrated in already established population surveys, however, it is important to standardize the information collected. We suggest including the following data regarding disease presentation: number, size, and location of tumours, weight of afflicted turtles, overall condition, and presence of parasites, and recommend more long-term monitoring, for reliable estimates of disease prevalence. The collection of biopsy samples from both affected and healthy tissues for molecular research is also desirable, as new molecular techniques are progressively becoming more available and may be key to understand the evolution of the ChHV5 and disease spread. A unified monitoring strategy could be achieved with little additional effort yet it would significantly improve the recognition of the implications of FP to marine turtle populations worldwide.

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Table 1. Summary of linear mixed effects models fitted to captures of immature green turtles from Puerto Rican foraging grounds. BCI = body condition index, FP = fibropapillomatosis, ID = turtle ID, TS = tumour score.

		Mixed effects			**Fixed effects**			
dataset	**Model**	**Covariate**	**Variance**	**SD**	**covariate**	**Estimate**	**SE**	**t value**
All captures	BCI~FP+(1|ID)	Turtle ID (Intercept)	6.69×10^{-11}	8.18×10^{-6}	Intercept	1.32×10^{-4}	6.09×10^{-7}	216.61
(n = 764)		Residual	1.01×10^{-10}	1.01×10^{-5}	FP	-2.67×10^{-7}	1.44×10^{-6}	-0.18
FP captures	BCI~TS+(1|ID)	Turtle ID (Intercept)	3.87×10^{-11}	6.22×10^{-6}	Intercept	1.37×10^{-4}	3.91×10^{-6}	34.91
(n = 85)		Residual	1.35×10^{-10}	1.16×10^{-5}	TS	-2.25×10^{-6}	2.48×10^{-6}	-0.91

Table 2. Summary of generalized additive mixed models (GAM) fitted to captures of immature green turtles from 2 Puerto Rican foraging grounds, Puerto Manglar and Tortuga Bay, to model the relationship between fibropapillomatosis expression (FP, response variable) and straight carapace length (SCL) and sampling year (predictor variables or covariates). edf: estimated degrees of freedom of smooth term, ref.df: estimated residual degrees of freedom of smooth term (1 = linear)

Dataset / site	**Model**	**Covariate**	**edf**	**ref.df**	**Chi^2**	**P-value**	**R^2**
Puerto Manglar (n = 443)	FP~SCL+Year	SCL	2.75	3.48	26.01	2.30×10^{-5}	0.42
		Year	5.17	6.20	71.25	4.02×10^{-13}	
Tortuga Bay (n = 321)	FP~SCL+Year	SCL	1.00	1.00	7.02	8.1×10^{-3}	0.18
		Year	2.18	2.74	11.43	8.0×10^{-3}	

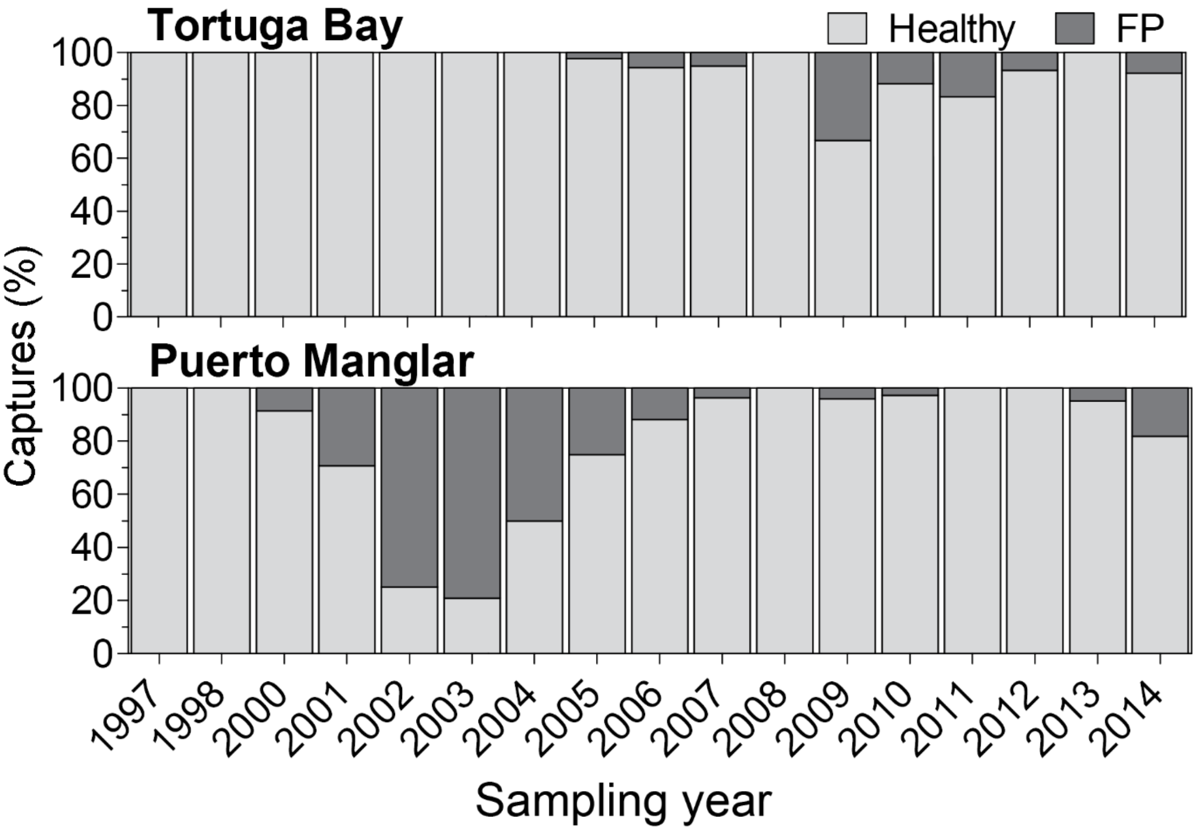

Figure 1. Percentage of captures of healthy green turtles (light grey) and those with fibropapillomatosis (FP; dark grey), at two juvenile turtle foraging grounds, Tortuga Bay (N = 321) and Puerto Manglar (N = 443), Puerto Rico, throughout 18 yr of capture-mark-recaptures.

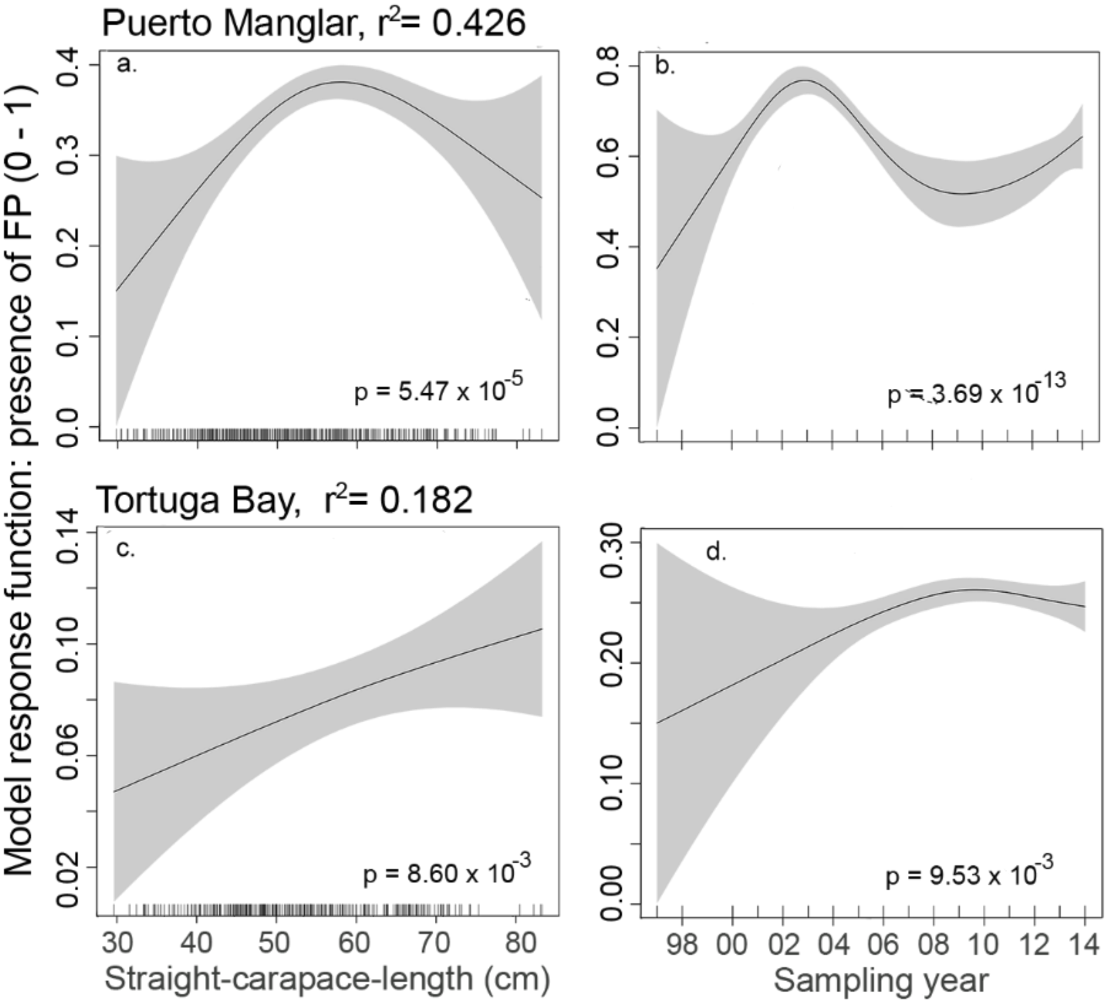

Figure 2. Graphical summary of generalized additive models fitted to an 18 yr green turtle mark-recapture dataset. Response variable: probability of fibropapillomatosis (FP) among immature green turtles from **(a,b)** Puerto Manglar and **(c,d)** Tortuga Bay foraging grounds, Culebra, Puerto Rico. Predictor variables: (a,c) straight carapace length and (b,d) year. *P*-values are displayed for significant effect of covariates in FP incidence.

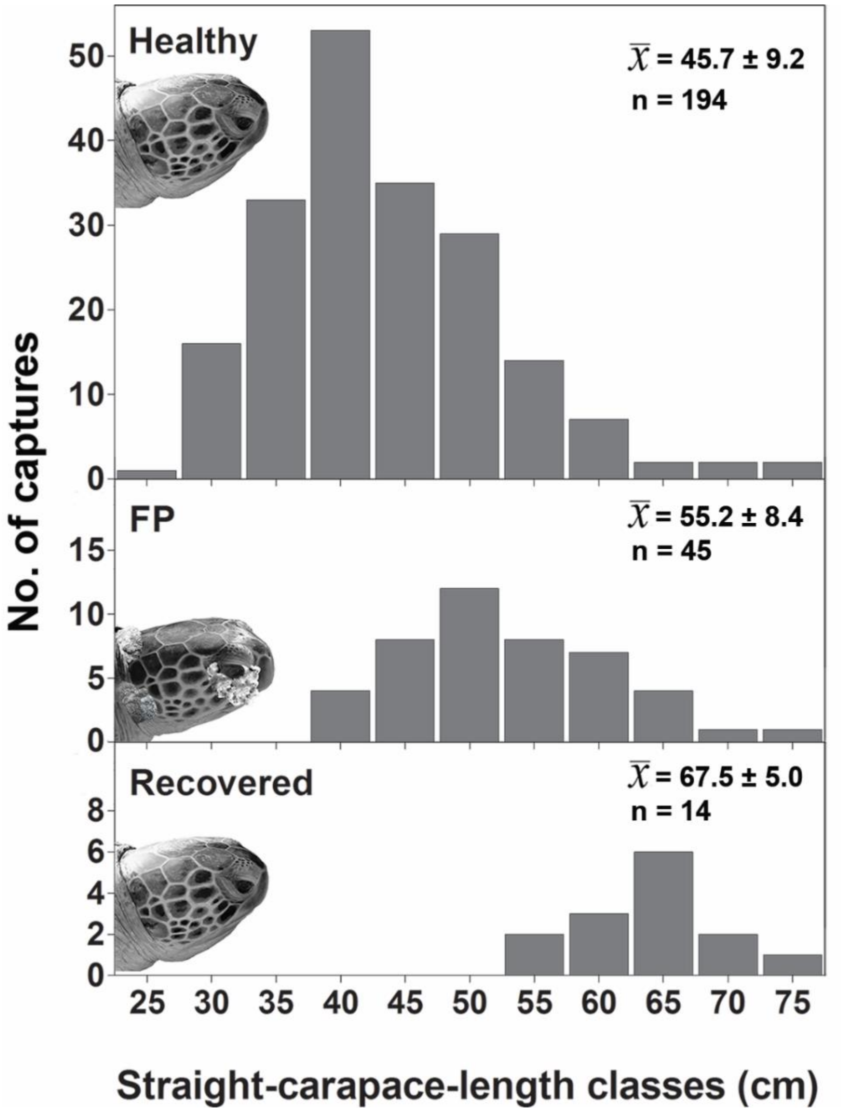

Figure 3. Distribution of straight carapace lengths (SCLs) at first capture of green turtles: **(a)** healthy, **(b)** with fibropapillomatosis (FP), and **(c)** after recovery from FP, at Puerto Manglar, Puerto Rico, throughout 18 yr of capture-mark-recaptures. Numbers on the x-axis represent the start of each 5cm SCL class.

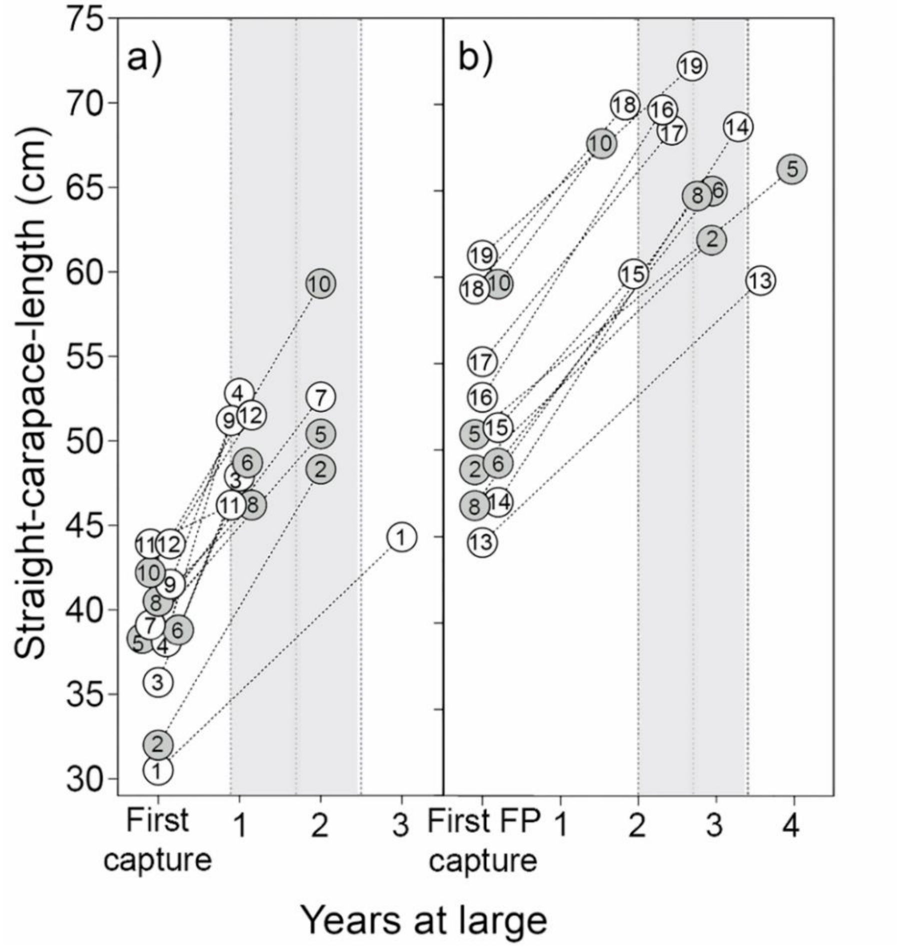

Figure 4. Straight carapace length at the first capture of resident green turtles at Puerto Manglar, Puerto Rico, that **(a)** were healthy and subsequently developed fibropapillomatosis (FP; n=12), and **(b)** had FP and later recovered from the disease (n=12). The x-axes show the time (in yr) for each transition. Circled numbers identify unique individuals, and grey circles highlight turtles for which both transitions were recorded (n = 5). Dashed vertical line: mean time for each transition (light grey bars: SD).

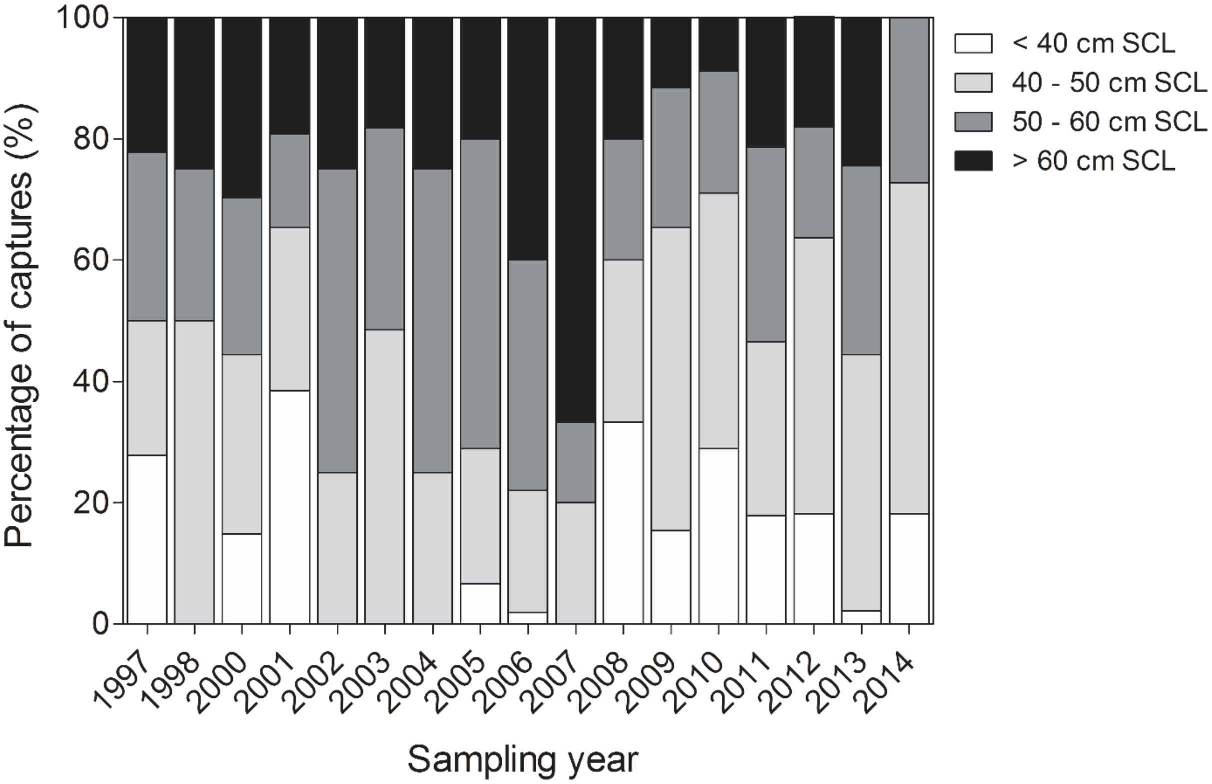

Figure 5. Percentage of captures of immature green turtles foraging at Puerto Manglar, Puerto Rico, corresponding to four straight carapace length (SCL) size classes (cm), throughout 18 yr of capture-mark-recaptures. The white size class (SCL<40cm) is indicative of recruitment.

Chapter 5: supplementary information

Table S1. Population parameters at two foraging grounds for immature green turtles: Puerto Manglar and Tortuga Bay, Puerto Rico. Ni: abundance.

Site	**Puerto Manglar**	**Tortuga Bay**
Year	**Ni***	**Ni***
1997	-	-
1998	19 (2 - 36)	48 (20 - 56)
2000	54 (37 - 71)	28 (41 - 79)
2001	111 (72 - 151)	97 (60 - 134)
2002	31 (3 - 59)	77 (33 - 122)
2003	56 (39 - 72)	70 (51 - 88)
2004	56 (38 - 74)	61 (42 - 80)
2005	48 (40 - 56)	61 (52 - 70)
2006	68 (52 - 84)	36 (24 - 48)
2007	56 (41 - 71)	41 (28 - 55)
2008	171 (88 - 254)	46 (3 - 88)
2009	86 (58 - 115)	31 (14 - 48)
2010	79 (60 - 98)	37 (24 - 50)
2011	104 (70 - 138)	46 (24 - 69)
2012	63 (29 - 97)	86 (47 - 126)
2013	116 (88 - 145)	31 (16 - 46)
2014	59 (27 - 90)	69 (35 - 104)
Mean growth rate* **($cm.y^{-1}$)**	6.1 ± 1.7 SD	4.2 ± 1.6 SD
Annual survival probability † (ϕ)	juveniles (CCL<65 cm) = 0.83 (0.79 - 0.87) subadults (CCL ≥ 65 cm) = 0.53 (0.39 - 0.67)	
Encounter probability † (ρ)	0.36 (0.31 - 0.41)	

* Patrício, Diez & van Dam 2014
† Patrício et al. 2011

Table S2. Number of individual captures per year of immature green turtles, at two foraging grounds in Puerto Rico; Puerto Manglar and Tortuga Bay, and annual prevalence of fibropapillomatosis (FP).

Site	**Puerto Manglar**			**Tortuga Bay**		
Year	**FP turtles**	**All turtles**	**FP prevalence**	**FP turtles**	**All turtles**	**FP prevalence**
1997	0	18	0.00	0	14	0.00
1998	0	4	0.00	0	10	0.00
2000	2	23	0.09	0	12	0.00
2001	7	24	0.29	0	21	0.00
2002	3	4	0.75	0	10	0.00
2003	19	24	0.79	0	30	0.00
2004	11	22	0.50	0	23	0.00
2005	9	36	0.25	1	46	0.02
2006	4	34	0.12	1	18	0.06
2007	1	27	0.04	0	20	0.00
2008	0	15	0.00	0	4	0.00
2009	1	25	0.04	3	9	0.33
2010	1	36	0.03	1	17	0.06
2011	0	27	0.00	2	12	0.17
2012	0	11	0.00	1	15	0.07
2013	2	41	0.05	0	11	0.00
2014	2	11	0.18	1	13	0.08

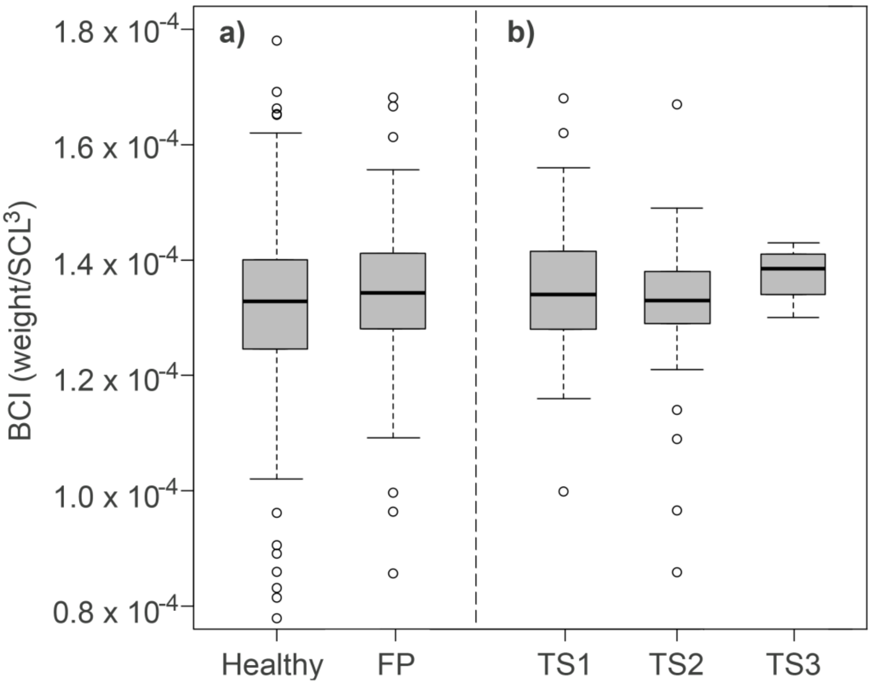

Figure S1. a. Body condition index (BCI, Bjorndal et al. 2000) at each capture of immature green turtles at Puerto Rican foraging grounds when: healthy (n=679) and with fibropapillomatosis (FP, n=85). **b.** BCI at each capture corresponding to turtles with FP (n=85), according to tumour score. TS1: mild FP, TS2: moderate FP and TS3: severe FP (Work & Balazs 1999).

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Final remarks

In this thesis I investigate several important population parameters of one of the largest green turtle populations globally, at the National Marine Park of João Vieira and Poilão (PNMJVP), in the Bijagós Archipelago, Guinea-Bissau. Among others, some interesting findings resulting from my research were that i) green turtles originating at the Bijagós archipelago disperse along West Africa and the Southwest Atlantic, emphasizing the regional importance of this rookery; ii) unlike most populations of sea turtles, which have female-biased primary sex ratios, at Poilão, the primary sex ratio is almost balanced; iii) green turtles show high repeatability in nesting habitat, suggesting potential for heritability of nest site selection; and iv) this population has medium to high resistance to predicted climate change, and potential for resilience. Additionally, using a juvenile aggregation from Puerto Rico as a case study, we found that, under current conditions, green turtles have remarkable resilience to Fibropapillomatosis.

In addition to the scientific findings, and publications originating from my PhD, this work also contributed directly to the conservation of marine turtles in Guinea-Bissau, and to the identification of knowledge gaps to be addressed in future research.

Conservation implications

The fieldwork in Guinea-Bissau was conducted in partnership and under the supervision of the Institute of Biodiversity and Protected Areas of Guinea-Bissau (IBAP-GB), and with the participation of the local communities from the Island of Canhambaque, at the Bijagós Archipelago. Sea turtles have always been important among the Bijagós people, often included in their ceremonial rituals, and used as a source of protein. At the present, the protection of these emblematic species and their habitats is contributing to the development of sustainable ecotourism in Guinea-Bissau, to the dissemination of environmental awareness, and to fundraising and support of biodiversity conservation. The local communities are deeply involved in the conservation management of sea turtles, which have become a source of income, through ecotourism activities and conservation jobs.

During my fieldwork campaigns we employed young men from the Bijagós communities to participate in monitoring, research, and conservation activities. They were trained in all sampling techniques, and during evening gatherings (‘djumbais’), we talked about the biology and conservation of sea turtles, problems of illegal captures in the national park, and other subjects of concern for them, and their way of living. These young men are key to biodiversity conservation, as they are the future decision makers. The IBAP always involves the community in all decisions affecting the use of their Protected Areas, so having these younger generations informed and willing to protect their natural resources will go a long way to improve management decisions and reduce conflict. Training of community members also led to the employment of some of them by the IBAP for permanent positions, therefore bringing income for the community, and contributing for an increased workforce on conservation in Guinea-Bissau. Other fieldwork collaborators were selected to receive additional training to join the ecotourism industry, as eco-guides.

Results from scientific research will also contribute directly to conservation management. The recognition of the importance of native vegetation for the resilience of sea turtles under future climate change is a compelling argument for the protection of the forest, not only at our study site, but in other Bijagós islands where nesting also occurs, which have been affected by slash-and-burn agricultural practices. We are currently collaborating with the IBAP for the implementation of updated regulations of use of the Bijagós national parks. Additionally, the indication that known green turtle juvenile foraging grounds in the Bijagós should be assessed to better understand the connectivity of this population, will lead to the establishment of a participatory in-water monitoring programme, managed by the IBAP, in collaboration with local fishers.

Lastly, the dissemination of the scientific outputs of this thesis, through peer-review publications, communications at international conferences, and social media platforms, will emphasize the importance of this major green turtle population, and of the Bijagós Archipelago, facilitating the establishment of future partnerships, the fundraising for biodiversity conservation, and the motivation of national staff, with prospects of continuation in the long term.

Future research

Results from **Chapter 1** to **Chapter 3** suggest that the green turtle population nesting at Poilão should be resilient to predicted climate change, which in fact, is expected to enhance population growth, through the production of more females. Given the already high nesting density at Poilão, it is plausible that density dependent processes, e.g. intraspecific nest destruction, or nesting failure due to intraspecific disturbance, will restrict population growth in this small island (Bustard & Tognetti 1969, Girondot et al. 2002), particularly if SLR reduces the available nesting habitat (Mazaris et al. 2009). This could potentially lead to spill-over to nearby islands, which currently support less than 10% of the population. Future research should look into the existence of density dependent processes, and model the carrying capacity of Poilão Island (Tiwari et al. 2006). Also, some of the work described here should be extended to the other islands of the PNMJVP, such as characterizing the nesting habitats, assessing the availability of spatial and temporal refugia, and estimating the potential impacts of sea level rise.

Several studies have explored which environmental variables better explain the incubation temperatures of sea turtle nests. The majority of these use mean air temperatures, usually collected by meteorological stations several km distant from study sites, to infer sand and incubation temperatures (Laloë et al. 2014, 2014, Santidrián Tomillo et al. 2015, Esteban et al. 2016), as is the case here, in **Chapter 1**. However, some authors have suggested that sea surface temperature (SST) is an important variable to further understand nest temperatures (Fuentes et al. 2009, Girondot & Kaska 2014). Additionally, the effect of protracted rainfall can also impact incubation temperatures (Houghton et al. 2007, Lolavar & Wyneken 2015). Recently, a permanent meteorological station has been deployed at João Vieira Island (within the PNMJVP, 17km from Poilão), recording daily temperature and precipitation data. These data, together with local measurements of SST, and incubation and sand temperatures, will allow to further explore the relationships between these environmental variables, and potentially find an improved model to predict incubation temperature of green turtle nests.

The finding of high repeatability in nest site selection, in **Chapter 2**, suggests potential for a genetic basis of this trait (Kamel & Mrosovsky 2005). Thanks to major advances in the field of molecular biology, it is now possible to test this hypothesis using genomic approaches (Rittschof & Robinson 2014). The cost of genomic analysis may have been prohibitive in the past, however this tool is becoming more cost-effective, and highly applicable to a broader set of conservation questions (Garner et al. 2016). Thus, the evolution of nest site choice could be one, among many other questions, to be addressed with genomics.

In **Chapter 4**, we emphasize the need to include more finely resolved markers in genetic analyses, and more genetic sampling from West African juvenile aggregations. Future research will include sampling of juvenile green turtles within the Bijagós, and from other known developmental sites, in continental Guinea-Bissau and Mauritania, for the identification of mitochondrial DNA haplotypes and new genetic markers (mtSTR, mitochondrial DNA short tandem repeats; Tikochinski et al. 2012, Shamblin et al. 2015), to further resolve the connectivity puzzle for this species in the Atlantic.

Adult connectivity also merits further research. A previous study has shown that some of the nesting females migrate to distant foraging grounds in Mauritania after breeding, while others might be residents at the Bijagós Archipelago (Godley et al. 2010). However, the sampling size in this study was limited (n=8), and it was only undertaken at the end of one nesting season. Satellite tracking along with stable isotope analysis (SIA) should be conducted, facilitating the analyses of larger sample sizes, more relevant for population studies (Zbinden et al. 2011). We aim to do this in the near future, extending the tracking to more individuals, multiple years, different periods along the nesting season, and across a range of size classes, to avoid inter-annual (Witt et al. 2011), seasonal (Rees et al. 2010), and phenotypic (Hawkes et al. 2006) biases in dispersal. We will additionally collect samples of potential prey items, from identified foraging sites, for SIA, to determine the dietary range of this population, a question that emerged in **Chapter 3**.

The finding that juvenile green turtles are likely to recover from FP was an encouraging one. However, as noted in **Chapter 5**, this may be subject to the existence of suitable environmental conditions, and, future climate change may enhance disease virulence (Harvell et al. 2002). It is therefore important to assess baseline values of disease prevalence, to enable the identification of outbreaks, and underlying causes. Nonetheless, FP prevalence remains unknown in many areas, particularly in West African juvenile aggregations, to which the nesting population of Poilão contributes to (Barnett et al. 2004, Formia et al. 2007, Duarte et al. 2012). Interestingly, during the course of my PhD I had the opportunity to collaborate with the sea turtle conservation project at Príncipe Island, Sao Tome and Principe, where we saw several afflicted animals, including two stranded juveniles with severe FP. Standardized data collection, potentially through the implementation of participatory capture-mark-recapture in-water programs, should be considered.

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