

**SEX RATIO ESTIMATION OF THE MOST EASTERN MAIN LOGGERHEAD SEA
TURTLE NESTING SITE: ANAMUR BEACH, MERSIN, TURKEY**

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ABSTRACT

The nesting activities of loggerhead turtles (*Caretta caretta*) at Anamur Beach, one of their main nesting grounds along the Mediterranean coast of Turkey, were investigated during the 2006 and 2007 nesting seasons. The mean sex ratios were estimated based on gonad histology of dead hatchlings and late stage embryos and were calculated as 72.1% and 79% as females for the years 2006 (n = 366) and 2007 (n = 271), respectively. The nest temperatures of two nests and sand temperatures at nest depths were also recorded by placing electronic temperature recorders into the nests. The recorded sand temperatures were lower when close to the sea and higher towards inland. The mean temperatures of two nests during the entire incubation period were 28.9 and 31.2 °C, with 8 days' difference in their incubation period. Based on mean temperature during the middle third of the incubation period, which ranged from 28.6 to 30.8 °C, the sex ratios were calculated as 49.3% and 79.9% females, respectively. These data are statistically significant when compared by t test ($t = 52.34$, $p < 0.0001$) and pair wise comparison ($p < 0.0001$). The sex ratios among the beach sections were also different ($\chi^2 = 16.5$, $df = 4$, $p < 0.002$). The mean incubation periods of nests were slightly shorter in 2007 compared to 2006, calculated as 48.97 and 52.51 days, respectively. According to overall sex ratio, based on incubation durations, 85.2% of the hatchlings were females and the yearly estimated sex ratio was 75.6% in 2006 and 87.8% in 2007, which is roughly similar to the histological values. The spatial and temporal variations of nests and the resulting sex ratios were due to the possible effects of global warming causing changes in the nesting site preferences of adult females.

Keywords: Sex ratio estimation, loggerhead sea turtle, Anamur Beach, Mersin, Turkey

INTRODUCTION

Sexual differentiation of sea turtle hatchlings is determined by egg incubation temperature, usually during the middle third of development (Yntema and Mrosovsky, 1980; Mrosovsky, 1994; Kaska et al., 2006) because of the fact that sea turtles show temperature-dependent sex determination (Bull, 1980; Wibbels et al., 2000). The temperature at which an equal sex ratio is produced is called the pivotal temperature; incubation above the pivotal temperature results in more females and below the pivotal temperature results in more males (Mrosovsky and Pieau, 1991; Kaska et al., 2006). For all sea turtle species, pivotal temperatures have a value around 29 °C, and different sex ratios were reported for different sea turtle populations but most have been reported as female-dominated (Mrosovsky, 1994).

Survival of sea turtle populations depends on incubation temperatures suitable for the production of enough hatchlings of both sexes. If the temperature of a nest during the middle third of development is known, the sex ratio of hatchlings from that nest can be estimated. If this information is known for all parts of a beach throughout the nesting season, the overall primary sex ratio can be predicted for all hatchlings produced from that beach (Standora and Spotila, 1985; Kaska et al., 1998).

The sex ratio can be determined by recording the nest temperature during the incubation period (Mrosovsky, 1994; Kaska et al., 1998, 2006). Estimates of the sex ratio have also been obtained by combining the nesting distribution with the sexing of samples of hatchlings from different times during the season (Mrosovsky, 1994) or from pivotal incubation durations (Marcovaldi et al., 1997). Nest temperatures can vary due to latitudinal variation, seasonal temperature changes, shading by vegetation, sand color, episodic events such as rain, and depth of the eggs (Hays et al., 2001; Matsuzawa et al., 2002). During particular parts of incubation in a clutch, eggs produce heat, called metabolic heat, and this metabolic heat causes temperature increases (Godfrey et al., 1997; Kaska et al., 1998). A large number of studies have been carried out on the estimates of the sex ratios of sea turtle hatchlings, and female-biased sex ratios up to 90% have been found for loggerhead turtle (*Caretta caretta*) hatchlings in the Mediterranean (Kaska et al., 1998; Godley et al., 2001; Mrosovsky et al., 2002; Öz et al., 2004; Kaska et al., 2006) and in Brazil and the USA (Marcovaldi et al. 1997; Hanson et al. 1998). Kaska et al. (2006) found an average sex ratio of 81.6% females, the pivotal temperature just below 29 °C, and the calculated incubation duration around 59.9 days, for the 1995 and 1996 nesting season in Cyprus and Turkey. At Fethiye beach, almost the same mean sex ratio of hatchlings (60–65% females) was found by using both histological study on dead hatchlings and mean nest temperatures during the middle third of incubation period (Kaska et al., 2006). This study suggests that Fethiye beach may be one beach contributing to populations with more males (35–40% males). Godley et al. (2001) reported very short incubation durations producing more females than long incubation durations (Mrosovsky et al., 1999) for loggerhead turtles in Cyprus (89–99% females). The pivotal temperatures in studies of loggerhead turtles all cluster within one degree of 29 °C (Mrosovsky, 1994; Marcovaldi et al., 1997; Hawkes et al., 2009). Mrosovsky et al.

(2002) reported the pivotal temperature for loggerhead turtles in the Mediterranean (by using two clutches from Greece) as 29.3 °C and the pivotal incubation duration as 52.6 days. For the eastern Mediterranean, Kaska et al. (1998) used mean temperatures in the middle third of incubation to indicate a pivotal temperature just below 29 °C and the pivotal incubation duration later calculated as 59.9 days, close to the values of 59.3 and 61.7 days for Brazil and the USA, respectively. Mrosovsky et al. (2002) also reported that hatchling sex ratio on some Mediterranean beaches is female-biased but probably varies within this region. Anamur Beach, which is located nearly at the midpoint of Turkey's southern (Mediterranean) coast, is a nesting beach for loggerhead turtle. Although it is one of the main nesting grounds in Turkey, there were not enough scientific studies carried out about the population size and no study about sex ratios of hatchlings.

Global research priorities for sea turtles: informing management and conservation in the 21st century by Hamann et al. (2010), we believe that global warming and sex ratio studies such as this study will contribute to conservation programs of sea turtle populations nesting on the Mediterranean coasts. In this paper, we investigated the population size in two consecutive years and also examined the nest temperatures together with the gonadal histology of dead hatchlings and embryos in order to find out the hatchling sex ratio on Anamur Beach. The initial purpose was to obtain basic reproductive data on Anamur Beach and further examine the data on nest temperatures, to predict the sex ratios in these nests, to examine the sexes of dead hatchlings from the nests histologically and then to compare these two sex ratios for Anamur Beach as intra-beach, seasonal, and inter-annual variations.

MATERIAL AND METHODS

Anamur Beach is located south of Anamur, Mersin, Turkey. The historic town of Ören (Anamurium) (36°01'20.1"N, 32°48'21.6"E) is located at the most western part of the beach and Pullu Forest Camp (36°05'26.0"N, 32°54'86.8"E) at the eastern end of the beach, for a total of 12.7 km in length. The beach is divided into 5 sectors from south-east to northeast by Sultansuyu (Sultançayı, rivulet), İskele (the wharf), Dragonçayı (Kocaçay, rivulet) and Mamure Castle (Fig. 1). Sector I consists of gravelly sand. The gravelly sand gives way to fine-grained sand in sectors II, III, IV, and V. In Sector II, there are sand dunes in the area close to Sultansuyu, where there is a refining plant and where the human settlement is less than in the rest of Sector II. The housing, artificial light sources, and the human density behind the beach increase from this area towards Dragonçayı, with the highest density in Sector III (summer houses, hotels, cafes, parks, and walking-track). In Sector IV, most nests face inundation due to the high tides since the beach has a lower slope in this section. In addition, the vegetation-covered area of Section IV is an archaeological site because of Mamure Castle. The Gazipaşa–Silifke highway passes by Sector V. In summer, a lot of people do tent camping on the pine-covered slope behind the bay in Pullu Forest Camp.

All field observations and consequent data collections were conducted between 07 July and 28 September of the 2006 nesting season, and between 15 June and 30 Septem-

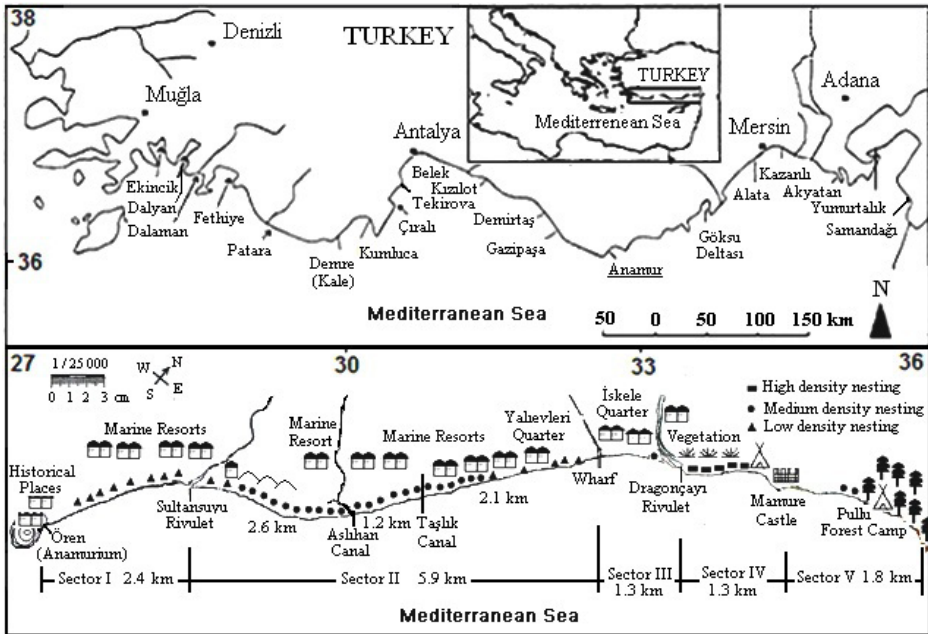


Fig. 1. Map of Anamur Beach showing the sub-sectors, beach-back structures, and nest density.

ber of the 2007 nesting season by daily patrols of the beach (without interruption). The nests dug before our patrols were identified either from recent adult tracks, from predation attempts, or at the time of hatchling emergences. In our daily patrols, the nesting and non-nesting emergences were also recorded. In order to find the number of non-nesting emergences, we used the estimation of “one in three emergences can result in a nest” (Groombridge 1990) as stated by Öz et al. (2004, p. 99), and the number of nests was multiplied by two in order to find the non-nesting emergences of the adults.

The nesting dates of the nests dug before our field work started were estimated by taking into consideration the mean incubation period for the nests whose incubation period was known exactly, for each nesting season. The mean incubation period was 53 days for 2006, and 49 days for 2007. For the calculation, this formula was used: Calculated nesting date = Hatching dates – Mean incubation period. From the nests whose hatching dates were known exactly, nests dug up by the predators before the hatching dates and those whose partial predation were excluded. The number of remaining nests was multiplied by two in order to find the non-nesting emergences of the adults for the temporal (Fig. 2a) and spatial (Fig. 2b) distribution of emergences.

Daytime (05:30–10:00 hrs) and nighttime (21:00–01:00 hrs) beach surveys and collection of data were done by teams of 5 or 6 persons (researchers and students). During the night study, the turtles emerging for laying eggs were observed without disturbing them and electronic temperature recorders were placed in two nests during egg-laying

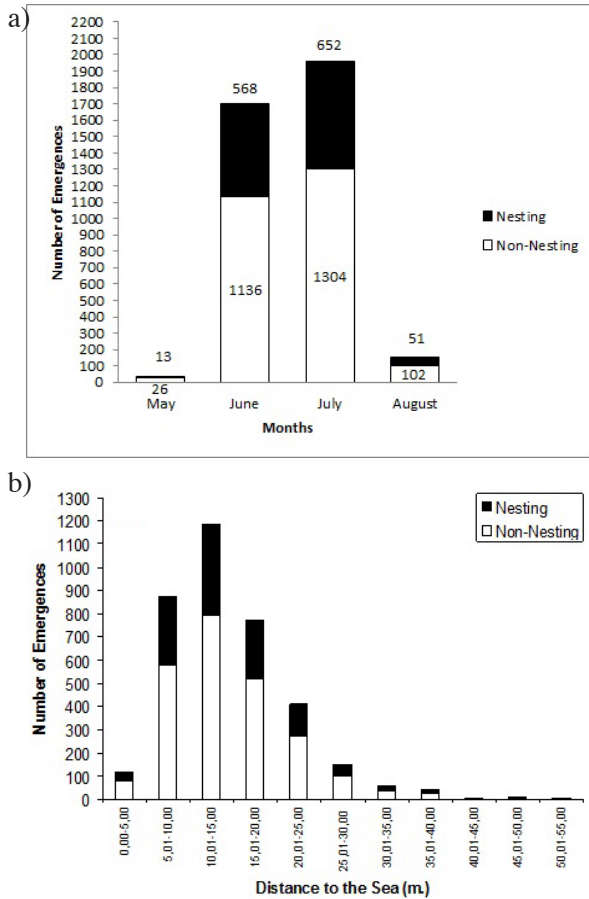


Fig. 2. Distribution of emergences (nests and non-nesting emergences). a) Temporal distribution of emergences. b) Spatial distribution of emergences.

on 10 June 2006. These nests were protected with wire cages against predation. After the sea turtles had completed their nesting process, nests were marked and their coordinates were taken by means of Global Positioning System (GPS). All the dates of nesting emergences were recorded on the day they were determined.

During the morning patrols, the turtle crawls and the places which were thought to be nests were checked. Then all the tracks in the area were cleaned. The nesting and non-nesting emergences, nest predation, and any hatchling emergences were recorded. The survey was conducted in the early hours in order to find the turtles' tracks while they were still intact and clear. Each track was examined in detail to determine whether or not there was a nest at the end of the track or not. If no nest resulted from a crawl, it

was recorded as a “non-nesting emergence”. After recording, all tracks of females were marked by drawing a horizontal line across the track in the sand to avoid duplicate counting. The nests were confirmed by carefully probing with a wooden stick until the egg canal was reached. The nests were not opened in order to not affect egg development. The locations of nests were recorded and measurements were taken. The distances of nests from the sea were measured when the nests were excavated one week after the first emergence of hatchlings.

Temperatures in two loggerhead turtle nests were measured by using electronic Tiny Talk temperature recorders (Gemini Data Loggers (UK) Ltd.). The device fits into a 35-mm film case. The accuracy of the device was tested under laboratory conditions against a standard mercury thermometer, and they were found to have a mean resolution of 0.35 °C (min. 0.3 °C, max. 0.4 °C) for temperatures between 4 and 50 °C. We programmed the Tiny Talk by computer for a recording period of 60 days; readings were taken at 90 min intervals.

The middle third of the incubation period was calculated from the total incubation period, that is, the numbers of days from the date of egg deposition to the date of the first emergence. Nests were excavated after the last hatchlings emerged; Tiny Talks were taken from the nests and nest depths were measured. The total number of eggs was calculated by counting unhatched eggs and hatched shell fragment. The information gathered by the Tiny Talks was off-loaded to a computer. The sex ratios of the loggerhead turtles were estimated using two methods. One method used the mean temperature during the middle third of the incubation period to predict sex ratio for the beach. In this method, the plots of mean temperatures during the middle third incubation period and corresponding sex ratio (% female), together with the pivotal incubation durations (duration that yield 50% of each sex) of the mean incubation temperatures, were obtained by using the data from Kaska et al. (1998). The sex ratios of the other nests, where laying dates were known, were calculated via incubation durations as given in the literature (Marcovaldi et al., 1997; Godfrey and Mrosovsky, 1999; Godley et al., 2001). The second method was to sex all dead hatchlings and late-stage embryos (>stage 25) (Kaska and Downie, 1999) found in favorable condition, by histological analysis of the gonads.

All the dead hatchlings found during patrolling or checking the nest surfaces and in nests during their excavation were collected, as were and late-stage dead embryos found during excavation of the nests. All samples in each nesting season were assigned to two-week hatching periods (15 July–1 August, 1–15 August, 15–31 August, 1–15 September, 15–30 September), and all samples were divided according to the beach sectors for the hatching season for both nesting seasons.

One gonad from each hatchling was cut in half transversely and embedded in paraffin wax, sectioned at 8–10 µm from the middle of the gonad, and stained with the periodic acid—Schiff’s reagent and Harris’s haematoxylin. We examined the gonads under a light microscope. The sex of each hatchling was assigned by the criteria of Yntema and Mrosovsky (1980).

RESULTS

NEST DATA

The overall number of nests recorded during the study period was 1581 (674 and 907 in 2006 and 2007, respectively). Temporal distribution of emergences is shown in Fig. 2a ($n = 1284$ [$n = 484$ and 800 nests in 2006 and 2007, respectively] of the 1581 nests in both nesting seasons) and spatial distribution perpendicular to the sea are given in Fig. 2b ($n = 1209$ of 1581 nests in both nesting seasons). As can be seen from these figures, the majority of nesting occurred in June and July and the nests were mainly distributed between 5 and 25 m from sea.

When these nests were excavated, the average clutch sizes determined for intact nests (440 nests in 2006 and 683 nests in 2007) were calculated as $76,4123 \pm 19,28915$ eggs (min. 14, max. 167, for 1123 nests). These clutch sizes were calculated as $76,1682 \pm 20,13053$ eggs (min. 17, max. 155, for 440 nests) in 2006, and $76,5695 \pm 18,74045$ eggs (min. 14, max. 167, for 683 nests) in 2007.

TEMPERATURE AND HISTOLOGICAL STUDY

By analyzing the nest temperatures during the middle third of the incubation period, the mean temperatures during the entire incubation period were determined to be 28.9 to 31.2 °C. The mean temperatures during the middle third of the incubation period were 28.6 and 30.8 °C. On average, there were higher increase in the first third of the incubation period (maximum increase 3.3 °C) and a lower increase in the middle third (maximum increase 1.8 °C); in the last third, values initially increased and then dropped (Fig. 3). The sex ratios of hatchlings for these nests were estimated according to the

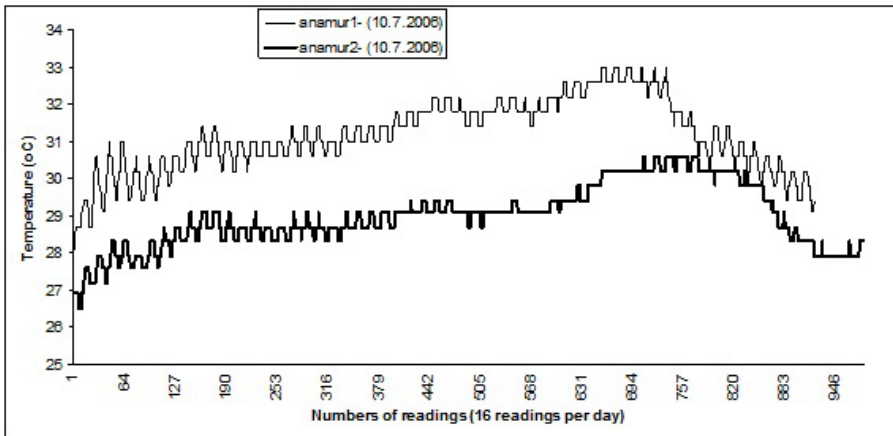


Fig. 3. The two nest temperatures recorded on Anamur Beach, showing the differences in temperature and therefore the sex ratio.

nest temperatures during the middle third of the incubation period and were found 49.3% and 79.9% females. The incubation periods of these nests were 46 (anamor 1) and 54 (anamor 2) days; the ± 2 °C differences in the mean incubation temperature correlates to 8 days' differences in the incubation period, which is confirmed by the field observations. These data are statistically significant when compared by t test ($t = 52.34$, $p < 0.0001$) and pair-wise comparison ($p < 0.0001$).

The two nest temperatures investigated, shown in Fig. 3, provided very interesting information. The eggs in both nests (anamor 1 and anamor 2) were laid on the same day, at almost the same distance (11.90 and 10.90 m, respectively) from the sea and almost the same depth of nest (53 and 54 cm, respectively), but with clutch size of 78 and 52, respectively. The difference between the mean temperatures over the entire incubation period was 2.1 °C, and the difference between the incubation periods was 8 days. Thus, a 1 °C change effects a 4 day change in incubation period and roughly 30% sex ratio differences between them.

The 637 gonads were sexed histologically for both years. The dead hatchlings and late stage embryos found in the nests showed a female sex ratio of 72.1% in 2006 ($n = 366$) and 79% in 2007 ($n = 271$). There was nearly one sample of gonad for every three nests, but the maximum number was 12 per nest.

TEMPORAL SEX RATIO VARIATIONS

Although our sample size was not high, when we divided these hatchlings into two-week periods according to date of sample collection, there were no statistically differences between these periods ($\chi^2 = 1.6$; $df = 4$; $p > 0.05$), but there was a slight increase in the male percentages at the beginning and the end of hatching seasons compare to the middle hatching season. The hatching seasons extended slightly before and after of these two-week periods, but there were no samples for those periods. The temporal distributions of sex ratio appears in Fig. 4.

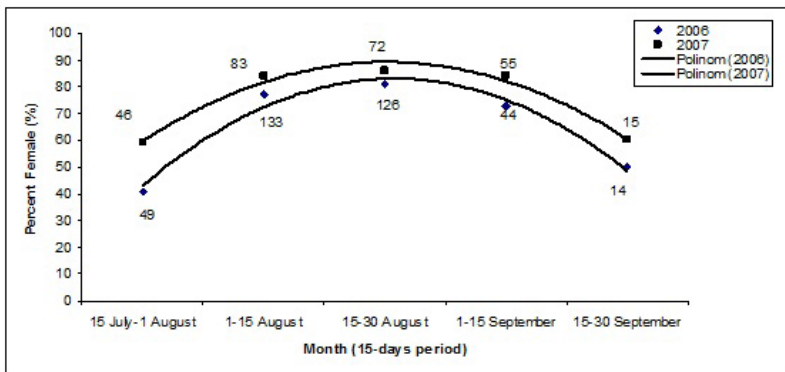


Fig. 4. The temporal distribution of sex ratio on Anamor Beach. The numbers of gonads sexed are given as sample sizes.

SPATIAL SEX RATIO VARIATIONS

The number of turtle emergences was also analyzed for two years for each sub-section of the Anamur Beach (Fig. 5a,b). (In Fig. 5a and 5b only the numbers of nesting emergences were given, and the number of nests was multiplied by two in order to find the non-nesting emergences of the adults). These turtle emergences were compared between the sub-sections and found statistically different for both nests ($\chi^2 = 34.5$, $df = 6$, $p < 0.0001$) and non-nesting emergences ($\chi^2 = 69.0$, $df = 6$, $p < 0.0001$), for both years.

The temperatures of nests close to the sea may be cooler, and therefore may potentially produce more males; nests further inland may be exposed to warmer temperature conditions and therefore produce more females. The sex ratio hatchlings produced from each beach sectors were given in Fig. 5c. There was a statistically significant difference in sex ratios among the beach sections ($\chi^2 = 16.5$, $df = 4$, $p < 0.002$).

When the gonads were analyzed according to the beach sectors, the Mamure-Dragon sector with 58.4% females in 2006 and 74% in 2007 was shown to be the highest male hatchling producer. The highest percentage of females in 2006, 85%, was in İskele-Sultansuyu, whereas the highest percentage of females for 2007, 83%, was at the Pullu-Mamure sector. These differences might be due to low sample size but clearly show the spatial and annual differences in sex ratio produced ($\chi^2 = 16.5$, $df = 4$, $p < 0.002$).

INCUBATION DURATION

In 2006, the average incubation period in Anamur Beach was estimated as $52,5104 \pm 5,34296$ days (min. 44, max. 67, for 96 nests). In 2007, the average incubation period in Anamur Beach was estimated as $48,9731 \pm 4,16123$ days (min. 42, max. 67, for 260 nests). The mean incubation period for the entire study period on Anamur Beach was $49,9270 \pm 4,76906$ days (min. 42, max. 67, for 356 nests). With such short durations, it is evident that sex ratios must be highly skewed toward females. After calculating the sex ratio for each incubation duration from this curve, and taking the nesting distribution into account, the overall sex ratio based on incubation durations is 85.2% female. The sex ratio estimated from incubation durations were 75.6% in 2006 and 87.8% in 2007.

DISCUSSION

With between 600 and 900 nests annually, Anamur Beach is one of the three most important loggerhead nesting site in Turkey (Türkozan and Kaska, 2010). The nesting season, and the spatial distributions of nests can vary depending on the beach width, but as can be seen from these results, the majority of the nests are located between 10 and 35 m from the sea. The Mediterranean Sea turtle population may be the first to be impacted by climatic changes and rising sea level (Roether et al., 1996). From this perspective, those beaches that produce sex ratios sufficient for reproduction – in this case, Anamur Beach – would gain additional importance in helping this species to survive in the Mediterranean. The beaches to the east of Anamur Beach are main green turtle nesting beaches, with a very low number of loggerhead nests.

The clutch size and nest frequency have been discussed extensively in the literature.

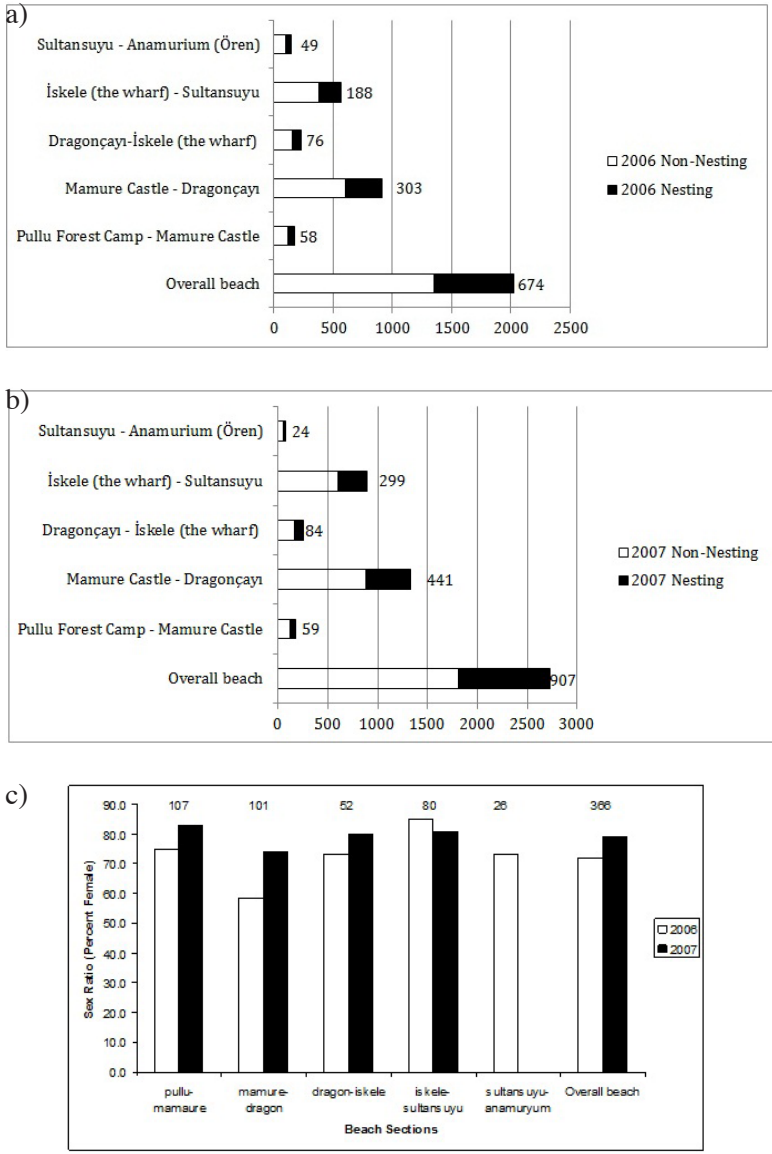


Fig. 5. a) Spatial distribution of turtle emergence on Anamur Beach for 2006; b) Spatial distribution of turtle emergence on Anamur Beach for 2007; c) The sex ratio of hatchlings from each beach sector. Data were obtained via gonad histology of dead hatchlings and embryos. The number of gonads sexed is shown as sample sizes for each beach section.

Mean clutch sizes were found to be similar on near-by beaches. The mean clutch size on Dalaman-Sarıgerme was 79.0 eggs (Kaska et al., 2010). The mean values have been reported as 76.0 eggs for Dalyan (Türkozan and Yılmaz, 2008), 80.1 eggs for Patara (Taşkın and Baran, 2001), 64.3–64.7 eggs for Egypt (Campbell et al., 2001), and 70.0 eggs for Northern Cyprus (Broderick and Godley, 1996). The Dalaman-Sarıgerme population was observed to be in the mid-range in terms of clutch size for the Mediterranean. Although Tiwari and Bjørndal (2000) could not find a correlation between clutch size and latitude, they hypothesized that there was an inverse relationship between annual numbers of nests and mean clutch size. Turtles may lay more nests with lower clutch sizes as a possible adaptation to global warming.

The sex ratio estimation for such an important beach is crucial for the survival of loggerhead turtles in the Mediterranean, since Anamur is one of the main nesting sites in the far eastern Mediterranean. Obtaining direct temperature measurements for a large sample of nests is a step forward for estimating the sex ratios of sea turtle hatchlings on a nesting beach. Kaska et al. (1998) compared the sex ratios at different levels and showed that the results at middle levels (82.7%) are very close to the general mean (81.6%) and the mean of the top and bottom levels (80.9%). Therefore, recording only middle levels of the nests would yield the best estimation of sex ratios (Hanson et al., 1998; Kaska et al., 1998). The temperature differences for the two nests we measured showed possible differences in nest temperatures due to clutch size. Apparently, the higher clutch size causes a warmer incubation environment than relatively smaller clutch sizes.

The reported sex ratio for loggerhead sea turtles is generally female dominated (Mrosovsky, 1994; Kaska et al., 1998; Godley et al., 2001; Öz et al. 2004). Marcovaldi et al. (1997) estimated the sex ratios of loggerhead turtles in Brazil from pivotal incubation durations and found that 82.5% of the hatchlings were female. Godley et al. (2001), however, have suggested that, based on incubation durations in Cyprus and published data on incubation durations, this ratio is likely to be female-biased. The laboratory work for determining the pivotal incubation period for Mediterranean loggerheads has suggested that there is a female-biased sex ratio (Mrosovsky et al., 2002). In contrast to the apparent global and Mediterranean-wide female bias in loggerhead sea turtle hatchlings, there are significant differences in clutch incubation durations among the six nesting beaches of Zakynthos where two beaches (Marathonissi and Laganas) produce a high proportion of males and the other four beaches (Kalamaki, Sekania, Daphni, and Gerakas) produce a female-biased hatchling sex ratio (Zbinden et al., 2007).

Sex ratio estimations and their biological and ecological implications are clearly a complex issue of intricate interplay between nest location, nest depth, nest temperature, duration of hatchlings, selective predation, and other mortalities within and outside the nest, along with changing conditions from year to year and from beach to beach. In Brazil, most sites are largely female-producing, but some key sites have conditions that are biased toward the production of male hatchlings (Baptistotte et al., 1999). Kaska et al. (2006) estimated that a lower percentage (60–66%) of the hatchlings produced on Fethiye beach were females. The sex ratios of our results from the two nests on Anamur Beach were also female-dominated but with slight differences in different beach sectors.

The relatively high number of loggerheads nesting on Anamur Beach shows that this beach is producing both sexes, and the sex ratio differences between the sectors might be an additional important characteristic of the beach. The humidity and sand characteristics of the different sectors of the beach could be different. There may be some ecological differences such as humidity in the subsections of the Anamur Beach. These and other possible different ecological parameters need more attention in order to further investigate the intra-beach sex ratio differences on Anamur Beach.

The general pattern of metabolic heating and the comparison of nest center compared favorably with previous studies: metabolic heating was recorded during the second half of the incubation (i.e., Godfrey et al., 1997), with a peak followed by a gradual decline in nest temperatures toward the end of incubation (Fig. 5b). The longer hatching durations may be an indication of cool temperatures, late development, and more male hatchlings. The longer the incubation period, the more chance a nest has to be predated since those hatchlings emerging last are most likely to suffer predation (Kaska, 2000). The smell of the first group of hatchlings may provide clues for predators about the location of a nest and those emerging lastly may suffer predation (Kaska, 2000).

As a main loggerhead sea turtle nesting beach, Anamur Beach, located in the far eastern Mediterranean, holds relatively important nests. One of the explanations for its relatively high numbers could be that there are enough adult males and females and enough different beach sectors for the continuation of the population dynamics. The sea turtles in extreme regions could be adaptive in different climatic conditions, and laying more nests with relatively small clutch sizes could be one of the results of this adaptation. The clutch size and nest temperature, and the associated sex ratio of hatchlings, could be very important parameters to record. The seasonal variation of sex ratios shows that there may also be a nesting season shifts as adaptation of loggerhead turtles under the threat of global warming. The possibility of nesting season shifts and of sex ratio differences among the beaches needs more attention, especially for the small beaches having different ecological characteristics, such as the beaches along the Mediterranean.

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