

Hatchling sex ratio, body weight and nest parameters for *Chelonia mydas* nesting on Sugözü beaches (Turkey)

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Abstract

Hatchling sex ratio, body weight and nest parameters for Chelonia mydas nesting on Sugözü beaches (Turkey).— We investigated the relationship between nest parameters, hatchling body mass, and sex ratio of green turtle, *Chelonia mydas*, embryos and hatchlings at the temperate nesting rookery of Sugözü Beach (Adana–Turkey). Mean nest temperature and distance from the sea were correlated, while mean nest temperature and incubation period were inversely related. There was no apparent relationship between incubation period and hatchling mass. Hatchling and embryo sex ratios, determined by histological examination, showed a 70.5% and 93.5% female bias, respectively. There was no correlation between sex and body weight of hatchlings, but mean hatchling bodyweight of females (16.8 g) was slightly higher than that of males (16.2 g).

Key words: *Chelonia mydas*, Hatchling mass, Nest parameters, Sex ratio, Sugözü beaches

Resumen

La proporción de sexos y el peso corporal de las crías y los parámetros del nido en la población de Chelonia mydas que anida en las playas de Sugözü (Turquía).— Estudiamos la relación entre los parámetros del nido y el peso corporal y la proporción de sexos de los embriones y las crías de tortuga verde, *Chelonia mydas*, en la colonia de cría templada de la playa de Sugözü (Adana, Turquía). Se observó que la temperatura media del nido y la distancia al mar estaban correlacionadas, mientras que la temperatura media del nido y el período de incubación estaban inversamente relacionados. No hubo relación aparente entre el período de incubación y el peso de las crías. La proporción de sexos en las crías y los embriones, que se determinó mediante examen histológico, mostró una desviación en favor del sexo femenino del 70,5 y el 93,5%, respectivamente. No hubo correlación entre el sexo y el peso corporal de las crías, pero el promedio del peso corporal de las hembras (16,8 g) fue ligeramente superior al de los machos (16,2 g).

Palabras clave: *Chelonia mydas*, Peso de las crías, Parámetros del nido, Proporción de sexos, Playas de Sugözü

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Introduction

The sex of a sea turtle depends on incubation temperature (Bull, 1980). The temperature at which hatchling sex ratios are 1:1 is known as the pivotal temperature; when incubation temperature is higher than the pivotal temperature, hatchlings become female, and when it is lower than the incubation temperature, hatchlings become male (Yntema & Mrosovsky, 1980).

Valenzuela et al. (2004), using landmark-based geometric morphometric methods, found that morphological methods, while producing high accuracy when compared with 'true sex' in *Chrysemys picta* (98%) and *Podocnemis expansa* (90%), had a discriminant function analysis cross-validation rate of only 85% when used to determine sex in hatchling turtles. Besides carapace length and width, weights of hatchlings, temperature profiles, nest parameters, and histological examination (Glen et al., 2003; Ischer et al., 2009; Sönmez et al., 2011) have been considered to determine the sex of hatchling turtles in the wild. A non-invasive, accurate methodology relating duration of incubation to hatchling sex ratio has also been developed for artificially incubated eggs (Mrosovsky et al., 2002; Zbinden et al., 2007; Katselidis et al., 2012). Although various methods may be used to determine hatchling sex ratios, their accuracy is variable (Ceriani & Wyneken, 2008) and only histological examination ensures accurate sex determination (Mrosovsky & Benabib, 1990; Mrosovsky & Godfrey, 1995).

We studied the relationship between nest parameters of *Chelonia mydas* L., 1758 on Sugözü beaches during the 2012 nesting season. Temperatures in eight *Chelonia mydas* nests were determined using data loggers, sex of hatchlings was determined by histological examination, and the relationship between nest temperatures, nest parameters, hatchling weights, and sexes was evaluated statistically.

Material and methods

Study site

Chelonia mydas were studied at the nesting site on Sugözü beaches (36° 48.677' N – 35° 51.068' E, 36° 52.795' N – 35° 56.017' E), located in the Adana province within the borders of Yumurtalık and Ceyhan counties. The site consists of four subsections (from west to east): Akkum (1.4 km), Sugözü (1.0 km), Botaş (0.6 km), and Hollanda (0.4 km), and consists of a total of 3.4 km suitable beach habitat. In 2012, the nesting season was between June and September (fig. 1).

Nest parameters

Gemini Data Loggers (Tinyalk Temperature Range H–30°C/+50°C Part No: TK–0040, UK) used to determine nest temperatures were placed in the middle of nests during nesting and programmed to provide hourly readings. Daily nest temperatures were determined by averaging 24 hourly measurements each day. Middle third and whole incubation temperatures were calculated.

Five parameters, incubation duration (time between date of egg laying and first day of hatchling emergence), clutch size (total number of eggs in the nest), hatching success (ratio of empty eggshells to total number of eggs [%]), nest depth (distance [cm] from surface of the sand to the bottom of the nest), and nest distance (distance [m] from high tide line to nest) from the sea, were used.

Weighing hatchlings

Dead hatchlings ($n = 88$) were collected in sterile plastic bags, and washed, dried, and weighed using a Densa JW precision scale ($600 \text{ g} \pm 0.01 \text{ g}$).

Histological determination of sex

After removing the genitourinary complex from hatchlings ($n = 88$) and late embryos ($n = 62$), the complex was preserved in 4% buffered paraformaldehyde. Samples to undergo histological examination were then embedded in paraffin blocks, further prepared at a thickness of $5 \mu\text{m}$ with a Thermo–Shandon microtome and stained with Haematoxylin and Eosin (H&E), and sealed with Entellan. Histological examination was carried out with a Nikon E 100 light microscope using both 10X and 40X objectives following the Yntema & Mrosovsky (1980) criteria for distinguishing ovaries and testicles.

Data analyses

The Pearson product–moment correlation coefficient (r) and simple linear regression coefficient of determination (r^2) were calculated and used to assess statistical significance among variables. The relationship between middle third temperature and sex ratio was evaluated by the Mann–Whitney (U) test. All statistical analyses were executed using an SPSS packaged program (SPSS Inc., Released 2006, Version 15.0. Chicago, USA).

Results

Nest temperatures and parameters

Table 1 presents nest temperatures and other parameters. It shows temperatures for the whole period and middle third were similar. There was no relationship between nest temperature and nest depth ($n = 8$, $r^2 = 0.38$, $P > 0.05$) and nest temperature with clutch size ($n = 8$, $r^2 = 0.11$, $P > 0.05$). The relationship between nest temperature and distance from the sea ($n = 8$, $r^2 = 0.85$, Pearson's $r = 0.927$, $P = 0.001$) was positive and the relationship between nest temperature and incubation duration was negative ($n = 8$, $r^2 = 0.71$, Pearson's $r = -0.846$, $P < 0.01$). We found a statistically significant difference between middle third temperature and hatchling sex ratio ($U = 12.500$, $P < 0.01$).

Hatchling weights and nest parameters

Weights and nest parameters were recorded for 88 hatchlings gathered from 31 nests throughout the nesting

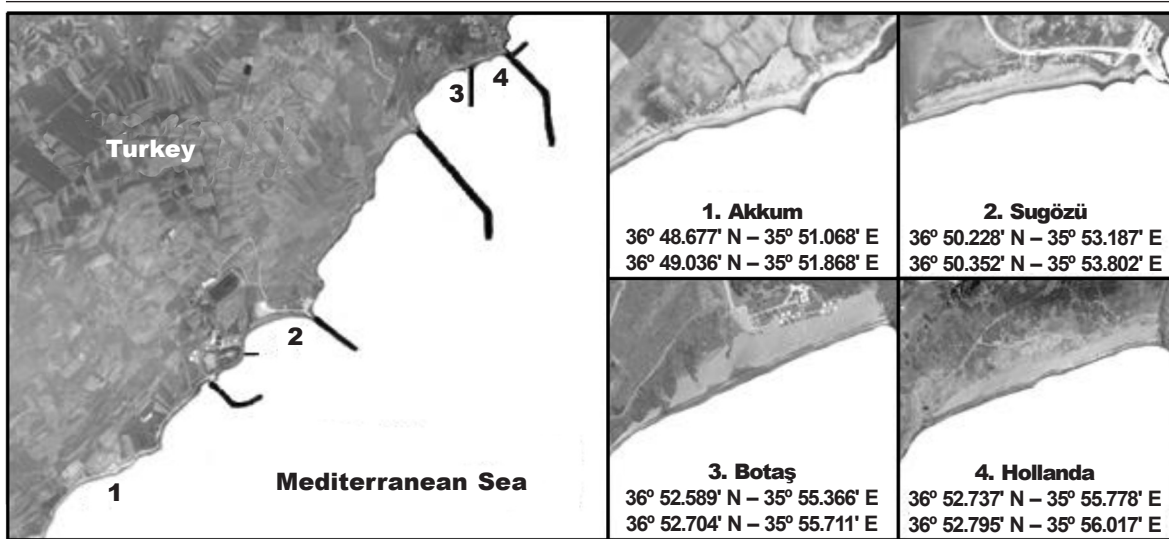


Fig. 1. Study site.

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season. No relationship was found between hatchling weight and duration of incubation ($n = 31, r^2 = 0.01, P > 0.05$), nest depth ($n = 31, r^2 = 0.01, P > 0.05$), distance from the sea ($n = 31, r^2 = 0.02, P > 0.05$), and hatching success ($n = 31, r^2 = 0.02, P > 0.05$). When relationships within nest parameters were assessed, only clutch size and nest depth appeared related ($n = 31, r^2 = 0.24, P < 0.01$).

Determination of sex ratios

Histological examination of 29 gonad samples taken from nests for which temperature data were available showed that female hatchling development occurred in 86% (table 1). When all gonad samples (150) were examined, 120 (80%) were determined to be female. When embryos and hatchlings were

Table 1. Nest temperatures and nest parameters ($n = 8$).

Tabla 1. Temperaturas y parámetros del nido ($n = 8$).

Nest number	Mean nest temperature		Incubation duration (day)	Clutch size (number)	Nest depth (cm)	Distance from sea (m)
	Whole period (°C)	Middle third (°C)				
1	30.5	30.4	54	103	75	31.4
2	32.6	32.5	48	134	86	35.5
3	32.1	32	45	103	84	40.4
4	31.1	31.3	49	73	82	36.5
5	31.4	31.3	44	46	62	36
6	28.1	27.9	61	146	102	12.9
7	29.6	29.7	57	142	90	19.8
8	29.3	29.2	51	88	99	24.9
Mean	30.6	30.5	51	104	85	29.7

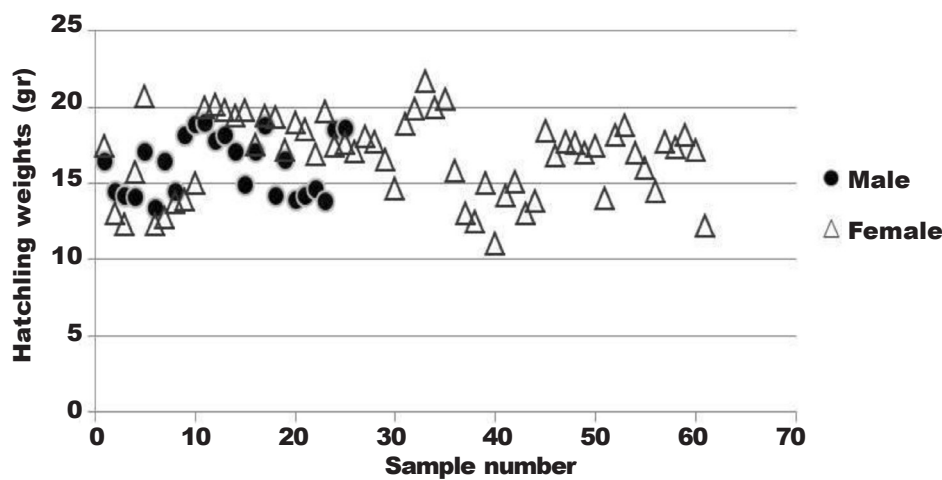


Fig. 2. Female and male hatchling weights.

Fig. 2. Pesos de las crías macho y hembra.

considered separately, 93.5% (58 of 62) of embryos were female, and 70.5% (62 of 88) of hatchlings were female.

Sex and hatchling weights

There was no significant difference in weight between male (mean = 16.2 g) and female (mean = 16.8 g) hatchlings (fig. 2).

Discussion

Most sex ratio studies performed on hatchling sea turtles (Öz et al., 2004; Candan 2006; Kaska et al., 2006; Katselidis et al., 2012; Uçar et al. 2012) along the Mediterranean coastline of Turkey concern nest and sand temperatures (Baran & Kasparek, 1989; Canbolat, 1991; Ergene et al., 2009), marine predation (Türkecan & Yerli, 2007), and morphometric diversification (Türkecan et al., 2008). Studies about sex ratio, body weight, and nest parameters among hatchlings are scarce (Sönmez et al., 2011).

The duration of incubation is reduced by increasing nest temperatures (Yntema & Mrosovsky, 1980; Godley et al., 2001; Wood et al., 2014). Our findings also support an inverse relationship between temperature and incubation duration. We found a positive relationship between nest temperature and nest distance from the sea. Candan (2006) found a similar positive relationship on the same beach. The greater the distance a nest is from the sea, the higher the nest temperature (Uçar et al., 2012).

Incubation duration and hatchling weight were not directly affected by nest temperature, supporting findings by Ischer et al. (2009) and Booth et al. (2013) who reported that *Chelonia mydas* hatchling weights

are not directly affected by nest temperatures. Average weights of female and male hatchlings were similar, and we found no significant relationship between hatchling sex and weight. A similar lack of significance was found in a study at Samandağ Beach (Sönmez, 2011), and in a laboratory study of *Chelonia mydas* hatchlings in Oman's Ras Al-Hadd Reserve (Mahmoud et al., 2005).

The sex ration of hatchlings in the Eastern Mediterranean is heavily skewed in favor of females (80%) in studies of natural temperature regimes (Kaska et al., 1998; Broderick et al., 2000; Casale et al., 2000). Katselidis et al. (2012) suggested that a sex ratio heavily biased in favor of females could be obtained from analysis of data from 1, 2 or 3 nesting seasons. When longer time frames, such as 20 or 150 years, are considered, results are likely to produce a 1:1 sex ratio. In our study, middle third (30.6°C) and whole incubation (30.5°C) temperatures were (table 1) greater than the pivotal temperature (28.9°C) defined by Kaska et al. (1998). When all sample gonads taken from nesting pre-hatchlings were examined histologically, the hatchling sex ratio was greater than 80% female, 20% male. Our findings are comparable to those of Kaska et al. (1998) and Elmas (2008), in which female percentages were 78.8% and 82.8% respectively, but they are lower than those of Broderick et al. (2000), at 96% female. While our results were significantly higher than those of Candan (2010), when considered separately, our embryo and hatchling sex ratios were significantly variable. This difference should be considered in other estimates of sex ratios.

Sex ratio varies between seasons, populations, and nesting sites (Merchant-Larios, 1999), and additional studies on sex ratio estimation are required to understand population dynamics in marine turtles. Although we distinguished between hatchling sex, weight, and

nest parameters in our study, the relationship between nest temperature and nest distance from the sea with duration of incubation is strong. It is encouraging that our results can be used to estimate sex ratio. Perhaps more precise results would be obtained by performing additional studies using additional samples over several nesting seasons.

References

- Baran, I. & Kasparek, M., 1989. *Marine Turtles Turkey, Status survey 1988 and recommendations for conservation and management*. WWF, Verlag, Heidelberg.
- Booth, D. T., Feeney, R. & Shibata, Y., 2013. Nest and maternal origin can influence morphology and locomotor performance of hatchling green turtles (*Chelonia mydas*) incubated in field nests. *Marine Biology*, 160: 127–137.
- Broderick, A. C., Godley, B. J., Reece, S. & Downie, J. R., 2000. Incubation periods and sex ratios of green turtles: highly female biased hatchling production in the eastern Mediterranean. *Marine Ecology Progress Series*, 202: 273–281.
- Bull, J. J., 1980. Sex Determination in Reptiles. *Quarterly Review of Biology*, 55: 3–21.
- Canbolat, A. F., 1991. The investigation on the population of the loggerhead sea turtle, *Caretta caretta* (Linnaeus 1758) nesting in Dalyan Beach, Mugla. Turkey. Doğa. *Turkish Journal of Zoology*, 15: 255–274.
- Candan, O., 2006. The sex–temperature relation on hatchlings of green sea turtles (*Chelonia mydas*) nesting in hollanda beach (Ceyhan–Adana). M. Sc. Thesis, Hacettepe University.
- 2010. Sex–temperature relation and histological investigation of sex on hatchlings of green sea turtle (*Chelonia mydas*) nesting in sugözü (Ceyhan–Adana) and Kazanlı (Kazanlı–Mersin) beaches. Ph. D. Thesis, Hacettepe University.
- Casale, P., Gerosa, G. & Yerli, S. V., 2000. Female–biased Primary Sex Ratio of the Green Turtle, *Chelonia mydas*, Estimated Through Sand Temperatures at Akyatan, Turkey. *Zoology in the Middle East*, 20: 33–42.
- Ceriani, S. A. & Wyneken, J., 2008. Comparative morphology and sex identification of the reproductive system in formalin–preserved sea turtle specimens. *Zoology*, 111: 179–187.
- Elmas, M., 2008. Early gonadal development and sexual differentiation in green turtle, *Chelonia mydas*. M. Sc. Thesis, Mustafa Kemal University.
- Ergene, S., Aymak, C., Uçar, A. H. & Kaçar, Y., 2009. The research on the population of *Chelonia mydas* and *Caretta caretta* nesting on Kazanlı Beach (Mersin) in 2005 nesting season. *Ege University Journal of Fisheries and Aquatic Sciences*, 26: 187–196.
- Glen, F., Broderick, A. C., Godley, B. J. & Hays, G. C., 2003. Incubation environment affects phenotype of naturally incubated green turtle hatchlings. *Journal of the Marine Biological Association of the United Kingdom*, 83: 1183–1186.
- Godley, B. J., Broderick, A. C., Downie, J. R., Glen, F., Houghton, J. D., Kirkwood, I., Reece, S. & Hays, G. C., 2001. Thermal conditions in nests of loggerhead turtles: further evidence suggesting female skewed sex ratios of hatchling production in the Mediterranean. *Journal of Experimental Marine Biology and Ecology*, 263: 45–63.
- Ischer, T., Ireland, K. & Booth, D. T., 2009. Locomotion performance of green turtle hatchlings from the Heron Island Rookery, Great Barrier Reef. *Marine Biology*, 156: 1399–1409.
- Kaska, Y., Downie, R., Tippett, R. & Furness, R. W., 1998. Natural temperature regimes for loggerhead and green turtle nests in the eastern Mediterranean. *Canadian Journal of Zoology*, 76: 723–729.
- Kaska, Y., Ilgaz, C., Ozdemir, A., Baskale, E., Turkoz, O., Baran, I. & Stachowitsch, M., 2006. Sex ratio estimations of loggerhead sea turtle hatchlings by histological examination and nest temperatures at Fethiye beach, Turkey. *Naturwissenschaften*, 93: 338–343.
- Katselidis, K. A., Schofield, G., Stamou, G., Dimopoulos, P. & Pantis, J., 2012. Females first? Past, present and future variability in offspring sex ratio at a temperate sea turtle breeding area. *Animal Conservation*, 15: 508–518.
- Mahmoud, I. Y., AlKindi, A. Y., Ba–Omar, T. A., Al–Siyabi, S., Al–Bahry, S. N., Elshafie, A. Q. & Bakheit, C. S., 2005. Emergence pattern of the Green Turtle, *Chelonia mydas*, hatchlings under laboratory and natural conditions. *Zoology in the Middle East*, 35: 19–27.
- Merchant–Larios, H., 1999. Determining hatchling sex. In: *Research and Management Techniques for the Conservation of Sea Turtles*: 130–136 (K. L. Eckert, K. A. Bjorndal, F. A. Abrer–Grobois & M. Donnelly, Eds.). IUCN/SSC Marine Turtle Specialist Group Publication, Pennsylvania, USA.
- Mrosovsky, N. & Benabib, M., 1990. An Assessment of 2 Methods of Sexing Hatchling Sea–Turtles. *Copeia*, 2: 589–591.
- Mrosovsky, N. & Godfrey, M. H., 1995. Manipulating sex ratios: turtle speed ahead. *Chelonian Conservation and Biology*, 1: 238–240.
- Mrosovsky, N., Kamel, S., Rees, A. F. & Margaritoulis, D., 2002. Pivotal temperature for loggerhead turtles (*Caretta caretta*) from Kyparissia Bay, Greece. *Canadian Journal of Zoology*, 80: 2118–2124.
- Öz, M., Erdoğan, A., Kaska, Y., Düşen, S., Aslan, A., Sert, H., Yavuz, M. & Tunç, M. R., 2004. Nest temperatures and sex–ratio estimates of loggerhead turtles at Patara beach on the South western coast of Turkey. *Canadian Journal of Zoology*, 82: 94–101.
- Sönmez, B., Turan, C. & Yalçın–Özdilek, Ş., 2011. The effect of relocation on the morphology of Green Turtle, *Chelonia mydas* (Linnaeus, 1758), hatchlings on Samandağ beach, Turkey. *Zoology in the Middle East*, 52: 29–38.
- Türkecan, O., Turkoz, O., Oruc, A., Mangit, F., Demirayak, F. & Yerli, S. V., 2008. A Preliminary Study on The Morphometric Variation of *Chelonia mydas* in Three Different Beaches of Turkey. *NOAA Technical Memorandum NMFS SEFSC*, 569: 1–251.

- Türkecan, O. & Yerli, S. V., 2007. Marine Predation on Loggerhead Hatchlings at Beymelek Beach, Turkey. *Israel Journal of Ecology and Evolution*, 53: 167–171.
- Uçar, A. H., Kaska, Y., Ergene, S., Aymak, C., Kaçar, Y., Kaska, A. & İli, P., 2012. Sex Ratio Estimation of the Most Eastern Main Loggerhead Sea Turtle Nesting Site: Anamur Beach, Mersin, Turkey. *Israel Journal of Ecology and Evolution*, 58: 87–100.
- Valenzuela, N., Adams, D. C., Bowden, R. M. & Gauger, A. C., 2004. Geometric morphometric sex estimation for hatchling turtles: A powerful alternative for detecting subtle sexual shape dimorphism. *Copeia*, 2004: 735–742.
- Wood, A., Booth, D. T. & Limpus, C. J., 2014. Sun exposure, nest temperature and loggerhead turtle hatchlings: Implications for beach shading management strategies at sea turtle rookeries. *Journal of Experimental Marine Biology and Ecology*, 451: 105–114.
- Yntema, C. L. & Mrosovsky, N., 1980. Sexual-Differentiation in Hatchling Loggerheads (*Caretta Caretta*) Incubated at Different Controlled Temperatures. *Herpetologica*, 36: 33–36.
- Zbinden, J. A., Davy, C., Margaritoulis, D. & Arlettaz, R., 2007. Large spatial variation and female bias in estimated loggerhead sea turtle hatchling sex ratio of Zakynthos (Greece). *Endangered Species Research*, 3: 305–312.
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