Balanced primary sex ratios and resilience to climate change in a major marine turtle population

Running title: Green turtle primary sex ratios

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Abstract

Global climate change is expected to have major impacts on biodiversity. Marine 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 looked at one of the world's largest green turtle rookeries, in West Africa, to assess the population's primary sex ratio and resilience to climate change. In 2013 and 2014, we deployed dataloggers recording nest (n=101) and sand (n=30) temperatures, and identified hatchling sex (n=131) 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, and both sexes were produced within incubation temperatures from 27.6ºC to 31.4ºC: transitional range of temperatures (TRT). Primary sex ratio changed from male to female dominated across relatively small temporal and spatial scales, but was overall balanced. 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 populations' response 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.

Key-words: sex ratio; climate change; green turtle; pivotal temperature; transitional range of temperatures; thermosensitive period

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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 and Andrews, 1994), sphenodontians (Mitchell et al. 2010), some lizards (Viets et al., 1994), and most turtle species (Mrosovsky & Yntema 1980).

Among marine turtles, clutches demonstrate a thermal tolerance of 23 ºC to 35 ºC during incubation (Ackerman, 1997). During the TSP, higher incubation temperatures produce female offspring, whereas lower incubation temperatures produce males (Mrosovsky and Yntema, 1980). Between these extremes, there is a transitional range of temperatures (TRT) at which both sexes can be produced (Mrosovsky and 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). Because under natural conditions the incubation temperatures fluctuate, typically associated with rainstorm events (Godfrey et al., 1996; Houghton et al., 2007; Lolavar and Wyneken, 2015; Matsuzawa et al., 2002) or diel temperature variation (Georges, 2013), the equivalent of the pivotal temperature is given as the mean of the temperatures experienced during the middle third of incubation leading to a balanced sex ratio (Mrosovsky and Pieau, 1991). 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, marine 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. 2010; 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 2006; 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 and Janzen, 2010). Although sea turtles have endured pronounced past climate variations (Poloczanska et al. 2010), 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 marine 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 (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 myd*as), 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. 2001b; 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; Braun McNeill et al. 2016; Laloë 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).

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 (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, these differences likely being explained by rainfall (Rebelo et al., 2011). Although their study importantly detected a temporal variation in male production, it did not encompass the duration of the nesting season, nor the diversity of nesting habitats. We aim 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 at Poilão Island throughout the nesting season, and 3) predict the current primary sex ratio.

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). Poilão has a total area of 43 hectares, is covered by undisturbed tropical forest, and sandy beaches extend for 2 km of the *ca.* 4 km coastline (Fig. 1b). The nesting season (mid-June to mid-December, Catry et al. 2002), largely coincides with the rainy season (May to November, peaking in August and September), although sporadic nesting occurs year-round.

Temporal nesting distribution

To assess the number of adult female emergences we conducted systematic track counts from 7 August to 21 November in 2013 (106 days), and from 10 August to 28 November in 2014 (111 days). 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. 2001c). 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 here represent < 5% of the overall numbers (Castro 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 – 1 m of the vegetation and experienced partial shade, and the 'open sand' corresponded to the area from > 1 m of the vegetation to the high tide line, 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), and determined the habitat and beach section for all 1,559 nests. During these focused assessments we surveyed all four beach sections (2 km), at high tide (see Catry et al. 2002), as fast 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. 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, we recorded hourly nest temperatures with Tinytag-TGP-4017 dataloggers (Gemini Data Loggers, Chichester, UK, ± 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. For a subset of nests (n=30; 16 and 14 for 2013 and 2014 respectively), control dataloggers were deployed 1 metre from the clutch, at a mean mid-clutch depth of ~ 70 cm (local data, unpublished), 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 (24 hours at 28 ºC) and used only if accuracy was ≤ 0.3 ºC. 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 ($\mathrm{E\%} = \mathrm{(n\ egg\ shells - \ n\ dead\ and\ live\ hatchlings\ found\ inside\ nest\ chamber)/clutch\ size}$).

A control data logger at the ‘forest border’ was left to measure sand temperature from March 2013 to March 2015, enabling comparisons with local air temperature obtained from the National Climatic Data Centre (http://cdo.ncdc.noaa.gov/CDO/cdo). 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 ºC mean value for this study). Sand temperature was regressed against air temperature in Bissau 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 (50 cm diameter x 30 cm height, wire mesh 1 cm^2) above 27 nests, 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 0.01 cm 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, 1 ml for each 15 ml of resin). The cross sections were then sectioned further into 3 mm-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 thermosensitive period, however, as gonad differentiation depends on embryonic development rather than incubation duration, the thermosensitive period (TSP) in nests with fluctuating temperatures may differ from the IP_{mid} (Girondot and Kaska, 2014). We thus used R package embryogrowth v.6.4 (see Girondot and 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 other 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 IP_{mid} 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 we counted 48,696 green turtle tracks in 2013, and 83,304 in 2014, from early August to late November, 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 the ‘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, we assumed that 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 $T\text{sand} = 0.94T\text{air\_Bissau} + 3.04$ (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 (IP)

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 (n=88). For the remaining 13 nests we estimated the IP by subtracting to 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 of 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), and to the 'forest' habitat (42.2 ± 41.6%, range: 0.0 – 96.2%, n = 16). We believe this is a consequence of the presence of roots at the forest habitat, as entangled hatchlings were frequently observed upon nest excavations. 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, with an average of 4.9 ± 0.4 hatchlings per nest (Table S3). Male hatchlings were significantly larger (4.95 ± 0.19 cm, range: 4.44 – 5.33 cm, N = 83) than females (4.73 ± 0.18 cm, range: 4.26 – 5.11 cm, 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 ± 0.1 ºC (range: 0.0 – 0.5 ºC). All three covariates: i) IP_{mid} mean incubation temperature, ii) TSP mean incubation temperature, and iii) IP (to hatching) had a significant effect on expected sex ratio, with $P < 0.0001$. 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 29.4 ºC, and the TRT ranged from 27.6 – 31.4 ºC (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, largest in the Southern Atlantic distinct population segment (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. This 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 on the conservation management of marine 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; Godfrey and Mrosovsky, 2006; Godley et al., 2002). This parameter alone however, is insufficient to predict primary sex ratios; accounting for the TRT is critical to characterize the population's response to incubation temperatures (Mrosovsky and 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 and 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), 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 3.8 ºC, suggesting that even with substantial increases in incubation temperatures (i.e. 2 – 3 ºC, IPCC 2013) some nests would continue to produce males.

Within-population variability on 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 (Godfrey and Mrosovsky, 1997; Godley et al., 2002; King et al., 2013; Mrosovsky et al., 1999; Spotila et al., 1987; Wyneken and Lolavar, 2015). Such variation has been attributed to the effect of fluctuating temperatures on embryo 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 thermosensitive periods (TSP, Girondot & Kaska, 2014). Interestingly, these were mostly coincident with the middle third of the 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 30.3 ºC). 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 and 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. 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 even 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), and beach orientation (Booth &

Freeman 2006; Fuentes et al. 2010a). At Poilão there is no marked difference in sand color between west and east sections, however, and beach orientation changes within each section. Instead, we hypothesize that this variation is driven 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 (Kamel, 2013; Janzen and Janzen, 2016), and rainfall (Godfrey et al., 1996; Houghton et al., 2007; Lolavar and 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, adjusting the start of the nesting season, to have peak activity coinciding with the colder months (December and January) would enhance male hatchling production, and likely clutch survival under future global warming scenarios, as high incubation temperatures have been shown to lower survival of clutches (Godley et al. 2001a; 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., 2016). Interestingly, individual inter-annual consistence in nest-site selection has been observed in sea turtles (Kamel & Mrosovsky 2006). 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 vegetated areas.

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' OSRs (Wright et al. 2012a; Rees et al. 2013; Stewart & Dutton 2014). These discrepancies resulting to some extent from males breeding more frequently than females (James et al., 2005; Hays et al., 2014), compensating partially for female-biased effective population sex ratios. However, at least one study has shown that a balanced OSR from a rookery with highly female-biased hatchling sex ratio was not due to sex-specific migratory strategies (Wright et al. 2012b). Moreover, 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 and Evans, 2011; Kobayashi et al. 2017). Males were also larger in our study, and ghost crabs preferentially prey on smaller hatchlings here (Rebelo et al., 2011). On the other hand, clutches laid under the vegetation are at greater distances from the water line, and emerging hatchlings may have poorer orientation skills (Kamel and Mrosovsky 2004), further increasing the length of time they spend exposed to land predators (e.g. ghost crabs, palm nut vultures; Rebelo et al., 2011, Carneiro et al., 2017). 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 TRT. At any rate Poilão 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. 2014), 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 marine 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 of clutches are also laid annually (IBAP unpublished 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).

Lastly, although the nesting population at Poilão seems resilient to warming temperatures, 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 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 neighboring islands, to fully understand how resilient this population may be to climate change.

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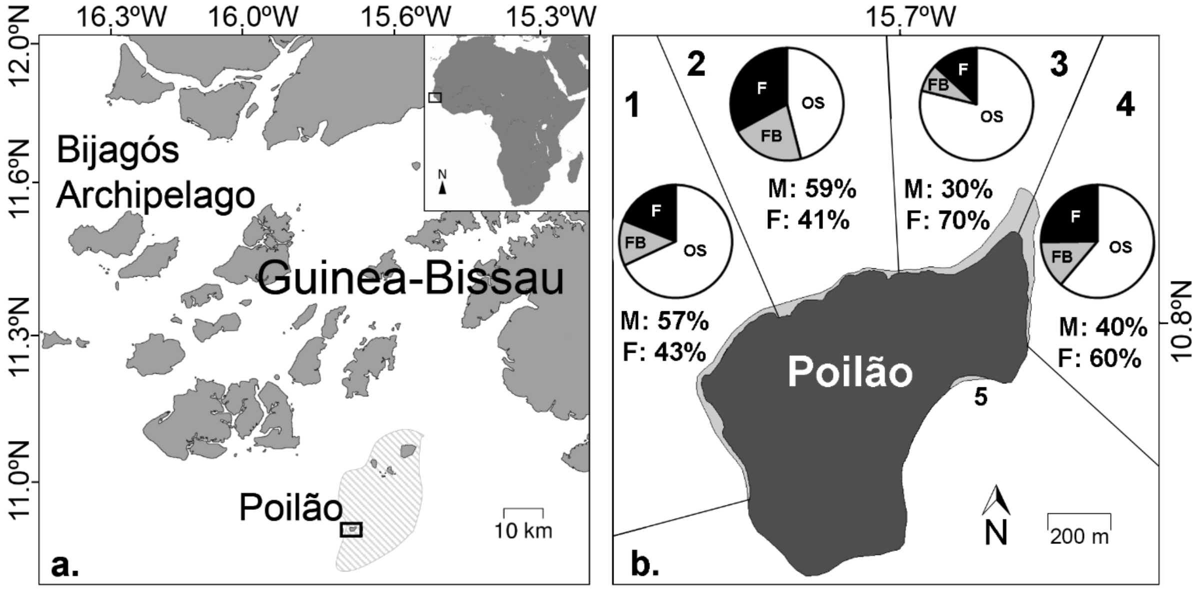

Figure 1a. Map of the Bijagós Archipelago, Guinea-Bissau: the João Vieira and Poilão Marine National Park is represented by the striped area, and the black frame depicts Poilão Island; **b.** Map of Poilão Island showing the four green turtle nesting beach sections monitored in this study (1 – Farol, 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 in 2013 and 2014 are given. 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).

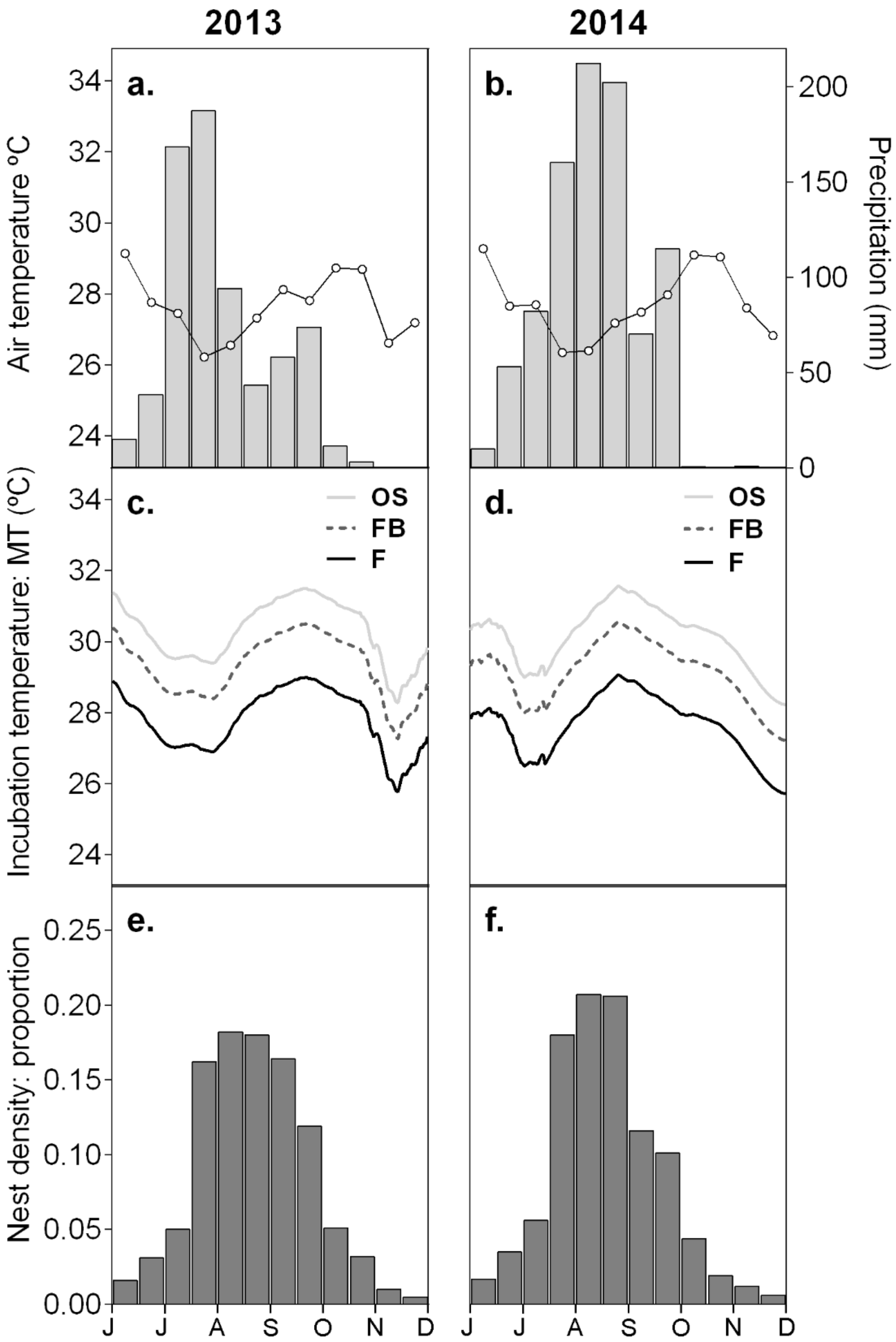

Figure 2a, b. Mean bi-weekly air temperature (open circles) and precipitation (bar) at Bissau (http://cdo.ncdc.noaa.gov/CDO/cdo); **c, d.** estimated middle third mean incubation temperatures experienced by green turtle clutches laid from 15 June to 15 December at Poilão Island, at three habitats (OS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

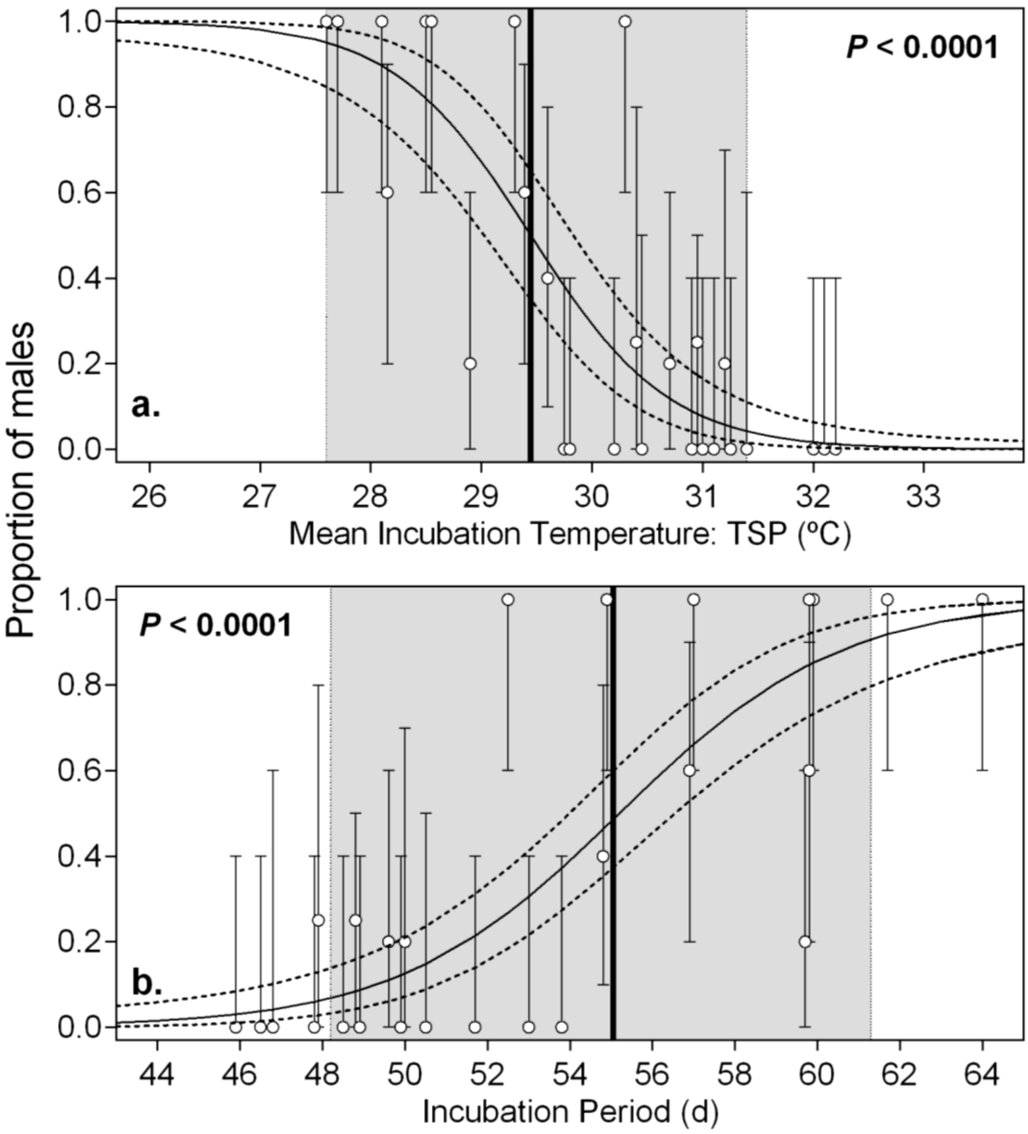

Figure 3. Logistic function (solid curve) and 95% confidence intervals (CI, dashed curves) showing expected proportion of green turtle male hatchlings, as a function of **a.** thermosensitive period (TSP) mean incubation temperatures, and **b.** incubation duration, at Poilão Island, Guinea-Bissau. Open 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 – 31.4 ºC) 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 (29.4 ºC) in **a.**, and incubation length equivalent (55.1 days) in **b.**

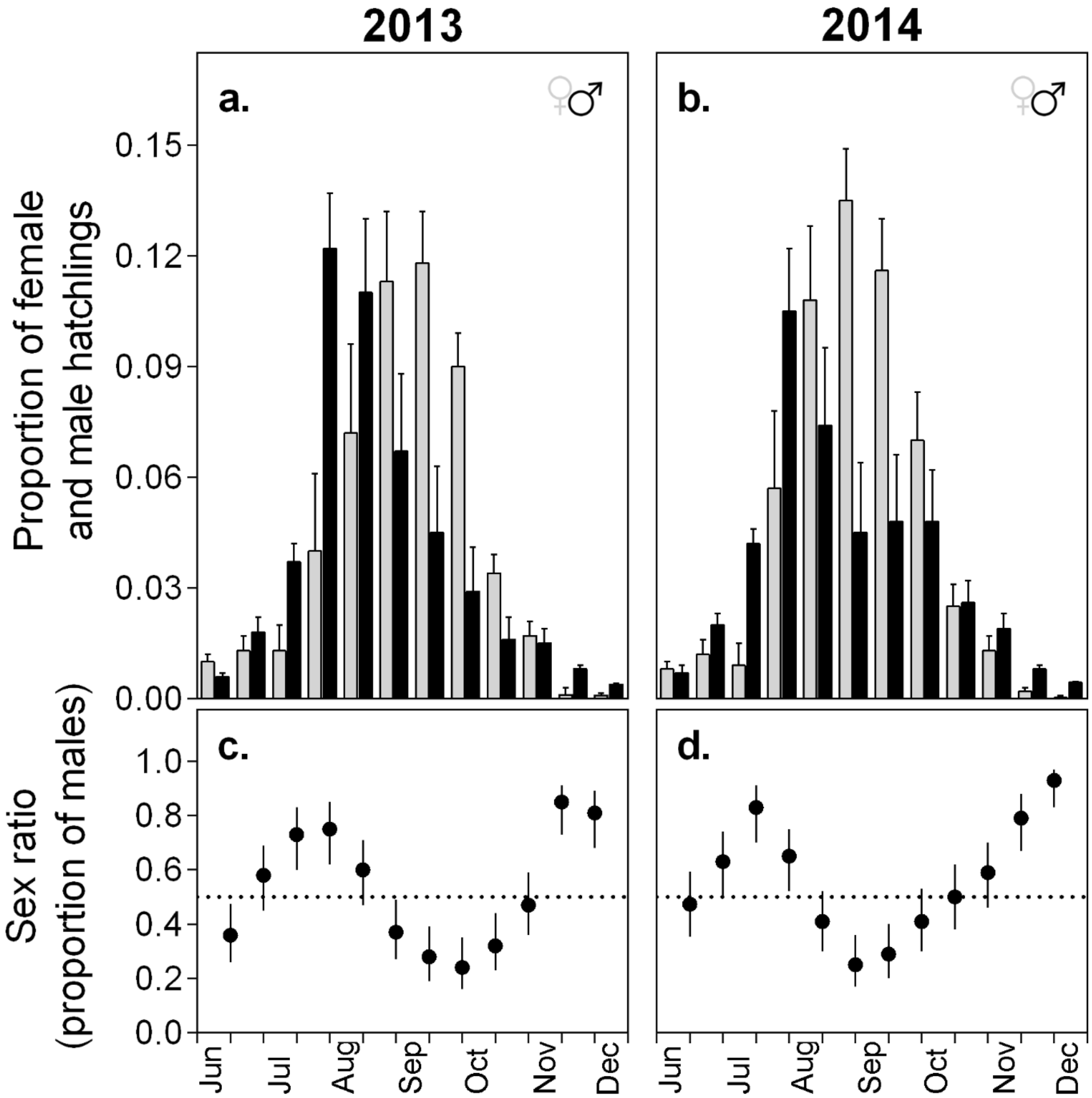

Figure 4a, b. Bi-weekly proportion of female (light grey) and of male (dark grey) green turtle hatchlings predicted to have been produced in Poilão, with error bar showing upper 95% confidence interval (CI); **c.d.** estimated mean sex ratio, with 95% CI, throughout the nesting season in 2013 2014.

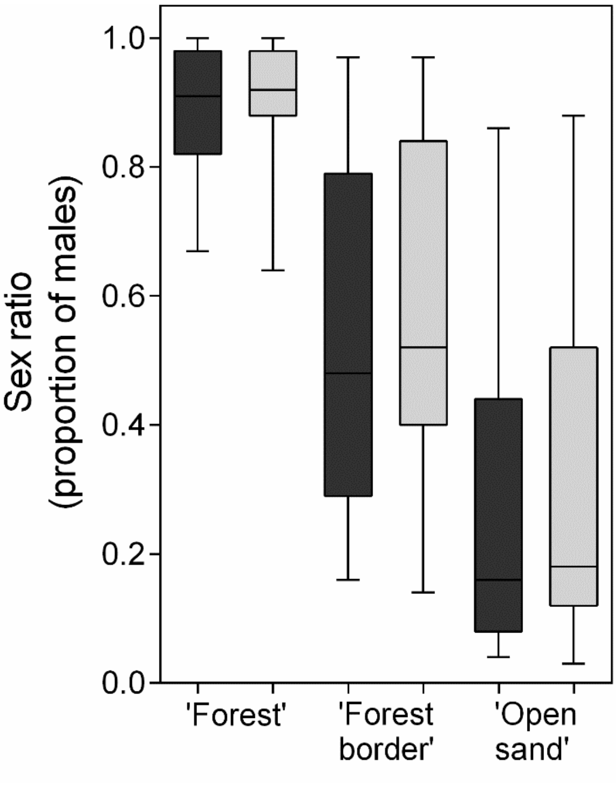

Figure 5. Estimated mean sex ratios (proportion of males) of green turtle hatchlings in each of three habitats: ‘Forest’, ‘Forest border’ and ‘Open sand’, at Poilão Island, Guinea-Bissau, for 2013 (dark grey) and 2014 (light grey). Boxes show median, upper and lower quartile, and whiskers show highest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

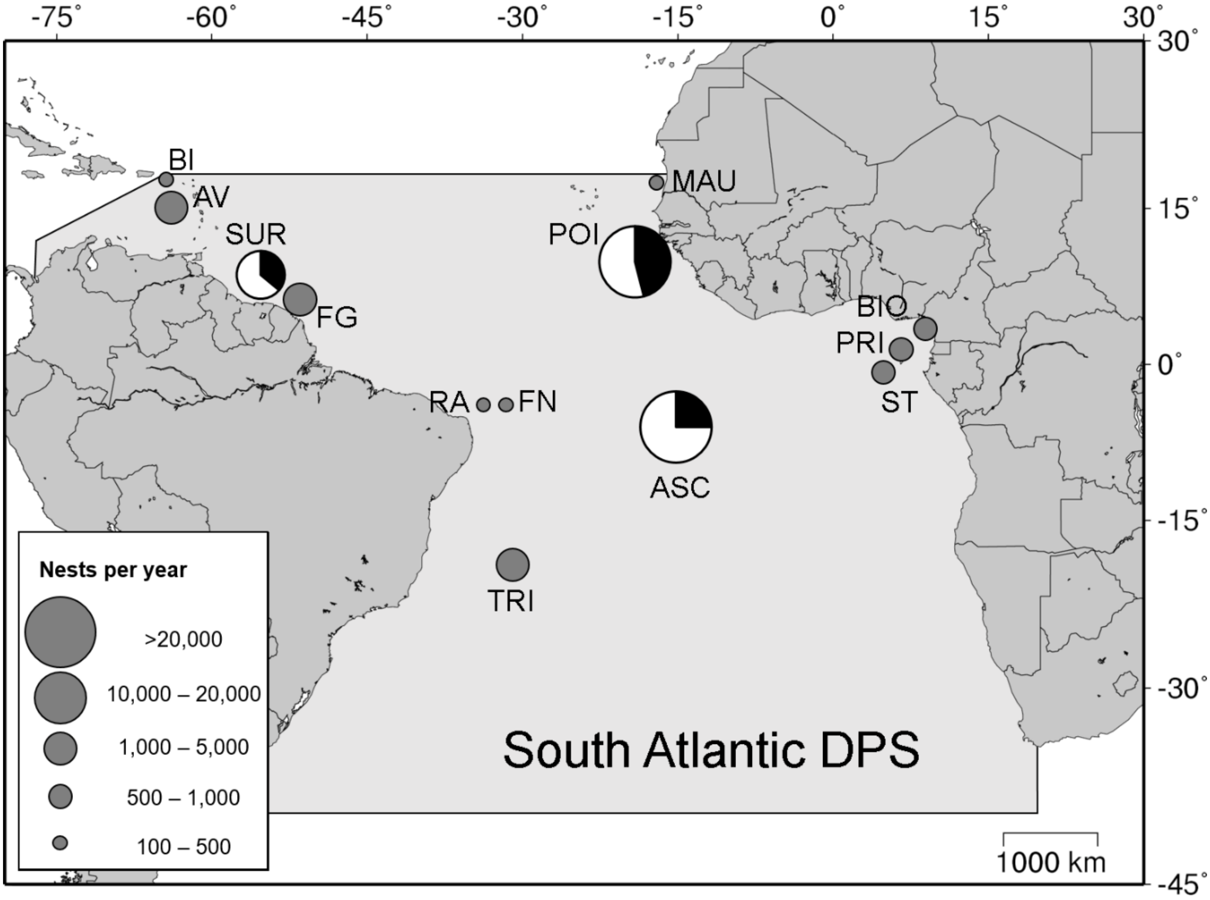

Figure 6. Limits of green turtle South Atlantic distinct population segment (DPS), showing rookeries with 100 or more nests. Pie charts indicate primary sex ratio (females: white, males: black), estimated for the three main nesting sites: Suriname (SUR, Godfrey et al. 1996, Seminoff et al. 2015), Ascension 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, Bellini, Centro Tamar, *pers. comm.*), Trindade Island, Brazil (TRI, Almeida et al. 2011), Mauritania (MAU, 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).

Supplemental 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.

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. Tukey HSD test – differences in mean incubation temperature during the middle third at four beach sections (see Fig.1b) and three habitats: ‘open sand’ from ≥ 1 m of vegetation or tree canopy to high tide line, ‘forest border from 0 – 1 m 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		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		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		-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. Habitat and beach section, incubation period ti hatching (IP), mean incubation temperatures during both the middle third (MT) of incubation and the thermo-sensitive period (TSP), and difference in days of between start and end of the MT of incubation and estimated TSP (using embryogrowth v.6.4 R package, Girondot and Kaska 2014), of 27 green turtle nests, incubated under natural conditions at Poilão Island, Guinea-Bissau, and number and proportions of male hatchlings sexed from each nest. CI: confidence interval. Habitat definitions can be found in the methods section. For beach section definitions see Fig.1b.

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

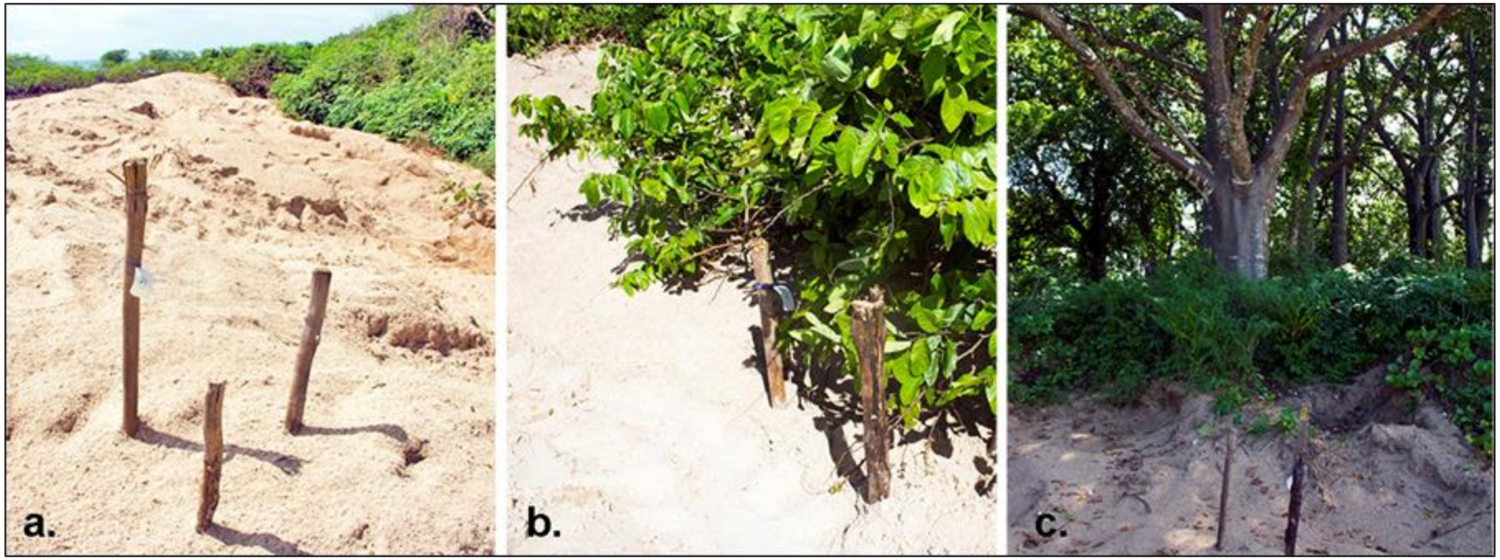

Figure S1. Nesting habitats utilized by green turtles at Poilão Island, Guinea-Bissau, according to vegetation cover: **a.** ‘open sand’ habitat, from > 1m of the vegetation to high tide line, completley exposed to the sun; **b.** ‘forest border’, comprised between 0 – 1m of the vegetation line, with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

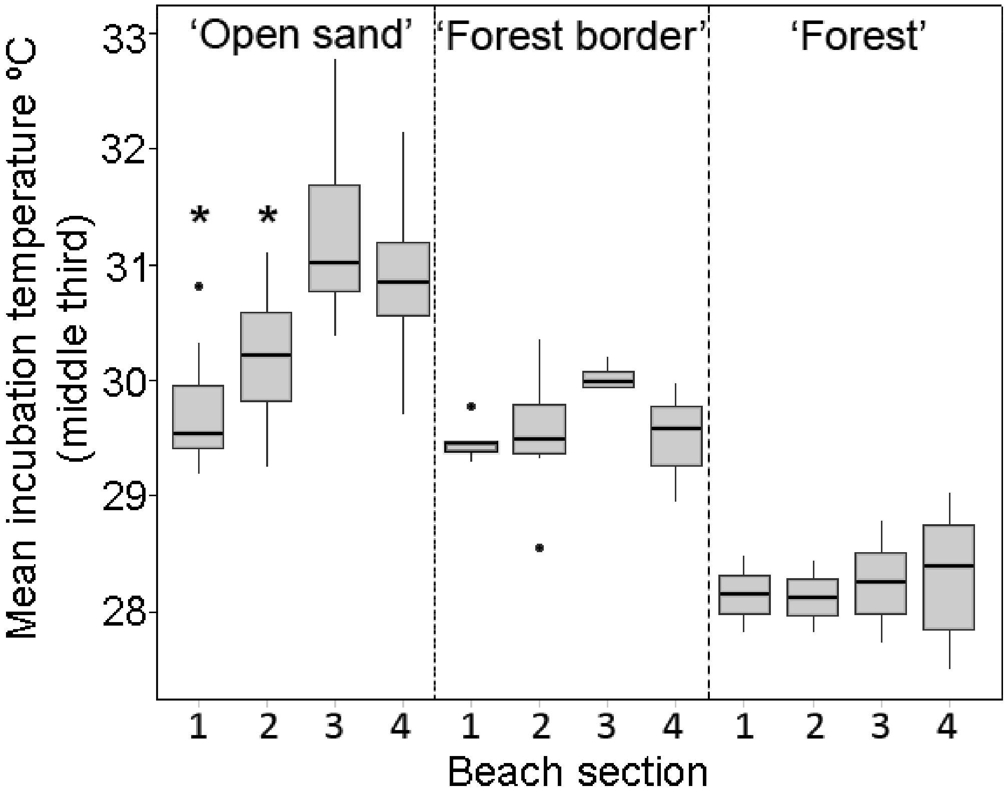

Figure S2. Middle third mean incubation temperatures of green turtle nests in three different habitats and four beach sections, at Poilão Island, Guinea-Bissau. Asterisks indicate significant differences within same habitat. For beach sections see Fig.1b. Habitat definitions can be found in the methods section.

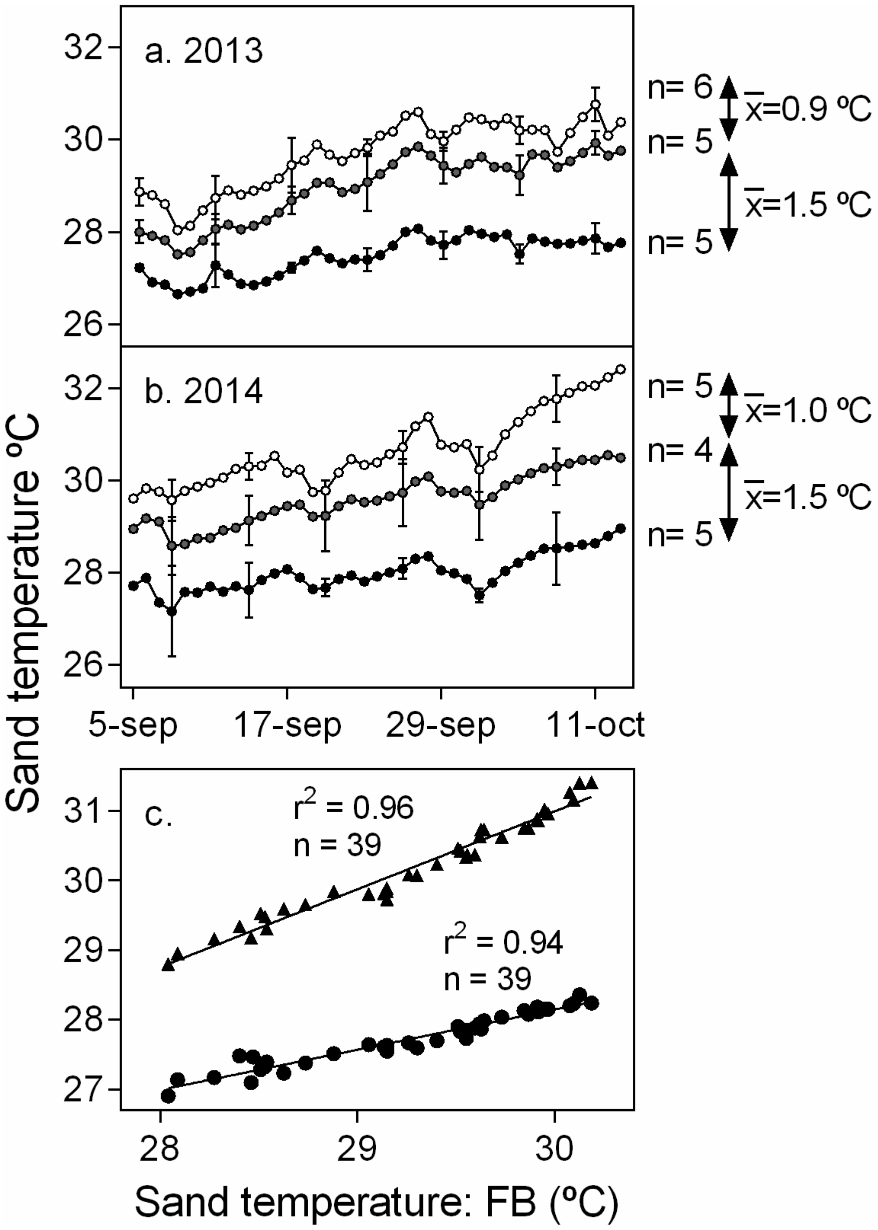

Figure S3. Sand temperature at Poilão Island, a green turtle nesting beach, in three habitats: ‘open sand’ (open circles), from > 1m of vegetation line to high tide line, ‘forest border’ (grey circles), 0-1m of vegetation line, and ‘forest’ (black circles), under vegetation, for 2013 (**a**) and 2014 (**b**). ‘n’ is the number of data loggers recording temperature at each habitat (0.3 ºC resolution), and $\bar{x}$ denotes mean difference between habitats. **c.** Linear regressions between mean sand temperatures at the ‘forest border’ and the ‘open sand’ (triangles), and the ‘forest’ (circles) habitats.

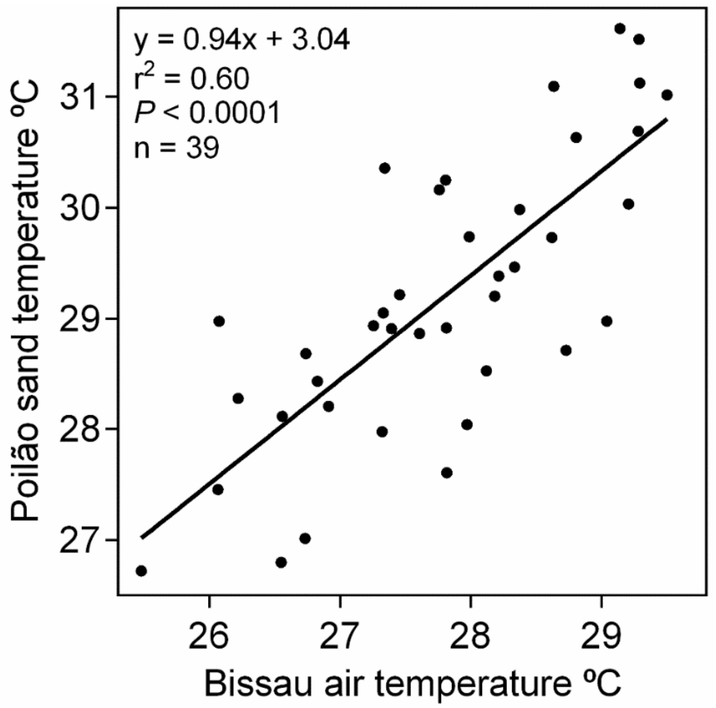

Figure S4. Linear regression between mean bi-weekly sand temperatures at Poilão (reference data loggers) and air temperatures in Bissau (http://cdo.ncdc.noaa.gov/CDO/cdo).