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Sex ratios and natal origins of green turtles from feeding grounds in the Southwest Atlantic Ocean

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Potential effects of climate change on living species are a widely debated topic. Species with temperature-dependent sex determination can be particularly impacted by warmer temperatures because unbalanced sex ratios could threaten population viability. In sea turtles, sex ratio estimates have highlighted the potential feminization of current populations, which tends to increase since warmer temperatures would generate more females. Here, we evaluated temporal variation in sex ratios of green turtles from feeding grounds of the Southwest Atlantic Ocean (SWA) using data from a 7-year time frame, from 2010 to 2016. We also evaluated natal origins of female and male green turtles from SWA based on mitochondrial DNA. Sex ratios of juvenile and adult green turtles were generally female-skewed across collection years. We identified 11 haplotypes in northeast SWA, and the haplotype composition of females and males was slightly different. Likewise, the estimated the natal origins of females and males were divergent. Ascension Island was estimated to be the main source of females while Guinea Bissau was estimated to be the main source of males. Studies evaluating natal origins of females and males independently are rare, this study provides one of the first assessments of the kind for green turtles in the SWA.

Keywords: Brazil, *Chelonia mydas*, population genetics, sea turtles, warming temperatures

Introduction

Marine ecosystems have been historically transformed by human activities; no area in the world is unaffected and many are strongly impacted by multiple threats (Halpern *et al.*, 2008). As a result, marine wildlife populations have been deeply affected both at local and global scales (McCauley *et al.*, 2015). In addition to widespread habitat degradation and overexploitation, climate change will likely accelerate population declines in the next decades (Harnik *et al.*, 2012). Although a pattern of historical decline is shared among marine animals (Lotze *et al.*, 2006), species susceptibility to anthropogenic threats may vary.

The impact of this rapidly changing world on migratory species such as sea turtles might be aggravated by aspects of their biology (Robinson *et al.*, 2009). Sea turtles are long-lived animals with a life cycle characterized by multiple habitat shifts, including direct land use during nesting activities, which increases their vulnerability (McCauley *et al.*, 2015). Additionally, sea turtles tend to use the same reproduction sites across mating seasons and females tend to nest on the same beaches they were born (philopatry), which can make them bound to potentially threatened areas (Hamann *et al.*, 2013). They also have temperature-dependent sex determination, where warmer incubation temperatures generate more females (Hamann *et al.*, 2013). Thus, rising temperatures at nesting beaches can potentially affect sex ratios, which could eventually compromise population viability (Laloë *et al.*, 2016).

Understanding sex ratio variations is key to comprehend sea turtle population dynamics, especially in a world threatened by climate change. However, determining the sex of individuals is logistically difficult and cannot always be determined through morphology (Wibbels, 2003). Because of these limitations, most of the information on the sex ratio available for sea turtles is based on indirect methods, such as measuring of sand and nest temperatures (e.g. Godfrey *et al.*, 1996; Laloë *et al.*, 2016, 2020). So far, sex ratios at nesting sites (NS) around the globe are usually female-skewed, especially in warmer beaches where females can represent over 90% of new hatchlings (e.g. Godley *et al.*, 2002; Hays *et al.*, 2014). Only a few NSs are reported to have unbiased or male-biased sex ratios (e.g. Esteban *et al.*, 2016; Patrício *et al.*, 2017b; Laloë *et al.*, 2020).

Sex ratios in feeding grounds (FG) are more difficult to access, but they can also provide important information about the population dynamics of sea turtles (e.g. Casale *et al.*, 2006; Maffucci *et al.*, 2013). While assessments of NSs provide sex ratios of hatchlings on specific nesting seasons; FGs harbour individuals of different age classes, which allows the investigation of sex ratios on a wider temporal spectrum (Maffucci *et al.*, 2013). Furthermore, because of the philopatric behaviour of females, a genetic structure can usually be detected in the matrilineal inherited mitochondrial DNA (mtDNA). This genetic structure can be used to trace the natal origins of individuals found in FGs. Hence, when the sex of individuals in FGs is known, it is possible to estimate the natal origins of females and males independently and thus help to investigate the sex ratios in local NSs.

Recently, Jensen *et al.* (2018) used genetic data of individuals with known sex to investigate sex ratio of green turtles, *Chelonia mydas* (Linnaeus, 1758), using samples from FGs in the Great Barrier Reef (GBR), Australia. They found that the sex ratio of green turtles from the northern GBR, one of the largest nesting areas for green turtles in the world, was highly female-skewed (Jensen *et al.*, 2018). This result raises concerns about other green turtle nesting sites but also demonstrates the usefulness of using data from FGs to investigate local sex ratios.

The Southwest Atlantic Ocean (SWA) is an important region for green turtles, harbouring two of the main NSs in the Atlantic (Ascension Island and Surinam), and it is considered to have high levels of threat to sea turtles (Wallace *et al.*, 2011). Until now, genetic studies of green turtles in the SWA using individuals with known sex are scarce. Studying population genetics of females and males independently can provide important information on population dynamics and help to identify male-producing NSs in the region, which are increasingly important due to the current trend of feminization of populations (Hays *et al.*, 2014). The identification of NSs that produce mainly or exclusively females is also important to help understand local environmental conditions surrounding extremely skewed sex ratios. This information can be valuable to planning conservation strategies that take into consideration the effects of climate change on green turtle populations (Laloë *et al.*, 2020).

Here, we compile historical data of green turtles from FGs in the SWA and discuss spatiotemporal variations in sex ratios. Additionally, we evaluate genetic data and determine natal origins of sexed specimens of an FG in north-eastern SWA. Our main goals were to evaluate temporal changes in female-male proportions as well as to determine the genetic composition of green turtles from north-eastern SWA in order to answer the following questions: (i) did sex ratios in the SWA change in the recent years?; (ii) is there geographic variation in sex ratios within the SWA?; and (iii) are there differences in natal origins of female and male green turtles feeding in north-eastern SWA? And if so, could that be an indicative of biased sex ratios at source NSs?

Material and methods

Feeding grounds sex ratios

Data from stranded green turtles were obtained from beach monitoring projects performed between 2010 and 2016 in FGs of two geographical regions in north-eastern and one in south-eastern Brazil (Figure 1). These projects were established by the *Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis* (IBAMA) as a measure to evaluate environmental impact during the implementation of activities that might alter the surrounding natural environment. Feeding grounds in north-eastern coast encompassed five states and are henceforth denominated Northeast 1 (along the coast of Ceará and Rio Grande do Norte States) and Northeast 2 (along the coast of Alagoas, Sergipe, and Bahia States). Feeding grounds in south-eastern, henceforth Southeast, encompassed the coast of Espírito Santo State (Figure 1, Supplementary Table S1). In total, we used 3465 records with information on location, date, sex (determined through gonadal inspection), and curved carapace length (CCL) of each stranded specimen.

We evaluated sex ratio variations throughout collection years by estimating the mean sex ratio among all FGs within each year and applying a Pearson chi-squared test. Subsequently, as we did not detect differences in sex ratios throughout the years (see Results section), we combined the data from different collection years and grouped females and males into three size classes: juveniles recently recruited to the neritic zone (CCL <40 cm), older juveniles (CCL 40.1–90 cm) and adults (CCL >90 cm). Sizes of recently recruited juveniles were determined conservatively based on the approximate CCL of the smallest individuals recorded in different FGs in the Atlantic (19–36 cm; Reich *et al.*, 2007; Goshe *et al.*, 2010; Lenz *et al.*, 2017). As estimated growth rates of South Atlantic green turtles with CCL between 30 cm and 39.9 cm is around 3.89 cm per year (Lenz *et al.*, 2017), we expect that individuals with CCL <40 cm had been recruited to the neritic zone only over the past few years. The size of adults was determined based on information of the smallest nesting females at Trindade NS (around 90 cm; Almeida *et al.*, 2011).

Data from age estimation and growth rates indicate that green turtles in the Atlantic are usually 2–7 years old when they recruit to the neritic zone and over 30 years old when they reach sexual maturity (Goshe *et al.*, 2010; Lenz *et al.*, 2017). Thus, by grouping our samples within these three size classes we should have sex ratio estimates for recent years (specimens with CCL <40 cm), for around 7–30 years (specimens with CCL 40.1–90 cm), and for over 30 years (specimens with CCL >90 cm). It is important to highlight that the size classes we used are not definitive and that the size at which green turtles recruit to neritic zones or reach sexual maturity might vary in different regions (Goshe *et al.*, 2010).

We estimated the mean sex ratio in the SWA for each size class using data from all FGs. We then evaluated differences in sex ratios among size classes using a chi-square goodness of fit test. Finally, we tested if the sex ratio within each FG differed from the estimated mean sex ratio for the SWA for each size class also using chi-squared goodness of fit tests. All analyses were performed using the software R 3.5.1 and the native package *stats* (R Core Team, 2018).

Genetic composition and natal origins of female and male green turtles

For molecular analyses, we used muscle samples from 146 specimens (CCL 23–115 cm), of which 89 were from females and 57 were from males. Samples were collected from stranded green

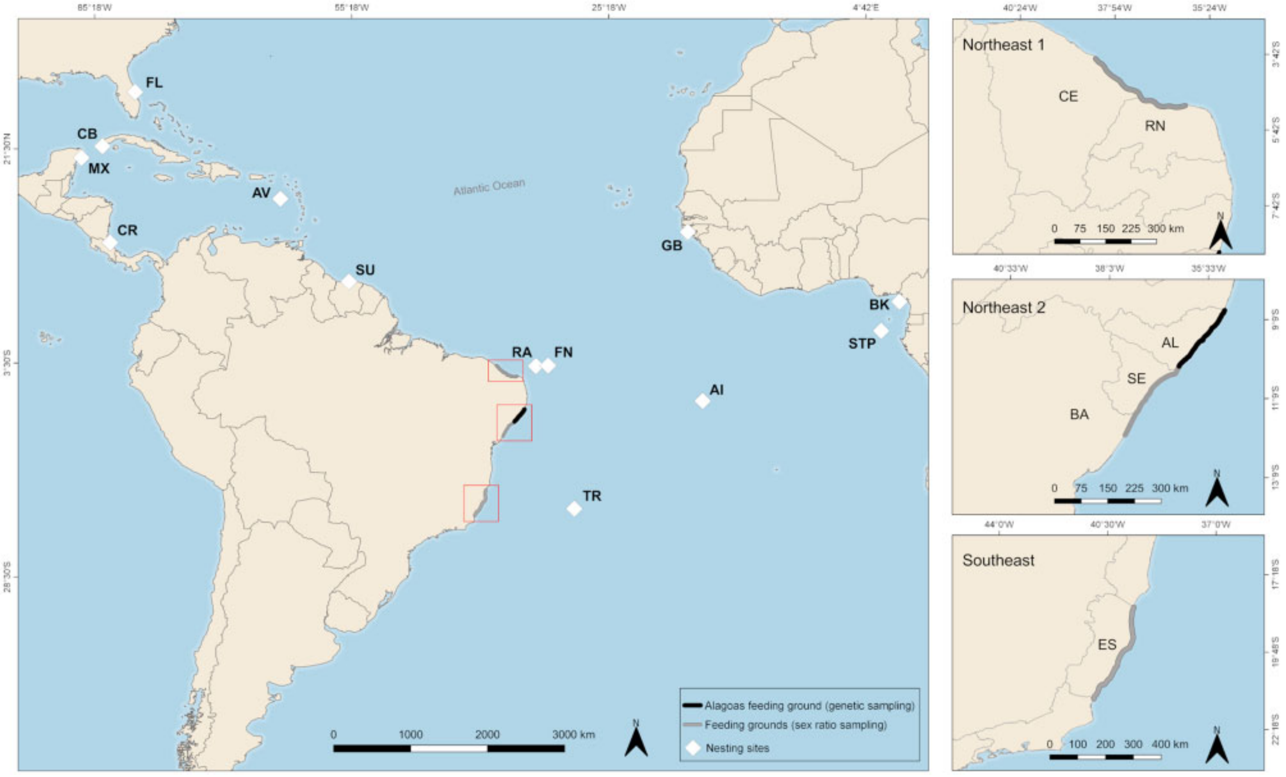

Figure 1. Green turtle sampling included in this study. Areas in grey represent feeding grounds included in sex ratio analyses. Area in black denotes feeding ground included for genetic analyses. White polygons indicate nesting sites used as possible sources in genetic analyses. Feeding grounds: AL, Alagoas; BA, Bahia, CE, Ceará; ES, Espírito Santo; RN, Rio Grande do Norte; SE, Sergipe. Nesting sites: AI, Ascension Island; AV, Aves Island; BK, Bioko; CB, Cuba; CR, Costa Rica; FL, Florida; FN, Fernando de Noronha; GB, Guinea Bissau; MX, Mexico; RA, Rocas Atoll; STP, São Tomé and Principe; SU, Surinam; TR, Trindade Island.

turtles found along the coast of Alagoas State between May 2018 and March 2020, covering ∼230 km of coastline in north-eastern Brazil (Figure 1). All specimens were dead, which allowed sex determination through direct inspection of the gonads. Samples were stored in ethanol 92% and kept at −18°C.

Total genomic DNA was extracted using phenol-chloroform method (Sambrook *et al.*, 1989), and a fragment of approximately 800 base pairs (bp) of the mitochondrial control region was amplified through 25 µl polymerase chain reactions using the primers LCM15382 and H950 (Abreu-Grobois *et al.*, 2006). Reactions consisted of 20.8 µl of 1XMaster Mix PCR Buffer with 0.4 mM of each dNTP and 3 mM of $MgCl_2$, 1.0 µl of each primer (10 pmol); 2 µl of DNA template (>20 ng/µl); and 0.2 µl of Taq DNA polymerase (5 U/µl). Amplifications were performed as follows: initial denaturation at 94°C for 7 min followed by 35–40 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, extending at 72°C for 1 min and a final extending at 72°C for 5–7 min. Posteriorly, samples were purified with sodium acetate and isopropanol to remove PCR residuals and sequenced in both directions using Sanger sequencing.

DNA sequences were submitted to the BLAST tool at GenBank database to check for contamination and subsequently edited using BioEdit 7.0.5.3 (Hall, 2011). Sequences were aligned using MAFFT 7.310 (Katoh and Standley, 2013), and trimmed to a 490 bp fragment for better comparison with nesting sites. Haplotypes were identified based on the Archie Carr Center for Sea Turtle Research haplotype database (https://accstr.ufl.edu/, last accessed 18 April 2021). Haplotype (h) and nucleotide (θ_π) diversities were estimated using DnaSP 5.10 (Librado and Rozas, 2009), and haplotype relationships were evaluated with median-joining haplotype networks (Bandelt *et al.*, 1999) reconstructed with Network 10.1 (https://www.fluxus-engineering.com, last accessed 10 December 2020). Genetic differentiation between females and males was evaluated using an Analysis of Molecular Variance (AMOVA) with 10 000 permutations implemented in Arlequin 3.5.2.2 (Excoffier and Lischer, 2010).

Natal origins of female and male green turtles were estimated independently based on many-to-one mixed-stock analyses (MSA), which allows to determine the relative contribution of multiple sources (NSs) to a single mixed population (FG) based on the frequency and relative proportions of haplotypes using Bayesian methods (Pella and Masuda, 2001). Data from the following nesting sites were used: Florida and Mexico (Encalada *et al.*, 1996), Aves Island and Surinam (Bjorndal *et al.*, 2006; Shamblin *et al.*, 2012); Ascension Island (Formia *et al.*, 2007); Costa Rica (Bjorndal *et al.*, 2005); Cuba (Ruiz-Urquiola *et al.*, 2010); Guinea Bissau (Patrício *et al.*, 2017a); Rocas Atoll, Fernando de Noronha, and Trindade Island (Bjorndal *et al.*, 2006); São Tomé and Principe and Bioko (Formia *et al.*, 2006). Data from Rocas Atoll and Fernando de Noronha were pooled because of the small sample size and because these two NSs are geographically close and genetically similar (Bjorndal *et al.*, 2006).

We performed MSA analyses using females and males independently. First, we used the size of each NS (MSA1), based on the number of nesting females, as a prior for the analyses (see Supplementary Table S2; also Seminoff *et al.*, 2015). Second, we considered equal weights for all NSs (MSA2), i.e. without the effect of size of NS. Analyses were performed using BAYES (Pella and Masuda, 2001), with 12 chains per run [equal to the number of sources (NSs)] and 50 000 iterations per chain. As default, half of these iterations were discarded as burn-in. Chains convergence was checked using the Gelman–Rubin criterion, considering that convergence has been achieved if values were below 1.2 (Gelman and Rubin, 1992).

Results

Feeding grounds sex ratios

From the 3465 records of stranded green turtles, 2630 were females and 835 were males (for a detailed description see Supplementary Table S1). Sex ratios in the SWA across collection years were similar, with slightly lower proportions of females in 2015 and 2016 (Figure 2). Variation observed among years was not significant ($X^2 = 10.318$, $p = 0.112$). Comparison of sex ratios among size classes revealed that sex ratios of recently recruited (mean 3.10 F:1 M) and older juveniles (mean 3.27F:1M) were similar and did not differ significantly ($X^2 = 0.976$, $p = 0.323$). Sex ratio of adults was slightly less female-biased (mean 3.03F:1M) but also did not differ significantly from recently recruited ($X^2 = 0.097$, $p = 0.7545$) or older juveniles ($X^2 = 0.658$, $p = 0.417$).

When we compared sex ratios within each FG to mean sex ratios estimated for the SWA for each size class, we found that in recently recruited juveniles sex ratios were not different from the average value for the SWA in Northeast 2 ($X^2 = 0.891$, $p = 0.345$) and Southeast ($X^2 = 1.958$, $p = 0.161$), but in Northeast 1 there were fewer females than average ($X^2 = 8.199$, $p = 0.004$; Figure 3). The number of older juvenile females was significantly higher than the expected in Northeast 1 ($X^2 = 6.036$, $p = 0.014$), significantly lower in Northeast 2 ($X^2 = 10.825$, $p = 0.001$), and no different from the average in Southeast ($X^2 = 1.566$, $p = 0.210$). The number of adult females was no different from the expected in Northeast 1 ($X^2 = 3.746$, $p = 0.053$), but lower in Northeast 2 ($X^2 = 13.531$, $p = 0.0002$, Figure 3). We did not compare the proportion of females in the Southeast to the SWA because of the low number of adults in this region (five females and five males).

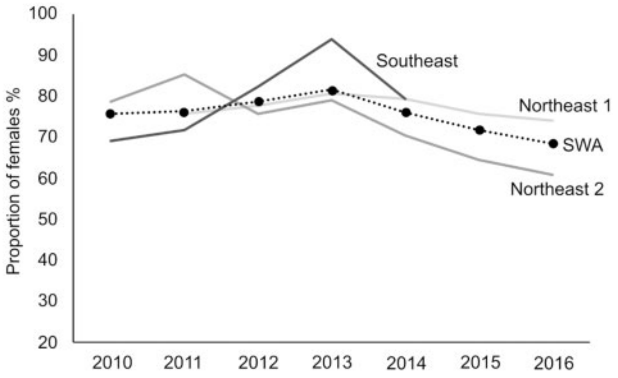

Figure 2. Proportion of female green turtles in Southwest Atlantic Ocean feeding grounds between 2010 and 2016, based on 3465 records.

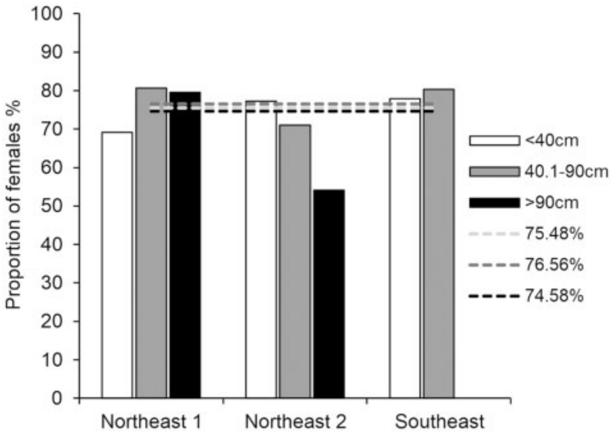

Figure 3. Proportions of female green turtle in Southwestern Atlantic Ocean feeding grounds according to size classes. Dashed light grey, dark grey, and black lines indicate mean proportion of females in recently recruited juveniles, older juveniles, and adults in the SWA, respectively.

Genetic composition and natal origins of female and male green turtles

We identified eleven haplotypes, of which nine were found in females and seven in males. Five haplotypes were shared between females and males (Figure 4, Supplementary Table S2). The most common haplotype was CM-A8, 76.4% of females and 64.9% of males, followed by CM-A5, 10.1% of females and 24.5% of males. Nucleotide and haplotype diversities were slightly larger in males ($\theta_\pi = 0.00234 \pm 0.00058$, $h = 0.502 \pm 0.062$) than in females ($\theta_\pi = 0.00127 \pm 0.00023$, $h = 0.389 \pm 0.063$). The AMOVA analysis indicated that most genetic variation was within females and males (95.83%), but variation between them was still significant ($F_{ST} = 0.0417$, $p = 0.017$). We obtained the longer fragment of the control region (~800 bp) from 106 of 146 specimens and only found more than one variant haplotype among CM-A8 samples: CM-A8.1 (48 females, 28 males), CM-A8.2 (two females), and CM-A8.3 (one male). A full list of long haplotypes is provided in Supplementary Table S3.

Mixed-stock analyses considering the number of nesting females (MSA1) estimated slightly different natal origins for females and males (Table 1). Ascension Island was the main contributor to the composition of females (62.28%), while Guinea Bissau contributed the most to the composition of males (40.28%). Guinea Bissau was the second-highest contributor to the composition of females (25.44%), followed by Surinam (8.83%). For males, Ascension Island (30.44%) and Surinam (23.26%) also had large contributions. All other NSs contributed with less than 5% for both females and males (Table 1). When considering equally weighted priors (MSA2), contributions of NSs were similar to MSA1, Ascension Island and Guinea Bissau contributed the most to the composition of females (53.83% and 25.93%, respectively) and males (20.90% and 37.33%, respectively).

Discussion

Feeding grounds sex ratios

Sex ratios in SWA feeding grounds were generally female-skewed (Figure 3), which is in line with the most reported sex ratios for

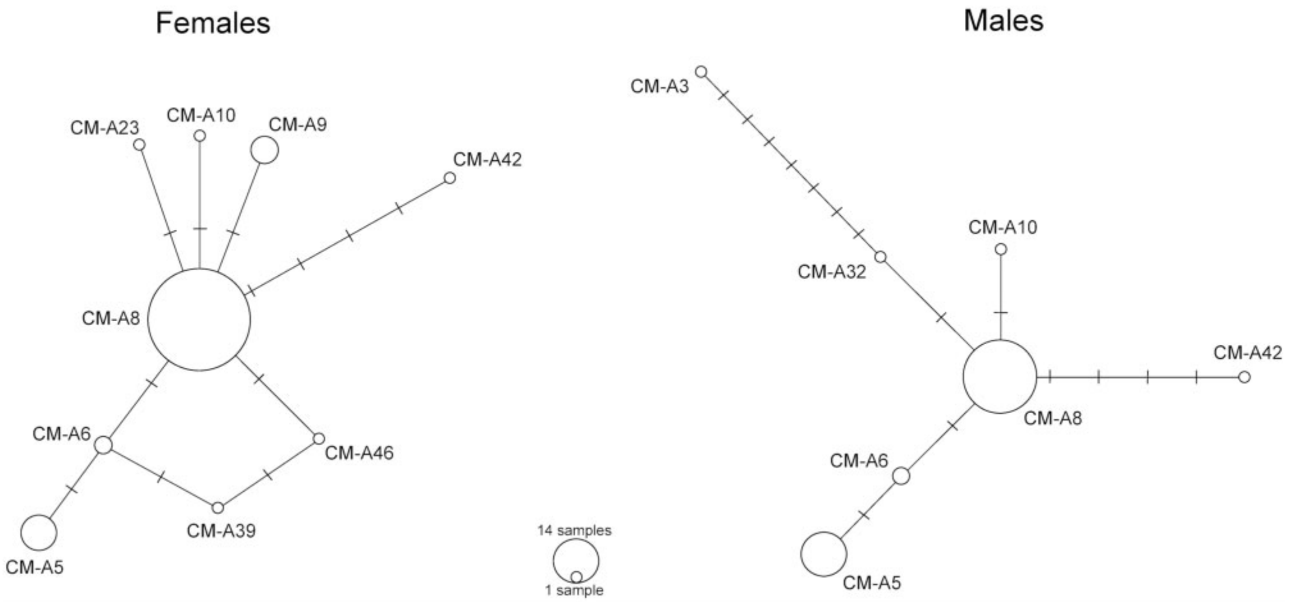

Figure 4. Haplotype networks of female and male green turtles from Alagoas feeding ground, based on 490 bp of the control region of the mitochondrial DNA.

Table 1. Mixed-stock analyses of female and male green turtles from Alagoas feeding ground.

	Females		**Males**	
Stock	**MSA1**	**MSA2**	**MSA1**	**MSA2**
Florida	0.05 (0–0.58)	0.11 (0–1.16)	0.11 (0–1.29)	0.29 (0–2.94)
Mexico	0.13 (0–1.28)	0.10 (0–1.07)	0.26 (0–2.51)	0.22 (0–2.31)
Costa Rica	0.70 (0–3.27)	0.10 (0–1.06)	2.19 (0.01–7.97)	0.38 (0–3.54)
Aves Island	1.38 (0–12.43)	4.38 (0–15.11)	2.88 (0–28.85)	10.97 (0–34.31)
Surinam	8.83 (0–17.17)	5.88 (0–16.20)	23.26 (0–38.43)	15.57 (0–37.30)
Rocas/Noronha	0.03 (0)	1.13 (0–11.68)	0.17 (0)	4.23 (0–26.04)
Trindade Island	1.06 (0–15.60)	6.22 (0–28.95)	0.19 (0–2.14)	1.36 (0–11.79)
Ascension Island	**62.28 (28.99–88.07)**	**53.83 (17.27–85.80)**	30.44 (1.67–66.65)	20.90 (0–63.33)
Guinea Bissau	25.44 (1.76–55.47)	25.93 (1.27–56.36)	**40.28 (5.94–68.31)**	**37.33 (3.28–67.13)**
Bioko	0.08 (0–0.02)	1.80 (0–18.38)	0.18 (0–0.07)	7.55 (0–77.74)
São Tomé and Principe	0.01 (0)	0.43 (0–4.35)	0.02 (0)	0.95 (0–10)
Cuba	0.01 (0–0.05)	0.10 (0–0.98)	0.03 (0–0.15)	0.27 (0–2.73)

Analyses were performed using size of nesting sites as prior (MSA1) and equal weights priors (MSA2). Contributions of nesting sites are in % and 2.5% and 97.5% confidence intervals are in parenthesis. Nesting sites with the highest contribution are in bold in each MSA.

green turtles worldwide (Hays *et al.*, 2014). There was no significant change in sex ratios throughout collection years, suggesting that the proportion of females and males have been somewhat constant in the SWA in recent years. Sex ratios of green turtles in the SWA have been historically investigated through the evaluation of nesting beach temperatures within or between nesting seasons (e.g. Broderick *et al.*, 2001; Godley *et al.*, 2002). Studies are mainly focused on two major NSs, Ascension and Surinam (Mrosovsky *et al.*, 1984; Godfrey *et al.*, 1996; Godley *et al.*, 2002). Sex ratios in Ascension have been repeatedly reported as female-skewed (Godley *et al.*, 2002, Pintus *et al.*, 2009) and this NS is usually reported as the main contributor to the composition of individuals in FGs in the SWA (e.g. Naro-Maciel *et al.*, 2012; Proietti *et al.*, 2012). Thus, we would expect that this high prevalence of females would be reflected in the composition of individuals in FGs. Our results support this observation, as the proportion of females generally exceeded 70%. Nevertheless, the implementation of genetic analyses is still required to corroborate the natal origins of these individuals.

The sex ratio of adult green turtles in SWA FGs was slightly less female-biased (2.97F:1M), but not significantly different from juveniles. However, FGs in Northeast 2, exhibited a noticeable lower proportion of adult females (Figure 3). As green turtles in the Atlantic usually take 30–40 years to reach sexual maturity (Goshe *et al.*, 2010), the similarity between juveniles and adult sex ratios could indicate that female output in local NSs has been constantly high in the recent decades. This result is in agreement with some studies that indicate that female-biased sex ratios were predominant during the last decades in the largest NS in SWA, Ascension Island (Godley *et al.*, 2002; Pintus *et al.*, 2009). However, other extrinsic factors, such as different migration periodicity and differential death rates in female and male adults, could also be playing a role in sex ratio variation between juveniles and adults (Maffucci *et al.*, 2013). Furthermore, the cause of death in stranded turtles is not always possible to determine accurately, making it difficult to determine if local factors such as fisheries and pollution could bias our results by having distinct impacts in females and males or even juveniles and adults.

Genetic composition and natal origins of female and male green turtles

Haplotype composition of females and males was slightly divergent, but the most common haplotypes were CM-A8 and CM-A5, similarly to what is found in other FGs in the SWA (Naro-Maciel *et al.*, 2012; Proietti *et al.*, 2012; Prosdocimi *et al.*, 2012). Nesting sites in the South Atlantic also exhibit a high prevalence of CM-A8 (e.g. Naro-Maciel *et al.*, 2014; Patrício *et al.*, 2017a), while CM-A5 is more frequently found in NS closer to the Caribbean (Shamblin *et al.*, 2012). The occurrence of two specimens with the haplotype CM-A42 is also noteworthy, since Guinea Bissau is the only NS to which this haplotype was reported so far (Patrício *et al.*, 2017a), and the presence of this haplotype might have influenced MSA results. Considering the longer fragments, the predominant variant of the CM-A8 ($N = 79$) haplotype was CM-A8.1 (96.2%, $N = 76$, 48 females and 28 males), similar to what was found in Rocas Atoll, Fernando de Noronha, and Trindade Island NSs (Shamblin *et al.*, 2015), all in the SWA, as well as Guinea Bissau (Patrício *et al.*, 2017a). Likewise, the predominant variant of the CM-A5 ($N = 14$) haplotype was CM-A5.1 (100%, ten females and four males), which is also the primary variant of this haplotype in Suriname, Aves Island, and Costa Rica NSs (Shamblin *et al.*, 2012).

Relative contributions of NSs to our study area were slightly divergent between female and male green turtles (Table 1). Considering the size of NSs (MSA1), the major contributors to female composition were Ascension Island and Guinea Bissau. The situation was inverted for males, with Guinea Bissau as the highest contributor followed by Ascension Island (Table 1). While Ascension is usually reported as the major contributor to the composition of individuals in FGs in the SWA, contributions of Guinea Bissau are usually considered unlikely. Data from satellite tracking, tag return, and particle dispersion suggest that green turtles from Guinea Bissau most likely feed on coastal areas of West Africa (Godley *et al.*, 2010). However, the contributions of Guinea Bissau can still be substantial in some cases (Naro-Maciel *et al.*, 2007; Proietti *et al.*, 2012). A recent report estimated that foraging aggregations in north-eastern Brazil could receive up to 25% contribution from Guinea Bissau in foraging-centric MSAs (Patrício *et al.*, 2017a). This finding reinforces that individuals from this NS could reach Alagoas FG as well. Additionally, data from hawksbill turtles, *Eretmochelys imbricata* (Linnaeus, 1766), also support transatlantic migration from African NSs to FGs in north-eastern Brazil (Proietti *et al.*, 2014).

Our results using both weighted (MSA1) and equal priors (MSA2) support a high contribution of Guinea Bissau to the composition of individuals, particularly males, in our study area. These results are also in agreement with estimates of balanced sex ratios in this NS (Rebelo *et al.*, 2012; Patrício *et al.*, 2017b), where environmental conditions seem to contribute to cooler nest temperatures and consequently to a higher proportion of male hatchlings when compared to other NSs (see Patrício *et al.*, 2019 for a detailed discussion). In contrast, sex ratios in Ascension Island have been reported to be female-skewed, with estimations varying between 54.2% and 99.6% of females, depending on the specific beach (Godley *et al.*, 2002). This is reflected in the contributions of this NS to the composition of females in our study area, which were high in both MSA1 and MSA2 (Table 1).

Contributions from Surinam to the composition of females and males were also large (9.26% and 23.26%, respectively), which is in agreement with reports from other FGs (Naro-Maciel *et al.*, 2012; Proietti *et al.*, 2012). Nevertheless, the noticeably greater incidence of males from this NS is noteworthy, and it may be a result of the large relative proportion of CM-A5 haplotype in male samples (24.5%) since this haplotype is predominant in Surinam (Bjorndal *et al.*, 2006; Shamblin *et al.*, 2012). Sex ratios in Surinam have also been reported to be slightly more balanced (68.4%) in relation to some of the largest female-biased beaches in Ascension Island, although it can vary throughout the same nesting season (Godfrey *et al.*, 1996). Our results are concordant with a larger output of males from this NS and seem to reinforce the hypothesis that Surinam could be an important source of males for green turtle FGs in the SWA, although more studies are still needed.

Male-producing NSs are sparsely scattered around the globe and most NSs of green and other sea turtles usually exhibit a prevalence of females (for a detailed discussion see Hays *et al.*, 2014). In fact, one of the largest green turtle NS in the world, the northern Great Barrier Reef, was recently revealed to be producing over 86.8% females for the past two decades due to increased sand temperatures (Jensen *et al.*, 2018). This raises concerns about population status and sea turtles' response to climate change (Laloë *et al.*, 2020), particularly on beaches where eggs are already incubated above the pivotal temperature, and where effects of a warming climate can be even more accentuated. With the imminent rise of global temperatures which, under different scenarios, can increase over 2.0°C above current temperatures until 2100 according to data from the Intergovernmental Panel on Climate Change (IPCC, 2014), production of females in sea turtle NSs is expected to rise as well, which may promote the feminization of some populations (Hays *et al.*, 2014; Jensen *et al.*, 2018).

The extent to which this feminization process would affect sea turtle populations is still not completely clear (Hays *et al.*, 2017). Undoubtedly, factors like extremely high incubation temperatures and changes in sea surface temperature will likely affect the population dynamics of sea turtles (Hamann *et al.*, 2013). However, some studies suggest that the higher reproduction frequency of males and higher availability of nesting females could, to a certain degree, compensate for female-skewed primary sex ratios, at least while complete feminization is not reached (Hays *et al.*, 2017; Tomillo and Spotila, 2020). Additionally, local environmental features such as the presence of vegetation and sand colour seem to play a role in the regulation of nest temperatures (Patrício *et al.*, 2019) and can be valuable tools for the management of populations.

Finally, besides the direct effect of rising temperatures on sex determination of sea turtles, a warming climate also poses other challenges, such as (i) the sea level rise, which can compromise nesting activities by reducing the total area available for nests; (ii) increased frequency of lethal incubation temperatures; and (iii) changes in sea surface temperature and ocean pH that can also affect sea turtles and associated marine communities, such as coral reefs (Fuentes *et al.*, 2010; Hoegh-Guldberg *et al.*, 2019).

Moreover, other anthropogenic pressures, such as pollution of marine and coastal environments, must also be taken into account as they can act synergistically as threats to sea turtle populations (Fuentes *et al.*, 2011). Thus, conservation actions that include identification and protection of male-producing rookeries, protection of coastal environments, and ultimately direct management of nests could help mitigate some of these harmful effects.

Concluding remarks

Studies distinguishing female and male sea turtles are rare because determining the sex of individuals is not always possible. Yet, these studies provide insights on current population dynamics and help developing efficient conservation plans (Jensen *et al.*, 2018). Here, we provided a spatiotemporal evaluation of green turtle sex ratios in FGs in the SWA revealing that a general pattern of female-biased sex ratios has likely been prevalent during the last decades.

We also provided the first independent evaluation of natal origins of female and male green turtles from a feeding ground in the SWA. We were able to detect slightly divergent natal origins in females and males and found a high influence of Guinea Bissau supporting previously proposed transatlantic migrations from this NS in Africa to FGs in the SWA. Furthermore, a prevalence of males from Guinea Bissau and Surinam highlighted the importance of these sites from a conservation standpoint. Nevertheless, more data are still needed to shed more light on the implications of climate change on the population dynamics of green turtles.

Supplementary material

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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Data availability

Raw data used in sex ratio analyses is detailed in supplementary material. All haplotypes used in genetic analysis were previously described by other studies and are available in GenBank.

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