



Spatio-temporal variation in the incubation duration and sex ratio of hawksbill hatchlings: Implication for future management



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ABSTRACT

Climate change poses a unique threat to species with temperature dependent sex determination (TSD), such as marine turtles, where increases in temperature can result in extreme sex ratio biases. Knowledge of the primary sex ratio of populations with TSD is key for providing a baseline to inform management strategies and to accurately predict how future climate changes may affect turtle populations. However, there is a lack of robust data on offspring sex ratio at appropriate temporal and spatial scales to inform management decisions. To address this, we estimate the primary sex ratio of hawksbill hatchlings, *Eretmochelys imbricata*, from incubation duration of 5514 in situ nests from 10 nesting beaches from two regions in Brazil over the last 27 years. A strong female bias was estimated in all beaches, with 96% and 89% average female sex ratios produced in Bahia (BA) and Rio Grande do Norte (RN). Both inter-annual (BA, 88 to 99%; RN, 75 to 96% female) and inter-beach (BA, 92% to 97%; RN, 81% to 92% female) variability in mean offspring sex ratio was observed. These findings will guide management decisions in Brazil and provide further evidence of highly female-skew sex ratios in hawksbill turtles.

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1. Introduction

Predicted increases in temperature (IPCC et al., 2013) pose a unique threat to species, such as marine turtles, that have temperature dependent sex determination (TSD), where increases in temperature can result in extreme sex ratio biases (Mitchell and Janzen, 2010). A forced bias in sex ratio can alter reproductive factors (e.g. intra- and intersexual competition, sperm competition, and multiple paternity) and possibly lead to reduced rates of fertilization and loss of genetic variation, thereby jeopardizing the ability of turtle populations to adapt to climate change (Hamann et al., 2010; Wibbels, 2003). Concern over the potential impacts from increased temperature on turtle species with TSD has generated a call for more research that describes and predicts the sex ratios of various populations (e.g., Fuentes et al., 2010; Hamann et al., 2010; Hawkes et al., 2007).

Knowledge of the natural sex ratios of populations with TSD improves our understanding of population viability and demography and also provides a baseline in advance of climate change, all of which are key for conservation planning (Maffucci et al., 2013; Mrosovsky, 1994; Mrosovsky et al., 2009; Zbinden et al., 2007). However, many TSD offspring sex ratio studies published to date generally are focused on limited spatio-temporal scales, which is inadequate to accurately understand the variability of offspring sex ratios within and between seasons and nesting grounds at a population scale (Fuller et al., 2013; Godfrey and Mrosovsky, 1999). Fuller et al. (2013) recommended that offspring sex ratio estimates are most informative when based on at least 6 years of data from multiple beaches that cover a range of physical aspects; yet most published studies do not meet this standard.

The lack of robust data on offspring sex ratio can be attributed largely to the difficulties associated in classifying sex of marine turtle hatchlings. Marine turtle hatchlings lack sexually dimorphic external characteristics (Mrosovsky et al., 1999b), and therefore the most reliable method for sexing hatchlings is through the histological examination of gonadal tissue (Mrosovsky et al., 2009; Wibbels, 2003; Yntema and Mrosovsky, 1980). However, this

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technique requires the sacrifice of hatchlings, which is difficult to justify for endangered species (Fuller et al., 2013; Rebelo et al., 2012). As a result, the estimation of clutch sex-ratios has been based in indirect methods, where researchers use temperature or incubation period of clutches to estimate sex ratio (e.g. Glen and Mrosovsky, 2004; Hawkes et al., 2007; Marcovaldi et al., 1997). The latter technique is based upon the negative relationship between nest temperature and incubation duration (Mrosovsky et al., 1999b). One benefit of using incubation period to indirectly estimate hatchling sex ratios is that these data are often available on larger spatio-temporal scales, because most conservation projects record them. A drawback is that the relationship between sex ratio and incubation period may become uncoupled in some nests, especially those producing mixed-sex offspring (Mrosovsky et al., 2009; Rebelo et al., 2012). Further, the variability in the period between hatching and nest emergence can add error to estimation of sex ratio using incubation duration. Nevertheless, the relationship remains robust when eggs are incubated at temperatures that produce strongly biased sex ratios (Mrosovsky et al., 2009), and if samples are large, some approximate estimates

can be obtained because sampling errors should cancel each other out (Mrosovsky et al., 2009). Nevertheless many studies have used incubation duration to infer the sex ratio of marine turtle clutches (see Godley et al., 2001; Marcovaldi et al., 1997; Zbinden et al., 2007).

The majority of studies to date on the sex ratios of hatchling marine turtles report a female-biased sex ratio (for reviews see Hawkes et al., 2009; Wibbels, 2003). Research conducted in the 1990s indicated that for hawksbill, *Eretmochelys imbricata*, hatchlings produced at key nesting grounds in Brazil, more than 90% were females (Godfrey et al., 1999). Godfrey et al. (1999) estimated hawksbill hatchling sex ratio, based on incubation durations, pivotal temperature, and pivotal incubation duration, which were determined by the study. Since these studies were conducted, the thermal profile and incubation duration of hawksbill nests in Brazil have not been revisited. Consequently, there is no information on whether the extremely biased sex ratios previously found at these hawksbill nesting sites have changed over time and whether this female-bias is mirrored in other important but unstudied nesting areas in Brazil. To address these knowledge

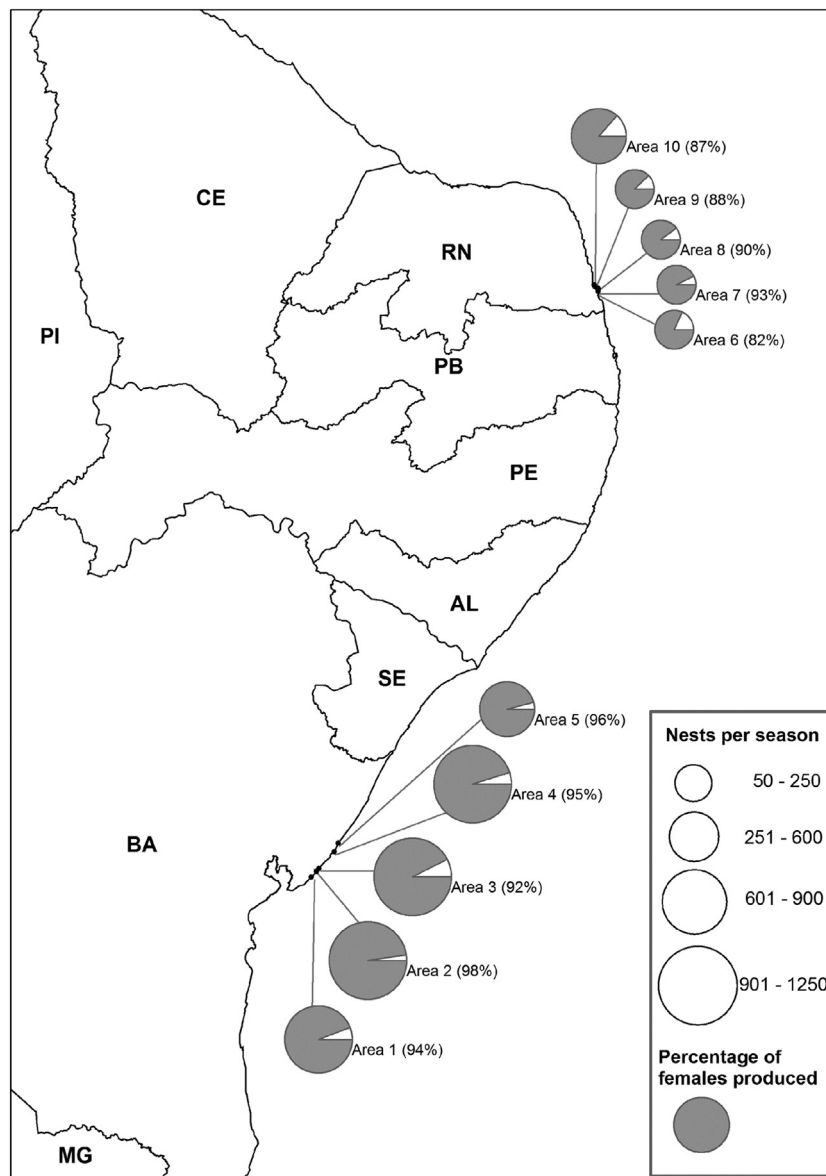


Fig. 1. Study sites with estimated proportion of females (grey) and male (white) hawksbill hatchlings in each area. Pie diameter is scaled according to magnitude of clutches laid each year in each area.

gaps, we expand on the earlier study and estimate sex ratios from incubation duration at 10 hawksbill nesting areas on a broader temporal scale (up to 27 nesting seasons, depending on nesting areas) and a wider spatial extent that includes two subpopulations of hawksbill turtles in Brazil. To better inform future assessments of impacts of climate change on these two subpopulations, we explored the variability of sex ratio produced between nesting beaches and years.

2. Material and methods

2.1. Study species and location

Two major hawksbill nesting sites have been identified on the coast of Brazil: northern Bahia (BA) and southern Rio Grande do Norte (RN) (Marcovaldi et al., 2007), which represent the spatial extent of our study (Fig. 1). Nesting grounds in these two regions are part of the Southwest Atlantic Hawksbill Regional Management Unit (RMU) (Wallace et al., 2010), although turtles nesting in each region are genetically distinct and represent two subpopulations (Vilaça et al., 2013). The majority of hawksbill nests in Brazil are laid in BA. Nesting in BA occurs mostly during the austral summer, generally from November to March, with an average of 1300 nests per season (Marcovaldi et al., 2007). A high proportion of hawksbill turtles nesting in BA are hybrids (42% with loggerheads and 2% with olive ridley) (Lara-Ruiz et al., 2006). Although fewer hawksbill turtles nest in RN (approximately 760 nests per season), the region has the highest density of hawksbill clutches per kilometre in the South Atlantic, with 48.5 nests per km/per season in some areas (Santos et al., 2013). Nesting in RN occurs mostly between November and May (Santos et al., 2013). No hybrids have been detected in this subpopulation (Vilaça et al., 2013).

Data on incubation durations were collected from 5 nesting locations for each region (Fig. 1 and Table 1); beach descriptions can be found in Marcovaldi and Laurent (1996), Marcovaldi et al. (2007), and Santos et al. (2013). Nesting locations were selected on the basis of being important nesting sites and of having distinct characteristics from each other (e.g. sand colour and grain size). All sites are intensive study areas and are patrolled daily during the nesting season by TAMAR staff (for information on TAMAR see Marcovaldi and dei Marcovaldi, 1999). During patrols, all freshly laid nests were marked; nests were checked again towards the expected end of the incubation period and excavated the morning after emergence when all eggs were examined to determine species and hatching success. Incubation duration was calculated

as the time in days between laying (the date of the morning when a freshly laid nest was first found) and the date of the morning of the first day when hatchling emerge from the nest. The temporal scale of available incubation duration data varies across the different regions as a reflection of TAMAR's work in the area (for a summary see Table 1).

2.2. Estimating sex ratios from incubation duration

The relationship between incubation duration and sex ratio derived from constant temperature laboratory experiments for hawksbill turtle eggs from BA (Godfrey et al., 1999) was used to convert nest incubation duration (Table 1) into nest hatchling sex ratio after adding 4 days to account for the hatch–emergence interval that occurs in naturally incubating marine turtle nests (see Godfrey et al., 1999). To calculate the overall sex ratio for each season and year we took into account the number of clutches deposited at half-month periods within the nesting season (as per Godfrey et al., 1999; Godley et al., 2001; Marcovaldi et al., 1997).

3. Results

3.1. Incubation duration

Incubation duration values were available for 5514 in situ clutches that successfully hatched (Table 1). The majority of nests incubated in less time than the pivotal incubation duration (62.9 days, see Godfrey et al., 1999), ranging from 45–70 in BA (mean: 55 ± 4 SD days) and from 49–70 (mean: 57 ± 3 SD days) in RN (Figs. 2 and 3A), with eggs in RN generally taking longer to incubate. Incubation duration was significantly different across nesting beaches (One-way ANOVA, $DF=4606$, $F=50.91$ (BA), $DF=908$, $F=11.56$ (RN), $P < 0.05$). In BA, eggs took the longest time to hatch in Imbassaí to Sauipe (range 45–66, mean 55 ± 3 SD days) and the shortest time to hatch in Santa Maria (range 47–68, mean 53 ± 3 SD days). Santa Maria presented the most distinct incubation durations, being significantly shorter to all other nesting in Bahia beaches ($N=4609$, Tukey–Kramer HSD post-hoc test, $q = -2.18$, $q = -1.33$, $q = -1.15$, $q = -1.90$, $P < 0.05$ respectively). In RN, Sibauma had the longest incubation duration (range 51–70, 59 ± 4 SD days) and Minas had the shortest incubation durations (range 50–64, 56 ± 3 SD days). Minas was found to be significantly different from most of the other beaches in RN (Cacimbinhas, Madeiro and Sibauma) ($N=908$, Tukey–Kramer HSD post-hoc test, $q = -1.42$, $q = -1.56$, $q = -2.31$, $p < 0.05$ respectively).

Table 1
Incubation duration data available from each study location.

Region	Area	Beach	Beach length (km)	Seasons of data	Years monitored	Records of nests
Bahia (BA)	Area1	Busca Vida	6	18	95/96–12/13	801
	Area 2	Santa Maria	6	27	86/87–12/13	928
	Area 3	Arembepe to Berta	11	18	95/96–12/13	1203
	Area 4	Praia do Forte	14	27	86/87–12/13	1089
	Area 5	Imbassaí to Sauipe	11	18	95/96–12/13	585
Total BA						4606
Rio Grande do Norte (RN)	Area 6	Sibauma	1.2	10	03/04–12/13	70
	Area 7	Minas	2.3	10	03/04–12/13	238
	Area 8	Chapadão	0.4	10	03/04–12/13	69
	Area 9	Madeiro	2	10	03/04–12/13	188
	Area 10	Cacimbinhas	3	10	03/04–12/13	343
Total RN						908
Grand total						5514

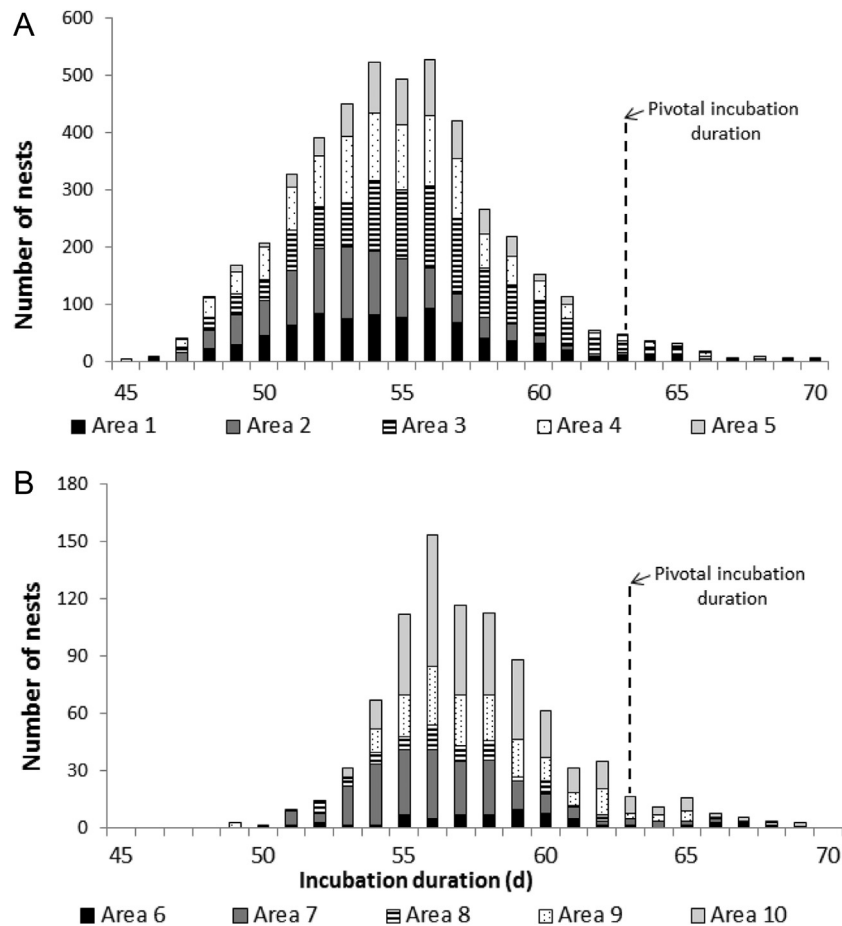


Fig. 2. Incubation period of hawksbill turtle clutches at nesting beaches in A) Bahia (1986/87 to 2012/13) and B) Rio Grande do Norte (2003/04-2012/13).

No significant increase or decrease was observed with annual mean incubation durations over time for either BA or RN (Fig. 3A); however there was an observed increase in maximum incubation duration, of about 10 days, and a decrease in minimum incubation duration, of about 2 days, between 1986 and 2012 at BA.

3.2. Sex ratio estimates

A strong female bias was estimated at all beaches, with $96\% \pm 13$ SD female offspring in BA and $89\% \pm 20$ SD in RN, (Fig. 3B). On average, all 10 study beaches produced highly biased ($> 80\%$) female sex ratios, although it should be noted that there was both inter-annual (BA, 88 to 99%; RN, 75 to 96% female) and inter-beach (BA, 92% to 97%; RN, 81% to 92% female) variability in mean offspring sex ratios (Figs. 1 and 3B). Beaches in RN generally produced a lower proportion of female hatchlings than beaches in BA (Fig. 1), with Sibauma producing the lowest proportion of female hatchlings (81%) of all the beaches studied. Inter-monthly variation in mean offspring sex ratio was also observed, with higher proportion of male hatchlings being produced from nests laid towards the end of the nesting season (from March onwards) (Fig. 4).

4. Discussion

We estimate that hawksbill nesting beaches in Bahia continue to produce highly female biased hatchling sex ratios ($> 90\%$ female) and that important nesting beaches in Rio Grande do

Norte (previously not studied) also produce predominately female hatchlings. By expanding our offspring sex ratio estimates to a wider temporal (up to 27 years, see Table 1) and spatial scale (10 nesting beaches, over 57 km) than the previously conducted study in Bahia by Godfrey et al. (1999), we were able to further explore the temporal trends in sex ratio production, how representative our results are (e.g., whether it accounted for typical and extreme years) and to identify the variability of sex ratio at multiple beaches. Despite the long temporal coverage of our dataset (27 nesting seasons at some beaches), we found no apparent trend, from potential changes in climate, in incubation duration. However, despite mean incubation duration being similar among years, there was an increase in the maximum incubation duration of nests observed in Bahia and Rio Grande do Norte, increasing the range of incubation durations observed over a given season. Exploring the reasons behind longer maximum incubation duration was outside the scope of this project and prompts further study; however it could be a result of several factors including: 1) some turtles nesting earlier and later in the season when temperatures are cooler (see Santos et al., 2013, Supplementary material 2) some turtles selecting cooler regions to nest within a beach, and 3) bigger fluctuations in temperatures during the nesting season. A slight increase in incubation duration of loggerhead nests at beaches in Cyprus was also observed between 2000 and 2006, however no apparent reason for this was given (Fuller et al., 2013).

Mean offspring sex ratios varied across the different nesting beaches, with beaches in Bahia generally producing a higher proportion of females than those in Rio Grande do Norte. This

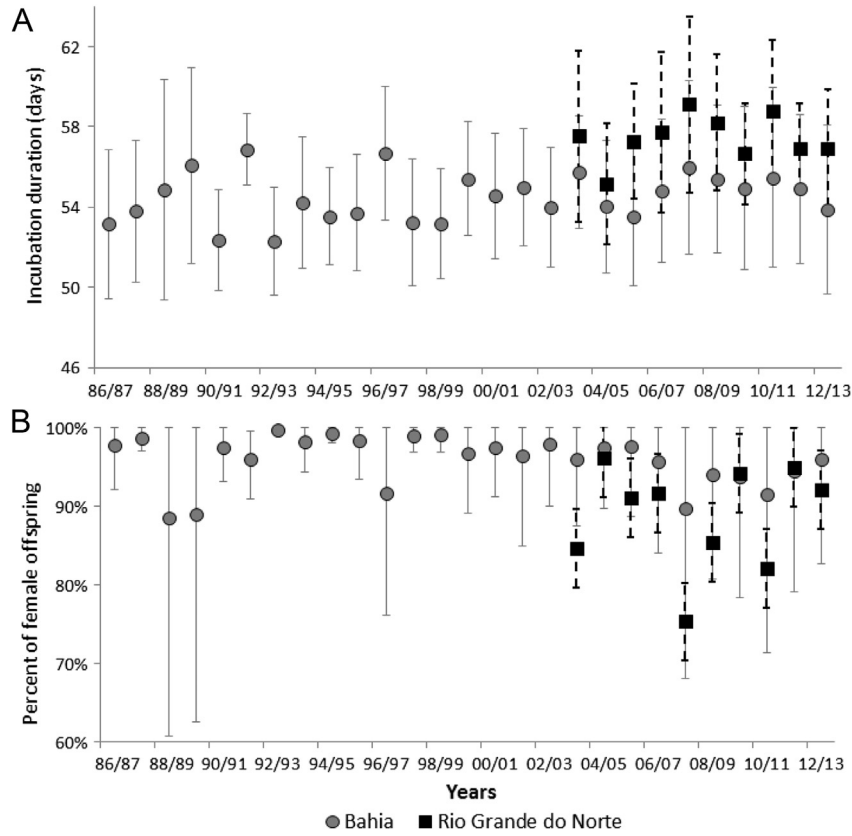


Fig. 3. Inter-annual A) mean (\pm SD), incubation duration of hawksbill clutches, and B) mean percentage of females produced (\pm SD) at Bahia and Rio Grande do Norte from the combined dataset as estimated using the curve from Godfrey et al. (1999).

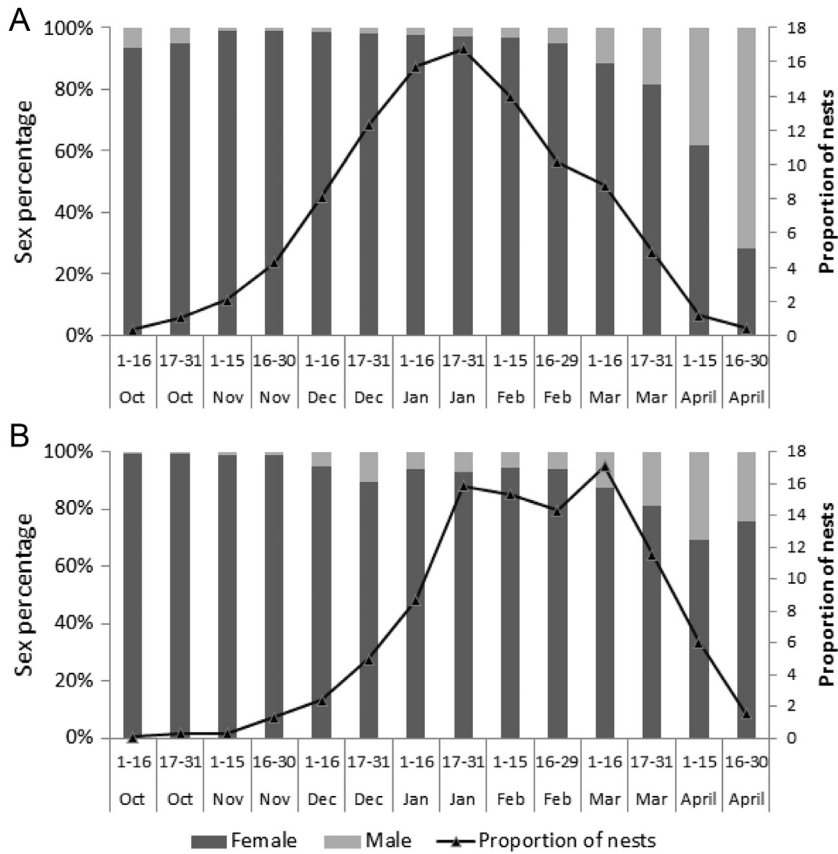


Fig. 4. Sex ratios as % female (bars) and percentage of hawksbill turtle nests per half month relative to the season's total (line) during all nesting seasons from A) 1986/87–2012/13 in Bahia and B) from 2003/04 to 2012/13 in Rio Grande do Norte.

difference might be a result of a few factors: 1) the majority of hawksbill eggs in Bahia are exposed to warmer temperatures since peak nesting in this region occurs earlier in the year coinciding with warmer summer months, and 2) nesting in Bahia generally occurs in open areas exposed to the sun (Serafini et al., 2009), while the areas studied in Rio Grande do Norte are shaded by cliff structures and have a small beach area where nests are closer to the high water mark (Santos et al., 2013). By determining the sex ratio at multiple beaches we were able to identify those that currently produce more male hatchlings in BA (Imbassaí to Sauipe) and RN (Sibauma), which may require specific conservation initiatives in the future to ensure continued production of male hatchlings (Baptistotte et al., 1999; Zbinden et al., 2007). However, our study indicates that the difference between the areas producing the most and least proportion of males was not high (10% in RN and 5% in BA, with RN producing in average 5% more male hatchlings than BA). This suggests that future studies should aim to identify other beaches in each region that are not currently major nesting areas but might be of importance for male hatchling production in the future if sand temperatures become warmer. Similarly, conservation efforts should ensure that clutches laid at the beginning and end of the nesting season are suitably protected, as these clutches tend to produce a higher proportion of rarer male hatchlings. Finally, while there is evidence that at Brazilian rookeries hawksbill turtles prefer to nest in vegetation, more research is needed to understand the influence that different physical aspects of the beach (such as albedo, orientation, vegetation) have on nest temperature and therefore hatchling sex ratio at these rookeries.

Identifying and improving the protection and population levels of male-producing beaches has been highlighted as high priority to mitigate potential impacts from projected increases in temperature (Fuentes et al., 2012). More active strategies such as shading, hatcheries, re-vegetation and water sprinkling, have also been identified as potential mitigation strategies (Fuentes et al., 2012; Patino-Martinez et al., 2012; Wood et al., 2014). However, even though nest shading has shown to decrease nest temperature and thus reduce female bias at some marine turtle rookeries (Patino-Martinez et al., 2012; Wood et al. 2014) – but see Jourdan and Fuentes (2013). It is suggested that such strategies should only be implemented when impacts are extreme (near zero male production and/ or high levels of mortality due to extreme temperatures, e.g.,) and we have a better knowledge of how effective they are (Fuentes et al., 2012; Hawkes et al., 2009; Jourdan and Fuentes, 2013). Selection of sex ratio manipulation strategies and their implementation will be guided by our understanding of what is the “desired” sex ratio of hatchlings produced at each area; this will be determined by information on historical sex ratio production, the current sex ratio entering the population and the operational sex ratio for that population (Jourdan and Fuentes, 2013). In Bahia, for example, open air hatcheries located in various nesting sites are used to protect nests that would not otherwise survive. The thermal condition of hatcheries generally matches with those of natural nesting beaches and produce sex ratios similar to what is produced in situ (Naro-Maciel et al., 1999). In the future, as temperatures increase and female bias becomes extreme, the thermal condition of these hatcheries could be actively manipulated to ensure production of hatchling sex ratios similar to historical ones (Wood et al., 2014).

Despite the importance of estimating natural sex ratios to provide a baseline in advance of climate change and to assess and select management practices, there is a lack of robust data on offspring sex ratio at appropriate temporal and spatial scales to inform management decisions. Hawksbill sex ratios have been less extensively studied than for other species of marine turtles, such as loggerheads (Kamel and Mrosovsky, 2006). Indeed, to date, less

than 10 published studies have estimated the sex of hawksbill hatchlings at nesting grounds (see Dobbs et al., 2010; Glen and Mrosovsky, 2004; Godfrey et al., 1999; Kamel and Mrosovsky, 2006; Mrosovsky et al., 1992; Wibbels et al., 1999) of these only Godfrey et al. (1999) estimated hatchling sex ratio across a variety of beaches for multiple years. The lack of robust data on offspring sex ratio can be attributed to the difficulties and ethical constraints associated in sexing marine turtle hatchlings. As a result, this study, like many others, based its estimates of clutch sex-ratios on an indirect measure (e.g. incubation duration see Glen and Mrosovsky, 2004; Hawkes et al., 2007; Marcovaldi et al., 1997). Although these methods have been validated for loggerhead marine turtles (see Mrosovsky et al., 1999a), more validation work is needed. Recently, Mrosovsky et al. (2009) suggested that incubation temperature during the thermo-sensitive period of sexual differentiation is a better estimator of hatchling sex ratios for hawksbill marine turtles. Nevertheless, that same study showed that for nests with short incubation periods (< 55 days), there was strong concurrence between sex ratios estimated from incubation period vs. histology. Another possible limitation in our study is that the relationship between sex ratio and incubation duration was based on only two clutches from Bahia (see Godfrey et al., 1999). Although it is possible that there is variation in pivotal temperatures (and hence pivotal incubation durations) for the population of hawksbill turtles in Bahia, and that these are different in Rio Grande do Norte, little variation has been found generally in pivotal temperatures of marine turtles (Mrosovsky, 1994). Variation in the estimates of sex ratio presented here could also exist as an artefact of changes in how many days it takes from when eggs hatch to hatchling emergence. Because it is estimated to take on average 4 days for hatchling marine turtles to emerge from the nest after hatching (Godfrey and Mrosovsky, 1997), we used a 4 day correction factor to the sex incubation curves generated from laboratory data (as per Godfrey et al., 1999). However, if the average hatching-to-emergence interval for hawksbill turtles in Bahia or Rio Grande do Norte is actually shorter than 4 days, then we underestimated the number of males hatchlings produced. This highlights the need for more sex ratio data based on direct sexing methods, regardless of the ethical and legal impediments, and we encourage more work to be done in this area. Nevertheless, these types of limitations in the present study would likely account for small variations from the estimated sex ratio, meaning that the predicted sex ratio of turtle hatchlings produced in the most important hawksbill nesting grounds in Brazil are extremely female-biased. Extreme female bias highlights the need for continued monitoring of the thermal profile and sex ratio being produced at these regions and for identification of areas that will have suitable incubating environment and produce high proportion of males in the future.

5. Conclusion

Hawksbill nesting beaches, in Brazil, have produced extremely female biased (> 90%) hatchlings for at least 27 years. The implication from this extreme bias in female production is unknown. Unfortunately, no data on the sex ratio of adult population and paternity studies exists to explore whether this pattern is reflected in the adult population. Thus, the full impact of feminization of turtle nesting grounds is not fully understood (Hamann et al., 2013). Some nesting beaches have persisted with strong female biases over a few decades or even longer (Hays et al., 2003; Marcovaldi et al., 1997) and there is no evidence that a low production of male hatchlings has resulted in a low reproductive success within populations (Broderick et al., 2000). To fully understand the implications of predicted extreme female biases in

hatchling production in marine turtle populations it is necessary to know the lower viable limits of the male to female ratio (Lasala et al., 2013). These limits are typically deduced by knowing marine turtles' effective population sizes (total number of individuals that pass on their genes to the next generation) and/or their operational sex ratios (Fuller et al., 2013). Insights into these indices can be obtained by understanding turtles' mating systems, since male breeding periodicity may be shorter than that of females and males court with females that nest on sites spread throughout the range of nesting sites for a particular population (Lasala et al., 2013; Wright et al., 2012). An understanding of the "optimal" and "historical" sex ratios will help identify the "desired" sex ratio, which will be necessary to guide future management strategies.

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