Sex ratio estimation for Green Turtle, *Chelonia mydas*, hatchlings at Akyatan Beach, Turkey

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(Received 23 June 2022; accepted 30 August 2022; first published online 13 September 2022)

Marine turtles have temperature-dependent sex determination and the increase in global temperature could cause female predominance due to higher nest temperatures. The temperatures of 163 Green Turtle nests were measured with temperature data loggers at Akyatan beach, Turkey. The mean entire incubation duration (EID) and the sex-determination period (SPD) temperatures of the Green Turtle nests were 31.03°C and 30.81°C respectively. The mean incubation duration (ID) was 51.94 days. Significant differences were found in terms of ID among nesting seasons, and ID had a negative relationship with the nesting seasons. The mean ID for the nesting seasons had a statistically significant negative correlation with EID temperature. The mean female ratio estimation for the nesting seasons was 89%. Significant differences were found among the female ratio estimations for the nesting seasons. The mean female ratio estimation for the nesting seasons showed a statistically significant negative correlation with ID and nest depth. Long-term temperature increase may pose a threat to the Green Turtle populations.

**Keywords:** Entire incubation duration temperature; sex-determination period temperature; incubation duration; female ratio

**Introduction**

The temperature during the incubation period induces sexual differentiation of gonads during the critical period of embryonic development in many reptile species (Pieau, 1996). Temperature-dependent sex determination (TSD) is often characterized by a pivotal temperature, which is the constant temperature producing equal numbers of each sex or transitional range of temperature (TRT), which consists of those incubation temperatures that produce both sexes (Godfrey & Mrosovsky, 2006; Mrosovsky & Pieau, 1991), and all marine turtle species exhibit TSD (Wibbels, 2003). In most marine turtles, while females are produced in high-temperature nests (>29.0°C), males are produced by cooler nests (<29.0°C) (Mrosovsky, 1994). The temperature for one-third of incubation is critical for sexual development and sex ratios of marine turtle populations are affected by latitudinal variation, seasonal temperature changes, shading by vegetation, sand colour, rain and metabolic heat that the most influential factor on nest temperature (Broderick et al., 2000; van de Merwe et al., 2006). The determination of the sex ratio in marine turtle hatchlings is significant basic information for population dynamics due to a necessary parameter when estimating population size and productivity (Casale et al., 2006; Hamann et al., 2010). The sex ratio of hatchling production in marine turtle populations can be determined by gonadal mor-
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The determination of sex ratio in marine turtles is largely controlled by environmental parameters including the nest temperature (Jribi & Bradai, 2014). The increase in global temperature may cause the prevalence of a single sex, forcing a species toward extinction (Calderón Peña et al., 2020). Recent studies related to sex ratios of marine turtles indicate a prevalence of females across most marine turtle species (LeBlanc et al., 2012; King, et al., 2013; Marcovaldi et al., 2014; Tomillo et al., 2014; Tanner et al., 2019).

The 13 major Green Turtle rookeries in the Mediterranean are located in Turkey, Cyprus, and Syria, and almost 99% of the overall nesting activity of Green Turtles occurs in Turkey and Cyprus (Kasperek et al., 2001; Türkozan & Kaska, 2010; Casale et al., 2018). Studies of Green Turtle populations in North Cyprus and Turkey provided evidence that the Mediterranean Green Turtle population is highly female biased. These results are based on gonadal histology and nest temperatures in Northern Cyprus (Kaska et al., 1988), sand temperatures at Akyatan Beach, Turkey (Casale et al., 2000), gonadal histology and nest temperatures at Alagadi Beach, Northern Cyprus (Broderick et al., 2000), gonadal histology and nest temperatures at Sugözü Beach, Turkey (Kılıç & Candan, 2014; Candan & Kolonkaya, 2016) and histological examination and nest temperatures at Samandağ Beach, Turkey (Yalçın Özdilek et al., 2016).

Akyatan Beach hosts about 20% of the total number of clutches recorded in the Mediterranean and is the largest nesting rookery for Green Turtles in the Mediterranean (Casale et al., 2018). The determination of the sex ratio of hatchlings at Akyatan rookery will provide a significant contribution to the Mediterranean population. Therefore, we evaluated the sex ratio estimate for Green Turtle hatchlings and the effect of nest parameters (nest depth and distance from the sea) and nest temperatures on sex ratio estimate. Also we tested correlations between incubation duration temperature, SDP (sex-determination period) temperature, and incubation duration of Green Turtle nests at Akyatan beach during seven consecutive reproduction seasons.

Material and Methods

This study was carried out from May to September over 7 years (2014–2020) on Akyatan beach, Turkey. The beach is located in a delta between Akyatan lagoon and the Mediterranean Sea in Adana province, southern Turkey. It is almost 22 km long and both Green and Loggerhead Turtles (Caretta caretta) nest on the beach.

Akyatan beach was monitored every day during the nesting seasons. The distance of the nests from the high tide line was measured with a tape meter. Temperatures in Green Turtle nests were measured by using electronic TinyTalk temperature data loggers (Ori-on Components, Chichester, UK, TK-4014-MED monitors temperatures from -40 to +85°C using a built-in sensor). These data loggers were placed in the center of the clutch within a few hours following oviposition and synchronized to record the temperature at 5-min intervals. We excluded datalogger records taken during the first 24 h during equilibration with the surrounding sand. After hatchling emergence was completed, the depth of nests was measured with a tape meter and the temperature data from each data logger, programmed to measure temperature every five minutes synchronously, was downloaded using the Tinytaq software ver. 2.3.1.
The incubation duration (ID) was calculated as the number of days from the date of egg deposition to the date of first emergence (Godley et al., 2002). Four days were subtracted from the IDs of the nests (Hendricson, 1958; Calderón Peña et al., 2020), because marine turtle hatchlings need some days to reach the surface after emerging from the egg (Hendricson, 1958). The adapted IDs were used to estimate sex ratio. The sex ratio in the nests was estimated from the entire incubation duration (EID) temperatures (Yntema & Mrososvsky, 1982). EID was divided into three equivalent periods as the first third of the incubation period, the middle third of the incubation period (sex-determining period: SDP), and the last third of the incubation period. The sex ratio estimate was determined with temperature taken during the middle third of the EID. The mean daily temperatures within the SDP and EID were used to compare nest temperatures between years. The pivotal temperature, which varies among species of turtles and between different populations, is the developmental temperature for which the sex ratio of hatchlings is 1:1. The pivotal temperature for Turkey’s rookeries of Green Turtles was determined only for Sugözü beach (Candan & Kolonkaya, 2016). Therefore, to determine the female ratio of the nests containing data loggers, we used this value (29.0°C) determined for Sugözü beach: Female ratio = 1 / (1 + exp (T - 29.0)/0.6)) (T: middle third of the incubation temperature, exp: exponential functions) (Calderón Peña et al., 2020).

Non-parametric tests were used for all statistical analyses because the nesting data were not homogeneous and normally distributed according to Levene’s and Kolmogorov-Smirnov tests (p<0.05). The EID and SDP temperatures, ID, and sex ratio estimate for hatchlings between nesting seasons were compared with the Kruskal-Wallis H test. Correlation between variables was tested with the Spearman rho and results were considered significant at 0.01 level. All analyses were conducted with IBM SPSS Statistics 20 software program. All means are presented ± standard deviation (SD).

Results
The temperature of 163 nests were measured, 90 in June and 73 in July. The mean EID temperature of the Green Turtle nests during the seven nesting seasons were 31.03±1.08°C (28.18–36.07°C), the SDP temperature 30.81±1.09°C (28.36–35.75°C) (Table 1). The mean EID and SDP temperatures of nests during the nesting seasons were higher than 30.0°C (Table 1). The mean SDP temperature of 95% of the nests (155 nests) was above the pivotal temperature (29.0°C). The mean EID and SDP temperatures showed a significant difference between the nesting seasons (EID temperature: p<0.0001, SDP temperature: p<0.0001) (Figure 1a, b). There was no significant correlation between the EID and SDP temperatures of the nesting seasons (EID temperature: r=0.15, p>0.05; SDP temperature: r=0.05, p>0.05). Also, SDP temperature had a statistically significant negative correlation with nest depth (r=-0.022, p<0.01) (Figure S1).

The mean ID during the seven nesting seasons was 51.94±3.46 days (range=44–62 day) and the lowest incubation durations were recorded in the 2016 (48.73±1.88 days), 2018 (50.44±3.93 days), and 2020 (49.94±3.05 days) nesting seasons (Table 1). A significant difference was found between ID of the nesting seasons (p<0.0001), and interannual variation of ID with a 95% confidence interval is shown in Figure 1c. According to nesting seasons, ID had a significant negative correlation (r=-0.221, p<0.01) (Figure S2). Also, ID had a statistically significant negative correlation with EID temperature (r=-0.539, p<0.001). When EID temperature was high, the ID was shorter (Figure S3).
Table 1. Descriptive statistics for temperatures of the nest, incubation durations, and female ratio in the nesting seasons (ID: incubation duration; EID: entire incubation duration; SDP: sex-determining period; N: Number of nests).

|          | ID (day) | EID temperature (ºC) | SDP temperature (ºC) | Female ratio (%) |
|----------|----------|-----------------------|-----------------------|------------------|
|          | N  | Mean ± Range | Mean ± Range | Mean ± Range | Mean ± Range |
| 2014     | 47 | 53.21±2.80  | 30.69±1.02 | 30.60±0.99 | 86±0.16 | 25-99 |
| 2015     | 16 | 52.50±2.03  | 30.65±0.55 | 29.02-31.74 | 89±0.12 | 51-99 |
| 2016     | 22 | 48.73±1.88  | 31.68±0.77 | 29.89-33.13 | 96±0.04 | 81-99 |
| 2017     | 24 | 54.00±3.81  | 30.61±0.94 | 28.76-32.23 | 84±0.17 | 40-99 |
| 2018     | 16 | 50.44±3.93  | 31.85±1.12 | 30.20-34.30 | 98±0.02 | 94-99 |
| 2019     | 17 | 52.59±3.55  | 31.22±0.87 | 29.10-33.48 | 90±0.12 | 46-99 |
| 2020     | 21 | 49.94±3.05  | 30.61±1.41 | 28.41-35.75 | 86±0.16 | 27-99 |
| Total    | 163| 51.94±3.46  | 31.03±1.08 | 28.36-35.75 | 89±0.14 | 25-99 |

The mean female ratio estimation for the nesting seasons was 89±0.14% (25–99%) (Table 1). The highest female ratio was observed in the 2018 (98%) nesting season. A significant difference was found between the female ratio estimation of the nesting seasons (p<0.0001) and inter-annual variation of female ratio with 95% confidence interval is shown in Figure 1d. The mean female ratio estimation had no statistically significant correlation with distance from the sea of the nests (r=0.097, p>0.01). Also, the mean female ratio estimation had a statistically significant negative correlation with incubation duration (r=-0.475, p<0.001) and nest depth (r=-0.234, p<0.01). When the incubation period and nest depth increased, the female ratio estimations decreased (Figure S4a, b).

Discussion

Determination of sex ratios of endangered species such as marine turtles is important to achieve the self-sustainability of populations. The increase in global temperature represents a major threat to sea turtle populations that have temperature-dependent sex determination (TSD) and female predominance with an increase in temperature may endanger the long-term sustainability of some sea turtle populations.

In our study, the mean EID (31.03±1.08ºC) and SDP (30.81±1.09ºC) temperatures of Green Turtle nests were similar to those reported by Kaska et al. (1998), Kılıç and Candan (2014), Yalçın-Özdilek et al. (2016) and Calderón Peña et al. (2020) (SDP temperature), Calderón Peña and Azanza Ricardo (2021) (EID temperature) (Table S1). However, while these values were higher than those reported at Long Beach, Ascension Island by Broderick et al. (2001), they were lower than those reported at Northeast Bay, Ascension Island by Broderick et al. (2001) (Table S1). The significant difference between the mean EID temperatures during the nesting seasons might result from local temperature variations between the nesting seasons. Also, the difference or similarity of nest temperature between nesting seasons on the same beach and at different nesting beaches could be affected by many environmental factors related to the nest location (distance from the sea, proximity to vegetation, being under dunes, sand albedo, climatic conditions like heavy rain and storms), the climate and geomorphology of the beach.
Figure 1. Interannual variation of Green Turtle nest EID (entire incubation duration) temperature (a), SDP (sex-determining period) temperature (b), incubation duration (c), and female ratio (d) on Akyatan Beach during seven nesting seasons (95% confidence interval in the error-bar plot).

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populations. Differences in the female ratio between these populations could be related to latitude, season, shading by nearshore forest, sand color, precipitation, nest depth, and anthropogenic events like beach nourishment (adding sand) and adjacent housing projects (King et al., 2013). Significant differences (p<0.0001) between female ratio estimations for the nesting seasons resulted from SDP temperature variations (Table 1, Figure 1b) between the nesting seasons. The highest proportion of females were present in the 2016 and 2018 nesting seasons with the highest mean SDP temperature (Table 1). The higher female ratio estimations may be due to higher temperatures during these nesting seasons and the higher temperatures recorded on data loggers that were placed in the nests in July (90.91%, 2016; 100%, 2018). In our study, the female ratio estimations showed no statistically significant correlation with distance from the sea. However, Türkozan et al. (2003) stated that distance from sea of Loggerhead Turtle nests was inversely related to nest temperature at Fethiye beach, Turkey, and Kılıç and Candan (2014) found a positive correlation between nest temperature and distance from sea for Green Turtle nests on Sugözü Beach, Turkey. Moreover, the female ratio estimations had a statistically significant negative correlation with ID and nest depth. ID and nest depth were negatively correlated with ID temperature (Mrosovsky et al., 1999; Matsu-zawa et al., 2002; Santidrián Tomillo et al., 2017) and when ID and nest depth increased, the female ratio decreased. In this study, we observed a female-biased sex ratio in Green Turtle hatchlings on Akyatan Beach, as in Heron Island, Southern Great Barrier Reef (Booth & Freeman, 2006), Akdeniz, Karpaz Beach and Alagadi beaches, Northern Cyprus (Kaska et al., 1998; Broderick et al., 2000), Sugözü and Samandag beaches, Turkey (Kılıç & Candan, 2014; Candan & Kolonkaya, 2016; Yalçın-Özdilek et al., 2016). Casale et al. (2000) estimated a female-biased sex ratio in Green Turtle hatchlings on Akyatan Beach according to subsurface sand temperatures. Determining the hatching sex ratio in Green Turtles that have strong nest site fidelity is important for the maintenance of viable population size (King et al., 2013). Sea turtles with TSD are particularly vulnerable to climate change and incubation temperatures will significantly increase over the next years, following the overall warming patterns of the Mediterranean region (Türkozan et al., 2021). As the global warming trend progresses, the female ratio of sea turtle hatchlings will be increased. To maintain populations and sex ratio of sea turtles, shading or sprinkling of nests can be applied during incubation (Fuentes et al., 2009; Poloczanska et al., 2009). However, for adaptation of marine turtles to global environmental changes in the face of long-term temperature increases strategies could be developed such as changes in nesting phenology (Mazaris et al., 2009), spatial shifts of nesting sites (Pike, 2013), and preference for cooler shady areas or laying deeper nests (Mainwaring et al., 2017).

**Supplementary Material**

Supplementary Material is given as a Supplementary Annex, which is available via the “Supplementary” tab on the article’s online page.

**Acknowledgements**

The authors would like to thank WWF-Turkey Marine Officer Ebru Tural, Wildlife Officer Mehmet Tural and volunteers who contributed field activities in the study.
Funding
This study is part of an ongoing project funded by WWF Turkey through a cooperation with the 7th Regional Directorate of Nature Conservation and National Parks of Turkish Ministry of Agriculture and Forestry, Adana Office.

Disclosure Statement
No potential conflict of interest was reported by the authors.

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