

1 **Incubation duration and predicted hatchling sex ratios of leatherback turtles**  
2 **(*Dermochelys coriacea*) in the Southwestern Atlantic Ocean (1988-2021)**

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17 **Running page head:** Leatherback turtle offspring sex ratios

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19

20 **ABSTRACT**

21 Knowledge of sex ratios of species with temperature-dependent sex determination (TSD) is key to  
22 provide baseline information which can be used to inform management strategies and predictions of  
23 how climate change can affect populations. In marine turtles, increased incubation temperatures can  
24 lead to extreme biases in sex ratios and reduced hatching success. Here we present a long-term  
25 analysis (34 years) of incubation durations of leatherback turtles (*Dermochelys coriacea*) in Brazil,

26 the only population of this species breeding in the Southwest Atlantic, and estimate offspring sex  
27 ratios. We deployed data loggers recording nest temperatures in 2015, 2016, 2017 and 2019 ( $n = 28$   
28 clutches), to predict offspring sex ratios based on incubation temperatures during the thermosensitive  
29 period when sex is determined. The overall mean incubation duration for leatherback turtle clutches  
30 in Brazil (1988-2021) was 66.3 days (range 52-91,  $SD = 6.4$ ,  $n = 867$ ), decreasing by 4.4 days between  
31 the first and last 10 years of monitoring and varying latitudinally across the nesting range of the  
32 population. When modelled to the overall nesting season and accounting for nesting seasonality, we  
33 estimated the current (2012-2021) mean season-wide primary sex ratio to be 66.5% female (range  
34 55.4% to 90.2%). Hindcasting for the first ten years of monitoring (1988-1997) showed the average  
35 predicted offspring sex ratios would have been 58.4% female (range 41.3-79.9). This population has  
36 not shown a phenological shift in the timing of nesting over the period 1988-2021. These findings  
37 suggest that, although the primary sex ratio of this population has likely become more female-biased  
38 in recent decades, the spatial and temporal variation observed demonstrates resilience to the effects  
39 of increasing temperatures under climate change.

40

41 **Keywords:** incubation duration, sex ratios, thermal ecology, reptile, *Dermochelys coriacea*

42

## 43 INTRODUCTION

44 Knowledge of primary sex ratios is key to assessing population viability and resilience (Melbourne  
45 and Hastings 2008, Mitchell et al. 2010). In species with temperature-dependent sex determination  
46 (TSD), offspring sex is determined by the incubation temperature during the thermosensitive period  
47 (TSP; Bull 1983, Binckley et al. 1998). Temperature is known to be the main environmental driver  
48 of sexual differentiation in many reptiles (Wyneken and Lolavar 2015), including alligators (Yatsu et  
49 al. 2015), most turtles (Pieau et al. 1994) and some lizards (Charnier 1966). In marine turtles, all  
50 species exhibit temperature-dependent sex determination with females produced at warmer

51 temperatures (Wibbels 2003), usually above 29°C, within a thermal range of 23 - 35 °C (Ackerman  
52 1997, Godley et al. 2001, Howard et al. 2014). The adaptive significance of TSD is not fully  
53 understood, and theoretical models suggest that such environmental sex determination would be  
54 favoured by selection over genotypic sex determination (chromosome-based) when the environment  
55 during development distinctly influences fitness of females and males (Charnov-Bull model; Warner  
56 & Shine 2008). A more recent study suggests that TSD could be selected over genotypic sex  
57 determination simply if there are demographic differences in age at maturity (Schwanz et al. 2016).  
58

59 Primary sex ratio in marine turtles is determined by the incubation temperature, and thus the factors  
60 that influence this parameter play a central role in the dynamics of marine turtle populations (Hays et  
61 al. 1999). Extreme temperatures could result in the production of hatchlings of a single sex, making  
62 marine turtles particularly vulnerable to the impacts of projected global warming (Poloczanska et al.  
63 2009, Fuentes et al. 2010, Mitchell and Janzen 2010, Fuentes et al. 2011). Most studies at marine  
64 turtle rookeries have estimated female-biased hatchling sex ratios, which are expected to be  
65 exacerbated under current predictions of climate change (Hawkes et al. 2007, Patino-Martinez et al.  
66 2012, Fuentes and Porter 2013, Hays et al. 2014, Marcovaldi et al. 2016). Few studies have reported  
67 male-biased or balanced primary sex ratios (Steckenreuter et al. 2010, Esteban et al. 2016, Patrício et  
68 al. 2017, Laloë et al. 2020), and highlight the importance of these rookeries for the future conservation  
69 of marine turtles. It is yet uncertain how well marine turtles will be able to adapt to the pace of future  
70 climate change. Highly female-skewed sex ratios being produced across several generations could  
71 lead to adaptive responses which would allow population persistence, or lead to population extinction  
72 (Mitchell and Janzen 2010). Many marine turtle populations have been reported to have increasing  
73 or stable population trends (Mazaris et al. 2017), many recovering from past overexploitation  
74 (McClenachan et al. 2006). However, impacts of climate change may act in combination with other

75 threats, such as fisheries bycatch, pollution and coastal development (Soykan et al. 2008, Wallace et  
76 al. 2010b, Duncan et al. 2017), hampering population growth (Saba et al. 2012).

77  
78 To understand the potential impacts from climate change and increased temperatures on species with  
79 TSD, more research is required to describe and predict the primary sex ratios of marine turtle  
80 populations (e.g. Hamann et al. 2013). Whilst there are a growing number of studies involving TSD  
81 and marine turtles (reviewed by Patrício et al. 2021), there are still several basic and applied research  
82 questions concerning TSD which remain unanswered for leatherback turtles (*Dermochelys coriacea*).  
83 Few studies of this species have considered long-term data to reconstruct past sex ratios, with most  
84 research using data that spans no more than one or two seasons (but see Sieg et al. 2011, Santidrián  
85 Tomillo et al. 2015). Studies describing the TSD curve and presenting pivotal temperatures and the  
86 range of intermediate temperatures producing both sexes, called the transitional range of temperatures  
87 (TRTs), are described from only three leatherback turtle rookeries: French Guiana in the Atlantic  
88 Ocean (Rimblot-Baly et al. 1987), Costa Rica in Eastern Pacific (Binckley et al. 1998) and Malaysia  
89 in the Western Pacific (Chen and Liew 1995). Recent studies have highlighted that research into  
90 thermal conditions in nests from additional rookeries is needed to understand TSD in leatherback  
91 turtles (Binckley & Spotila 2015).

92  
93 Marine turtles have a wide geographical distribution, however the pivotal temperature (where a 1:1  
94 sex ratio is produced) is believed to be relatively conserved among species and geographic locations,  
95 ranging from 28°C to 30°C (Mrosovsky 1994, Wibbels et al. 1998, Godley et al. 2002). For  
96 leatherback turtles, studies have shown a conservative range of pivotal temperatures (within 29.4-  
97 29.8 °C) in the Atlantic Ocean, Eastern Pacific and Western Pacific. Chevalier et al. (1999) suggested  
98 that the pivotal temperatures in the Atlantic and East Pacific rookeries were not significantly different,

99 but the TRT was significantly narrower for the French Guiana population, with other research  
100 showing that TRT becomes wider in cooler areas (Bentley et al. 2020).

101  
102 In Brazil, leatherback turtles nest mainly along the northern coast of Espírito Santo state, in eastern  
103 Brazil (Thomé et al. 2007, Colman et al. 2019). Nesting also occurs, in smaller numbers, on the  
104 northeast coast, in Piauí (Magalhães et al. 2021) and sporadic nesting occurs along a large range of  
105 the Brazilian coastline (Soto et al. 1997, Barata and Fabiano 2002, Loebmann et al. 2008, Bezerra et  
106 al. 2014, Gandu et al. 2014). The rookery in Espírito Santo is an unique Regional Management Unit  
107 (Wallace et al. 2023), genetically distinct from other rookeries in the Atlantic (Dutton et al. 2013,  
108 Vargas et al. 2017) and is currently classified as Critically Endangered by the IUCN (Wallace et al.  
109 2013). In Brazil, the species is included on the federal government's official list of endangered fauna  
110 (Machado et al. 2008). Despite its small population size - on average < 100 clutches laid per year  
111 (Colman et al. 2019) – this rookery is key, since it is located on the southernmost end of the species  
112 range in the Western Atlantic Ocean. Studies have investigated the sand temperatures and the primary  
113 sex ratios of loggerhead turtles (*Caretta caretta*) being produced in Espírito Santo and highlight the  
114 importance of these male-producing beaches for the species (Baptistotte et al. 1999, Marcovaldi et al.  
115 2016). Considering the predicted scenarios of climate change, this region may also become  
116 increasingly important for leatherback turtles, as other areas may become extremely female-biased or  
117 too hot to sustain hatchling production (Hays et al. 2017).

118  
119 To date, no studies have investigated the offspring sex ratios produced by the southwest Atlantic  
120 leatherback turtle population. Gonadal histology is currently the only reliable method to determine  
121 hatchling sex (but see Bozak et al. (2020) for recent developments), however it raises ethical concerns  
122 for studies of very small population units. Incubation duration has often been used as an alternative  
123 to infer hatchling sex ratios, as the metric is highly correlated with incubation temperature

124 (Marcovaldi et al. 1997, Marcovaldi et al. 2016, Fuentes et al. 2017). A study by Thomé et al. (2007)  
125 presented the incubation duration parameters for this population between 1988 and 2003 but did not  
126 estimate hatchling sex ratios. Here we build on this dataset, provide further ecological information  
127 regarding this parameter, and explore whether it has changed over time. We used indirect estimation  
128 methods based on the temperature-sex ratio relationship determined in the laboratory for leatherback  
129 turtles (Binckley et al. 1998). Finally, we discuss the resilience of this rookery to predicted rising  
130 temperatures and the conservation implications for the population.

131

## 132 **METHODS**

### 133 **Study site and nest monitoring**

134 The state of Espírito Santo is located on the coast of Brazil between latitudes -19.6667 and -18.4167  
135 (Figure 1a). The leatherback turtle nesting areas comprise 160 km of dynamic, high-energy beaches,  
136 with coarse sand influenced by discharge from the Doce River in its southern extent (Figure 1b).  
137 There is no natural shading along the beach and virtually no human occupation or anthropogenic  
138 structures. Most nests (~ 80%) are laid on the southernmost 80 km between September and March,  
139 with the main nesting activity concentrated between October and January and hatching occurring  
140 from November to March (Colman et al. 2019). As nesting seasons span two calendar years, we refer  
141 to a season as occurring in the first of those two years, e.g. the season 2005-2006 is called the 2005  
142 season. Leatherback turtle nesting activity on northern Espírito Santo has been monitored since 1982,  
143 systematically since 1988 (Marcovaldi and Marcovaldi 1999, Thomé et al. 2007). Early-morning  
144 patrols of the 160 km area using quadbikes are conducted daily from 1<sup>st</sup> September to 31<sup>st</sup> March.  
145 Nest locations are recorded and marked with a numbered wooden stake, monitored during the entire  
146 incubation period, and excavated after most hatchlings have emerged. Incubation duration (ID) was  
147 calculated as the number of days between oviposition and the emergence of the first hatchlings  
148 (Thomé et al. 2007). The climate in the area is predominantly tropical with hot and rainy summers

149 and the dune vegetation mainly includes low-lying grasses and bayhops *Ipomea pes-caprae*  
150 (Baptistotte et al. 2003).

151

## 152 **Field data**

153 During the 2015, 2016, 2017 and 2019 nesting seasons, a sample of 28 clutches were individually  
154 monitored to gather baseline data on incubation temperature, duration and hatching success. In each  
155 monitored clutch, hourly nest temperatures were recorded with a temperature datalogger (Tinytag,  
156 TGP 401, TGP 4005, Gemini Dataloggers Ltd, Chichester, UK;  $\pm 0.2^{\circ}\text{C}$  accuracy,  $0.1^{\circ}\text{C}$  resolution).  
157 Data loggers were deployed in the centre of the clutch during oviposition, at beaches on the  
158 southernmost 60 km of the study area (Comboios and Povoação beaches, Figure 1b) and retrieved  
159 post-hatching upon nest excavation. The initial four hours of temperature records were not included,  
160 to enable data loggers to equilibrate with the surrounding sand (Broderick et al. 2001). All data  
161 loggers were compared to a calibrated datalogger in a constant temperature room. For each nest we  
162 also recorded clutch size (by counting eggshells and unhatched eggs, with shelled albumin gobs  
163 (SAGs) frequently found in leatherback turtle nests, counted separately from yolked eggs and not  
164 considered in clutch size) and estimated hatching success (percentage of yolked eggs that produced  
165 live hatchlings, including live hatchlings encountered in the nest during excavation; Thomé et al.  
166 2007).

167

## 168 **Incubation duration analysis**

169 Only *in situ* nests laid between 1988 to 2021, where both oviposition and hatching dates were  
170 recorded, were used in this analysis. During that period, 22.3% of nests were translocated, however  
171 those translocated nests were not included in the incubation duration analysis as translocation can  
172 impact this metric (Pintus et al. 2009). The variation of incubation duration over the years was  
173 analysed with a generalized additive model (GAM), using the R-package *mgcv* (Wood 2017). To test

174 for the difference among years, a Kruskal-Wallis test for non-parametric data was used (Hollander  
175 & Wolfe 1999). To analyse the relationship of incubation duration with geographical location of nests  
176 and the day in the season, we used loess regressions (Cleveland et al. 1993). Generalized Linear  
177 Models (GLMs) with Gaussian error structure and identity link function were used to test for the  
178 effect of clutch size (independent variable) on (1) mean TSP temperature and (2) hatching success  
179 (response variables). Estimates are presented as mean  $\pm$  SD unless stated otherwise.

180

### 181 **Reconstructing current nest temperatures**

182 Daily nest temperatures were reconstructed for Espírito Santo between January 1979 and June 2021  
183 using a correlative approach with sea surface temperature and air temperature (Girondot and Kaska  
184 2015, Monsinjon et al. 2017b, Laloë et al. 2020). Sea surface and air temperatures were extracted  
185 from the European Centre for Medium-Range Weather Forecasts (ECMWF) climate reanalysis v5  
186 (ERA5; Hersbach et al. 2020) for the Southwest Atlantic region. A linear mixed-effect model was  
187 fitted to the nest temperature data using the R package *nlme* (Pinheiro et al. 2022), with an ARMA  
188 correlation structure and nest identity as random effect. To estimate metabolic heating (i.e., the  
189 difference in nest temperature compared to the surrounding sand), we used the proportion of  
190 incubation time as an additional predictor. This produces a proxy for metabolic heating specific to  
191 this nesting site (Monsinjon et al. (2017a) for details). The best model was selected based on the  
192 lowest AICc (Burnham and Anderson 2002) from models using a daily lag with air or sea temperature  
193 varying from 0 (i.e., synchronous relationship with nest temperature) to 5 days (i.e., lagged  
194 relationship with nest temperature at day+5). We used the standard deviation of the coefficients of  
195 the random effect as a proxy of nest thermal heterogeneity at the nesting beach scale (Monsinjon et  
196 al. 2019). The coefficients of each predictor (sea surface temperature, air temperature and proportion  
197 of incubation time) were estimated for the selected model within a standard generalized linear model  
198 framework and with Gaussian link function. Daily thermal fluctuations were reconstructed by



199 computing daily maximum and minimum temperatures as the average daily temperature +/- average  
200 daily amplitude (as defined by daily maxima – daily minima). Those values were set at the average  
201 time of day when they occurred. Metabolic heating, which is the difference between nest temperature  
202 and the surrounding sand, attributed to the heat produced by the incubation of marine turtle clutches  
203 (Broderick et al. 2001) was accounted for, and daily thermal fluctuations were estimated.

204

### 205 **Modelling embryonic development**

206 Embryo development and the dates of each embryonic stage (Miller 1985) across the nest temperature  
207 time series were estimated using the thermal reaction norm of embryonic growth rate and a growth  
208 function of incubation time (Supplemental Figure S1; Girondot and Kaska 2014, Fuentes et al. 2017,  
209 Monsinjon et al. 2017b, Girondot et al. 2018). Those were implemented with the R package  
210 *embryogrowth* (Girondot, 2022). We used hatchling measurements from the literature (mean SCL =  
211  $59.2 \pm 2.4$  mm; Banerjee et al. 2020), assumed a Gompertz model for embryo growth and estimated  
212 model parameters using maximum likelihood (Girondot and Kaska 2014). To compute confidence  
213 intervals, we identified the posterior distributions using Bayesian MCMC with the Metropolis-  
214 Hastings algorithm (Chib and Greenberg 1995) on 10,000 iterations, assuming a uniform distribution  
215 for priors. To ensure an optimal acceptance rate across iterations, we applied the adaptive proposal  
216 distribution procedure described in Rosenthal (2011) and implemented in the R package *HelpersMG*  
217 (Girondot 2022). Once calibrated, we ran the embryonic growth model along reconstructed nest  
218 temperatures to estimate, for any given day a clutch would be laid, the duration of incubation (i.e.,  
219 when embryo size reaches hatchling size) and the point of the thermosensitive period of development  
220 for sex determination (Girondot et al. 2018).

221

### 222 **Estimating primary sex ratios in study years**

223 We estimated the sex ratio thermal reaction norm (i.e., the temperature response curve for offspring  
224 sex ratios calculated using constant temperature during incubation) using data from constant  
225 temperature experiments for leatherback turtles conducted in the Atlantic Ocean (Rimblot-Baly et al.  
226 1987) and Eastern Pacific (Binckley et al. 1998) (Supplemental Figure S2). We used logistic models  
227 fitted using maximum likelihood (Abreus-Grobois et al. 2020), implemented with the R package  
228 *embryogrowth* (Girondot 2022). The nest temperature profiles from *in situ* monitored nests were used  
229 to estimate sex ratios. We accounted for the nonlinear development within incubation (Fuentes et al.  
230 2017) by calculating a Constant Temperature Equivalent (CTE), which is the mean temperature  
231 weighted by the differential embryo growth during the TSP (Monsinjon et al. 2019).

232

### 233 **Predicting season-wide sex ratios**

234 To scale up incubation temperatures and predicted sex ratios at the scale of a clutch to the whole  
235 nesting season, we estimated the overall nesting dynamics based on series of nest counts. We  
236 standardised the nesting distribution (number of nests per day) between 0 and 1 to have a daily  
237 proportion of nesting. We calculated the mean predicted Sex Ratio (SR) as being the mean sex ratio  
238 during a nesting season, corrected by the mean hatching success and weighted by the proportion of  
239 nests laid (Eq. (1)).

240

$$241 \quad SR = \sum_{i=k}^N SR_i \times Prop.Nest_i \times HS \quad (1)$$

242

243  $SR_i$  is the sex ratio (male proportion) for nests laid at the day  $i$  of the season with  $k$  being the first date  
244 of the season.  $Prop.Nest_i$  is the proportion of nests laid at the day  $i$  of the season.  $HS$  is the mean  
245 hatching success for nests laid at Espirito Santo (67.1%).  $N$  is the last date of the season.

246

247 Air temperature data was extracted from the European Centre for Medium-Range Weather Forecasts  
248 (ECMWF) climate reanalysis v5 (ERA5; Hersbach et al. 2020) for the Southwest Atlantic region. We  
249 then determined the relationship between air temperature and the parameters: predicted proportion of  
250 female offspring, mean incubation duration, annual onset of nesting (5<sup>th</sup> percentile nesting) and year.  
251 We used the mean temperature in December since this month has the greatest proportion of incubating  
252 clutches.

253

## 254 **RESULTS**

### 255 **Incubation duration**

256 The overall mean incubation duration for *in situ* clutches between 1988 and 2021 (34 years) was 66.3  
257  $\pm$  6.4 days (range 52-91,  $n$  = 867 clutches, Figure 2, 3a). Mean annual incubation durations ranged  
258 from 61.5 days (in 1994,  $n$  = 4 and 2015,  $n$  = 49) to 78 days (in 1988,  $n$  = 1; Supplemental Table S1).  
259 Incubation duration was significantly different among years (Kruskal-Wallis test,  $n$  = 867,  $p$  < 0.001)  
260 with a significant decline across the 34 years ( $F$  = 11.96,  $p$  < 0.001,  $n$  = 867; Figure 3b). The annual  
261 mean incubation duration was 69.5  $\pm$  7.4 days (range 56-76,  $n$  = 75) during the first ten years (1988-  
262 1997), and 65.1  $\pm$  5.7 days (range 53-91,  $n$  = 505) during the last ten years (2012-2021) of the study  
263 period. The incubation duration varied significantly with the day in the season, as no horizontal line  
264 (representing a constant mean incubation duration in the period) can be placed inside the 0.95  
265 simultaneous confidence band in Figure 3c. Considering the geographic location of nests, the  
266 incubation duration significantly increased with latitude (considered from south to north), as no  
267 horizontal line (representing a constant mean incubation duration in the area) can be placed inside the  
268 0.95 simultaneous confidence band in Figure 3d.

269

### 270 **Nest temperatures**

271 For clutches with temperature data loggers ( $n = 28$ ), the mean growth-weighted TSP temperature  
272 (CTE) varied between  $28.4^{\circ}\text{C}$  in 2019 and  $30.3^{\circ}\text{C}$  in 2015. (Table 1). The CTE varied slightly with  
273 the date during the nesting season, being relatively constant until mid-November, increasing until  
274 mid-December and then decreasing (Figure 4). Clutch size ( $72.0 \pm 13.6$  eggs, range = 36-94,  $n = 28$ ;  
275  $F_{1,26} = 0.9, p = 0.8$ ) was found to be a poor predictor of CTE.

276

### 277 **Hatching success**

278 Hatching success in nests with monitored temperature varied between 38.3 and 98.3%, with a mean  
279 of  $74.9 \pm 18.7\%$  and we found no significant relationship with clutch size ( $F_{1,26} = 2.0, p = 0.2, n =$   
280 28). Hatching success was higher when CTEs were between 30 and  $30.5^{\circ}\text{C}$  (Supplemental Figure  
281 S3), although the relationship was not significant ( $F_{1,26} = 1.4, p = 0.3, n = 28$ ).

282

### 283 **Predicted sex ratios**

284 The mean seasonal predicted primary sex ratio (proportion of males) for nests with monitored  
285 temperature varied between 0.1 in 2015 and 0.9 in 2019 (Table 1). The season starts with nests that  
286 are likely to be 100% males, peaks when approximately 60% males are expected, and ends when  
287 nearly 100% females are expected (Figure 5). When accounting for nesting seasonality, we estimated  
288 the season-wide leatherback turtle mean sex ratio to currently be 66.5% female (during the period  
289 between 2012-2021, representing the current scenario (most recent ten years of monitoring), varying  
290 between 55.4% females in 2019 and 90.2% in 2015. When hindcasting to the period between 1988-  
291 1997 (first ten years of monitoring), the overall predicted sex ratio would have been 58.4% female,  
292 varying between 41.3% females in 1992 and 79.9% in 1997.

293

294 There has not, however, been phenological adaptation at this site. The annual median day of nesting  
295 showed no significant trend (Supplemental Figure S4; linear regression,  $F_{1,32} = 0.53, R^2 = -0.02, P =$

296 0.5), similarly to the onset of nesting (5<sup>th</sup> percentile ordinal day) (Supplemental Figure S4; linear  
297 regression,  $F_{1,32} = 0.72$ ,  $R^2 = -0.01$ ,  $P = 0.4$ ). There was also no significant trend of nesting season  
298 duration (days elapsed between first and last nest) throughout the study period (Supplemental Figure  
299 S4; linear regression,  $F_{1,32} = 3.52$ ,  $R^2 = 0.07$ ,  $P = 0.07$ ).

300  
301 December mean air temperature was significantly positively correlated with the proportion of female  
302 offspring produced (Supplemental Figure S5a; Pearson's coefficient of correlation = 0.75,  $t = 6.36$ ,  $df$   
303 = 31,  $p < 0.001$ ; ), significantly negatively correlated with the incubation duration (Supplemental  
304 Figure S5b; Pearson's coefficient of correlation = -0.66,  $t = -4.94$ ,  $df = 31$ ,  $p < 0.001$ ) and not  
305 significantly correlated with the annual onset of nesting (Supplemental Figure S5c; Pearson's  
306 coefficient of correlation = -0.17,  $t = -0.97$ ,  $df = 31$ ,  $p = 0.34$ ). The mean December air temperature  
307 showed an increasing trend throughout the period (Figure 6; linear regression,  $F_{1,31} = 4.60$ ,  $R^2 = 0.10$ ,  
308  $P = 0.04$ ).

309  
310 **DISCUSSION**

311 Small populations are of conservation concern; however, it can be challenging to research key life-  
312 history parameters due to limited data volume and concerns regarding invasive research methods.  
313 Here we use long-term leatherback turtle nesting data to gain insights into predicted hatchling sex  
314 ratio production at the main nesting ground for the Critically Endangered Southwest Atlantic Ocean  
315 leatherback turtle subpopulation (Wallace et al. 2013). Our major findings offer clear insights that  
316 lead to recommendations on how to augment this work going forward to better understand the scope  
317 of population recovery, resilience and potential for adaptation in the face of climate change.

318  
319 Leatherback turtle nesting populations worldwide are believed to vary greatly in the primary sex  
320 ratios produced. While the populations in the Pacific Ocean produce overall female-biased sex ratios

321 (83.2% females estimated in Costa Rica from 1998 to 2007, Sieg et al. 2011), a well-studied  
322 leatherback turtle nesting colony in the western Atlantic Ocean, Suriname, when researched two  
323 decades ago, was estimated to be producing modestly female-biased sex ratios (60.5 - 69.4% females,  
324 Godfrey et al. 1996, Mrosovsky et al. 1984). Our estimates of 66.5% female hatchling sex ratios are  
325 more similar to the ones reported in Suriname. Incubation studies suggest that most field temperatures  
326 produce either all females or males, given the narrow TRT of leatherback turtles (Binckley & Spotila  
327 2015). This could also be the case for leatherback turtles in Brazil, where most nests were estimated  
328 to have produced predominantly female or male hatchlings with few nests producing balanced sex  
329 ratios.

330  
331 The increase seen on leatherback turtle nesting numbers in Espírito Santo is encouraging (Colman et  
332 al. 2019). The decrease in mean incubation duration, together with a modestly female-biased  
333 hatchling sex ratio estimated over the decades may be influencing the recovery of this population.  
334 The female-biased sex ratios could contribute to population growth, since it would ultimately increase  
335 the number of nesting females (Hays et al. 2017, Patrício et al. 2017). A highly biased sex ratio could  
336 however decrease the effective population size (the adults that effectively contribute to the next  
337 generations).

338  
339 Incubation duration varied both temporally and spatially, with the decrease in the mean annual  
340 incubation duration throughout the years being suggestive of potential future feminization of the  
341 population. The lack of a significant trend on the phenology of nesting suggests this population is not  
342 yet adjusting its time of breeding to recent increases in sea surface temperature (Neeman et al. 2015),  
343 or that population growth makes it more challenging to detect trends. The spatial variability in  
344 incubation duration indicates the potential for leatherback turtles to use nest-site selection to produce

345 a broader range of offspring sex ratios and consequently be more resilient to climate change (Fuentes  
346 et al. 2013, Abella Perez et al. 2016).

347  
348 The decrease in incubation durations seen towards the northern part of the nesting beach could be  
349 caused by variation in sediment characteristics, such as colour and grain size (Hays et al. 1999, Fadini  
350 et al. 2011). The fact that our monitored nests were all in the southernmost part of the nesting beach  
351 could add a modest bias, considering that the northern part probably produces less males most likely  
352 due to differences in sand properties such as grain size. That could have influenced our temperature  
353 logger estimates of female production. It is also important to consider that other factors such as nest  
354 moisture can also influence sexual determination (Wyneken & Lolavar 2015) likely due to  
355 evaporative cooling, not a direct effect of moisture on gene expression.

356  
357 In the present study, we used models considering the stages of embryonic development in response  
358 to temperature to estimate the mean growth-weighted incubation temperatures during the TSP  
359 (Girondot & Kaska 2014, Fuentes et al. 2017, Girondot et al. 2018). The lack of locally derived  
360 empirical data from constant temperature incubation experiments studying the effects of temperature  
361 on sex ratios and hatching success currently limit the reliability of future predictions for this region.  
362 It also hampers our ability to estimate how much of phenological change would be needed in order  
363 to keep current sex ratios considering predicted scenarios of climate change (Fuentes et al. 2023).  
364 Further research with more representative sample sizes and spanning more seasons, together with  
365 information on other important Atlantic rookeries such as Gabon, in Central Africa (Witt et al. 2009),  
366 would help us to better understand patterns of primary sex ratios for leatherback turtles across the  
367 Atlantic. Furthermore, novel methods to estimate the sex of hatchlings based on blood samples (Tezak  
368 et al. 2020) are promising and should contribute to more reliable long-term sex ratio predictions.

369

370 Better knowledge of beach temperatures and records of incubation duration contribute to an increase  
371 in our understanding of natural sex ratios for marine turtle populations and have implications for  
372 conservation practices (Mrosovsky and Yntema 1980), such as the potential effects of nest relocation.  
373 This practice could interfere with the incubation process and embryonic development, altering the  
374 sex ratios produced (Sieg et al. 2011). For a Critically Endangered subpopulation as the one in the  
375 present study, such conservation practices, when used cautiously, are still valuable as they reduce the  
376 loss of egg clutches and potentially contribute to population recovery. Future studies should assess  
377 the primary sex ratios of relocated nests to better understand the impacts of this management strategy  
378 over the nesting population (e.g Sieg et al. 2011). Irrespective, our results present baseline data on  
379 nest temperatures and estimated sex ratios for the region and are important to guide future  
380 management decisions for the southwestern Atlantic leatherback turtle population.

381

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394



395 **ETHICAL STANDARDS**

396 This research was submitted to SISBIO in Brazil (permit no. 47845-3) and subjected to approval by  
397 the ethical committee at the University of Exeter, UK, with ethical clearance(s) received.

398

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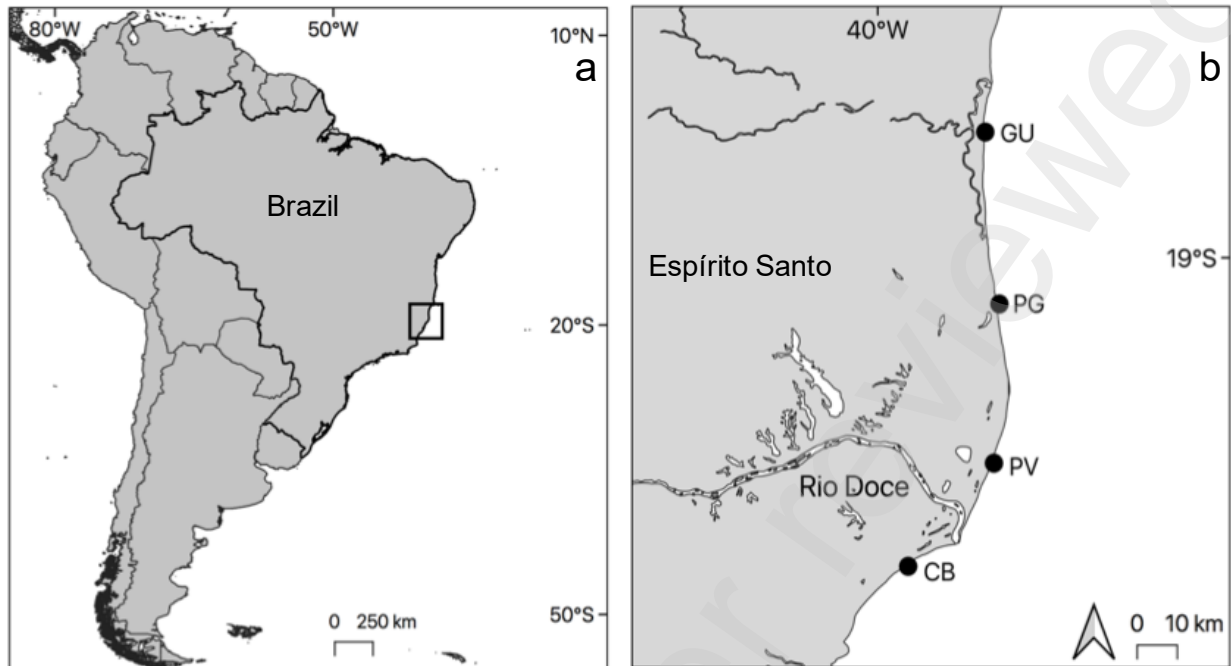
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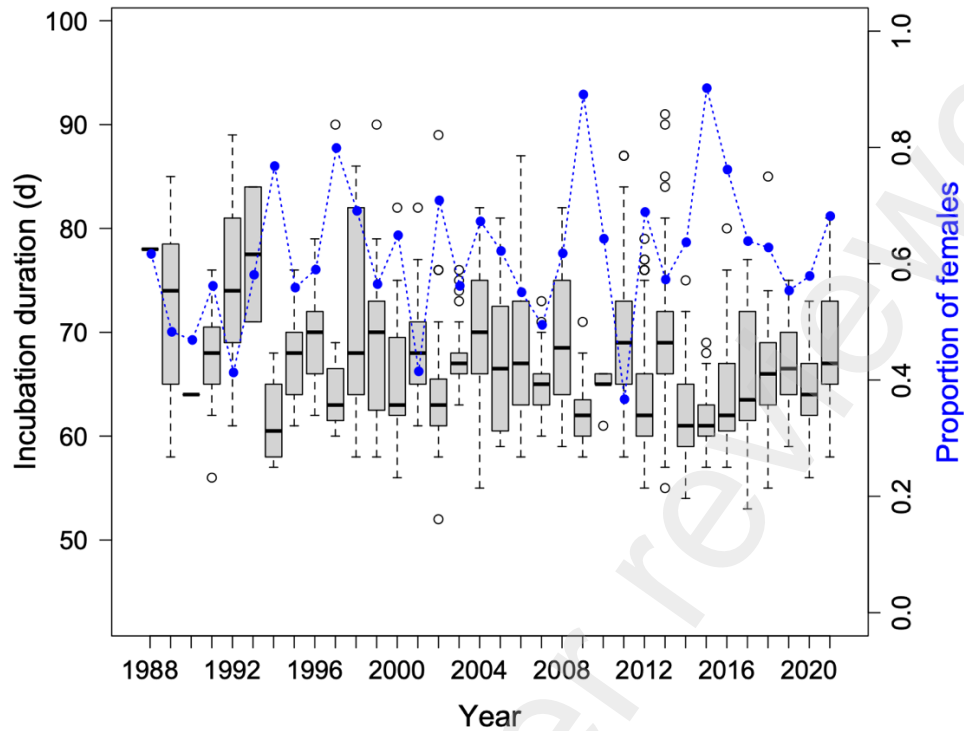
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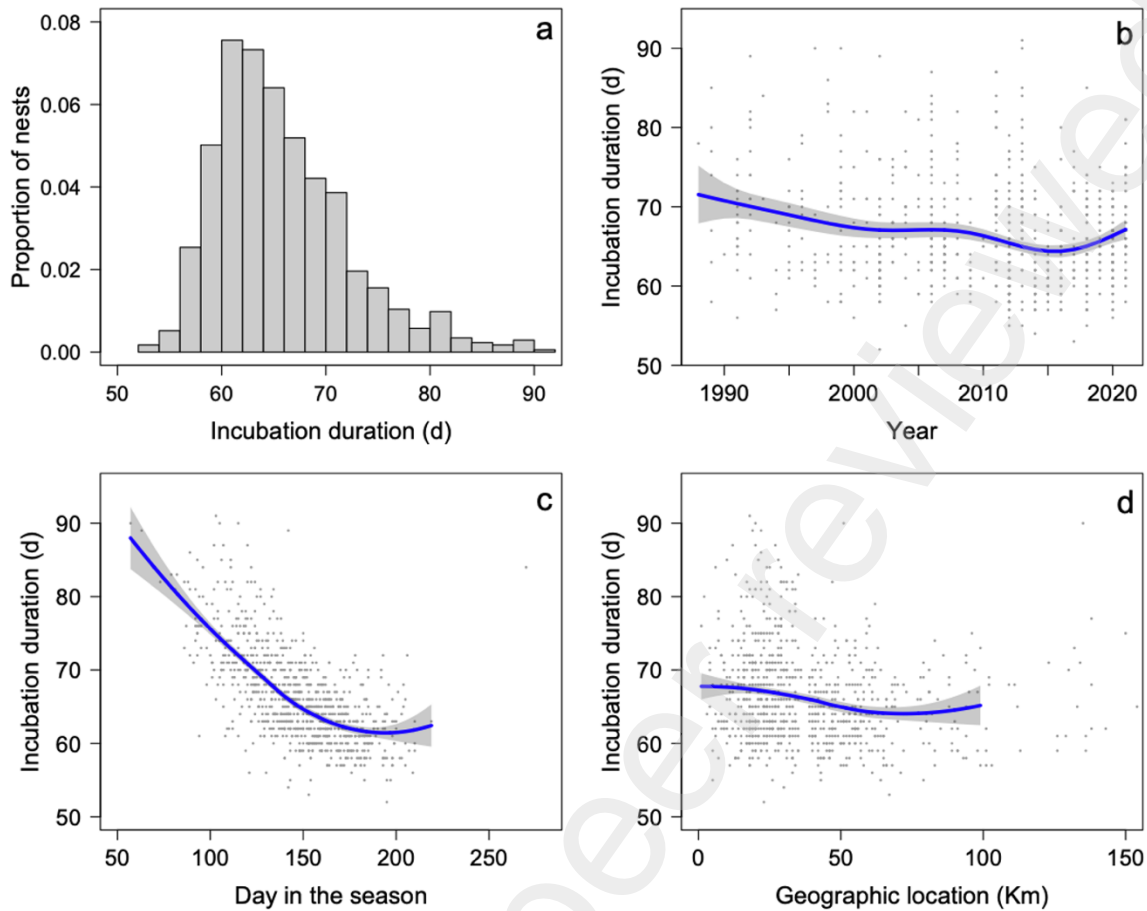
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668 **Figure 1. (a)** Map of Brazil: the leatherback turtle nesting areas in Espírito Santo state are depicted  
669 by the black frame. **(b)** Map of the coast of Espírito Santo state, Brazil. Black circles represent the  
670 TAMAR stations where the data were collected. From south to north: CB = Comboios, PV =  
671 Povoação, PG = Pontal do Ipiranga, GU = Guriri. Rio Doce = Doce River.

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 677 **Figure 2.** Incubation duration (days) of *in situ* leatherback turtle nests from Espírito Santo, Brazil  
 678 (1988- 2021). The black vertical lines represent median values (50<sup>th</sup> percentile). The grey boxes  
 679 contain the 25<sup>th</sup> to 75<sup>th</sup> percentiles of dataset. The black whiskers mark the 5<sup>th</sup> and 95<sup>th</sup> percentiles,  
 680 and values beyond these upper and lower bounds are considered as outliers, represented by black  
 681 open circles. The mean annual predicted proportion of females is shown by blue filled circles.

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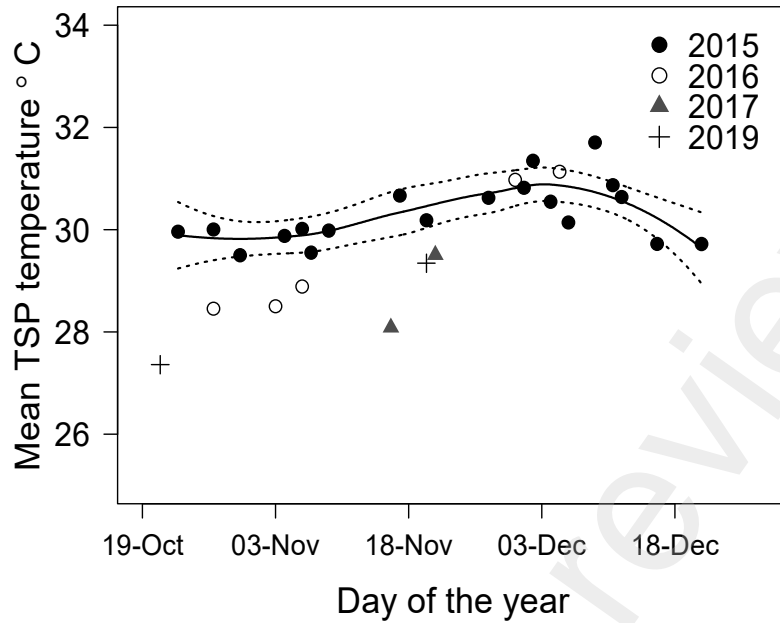


691  
 692 **Figure 3.** Incubation duration (days) of *in situ* leatherback turtle nests from Espírito Santo, Brazil  
 693 (1988- 2021). **(a)** Proportion of nests and incubation duration ( $n = 867$ ). **(b)** Incubation duration by  
 694 year, 1988-2021 ( $n = 867$ ). The blue curve is a GAM regression, and the grey area delimits a 0.95  
 695 simultaneous confidence band. In the graph, to increase clarity, the data points were jittered on the  
 696 y-scale. **(c)** Incubation duration by day in the season (July 1 = day 1). **(d)** Incubation duration by  
 697 geographical location. In c-d) The blue line curve is a loess regression, grey shaded show  
 698 approximate pointwise 95% confidence intervals. These are truncated so as only to include  
 699 categories with sufficient data points c)  $n = 866$ ; d)  $n = 836$ .

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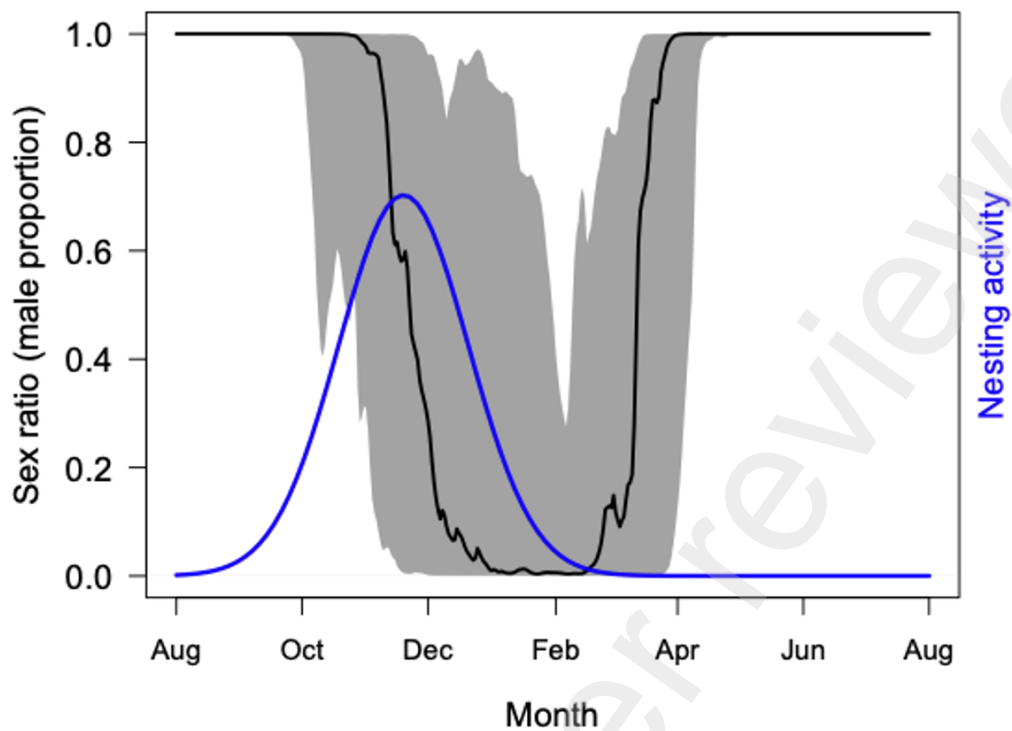
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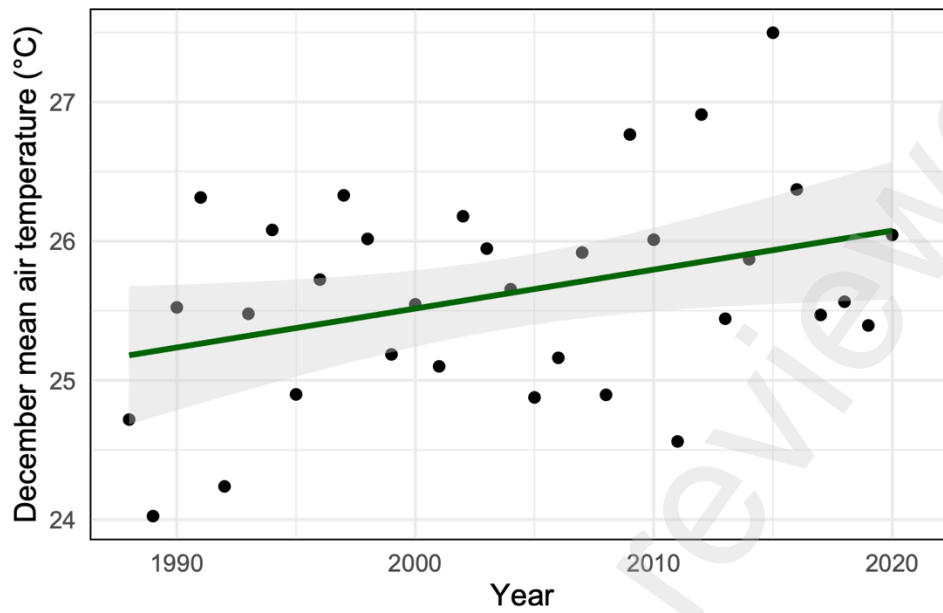
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 704 **Figure 4.** Mean growth-weighted TSP temperature for leatherback turtle nests from Espírito Santo,  
 705 Brazil, with monitored temperature by day of the year ( $n = 28$ ). The solid line curve is a loess  
 706 regression and outer curves (dashed lines) show approximate pointwise 95% confidence intervals.  
 707 The loess regression curve was calculated with the 2015 data points only ( $n = 19$ ).

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 720 **Figure 5:** Current sex ratios estimates (quantiles at 50%, black line) throughout the year with lower  
 721 and upper quantiles (at 2.5% and 97.5% respectively, shaded in grey) for the leatherback turtle  
 722 population nesting in Espírito Santo, Brazil. Quantiles were calculated based on 1979–2020 time  
 723 series. Standardized nesting activity (proportion of nests laid throughout the season based on the  
 724 description of nesting activity) is indicated by the blue line.

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735 **Figure 6.** Annual December mean air temperature at a leatherback nesting site, in Espírito Santo,  
736 Brazil (1988-2020). Green line: linear regression. Grey shaded areas: 95% CI.

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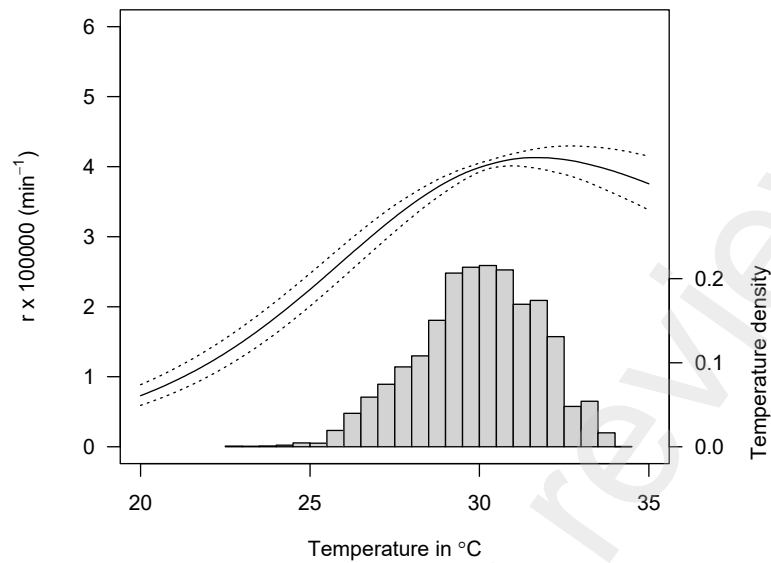


750 **Table 1.** Summary incubation temperatures and estimated primary sex ratios of leatherback turtle  
 751 nests with monitored temperature per year in Espírito Santo, Brazil, between 2015 and 2019. TSP  
 752 = thermosensitive period.

Year	TSP Temperature		Sex ratio (male proportion )		n
	Mean	Range $\pm$ SD	Mean	Range $\pm$ SD	
2015	30.3	29.5-31.7 $\pm$ 0.6	0.1	0.0-0.6 $\pm$ 0.2	19
2016	29.6	28.5-31.1 $\pm$ 1.2	0.6	0.0-1.0 $\pm$ 0.5	5
2017	28.8	28.1-29.5 $\pm$ 0.7	0.8	0.6-1.0 $\pm$ 0.2	2
2019	28.4	27.4-29.3 $\pm$ 1.0	0.9	0.8-1.0 $\pm$ 0.1	2
Overall	29.9	27.4-31.7 $\pm$ 1.0	0.3	0.0-1.0 $\pm$ 0.4	28

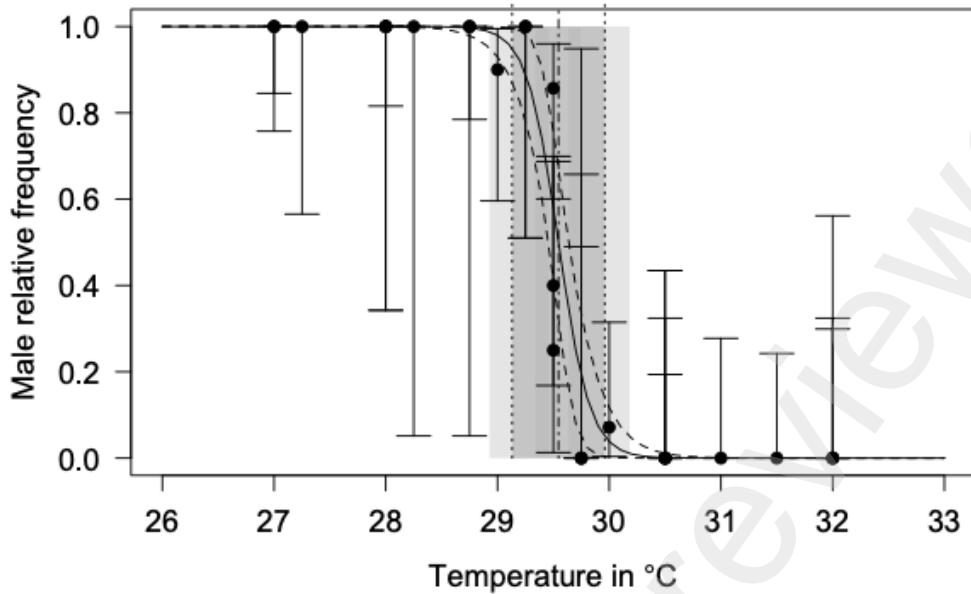
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769 **Supplemental Material**

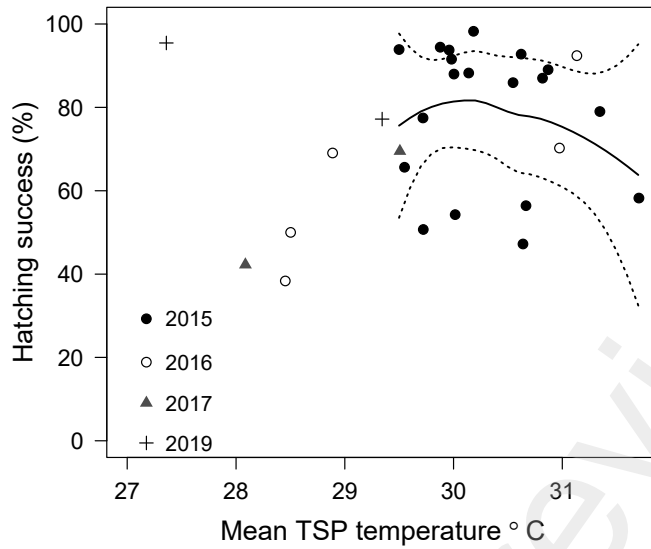


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771 **Supplemental Figure S1:** Growth rate thermal reaction norm (GRTRN). Fitted growth rate  $r(T)$  of  
772 straight carapace length (SCL) of leatherback turtles depending on incubation temperature  $T$ . The  
773 area with the dashed lines shows the 95% confidence interval. The histogram shows the density of  
774 temperatures within 28 monitored nests in Espírito Santo, Brazil.

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 788 **Supplemental Figure S2:** Relationship between constant incubation temperature and sex ratio  
 789 modelled as a logistic equation for leatherback turtles using data from: Atlantic Ocean (Rimblot-  
 790 Baly et al. 1987) and Eastern Pacific (Binckley et al. 1998). The solid line represents the mean  
 791 expected sex ratio and dashed lines display the 95% confidence interval. Black filled circles are the  
 792 observed sex ratios from constant temperature experiments and error bars indicate their 95%  
 793 confidence intervals. The darker grey area between the vertical dotted lines indicates the transitional  
 794 range of temperatures (TRT) defined as the temperatures that produce sex ratio between 5% and 95%  
 795 males. The vertical dash-dot-dashed line within the TRT indicates the pivotal temperature (P). The  
 796 lighter grey areas indicate the 95% credibility region of TRT limits and P obtained from the  
 797 maximum likelihood method.  
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800 **Supplemental Figure S3:** Relationship of hatching success of leatherback turtle nests with mean  
 801 TSP incubation temperature for monitored nests in Espírito Santo ( $n = 28$ ). Curves show fitted  
 802 polynomial regression and dashed lines represent 95% confidence interval (CI). Significance of  
 803 fit:  $p = 0.48$ .

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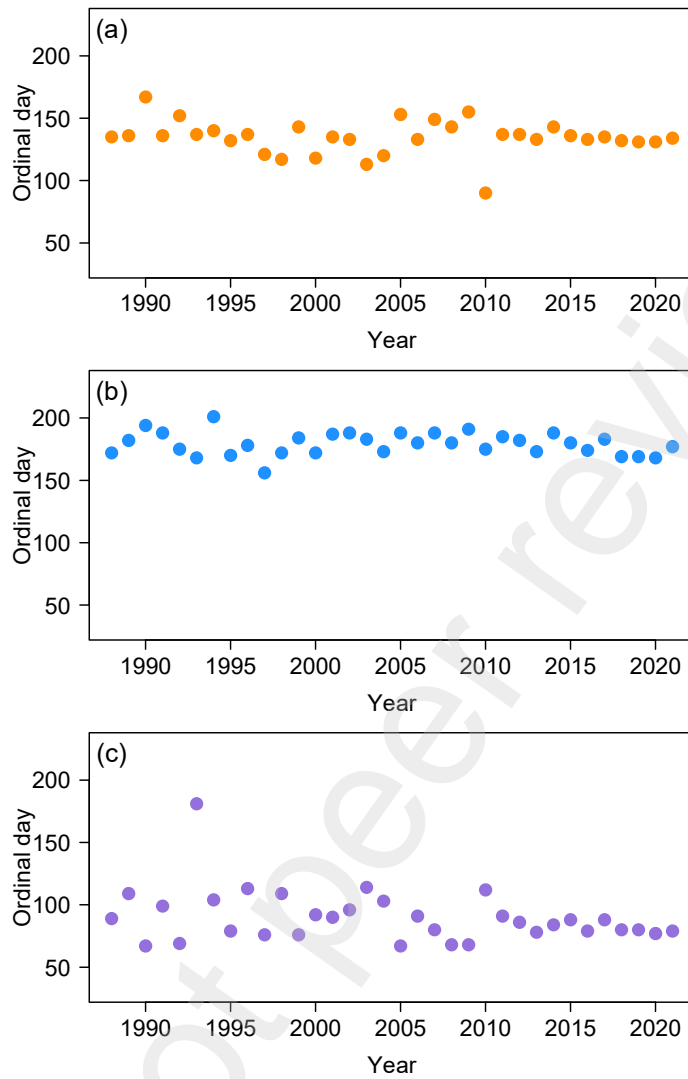
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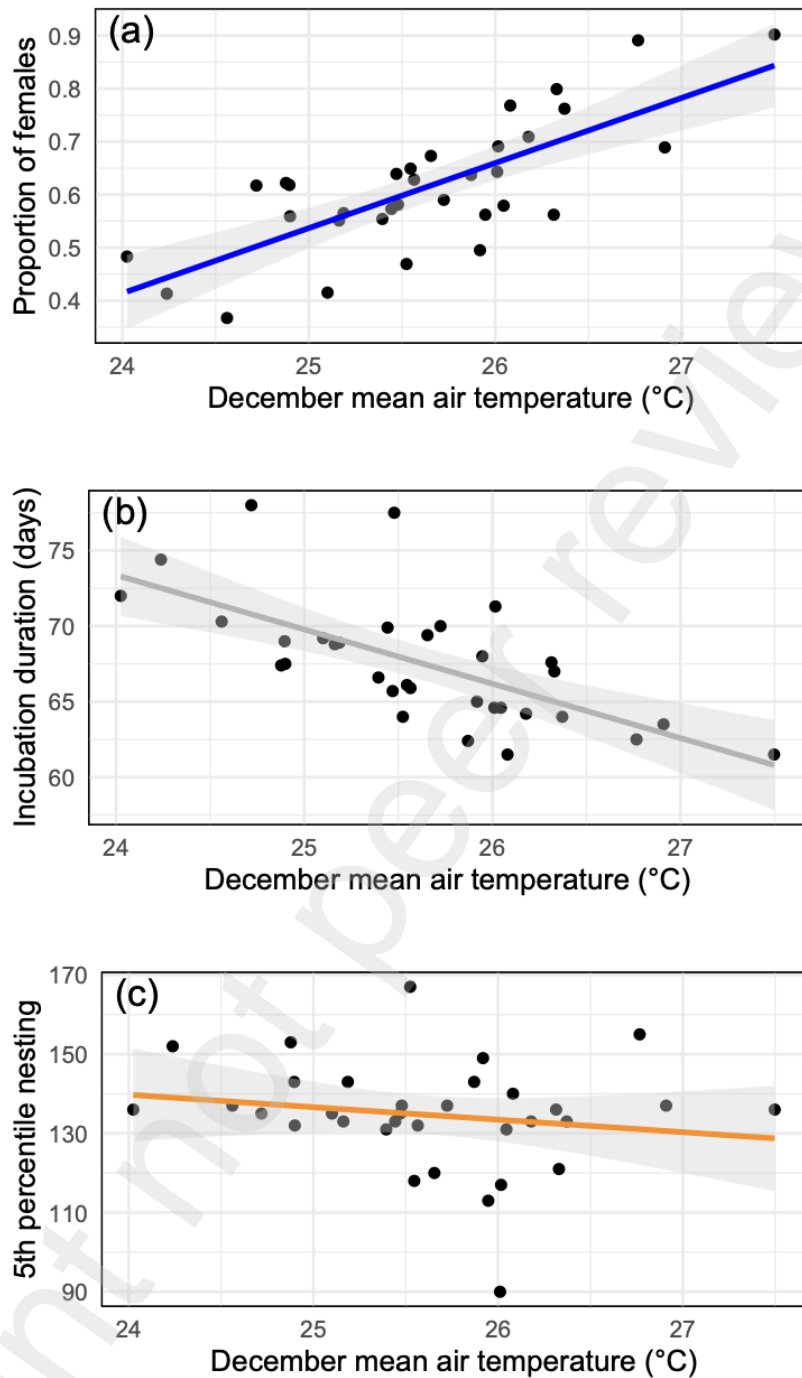
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 818 **Supplemental Figure S4:** Phenology of leatherback turtles in Espirito Santo, Brazil, 1988-2021.  
 819 (a) 5<sup>th</sup> percentile day of annual nesting onset , (b) Population annual median day of nesting (c)  
 820 nesting season duration in days.

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 829 **Supplemental Figure S5:** Responses of leatherback turtles to mean December air temperature at  
 830 the nesting site and demography. (a) predicted proportion of females, (b) mean annual incubation  
 831 duration and (c) onset of nesting (5<sup>th</sup> percentile of nesting) per year. Coloured lines: fit of respective  
 832 models. Grey shaded areas: 95% CI.  
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834 **Supplemental Table S1.** Incubation duration of leatherback turtle nests per year (1988- 2021,  $n =$   
 835 867).

Season	Incubation Duration (days)	$n$	predicted sex ratios (% female)
1988-1989	78.0 ± NA	1	61.7
1989-1990	72.0 ± 9.7 (58-85)	7	48.3
1990-1991	64.0 ± NA	1	46.9
1991-1992	67.6 ± 4.6 (56-76)	19	56.2
1992-1993	74.4 ± 7.8 (61-89)	14	41.3
1993-1994	77.5 ± 9.2 (71-84)	2	58.1
1994-1995	61.5 ± 4.8 (57-68)	4	76.8
1995-1996	67.5 ± 4.5 (61-76)	11	55.9
1996-1997	70.0 ± 5.9 (62-79)	9	59.0
1997-1998	67.0 ± 10.6 (60-90)	7	79.9
1998-1999	71.3 ± 9.9 (58-86)	9	69.1
1999-2000	68.9 ± 8.2 (58-90)	19	56.5
2000-2001	66.1 ± 7.4 (56-82)	11	64.9
2001-2002	69.2 ± 6.8 (61-82)	9	41.5
2002-2003	64.2 ± 6.0 (52-89)	43	70.9
2003-2004	68.0 ± 3.7 (63-76)	21	56.2
2004-2005	69.4 ± 7.1 (55-82)	13	67.3
2005-2006	67.4 ± 7.9 (59-81)	8	62.2
2006-2007	68.8 ± 7.2 (58-87)	45	55.1
2007-2008	65.0 ± 2.7 (60-73)	34	49.5
2008-2009	69.0 ± 6.9 (59-82)	20	61.8
2009-2010	62.5 ± 3.9 (58-71)	11	89.1
2010-2011	64.6 ± 2.1 (61-66)	5	64.3
2011-2012	70.3 ± 7.4 (58-87)	39	36.7
2012-2013	63.5 ± 5.4 (55-79)	118	68.9
2013-2014	69.9 ± 7.3 (55-91)	53	57.3
2014-2015	62.4 ± 5.1 (54-75)	32	63.7
2015-2016	61.5 ± 2.4 (57-69)	49	90.2
2016-2017	64.0 ± 5.5 (57-80)	28	76.2
2017-2018	65.7 ± 6.3 (53-77)	20	63.9
2018-2019	65.9 ± 5.2 (55-85)	65	62.8
2019-2020	66.6 ± 3.8 (59-75)	30	55.4
2020-2021	64.6 ± 4.1 (56-73)	64	57.9
2021-2022	68.8 ± 5.6 (58-81)	46	68.2

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