Commagene Journal of Biology

Karaman, (2026) *Comm. J. Biol.* 10(1), 273-280.
DOI: 10.31594/commagene.1947796

e-ISSN 2602-456X

Research Article/Araştırma Makalesi

Primary Sex Ratio Estimation and the Feminizing Effect of Metabolic Heating in Green Sea Turtle (*Chelonia mydas*) Nests at Samandağ Beach

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Received: 09.05.2026 **Revised:** 17.06.2026 **Accepted:** 30.06.2026 **Published:** 14.07.2026

Abstract: This study investigated the primary sex ratios of green sea turtle (*Chelonia mydas*) nests at Samandağ Beach, Türkiye during the 2018 nesting season. Sex ratios were estimated using a symmetric logistic Temperature-Dependent Sex Determination (TSD) model applied to temperature data recorded throughout the thermosensitive period (TSP). In addition, the contribution of embryonic metabolic heating and its feminizing effect on sex ratio were evaluated. Nest temperatures were monitored in 12 nests; in five of these nests, additional temperature loggers were placed in adjacent control sand at the same depth and recorded simultaneously to quantify metabolic heat production. Incubation temperatures increased significantly over the course of embryonic development ($p < 0.001$), rising from 27.50–30.06°C during the first third of incubation to a mean of 32.27°C in the final third, with a maximum of 33.39 ± 0.66°C. Estimated primary sex ratios showed a strong female bias (mean: 87.71% ± 16.47), with only one nest producing a male-biased outcome (42.22% female at 29.07°C). While metabolic heating was negligible during early incubation, it increased markedly in later stages, elevating nest temperatures by an average of 1.99 ± 0.78°C above ambient sand temperatures during the final third. The feminizing effect of metabolic heating averaged 8.75% ± 10.59 and ranged from 1.14% to 27.36% among nests. This effect was most pronounced when ambient sand temperatures were close to the transitional range of the TSD reaction norm. Overall, the findings confirm a strongly female-biased sex ratio at the Samandağ Beach and demonstrate that metabolic heating can alter hatchling sex ratios beyond estimates based solely on sand temperature. These results highlight the importance of considering both environmental and metabolic factors in conservation assessments of Eastern Mediterranean *C. mydas* populations.

Keywords: Incubation temperature, temperature-dependent sex determination, metabolic heating, *Chelonia mydas*.

Samandağ Kumsalı'ndaki Yeşil Deniz Kaplumbağası (*Chelonia mydas*) Yuvalarında Birincil Eşey Oranının Tahmini ve Metabolik Isınmanın Dişileştirici Etkisi

Öz: Bu çalışmada, 2018 yuvalama sezonunda Samandağ Kumsalı'ndaki yeşil deniz kaplumbağası (*Chelonia mydas*) yuvalarının birincil eşey oranları incelenmiştir. Eşey oranları, termosensitif periyot (TSP) boyunca kaydedilen sıcaklık verileri kullanılarak simetrik lojistik TSD modeli ile tahmin edilmiştir. Ayrıca, embriyonik metabolik ısınmanın katkısı ve bu etkinin eşey oranı tahminleri üzerindeki dişileştirici etkisi değerlendirilmiştir. Yuva sıcaklıkları 12 yuvada izlenmiş; bunların beşinde metabolik ısı üretimini belirlemek amacıyla aynı derinliğe yerleştirilen eşzamanlı kontrol kum sıcaklığı veri kaydedicileri kullanılmıştır. Kuluçka sıcaklıklarının embriyonik gelişim süresince anlamlı düzeyde arttığı belirlenmiştir ($p < 0.001$); sıcaklıklar kuluçkanın ilk üçte birlik döneminde 27.50–30.06°C aralığından, son üçte birlik dönemde ortalama 32.27°C'ye yükselmiş ve 33.39 ± 0.66°C ile en yüksek değere ulaşmıştır. Tahmin edilen birincil eşey oranları güçlü bir dişi baskınlığı göstermiştir (ortalama: %87.71 ± 16.47); yalnızca bir yuvada erkek baskın eşey oranı belirlenmiştir (%42.22 dişi, 29.07°C). Metabolik ısınma kuluçkanın erken evresinde ihmal edilebilir düzeydeyken ilerleyen evrelerde belirgin şekilde artmış ve son üçte birlik dönemde yuva sıcaklıklarını çevresel kum sıcaklığına göre ortalama 1.99 ± 0.78°C yükseltmiştir. Metabolik ısınmanın dişileştirici etkisi ortalama %8.75 ± 10.59 olarak bulunmuş ve yuvalar arasında %1.14 ile %27.36 arasında değişmiştir. Bu etkinin, çevresel kum sıcaklıklarının TSD tepki eğrisinin geçiş aralığına yakın olduğu durumlarda en belirgin olduğu görülmüştür. Sonuç olarak, bulgular Samandağ kumsalında güçlü bir dişi ağırlıklı eşey oranını doğrulamakta ve metabolik ısınmanın, yalnızca kum sıcaklığına dayalı tahminlerin ötesinde yavru eşey oranlarını değiştirebildiğini göstermektedir. Bu durum, Doğu Akdeniz *C. mydas* popülasyonlarının korunmasına yönelik değerlendirmelerde hem çevresel hem de metabolik faktörlerin birlikte dikkate alınması gerektiğini vurgulamaktadır.

Anahtar kelimeler: İnkübasyon sıcaklığı, sıcaklığa bağlı cinsiyet belirlenmesi, metabolik ısınma, *Chelonia mydas*.

1. Introduction

Sea turtles are marine reptiles known for their complex life cycles and key ecological roles in marine ecosystems (Wyneken et al., 2013). Despite their wide global distribution, most species are currently classified as threatened or endangered, reflecting ongoing environmental pressures and conservation challenges (Wallace et al., 2011). Among them, *C. mydas* represents a key species due to its ecological importance and widespread distribution. Although it has recently been classified as Least Concern at the global scale (Wallace & Broderick, 2025), regional populations show considerable variation in conservation status. In the Mediterranean, *C. mydas* is currently listed as Near Threatened (Broderick et al., 2024), highlighting the importance of understanding population-specific responses to environmental change.

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Temperature is one of the primary environmental drivers of reproductive success in sea turtles, particularly in species exhibiting temperature-dependent sex determination (TSD). In such species, incubation temperature directly determines offspring sex ratios, thereby influencing population structure and long-term viability (Santidrian Tomillo & Spotila, 2020). *C. mydas* exhibits TSD, with a pivotal temperature of approximately 29°C producing balanced sex ratios, while higher temperatures result in female-biased populations (Mrosovsky, 1994; Mrosovsky & Pieau, 1991). Under ongoing climate change, increasing sand temperatures in nesting environments are intensifying the risk of feminization, a phenomenon that may severely compromise population persistence (Hawkes et al., 2007; Smith et al., 2021). Temperature-dependent sex determination in sea turtles is typically described using sigmoid thermal reaction norms that mathematically relate constant incubation temperatures to resulting sex ratios. The development of these models has progressed over the decades, beginning with the symmetric logistic equation (Girondot, 1999), originally derived from population growth functions and defined by two biologically interpretable parameters: the pivotal temperature (P), at which a 1:1 sex ratio is produced, and the scale parameter (*S*), which characterizes the slope of the transition. To accommodate asymmetry in the thermal response, alternative formulations have subsequently been introduced, including the Hill equation (Hill, 1910), the A-logistic model (Godfrey et al., 2003), and the four-parameter Hulin model (Hulin et al., 2009). However, the Hill equation has been considered insufficiently flexible to capture asymmetric TSD patterns (Godfrey et al., 2003), the A-logistic model controls the transitions toward the lower and upper asymptotes through a single parameter and therefore cannot characterize each asymptote independently, and the Hulin model has been described as challenging to fit due to convergence to local minima during maximum likelihood optimization (Abreu-Grobois et al., 2020). More recently, the flexible-logistic (flexit) model has been introduced to overcome these constraints by enabling independent control of the lower and upper asymptotes through four parameters (Abreu-Grobois et al., 2020). Comparative analyses in *C. mydas* have consistently identified the logistic model as providing robust fits to empirical sex ratio data (Godley et al., 2002; Stubbs & Mitchell, 2018), making it a widely accepted framework for describing TSD patterns.

In addition to ambient environmental conditions, nest temperature dynamics are also influenced by metabolic heating generated during embryonic development (Gammon et al., 2020; Önder & Candan, 2016; Sönmez, 2018). A recent systematic review of all sea turtle species reported that metabolic heating is strongly stage-dependent, with mean values of approximately 0.5°C in the first third of incubation, 0.9°C in the middle third, and peaking at 2.6°C in the final third (Gammon et al., 2020). Although metabolic heating typically peaks after the thermosensitive period (TSP), contributions exceeding 1°C during the TSP have been documented in approximately 20% of reviewed studies (Gammon et al., 2020), with feminizing effects of 5–10% reported for *C. mydas* nests in the eastern Mediterranean (Önder & Candan, 2016; Sönmez, 2018). The biological significance of metabolic heating is strongly moderated by ambient sand temperature, as small thermal increments can rapidly shift primary sex ratios when sand temperatures fall within the steep portion of the TSD reaction norm (Gammon et al., 2020). Quantifying this contribution is therefore essential for accurately characterizing the thermal environment of developing embryos and for refining sex ratio predictions in regional populations.

The Eastern Mediterranean basin represents a critical nesting region for *C. mydas*, with nesting activity largely concentrated along the coasts of Türkiye and Cyprus (Casale et al., 2018). Among these, Samandağ Beach (Hatay, Türkiye) is one of the most important nesting sites in the Mediterranean, supporting a substantial proportion of the regional population (Sönmez et al., 2024). Given its importance and the increasing risk of temperature-driven feminization, a better understanding of nest thermal dynamics and their implications for sex ratio estimation is needed. This study characterizes nest thermal profiles during the 2018 nesting season and estimates primary sex ratios based on temperatures during the middle third of incubation using a logistic TSD framework. In addition, the contribution of metabolic heating to nest thermal conditions and its potential influence on feminization patterns are evaluated by comparing nest and ambient sand temperatures.

2. Material and Method

2.1. Study Area and Thermal Monitoring Data

Data were collected on Samandağ Beach, Türkiye (36°07′ N, 35°55′ E) during the 2018 nesting season. The beach extends approximately 14 km from Çevlik Harbour in the north to the Sabca Promontory in the south and is divided into three sub-sections: Çevlik (5.5 km), Şeyh-Hızır (4.1 km), and Meydan (4.4 km) (Sönmez et al., 2024; Yalçın Özdilek, 2007). The Şeyh-Hızır sub-section (Fig. 1), located between the Şeyh-Hızır Tomb and the mouth of the Asi River, was selected for analysis due to its high nesting density (Sönmez, 2018; Sönmez et al., 2024).

Figure 1.Sampling area of nesting beach.

Nest temperatures were monitored in 12 *C. mydas* nests using temperature data loggers (Tinytag Talk 2, Gemini Data Loggers, Chichester, UK; resolution = 0.01°C, accuracy = ±0.5°C, IP54 splash-proof housing). Loggers were placed at the center of the clutch during oviposition, after approximately half of the eggs had been deposited, and retrieved immediately after hatchling emergence. Temperature was recorded at 5-minute intervals

throughout the incubation period. Incubation duration was defined as the period between egg deposition and the emergence of the first hatchlings (Broderick et al., 2000). To assess the contribution of metabolic heating, an additional control logger was deployed in five of the monitored nests. Control loggers were placed approximately 50 cm laterally from each nest at the same depth to record ambient sand temperature representative of the nest's thermal environment.

2.2. Incubation Stages, Metabolic Heating, and Feminization Effect

The total temperature record of each nest was divided into three temporally equal phases corresponding to canonical embryonic developmental stages (Mrosovsky & Pieau, 1991): First-Third (FT), Mid-Third (MT) coinciding with the TSP for sex determination, and Last-Third (LT). For each of the 12 monitored nests, mean nest temperature, standard deviation, and range (min–max) were computed within each phase (Table 1). All individual 5-minute recordings were pooled across the 12 nests within each phase ($n \approx 149\,000$ total observations) and compared using the Kruskal–Wallis rank sum test. Statistical significance was set at $\alpha = 0.05$. Analyses were performed in R v4.5.2 using the *tidyverse* (Wickham et al., 2019) and *ggpubr* (Kassambara, 2018) packages.

Metabolic heating was quantified for the five nests with paired control loggers as the difference between the nest center temperature (T_nest) and the corresponding ambient sand temperature (T_sand) recorded at the same time interval (ΔT = T_nest − T_sand). Mean ΔT values were calculated for each incubation phase (FT, MT, and LT) and reported as mean ± standard deviation (Sönmez, 2018). The feminizing effect of metabolic heating was estimated for each of the five paired-logger nests as the difference between the predicted female ratio based on the mean nest temperature during the TSP and the predicted female ratio (Stubbs & Mitchell, 2018) based on the corresponding mean sand temperature over the same period (Broderick et al., 2001; Önder & Candan, 2016; Sönmez, 2018).

2.3. Sex Ratio Estimation

Hatchling sex ratios were estimated for each nest by applying a symmetric logistic TSD model (Girondot, 1999) to the mean temperature recorded during the TSP. The proportion of females produced at temperature T was calculated as:

$$\mathrm{sr}(T) = \frac{1}{1 + exp\left(\frac{T - P}{S}\right)}$$

where P is the pivotal temperature (the constant temperature producing a 1:1 sex ratio) and S is the scale parameter controlling the slope of the transition. Following Stubbs and Mitchell (2018), species-specific parameters derived from controlled constant-temperature incubation experiments on *C. mydas* were adopted: P = 29.2°C, S = -0.421 and TRT= 2.5°C.

All analyses were performed in R v4.5.2 (R Core Team, 2016) using the *tidyverse* (Wickham et al., 2019), *lubridate* (Grolemund & Wickham, 2011), *ggpubr* (Kassambara, 2018), *patchwork* (Pedersen, 2019), and *scales* (Wickham et al., 2016) packages.

3. Results

3.1. Thermal Conditions and Incubation Stages

A total of 12 *C. mydas* nests were monitored during the 2018 nesting season (laying dates ranging from June 6 to July 15). The incubation periods varied considerably, ranging from a minimum of 42 days (Nest 8) to a maximum of 57 days (Nest 1). As detailed in Table 1, the overall mean incubation temperatures (TM) across the entire developmental period ranged from 28.93 ± 1.17°C (Nest 1) to 31.32 ± 1.48°C (Nest 7). When the incubation period was mathematically divided into three equal temporal stages (First, Mid, and Last-Third), a consistent and highly significant thermal progression was observed across all nests (Fig. 2). A Kruskal-Wallis test confirmed that the mean temperatures differed significantly among the three developmental stages ($p < 0.001$) (Fig. 2). During the first third of incubation, nest temperatures were relatively low and stable, largely reflecting ambient sand temperatures. Mean temperatures during this stage ranged from 27.50°C (Nest 1) to 30.06°C (Nest 13). During the middle third of incubation, corresponding to the thermosensitive period for sex determination, temperatures increased and ranged from 29.09°C to 31.15°C. During the final third of incubation, nest temperatures reached their highest values as a result of embryonic metabolic heating, with mean temperatures reaching up to 33.39 ± 0.66°C in Nest 3 (Table 1).

3.2. Sex Ratio Estimation

Mean TSP temperatures across the 12 monitored nests ranged from 29.07°C to 31.16°C (Table 1), with an overall mean of 30.32 ± 0.64°C. Applying the symmetric logistic TSD model to these temperatures yielded predicted female ratios from 42.22% (Nest 1) to 99.07% (Nest 13), with an overall mean female proportion of 87.71 ± 16.47% (Fig. 3).

Nest 1 was the only male-biased nest in the dataset, with a TSP temperature of 29.07°C and a predicted female ratio of 42.22%. Three nests (Nests 2, 9, and 12) produced female ratios between 70% and 90% (72.87%, 78.87%, and 89.93%, respectively), while the remaining eight nests yielded female ratios of ≥ 90%. Among these, the three warmest nests (Nests 5, 7 and 13) exceeded 98% female (Fig. 3).

Table 1. Summary of incubation parameters and stage-specific thermal profiles for *Chelonia mydas* nests at Samandağ Beach.

Nest	Laying Date	ID	FTT + SD	Min-Max	MTT + SD	Min-Max	LTT + SD	Min-Max	TMT + SD	Min-Max
N1	6.6.2018	57	27.50 ± 0.39	26.5 - 28.7	29.07 ± 0.47	28.0 - 29.8	30.20 ± 0.22	29.8 - 30.6	28.93 ± 1.17	26.5 - 30.6
N2	8.6.2018	53	28.13 ± 0.26	27.7 - 28.6	29.57 ± 0.64	28.5 - 30.8	31.65 ± 0.38	30.8 - 32.2	29.78 ± 1.52	27.7 - 32.2
N3	14.6.2018	47	28.13 ± 0.52	27.2 - 29.2	30.30 ± 0.82	29.1 - 31.8	33.39 ± 0.66	31.8 - 34.2	30.61 ± 2.26	27.2 - 34.2
N4	16.6.2018	49	28.82 ± 0.47	28.0 - 29.8	30.56 ± 0.67	29.7 - 32.0	32.46 ± 0.37	31.3 - 32.9	30.61 ± 1.57	28.0 - 32.9
N5	19.6.2018	49	29.29 ± 0.64	24.5 - 30.2	31.07 ± 0.61	30.1 - 32.2	32.99 ± 0.26	32.2 - 33.4	31.12 ± 1.60	24.5 - 33.4

Nest	Laying Date	ID	FTT + SD	Min-Max	MTT + SD	Min-Max	LTT + SD	Min-Max	TMT + SD	Min-Max
N6	24.6.2018	48	29.19 ± 0.36	28.6 - 29.8	30.56 ± 0.51	29.8 - 31.5	31.92 ± 0.18	31.4 - 32.2	30.56 ± 1.18	28.6 - 32.2
N7	27.6.2018	43	29.73 ± 0.51	28.5 - 30.5	31.10 ± 0.50	30.5 - 32.2	33.14 ± 0.38	32.2 - 33.6	31.32 ± 1.48	28.5 - 33.6
N8	29.6.2018	42	29.07 ± 0.36	28.6 - 29.7	30.30 ± 0.43	29.7 - 31.2	32.17 ± 0.50	31.2 - 32.8	30.51 ± 1.35	28.6 - 32.8
N9	1.7.2018	50	28.55 ± 0.23	28.3 - 28.9	29.73 ± 0.52	28.9 - 30.8	32.08 ± 0.54	30.8 - 32.7	30.12 ± 1.54	28.3 - 32.7
N11	6.7.2018	53	29.19 ± 0.35	28.5 - 29.7	30.28 ± 0.48	29.7 - 31.4	32.51 ± 0.38	31.3 - 32.9	30.66 ± 1.44	28.5 - 32.9
N12	12.7.2018	49	28.88 ± 0.29	28.4 - 29.3	30.13 ± 0.62	29.3 - 31.6	32.22 ± 0.68	30.4 - 32.8	30.41 ± 1.48	28.4 - 32.8
N13	15.7.2018	53	30.06 ± 0.26	29.0 - 30.5	31.16 ± 0.50	30.3 - 32.0	32.46 ± 0.24	31.9 - 35.6	31.22 ± 1.04	29.0 - 35.6

ID: Incubation Duration (days); FTT: First-Third Mean temperature (°C); MTT: Mid-Third Mean temperature (°C); LTT: Last-Third Mean temperature (°C); TMT: Total Incubation Mean temperature (°C).

Table 2. Descriptive statistics of nest and sand temperatures (°C) and metabolic heating across the three thirds of incubation. FT: first third; MT: middle third; LT: last third of incubation.

Period	Nest	Nest Temperature°C ± SD (Min-Max)	Sand Temperature°C ± SD (Min-Max)	Metabolic Heating°C ± SD (Min-Max)
FT	3	28.13 ± 0.52 (27.2-29.2)	28.14 ± 0.52 (27.2-29.1)	-0.02 ± 0.14 (-0.3-0.2)
	4	28.82 ± 0.47 (28.0-29.8)	29.07 ± 0.49 (28.3-30.1)	-0.25 ± 0.05 (-0.4--0.1)
	6	29.19 ± 0.36 (28.6-29.8)	29.34 ± 0.31 (28.8-29.9)	-0.15 ± 0.06 (-0.3--0.1)
	7	29.73 ± 0.51 (28.5-30.5)	29.49 ± 0.33 (28.8-30.1)	0.24 ± 0.19 (-0.3-0.4)
	8	29.07 ± 0.36 (28.6-29.7)	29.17 ± 0.32 (28.6-29.8)	-0.10 ± 0.05 (-0.2-0.1)
MT	3	30.30 ± 0.82 (29.1-31.8)	29.48 ± 0.28 (29.0-29.9)	0.82 ± 0.58 (0.1-2.1)
	4	30.56 ± 0.67 (29.7-32.0)	30.45 ± 0.28 (30.0-30.9)	0.11 ± 0.41 (-0.3-1.1)
	6	30.56 ± 0.51 (29.8-31.5)	30.27 ± 0.24 (29.9-30.7)	0.29 ± 0.27 (-0.1-0.9)
	7	31.10 ± 0.50 (30.5-32.2)	30.32 ± 0.18 (30.1-30.6)	0.78 ± 0.32 (0.4-1.5)
	8	30.30 ± 0.43 (29.7-31.2)	29.99 ± 0.16 (29.7-30.2)	0.31 ± 0.28 (-0.0-1.0)
LT	3	33.39 ± 0.66 (31.8-34.2)	30.28 ± 0.23 (29.7-30.6)	3.11 ± 0.52 (1.6-3.7)
	4	32.46 ± 0.37 (31.3-32.9)	30.96 ± 0.07 (30.8-31.1)	1.49 ± 0.32 (0.5-1.9)
	6	31.92 ± 0.18 (31.4-32.2)	30.80 ± 0.07 (30.7-30.9)	1.12 ± 0.17 (0.5-1.3)
	7	33.14 ± 0.38 (32.2-33.6)	30.76 ± 0.07 (30.6-30.9)	2.38 ± 0.34 (1.5-2.8)
	8	32.17 ± 0.50 (31.2-32.8)	30.33 ± 0.10 (30.2-30.5)	1.84 ± 0.43 (1.0-2.3)
Total	3	30.61 ± 2.26 (27.2-34.2)	29.30 ± 0.95 (27.2-30.6)	1.31 ± 1.40 (-0.3-3.7)
	4	30.61 ± 1.57 (28.0-32.9)	30.16 ± 0.86 (28.3-31.1)	0.45 ± 0.81 (-0.4-1.9)
	6	30.56 ± 1.18 (28.6-32.2)	30.14 ± 0.64 (28.8-30.9)	0.42 ± 0.56 (-0.3-1.3)
	7	31.32 ± 1.48 (28.5-33.6)	30.19 ± 0.57 (28.8-30.9)	1.13 ± 0.95 (-0.3-2.8)
	8	30.51 ± 1.35 (28.6-32.8)	29.83 ± 0.53 (28.6-30.5)	0.68 ± 0.89 (-0.2-2.3)
Overall	All Nests (N=5)	30.72 ± 1.65 (27.2-34.2)	29.92 ± 0.81 (27.2-31.1)	0.79 ± 1.03 (-0.4-3.7)

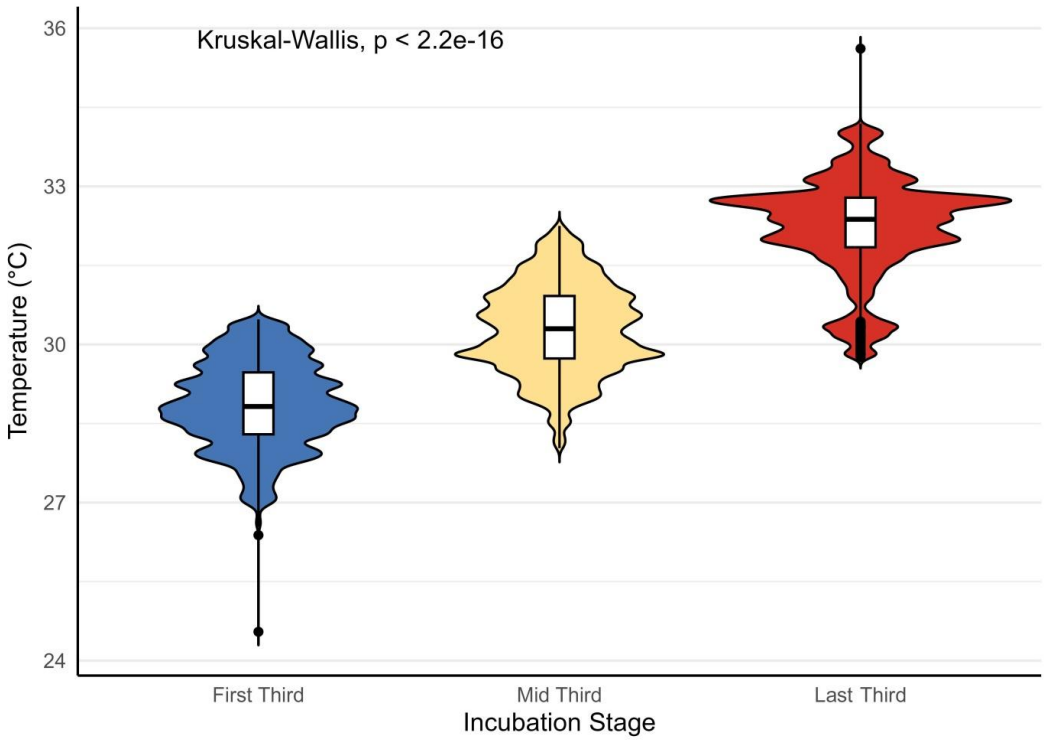

Figure 2. Temperature evolution across the three incubation stages of *Chelonia mydas* nests. Violin plots showing temperature distributions across the three incubation stages (FT: first third; MT: middle third; LT: last third) of *Chelonia mydas* nests. Internal boxplots indicate the median, interquartile range, and outliers.

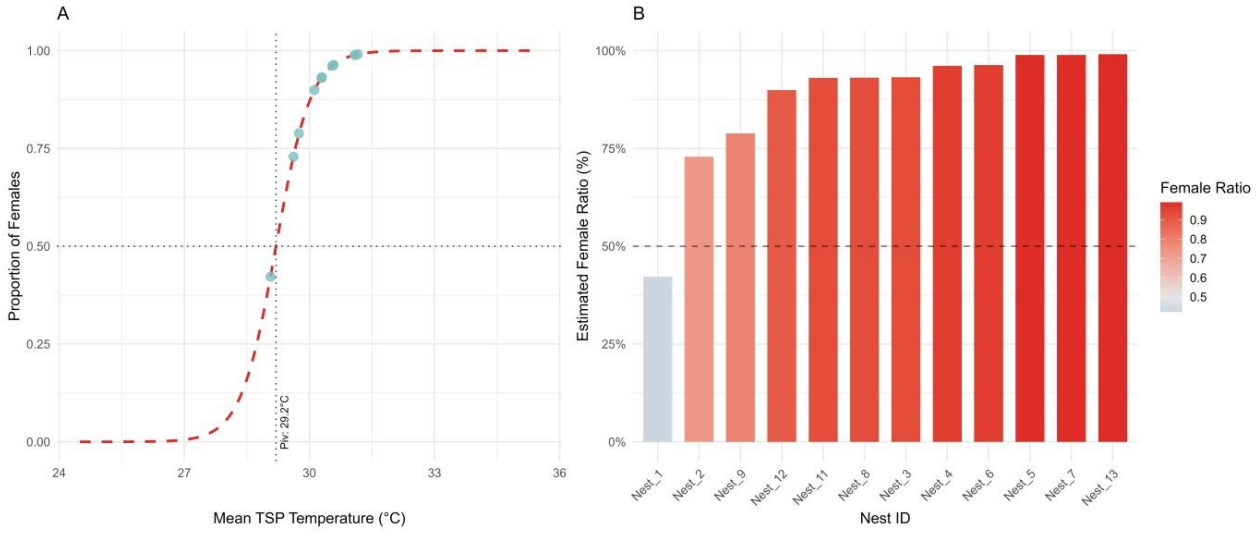

Figure 3. Temperature-Dependent Sex Determination (TSD) modeling and estimated sex ratios for the monitored *Chelonia mydas* nests. (A) Thermal reaction norm showing the relationship between incubation temperature and the proportion of females, modeled using a symmetric logistic function (dashed red line). The light blue scatter points represent the mean temperatures recorded during the thermosensitive period (TSP) for each nest, projected onto the model. The vertical and horizontal dotted lines indicate the pivotal temperature (P_{iv} = 29.2°C), where a 1:1 sex ratio is expected. (B) Estimated female proportions for individual nests (identified by Nest ID). Bar color indicates the estimated female proportion.

3.3. Metabolic Heating and Its Feminizing Effect

To quantify embryonic heat production, paired control loggers were deployed alongside five nests. Metabolic heating (ΔT) exhibited a strongly stage-dependent progression (Table 2; Fig. 4). During the FT, ΔT was practically negligible (mean −0.06 ± 0.19°C, range -0.25-0.24°C), indicating that early-stage embryos lacked sufficient biomass to influence their microclimate. Active heat production emerged during the MT, with mean ΔT shifting to consistently positive values (0.46 ± 0.32°C, range 0.11–0.82°C). The most pronounced thermal divergence occurred during the LT, when nest temperatures sharply decoupled from the surrounding sand (mean ΔT = 1.99 ± 0.78°C, range 1.12–3.11°C). Across the entire incubation period, the five nests maintained an overall mean temperature 0.79 ± 1.03°C above the adjacent sand (Table 2).

To evaluate the contribution of metabolic heating to feminization, predicted female ratios derived from nest temperatures were compared with those estimated from corresponding sand temperatures during the TSP (Table 3). The feminizing effect averaged 8.75 ± 10.59%, ranging from 1.14% to 27.36%. The strongest feminizing effect was observed in Nest 3, where the sand and nest temperatures during the TSP were 29.48°C and 30.30°C, respectively; this 0.82°C difference corresponded to a shift in the predicted female ratio from 65.83% (sand-based) to 93.19% (nest-based) (Table 3). In Nest 4, the sand and nest temperatures were 30.45°C and 30.56°C, and the corresponding 0.11°C difference produced a much smaller shift in female ratio (95.00% to 96.14%).

Table 3. Feminizing effect of metabolic heating.

Nest No	MTT Nest (°C)	MTT Sand (°C)	ΔT (°C)	Female % (nest)	Female % (sand)	Feminization %
3	30.30	29.48	0.82	93.19	65.83	27.36
4	30.56	30.45	0.11	96.14	95	1.14
6	30.56	30.27	0.29	96.33	92.81	3.52
7	31.10	30.32	0.78	98.90	93.49	5.41
8	30.30	29.99	0.31	93.08	86.77	6.31

4. Discussion

The female-biased primary sex ratio estimated in this study (mean = 87.71 ± 16.47%, n = 12) aligns closely with the high female proportions reported for major *C. mydas* rookeries in the Eastern Mediterranean, such as Alagadi in Northern Cyprus (86–96%; Broderick et al., 2000) and Akyatan in Türkiye (89%; Yılmaz & Oruç, 2022). An earlier study conducted in Samandağ between 2003 and 2007 reported a temperature-based mean female proportion of 73.7 ± 15.8% (n = 14 nests), together with a histology-based estimate of 85% derived from 190 dead hatchlings (Yalçın Özdilek et al., 2016). Although that study reached a similar overall conclusion of strong female bias, it differed from the present study in its approach to sex-ratio estimation. Female proportions in that study were estimated using the linear regression equation of Kaska et al. (1998), whereas the present study applied the symmetric logistic TSD model (Girondot, 1999; Stubbs & Mitchell, 2018), which more closely reflects the nonlinear relationship between incubation temperature and sex ratio. Despite these methodological differences, the mean female proportion estimated in the present study (87.71%) was similar to the histology-based estimate reported by Yalçın Özdilek et al. (2016) (85%), supporting the applicability of the logistic model for sex-ratio prediction at Samandağ Beach.

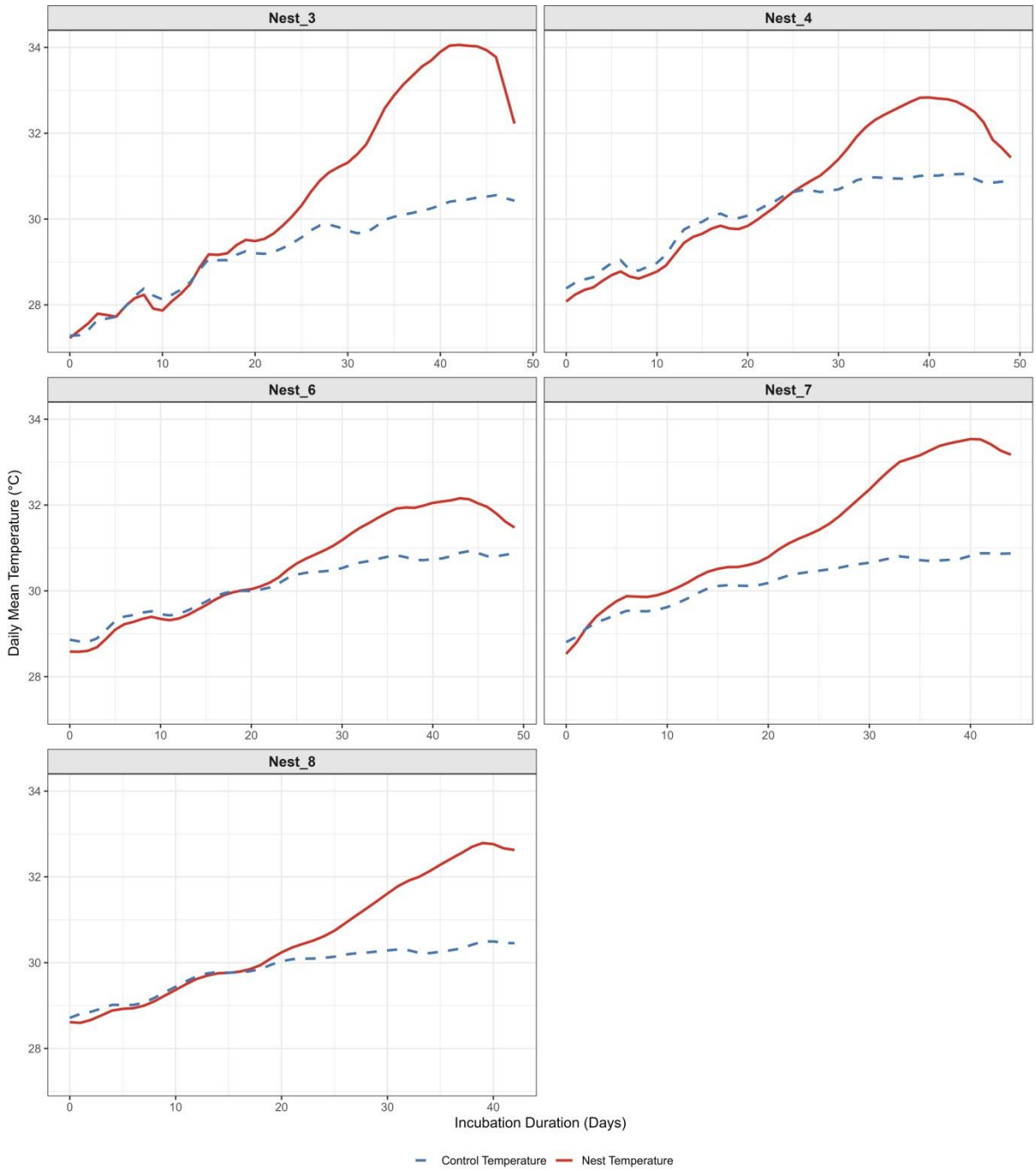

Figure 4. Daily mean nest temperature and control temperature throughout the incubation period in five nests of *Chelonia mydas*. Solid red lines represent nest temperatures, and dashed blue lines indicate control temperatures. The x-axis shows incubation duration (days), and the y-axis shows daily mean temperature (°C).

In contrast, lower female proportions have been reported from other regional nesting sites, including Sugözü Beach (54.9–56.5%; Candan & Kolankaya, 2016) and Kazanlı Beach (67%; Candan, 2023). At Sugözü, sex ratios were estimated from pivotal incubation duration, whereas the Kazanlı estimate was based on histological examination. Such variation among nesting sites, even within the same region, likely reflects differences in local thermal conditions. However, methodological differences among studies should also be considered when comparing sex-ratio estimates derived from different proxies.

Beyond environmental thermal variability, the thermal regime determining hatchling sex is also shaped by metabolic heat generated by developing embryos, which contributes substantially to incubation temperatures. In five nests monitored throughout incubation alongside paired sand-temperature controls, metabolic heating increased progressively across developmental phases. Across the entire incubation period, the overall mean metabolic heating was 0.79 ± 1.03°C (Table 2). These values closely correspond to those previously reported for the region. At Samandağ during the 2016–2017 seasons, the overall mean metabolic heating was reported as 0.68 ± 0.34°C, with stage-specific values of −0.02, 0.37, and 1.69°C for FT, MT, and LT, respectively (Sönmez, 2018), while a similar progression (0.3, 0.6, and 1.6°C) was reported at the neighbouring Sugözü beach (Önder & Candan, 2016). Beyond these absolute thermal increments, the biological significance of metabolic

heating arises from its capacity to modify primary sex ratios. The mean feminizing effect observed in the present study (8.75 ± 10.59%) lies between the values reported for Sugözü (10.4%; Önder & Candan, 2016) and Samandağ (6.8%; Sönmez, 2018), supporting the view that metabolic heating is a consistent and biologically meaningful contributor to female-biased sex ratios in Eastern Mediterranean *C. mydas* populations. Collectively, these findings position the Samandağ population among the strongly feminized nesting sites of the Eastern Mediterranean. The mean TSP temperature across the 12 monitored nests (MTT = 30.32 ± 0.64°C) lies approximately 1.1°C above the 29.2°C pivotal threshold, indicating that the majority of nests develop on the feminizing slope of the TSD reaction norm a pattern that directly explains the observed female bias. The 35.6°C maximum recorded in Nest 13 during the LT phase exceeds the 33–35°C upper tolerance range generally reported for sea turtle embryos; however, Howard et al. (2014) emphasized that exposure duration may be a more decisive determinant than peak temperature alone. In a 5-year study at Akyatan, Turkozan et al. (2021) demonstrated that exceeding the 33°C threshold for more than approximately two-fifths of the incubation period was associated with a marked decline in hatching success; the same study projected that under the RCP4.5 scenario the proportion of days exceeding 35°C could rise from 1% to 19.3%. At the same time, female-biased primary sex ratios do not necessarily translate directly into proportionally biased operational sex ratios (OSR), as more frequent breeding migrations by males can substantially compensate for hatchling-level female bias at the OSR level (Hays et al., 2010). These findings underscore the importance of incorporating metabolic heating into sex ratio estimation, particularly for nests in which mean temperatures lie close to the pivotal threshold, where metabolic contributions can substantially alter primary sex ratios. Given that the Mediterranean is projected to warm at rates exceeding the global average (IPCC, 2021; Lionello & Scarascia, 2018) and that nest temperatures in the region are anticipated to rise substantially over this century (Turkozan et al., 2021). Under these conditions, an increasing proportion of nests is likely to produce exclusively female hatchlings, with a corresponding decline in male hatchling production (Jensen et al., 2018). Long-term monitoring of these dynamics, together with the systematic incorporation of metabolic heating into sex ratio models, will be critical for accurately assessing the vulnerability of regional *C. mydas* populations to ongoing climate change.

Acknowledgement: I would like to thank Prof. Dr. Bektaş Sönmez for granting access to the Samandağ nesting beach and for his support during fieldwork. This study was conducted under the authorization of the Republic of Türkiye Ministry of Agriculture and Forestry, 7th Regional Directorate (Hatay Province).

Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The author declares that there is no conflict of interest.

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