Incubation duration and predicted hatchling sex ratios of leatherback turtles (*Dermochelys coriacea*) in the southwestern Atlantic Ocean (1988–2021)

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A R T I C L E   I N F O

A B S T R A C T

Knowledge of sex ratios of species with temperature-dependent sex determination (TSD) is key to provide baseline information which can be used to inform management strategies and predictions of how climate change can affect populations. In marine turtles, increased incubation temperatures can lead to extreme biases in sex ratios and reduced hatching success. Here we present a long-term analysis (34 years) of incubation durations of leatherback turtles (*Dermochelys coriacea*) in Brazil, the only population of this species breeding in the Southwest Atlantic, and estimate offspring sex ratios. We deployed data loggers recording nest temperatures in 2015, 2016, 2017 and 2019 ($n = 28$ clutches), to predict offspring sex ratios based on incubation temperatures during the thermosensitive period when sex is determined. The overall mean incubation duration for leatherback turtle clutches in Brazil (1988–2021) was 66.3 days (range 52–91, SD = 6.4, $n = 867$), decreasing by 4.4 days between the first and last 10 years of monitoring and varying latitudinally across the nesting range of the population. When modelled to the overall nesting season and accounting for nesting seasonality, we estimated the current (2012–2021) mean season-wide primary sex ratio to be 46.9 % female (range 32.7 % to 84.8 %). Hindcasting for the first ten years of monitoring (1988–1997) showed the average predicted offspring sex ratios would have been 34.6 % female (range 7.7 % to 68.1 %). This population has not shown a phenological shift in the timing of nesting over the period 1988–2021. These findings suggest that, although the primary sex ratio of this population has likely become more female-biased in recent decades, the spatial and temporal variation observed demonstrates some resilience to the effects of increasing temperatures under climate change.

1. Introduction

Knowledge of primary sex ratios is key to assessing population viability and resilience (Melbourne and Hastings, 2008; Mitchell et al., 2010). In species with temperature-dependent sex determination (TSD), offspring sex is determined by the incubation temperature during the thermosensitive period (TSP; Bull, 1983, Vetere et al., 2025). Temperature is known to be the main environmental driver of sexual differentiation in many reptiles (Wyneken and Lolavar, 2015), including alligators (Yatsu et al., 2015), most turtles (Pieau et al., 1994) and some lizards (Charnier, 1966). In marine turtles, all species exhibit TSD (Yntema and Mrosovsky, 1980; Ackerman, 1997), with a higher proportion of females produced above the pivotal temperature, tipically around 29 °C. The adaptive significance of TSD is not fully understood, and theoretical models suggest that such environmental sex determination would be favoured by selection over genotypic sex determination

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(chromosome-based) when the environment during development distinctly influences fitness of females and males (Charnov-Bull model; Charnov and Bull, 1977, Warner and Shine, 2008). A more recent study suggests that TSD could be selected over genotypic sex determination simply if there are sex differences in age at maturity (Schwanz et al., 2016).

Primary sex ratio in marine turtles is determined by the incubation temperature, and thus the factors that influence this parameter play a central role in the dynamics of marine turtle populations (Hays et al., 1999). Extreme temperatures could result in the production of hatchlings of a single sex, making marine turtles particularly vulnerable to the impacts of projected global warming (Janzen, 1994; Poloczanska et al., 2009; Fuentes et al., 2010; Mitchell and Janzen, 2010; Fuentes et al., 2011). Most studies at marine turtle rookeries have estimated female-biased hatchling sex ratios, which are expected to be exacerbated under current predictions of climate change (Hawkes et al., 2007; Patino-Martinez et al., 2012; Fuentes and Porter, 2013; Hays et al., 2014; Marcovaldi et al., 2016). Few studies have reported male-biased or balanced primary sex ratios (Patrício et al., 2017; Laloë et al., 2020), and highlight the importance of these rookeries for the future conservation of marine turtles. It is yet uncertain how well marine turtles will be able to adapt to the pace of future climate change. Highly female-skewed sex ratios being produced across several generations could lead to adaptive responses which would allow population persistence, or lead to population extinction (Mitchell and Janzen, 2010). Many marine turtle populations have been reported to have increasing or stable population trends (Mazaris et al., 2017), many recovering from past over-exploitation (McClenachan et al., 2006). However, impacts of climate change may act in combination with other threats, such as fisheries bycatch, pollution and coastal development (Soykan et al., 2008; Wallace et al., 2010; Duncan et al., 2017), hampering population growth (Saba et al., 2012).

To understand the potential impacts from climate change and increased temperatures on species with TSD, more research is required to describe and predict the primary sex ratios of marine turtle populations (e.g. Hamann et al., 2013). Whilst there are a growing number of studies involving TSD and marine turtles (reviewed by Patrício et al., 2021), there are still several basic and applied research questions concerning TSD which remain unanswered for leatherback turtles (*Dermochelys coriacea*). Few studies of this species have considered long-term data to reconstruct past sex ratios, with most research using data that spans no more than one or two seasons (but see Sieg et al., 2011, Santidrían Tomillo et al., 2015). Studies describing the TSD curve and presenting pivotal temperatures and the range of intermediate temperatures producing both sexes, called the transitional range of temperatures (TRTs), are described from only three leatherback turtle rookeries: French Guiana in the Atlantic Ocean (Rimblot-Baly et al., 1987), Costa Rica in Eastern Pacific (Binckley et al., 1998) and Malaysia in the Western Pacific (Chan and Liew, 1995). Recent studies have highlighted that research into thermal conditions in nests from additional rookeries is needed to understand TSD in leatherback turtles (Binckley and Spotila, 2015).

Marine turtles have a wide geographical distribution, however the pivotal temperature (where a 1:1 sex ratio is produced) is believed to be relatively conserved among species and geographic locations, ranging from 28 °C to 30 °C (Mrosovsky, 1994; Wibbels et al., 1998; Godley et al., 2002). For leatherback turtles, studies have shown a conservative range of pivotal temperatures (within 29.4–29.8 °C) in the Atlantic Ocean, Eastern Pacific and Western Pacific. Chevalier et al. (1999) suggested that the pivotal temperatures in the Atlantic and East Pacific rookeries were not significantly different, but the TRT was significantly narrower for the French Guiana population, with other research showing that TRT becomes wider in cooler areas (Bentley et al., 2020). In a more recent paper, Girondot et al. (2024) found that sex-ratio reaction norms do not vary among leatherback RMUs, further supporting the idea of conserved pivotal temperatures across populations.

Leatherback turtle nesting populations exhibit considerable variation in primary sex ratios across the globe, although there are few studies. In the Pacific Ocean, populations are generally characterized by a strong female bias, with an estimated 83.2 % of hatchlings being female in Costa Rica between 1998 and 2007 (Sieg et al., 2011). In contrast, a well-documented nesting colony in Suriname, located in the western Atlantic Ocean, was found likely to be producing moderately female-biased sex ratios two decades ago, ranging from 60.5 % to 69.4 % female (Godfrey et al., 1996; Mrosovsky et al., 1984).

In Brazil, leatherback turtles nest mainly along the northern coast of Espírito Santo state, in eastern Brazil (Thomé et al., 2007; Colman et al., 2019). Nesting also occurs, in smaller numbers, on the northeast coast, in Piauí (Magalhães et al., 2021) and sporadic nesting occurs along a large range of the Brazilian coastline (Soto et al., 1997; Barata and Fabiano, 2002; Loebmann et al., 2008; Bezerra et al., 2014; Gandu et al., 2014). The rookery in Espírito Santo is a unique Regional Management Unit (Wallace et al., 2023), genetically distinct from other rookeries in the Atlantic (Dutton et al., 2013; Vargas et al., 2017) and is currently classified as Critically Endangered by the IUCN (Wallace et al., 2013). In Brazil, the species is included on the federal government's official list of endangered fauna (Machado et al., 2008). Despite its small population size - on average < 100 clutches laid per year (Colman et al., 2019) – this rookery is key, since it is located on the southernmost end of the species range in the Western Atlantic Ocean. Studies have investigated the sand temperatures and the primary sex ratios of loggerhead turtles (*Caretta caretta*) being produced in Espírito Santo and highlight the importance of these male-producing beaches for the species (Baptistotte et al., 1999; Marcovaldi et al., 2016). Considering the predicted scenarios of climate change, this region may also become increasingly important for leatherback turtles, as other areas may become extremely female-biased or too hot to sustain hatchling production (Hays et al., 2017).

To date, no studies have investigated the offspring sex ratios produced by the southwest Atlantic leatherback turtle population. Gonadal histology is currently the only reliable method to determine hatchling sex, however this method raises ethical concerns for studies of very small population units. Incubation duration has often been used as an alternative to infer hatchling sex ratios, as the metric is highly correlated with incubation temperature (Marcovaldi et al., 1997; Marcovaldi et al., 2016; Fuentes et al., 2017). A study by Thomé et al. (2007) presented the temporal and geographical incubation duration parameters for this population between 1988 and 2003, when the incubation duration was found to be on average 67.8 days, varying significantly among years and latitudinally. This study did not, however, estimate hatchling sex ratios. Here we build on this dataset, provide further ecological information regarding this parameter, and explore whether it has changed over time. We used indirect estimation methods based on the temperature-sex ratio relationship determined in the laboratory for leatherback turtles (Binckley et al., 1998). Finally, we discuss the resilience of this rookery to predicted rising temperatures and the conservation implications for the population.

2. Methods

2.1. Study site and nest monitoring

The state of Espírito Santo is located on the coast of Brazil between latitudes 19.6667°S and 18.4167°S (Fig. 1a). The leatherback turtle nesting areas comprise 160 km of dynamic, high-energy beaches, with coarse sand influenced by discharge from the Doce River in its southern extent (Fig. 1b). There is no natural shading along the beach and virtually no human occupation or anthropogenic structures. The area is divided in kilometres, being monitored from south to north. Most nests (~ 80 %) are laid on the southernmost 80 km between September and March, with the main nesting activity concentrated between October and January and hatching occurring from November to March (Colman et al., 2019). As nesting seasons span two calendar years, we refer to a
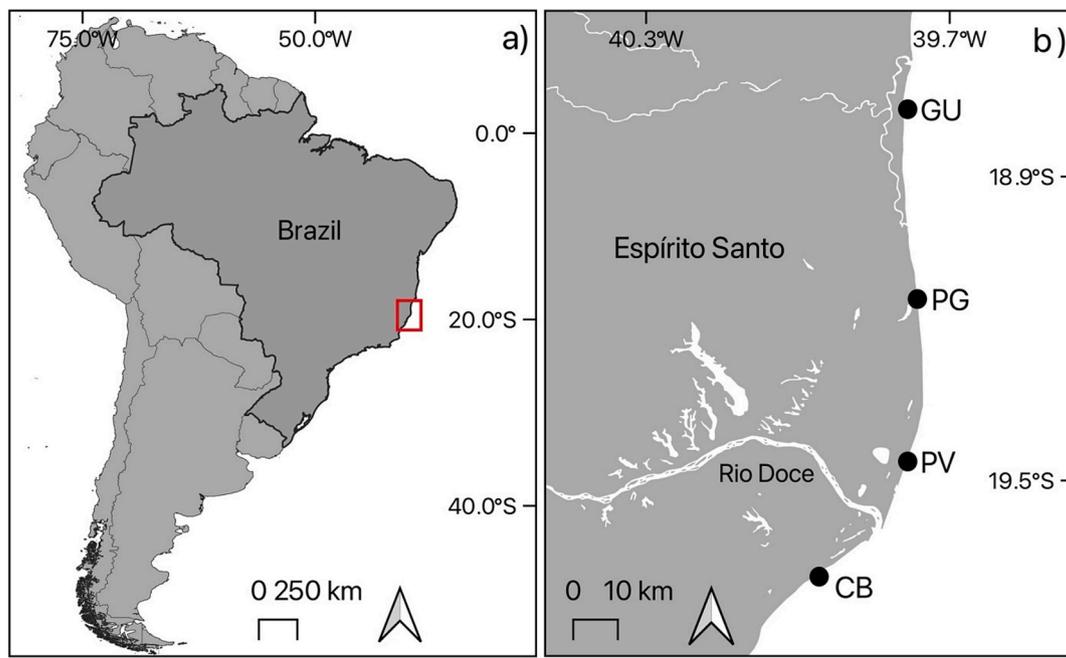
Fig. 1. **(a)** Map of Brazil: the leatherback turtle nesting areas in Espírito Santo state are depicted by the red frame. **(b)** Map of the coast of Espírito Santo state, Brazil. Black circles represent the TAMAR stations where the data were collected. From south to north: CB = Comboios, PV = Povoação, PG = Pontal do Ipiranga, GU = Guriri. Rio Doce = Doce River. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

season as occurring in the first of those two years, e.g. the season 2005–2006 is called the 2005 season. Leatherback turtle nesting activity on northern Espírito Santo has been monitored since 1982, systematically since 1988 (Marcovaldi and Marcovaldi, 1999; Thomé et al., 2007). Early-morning patrols of the 160 km area using quadbikes are conducted daily from 1st September to 31st March. Nest locations are recorded and marked with a numbered wooden stake, monitored during the entire incubation period, and excavated after most hatchlings have emerged. Incubation duration (ID) was calculated as the number of days between oviposition and the emergence of the first hatchlings (Thomé et al., 2007). The climate in the area is predominantly tropical with hot and rainy summers and the dune vegetation mainly includes low-lying grasses and bayhops *Ipomea pes-caprae* (Baptistotte et al., 2003).

2.2. Incubation duration analysis

Only in situ nests laid between 1988 to 2021, where both oviposition and emergence dates were recorded, were used in this analysis. During that period, 22.3 % of nests were translocated, however those translocated nests were not included in the incubation duration analysis as translocation can impact this metric (Pintus et al., 2009). A Generalized Additive Model (GAM) was chosen to analyse the variation of incubation duration over the years due to its flexibility in modelling non-linear relationships. GAMs can handle non-parametric data and do not assume a specific functional form, making them particularly suitable given the small sample sizes and potential non-linearity in temporal trends. We used the R-package *mgcv* (Wood, 2017) for all analysis. A Kruskall-Wallis test was employed to test for differences in incubation duration among years, since the data were non-parametric. The Kruskall-Wallis test is a rank-based method that is well-suited for detecting differences across groups when data distributions are skewed or contain outliers (Hollander and Wolfe, 1999). Loess regressions were used to analyse the relationships between incubation duration, nest latitude, and day in the season because this method is effective for capturing localized, non-linear patterns in data (Cleveland et al., 1993). Loess is particularly appropriate for exploring ecological relationships where the data may vary smoothly but unpredictably across gradients, such as spatial (latitude) or temporal (seasonal) scales. Estimates are presented as mean ±

SD unless stated otherwise. To analyse the relationship between the different parameters and environmental variables, we extracted air temperature from the European Centre for Medium-Range Weather Forecasts (ECMWF) climate reanalysis v5 (ERA5; Hersbach et al., 2020) for the Southwest Atlantic region. Air temperature was selected as a key variable because of its known influence on sea turtle nesting dynamics, particularly incubation duration and hatchling sex ratios. To explore these relationships, we focused on mean December temperatures, as this month contains the highest proportion of incubating clutches, making it the most representative period for examining temperature impacts. Linear models were used to assess the relationships between mean December air temperature and each parameter—mean incubation duration, annual onset of nesting (defined as the 5th percentile of lay dates to avoid the influence of sporadic early nesters), and year. Linear models were chosen due to their ability to quantify the strength and direction of these relationships, offering a clear and interpretable framework for evaluating potential trends. Additionally, correlation tests were employed to further explore associations between these variables, providing a complementary non-parametric assessment of their interdependence. This dual approach ensures robust and transparent insights into the connections between environmental factors and reproductive parameters.

2.3. Field data

During the 2015, 2016, 2017 and 2019 nesting seasons, a sample of 28 clutches were individually monitored to gather baseline data on clutch temperature, incubation duration and hatching success. In each monitored clutch, hourly nest temperatures were recorded with a temperature datalogger (Tinytag, TGP 401, TGP 4005, Gemini Dataloggers Ltd., Chichester, UK; ± 0.2 °C accuracy, 0.1 °C resolution). Data loggers were deployed in the centre of the clutch during oviposition, at beaches on the southernmost 60 km of the study area (Comboios and Povoação beaches, Fig. 1b) and retrieved post-hatching upon nest excavation. The initial four hours of temperature records were not included, to enable data loggers to equilibrate with the surrounding clutch contents (Broderick et al., 2001). All data loggers were compared to a calibrated datalogger in a constant temperature room. For each nest we also

recorded clutch size (by counting eggshells and unhatched eggs, with shelled albumin gobs (SAGs) frequently found in leatherback turtle nests, counted separately from yolked eggs and not considered in clutch size) and estimated hatching success (percentage of yolked eggs that produced live hatchlings, including live hatchlings encountered in the nest during excavation and those that had pipped but not emerged from the egg shell; Thomé et al., 2007).

2.4. Estimating primary sex ratios in study years

We estimated the sex ratio thermal reaction norm (i.e., the temperature response curve for offspring sex ratios calculated using constant temperature during incubation) using data from constant temperature experiments for leatherback turtles conducted in the Atlantic Ocean (Rimblot-Baly et al., 1987) and Eastern Pacific (Binckley et al., 1998) (Supplementary Figure S1). We used logistic models fitted using maximum likelihood (Abreu-Grobois et al., 2020), implemented with the R package *embryogrowth* (Girondot, 2022). The nest temperature profiles from in situ monitored nests were used to estimate sex ratios. We accounted for the nonlinear development within incubation (Fuentes et al., 2017) by calculating a Constant Temperature Equivalent (CTE), which is the mean temperature weighted by the differential embryo growth during the TSP (Monsinjon et al., 2019). The association between clutch size (independent variable) on (1) mean TSP temperature and (2) hatching success (response variables) were analysed. Prior to analysis, the normality of the response variables (mean TSP temperature and hatching success) was assessed using the Shapiro-Wilk test. Results indicated that neither variable followed a normal distribution. Consequently, Spearman's rank correlation, a non-parametric method robust to non-normality, was used.

2.5. Reconstructing current nest temperatures

Daily nest temperatures were reconstructed for Espírito Santo between January 1988 and June 2021 using a correlative approach with sea surface temperature and air temperature (Girondot and Kaska, 2015; Monsinjon et al., 2017b; Laloë et al., 2020). Sea surface and air temperatures were extracted from the ECMWF climate reanalysis v5 (ERA5; Hersbach et al., 2020) for the Southwest Atlantic region. A linear mixed-effect model was fitted to the nest temperature data using the R package *nlme* (Pinheiro et al., 2022), with an ARMA correlation structure and nest identity as random effect. To estimate metabolic heating (i.e., the difference in nest temperature compared to the surrounding sand), we used the proportion of incubation time as an additional predictor. This produces a proxy for metabolic heating specific to this nesting site (Monsinjon et al. (2017a) for details). The best model was selected based on the lowest AICc (Burnham and Anderson, 2002) from models using a daily lag with air or sea temperature varying from 0 (i.e., synchronous relationship with nest temperature) to 5 days (i.e., lagged relationship with nest temperature at day+5). We used the standard deviation of the coefficients of the random effect as a proxy of nest thermal heterogeneity (TH) at the nesting beach scale (Monsinjon et al., 2019). Data was checked for normality and the coefficients of each predictor (sea surface temperature, air temperature and proportion of incubation time) were estimated for the selected model within a standard generalized linear model framework and with Gaussian link function. Daily thermal fluctuations were reconstructed by calculating daily maximum and minimum temperatures as the average daily temperature ± half of the average daily thermal amplitude (calculated as the difference between observed daily maxima and minima). These reconstructed maximum and minimum temperatures were then assigned to the average times of day when daily maxima and minima typically occur, respectively. Metabolic heating (MH), attributed to the heat produced by the incubation of marine turtle clutches (Broderick et al., 2001) was also accounted for. Specific parameters used to predict nest temperatures are presented in Supplementary Table A1 (see also Supplementary Note A1)

and model results are presented in Supplementary Table A2. This modelling approach allowed for an accurate prediction of nest temperature (Supplementary Note A1, Supplementary Figure S2).

2.6. Modelling embryonic development

Embryo development and the dates of each embryonic stage (Miller 1985) across the nest temperature time series were estimated using the thermal reaction norm of embryonic growth rate and a growth function of incubation time (Supplemental Figure S3; Girondot and Kaska, 2014, Fuentes et al., 2017, Monsinjon et al., 2017b, Girondot et al., 2018). Those were implemented with the R package *embryogrowth* (Girondot, 2022). We used hatchling measurements from the literature (mean SCL = 59.2 ± 2.4 mm; Banerjee et al., 2020), assumed a Gompertz model for embryo growth and estimated model parameters using maximum likelihood (Girondot and Kaska, 2014). To compute confidence intervals, we identified the posterior distributions using Bayesian MCMC with the Metropolis-Hasting algorithm on 10,000 iterations, assuming a uniform distribution for priors. To ensure an optimal acceptance rate across iterations, we applied the adaptive proposal distribution procedure described in Rosenthal (2011) and implemented in the R package *HelpersMG* (Girondot, 2022). Once calibrated, we ran the embryonic growth model along reconstructed nest temperatures using the function *MovingIncubation()* from the R package *embryogrowth* (Girondot, 2022) to estimate, for any given day a clutch would be laid, the duration of incubation (i.e., when embryo size reaches hatchling size), the point of the thermosensitive period of development (TSP) for sex determination (Girondot et al., 2018), the CTE during the TSP and the sex ratio. The function considers metabolic heating (MH) and temperature heterogeneity (TH). CTE was not estimated for nests with reconstructed temperatures since reconstructed TSP temperatures are more prone to uncertainties, because they also depend on the accuracy of the model used to reconstruct nest temperature.

2.7. Predicting season-wide sex ratios

To scale up incubation temperatures and predicted sex ratios at the scale of a clutch to the whole nesting season, we estimated the overall nesting dynamics based on series of nest counts. We used the general nesting curve, where p is the proportion of nests laid on each day of the season/year, with credible intervals, calculated from the period between 2015 and 2019. The curve was fit using the model described in Omeyer et al. (2022). We calculated the season-wide Sex Ratio (SR) as being the mean sex ratio during a nesting season weighted by the proportion of nests laid (Eq. (1)).

$$SR = \sum_{i=k}^{N} SR_i \times Prop.Nest_i \tag{1}$$

SR_i is the predicted sex ratio (male proportion) for nests laid at the day i of the season with k being the first date of the season. Prop.Nest$_i$ is the proportion of nests laid at the day i of the season. N is the last date of the season. The relationship between the annual predicted sex ratio and the December air temperature (chosen as explained in the section Methods – incubation duration analysis) for each year was analysed using a linear model and a correlation test.

3. Results

3.1. Incubation duration

The overall mean incubation duration for in situ clutches between 1988 and 2021 (34 years) was 66.3 ± 6.4 days (range 52–91, $n = 867$ clutches, Fig. 2, 3a). Mean annual incubation durations ranged from 61.5 days (in 1994, $n = 4$ and 2015, $n = 49$) to 78 days (in 1988, $n = 1$; Supplemental Table A3). Incubation duration was significantly different among years (Kruskal-Wallis test, $n = 867$, $p < 0.001$) with a significant
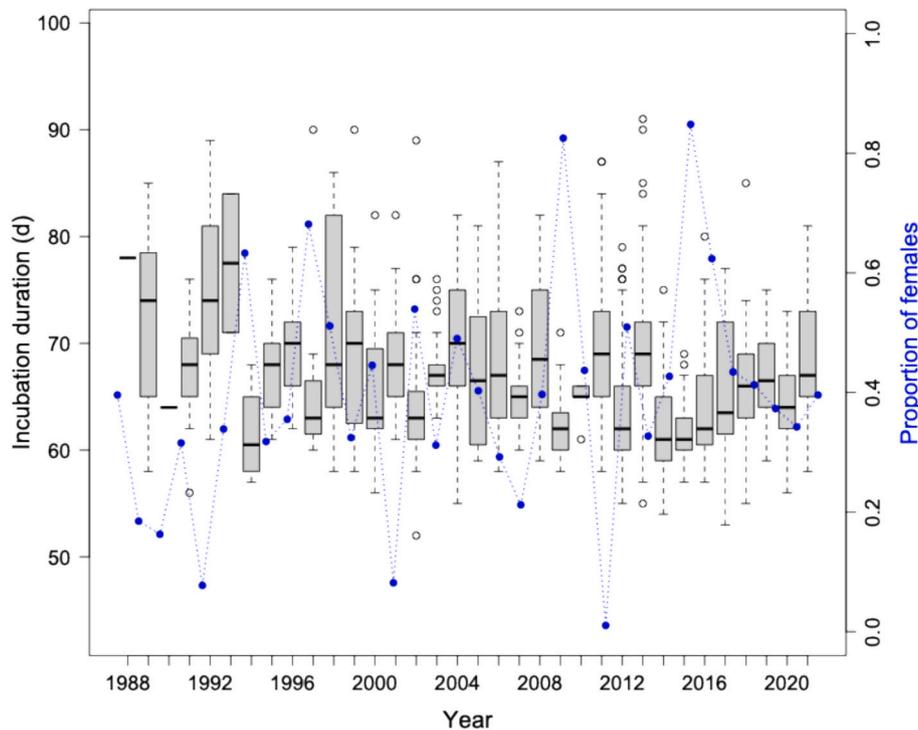
Fig. 2. Incubation duration (days) of in situ leatherback turtle nests from Espírito Santo, Brazil (1988–2021). The black vertical lines represent median values (50th percentile). The grey boxes contain the 25th to 75th percentiles of dataset. The black whiskers mark the 5th and 95th percentiles, and values beyond these upper and lower bounds are considered as outliers, represented by black open circles. The mean annual predicted proportion of females is shown by blue filled circles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

decline across the 34 years (F = 11.96, p < 0.001, n = 867; Fig. 3b). The annual mean incubation duration was 69.5 ± 7.4 days (range 56–76, *n* = 75) during the first ten years (1988–1997), and 65.1 ± 5.7 days (range 53–91, *n* = 505) during the last ten years (2012–2021) of the study period. The incubation duration varied significantly with the day in the season, as no horizontal line (representing a constant mean incubation duration in the period) can be placed inside the 0.95 simultaneous confidence band in Fig. 3c. Considering the geographic location (latitude) of nests, the incubation duration significantly increased with latitude (considered from south to north), as no horizontal line (representing a constant mean incubation duration in the area) can be placed inside the 0.95 simultaneous confidence band in Fig. 3d.

3.2. Nest temperatures and hatching success

For clutches with temperature data loggers (*n* = 28), the mean growth-weighted TSP temperature (CTE) varied between 28.4 °C in 2019 and 30.3 °C in 2015. (Table 1). The CTE varied slightly with the date during the nesting season, being relatively constant until mid-November, increasing until mid-December and then decreasing (Supplementary Figure S4). Clutch size (72.0 ± 13.6 eggs, range = 36–94, *n* = 28; showed a weak positive relationship with CTE (Spearman's ρ = 0.2, *p* = 0.3), indicating that clutch size was found to be a poor predictor of CTE. Hatching success in nests with monitored temperature varied between 38.3 and 98.3 %, with a mean of 74.9 ± 18.7 % and we found no significant relationship with clutch size ($F_{1,26}$ = 2.0, *p* = 0.2, *n* = 28). Hatching success was higher when CTEs were between 30 and 30.5 °C (Supplemental Figure S5), although the relationship with clutch size was weak and not significant (Spearman's ρ = −0.1, *p* = 0.5, *n* = 28).

3.3. Predicted sex ratios

The mean seasonal predicted primary sex ratio (proportion of females) for nests with monitored temperature varied between 0.9 in 2015

and 0.1 in 2019 (Table 1). The season starts with nests that are likely to be 100 % males, peaks when approximately 60 % males are expected, and ends when nearly 100 % females are expected (Fig. 4). When accounting for nesting seasonality, we estimated the season-wide leatherback turtle mean sex ratio to currently be 46.9 % female (during the period between 2012 and 2021, representing the current scenario (most recent ten years of monitoring), varying between 32.7 % females in 2013 and 84.8 % in 2015. When hindcasting to the period between 1988 and 1997 (first ten years of monitoring), the overall predicted sex ratio would have been 34.6 % female, varying between 7.7 % female in 1992 and 68.1 % female in 1997.

There has not, however, been phenological adaptation at this site. The annual median day of nesting showed no significant trend (Supplemental Figure S6; linear regression, $F_{1,32}$ = 0.53, R^2 = −0.02, *P* = 0.5), similarly to the onset of nesting (5th percentile ordinal day) (Supplemental Figure S6; linear regression, $F_{1,32}$ = 0.72, R^2 = −0.01, *P* = 0.4). There was also no significant trend of nesting season duration (days elapsed between first and last nest) throughout the study period (Supplemental Figure S6; linear regression, $F_{1,32}$ = 3.52, R^2 = 0.07, *P* = 0.07).

December mean air temperature was significantly positively correlated with the proportion of female offspring produced (Supplemental Figure S7a; Pearson's coefficient of correlation = 0.75, *t* = 6.36, df = 31, *p* < 0.001;), significantly negatively correlated with the incubation duration (Supplemental Figure S7b; Pearson's coefficient of correlation = −0.66, *t* = −4.94, df = 31, p < 0.001) and not significantly correlated with the annual onset of nesting (Supplemental Figure S7c; Pearson's coefficient of correlation = −0.17, *t* = −0.97, df = 31, *p* = 0.34). The mean December air temperature showed an increasing trend throughout the period (Fig. 5; linear regression, $F_{1,31}$ = 4.60, R^2 = 0.10, *P* = 0.04).

4. Discussion

Small populations are of conservation concern; however, it can be
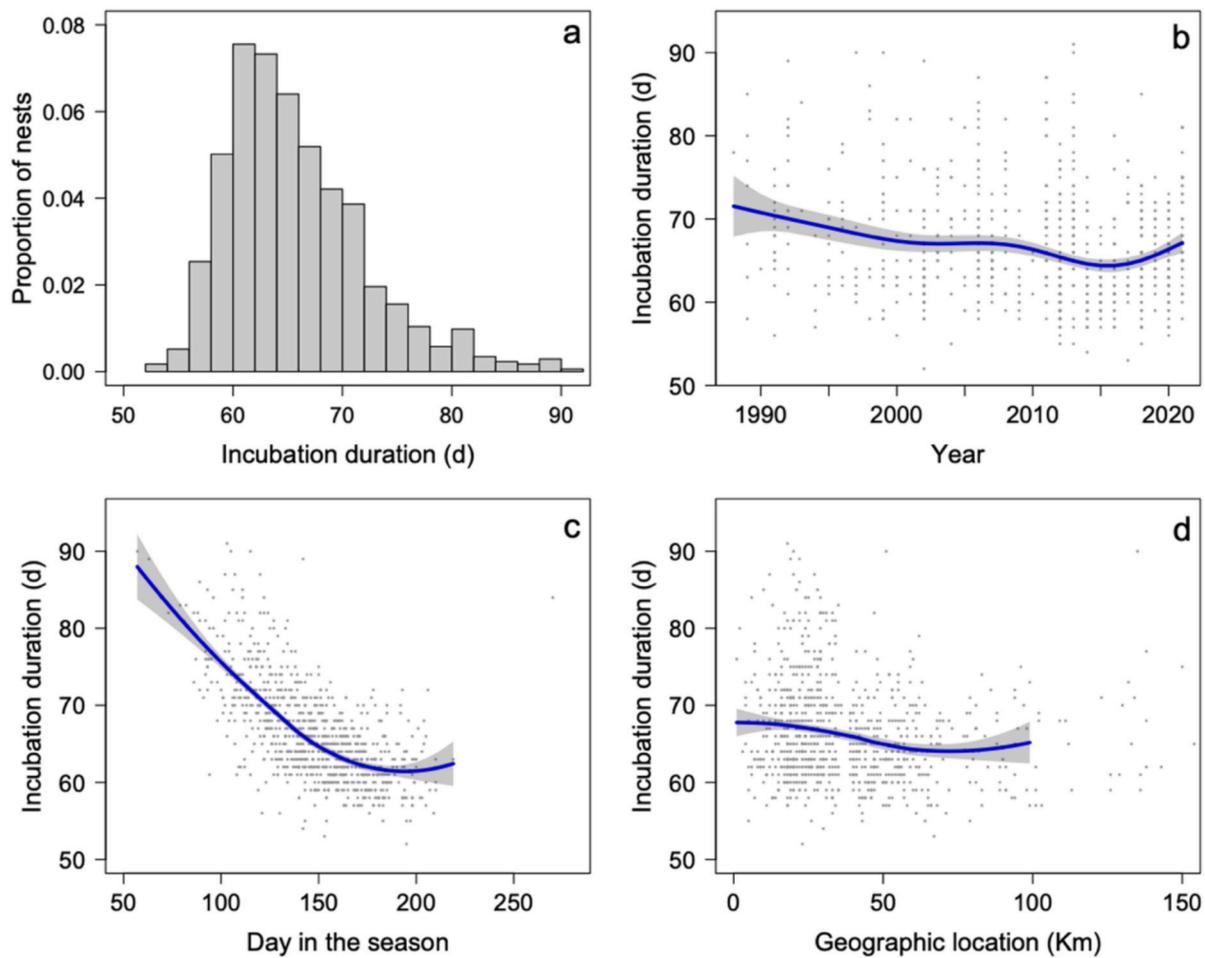
Fig. 3. Incubation duration (days) of in situ leatherback turtle nests from Espírito Santo, Brazil (1988–2021). **(a)** Proportion of nests and incubation duration ($n = 867$). **(b)** Incubation duration by year, 1988–2021 ($n = 867$). The blue curve is a GAM regression, and the grey area delimits a 0.95 simultaneous confidence band. In the graph, to increase clarity, the data points were jittered on the y-scale. **(c)** Incubation duration by day in the season (July 1 = day 1). **(d)** Incubation duration by geographical location. Km 0 is the southernmost point of the study area. In c-d) The blue line curve is a loess regression, grey shaded show approximate pointwise 95 % confidence intervals. These are truncated so as only to include categories with sufficient data points c) $n = 866$; d) $n = 836$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Summary growth-weighted TSP temperatures and estimated primary sex ratios of leatherback turtle nests with monitored temperature per year in Espírito Santo, Brazil, between 2015 and 2019. TSP = thermosensitive period. Mean annual sex ratio is weigthed by the proportion of nests.

| Year	Growth-weighted TSP Temperature		Sex ratio (female proportion)		
	Range	Mean ± SD	Range	Mean (2.5th – 97.5th percentiles)	n
2015	29.5–31.7	30.3 ± 0.6	0.4–1.0	0.9 (0.5–1.0)	19
2016	28.5–31.1	29.6 ± 1.3	0.0–1.0	0.4 (0.0–1.0)	5
2017	28.1–29.5	28.8 ± 1.0	0.0–0.5	0.2 (0.0–0.4)	2
2019	27.4–29.3	28.4 ± 1.4	0.0–0.2	0.1 (0.0–0.2)	2
Overall	27.4–31.7	29.9 ± 1.0	0.0–1.0	0.7 (0.0–1.0)	28

challenging to research key life-history parameters due to limited data volume and concerns regarding invasive research methods. Here we use long-term leatherback turtle nesting data to gain insights into predicted hatchling sex ratio production at the main nesting ground for the

Critically Endangered Southwest Atlantic Ocean leatherback turtle subpopulation (Wallace et al., 2013). Our major findings offer clear insights that lead to recommendations on how to augment this work going forward to better understand the scope of population recovery, resilience and potential for adaptation in the face of climate change.

Leatherback turtle nesting populations worldwide are believed to vary greatly in the primary sex ratios produced. While the populations in the Pacific Ocean produce overall female-biased sex ratios (83.2 % females estimated in Costa Rica from 1998 to 2007, Sieg et al., 2011), a well-studied leatherback turtle nesting colony in the western Atlantic Ocean, Suriname, when researched two decades ago, was estimated to be producing modestly female-biased sex ratios (60.5–69.4 % females, Godfrey et al., 1996, Mrosovsky et al., 1984). Our estimated female hatchling sex ratio of 46.9 % is less female-biased than what has been reported for other locations. However, our study site in Espírito Santo, Brazil, is situated further south in latitude, where cooler temperatures are expected. This region has already been identified as an important male-producing area for loggerhead turtles (Baptistotte et al., 1999), which may similarly contribute to the less female-biased sex ratios observed in our study. Incubation studies suggest that most field temperatures produce either all females or males, given the narrow TRT of leatherback turtles (Binckley and Spotila, 2015). This could also be the case for leatherback turtles in Brazil, where most nests were estimated to have produced predominantly female or male hatchlings with few nests
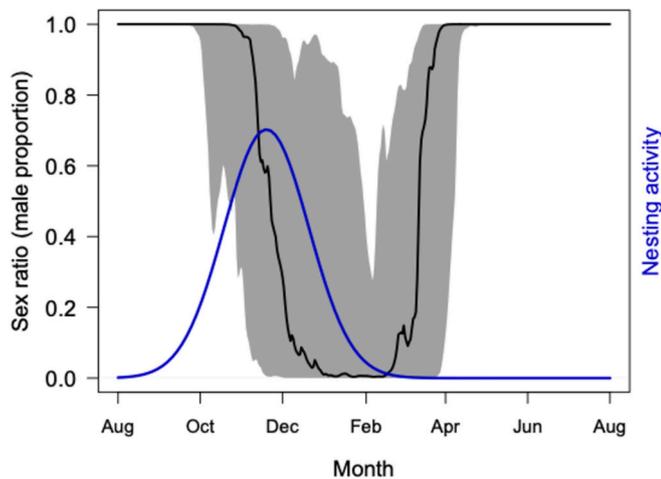
Fig. 4. Current sex ratios estimates (quantiles at 50 %, black line) throughout the year with lower and upper quantiles (at 2.5 % and 97.5 % respectively, shaded in grey) for the leatherback turtle population nesting in Espírito Santo, Brazil. Quantiles were calculated based on 1988–2020 time series. Standardized nesting activity (proportion of nests laid throughout the season based on the description of nesting activity) is indicated by the blue line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
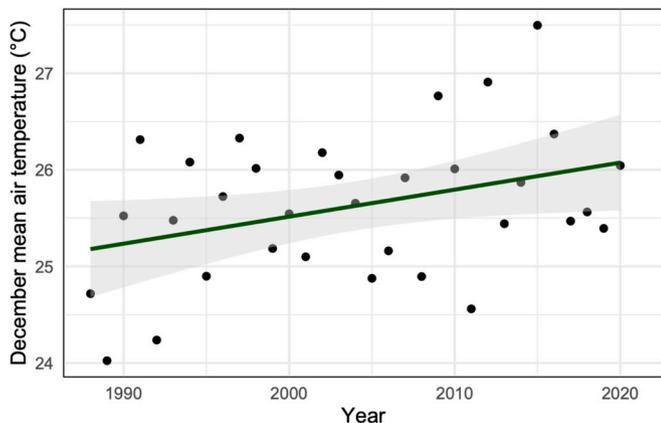
Fig. 5. Annual December mean air temperature at a leatherback nesting site, in Espírito Santo, Brazil (1988–2020). Green line: linear regression. Grey shaded areas: 95 % CI. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

producing balanced sex ratios.

The increase seen on leatherback turtle nesting numbers in Espírito Santo is encouraging (Colman et al., 2019). The decrease in mean incubation duration, together with a modestly female-biased hatchling sex ratio estimated over the decades may be influencing the recovery of this population. The female-biased sex ratios could contribute to population growth, since it would ultimately increase the number of nesting females (Hays et al., 2017; Patrício et al., 2017). A highly biased sex ratio could however decrease the effective population size (the adults that effectively contribute to the next generations).

Incubation duration varied both temporally and spatially, with the decrease in the mean annual incubation duration throughout the years being suggestive of potential future feminization of the population. The lack of a significant trend on the phenology of nesting suggests this population is not yet adjusting its time of breeding to recent increases in sea surface temperature (Neeman et al., 2015), or that population growth makes it more challenging to detect trends. The spatial variability in incubation duration indicates the potential for leatherback

turtles to use nest-site selection to produce a broader range of offspring sex ratios and consequently be more resilient to climate change (Fuentes et al., 2013; Abella Perez et al., 2016).

The seasonal decrease in incubation duration could be attributed to a seasonal increase in air temperature, as the nesting season in the region coincides with the austral summer (Colman et al., 2019), characterized by higher temperatures. The decrease in incubation durations seen towards the northern part of the nesting beach could be attributed to variations in sediment characteristics, such as colour and grain size (Hays et al., 1999; Fadini et al., 2011). Fine-grained sands have been shown to reach higher temperatures compared to coarse-grained sands (Torres-Rodríguez et al. 2019), which could lead to warmer nest temperatures and a higher proportion of females. Our monitored nests were all located in the southernmost part of the nesting beach (Comboios and Povoação), where the sand is coarse (Albino and Suguio, 2010; Fadini et al., 2011). This spatial bias in monitoring could influence our sex ratio estimates, as the relationship between air or sea surface temperatures and nest temperatures depends on sediment properties. Specifically, nests in the northern part of the beach (Pontal do Ipiranga), with fine-grained sand (Fadini et al., 2011), may produce fewer males due to higher incubation temperatures caused by sediment characteristics. This potential bias should be considered when interpreting our temperature logger estimates of female production. Additionally, other factors, such as nest moisture, may also play a role in influencing sexual determination (Wyneken and Lolavar, 2015) likely through evaporative cooling rather than a direct effect on gene expression.

Our results showed no significant relationships between clutch size and nest temperature, clutch size and hatching success, or hatching success and nest temperature. Hatching success in monitored nests varied widely, and while we observed a trend of higher hatching success when CTEs were between 30 and 30.5 °C, this relationship was not statistically significant. This suggests that although specific temperature ranges may favour hatching success, other factors could be contributing to the variability observed in our dataset. Our findings contrast with Wallace et al. (2004), who reported a significant positive correlation between clutch size and nest temperature in leatherback turtles. However, their study focused on maximum nest temperatures, which typically occur during the latter stages of incubation due to metabolic heating from developing embryos. In contrast, our study concentrated on temperatures during the thermosensitive period (TSP), occurring in the middle third of embryonic development. The focus on different temporal windows could explain the discrepancy, highlighting the importance of distinguishing between TSP temperatures and those occurring later in the incubation period when evaluating their effects on hatching success. Similarly, Fadini et al. (2011) found a significant relationship between clutch size and hatching success in loggerhead turtles in Brazil. This difference could be attributed to interspecific variations in biology, including differences in thermal tolerance, clutch size, and reproductive strategies between loggerhead and leatherback turtles. The lack of significant relationships in our study may also be due to the relatively small sample size of 28 nests, which limits statistical power and the ability to detect subtle trends. Moreover, environmental variables such as sand characteristics, humidity, or predation, which were not accounted for in our study, may further influence hatching success. Future studies incorporating larger sample sizes and accounting for a broader range of environmental factors would be valuable in providing a more comprehensive understanding of the determinants of hatching success in leatherback turtles. Lastly, while our results do not establish significant relationships, the observed trend of higher hatching success at CTEs between 30 and 30.5 °C aligns with the notion that there is an optimal thermal range for successful embryonic development. Further investigation into this trend, particularly in conjunction with environmental factors and inter-clutch variability, could yield insights into the resilience and adaptability of leatherback turtle populations under varying climatic conditions.

In the present study, we used models considering the stages of

embryonic development in response to temperature to estimate the mean growth-weighted incubation temperatures during the TSP (Girondot and Kaska, 2014; Fuentes et al., 2017; Girondot et al., 2018). While the lack of locally derived empirical data from constant temperature incubation experiments studying the effects of temperature on sex ratios and hatching success currently limits the reliability of future predictions for hatching success, the findings of Girondot et al. (2024) suggest that sex ratio reaction norms do not vary among leatherback RMUs. This implies that the absence of local data may not significantly affect the reliability of sex ratio estimates, though continued research would still be valuable for regional validation and understanding. It also challenges our ability to estimate how much of phenological change would be needed in order to keep current sex ratios considering predicted scenarios of climate change (Fuentes et al., 2023). Further research with more representative sample sizes and spanning more seasons, together with information on other important Atlantic rookeries such as Gabon, in Central Africa (Witt et al., 2009), would help us to better understand patterns of primary sex ratios for leatherback turtles across the Atlantic. Furthermore, novel methods to estimate the sex of hatchlings based on blood samples (Tezak et al., 2020) are promising and should contribute to more reliable long-term sex ratio predictions.

Better knowledge of beach temperatures and records of incubation duration contribute to an increase in our understanding of natural sex ratios for marine turtle populations and have implications for conservation practices (Mrosovsky and Yntema, 1980), such as the potential effects of nest relocation. Relocating nests can influence nest temperatures, potentially altering the incubation process, embryonic development and the resulting sex ratios (Sieg et al., 2011). While this practice may impact temperature-sensitive outcomes, it remains a valuable strategy for Critically Endangered populations like the one in this study, as it helps reduce the loss of egg clutches and supports population recovery. Future studies should evaluate the impacts of nest relocation on nest temperatures, sex ratios and hatching success to better understand the impacts of this management strategy over the nesting population (e. g Sieg et al., 2011) and better inform the use of this management strategy and optimise its benefit for population conservation.

In conclusion, future research should focus on addressing key gaps in understanding leatherback turtle population dynamics and resilience to climate change. Expanding studies on primary sex ratios with larger, regionally representative datasets and incorporating novel sexing techniques like blood-based methods would improve the accuracy of predictions and long-term monitoring. Investigating spatial and temporal variability in nesting conditions, particularly the role of environmental factors like sand characteristics and moisture, could clarify their influence on incubation temperatures and sex ratios. Comparative studies across Atlantic rookeries and modelling the potential for phenological shifts in response to warming would provide critical insights into adaptive strategies. Together, these efforts would enhance our understanding of population recovery and inform conservation practices to promote resilience in the face of ongoing climatic changes.

CRediT authorship contribution statement

Liliana P. Colman: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Cecília Baptistotte:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Brendan J. Godley:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **João C.A. Thomé:** Writing – review & editing, Supervision, Project administration, Data curation, Conceptualization. **Ana C. Marcondes:** Methodology, Data curation. **Jonathan R. Monsinjon:** Writing – review & editing, Methodology, Formal analysis. **Alexsandro Santos:** Writing – review & editing, Methodology, Data curation. **Ana Rita Caldas Patrício:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis,

Conceptualization. **Gustavo Stahelin:** Writing – review & editing, Project administration, Data curation. **Annette C. Broderick:** Writing – review & editing, Supervision, Project administration, Methodology, Formal analysis, Conceptualization.

Ethical standards

Declaration of competing interest

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jembe.2025.152100.

Data availability

The authors do not have permission to share data.

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