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**RESEARCH ARTICLE**

# Sex determination of green sea turtle (*Chelonia mydas*) hatchlings on the bases of morphological characters

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**Abstract**

Morphological differences between female and male hatchlings of green sea turtle (*Chelonia mydas*) were investigated to identify key morphological characters for sex determination. A total of 152 dead hatchlings of green sea turtles were examined for 14 morphometric and seven meristic characters in the 2008 and 2009 nesting seasons on Samandag Beach in the north-eastern Mediterranean Sea, Turkey. The sex of dead hatchlings was determined with gonad histology. Multivariate statistics revealed significant differences in three morphometric characters between females and males. The males had a longer curved carapace width (CCW), hind limb length (HLL) and plastron–cloaca length (PCL) than the females. Principal component analysis also supported the detected differences between sexes.

**Key Words:** Green Turtle, sex determination, gonad histology, morphology

## Introduction

Knowledge on the sex ratio in sea turtles is necessary to determine population dynamics. Sex ratios at hatching may be different from adult sex ratios. Thus, comparing sex ratio between hatchings and adults can give us information about differential mortality, migration and dispersal pattern between sexes (Bulmer 1994). The sex ratio is a demographic variable and particularly vulnerable to environmental conditions because the sex of sea turtles is determined by their incubation temperature (temperature dependant sex determination, TDS). The relationship between sex and incubation temperature is characterized by a pivotal temperature, which is equal proportion of male and female individuals

(Bull 1980). For instance, embryos incubated at high temperature (>29.0°C) hatch as females at larger proportion. On the other hand, cooler nests (<29.0 °C) produce a greater proportion of males (Mrosovsky 1994). Therefore, current and future populations of sea turtles may be affected by global climate change. Knowledge on the sex ratio of nesting beaches is important for the prediction of the future of sea turtles as well as for effective conservation planning because long-term survival depends on both female and male production (Janzen 1994).

Morphological differences are observed between sexes in mature turtles (Hendrickson 1958; Berry and Shine 1980), however, few or no external sex differences are evident at hatching (Valenzuela *et al.* 2004). Researchers therefore employ different techniques to determine the sex of hatchlings. As in other reptiles with TSD, sea turtles do not have sex chromosomes (Bull 1980). Therefore, various methods such as gonadal histology (Merchant-Larios *et al.* 1989; Godfrey and Mrosovsky 2006) and radio-immunoassay (Owens *et al.* 1978; Gross *et al.*1995), laparoscopy (Wyneken *et al.* 2007), direct observations of the gonads *in situ* (McCoy *et al.* 1983), and clearing of gonads *in toto* (van der Heiden *et al.* 1985), histology and paramesonephric ducts (Ikonomopoulou *et al.* 2012) have been used to determine sex. On the other hand, gonadal histology is the most commonly used method to determine sex.

Differences between male and female hatchlings may be revealed by morphological measurements, which is an easy and cheap method in comparison to the methods formerly given. Therefore, in the present study, we aimed to find sexually distinguishable morphological characters for female and male *Chelonia mydas* hatchlings.

**Materials and methods**

*C. mydas* hatchlings (n = 152) were collected on Samandag Beach (36° 7.500' N 35° 55.100' E), located along the northeastern Mediterranean in Turkey during the 2008 and 2009 nesting seasons (Figure 1). All dead hatchlings were found on the way to the sea. Fresh carcasses of hatchlings without decomposition were chosen in the field and then transferred to a laboratory for gonad analysis and morphological data collection. First, 13 morphometric and 7 meristic characters from the dead hatchlings were taken (Figure 2). Straight measurements were taken using manual callipers with an accuracy range of 0.1 mm and curved measurements were taken using a plastic tape measure: straight carapace length (SCL); straight carapace width (SCW); curved carapace length (CCL); curved carapace width (CCW); forelimb length (FLL; the maximum forelimb length from the point of the humerus to the end of the forelimb when held flat with light finger pressure); hind limb length (HLL; the maximum hindlimb length from the point of the femur to end of hindlimb when held flat with light finger pressure); head length (HL); head width (HW); head circumference (HC); total tail length (TTL); length between the cloaca and the end of the tail (CETL);

length between the end of the plastron and cloaca (PCL); and body depth (BD; the maximum body depth between the first vertebral scute and the plastron). Meristic traits constituted carapace scute patterns including nuchals (N), vertebrals (V), costals from both the left and right (C), marginals from both the left and right (M), and supracaudals (S).

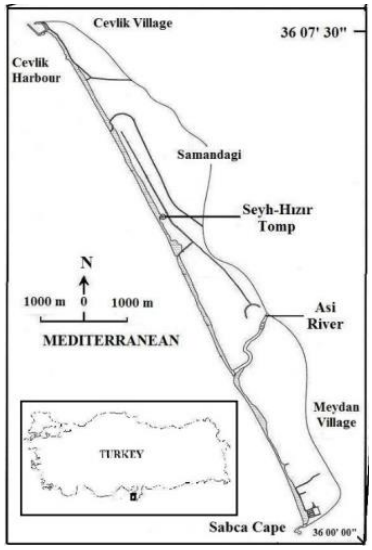


**Figure 1.** The map of the research area

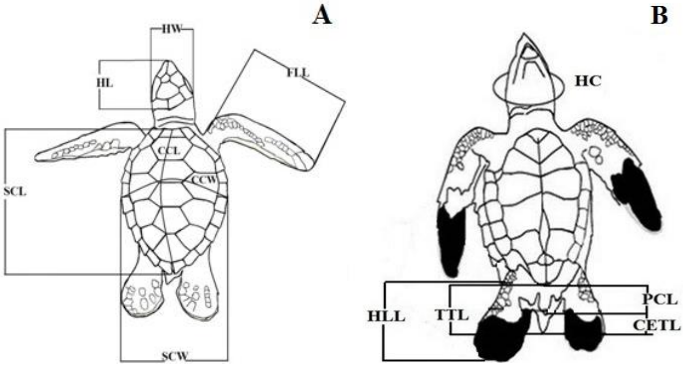


**Figure 2.** Morphometric measurements of the green turtle (*Chelonia mydas*) hatchlings (A: Dorsal side, B: Ventral side).

Measures of straight carapace length (SCL), straight carapace width (SCW), curved carapace length (CCL), curved carapace width (CCW), forelimb length (FLL), hind limb length (HLL), head length (HL), head width (HW), head

circumference (HC), total tail length (TTL), length between the cloaca and end of the tail (CETL), and length between the end of the plastron and cloaca (PCL). The sex of hatchlings was identified by gonadal histology (Figure 3). Dead hatchlings were dissected and their gonads were preserved in formalin solution. The gonads were cut in half transversely, with one-half embedded in paraffin wax, sectioned at 6–10 mm from the middle of the gonad, and stained with eosin and Harris hematoxylin. The sex of a hatchling was identified by microscopic examination of sections of the gonads for differentiation of the gonadal medulla and cortex or absence of seminiferous tubules (Yntema and Mrosovsky 1980). According to Yntema and Mrosovsky (1980), the ovary has the thick ventral germinal epithelium, the grooved surface and infolding of the germinal epithelium and the many small primary sex cords in the medulla. In males, the seminiferous tubules are embedded in a PAS positive stroma and tunica albuginea is covered by a thin layer of epithelium.

Multivariate analysis of variance (MANOVA) was carried out in order to test the significance of meristic and morphometric differences between sexes. In addition, morphometric and meristic data were standardised and submitted to principal component analysis (PCA) using SPSSv17 to identify morphometric characters that were important for identifying sexes.

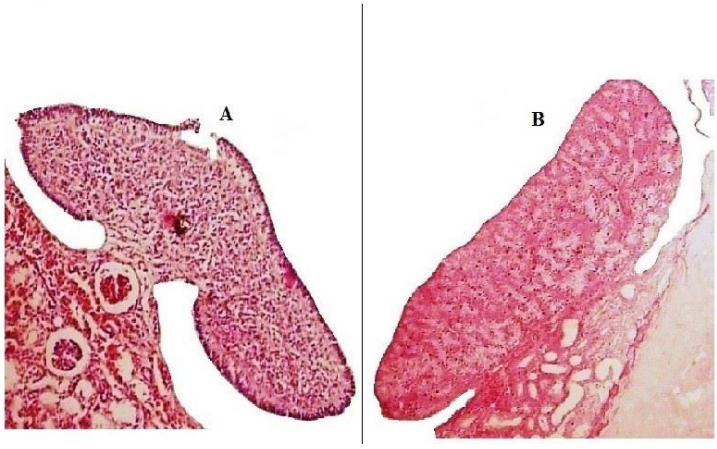


**Figure 3.** Sex identifications of *Chelonia mydas* on the basis of gonad histology (A: Female, B: Male)

## Results

Based on gonadal histology, we identified 30 male and 122 female hatchlings. Data on the variables taken for male and female hatchlings are given in Table 1. Multivariate statistics (MANOVA) identified overall significant differences in three variables (which are summarised in Table 2) and confirmed that these

three measurements were statistically different between sexes. The males were larger than the females for these three variables (Table 1).

**Table 1.** Summarised statistics of morphometric variables of female and male *Chelonia mydas* hatchlings.

| Character | Sex | n | Mean | SE | Min–Max |
|---|---|---|---|---|---|
| SCL | Male | 30 | 4.21 | 0.27 | 3.60–4.70 |
| | Female | 122 | 4.24 | 0.22 | 3.70–4.80 |
| SCW | Male | 30 | 3.27 | 0.29 | 2.70–3.80 |
| | Female | 122 | 3.22 | 0.26 | 2.60–3.80 |
| CCL | Male | 30 | 4.94 | 0.25 | 4.40–5.50 |
| | Female | 122 | 4.78 | 0.22 | 4.20–5.30 |
| CCW | Male | 30 | 4.24 | 0.47 | 3.80–6.00 |
| | Female | 122 | 4.02 | 0.25 | 3.40–5.00 |
| FLL | Male | 29 | 3.81 | 0.36 | 2.60–4.30 |
| | Female | 122 | 3.90 | 0.24 | 3.20–4.50 |
| HLL | Male | 14 | 2.34 | 0.14 | 2.10–2.50 |
| | Female | 85 | 2.26 | 0.16 | 1.90–2.70 |
| TTL | Male | 30 | 1.15 | 0.15 | 0.90–1.40 |
| | Female | 122 | 1.11 | 0.15 | 0.70–1.50 |
| CETL | Male | 30 | 0.36 | 0.11 | 0.20–0.50 |
| | Female | 122 | 0.38 | 0.10 | 0.20–0.60 |
| PCL | Male | 30 | 0.78 | 0.10 | 0.60–1.00 |
| | Female | 122 | 0.73 | 0.11 | 0.40–1.10 |
| BD | Male | 26 | 1.30 | 0.20 | 0.90–1.70 |
| | Female | 118 | 1.41 | 0.18 | 1.00–1.80 |
| HL | Male | 30 | 1.78 | 0.23 | 1.30–2.00 |
| | Female | 120 | 1.78 | 0.21 | 1.30–2.20 |
| HW | Male | 14 | 1.14 | 0.12 | 1.00–1.40 |
| | Female | 84 | 1.23 | 0.16 | 1.00–1.50 |
| HC | Male | 30 | 4.64 | 0.30 | 4.10–5.30 |
| | Female | 121 | 4.63 | 0.23 | 4.00–5.20 |
| W | Male | 30 | 17.23 | 2.30 | 12.00–22.00 |
| | Female | 121 | 17.59 | 3.42 | 12.00–26.60 |

The component-loading plot from the PCA, which allows a reduction of the number of variables in a data set by finding linear combinations that explain most of the variability, showed how distances were related in two-dimensional space. In the PCA, the first principal component accounted for 34% and the second principal component accounted for 24% of the shape variation between the sexes. The distance of the variables from the origin was mainly driven by head, limbs, curved measurements, and PCL, illustrating how these regions were important in discrimination of the sexes (Figure 4).

## Discussion

Multivariate analyses revealed that three out of twenty one morphometric measurements differed significantly between female and male *C. mydas* hatchlings. Previous studies reported similar results from *P. expansa* hatchlings (Valenzuela *et al.* 2004). Thus, these three morphological characters can facilitate sexual identification of hatchlings of *C. mydas*. Perhaps most

promising finding is the PCL character (cloaca length) (see Figure 2B), with male hatchlings having a longer PCL than females. Similarly, Casale *et al.* (2005) investigated six tail characters in mature loggerhead turtles and found that the Plastron-Cloaca (PCL) was a better indicator of sex than Cloaca-Tail and Plastron-Tail. Moreover, immature male loggerheads have longer tails than immature females (Wibbles *et al.* 1987), and adult male sea turtles have longer tails than females (Hendrickson 1958). Thus, sexual dimorphism of tail length in adult sea turtles may capture differentiation already initiated in the early life.

**Table 2.** Multivariate analysis of variance testing sex differences in all morphometric and meristic measurements of *Chelonia mydas* hatchlings.

| Character | Type III Sum of Squares | Mean Square | F | P |
|---|---|---|---|---|
| SCL | 0.028 | 0.028 | 0.497 | 0.482 |
| SCW | 0.046 | 0.046 | 0.627 | 0.430 |
| CCL | 0.126 | 0.126 | 0.166 | 0.685 |
| CCW | 1.097 | 1.097 | 11.395 | **0.001** |
| FLL | 0.191 | 0.191 | 2.570 | 0.111 |
| HLL | 0.147 | 0.147 | 5.845 | **0.017** |
| TTL | 0.024 | 0.024 | 1.044 | 0.309 |
| CETL | 0.006 | 0.006 | 0.519 | 0.472 |
| PCL | 0.054 | 0.054 | 4.490 | **0.036** |
| BD | 0.236 | 0.236 | 0.102 | 0.750 |
| HL | 0.003 | 0.003 | 0.041 | 0.841 |
| HW | 0.086 | 0.086 | 3.689 | 0.057 |
| HC | 0.004 | 0.004 | 0.070 | 0.792 |
| W | 4.111 | 4.111 | 0.381 | 0.538 |
| V | 0.419 | 0.419 | 2.431 | 0.121 |
| LEFT-C | 0.322 | 0.322 | 3.335 | 0.070 |
| RIGHT-C | 0.058 | 0.058 | 0.859 | 0.356 |
| N | 0.313 | 0.313 | 1.509 | 0.221 |
| S | 0.000 | 0.000 | a | a |

a: Cannot be computed because this is a constant variable.

Significant sex differences in other morphological characters have been reported in hatchlings of other sea turtles species (e.g., olive ridley; Michel-Morphin *et al.* 2001). In the present study, male hatchlings had wider carapaces than females. Sea turtles show TSD with low incubation temperatures producing mostly all male offsprings. Thus, nest temperature (and therefore moisture content) has an effect on the sex ratio between nests. Packard and Packard (1988) stated that turtle embryos exposed to wet conditions during development had longer incubation periods and grew larger than those exposed to drier conditions grow. In addition, Ackerman (1981) stated that when the capacity for gas exchange was decreased, the embryo grew more slowly. Growth rate may have an effect on differences in carapace dimensions between adults and hatchlings. Bjorndal *et al.* (2000) concluded that immature male green turtles had a faster growth rate than immature female green turtles in Union Creek on the north coast of Great Inagua. Similarly, significant sex-specific differences in

growth rate have been reported for green turtles in the southern Great Barrier Reef (Limpus and Chaloupka 1997).

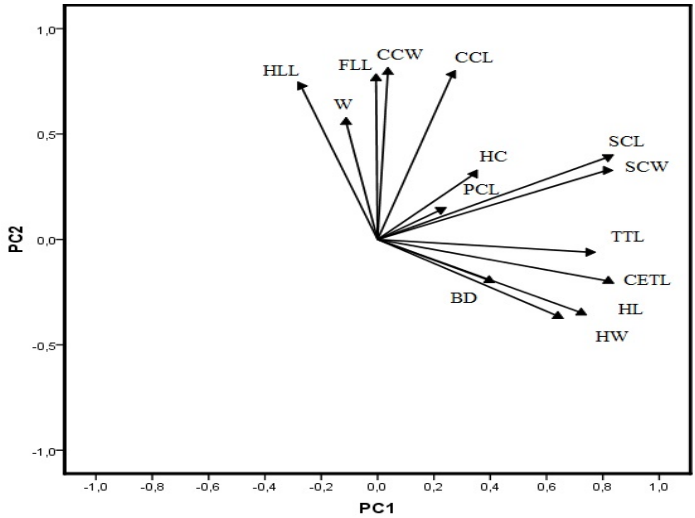


**Figure 4.** Contribution of morphometric characters to the first two principal components. Vector length is proportional to the loading scores for each variable on the first two principal component axes.

In the present study, male hatchlings had longer hind limbs than females. There is, up to date, no report on hind limb differences between females and males in both adult and hatchlings. There are a few reports on regional differences in hind limb area (Wyneken *et al.* 1999; Glen *et al.* 2003). One might argue that females should have longer hind flippers to dig a deeper egg chamber to provide a more favourable incubation environment. On the other hand, it might be argued that males should have longer hind flippers for grasping during mating. Therefore, sexual dimorphism of hind limbs between adult males and females should be investigated in future studies. A description of the actual pattern of sexual dimorphism would be of benefit.

Since the sex of hatchlings of most sea turtle species is not identifiable phenotypically, a more sophisticated method is required to distinguish males from females. In this study, we detected significant sexual dimorphism in the external morphology of *C. mydas* hatchlings. Especially, measuring the PCL could provide an alternative way to the current scholastic sexing techniques.

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# Yeşil deniz kaplumbağası (*Chelonia mydas*) yavrularında morfolojik karakterlere dayalı cinsiyet tayini

**Özet**

Ölü dişi ve erkek yeşil deniz kaplumbağa yavruları arasında morfolojik farklılıklar cinsiyet tayininde önemli karakterleri belirlemek için incelendi. Kuzeydoğu Akdeniz'de Samandağ kumsalında 2008 ve 2009 yuvalama sezonu içerisinde toplam 152 ölü yeşil deniz kaplumbağa yavrusu 14 morfometrik ve 7 meristik karakter bakımından incelendi. Ölü yavruların cinsiyeti, gonad histolojisi ile tespit edildi. Çok değişkenli istatistiksel analizler sonucunda dişi ve erkek yavrular arasında üç morfometrik karakter bakımından anlamlı farklılıklar saptandı. Erkek yavruların dişi yavrulara göre daha geniş karapasa (EKE), arka yüzgeç uzunluğuna (AYU) ve plastron-kloak (PKU) arası mesafeye sahip olduğu belirlendi. Ana bileşenler analizi de cinsiyetler arasındaki bu farklılıkları destekledi.

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