

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

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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 data loggers 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 29.4°C, with both sexes produced within incubation temperatures from 27.6 to 31.4°C: 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 populations' responses 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 · *Chelonia mydas* · Pivotal temperature · Transitional range of temperatures · Thermosensitive period

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INTRODUCTION

Sex ratio is an important parameter with which to assess population viability and resilience (Melbourne & 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

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thermosensitive period (TSP), corresponding approximately to the middle third of embryogenesis (Bull 1983). This is the mechanism of sex differentiation among crocodylians (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 to 35°C during incubation (Ackerman 1997, Godley et al. 2001a, 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°C 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, Matsuzawa et al. 2002, Houghton et al. 2007, Lolavar & Wyneken 2015) 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, Poloczanska et al. 2009, Mitchell & Janzen 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, 2010a, Katselidis et al. 2012, Reneker & Kamel 2016), and beachfront deforestation (Kamel & Mrosovsky 2006a, Kamel 2013). Feminizing 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 varia-

tions (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 (Hawkes et al. 2009, Fuller et al. 2013). 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. 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, 2014, 2016, 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, 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, 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% 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

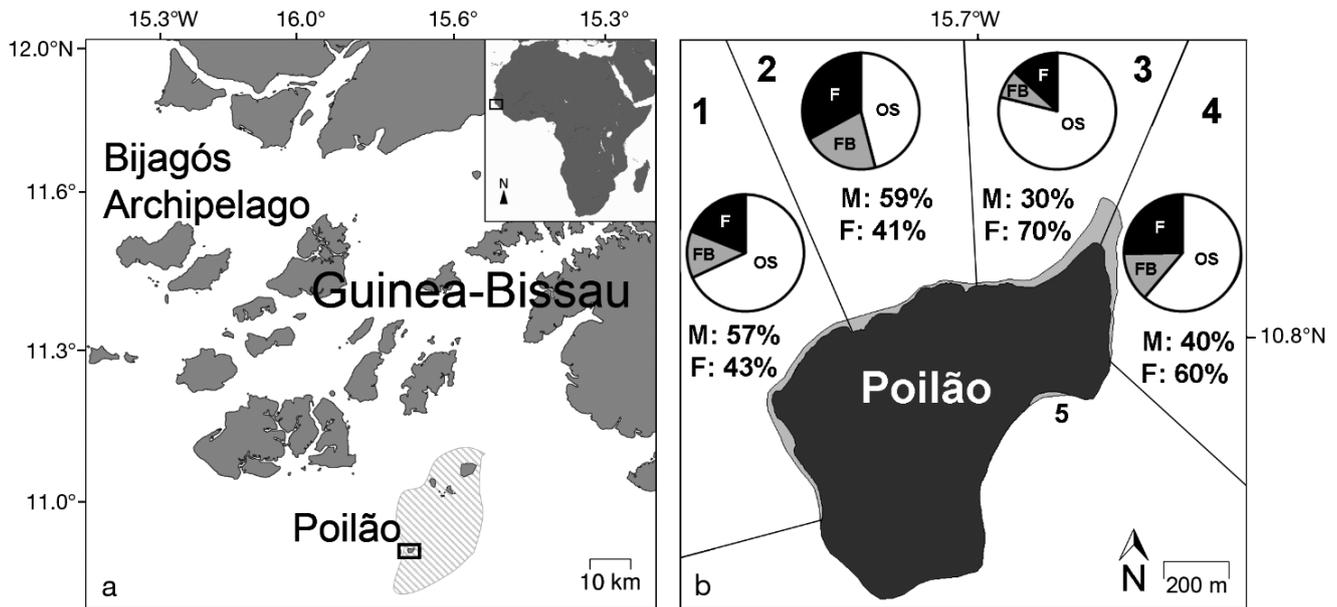


Fig. 1. (a) Bijagós Archipelago, Guinea-Bissau. Striped area: the João Vieira and Poilão Marine National Park; black frame: Poilão Island. (b) Poilão Island, showing the 4 green turtle *Chelonia mydas* nesting beach sections monitored in this study (1: Farol; 2: Acampamento Oeste; 3: Acampamento Este; 4: Cabaceira). Pie charts present the mean nesting distribution across 3 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 (average across 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

(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 *Chelonia mydas* 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, 2009), the smallest and southernmost island within the João Vieira and Poilão Marine National Park (JVPMNP; Fig. 1a). An estimate of 29000 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 2 km of the ca. 4 km 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 7 d (6.6% of the period covered) and 3 d (2.7% of the period covered) 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 reconstructed the nest density from 15 to 30 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 into 4 beach sections from west to east (1 to 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 3 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 1 m of the vegetation and experienced partial shade, and open sand corresponded to the area from >1 m of the vegetation to the high tide line, which was exposed to the sun throughout all or most of the day (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m577p189_supp.pdf).

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 specific nests, even on the subsequent morning. Thus, to determine nest distribution across habitats, we monitored turtle nesting activity at night, for 3 nights in 2013 ($n = 407$ nests identified) and 6 nights in 2014 ($n = 1152$ nests identified) during the peak of the nesting season, and determined the habitat and beach section for all 1559 nests. During these focused assessments we surveyed all 4 beach sections (2 km) at high tide (see Catry et al. 2002) and as quickly as possible (typically <1 h), 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 only 1 person, and in only 1 direction (i.e. on return no turtles were counted). Additionally, on wider beach sections with higher density, temporary marks were drawn in the sand to identify a counted animal. We used chi-squared 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 data loggers (Gemini Data Loggers; $\pm 0.3^\circ\text{C}$ accuracy, 0.1°C resolution). We placed

data loggers in the center 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 3 wooden poles to help prevent destruction by other nesting females. The data loggers had a long red string attached that 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 in 2013 and 2014, respectively), control data loggers were deployed 1 m from the clutch, at a mean mid-clutch depth of ~ 70 cm (local unpubl. 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 4 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 3 habitats identified (open sand: $n = 64$ nests, 11 control sites; forest border: $n = 21$, 9 forest: $n = 16$, 10). All data loggers were calibrated before and after each field season in a constant temperature room (24 h at 28°C) and used only if accuracy was $\leq 0.3^\circ\text{C}$. 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 4 h 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 nest 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 data logger was left to measure sand temperature from March 2013 to March 2015, to encompass both nesting seasons and enable comparisons with local air temperature. Due to the risk of data loggers being removed outside of the monitoring campaign (by turtles or people), the reference data logger 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 forest habitats against the forest border habitat (where we had the reference data logger), and used the latter as reference to extend sand temperature estimations at each habitat through the entirety of the nesting seasons. We esti-

mated IP_{mid} (mean incubation temperatures for nests laid from 15 June to 15 December 2013 and 2014) by calculating an 18 d moving average of sand temperature at each habitat (18 d corresponding to the mean duration of IP_{mid} in this study), and added mean metabolic heating ($0.5 \pm 0.4^{\circ}\text{C}$; 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, 50 km distant), 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 \times 30 cm height wire mesh, 1 cm²) above 27 of the monitored nests (i.e. nest with data loggers) from Day 45 of incubation, checking them daily for emergent hatchlings. Straight carapace length (SCL) of hatchlings was measured to 0.01 cm with a digital caliper. A random sample of 4 to 5 hatchlings nest⁻¹ (total 131 hatchlings) were sacrificed following procedures in Stocker (2005), for sex identification. 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 2 field seasons we saved over 2000 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 h in a 50:50 mix of resin (Kulzer; Technovit 7100 system) and 96% ethanol, followed by 24 h in 100% resin, and a further 24 h 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 h, stained with Toluidine Blue for 1 min 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 2 researchers (A.M. and R.R.). Consistency in sex identification was 95% (compared for 131 hatchlings); for mismatched as-

signments ($n = 7$), observers conferred until reaching agreement.

Data analysis

Generalized Linear Models (GLMs) 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 (1) IP_{mid} mean incubation temperature (response variable); and (2) 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 IP_{mid} (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 *Chelonia 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: (1) IP_{mid} mean incubation temperature, (2) TSP mean incubation temperature, and (3) 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 \pm SD, unless stated otherwise.

RESULTS

Nesting distribution

During our daily surveys from early August to late November we counted 48696 green turtle tracks in 2013 and 83304 in 2014, corresponding to 24348 and 41652 female emergences, respectively (each emergence corresponding to an ascending and a descending track). Following Catry et al. (2009), we multi-

plied 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 (Cтры et al. 2009). We estimate that in total 20785 clutches (95% CI: 18049–22855) were laid in 2013 and 35556 clutches (95% CI: 30877–39099) 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 ($\chi^2_{(3)} = 0.14$,

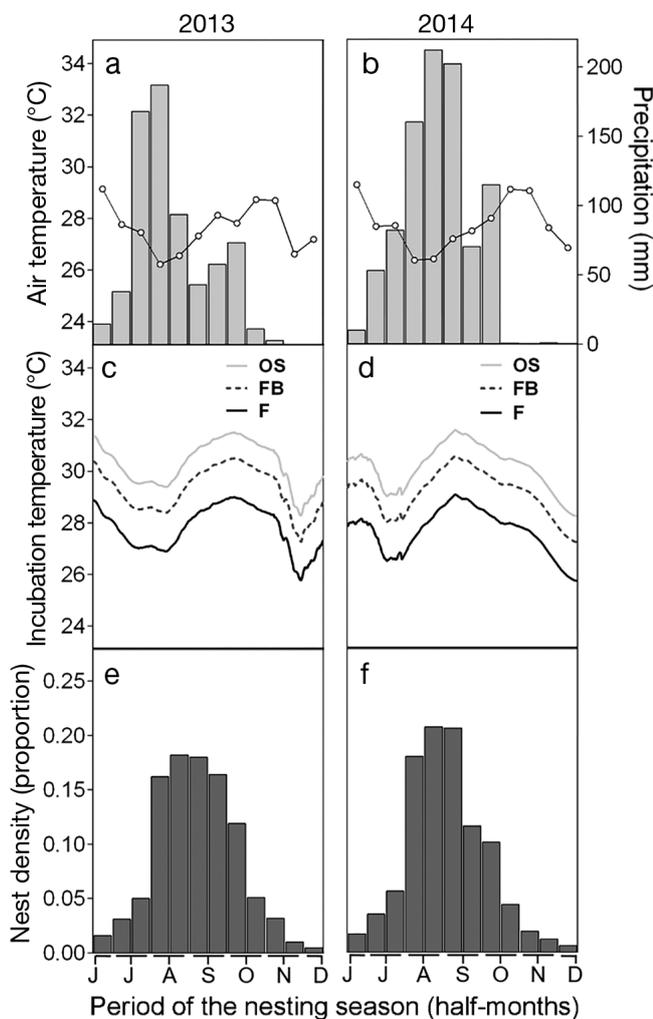


Fig. 2. (a,b) Mean bi-weekly air temperature (open circles) and precipitation (bars) at Bolama Island (<http://cdo.ncdc.noaa.gov/CDO/cdo>); (c,d) estimated mean incubation temperature during the thermosensitive period (TSP) experienced by green turtle clutches laid from 15 June to 15 December at Poilão Island, Guinea-Bissau, in 3 habitats (OS: open sand; FB: forest border; F: forest); (e,f) bi-weekly proportion of green turtle nesting distribution at Poilão

$p = 0.98$) or habitats (forest, forest border, open sand; see Table S1 in the Supplement) 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 ± 0.2 m, $n = 98$, $F_{1,97} = 0.8$, $p = 0.4$) 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 ($28.3 \pm 0.7^\circ\text{C}$; range: 27.5 to 29.0°C, $n = 16$), to the forest border ($29.7 \pm 0.7^\circ\text{C}$; range: 28.5 to 30.3°C, $n = 21$), and to the open sand ($30.6 \pm 0.8^\circ\text{C}$; range: 29.2 to 32.3°C, $n = 64$). 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's HSD test indicated that the IP_{mid} mean incubation temperature at the open sand habitat in eastern beach sections (Sections 3 and 4 in Fig. 1b) was significantly warmer ($31.1 \pm 0.6^\circ\text{C}$; range: 29.7 to 32.8°C, $n = 38$; see Fig. S2, Table S2 in the Supplement) than in the western sections (Sections 1 and 2 in Fig. 1b). In addition, IP_{mid} mean incubation temperatures of the open sand nests located in the western sections ($29.9 \pm 0.6^\circ\text{C}$; range: 29.2 to 31.1°C, $n = 25$) were not significantly different from the nests located in the forest border ($p = 0.45$).

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°C ; Fig. S3a,b in the Supplement) and at the forest habitat (-1.5°C , Fig. S3a,b) to the 18 d 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 July 2014, so we reconstructed these with air temperature using the equation $T_{sand} = 0.94T_{air} + 3.04$, where T = temperature (°C) ($F_{1,37} = 54.53$, $p < 0.0001$, $r^2 = 0.60$; Fig. S4 in the Supplement). Finally, we added 0.5°C of mean metabolic heating, estimated for the IP_{mid} ($0.5 \pm 0.4^\circ\text{C}$,

range: -0.4 to 1.2°C , $n = 20$). 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 d, with a mean of 53.5 ± 5.0 d. 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 d. The IP was inversely correlated with mean incubation temperature ($\text{IP} = -3.4644 \times \text{mean incubation temperature} + 156.92$; $r^2 = 0.87$, $p < 0.0001$). Consequently, mean IP decreased from the forest habitat (60.2 ± 5.1 d, $n = 13$) to the forest border (55.5 ± 3.9 d, $n = 16$) to the open sand (51.3 ± 3.5 d, $n = 59$).

Hatching and emergence successes

Hatching success ranged from 0 to 100% with a mean of $65.4 \pm 33.9\%$, 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 ($F_{2,95} = 2.2$, $p = 0.119$). 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 ($66.1 \pm 30.8\%$, range: 0.0 to 100%, $n = 62$), to the forest border ($51.9 \pm 38.3\%$, range: 0.0 to 98.2%, $n = 20$), to the forest habitat ($42.2 \pm 41.6\%$, range: 0.0 to 96.2%, $n = 16$). It should be noted that study nests 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 September and distributed across the 3 habitats and the 4 beach sections (Table S3 in the Supplement), with an average of 4.9 ± 0.4 hatchlings nest⁻¹. Male hatchlings were significantly larger (4.95 ± 0.19 cm, range: 4.44 to 5.33 cm, $n = 83$)

than females (4.73 ± 0.18 cm, range: 4.26 to 5.11 cm, $n = 48$; $t_{95} = -6.542$, $p < 0.0001$). The beginning of the TSP was 2.0 ± 0.7 d later than the start of the IP_{mid} (range: 0.8 to 3.2 d), and the end of the TSP was 3.3 ± 1.1 d later than the end of the IP_{mid} (range: 2 to 5 d). 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 d), justifying the use of the 18 d 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; $0.3 \pm 0.1^{\circ}\text{C}$ (range: 0.0 to 0.5°C). All 3 covariates, (1) IP_{mid} mean incubation temperature, (2) TSP mean incubation temperature, and (3) IP (to hatching) had a significant effect on expected sex ratio, at $p < 0.0001$. 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 [1] IP_{mid} mean temperatures = 56.8, [2] TSP mean temperatures = 56.0, and [3] IP = 62.9). The pivotal temperature was 29.4°C , and the TRT ranged from 27.6 to 31.4°C (Fig. 3a). Some nests behaved atypically; for instance, we sampled only males from a nest incubated at feminizing temperatures ($>30^{\circ}\text{C}$; Fig. 3a). The IP equivalent to the pivotal temperature was 55.1 d (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. 1b). Both 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 more 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 TRT, from one of the major green turtle nesting rookeries

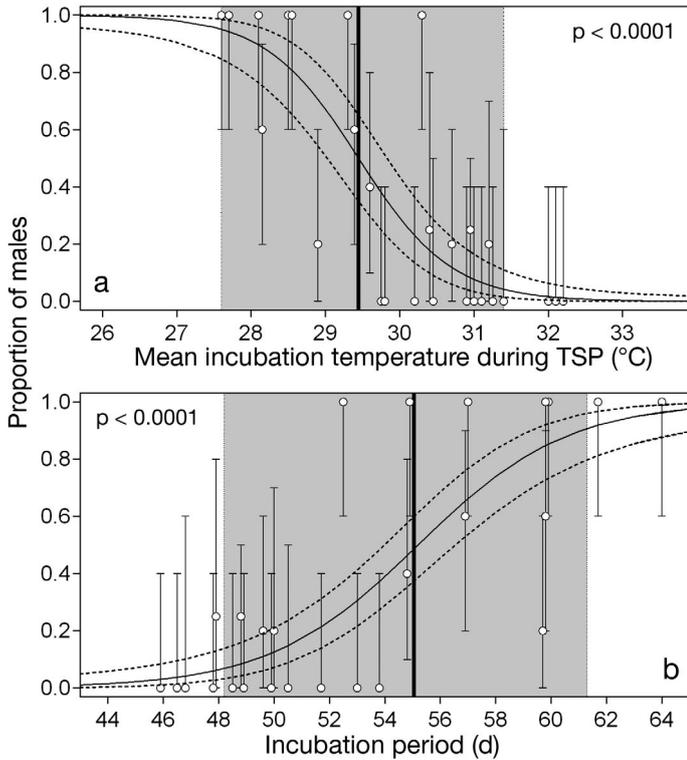


Fig. 3. Logistic function (solid curve) and 95 % confidence intervals (CI, dashed curves) showing expected proportion of green turtle male hatchlings as a function of (a) thermosensitive period (TSP) mean incubation temperatures, and (b) incubation duration, at Poilão Island, Guinea-Bissau. Open circles and 95 % CI error bars show the proportion of males found in natural nests ($n = 27$), with a mean (\pm SD) sample size of 4.9 ± 0.4 hatchlings nest⁻¹. Shaded areas: limits of transitional range of temperatures (TRT; 27.6 to 31.4°C) in (a), and corresponding limits of incubation periods (48.1 to 61.3 d, $y = -3.4644x + 156.92$, $r^2 = 0.87$) in (b). Thick vertical line: the pivotal temperature (29.4°C) for this population in (a), and incubation length equivalent (55.1 d) in (b)

worldwide, and the largest in the Southern Atlantic DPS (Fig. 6) (Seminoff et al. 2015). 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

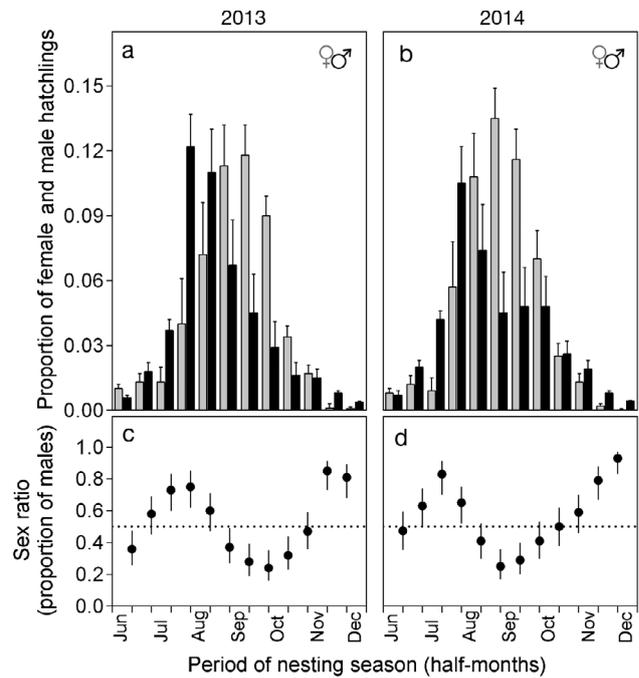


Fig. 4. (a,b) fortnightly proportions of female (light grey) and male (dark grey) green turtle hatchlings predicted to have been produced in Poilão Island, Guinea-Bissau, with error bar showing upper 95 % confidence interval (CI); (c,d) estimated mean (\pm 95 % CI) sex ratio throughout the nesting season; in (a,c) 2013 and (b,d) 2014

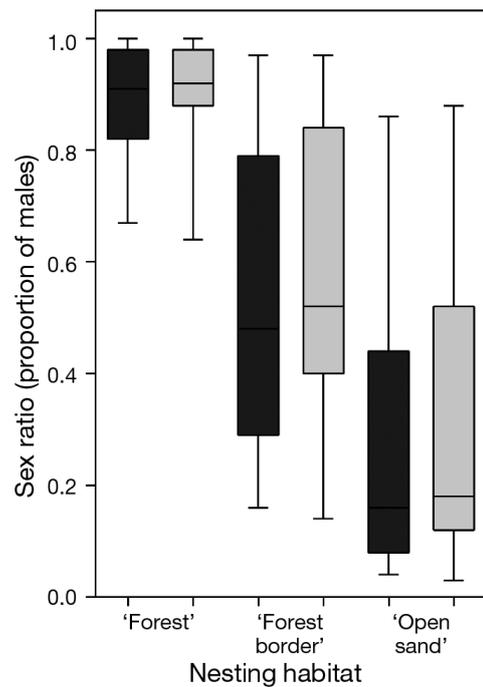


Fig. 5. Estimated mean sex ratios (proportion of males) of green turtle hatchlings in each of 3 nesting habitats at Poilão Island, Guinea-Bissau, during 2013 (dark grey) and 2014 (light grey). Boxes: median, upper and lower quartile; whiskers: highest and lowest observation

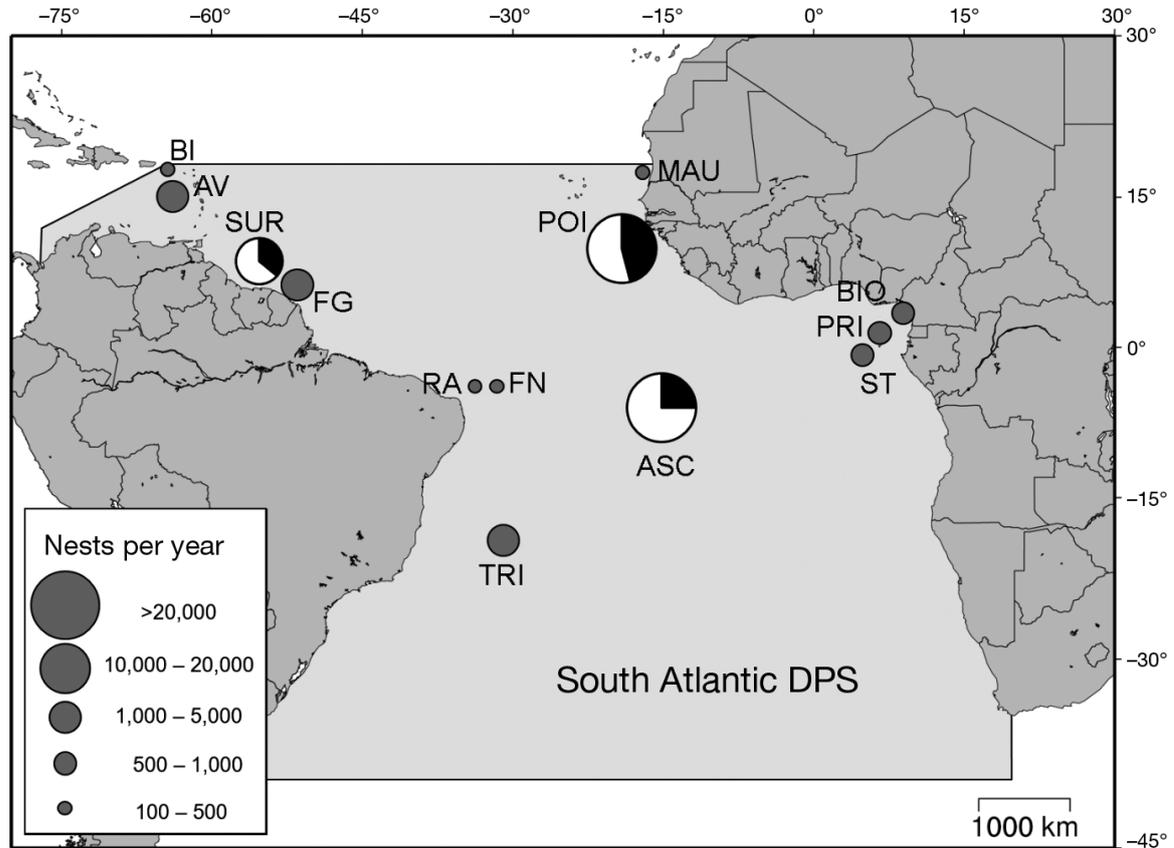


Fig. 6. Limits of green turtle South Atlantic distinct population segment (DPS), showing rookeries with 100 or more nests yr⁻¹. Pie charts indicate primary sex ratio (females: white; males: black), estimated for the 3 main nesting sites: Suriname (SUR; Godfrey et al. 1996, Seminoff et al. 2015), Ascension Island, UK (ASC; Godley et al. 2002, Weber et al. 2014), and Poilão Island, Guinea-Bissau (POI; Catry et al. 2009, this study). 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; García 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 and 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), Sao Tome (ST; ATM/MARAPA 2016) and Principe (PRI; Principe Trust Foundation pers. comm.). Map created using www.seaturtle.org/maptool

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 the 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 TRTs 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 to 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 3.8°C, suggesting that even with substantial increases in incubation temperatures as predicted by the Intergovernmental Panel on Climate Change (i.e. 2 to 3°C; Stocker et al. 2013) some nests would continue to produce males.

Within-population variability in the primary sex ratio response

We found inter-clutch variation in 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 embryo development (Girondot et al. 2010). However, this should not be the case here, as we accounted for the embryos' 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 (<1°C, decreasing from the top to the bottom; Kaska et al. 1998, Booth & Astill 2001), and our small sample size (inherent in 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°C). 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 (~1 m). 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 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 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 minimized, 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 in 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 relatively 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 & Dut-

ton 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 that are 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. 2012). 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 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), making it of global importance for the future of the green turtle in a warming world, particularly given the scale of magnitude of this population (>1 million hatchlings produced every year). Considering that some 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 3 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 full protec-

tion of its habitat, thanks to national laws and its sacred status among the local communities (Cтры et al. 2009). However, on nearby islands where numerous clutches are also laid annually (Institute of Biodiversity and Protected Areas of Guinea-Bissau [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 neighboring islands, to fully understand how resilient this population may be to climate change.

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