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Present and Future Estimates of Hatchling Sex-ratio of
Loggerhead Turtles on Maio Island, Cabo Verde in the
Context of Global Warming



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Master of Marine and Coastal Systems (MaCS)

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2024

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Resumo

A aceleração das alterações climáticas tem afetado negativamente muitas espécies animais e a biodiversidade a uma escala global. Essa situação é alarmante, pois a biodiversidade é essencial para a saúde dos ecossistemas e para o bem-estar humano. A resiliência de uma espécie será o fator determinante para sua capacidade de persistir diante dessas mudanças adversas. No caso das tartarugas marinhas, que desempenham um papel crucial nos ecossistemas marinhos, estas apresentam determinação sexual dependente da temperatura (TSD). Isso significa que o sexo das suas crias é diretamente influenciado pela temperatura da areia em que os ovos são incubados, entre outros fatores ambientais. À medida que as temperaturas globais aumentam, há a possibilidade de alteração das proporções de sexo nessas populações, levando a uma maior produção de crias fêmeas e a um aumento da mortalidade das crias devido ao calor excessivo.

Este estudo é pioneiro na análise de um grande conjunto de dados coletados ao longo de um período de 2017 a 2023, focando nas temperaturas registradas em praias com diferentes cores de areia na Ilha do Maio, em Cabo Verde. A análise desses dados foi realizada com o objetivo de modelar as proporções de sexo das crias de tartaruga-cabeçuda (*Caretta caretta*) durante o período do estudo, oferecendo uma visão detalhada das possíveis mudanças nas populações locais em resposta às variações climáticas. Essas variações são de suma importância, pois as tartarugas marinhas enfrentam múltiplas ameaças, incluindo a perda de habitat, a poluição e a mudança climática.

A determinação sexual nas tartarugas marinhas é extremamente sensível às mudanças de temperatura, com temperaturas mais altas resultando num maior número de fêmeas, enquanto temperaturas mais baixas produzem um número maior de machos. Portanto, o aumento progressivo das temperaturas globais, causado pelas emissões de gases de efeito estufa, pode levar a um desequilíbrio nas proporções de sexo nas populações de tartarugas. Esse desequilíbrio é preocupante, pois pode comprometer a capacidade reprodutiva das populações a longo prazo. De acordo com o Intergovernmental Panel on Climate Change (IPCC), diferentes cenários de emissões podem impactar significativamente as condições de incubação destes animais, tornando-se crucial prever cenários futuros com base nas variações térmicas. A capacidade de modelar essas variações é fundamental para o planejamento de estratégias de conservação eficazes. Este estudo estimou as proporções primárias de sexos (a proporção de machos para fêmeas ao nascer) das tartarugas-cabeçudas com base nos dados de temperatura da areia. Além disso, as futuras proporções de sexo foram projetadas de acordo com três cenários de SSP (Caminhos Socioeconômicos Partilhados) previstos pelo IPCC: SSP1-2.6 (um caminho sustentável com baixas emissões), SSP3-7.0 (um caminho fragmentado com emissões moderadas) e SSP5-8.5 (um caminho com altas emissões e alta dependência de combustíveis

fósseis). Estes cenários fornecem um quadro abrangente sobre como diferentes políticas de mitigação e desenvolvimento sustentável podem influenciar a conservação das tartarugas marinhas.

Para entender melhor o impacto das mudanças de temperatura na produção de machos e fêmeas, foi realizada uma análise detalhada comparando as temperaturas da areia a diferentes profundidades— 27 cm, 35 cm, 45 cm e 55 cm—e entre tipos de areia e períodos do ano. O estudo focou principalmente a identificação de possíveis refúgios térmicos, tanto espaciais quanto temporais, onde as condições seriam mais favoráveis para a produção de filhotes machos. Essas áreas de refúgio podem desempenhar um papel vital na conservação das tartarugas marinhas, especialmente diante do aquecimento global. O reconhecimento destas áreas é crucial para a implementação de estratégias de gestão que visem a proteção dos ninhos. Os resultados obtidos até agora indicam que a população local de tartarugas cabeçadas na Ilha do Maio já mostra uma forte tendência para a produção de fêmeas, uma tendência que se deve intensificar no futuro à medida que as temperaturas aumentam. Esse desequilíbrio nas proporções de sexo pode ter consequências sérias para a sobrevivência a longo prazo da população. A redução do número de machos compromete a reprodução e, conseqüentemente, a viabilidade da população. Isso torna-se ainda mais preocupante em uma espécie que já enfrenta diversas ameaças devido à ação humana e às mudanças ambientais. No entanto, o estudo também identificou áreas e períodos específicos que podem ser considerados refúgios térmicos para a produção de crias machos. Observou-se que a uma profundidade de 55 cm, particularmente em areias de cor mais clara, no início e no final da temporada de nidificação, as temperaturas de incubação tendem a ser mais baixas, criando condições mais favoráveis para o desenvolvimento de machos. Assim, esses períodos e locais devem ser priorizados nas estratégias de conservação como forma de maximizar a produção de machos e equilibrar as proporções de sexo das populações.

Além disso, o estudo enfatiza a importância de ações de gestão adaptativa para mitigar os efeitos das alterações climáticas nas tartarugas marinhas. Uma das estratégias sugeridas é a transferência de ninhos para áreas com temperaturas mais adequadas para a produção de machos, como locais com areia de cor mais clara e a uma profundidade de 55 cm. Contudo, deve-se ter cautela ao implementar quaisquer mecanismos de mitigação induzidos antropogenicamente, pois estes podem atrasar a potencial adaptação da população local ao diminuir as pressões de seleção natural. Um gestão adequada da realocação de ninhos pode ser uma ferramenta essencial para garantir a continuidade das populações de tartarugas-cabeçadas na Ilha do Maio e em outras regiões afetadas pelas alterações climáticas. Outro aspecto importante abordado neste estudo é a limitação das tartarugas marinhas em responder rapidamente às mudanças climáticas por meio de adaptações fenológicas. A longa vida

destas espécies, combinada com sua maturidade tardia, dificulta a adaptação rápida a novas condições ambientais. Como tal, a intervenção humana por meio de estratégias de gestão adaptativa, embora necessária para aliviar ameaças imediatas, é provavelmente inevitável para evitar um declínio populacional ainda maior, embora possa não ser suficiente para prevenir completamente a extinção destas espécies a longo prazo.

Em suma, este estudo oferece uma contribuição importante para a compreensão dos impactos das alterações climáticas sobre as tartarugas marinhas, particularmente a tartaruga-cabeçuda, na Ilha do Maio. Através da análise detalhada dos dados de temperatura da areia e das projeções futuras das proporções de sexo, o estudo fornece insights valiosos para o desenvolvimento de estratégias de conservação eficazes. Proteger os ninhos durante períodos mais favoráveis para a produção de machos e implementar técnicas de gestão adaptativa, como a realocação de ninhos em profundidades e locais mais adequados, são ações que podem contribuir significativamente para a preservação desta espécie. A urgência destas ações torna-se ainda mais evidente perante estimativas de um aquecimento contínuo e da tendência de proporções de sexo altamente desequilibradas, que podem comprometer a sobrevivência a longo prazo das populações de tartarugas marinhas.

Palavras-chave: *Caretta caretta*, Tartaruga cabeçuda, Reprodução, Conservação, Proporção de sexo, Mudanças climáticas

Abstract

Accelerating climate change negatively affects many animal species and the biodiversity on a global scale. Resilience will be the fundamental determinant whether a particular species will be able to persist. Marine turtles exhibit temperature-dependent sex determination (TSD), hence their sex is influenced directly by the sand temperature (among other external factors). Thus, increasing temperatures can alter sex-ratios and may lead to a higher production of female hatchlings and hatchling mortality. The current study is the first one analysing a large dataset over a period of 7 years (2017-2023) consisting of temperatures collected from beaches (with distinct colours of the sand) on Maio Island (Cabo Verde). The sand temperature data are used to model the sex-ratios of loggerhead turtle hatchlings during the study period. The future primary sex-ratios are estimated based on three emissions scenarios according to the IPCC. Potential thermal spatial-temporal refuges for male production are explored by comparing sand temperatures and inferred sex-ratios across different depths, sand types and time periods. At present, highly female-biased sex-ratios are estimated in the local population, which is predicted also into the future. Potential for lower incubation temperatures is found at 55 cm depth in light-coloured sand in the beginning and end of nesting season. Protection of nests laid in these time periods should be of conservation priority if we want to reach a greater potential for male production. The rapid increase in incubation temperatures, coupled with the longevity of sea turtle species, hampers phenological changes and may lead to the extinction of these species. Finally, an adaptive management strategy to place the relocated hatchery nests at a depth of 55 cm (while maintaining the original depth of relocated in situ nests) is proposed.

Keywords: *Caretta caretta*, Loggerhead turtle, Reproduction, Conservation, Sex ratio, Climate change

Table of Contents

Acknowledgements.....	I
Resumo	II
Abstract.....	V
Table of Contents.....	VI
List of Figures	VII
List of Tables	VIII
List of Abbreviations	VIII
1. Introduction	1
2. Literature Review	3
2.1. Reproduction and TSD.....	3
2.2. Primary Sex-ratio Estimation Approaches.....	4
3. Case Study.....	8
3.1. Study Area	8
3.2. Maio Biodiversity Foundation (FMB).....	10
4. Methodology.....	12
4.1. Data Collection	12
4.1.1. Metabolic heating.....	12
4.1.2. Sand and air temperature.....	13
4.2. Data Analysis	13
4.2.1. Temperature analysis	13
4.2.2. Sex-ratio estimation.....	14
4.2.3. Future sex-ratio estimation	14
5. Results.....	16
5.1. Temperature Analysis.....	16
5.1.1. Interbeach variation.....	16
5.1.2. Interannual variation	18
5.1.3. Temperature variation across depth	20
5.1.4. Sand and air temperature relationship.....	22
5.2. Present Sex-ratio	24
5.2.1. Metabolic heating.....	24
5.2.2. Sex-ratio.....	25
5.3. Future Sex-ratio	28
6. Discussion.....	30
6.1. Female Skewed Sex-ratios and Mortality.....	30

6.2.	Capacity to Adapt	31
6.3.	Management Strategies and Conservation	32
6.4.	Methodological Limitations	33
7.	Conclusion.....	35
	References	36
	Appendix 1	43
	Appendix 2	44
	Appendix 3	45
	Appendix 4	46
	Appendix 5	47

List of Figures

Figure 1:	Generalised model of a TSD pattern in sea turtles.	4
Figure 2:	Map of the study area, Maio, Cabo Verde showing the three locations of interest as dots and the hatchery location highlighted in triangle.....	8
Figure 3:	Different types of sand present at the three study locations: a) Pajoana (dark-coloured beach), b) Ribeira Baia (mixed-sand beach), c) Morro (light-coloured beach).....	9
Figure 4:	Hatchery located on Beach Rocha (where the study nest for the metabolic heating assessment was placed).....	10
Figure 5:	Scheme showing how an optical interface is used for retrieving the collected data.	12
Figure 6:	Box on top of violin plot showing sand temperature data collected in the 7 study years. ...	16
Figure 7:	(A) Line graph comparing the daily average temperature of the 3 distinct colours of the sand across the nesting season (averaged from all of the depths and from the whole collecting period, i.e. 2017- 2023). (B) Comparison of the daily average temperature of the 3 types of sand shown for each study year separately.	17
Figure 8:	Comparison of average daily temperatures by the study years (sand types and depths averaged). The 3 coldest years (2018, 2019, 2022) are plotted separately.	18
Figure 9:	Decomposition of additive time series over the entire study period (20 Jul- 10 Nov across all years, i.e. frequency of 114 days/year is used) into trend, seasonal and random components.....	19
Figure 10:	Comparison of the daily average temperature (average of all study years) across 4 depths (27 cm, 35 cm, 45 cm, 55 cm) plotted for each type of sand separately.	20
Figure 11:	Air-sand temperature cross-correlation with the most significant lag highlighted (red dashed line) for different types of sand and depths shown separately.	22
Figure 12:	Scatter plots showing the correlation between the daily average air and sand temperatures (A) for all sand types and depths jointly (the group values are averaged) and (B) for each group (sand type and depth) separately.	23
Figure 13:	Metabolic heating measured in the study nest within the hatchery (01 Oct- 19 Nov 2023) at 55 cm depth.	24

Figure 14: Comparison of sex-ratio (female proportion) among different types of sand during the nesting season (averaged for all depths and the entire study period)..... 25

Figure 15: The sex-ratio (female proportion) during the nesting season shown separately for each type of sand and year, with depth differences indicated by colour 26

Figure 16: Comparison of the predicted sand temperature (2000 to 2100) according to the three IPCC scenarios (SSP1-2.6, SSP3-7.0, SSP5-8.5) based on the best models selected according to AIC scores. 28

Figure 17: Time-series comparison of sex-ratios among sand types (averaged across all depths) according to the IPCC emissions scenarios SSP1-2.6, SSP3-7.0 and SSP5-8.5 (2000-2100)..... 29

List of Tables

Table 1: Correlative model equations for the sand temperature calculation. C represents the intercept value of the given regression model. M and N define the linear constants. 15

Table 2: Temperature reduction averaged across the entire study period for each sand type separately, with corresponding standard deviations (SD) and number of observations (n)..... 21

List of Abbreviations

- AIC- Akaike Information Criterion
- AICc- Corrected Akaike Information Criterion
- AMH- Anti-Müllerian Hormone
- CTE- Constant-temperature Equivalent
- DP- Dissertation Plan
- FMB- Maio Biodiversity Foundation
- GHG- Greenhouse Gases
- GSD- Genetic Sex Determination
- IUCN- International Union for Conservation of Nature
- IPCC- Intergovernmental Panel on Climate Change
- MH- Metabolic Heating
- NGO- Non-Governmental Organization
- PT- Pivotal Temperature
- SST- Sea Surface Temperature
- TRT- Transitional Range of Temperatures
- TSD- Temperature Dependent Sex Determination
- TSP- Thermosensitive Period

1. Introduction

Global biodiversity is endangered due to climate change and the worst-case scenario predicts that the anthropogenic perturbations may lead to the sixth “mass extinction” (Bellard et al., 2012). Many species already exhibit diverse responses to climate change (e.g., have moved spatially or temporally possibly searching for their former thermal niches). The more able to adjust, the higher the probability to survive and respond to changes. However, the extent to which endangered species are able to cope with abrupt climate change is yet unknown (Moritz & Agudo, 2013). Resilience is the key term which can be assessed on a number of scales: from the base physiological responses to genomic changes and subsequent evolutionary development (Barshis et al., 2013; Pörtner, 2010; Sgrò et al., 2011). Ectothermic species are especially sensitive to any thermal alterations, which climate change may bring. Regulation of the body temperature, eggs development and basal metabolic rate of most ectotherms depends on the external environment (Paaijmans et al., 2013). Among reptiles, marine turtles have been extensively studied due to the fact that some colonies of the seven species have suffered severe declines and even recent extinctions. Their existence is threatened not only by climate change but also by other anthropogenic disturbances such as fisheries bycatch (Santos et al., 2013), poaching of either eggs or adult individuals (McClenachan et al., 2006), urban and tourism development on nesting beaches including artificial lighting (Sella & Fuentes, 2019) etc. Nevertheless, temperature is still the key factor and is tightly interconnected with their life affecting them from incubation to adulthood (Abella Perez et al., 2016). Hatching success, emergence, the physical condition (Patrício et al., 2019), the sex (Pieau, 1972) and the dispersal behaviour are all determined by temperature (Hamann et al., 2007). In adulthood, the breeding interval is also influenced by temperature, e.g. during the years with warmer spring, an earlier onset and peak of nesting activities and prolonged nesting seasons were detected (Weishampel et al., 2004).

The loggerhead sea turtle (*Caretta caretta*) is classified as Vulnerable at the species level (IUCN, 2023) and its North-East Atlantic subpopulation (studied in the present paper) as Endangered on the IUCN (International Union for Conservation of Nature) Red List (Casale & Marco, 2015). The island of Maio (Cabo Verde) is home to one of the largest nesting colonies in the world. An increase in nesting activities is detected in the past years (Patino-Martinez et al., 2021), although the species’ distribution may be increasing by decreasing elsewhere (Wallace et al., 2010). However, the excellent condition of its beaches makes the Island of Maio an important location for loggerhead turtle conservation efforts (Patino-Martinez et al., 2021). Although having a large attention by research groups, universities, non-governmental and also governmental organizations, information regarding populations based in relatively undeveloped areas are still scarce (Mazaris et al., 2017; Tomás et al., 2010). With the future

possible changes of thermal habitats caused by climate change, it is essential to monitor the population and understand the changes on global scale (including the relatively undisturbed areas). The phenological response along with spatial distribution changes in turtles are current topics of study. With abrupt increase in temperatures, however, sex ratio and nest survivorship are subjects to a change and may be jeopardized (M. M. P. B. Fuentes et al., 2010; Glen & Mrosovsky, 2004; Hawkes et al., 2007).

The objectives of this study are: (1) to estimate the sex-ratio of loggerhead turtle hatchlings for the period 2017-2023, (2) to model the future primary sex-ratio based on air and SST (sea surface temperature) sourced from emissions scenarios provided by the IPCC (2023), and (3) to explore potential spatial-temporal refuges for incubation at lower temperatures to produce male hatchlings.

2. Literature Review

2.1. Reproduction and TSD

Marine turtles do not exhibit any parental care; however, the nest site is selected in a way to reach the highest probability of the successful hatchlings' development (e.g. high humidity, low salinity, sufficient distance from the water mark to avoid inundation etc.). The breeding interval of males is recorded to be annual while females usually mate in a span of two to three years (and reaching maturity from 17 to 35 years of age). Throughout one nesting season, females usually lay several clutches. Generally, when assessing the sex-ratio of the population, we differentiate between two types: the operational sex-ratio, i.e. the ratio of the breeding population and the primary sex-ratio, i.e. at the point of the sex determination (Foley et al., 2006; Hawkes et al., 2009; Laloë et al., 2014). The focus of the paper is to estimate the primary one. Sand temperature is the most influential factor in determining the sex of developing embryos, among other environmental conditions (Hawkes et al., 2009). A more detailed explanation of how sex is determined is provided in the next section.

The first description of temperature-dependent sex determination (TSD) was assessed in an agamid lizard (Charnier, 1966). Most of the reptiles exhibit TSD rather than GSD (genetic sex determination). Turtles being part of this class are no exception. TSD present in turtles was firstly described by Pieau (1972). In theory, the pivotal temperature (PT) for most of the marine turtle species (producing a balanced sex-ratio, meaning 50% males & 50% females) is determined to be approximately 29°C (Mrosovsky, 1988). The TRT (transitional range of temperatures) in marine turtles is the temperature range during egg incubation in which both sexes are produced (Figure 1). Outside this range, only one sex is typically produced: males at cooler temperatures and females at warmer temperatures (Mrosovsky & Pieau, 1991). Moreover, hatchlings' mortality appears when temperatures go beyond a range between 25°C to 35°C. The review by Hawkes et al. (2009) supplies literature comparing the temperature threshold values (PT and TRT) of different turtle species. The TRT for loggerhead turtles is approximately 27.5°C to 30.5°C among populations. It is important to note that the PT and TRT varies not only among marine turtle species but also among populations of one species, i.e. genetically and spatially (Mrosovsky, 1988). TSD pattern present in marine turtles is depicted in Figure 1.

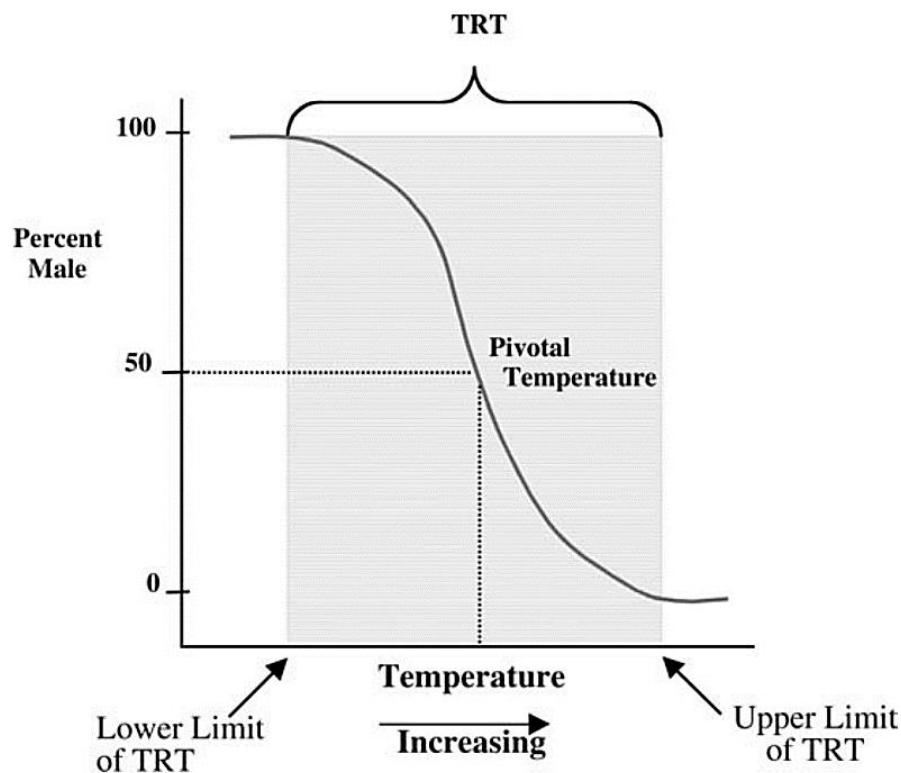


Figure 1: Generalised model of a TSD pattern in sea turtles.
Source: Wibbels et al. (2003).

2.2. Primary Sex-ratio Estimation Approaches

As there are no dimorphic characters according to which it would be possible to distinguish sex in sea turtle juveniles, a need to develop alternative methods has arisen. There are direct methods (Eckert et al., 1999; Patrício et al., 2021) which are the most reliable ones but are also invasive and mostly require sacrifice of the hatchlings. The most straightforward (and reliable) method is a dissection of a hatchling and subsequent histological study of the gonads. Nowadays, this method is not commonly used due to ethical and also practical reasons (Eckert et al., 1999; Mrosovsky & Yntema, 1980; Wibbels et al., 2003). Another approach is a laparoscopic examination (hatchlings remain alive). A morphology of an accessory and gonadal duct is examined. Although not lethal, this method is still considered slightly invasive, and labour and expertise intensive as the personnel has to undergo a specific training. Moreover, the turtles need to be raised for several months (in order to perform safe laparoscopy, turtles need to be an adequate size) and thus the method is not suitable for large-scale studies (Ligon et al., 2014; B. M. Tezak et al., 2017; Wyneken et al., 2007). Less invasive (and also non-lethal) approach is the blood/amniotic fluid samples collection. Various studies assess different hormones (e.g. estrogen-testosterone ratio- which may be overlapping for both sexes, Anti-Müllerian hormone

etc.) either in blood samples (B. Tezak et al., 2020, Wibbels et al., 2000) or amniotic fluid (Gross et al., 1995; Xia et al., 2011). Radioimmunoassay of both requires sufficiently large amount to be collected in order to be carried forward for analyses. For small turtle species this amount of blood taken may be too large. Moreover, in the case of amniotic fluid collection, handling of unhatched eggs is extensive and logistically difficult. Thus, the method is considered rather impractical for large-scale studies. Nevertheless, a fairly recent study used a Western Blot analysis to compare number of sex-specific proteins in two species (*Caretta caretta* among these). AMH (Anti-Müllerian hormone) was identified as the sex specific protein marker in the study (all hatchlings tested positive for AMH were later confirmed as males via laparoscopy and the rest of the hatchlings tested negative for AMH were subsequently identified as females also via laparoscopy). The hatchlings were tested for AMH again after the laparoscopy and the accuracy of the method went down to 90%. Therefore, more studies to confirm the reliability of the method need to be performed (B. Tezak et al., 2020).

As the direct methods listed in the previous section are either lethal, invasive or require sampling a large number of individuals, it is essential to develop indirect methods to estimate the sex-ratios. Variables such as (1) incubation duration or (2) sand and nest temperatures are used in various studies. (1) The incubation duration was extensively used as a proxy for estimation of the sex-ratio in the past. It is inversely proportional to the incubation temperature (i.e. the higher the temperature, the faster the embryonic development which results in shorter incubation period). Mrosovsky et al. (1999) found a high correlation between incubation duration and sex-ratio ($r= 0.85$). However, this correlation is perceived to be weak for several reasons. Higher temperature speeds up growth throughout the whole incubation period while it affects the sex only during the thermosensitive period (TSP) (Monsinjon et al., 2022), which is an interval during the middle third of embryogenesis when the sex is determined (Mrosovsky & Yntema, 1980). Moreover, high, near-lethal temperatures (which may seem highly feminizing) result in prolonged incubation durations (Monsinjon et al., 2022). Fuentes et al. (2017) compares eight different proxies used for sex-ratio estimations of *Caretta caretta* (six based on temperature, two on incubation duration). The study shows that the estimates differ considerably. The least reliable ones are those based on the incubation duration. Thus, the use of the incubation period is not perceived as a standalone method to be used for the sex-ratio estimations.

(2) Proxies based on temperature (either of a sand or a clutch itself) are considered substantially more accurate than those based on the incubation duration. Vast number of studies used this approach (Wibbels et al., 2003). When the data loggers are placed within the nest, the temperature is recorded for that exact clutch (e.g. can be done also in different depths as the temperature may vary) and there is no need for the data adjustment once collected. However, when loggers are placed in sand (i.e. at

the nest depth recording data generally for the whole beach), the adjustment for the metabolic heating (exerted by eggs increasing the overall nest temperature) needs to be accounted for (Eckert et al., 1999; Yntema & Mrosovsky, 1982). The sex of the hatchlings is determined during the thermosensitive period (TSP), i.e. during the middle third of embryogenesis (Mrosovsky & Yntema, 1980). The TSP does not necessarily equal to the middle third of the total incubation period (Lolavar & Wyneken, 2015). Most of the studies focus on an assessment of temperatures during this second third of the incubation (using the mean temperature for calculations). However, this method is accurate when the eggs are incubated in a constant temperature or in a narrow range of temperatures which is possible only under laboratory conditions (Mrosovsky & Yntema, 1980).

To determine the sex ratio (and also the TSP) in natural nests is a substantially more complex task as it depends also on other environmental factors such as degree of rainfall (Godfrey et al., 1996; Houghton et al., 2007, Lolavar & Wyneken, 2015), sand composition/colour/albedo (Hays et al., 2001), nest position, i.e. degree of sun exposure (Standora & Spotila, 1985), its depth etc. There are also studies suggesting that the measure of temperature variations are more reliable predictors of sex of the hatchlings (Georges et al., 2004; Girondot et al., 2010; Telemeco et al., 2013). The constant-temperature equivalent (CTE) coupled with degree hour model attempts to define a constant temperature that would have the same effect as the fluctuating temperature. An embryo development response above and below PT was considered to be deterministic rather than the relative time spent above or below PT. This has been assessed by incubating eggs in the same mean temperature but distinct temperature variance (Georges, 1989). There are number of ways of converting the fluctuating temperature into CTE. (Georges et al., 1994, 2004) uses the daily temperature basis during the TSP (females are produced when more than a half of the daily embryonic development occurs above the PT and males vice versa). Then the daily temperature at which half of the development appears is converted into CTE. If the CTE value oscillates around the PT (i.e. being in its near proximity above or below), the sex ratio is then more difficult to infer and the interpretation is not quantitative. Thus, the CTE method is only reliable when the CTE value exceeds/is below the PT consistently (Delmas et al., 2008; Georges et al., 2004). Girondot et al. (2010) uses a more refined model of CTE including the embryonic thermal reaction norm, gonadal growth and the aromatase activity (cytochrome P450). This study focuses on a freshwater turtle *Emys orbicularis*. As there is not a large amount of molecular and embryological data for other species, the model is dependent only on the constant temperature TSD pattern and the thermal reaction norm of embryonic growth. Monsinjon et al. (2022) lists number of reasons why the CTE model is perceived as suboptimal. The study tests new proxies against the commonly used ones. Availability of temperature data from 151 nests (linked with a verified sex of 10 individuals/nest from the total of 142 nests) allowed to compare

these and shows, that the model used in the current study is capable to interpret sex-ratios with a considerably higher accuracy (AIC, Akaike Information Criterion method was used for models' comparison). Thermal reaction norm for sexualization (Schoolfield et al., 1981) is used as it links temperature and the molecular mechanisms responsible for the sex determination of an embryo. The model also accounts for differences in the sensitivity of sexualization during the TSP, meaning the effect of temperature on sexualization may be different in the different points throughout the TSP (e.g. stronger at the beginning than at the end of the TSP). Moreover, the TSP is not strictly set as the middle third of the incubation period but the location as well as duration may vary. It is recommended to undertake similar studies using the model to verify its accuracy (as the study is first of its origin comparing the observed sex-ratios from natural clutches and the predicted sex-ratio values from the most widely used methods). the minimal data needed to incorporate the model are: (i) in situ temperature data throughout the whole incubation duration, (ii) consistently collected measurements of embryos/hatchlings (e.g. straight carapace length), (iii) sexed subsample of the hatchlings per nest, (iv) constant temperature (i.e. from laboratory experiments) sex-ratio data, while all the data are collected within the same population.

Mrosovsky et al. (2002) follows the traditional, widely used method including the mean temperature during the TSP and the PT of 29.3 °C. The method is conducted under laboratory conditions using the constant temperature, hence the use for natural nests may not be completely accurate. Although this is known, considerable number of studies is still using this method as the data availability for the use of other, more accurate methods, is scarce. There is also similar approach to assess the sex-ratio using the logistic function while fitting the data using maximum likelihood method (Girondot, 1999). It is also assumed to be used with constant incubation temperatures. Abreu-Grobois et al. (2020) describes several models to determine the sex-ratio (e.g. logistic, Hill, A-logistic, Hulin). A new asymmetric flexit model (4 parameters) is introduced and compared with the logistic model (2 parameters). According to AIC and the Akaike weight, the flexit model performs better. However, some parameters are challenging to fit and thus, only the logistic model results are further discussed in the study. As unable to derive PT and observe stages of embryogenesis of the local population in situ, the logistic model is used in the current study as well. Therefore, the results should be interpreted with caution as the real situation may differ. A thorough description of the method itself is provided in the methodology section.

3. Case Study

3.1. Study Area

The study was conducted on the Maio Island (part of the Cabo Verde archipelago consisting of 10 islands in West Africa). Florida, USA (northwest Atlantic) and Oman (northwest Indian Ocean) are the biggest known rookeries of *Caretta caretta*, with Cabo Verde being the third largest (Casale & Marco, 2015). However, findings by Patino-Martinez et al. (2021) suggest that the nesting subpopulation is far bigger than previously estimated. The nesting activity in the span 2016- 2019 of $95762 \pm \text{SD}$ nests is shown to be currently the biggest one globally. Boa Vista island hosts approximately 75% of the Cabo Verde archipelago’s nesting activity (Tanner et al., 2019) while Sal and Maio islands account for 16.6% (Laloë et al., 2019).

The island of Maio is relatively small in size (269 km²) and consists of 110 km long coastline out of which 38 km are sandy beaches (i.e. potential turtle nesting sites) with almost no anthropogenic

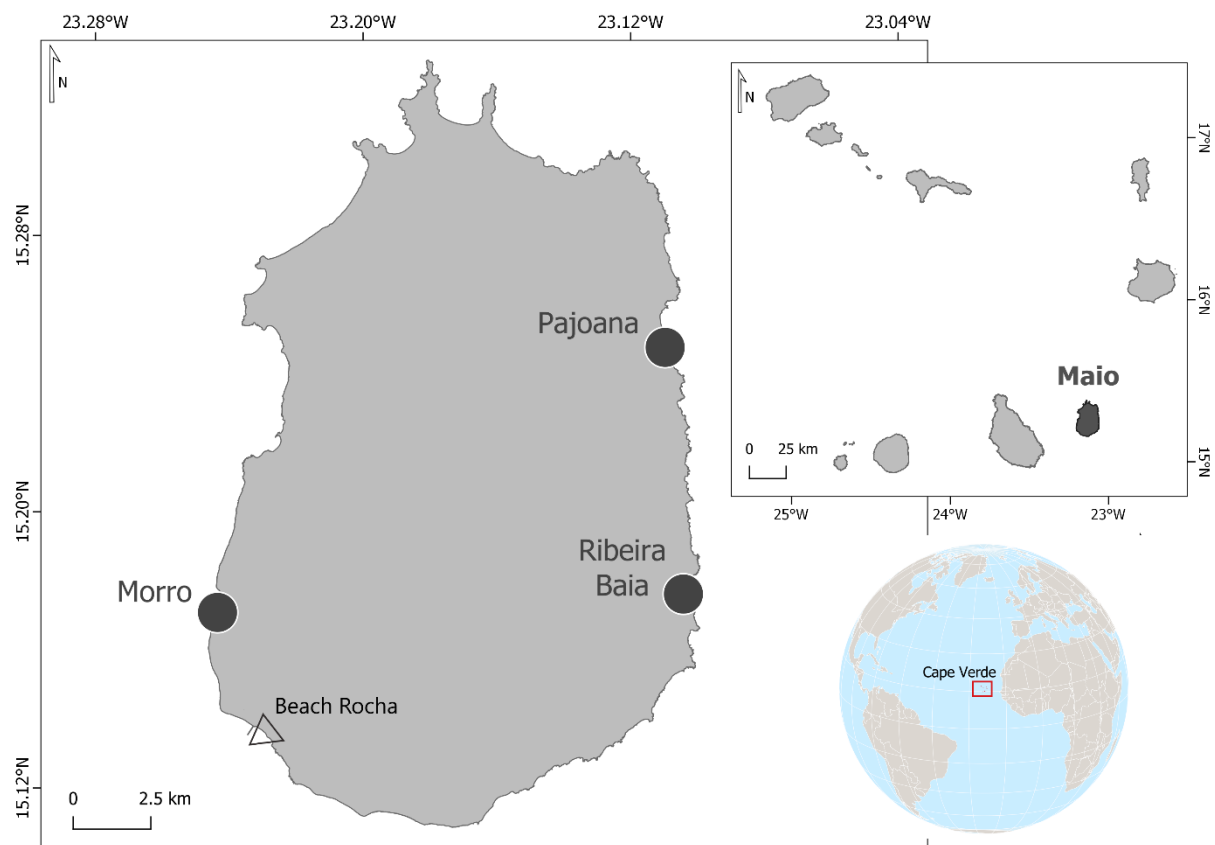


Figure 2: Map of the study area, Maio, Cabo Verde showing the three locations of interest as dots and the hatchery location highlighted in triangle (the location of the Cabo Verde archipelago is shown on the globe- West Africa, and the detailed map of the whole archipelago shows the location of Maio Island within).

disturbance. The beaches possess various sand colours as well as composition and grain sizes differ (Patino-Martinez et al., 2022). Therefore, the HOBO TidbiT v2 temperature data loggers were deployed in three distinct areas, each characterized by different sand type. The Figure 2 depicts the three study locations: Morro- white-coloured sand, Pajoana- dark-coloured sand, Ribeira Baia- mixed sand (the difference of the sand colours is shown in the Figure 3). Moreover, air temperature loggers were deployed near the Beach Rocha (location marked with a triangle in Figure 2). Lastly, the temperatures were analysed within the hatchery (Beach Rocha), including inside a nest. The hatchery (Figure 4) was protected by a fence (against predators) and was checked daily. A total of 92 loggers were deployed during the study period and 414,970 data points were analysed.



Figure 3: Different types of sand present at the three study locations: a) Pajoana (dark-coloured beach), b) Ribeira Baia (mixed-sand beach), c) Morro (light-coloured beach).
Photo: Juan Patino-Martinez archive.



Figure 4: Hatchery located on Beach Rocha (where the study nest for the metabolic heating assessment was placed).
Photo: Katarína Gajdošechová.

3.2. Maio Biodiversity Foundation (FMB)

The Maio Biodiversity Foundation is an NGO operating on the island of Maio. Turtle conservation and protection is one of its main projects. As the tradition of eating turtle meat still has not vanished completely, efforts to stop poaching on the island are exerted. Local awareness and educational programs, visits of the hatcheries and the nesting beaches, night patrols consisting of locals, local team leaders within each area are all examples of measures implemented to prevent the poaching. The situation has gotten better over the years also thanks to the new law (passed in 2018) criminalizing the capture, consumption and trade of sea turtles (or their parts) as well as any disturbance of their nesting habitat. Nevertheless, the collaboration with local natural leaders has had the biggest impact in terms of changing locals' view of turtles as a source of meat and tradition pursuance (Patino-Martinez et al., 2020).

Conservation efforts also include the hatchery, which is situated in the main city, Porto Inglês. Educational activities (not only for children from local schools) take place here. The nests at risk (e.g. of inundation laid in the risk zone too close to the water line) are mostly reburied in the safe zone

within the given beach and do not need to be relocated to the hatchery. FMB personnel made it feasible to collect and rebury the clutch in the study nest within the hatchery. The rest of the data used in the study are supplied from FMB as well and the collection and methods used are described in the following chapter.

4. Methodology

4.1. Data Collection

HOBO Tidbit v2 temperature loggers (with an accuracy of ± 0.2 °C and 12-bit resolution) were used to collect the data throughout the nesting seasons in the period 2017-2023. To ensure the accuracy, the loggers were placed in a box with a known temperature 48 hours before and after the deployment (if some logger recorded data differing from the mean of the tested loggers by more than 0.5°C, the logger was excluded and the data were not included in the analysis). The logging interval was set to record the temperature every 30 minutes and the data were recorded simultaneously (among all deployed loggers). Once the needed data were recorded, the loggers were collected and the data downloaded with the use of an optic USB base station and the HOBOWare software (www.onsetcomp.com, Figure 5).



Figure 5: Scheme showing how an optical interface is used for retrieving the collected data.
Source: www.onsetcomp.com

4.1.1. Metabolic heating

Metabolic heating (MH) is the additional temperature increase that occurs in a nest due to the developing eggs during the incubation period, compared to the sand temperature at the same depth. This increase varies throughout different stages of incubation and within the nest (Godfrey et al., 1997). The average MH for the Cabo Verde population has previously been determined to be 0.5°C on the islands of Sal and Boa Vista (Abella Perez et al., 2016; Laloë et al., 2014). Despite having this information, we assessed the MH in a nest relocated to the hatchery (a white sand beach located at Beach Rocha, Figure 2). The clutch (73 eggs) was relocated immediately after the turtle finished laying the eggs, and the process did not exceed 60 minutes. A data logger was placed within the egg chamber at a depth of 55 cm (after the first 20 eggs). The study nest was positioned in the centre of the hatchery

(with no shade during the day). The control logger was deployed at 55 cm depth, positioned 1 m from the study nest. Both loggers were aligned parallel to the waterline.

MH was calculated every 30 minutes as the difference in temperatures between the study nest logger and the control logger (using the period from October 1, burial day, to November 19, first emergence day, 2023). White sand and the depth of 55 cm were chosen for the MH study with the aim of seeking the highest possible value (Martins et al., 2020).

4.1.2. Sand and air temperature

Thermal variability between beaches was assessed using a total of 72 data loggers, distributed across 3 types of sand based on their colour (Figure 3). Temperatures at four different depths (27 cm, 35 cm, 45 cm, and 55 cm) were compared for each type of substrate except for the years 2017 (27 cm, 35 cm), 2018 (27 cm, 35 cm, 45 cm) and 2023 (35 cm, 45 cm, 55 cm). A total of 14 data loggers (one pair per year) were programmed to record temperature every 30 minutes, simultaneously with the sand data loggers. These were deployed in a shadow under the roof, approximately 150m from the water line. All temperatures were monitored annually from July 1 to November 17.

4.2. Data Analysis

The data were analysed and plotted with the use of RStudio (using R software version 4.3.3., R Core Development Team; www.r-project.org). A visual inspection of the q-q and the frequency distribution plots shown a close to normal distribution of the sand temperature data and non-normal distribution of the air temperature data (Appendix 1). Quantitative normality tests (e.g. Shapiro-Wilk test in Appendix 1) proved non-normality of both datasets. The sand temperature database was extensive (more than 370000 records) and thus, the numerical analysis may have been overly sensitive to any small deviations from normality. Nevertheless, non-parametric tests were used to assess the temperature as well as sex-ratio data. A significance value α of 0.05 was used throughout all tests.

4.2.1. Temperature analysis

To assess the interbeach variation, interannual variation and variation across depths, the sand temperature data were compared. The non-parametric Kruskal-Wallis test was used to assess a significant difference between the groups. Moreover, the sand and air temperature correlation was assessed by non-parametric Spearman's rank correlation coefficient. To find any lag between the air and sand temperature, the ccf R function (cross-correlation function) was used.

4.2.2. Sex-ratio estimation

The *embryogrowth* R-package (Girondot, 2023) was used to model the TSD pattern. As there were no data available for the current population, data from Marcovaldi et al. (1997) were used to construct the model. The database (sex-ratio data and the constant incubation temperature) was available within the *embryogrowth* R-package. Five models (Logistic, Hill, A-logistic, flexit and double A-logistic) were applied to the data from Brazilian population of *Caretta caretta*. The best model according to AICc (Burnham & Anderson, 2002) was the logistic while Hill model had $\Delta AICc$ of 0.02 and thus, these two were considered for further analysis.

The mean temperature of 18-day intervals (the calculated average length of the middle third of development, i.e. the TSP) was determined. The interval start date was always postponed by one day. The mean TSP temperatures (also adjusted for metabolic heating, i.e. +0.5°C) were then fitted to both models and the sex-ratio was derived. Although significant difference was confirmed by the paired t-test between sex-ratio values produced by Logistic and Hill model (high sensitivity of the test due to large dataset), the mean difference was only 0.00027 and thus the practical significance was not concluded. Therefore, the models were perceived as producing similar results for the given dataset and only the logistic model was used in further analysis (Equation 1). Sr being the sex-ratio at a given incubation temperature T (i.e. the TSP mean), PT is the pivotal temperature (29.125°C determined for the Brazilian population by the program TSD; Appendix 2) and S is the curve shape at the transition from masculinizing to feminizing temperature (Girondot, 1999). The Kruskal-Wallis test was used to analyse significant differences in sex-ratios, and a post-hoc Dunn's test was applied when significant differences were detected.

$$sr(T) = (1 + e^{S^{-1}(PT-T)})^{-1} \quad (1)$$

4.2.3. Future sex-ratio estimation

To identify the relationship between the sand temperature (the actual collected sand data), the air temperature and the SST (sea surface temperature) a linear regression analyses were used (Bentley et al., 2020). The surface air temperature and SST (only the monthly averages were available to download) were sourced from the Copernicus Interactive Climate Atlas (<https://atlas.climate.copernicus.eu/atlas>) which is a live evolution of the IPCC WGI Interactive Atlas (<https://interactive-atlas.ipcc.ch/>). The data from three IPCC (2023) emissions scenarios were used: SSP1-2.6, SSP3-7.0, and SSP5-8.5, corresponding to projected temperature increases of +1.5°C to 2°C, +3.2°C to 4.0°C, and +4.3°C to 5.7°C by 2100. Each model was run for all three scenarios (Table 1). The models' comparison was then made using AIC (Akaike, 1974). The R-squared was also calculated to

confirm the AIC results. Once the best model for each combination of sand type, depth and the given emissions scenario was selected, the sex-ratio was calculated using the estimation methodology described in the previous subchapter, *Sex-ratio estimation*.

Table 1: Correlative model equations for the sand temperature calculation. C represents the intercept value of the given regression model. M and N define the linear constants.

Model	Equation	Independent variables
1	$T(\text{sand}) = M * T(\text{air}) + C$	Air temperature according to the IPCC SSP1-2.6, SSP3-7.0 and SSP5-8.5 scenarios
2	$T(\text{sand}) = M * T(\text{sea}) + C$	SST according to the IPCC SSP1-2.6, SSP3-7.0 and SSP5-8.5 scenarios
3	$T(\text{sand}) = M * T(\text{air}) + N * T(\text{sea}) + C$	Air temperature and SST according to the IPCC SSP1-2.6, SSP3-7.0 and SSP5-8.5 scenarios

5. Results

5.1. Temperature Analysis

The sand temperature data were non-normally distributed in each study year, as indicated by the various shapes of the violins (Figure 6). The median and the density shown by the violin in 2018 and 2019 provided the first indication that the highest proportion of males may have been generated (as these were visibly the coldest years on average). The highest range of temperatures collected was in the years 2020 and 2022. The minimum half-hourly point sand temperature measured was 24.24°C and the maximum was 37.48°C. Data from 2023 showed the highest density at the warmest temperatures, indicating it was the warmest year of the entire study period.

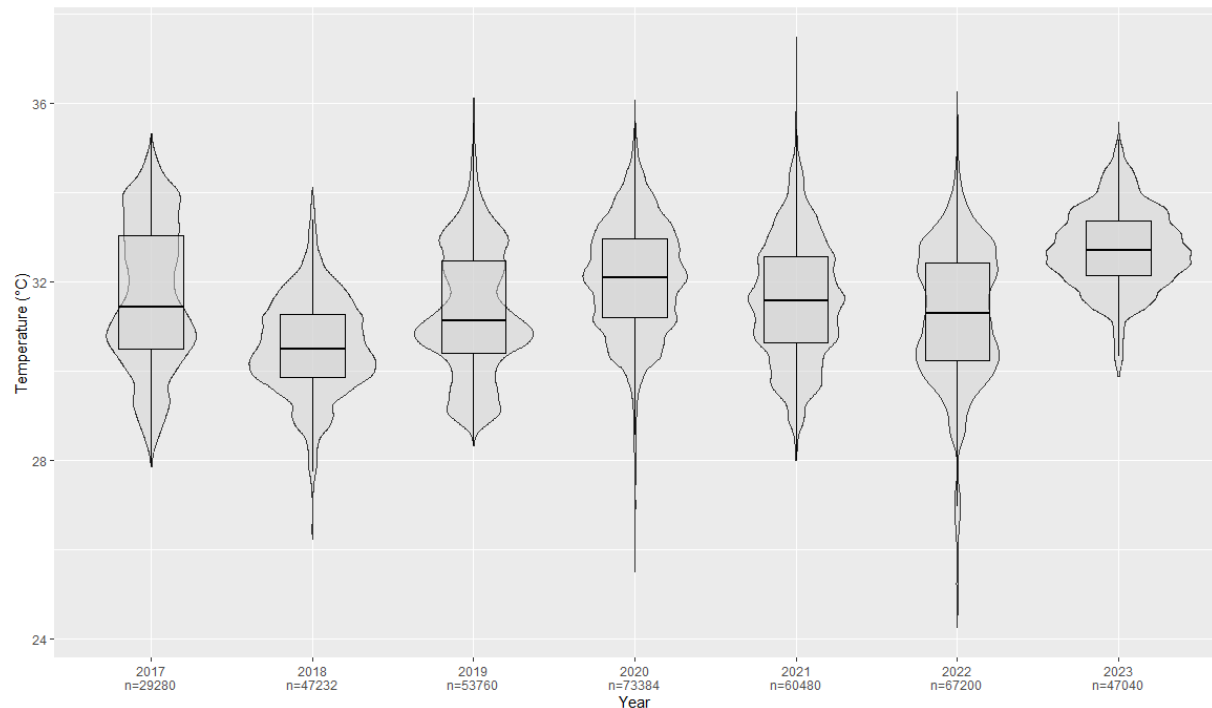


Figure 6: Box on top of violin plot showing sand temperature data collected in the 7 study years (the n below the years indicates the number of observations for the given year, the boxes represent the inter-quartile range with the horizontal line being the median and the whiskers show the range). The violins show the distribution of the temperatures for each year.

5.1.1. Interbeach variation

A significant difference in the daily average temperature was observed among the three sand types. The Kruskal-Wallis test was conducted separately for each year and depth combination. Chi-squared values ranged from $\chi^2 = 24.29$ to $\chi^2 = 250$, with p-values smaller than 0.05 for each combination of depth and year, providing evidence of their difference. The three types of sand followed a similar trend, but each within a different range of temperatures. When all years were averaged, the white sand was the coldest (mean = 30.6°C \pm SD 1.13), while dark sand was shown as the warmest (mean =

32.4 °C ± SD 1.25), with mixed sand following closely (mean = 32.2 °C ± SD 1.16; Figure 7A). The lowest average temperature was detected in white sand in 2022 (29.9 °C ± SD 1.18) while the highest average temperature was observed in the dark sand in 2023 (33.26 °C ± SD 0.76). Additionally, mid-September, when the most precipitation typically occurs in Cabo Verde, was associated with a decrease in sand temperatures (most markedly visible in mid-September in 2018 and 2020; Figure 7B). The highest concentration of temperatures close to the pivotal temperature (PT = 29.125°C) were observed in white (n = 6,672) and mixed (n = 251) sands in July while in dark sand the highest concentration was detected in September (n = 675).

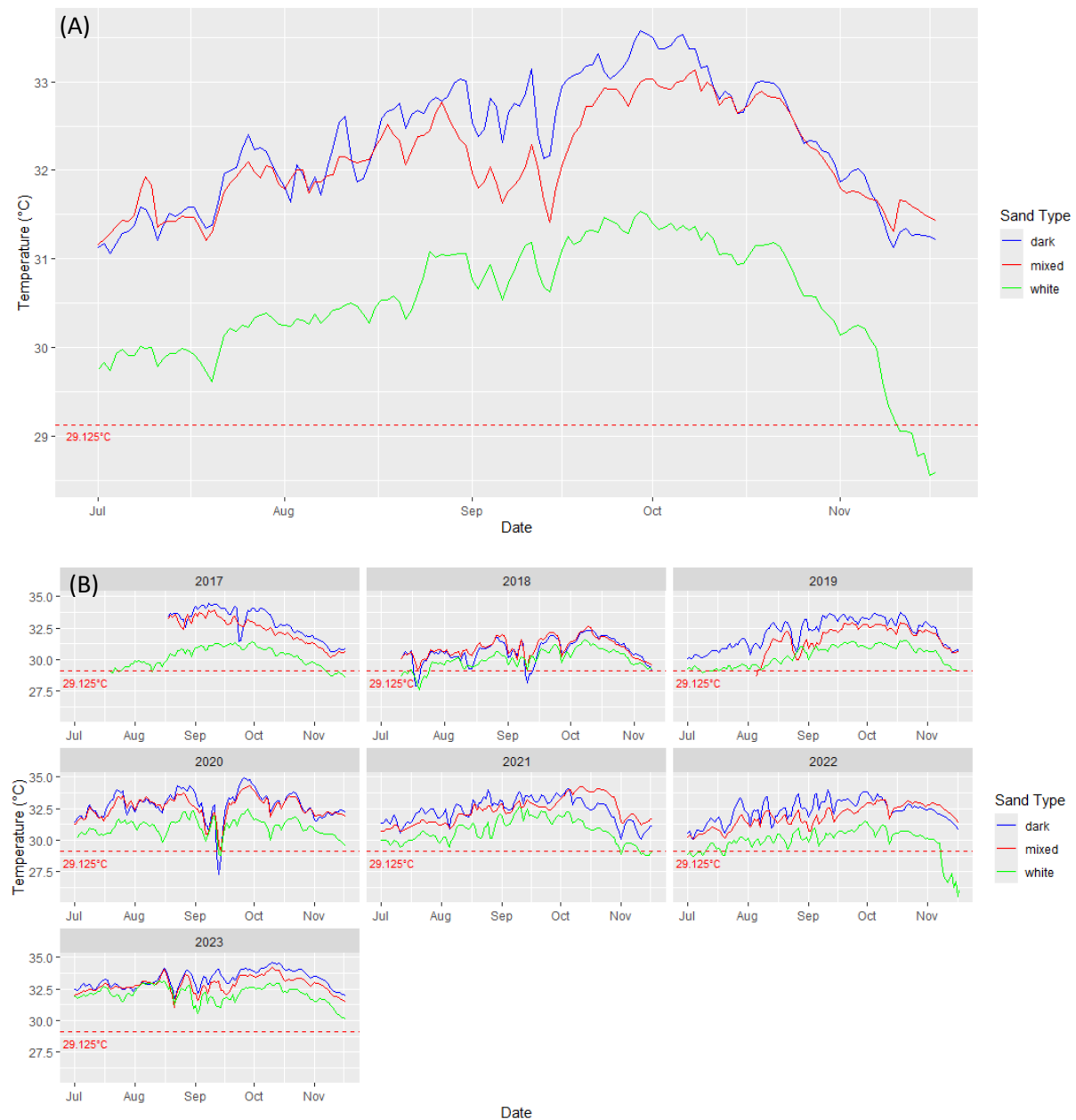


Figure 7: (A) Line graph comparing the daily average temperature of the 3 distinct colours of the sand across the nesting season (averaged from all of the depths and from the whole collecting period, i.e. 2017- 2023). (B) Comparison of the daily average temperature of the 3 types of sand shown for each study year separately.

5.1.2. Interannual variation

Since a significant difference between the temperatures of distinct sand types was confirmed, an assessment of the interannual variation was conducted for each sand colour separately. A significant difference was found among the years (for each combination of sand type and depth with chi-squared χ^2 from 15.76 to 425.12, $p < \alpha$). When all sand types averaged together, 2018 was the coldest year, with a mean temperature of $30.57^\circ\text{C} \pm \text{SD } 0.92$ (Figure 8). The lowest mean temperature (only temperature data with common dates among years included) in the white sand was recorded in 2018 (mean = $30.04^\circ\text{C} \pm \text{SD } 0.76$), followed by 2022 ($30.17^\circ\text{C} \pm \text{SD } 0.7$) and 2019 ($30.4^\circ\text{C} \pm \text{SD } 0.76$). The dark (mean = $30.73^\circ\text{C} \pm \text{SD } 0.96$) and mixed (mean = $30.95^\circ\text{C} \pm \text{SD } 0.78$) sands also detected the lowest mean temperature in 2018, with 2019 and 2022 being the subsequent coldest years.

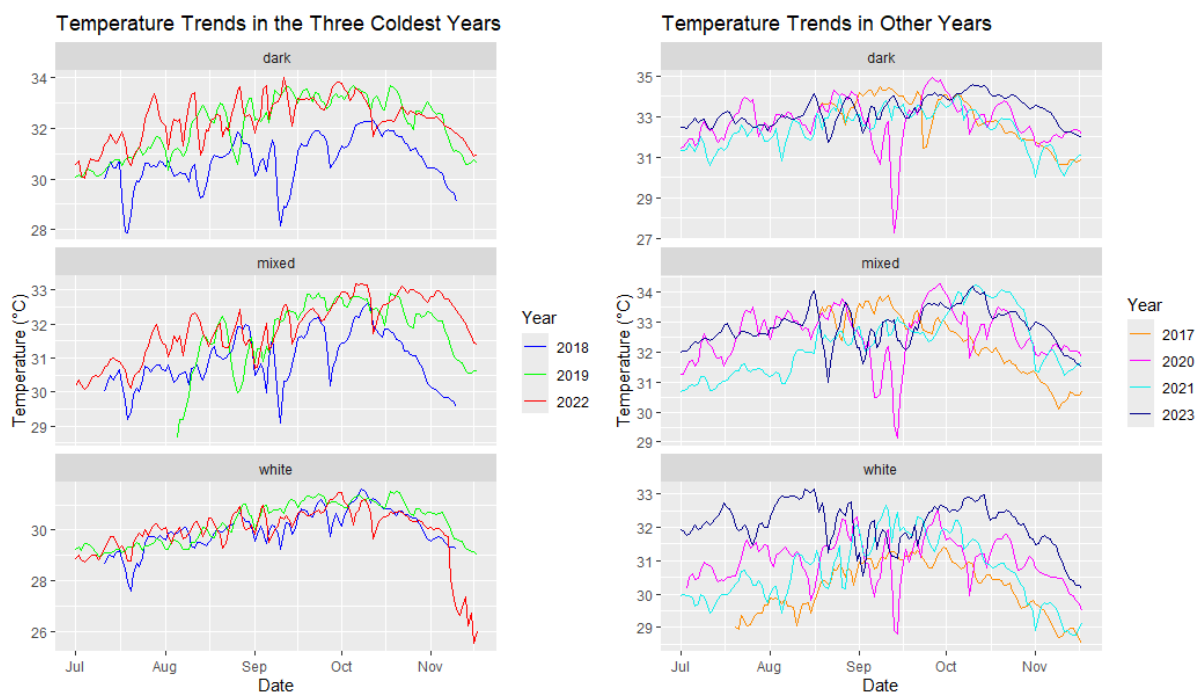


Figure 8: Comparison of average daily temperatures by the study years (sand types and depths averaged). The 3 coldest years (2018, 2019, 2022) are plotted separately.

Furthermore, the entire study period time series was analysed, focusing on the common period of all years (July 20- November 10, i.e. 114 days/year). The series was decomposed into trend and seasonal components, with the trend component removing the seasonality and vice versa, the seasonal component removing the trend (Figure 9). Both were present, with the trend showing an upward tendency throughout the years, with two notable troughs: an absolute minimum during 2018 and an additional local minimum during 2022. The seasonality revealed that the lowest temperatures were present at the beginning and end of the nesting seasons and the peak in temperatures was detected roughly after the middle of the season.

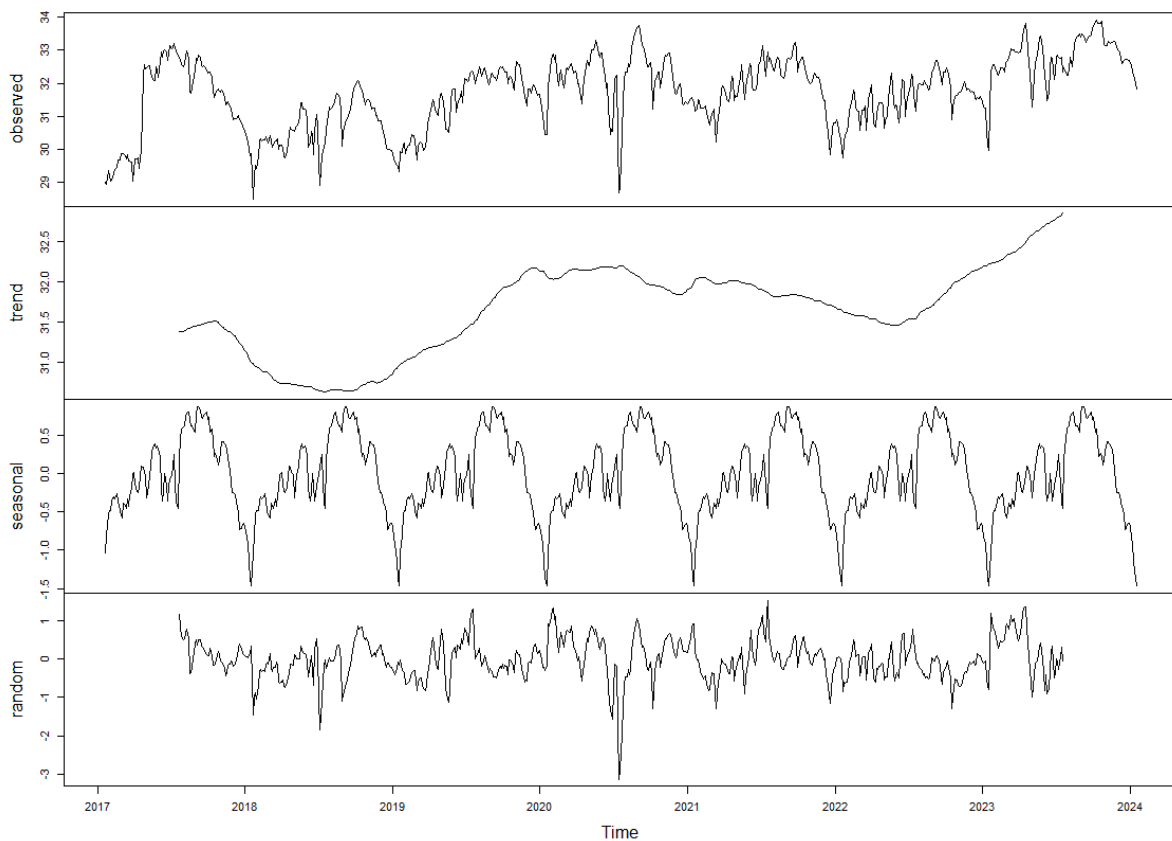


Figure 9: Decomposition of additive time series over the entire study period (20 Jul- 10 Nov across all years, i.e. frequency of 114 days/year is used) into trend, seasonal and random components.

5.1.3. Temperature variation across depth

The variation in temperatures among depths was also assessed for each sand type separately (Figure 9). No significant difference was detected during 2017 (in white and mixed sands, $df = 1$, depth difference < 11 cm) and at the end of the nesting seasons (mid-October onwards; 2019-2022) in white and dark sand. Nevertheless, significant differences among depths were observed in the majority of combinations of years and sand types (χ^2 from 4.11 to 136.73, $p < \alpha$). Therefore, it was concluded that significant differences among depths were generally present. On average (all years pooled and only data with corresponding date for each sand type and depth included), the coldest temperatures were recorded at a depth of 55 cm for dark sand ($32.12^\circ\text{C} \pm \text{SD } 0.8$), white sand ($30.28^\circ\text{C} \pm \text{SD } 1$) and mixed sand ($32.4^\circ\text{C} \pm \text{SD } 0.8$).

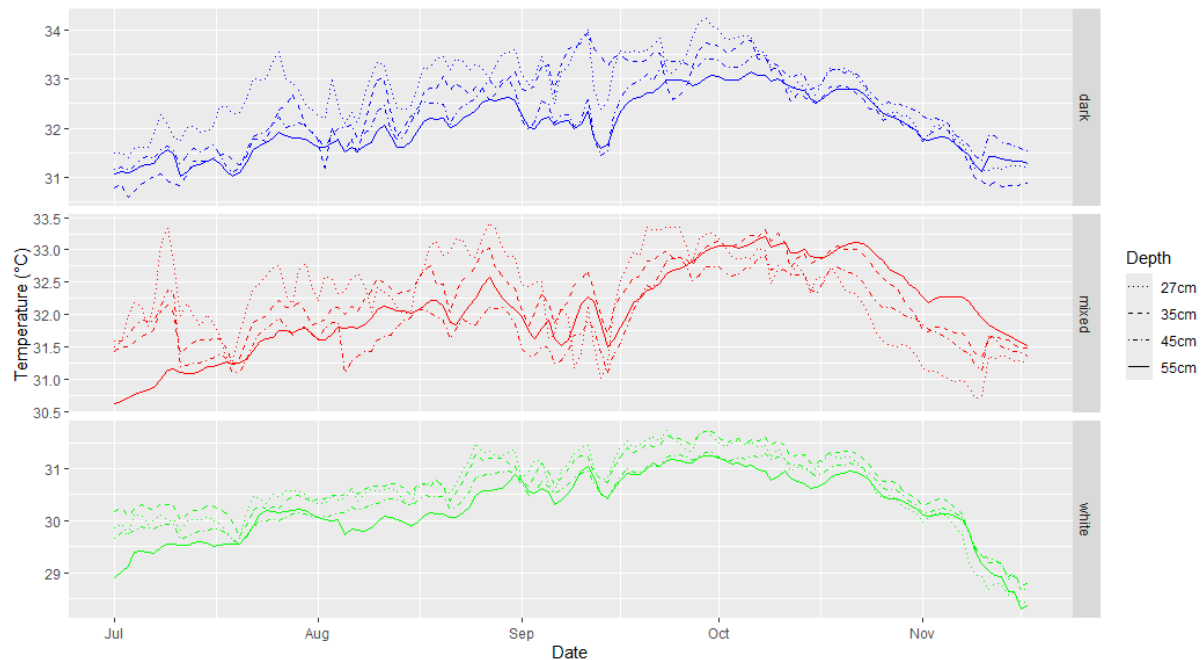


Figure 10: Comparison of the daily average temperature (average of all study years) across 4 depths (27 cm, 35 cm, 45 cm, 55 cm) plotted for each type of sand separately.

Moreover, temperature differences among depths were calculated for each date and time combination (i.e. for every 30-minute interval for each sand type separately and only records with corresponding logging time and date were analysed) and then averaged across all years (Table 2). From the reference point of 27 cm, the average temperature reduction in white sand was 0.21°C 8 cm deeper, 0.40°C 18 cm deeper and 0.55°C 28 cm deeper (i.e. an average reduction of 0.18°C by each 10 cm). In dark sand, similar reductions were observed: reduction of 0.18°C 8 cm deeper, 0.36°C 18 cm deeper and 0.58°C 28 cm deeper (i.e. an average reduction of 0.19°C by each 10 cm). The reduction values for mixed sand along with other depth comparisons are stated in the Table 2. Overall, the

highest reductions in temperature were recorded between 27 cm and 55 cm in dark sand (0.58°C) and white sand (0.55°C). Relatively high SD values were observed for all depth comparisons.

Table 2: Temperature reduction averaged across the entire study period for each sand type separately, with corresponding standard deviations (SD) and number of observations (n).

Sand type	Reference depth	Comparison depth	Mean temperature reduction (°C)	SD	n
dark	27cm	35cm	0.18	0.45	47040
dark	27cm	45cm	0.36	0.70	47040
dark	27cm	55cm	0.58	0.86	47040
dark	35cm	45cm	0.18	0.35	47040
dark	35cm	55cm	0.40	0.57	47040
dark	45cm	55cm	0.22	0.25	47040
mixed	27cm	35cm	0.14	0.27	26880
mixed	27cm	45cm	0.32	0.55	26880
mixed	27cm	55cm	0.39	0.63	26880
mixed	35cm	45cm	0.18	0.30	26880
mixed	35cm	55cm	0.24	0.39	26880
mixed	45cm	55cm	0.06	0.10	26880
white	27cm	35cm	0.21	0.41	80104
white	27cm	45cm	0.40	0.64	80104
white	27cm	55cm	0.55	0.75	80104
white	35cm	45cm	0.18	0.28	80104
white	35cm	55cm	0.34	0.41	80104
white	45cm	55cm	0.15	0.18	80104

5.1.4. Sand and air temperature relationship

A moderate to strong positive correlation was observed between the daily average sand and air temperatures, with rho values ranging from 0.42 to 0.67. This correlation was statistically significant and was confirmed both for overall averages across all sand types and depths (Appendix 3A) as well as separately for each combination of these variables (Appendix 3B).

Moreover, a lag of one day was observed between the air and sand temperatures in the shallower depths of the sand. At the depth of 55 cm, a two-day lag was noted for mixed and white sand, while in dark sand, the cross-correlation was nearly identical for both one- and two-day lags (Figure 10).

Subsequently, the correlation was reassessed by applying two-day lag to all sand types at 55 cm depth and one-day lag to the rest of the depths. A stronger positive correlation was detected compared to the initial correlation assessment, with the range of rho values shifting to 0.55-0.77. The significance was confirmed both for the averages of all sand types and depths combined (Figure 11A) and for each of these groups separately (Figure 11B).

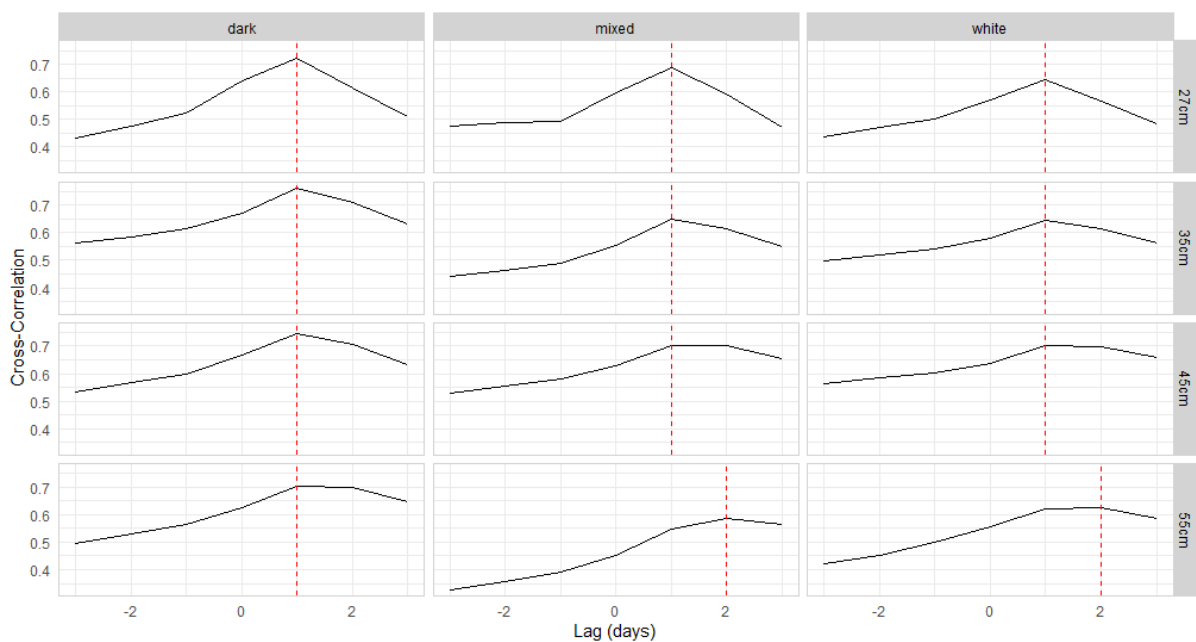


Figure 11: Air-sand temperature cross-correlation with the most significant lag highlighted (red dashed line) for different types of sand and depths shown separately.

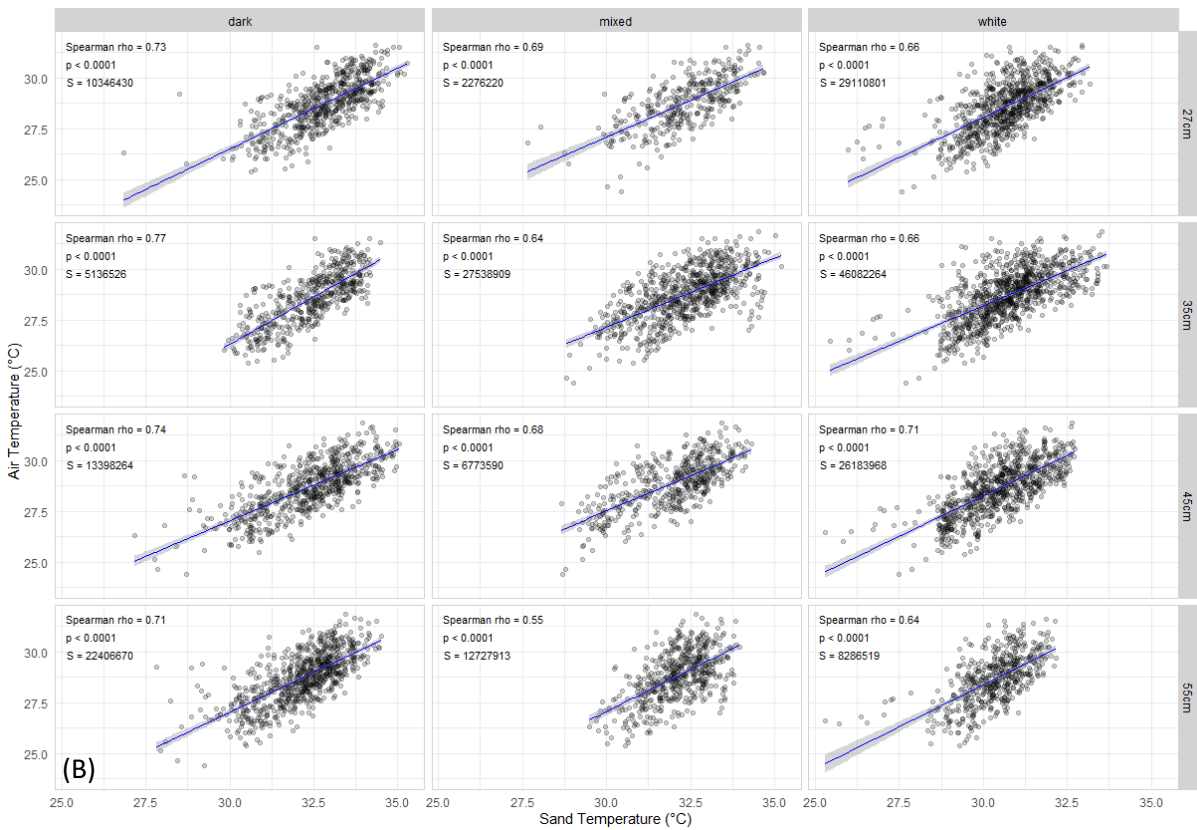
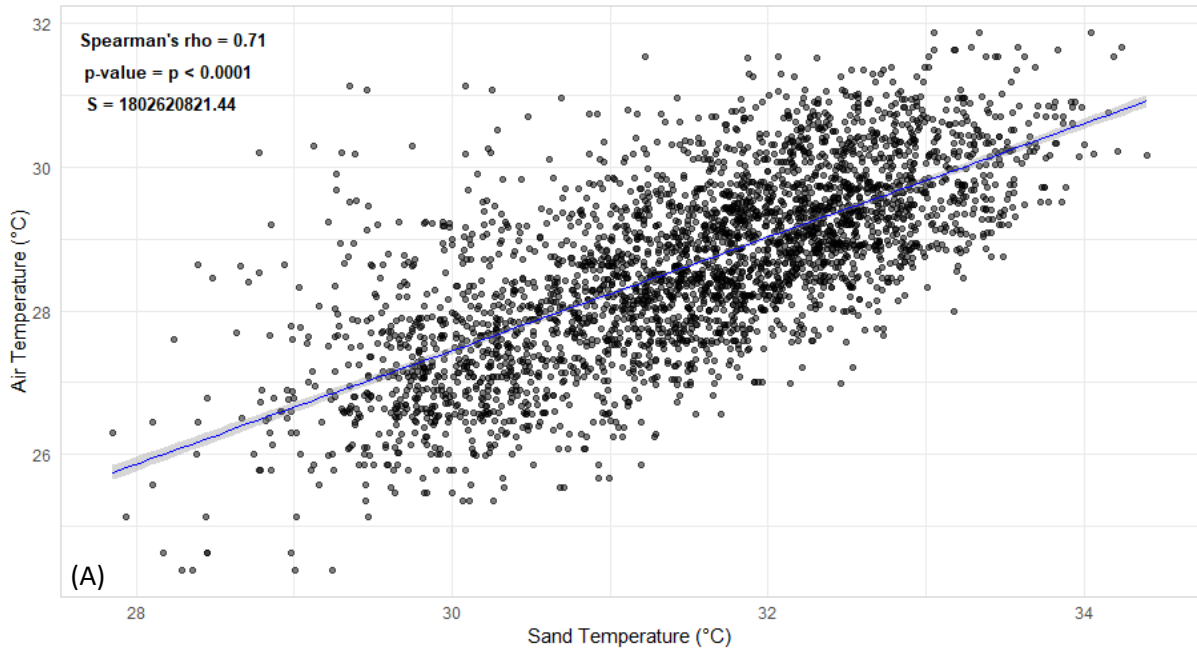


Figure 12: Scatter plots showing the correlation between the daily average air and sand temperatures (A) for all sand types and depths jointly (the group values are averaged) and (B) for each group (sand type and depth) separately. Caption in the upper left corner shows the Spearman's correlation test results. Regression line (blue) and the 95% confidence interval band (grey) depict the positive correlation trend.

5.2. Present Sex-ratio

5.2.1. Metabolic heating

Metabolic heating (MH) appeared to be negative at the beginning of the incubation period (the control logger was deployed earlier and had time to adjust to the sand temperature, whereas study logger was placed in the nest at the time of egg burial). A gradual increase was observed throughout the incubation, with the highest mean temperature observed in the final third (1.14°C ± SD 0.29). To focus on the sex deterministic period (i.e. the middle third of incubation), the mean MH at 55 cm depth was 0.45°C ± SD 0.17. Therefore, the estimated value of 0.5°C was carried forward to further analysis of the primary sex-ratio.

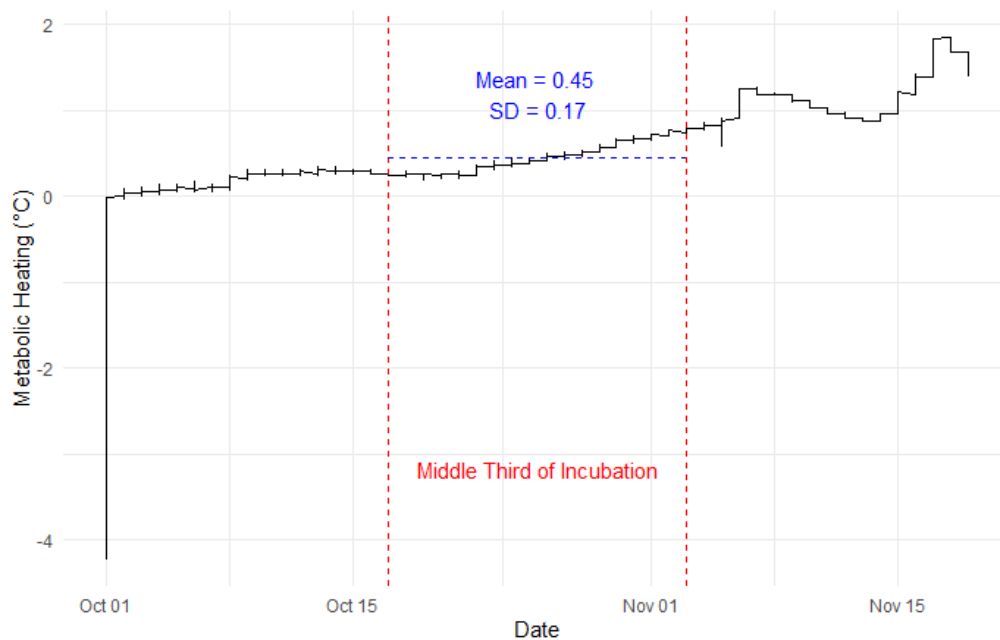


Figure 13: Metabolic heating measured in the study nest within the hatchery (01 Oct- 19 Nov 2023) at 55 cm depth. The red dashed lines indicate the middle third of the incubation period (33% to 66% of the total incubation) and the blue dashed line represents the mean MH during the middle third along with its value and SD.

5.2.2. Sex-ratio

The overall proportion of females found across all types of sand was 99.2%. A significantly smaller proportion of females was produced in the white sand (mean = 98% females; $\chi^2 = 197.99$, $df = 2$, $p < 0.05$) when compared to either the dark or mixed sand (approaching 100% of females produced in each, with no significant difference between them). Moreover, a potential to produce fewer females in the white sand in the beginning and end of the nesting season was detected (Figure 13). Significantly fewer females were produced in July (94% females; $\chi^2 = 145.73$, $df = 4$, $p < 0.05$) compared to the rest of the season. Additionally, no significant difference was observed between August (98.7% females) and the last month of the study period (mid-October to mid-November; 97% females), although both were significantly different from all other months. Thus, the highest probability of producing the least number of female hatchlings was observed in the white sand in the beginning of the monitoring season.

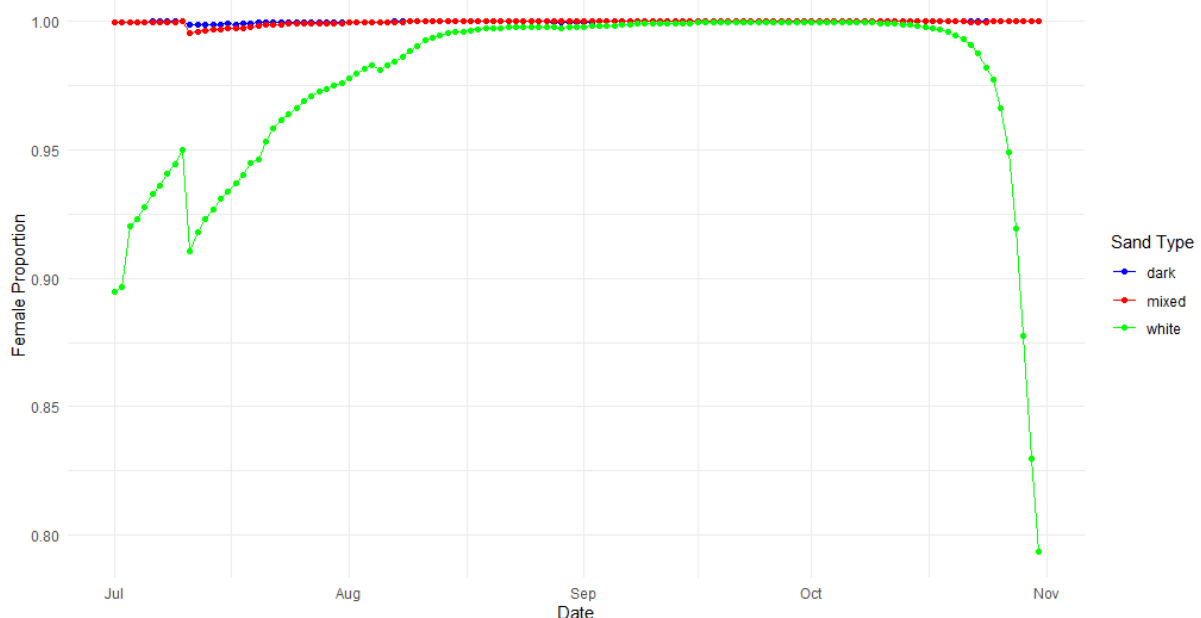


Figure 14: Comparison of sex-ratio (female proportion) among different types of sand during the nesting season (averaged for all depths and the entire study period). The dots indicate the start dates of the 18-day intervals used to calculate the sex-ratio.

Based on the visual inspection of sex-ratios assessed at different depths (Figure 14), significance tests were executed only for the white sand (for each year separately). In 2018, a significantly lower proportion of females was produced at the 45 cm depth (mean = 87% females, $\chi^2 = 145.25$, $df = 2$, $p < 0.05$), with the greatest difference noted between the 27 cm and 45 cm depths ($z = 11.71$). In 2019, the most substantial effect size ($z = 6.36$) was again found between the 27 cm (99.5%) and 45 cm (93.9%) depths ($\chi^2 = 43.17$, $df = 3$, $p < 0.05$). Although in 2020 and 2021 the visual analysis suggested no significant differences among depths (mean female production approaching 100% across all

depths), the high sensitivity of the statistical test given by high precision of the sex-ratio values revealed significant differences with the largest effect sizes ($z = 9.79$ and $z = 4.62$, respectively) between the 27 cm and 55 cm depths. In 2022, a significantly lower proportion of females was produced at the 55 cm depth ($\chi^2 = 120.77$, $df = 3$, $p < 0.05$), with the highest effect size ($z = 10.68$) noted between the 27 cm (97.7%) and 55 cm (91.8%) depths. In 2023, despite mean female percentages approaching 100% at both tested depths (35 cm and 45 cm), a significant difference was detected ($\chi^2 = 58.84$, $df = 1$, $p < 0.05$). Thus, years 2020, 2021 and 2023 were considered to have a very little practical significance among depths, while the highest significance was detected within 2018.

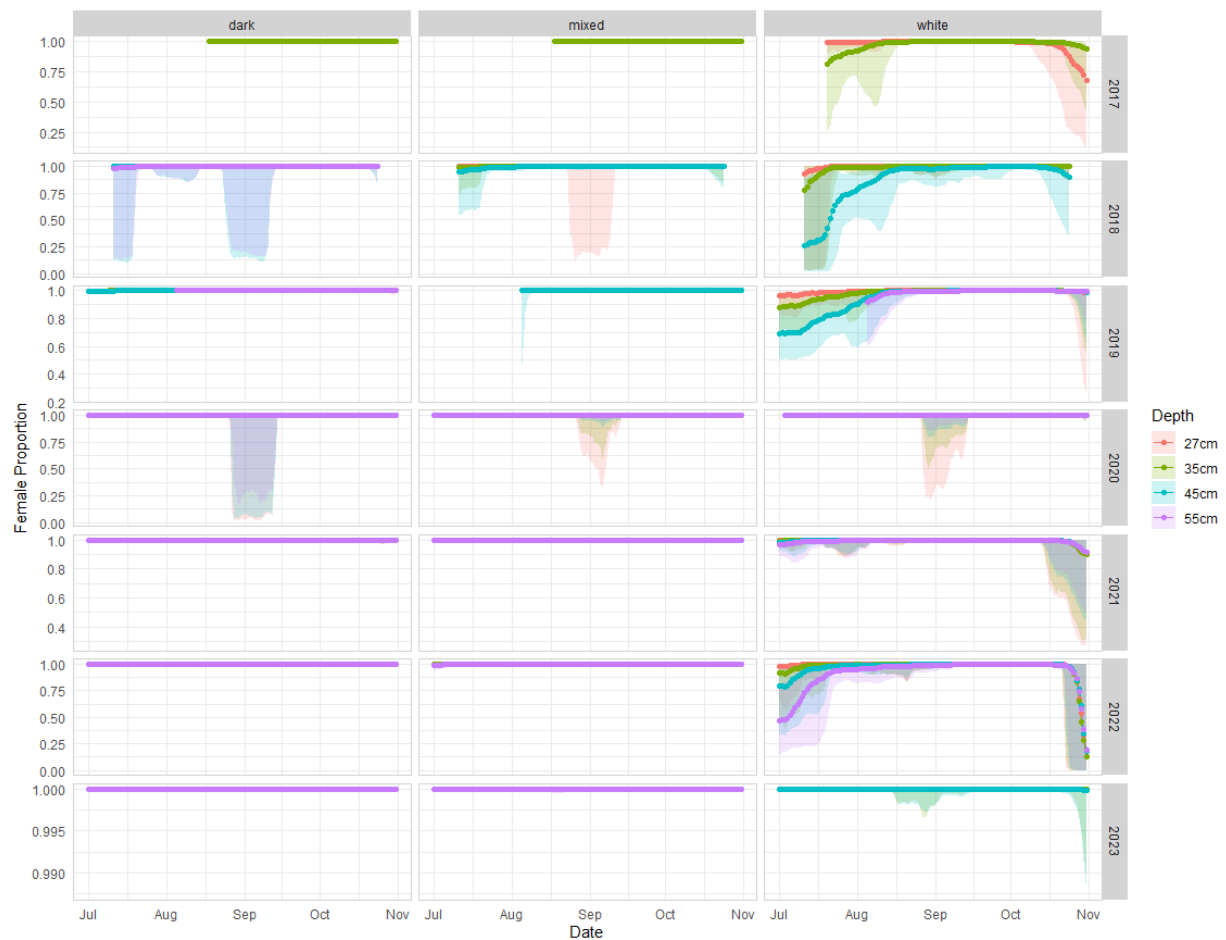


Figure 15: The sex-ratio (female proportion) during the nesting season shown separately for each type of sand and year, with depth differences indicated by colour. The corresponding colour bands represent quantiles from 0.025 to 0.975.

Furthermore, when examining the data averaged across the entire study period for white sand, a significant difference was found ($\chi^2 = 65.27$, $df = 3$, $p < 0.05$) except for two depth combinations: 27 cm – 35 cm and 45 cm – 55 cm. The greatest difference was observed among 27 cm (98.9%) and 45 cm (96.2%) with a z-value of 7.40. Although similar mean female percentage of 97% was observed at the 55 cm depth, the effect size between the 27 cm and 55 cm depths was smaller ($z = 5.27$). This

could be attributed to incomplete data for 55 cm across all years. To conclude, the highest effect sizes consistently occurred between the 27 cm and 45 cm or the 27 cm and 55 cm depths, indicating that these depths had the most significant differences, with deeper depths producing smaller proportion of females.

Significant differences were also observed among the years. When averaged across available depths, a significant difference was noted for 2018 (95.2% females; $\chi^2 = 459.78$, $df = 6$, $p < 0.05$) compared to all years except 2022 (95.6% females). Moreover, the proportion of females in 2022 did not differ significantly from those in 2019 (97.1%) or 2017 (97.8%). The variation in effect sizes (relative to 2022) suggested the following order: 2018 ($z = -1.51$), 2022, 2019 ($z = 2.68$) and 2017 ($z = 2.71$). 2018 had the highest negative deviation, reflecting the lowest female proportion. Conversely, 2019 and 2017 had positive z-value deviations, indicating a trend towards higher female proportions.

In conclusion, a significantly lower proportion of females was produced in 2018 when compared to the rest of the years. Additionally, in 2018 the highest statistical significance was present among depths, with the greatest effect size noted between 27 cm and 45 cm. This pattern of the strongest differences among the 27 cm and 45 cm or 27 cm and 55 cm was consistently observed in all years (in cases where these depths were available for analysis).

5.3. Future Sex-ratio

Future sand temperature was derived from correlative models using either future T(air), T(sea) or a combination of both, for each sand type and depth. The majority of the models selected (based on AIC) employed T(sea) as the primary predictor. The subsequent most successful one was T(air), while the model combining both variables was used only in three cases (Appendix 4).

Sand temperatures above the critical thermal threshold (35°C) for successful egg incubation were predicted for the first time at 35 cm depth in dark sand in September 2061. The absolute maximum temperature (37.29°C) was modelled at the same depth and sand type in September 2099 (SSP5-8.5 scenario). Temperatures consistently exceeding this threshold were projected for dark sand at depths of 27 cm (after 2080) and 35 cm (after 2075) under the SSP5-8.5 scenario. The 95% confidence intervals indicated that this limit could have been surpassed yearly in other sand types and scenarios as well, although primarily at shallower depths. Consequently, successful egg incubation may be increasingly jeopardised towards the end of the century, especially under the SSP5-8.5 scenario in dark sand and shallow depths. On the other hand, the sand temperature at 55 cm was shown to be the least likely to exceed the 35°C limit.



Figure 16: Comparison of the predicted sand temperature (2000 to 2100) according to the three IPCC scenarios (SSP1-2.6, SSP3-7.0, SSP5-8.5) based on the best models selected according to AIC scores. The temperature values are presented as yearly averages for each sand type and depth. The bands represent the 95% CI and the thermal threshold of 35°C is indicated by the dashed red line.

The sex-ratio was calculated based on modelled sand temperatures, averaged across depths and depicted separately for each type of sand and IPCC scenario (2000-2100: Figure 16; 1850-2000: Appendix 5). The lowest mean proportion of females in period 2000-2100 was predicted in white sand under the SSP3-7.0 scenario (95.6%), followed by the SSP1-2.6 (96.7%) and SSP5-8.5 scenarios (99.5%). The absolute minimum female proportion modelled was 39% in white sand under the SSP3-7.0 scenario in July 2009. The smallest female proportion consistently occurred in July for each combination of sand type and scenario, while the maximum proportion was observed always in September. The projections for white sand indicated that female proportions are expected to reach 100% by 2055 under the SSP5-8.5 and by 2085 under the SSP3-7.0. Only the SSP1-2.6 scenario consisted of predictions where the troughs detected in July of each year until 2100 were below 100% females (e.g. the last trough detected in July was 97% females). Thus, no other scenario or sand type, except for white sand under the SSP1-2.6 scenario, showed the potential for producing male hatchlings towards the end of the century.

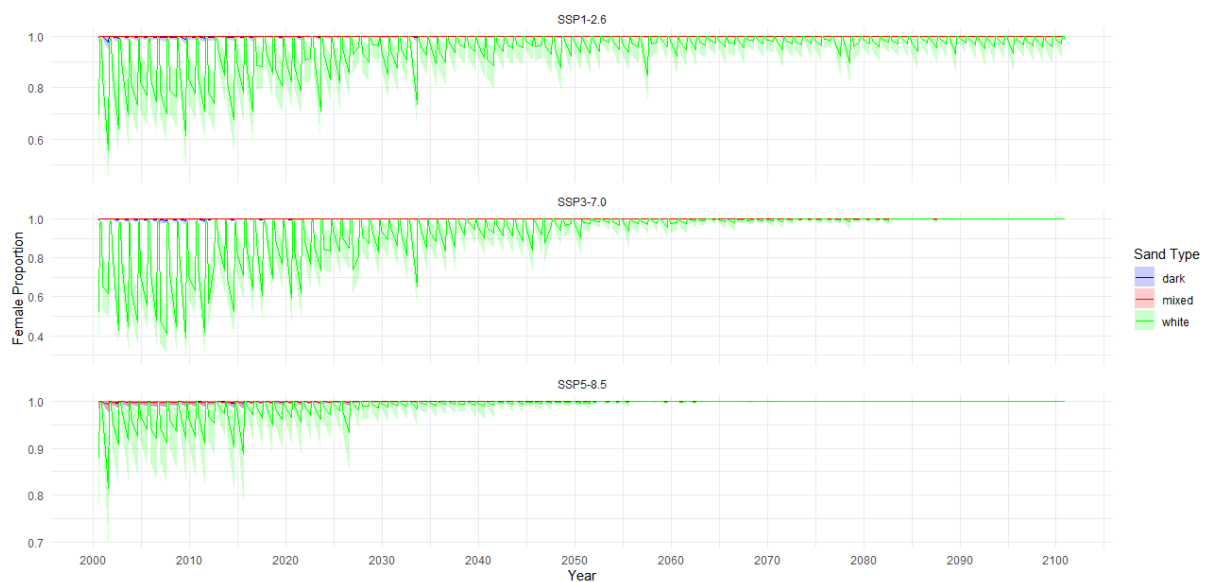


Figure 17: Time-series comparison of sex-ratios among sand types (averaged across all depths) according to the IPCC emissions scenarios SSP1-2.6, SSP3-7.0 and SSP5-8.5 (2000-2100). The sex ratio calculations are based on sand temperature data derived from the best-fitting models according to AIC. Shaded bands represent the 0.025 to 0.975 quantile range.

6. Discussion

The work aimed to assess present and future sex-ratio estimations and to identify potential spatial and temporal refuges for male hatchling production on Maio Island. Due to methodological limitations, the sex-ratio results should be interpreted with caution. The following chapter discusses the female skewed sex-ratios and mortality of hatchlings along with potential mechanisms through which loggerhead turtles could adapt to climate warming. Furthermore, management and conservation strategies are explored. Finally, the limitations of the study are highlighted, and implications for further research provided.

6.1. Female Skewed Sex-ratios and Mortality

The results of the current study indicated that the present primary sex-ratio was highly female skewed (99.2%). This is in consonance with other studies as majority of them reported female biased sex-ratios around the world (Patrício et al., 2021) as well as locally, on other islands in Cabo Verde archipelago (Abella Perez et al., 2016; Laloë et al., 2014; Tanner et al., 2019). Moreover, the sand temperatures throughout the study years suggested an upward shifting trend (Figure 9). This trend was also obvious in the future projections of sand temperatures (Figure 16) which were based on three (IPCC, 2023) emissions scenarios. These revealed that the local population is keen not only to complete feminisation but also indicated that the hatching success may be compromised towards the end of the century. This is due to temperatures reaching and exceeding the thermal threshold of 35°C, at which, on average, only 5 female and 0.002 male hatchlings per 100 eggs survive incubation (Hays et al., 2017). Other studies have reached similar conclusions, predicting that male production will be in jeopardy towards 2100, and the populations already showing high female skewness are especially vulnerable (Hawkes et al., 2007; Monsinjon et al., 2019; Tanner et al., 2019; Witt et al., 2010). On the other hand, the female-skewed sex-ratios were present also in the past when global warming was not the driving force (Laloë et al., 2014, 2019; Mazaris et al., 2017; prevalent female skewness in 1850-2000 modelled by the current study is shown in Appendix 5). The long persistence of sea turtle populations with skewed sex-ratios suggests that such skewness, up to a certain degree, may not necessarily compromise the viability of a population (Hays et al., 2017). Males have a shorter remigration interval (i.e. breed more frequently than females), actively search for mating partner and mate with multiple females from several nesting sites, i.e. male-mediated gene flow is present in sea turtles (Bowen & Karl, 2007; Hays et al., 2022). Thus, if we assume, that there are only a few adult males needed in a system to fertilize the eggs, the female skew can actually promote viability by maximizing the population growth (Boyle et al., 2014; Dyson & Hurst, 2004; J. Rankin & Kokko, 2007). That is, skewed primary sex ratios, in some scenarios, can be adaptive to ensure optimal (not

necessarily balanced) operational sex-ratios (Hays et al., 2014). Moreover, in some populations with female biased primary sex-ratios, a balanced or male biased operational sex-ratios were assessed (Lasala et al., 2018; Schofield et al., 2017). The question of the specific degree to which female skewness in primary sex-ratio benefits the viability needs to be investigated for each population individually. In the current study, the observed trend (Figure 9) suggested presence of significantly colder nesting season in 2018. Another smaller drop in temperatures was noted during 2022 (no significant difference in sex-ratio proportions was noted between these two years). This raises a question: could a cold year occurring periodically (e.g. every eight or four years) ensure the incorporation of males into the population and would this male proportion be sufficient to retain the population viability. If so, the female biased sex-ratios would be less of a threat to the population than the temperatures increasing above the thermal tolerance limit resulting in hatchling mortality in the current century (Hays et al., 2017). Therefore, it remains to be determined whether highly female-biased populations will become (or have already become) population sinks (Patrício et al., 2021). In the face of global warming and its accelerating speed (IPCC, 2023), both the complete feminisation and hatchling mortality pose significant threats to the local population (especially under the SSP5-8.5 IPCC emissions scenario). Inevitably, the population will need to exhibit, to some degree, capacity for adaptation.

6.2. Capacity to Adapt

Sea turtles inhabit the earth for millions of years (Hirayama, 1998) what proves their capacity to withstand paleo-climatic shifts including high fluctuations in temperatures (Hawkes et al., 2009). However, the current climate change drives temperature increase at a considerably higher speed than ever before (IPCC, 2023). Physiological adaptation on a genetic basis (i.e. micro-evolution, more favourable in long term) in terms of adjustment of pivotal temperature (PT) and critical thermal limits of incubation could be potential mechanisms for adaptation in loggerhead turtles (Patrício et al., 2021). The pivotal temperature threshold and its genetic basis is still not fully explored and it is a topic of ongoing research (Blechsmidt et al., 2020). Nevertheless, evidence of physiological response to heat stress in loggerhead embryos was found along with heritability of the heat shock genes expression (i.e. when and how much of the heat shock protein is produced). The plasticity of expression (i.e. ability to adjust the gene expression) was also proved to be heritable. These findings confirm that embryos are indeed capable to adapt to higher incubation temperatures at the molecular level; however, this evolutionary potential may still be outpaced by climate change (Tedeschi et al., 2016).

Additionally, there are other adaptation mechanisms which are more favourable in the short term, such as changes in phenology and phenotypic plasticity (e.g. timing of the breeding/nesting intervals, nest site selection, construction of deeper nests or range shifts, i.e. colonisation of new nesting areas; Hulin et al., 2007). The current study shows that an earlier onset of the nesting season would have the potential to increase the proportion of male hatchlings produced (a significantly lower proportion of females was, on average, produced in July, i.e. when the nesting season on Maio Island currently starts). Nesting seasons have already been observed to shift, with earlier onsets documented in other rookeries (Monsinjon et al., 2019; Weishampel et al., 2004). Nevertheless, the effectiveness of this phenological response varies among locations, thus these need to be evaluated individually (M. Fuentes et al., 2024; Monsinjon et al., 2019). Furthermore, the study results suggested that the sand temperature decreased significantly with greater depth (on average, 0.51 °C temperature reduction from 27 cm to 55 cm depth). Therefore, the phenotypic plasticity through deeper nest construction could be another potential adaptation mechanism. However, this response has not yet been evaluated in sea turtles except for studies relating body size to nest depth (Hulin et al., 2007; Marco et al., 2018). Lastly, although philopatric, the local population was shown to use bet-edging strategy (distributing nests among several beaches while choosing the most favourable area within each beach), which suggested an adaptive potential to colonise new nesting sites (Patino-Martinez et al., 2023). However, lack of shoreline continuity and the considerable distance to other nesting suitable areas (e.g. west African coast) limits this adaptation mechanism (Patino-Martinez et al., 2023; Tanner et al., 2019). Whether the local population is capable of exhibiting all the suggested phenological responses remains to be investigated.

6.3. Management Strategies and Conservation

Previous studies on Maio Island have shown higher hatching success, as well as greater size and vitality of hatchlings in light-coloured substrate. Thus, white sand beaches are a priority in terms of conservation (Patino-Martinez et al., 2022). This study shows that the thermal regime of light-coloured sand was the most favourable for producing hatchlings of both sexes while for darker-coloured beaches it was shown that male production is minimal. Assuming that nest relocation is a priority conservation strategy, it is recommended to choose light-coloured beaches. These areas are also priorities for avoiding urbanization and artificial lighting. In the case of Maio, it is recommended to prioritize the light-coloured beaches on the east coast as these were shown to host the largest number and density of nests along with the highest nesting success and re-nesting activities (Patino-Martinez et al., 2021, 2023). Moreover, significantly cooler temperatures were found in the beginning and end

of the nesting seasons in the study years and thus, nests laid during these periods should be of priority protection if we want to reach a greater potential for male production.

There is also implication for placing relocated nests deeper as given by the findings of the current study. In white sand, burying eggs in 55 cm depth instead of 35 cm has potential to reduce the temperature by 0.34°C with significantly lower proportion of females produced. On the other hand, it is important to note that there are also other biological (e.g. metabolic heating which varies within the nest chamber), chemical, environmental and physical factors that can influence the magnitude and significance of these temperature reductions with depth and these are often site specific (Van De Merwe et al., 2006). Thus, the implementation should be first applied only to a representative sample of study nests in the hatchery and also to relocated in situ nests, and its effectiveness should be assessed along with hatching success. Additionally, it is also important to assess whether greater depth represents an advantage in terms of lower temperatures also at the end of the nesting season (mid-October onwards) as no significant difference in temperatures among depths was spotted for some of the study years in white sand. Thus, more evidence of the effectiveness of this strategy needs to be gathered in order to implement it as an adaptive management strategy on a local scale. For now, it is recommended to place relocated nests into 55 cm depth in the hatchery only as this depth also exhibits higher hatching success (Marco et al., 2018) while in situ relocated nests should maintain the original depth of the nest chamber in order not to reduce natural selection pressures (Mrosovsky, 2006).

Further strategies such as hatcheries shading or watering of nests (Esteban et al., 2018; Hill et al., 2015; Jourdan & Fuentes, 2015) could be followed in local conditions. However, when implementing any human induced mitigation mechanisms caution needs to be taken as these may slow down potential adaption of the local population by lowering natural selection pressures (Mrosovsky, 2006).

6.4. Methodological Limitations

The pivotal temperature (PT) used in this study is sourced from a different loggerhead population (Marcovaldi et al., 1997), as the PT for the Cabo Verde population has not yet been determined. Thus, the sex-ratio results in the present study are indicative and further research on local PT is encouraged. Moreover, the mean temperature of the middle third of incubation was used to calculate the sex-ratios. This is a generalised approach used by a considerable number of studies (due to limited availability of other data), although proved inaccurate under in situ conditions with fluctuating temperatures. Instead, the methodological approach proposed by Monsinjon et al. (2022) is recommended, provided that sufficient data are available for the studied population. These include:

(i) in situ temperature data throughout the whole incubation duration, (ii) consistently collected measurements of embryos/hatchlings (e.g. straight carapace length- one of the variables needed to calculate CTE that is to be used instead of the mean TSP temperature), (iii) sexed subsample of the hatchlings per nest, (iv) constant temperature sex-ratio data (i.e. from laboratory experiments to be able to construct program TSD and determine PT of the local population). Furthermore, the intensity of nesting activities varies spatially and temporally throughout the nesting season. To provide more realistic sex-ratio estimates for Maio, future studies should account for these variations by weighting the estimated sex-ratio proportions accordingly.

Regarding the future sex-ratio projections, correlative models were used to predict future sand temperatures. A specific model was determined for three IPCC (2023) emissions scenario, sand type and depth separately to enhance precision. However, it should be noted that the R-squared values were relatively low for some of the combinations, indicating weak correlations (i.e. the actual sand temperature data did not fully correspond to the modelled temperatures during the study years). Alternative methods, such as mechanistic microclimate modelling (Bentley et al., 2020), require set of input variables: meteorological variables, sand physical properties, beach topography etc. Incorporating these is beyond the scope of this study. Additionally, the input data (SST and air temperature) for the correlative models in the study are sourced from IPCC emissions scenarios, which provide only mean monthly temperatures. These data slightly varied among emissions scenarios during the study years, as the median of several models is used for each scenario. This variation contributed to discrepancies between actual and modelled sand temperatures. Moreover, it is important to note that the sex can also be influenced by short-term exposures of high temperatures as well as by the amount of rainfall (Lolavar & Wyneken, 2015). Thus, incorporating daily temperature data into both mechanistic and correlative models should improve the precision of forecasts and hindcasts (Laloë et al., 2020; Monsinjon et al., 2019).

Lastly, it is advisable for future studies (if materially feasible) to use pairs of temperature loggers to collect data at each depth, as the current study lacks measurements at several depths in some years (due to logger's malfunction, inaccuracy, overwash etc.), which limits the analysis. Nevertheless, the study covers a significantly large time span, allowing for representative and robust comparisons.

7. Conclusion

The current study is the first to analyse an extensive sand temperature dataset gathered over 7 consecutive years (2017-2023) to estimate the present and future primary sex-ratio of the North-East Atlantic subpopulation of loggerhead turtles nesting on Maio Island. The results suggested exceptionally high female skewed hatchling production (99.2%). When assessed separately, white sand was the only type associated with a lower female proportion (98%). Additionally, the beginning and end of nesting season (July confirmed as the lowest female-producing month) along with depths of 45 cm and 55 cm, had a significant impact on reducing female proportion. It is important to note that the pivotal temperature (PT) used in the study was not sourced from the local population. Further research addressing variables (such as the PT, hatchling/embryo measurements and sexed samples of hatchlings from corresponding nests) assessed from the local population is needed. Additionally, weighing the sex-ratio values spatially and temporally (as the intensity of nesting activities and spatial distribution of nests vary throughout nesting season) could refine the sex-ratio estimates even further.

According to the IPCC scenarios, the projected sand temperatures suggested not only female skewed future sex-ratios but also revealed critically high values exceeding the thermal threshold of 35°C towards the end of the century, which would be detrimental to egg development. This effect was most vividly modelled in shallower depths of 27 cm and 35 cm, especially in dark sand under the SSP5-8.5 scenario (from 2075 onwards). The sex-ratio projections revealed that the potential to produce at least a small fraction of male hatchlings towards the end of the century was realistic only under the SSP1-2.6 scenario in white sand, where the troughs of female proportion were consistently detected in July (the last detected trough in 2100 exhibited 97% of females).

To conclude, potential refuge for generating male hatchlings is in the beginning of nesting season, i.e. in July, in white sand and at greater depths. The future primary sex-ratio projections also suggest July as the least female producing month, thus the species adaptive behaviour in terms of earlier onset of breeding activities along with deeper nests could possibly help to increase the male production. Nevertheless, other mitigation strategies such as artificially shaded hatcheries and nest watering will most likely be needed to preserve the local population. Further research is needed to assess the effectiveness of the mentioned strategies in the local conditions. For now, it is recommended to place the relocated hatchery nests in a depth of 55 cm. These, however, are only perceived as emergency responses and further population studies to raise awareness and incentives to policymakers to slow down the acceleration of climate change need to be in place to preserve the species on a global scale.

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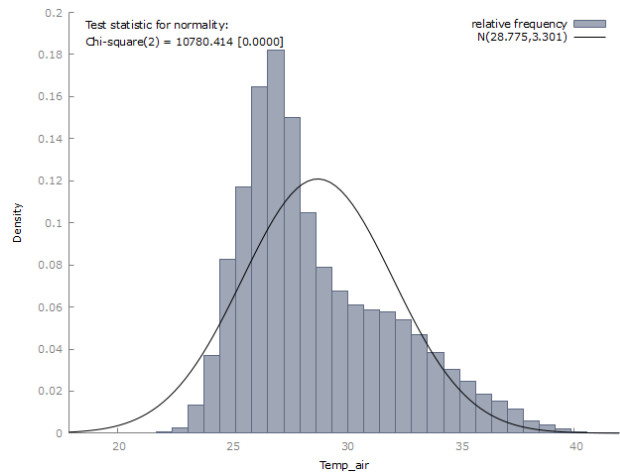
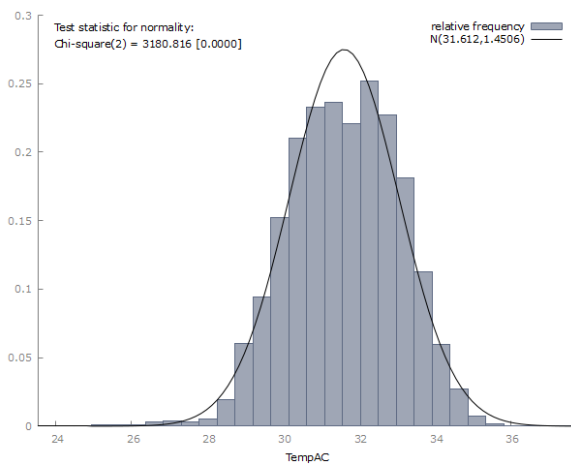
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Appendix 1

Normality tests of the sand and air temperature data, respectively. Frequency distribution plotted against the normal distribution imply the sand temperature data to be relatively normal (mean < median => small negative skewness present,) and the air temperature data to be non-normal. However, the quantitative normality tests (i.e., Shapiro-Wilk, Doornik-Hansen etc.; shown below) suggest both to follow a non-normal distribution ($p\text{-value} \leq \alpha$, $\alpha = 0.05$). Lastly, the summary statistics is provided. The plots and normality tests are produced in Gretl (<https://gretl.sourceforge.net/>).



Test for normality of TempAC:

Doornik-Hansen test = 3180.82, with p-value 0
 Shapiro-Wilk W = 0.993041, with p-value 4.51645e-069
 Lilliefors test = 0.0317262, with p-value \approx 0
 Jarque-Bera test = 3070.72, with p-value 0

Test for normality of Temp_air:

Doornik-Hansen test = 10780.4, with p-value 0
 Shapiro-Wilk W = 0.934869, with p-value 2.7642e-081
 Lilliefors test = 0.120569, with p-value \approx 0
 Jarque-Bera test = 4683.85, with p-value 0

Summary statistics, using the observations 1 - 377992 for the variable 'TempAC' (377992 valid observations)

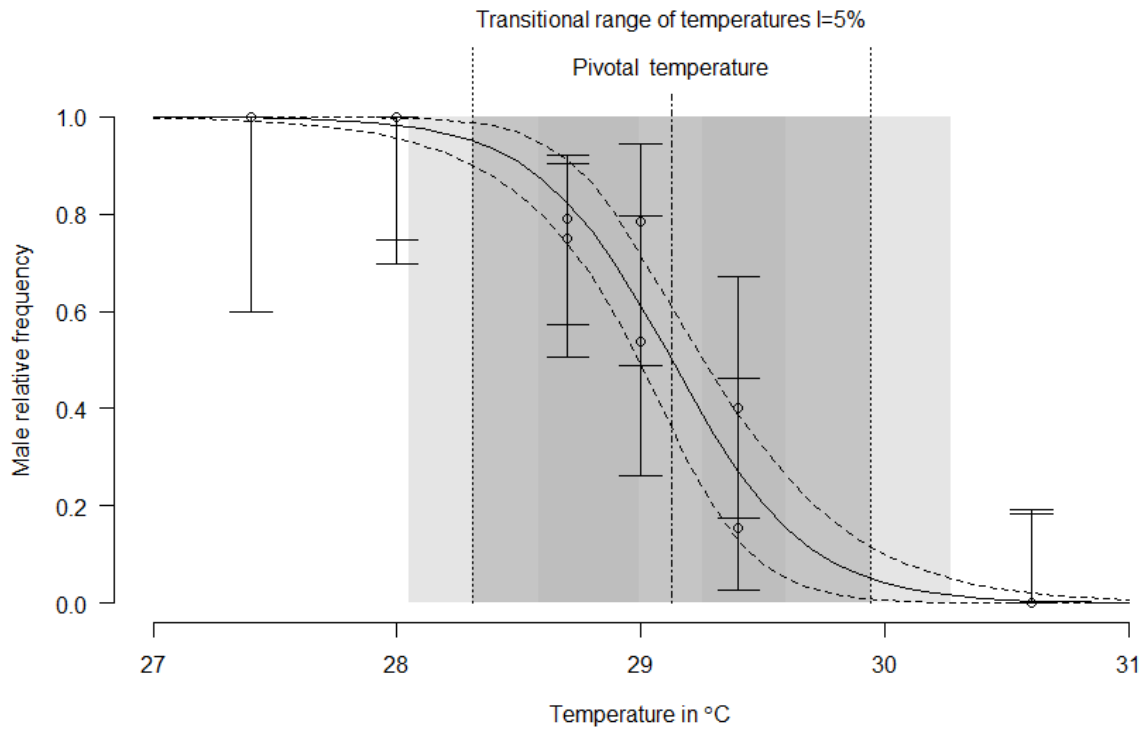
Mean	31.612
Median	31.637
Minimum	24.243
Maximum	37.480
Standard deviation	1.4506
C.V.	0.045887
Skewness	-0.22017
Ex. kurtosis	0.032622
5% percentile	29.265
95% percentile	33.887
Interquartile range	2.1200
Missing obs.	0

Summary statistics, using the observations 1 - 38994 for the variable 'Temp_air' (38994 valid observations)

Mean	28.775
Median	27.801
Minimum	22.046
Maximum	41.619
Standard deviation	3.3010
C.V.	0.11472
Skewness	0.84822
Ex. kurtosis	0.069873
5% percentile	24.677
95% percentile	35.288
Interquartile range	4.5595
Missing obs.	0

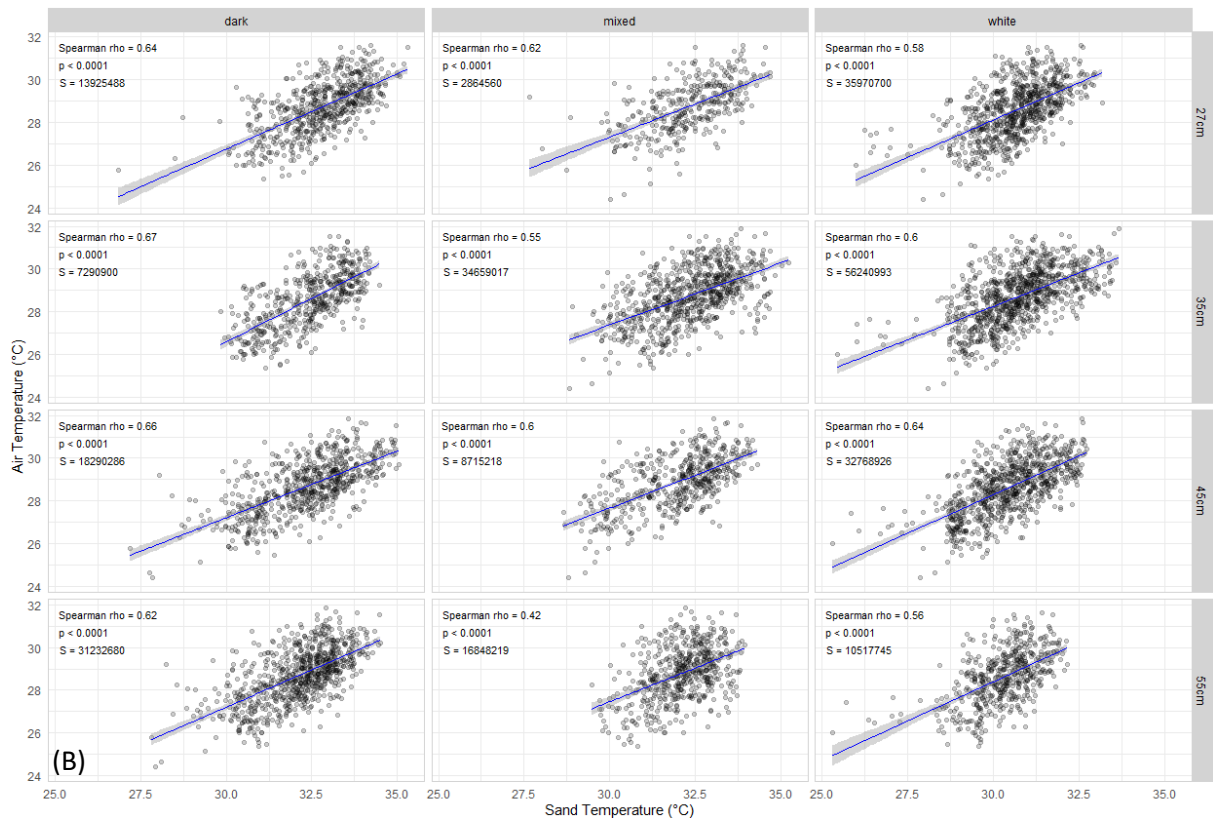
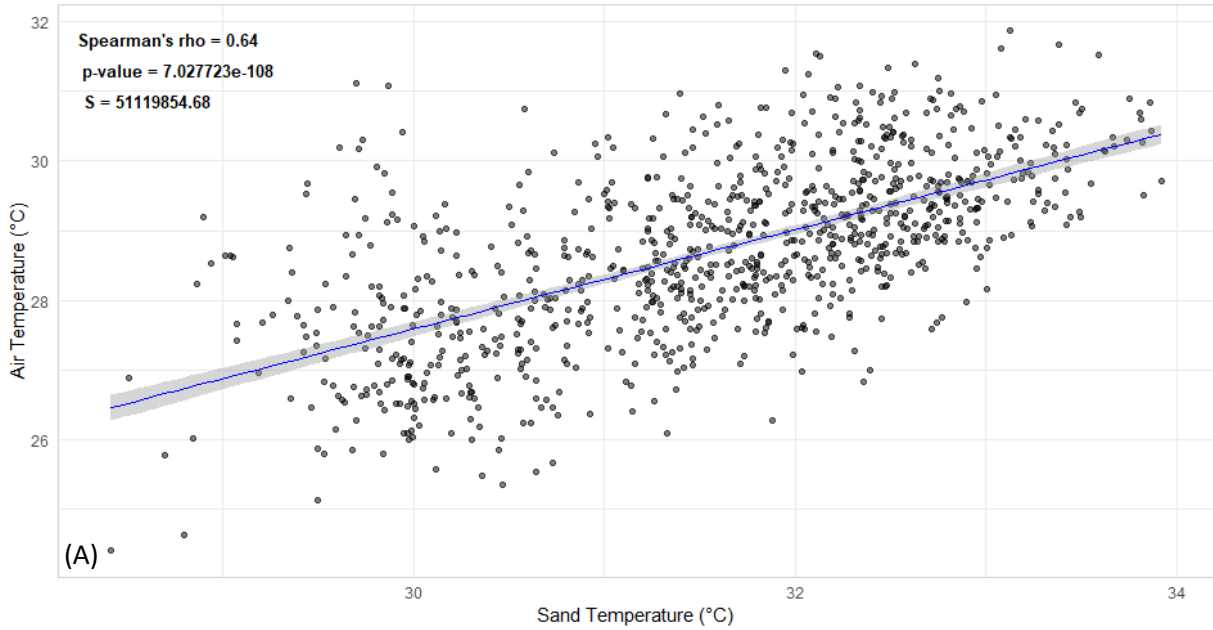
Appendix 2

The Program TSD determined for Praia do Forte, Bahia, Brazil (Marcovaldi et al., 1997) constructed with the use of the *embryogrowth* R-package (Girondot, 2023):



Appendix 3

Scatter plots showing the correlation between the daily average air and sand temperatures before the lag was applied: (A) for all sand types and depths jointly (the group values are averaged) and (B) for each group (sand type and depth) separately. Caption in the upper left corner shows the Spearman's correlation test results. Regression line (blue) and the 95% confidence interval band (grey) depict the positive correlation trend.



Appendix 4

Best correlative models based on AIC:

Depth	Sand type	Source	Model type	Equation	AIC	R ²
27cm	dark	SSP1-2.6	model_sea	$0.95 * T_SEA + 7.29$	37.88	0.63
27cm	dark	SSP3-7.0	model_sea	$1.03 * T_SEA + 5.06$	39.65	0.60
27cm	dark	SSP5-8.5	model_air	$0.98 * T_AIR + 7.57$	42.58	0.54
35cm	dark	SSP1-2.6	model_sea	$1.16 * T_SEA + 1.47$	20.55	0.83
35cm	dark	SSP3-7.0	model_sea	$1.22 * T_SEA + -0.26$	22.93	0.81
35cm	dark	SSP5-8.5	model_air	$1.22 * T_AIR + 0.88$	25.85	0.78
45cm	dark	SSP1-2.6	model_sea	$0.75 * T_SEA + 12.30$	77.25	0.22
45cm	dark	SSP3-7.0	model_air	$0.91 * T_AIR + 8.75$	77.03	0.23
45cm	dark	SSP5-8.5	model_sea	$0.96 * T_SEA + 6.86$	73.59	0.33
55cm	dark	SSP1-2.6	model_sea	$0.74 * T_SEA + 12.28$	77.96	0.28
55cm	dark	SSP3-7.0	model_air	$0.85 * T_AIR + 10.01$	78.18	0.27
55cm	dark	SSP5-8.5	model_sea	$0.94 * T_SEA + 7.16$	72.76	0.39
27cm	mixed	SSP1-2.6	model_both	$-2.79 * T_AIR + 3.17 * T_SEA + 19.04$	36.55	0.50
27cm	mixed	SSP3-7.0	model_sea	$0.68 * T_SEA + 14.08$	41.15	0.20
27cm	mixed	SSP5-8.5	model_sea	$0.81 * T_SEA + 10.57$	38.99	0.32
35cm	mixed	SSP1-2.6	model_sea	$0.70 * T_SEA + 13.58$	85.62	0.21
35cm	mixed	SSP3-7.0	model_air	$0.78 * T_AIR + 12.00$	86.74	0.18
35cm	mixed	SSP5-8.5	model_sea	$0.87 * T_SEA + 9.15$	82.94	0.28
45cm	mixed	SSP1-2.6	model_both	$-2.61 * T_AIR + 2.88 * T_SEA + 22.09$	61.49	0.25
45cm	mixed	SSP3-7.0	model_air	$0.61 * T_AIR + 15.92$	62.88	0.11
45cm	mixed	SSP5-8.5	model_sea	$0.82 * T_SEA + 9.93$	60.30	0.22
55cm	mixed	SSP1-2.6	model_sea	$0.52 * T_SEA + 18.32$	43.02	0.26
55cm	mixed	SSP3-7.0	model_air	$0.60 * T_AIR + 16.55$	42.28	0.29
55cm	mixed	SSP5-8.5	model_sea	$0.63 * T_SEA + 15.37$	40.33	0.36
27cm	white	SSP1-2.6	model_sea	$0.86 * T_SEA + 7.51$	65.51	0.46
27cm	white	SSP3-7.0	model_sea	$0.91 * T_SEA + 6.28$	63.84	0.49
27cm	white	SSP5-8.5	model_air	$0.94 * T_AIR + 6.31$	65.61	0.46
35cm	white	SSP1-2.6	model_sea	$0.71 * T_SEA + 11.82$	102.70	0.21
35cm	white	SSP3-7.0	model_sea	$0.88 * T_SEA + 7.20$	98.26	0.30
35cm	white	SSP5-8.5	model_air	$0.93 * T_AIR + 6.66$	97.84	0.31
45cm	white	SSP1-2.6	model_sea	$0.72 * T_SEA + 11.08$	86.53	0.23
45cm	white	SSP3-7.0	model_sea	$0.80 * T_SEA + 9.05$	85.57	0.26
45cm	white	SSP5-8.5	model_air	$0.92 * T_AIR + 6.66$	82.63	0.33
55cm	white	SSP1-2.6	model_both	$-1.18 * T_AIR + 1.83 * T_SEA + 11.69$	44.52	0.44
55cm	white	SSP3-7.0	model_sea	$0.85 * T_SEA + 7.43$	44.09	0.39
55cm	white	SSP5-8.5	model_air	$0.82 * T_AIR + 9.11$	44.70	0.37

Appendix 5

Time-series comparison of sex-ratios among sand types (averaged across all depths) according to the IPCC emissions scenarios SSP1-2.6, SSP3-7.0 and SSP5-8.5 (1850-2000). The sex ratio calculations are based on sand temperature data derived from the best-fitting models according to AIC. Shaded bands represent the 0.025 to 0.975 quantile range.

