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Sex Ratio Estimation of Green Turtle Hatchlings in Kazanlı Beach, Türkiye

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Abstract

Sea turtles are distributed worldwide and have temperature-dependent sex determination. For hatchlings with a female:male ratio of 1:1, the pivotal temperature is usually around 29°C, with female hatchlings being observed at higher values and male ones at lower values. It is expected that wildlife threatened by global warming will be more severely affected, especially species that sex is determined by temperature. This study was carried out by measuring air, sand and nest temperatures during 2008 and 2009 nesting seasons at Kazanlı Beach, one of the largest nesting sites of *Chelonia mydas* (Green turtle) in the Mediterranean. Sand grain size was also measured, and gonads of dead hatchlings were examined histologically. Both sand (n=4) and nest (n=6) temperatures were above the pivotal temperature (between 29.1-31.6°C) in the study area. According to the estimation based on temperatures, hatchlings were female (♀>50%) biased. Of the hatchling samples, 75 were examined histologically. According to histological examination, the female:male ratio was 2:1. According to the direct examination and indirect estimation; the sex ratio of green turtle hatchlings in Kazanlı Beach, one of the highest hatchling producing nesting sites of the Mediterranean, was reported for the first time in this study. There is a need to fill the data gaps regarding sex ratios, especially for major beaches with high hatchling production. Knowing the temperature profiles in and around the nest and sex ratios of hatchlings is critical for the development of conservation measures for endangered sea turtles.

1. Introduction

Climate change is one of the most serious problems faced by wildlife. Human-induced climate change shows its effects at every stage of wildlife [1]. In species with temperature-dependent sex determination (TSD), it is reported that population sex ratios have started to change, especially in places where temperature increases are high [2]. Sea turtles, which are globally endangered except Natator depressus, are among the species with TSD. In order to understand the effects of global warming, it is critical to know the temperature values of the nesting site and the sex ratios of the hatchlings for the survival of these species [3]. Because it may be difficult for long-lived species such as sea turtles to adapt to the rate of temperature increase [4].

Studies on the impact of global warming on sea turtles have increased rapidly, especially in the last two decades [5]. Terrestrial studies and especially nest-hatchling studies are the easiest to conduct [6]. According to the sexual dimorphism index, curved carapace length is reported to be longer in females than males for sea turtle adults [7], but sexual dimorphism is not observed in hatchlings [8]. However, it has also been reported that female hatchlings are slightly heavier than males [9]. There are direct methods (gonadal histology [10] radio immuno-assay [11], gonad gross morphology [12]) and indirect methods (morphometrics [13], nest

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temperature [14], incubation duration [15]) to determine hatchlings' sex ratio. The methods and accuracy rates used for sex determination vary.

Histologic examination is the most accurate way to determine sex in hatchlings. However, it is necessary to develop non-invasive methods with high accuracy rather than methods that require hatchling sacrifice [15]. For green turtle hatchlings, the temperature at which the female:male ratio is equal is the pivotal temperature. Above this temperature, female hatchling development is observed, while below this temperature, male hatchling development is observed. The pivotal temperature for sea turtles is around 29°C [16].

The eastern Mediterranean coast of Türkiye, including Kazanlı Beach, hosts important nesting sites for green turtle. [17]. Since the high number of nests increases the number of hatchlings joining the population, Kazanlı Beach is an important nesting site for the survival of the green turtle in the Mediterranean. However, there is a gap in the data on sex ratios for this beach. In this study, temperature values in and around the nest (air, sand and nest temperatures) are presented. The sex ratios of green turtle hatchlings on Kazanlı Beach are given according to these temperature values and histological examination results. In addition to the temperature values, sex ratio of hatchlings was also estimated based on the incubation period.

2. Material and Method

2.1. Study Area (BEU-FBD-Main Title)

This study was conducted in 2008 and 2009 nesting season at Kazanlı Beach, located in Mersin province (Figure 1). The beach is divided into four sub-sections but just sub-sections K1, K2 and K3 were studied. Sub-section K-1 is approximately 2 km in length from the D-7 drainage channel to the poles in front of the Onur Site. The dunes behind the beach are 1-1.5 m high and covered with dense vegetation. The sandy area continues behind the dunes. There is a road separating the beach from the greenhouses at the back of the sandy area. The K-2 sub-section is ~1.7 km in length from the poles in front of Onur Site to the wastewater channel near High School building. There are various facilities within this section. The coast narrows from east to west in this sub-section. K-3 sub-section is ~0.47 km section from school building to Soda-Chromium factory.

2.2. Air, Sand and Nest Temperatures with Incubation Duration

Data loggers (Gemini Data Loggers-Tinytalk Part No: TK-0040) were placed at a suitable point on the beach at the beginning of the nesting season and recorded the sand temperature at a constant depth (50 cm) throughout the nesting season. Considering that this depth from the sand surface to the bottom of the nest is approximately 80 cm [18] and that some of this depth is covered by eggs, it was found appropriate to deploy the data-logger at a depth of 50 cm. When selecting the locations where these data loggers deployed, areas with dense nesting were preferred. The area where the nests are densed in Kazanlı Beach is 22 m from the sea on average.

A total of four sand temperature data loggers were placed in Kazanlı Beach, two in each season (one in each sub-section of K-1 and K-2). Due to intense erosion and tide line, no data-loggers were placed in the K-3 sub-section of Kazanlı Beach to measure sand temperature.

The device was programmed with its original software (GLM v.2.8). After programming, the data-logger was deployed in the beach sand and a total of 1500 measurements were taken with 120 minutes' interval.

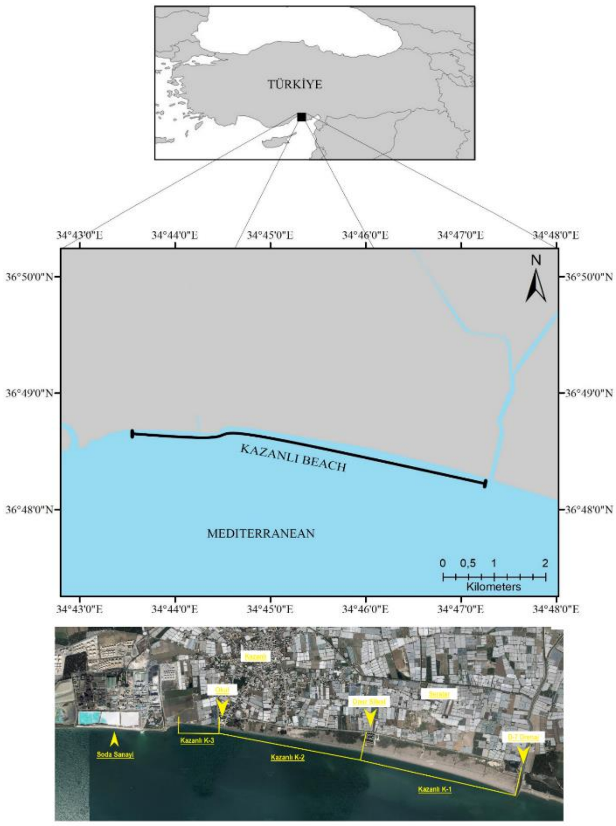

Figure 1. Site map and subsections.

Daily air temperature data for the 2008 and 2009 nesting seasons for Kazanlı Beach were obtained from the Turkish General Directorate of Meteorology. For Kazanlı Beach, Mersin station (Station Code: 17340) was chosen as the closest station to the area.

During the nesting period, the temperature data-logger was deployed into the centre of nest with two methods. The first method is after the female had laid the eggs in egg chamber reached a certain number (~ 50 eggs). In the second method, the datalogger was deployed after half of the eggs had been removed from the nest within 12 hours of egg laying. The time and date when the data logger was placed in the nest was recorded with the serial number of the device. These nests were caged against predation.

The data-logger was programmed with its original software and deployed in the nest. A total of 1500 measurements were taken at 60-minute' intervals during the incubation duration.

The nest temperature values were divided into three equal trimesters depending on the incubation duration [19]. The incubation duration is the time from the day the nest is laid to the day of the first hatchling emergence. Regression of the incubation period of the nests with histological results was calculated with gonad samples taken randomly. The regression equation was calculated by combining the data obtained for Kazanlı Beach with [20] Candan and Kolankaya (2016). Temperature differences between nesting seasons were evaluated by t-test.

2.3. Sand Sampling

When beach sand samples were taken, areas close to the sand temperature data loggers were preferred. A core was taken from the surface to the depth of the data logger with 10 cm diameter. The sand in the core was homogenized and tested. Thus, it was aimed to provide a comparison between beach temperature and sand grain structure. Samples were dried at 105 °C before grain size measurement. Sieving was performed on a Retsch shaker sieve at 60% shaking intensity for 10 minutes. Sand grain sizes were grouped into five different sizes (≥1.00 mm to ≤0.125 mm) and the results are presented as percentage.

2.4. Nest Parameters and Gonad Sampling

Hatchlings emerging from the eggs after the incubation period was over were identified by their tracks on the beach. The nests were uncovered for control after the completion of hatching, the number of hatchlings stucking in the nest (dead and alive), the number of hatchlings (empty eggshells), the number of damaged eggs and their embryonic development status were determined according to the Whitmore and Dutton [19]. The period from the day the nest was laid to the first hatchling emergence was calculated as the incubation duration. The number of eggs in the nest was recorded as the clutch size. The distance from the sand surface to the bottom of the nest was measured as nest depth. The distance from the nest to the high tide line was the distance from the sea.

Gonad samples were obtained from dead hatchlings and late-stage embryos found in the nest and/or on the beach (due to predation and sun exposure). The gonads were dissected as a complex together with the kidneys and placed in tubes with the nest number labels. Buffered para-formaldehyde (4%) prepared in advance for tissue fixation was added and the sample tubes were sealed via parafilm and stored at +4 °C until the histological preparation.

2.4. Histological examination and Sex ratios

The routine histologic procedures were applied to gonad samples after fixation. During this process, the specimens were vacuumed in paraffin passed through graded alcohol series. After these procedures, the tissues were embedded in paraffin blocks. Paraffin blocks were cut in 4µ and 5µ sizes (Thermo-Shandon Microtome) and stained with hematoxylin and eosin. The slides were covered with entellan for histological examination.

Histologic examination was performed with a light microscope (Leica). The criteria of [21]Yntema and Mrosovsky (1980) were used in the examinations. The primary follicle, medulla and thick cortex were used to identify the ovary, while a thin germinal epithelium, testicular cords and testicular tubules were used to identify the testis.

Sex ratios were calculated based on direct histological examination. The values in the literature were used for the calculation according to the regression equation (for details see [18]). According to the regression equation, values above 100% were accepted as 100%.

Table 1. Nest temperature, incubation duration and sex ratio estimation (WID: Whole incubation duration, MT: Middle Third, ID: Incubation duration)

		Temperature (ºC)			Sex ratio estimation (%♀) via		
Year	Nest No	WID	MT	ID (day)	WID	MT	ID
2008	K1	31.0	30.6	51	87.0	74.8	67.0
	K2	32.2	31.6	48	**100.0**	92.7	82.5
2009	K3	30.5	30.4	49	78.8	70.5	77.3
	K4	30.2	30.2	42	72.6	68.1	**100.0**
	K5	30.7	30.7	48	81.4	76.6	82.5
	K6	29.6	29.3	55	61.0	50.8	46.2

3. Results and Discussion

In Kazanlı Beach, which is one of the largest nesting sites for green turtles in the Mediterranean, it was determined that the hatchlings had a female biased (♀ ratio > 50%) sex ratio according to the results of air, sand and nest temperatures and histological examinations. However, there were differences between the values obtained in sex ratio estimation. Sex ratio estimates based on nest temperatures (whole incubation duration and middle third of incubation) differed by ~20% between seasons. However, the ratios are very close to each other based on incubation duration (Table 1). In the 2009 nesting season with higher sampling, it was calculated that a 1°C temperature increase shortened the incubation period by 5.7 days.

Air temperatures increase from June to August in both seasons. Except for June, air temperatures in the 2008 nesting season were higher than those in 2009 nesting season but difference is not significant (T-Value = 1,74 P> 0,05 DF = 241). The mean air temperature during the entire nesting season was 28.4 °C in the 2008 and 27.9 °C in the 2009 nesting season. July and August temperatures were above 29°C in both seasons (Table 2, Figure 2).

Table 2. Air temperature of Kazanlı Beach

Months	2008 nesting season		2009 nesting season	
	Mean ± SD	Min - Max	Mean ± SD	Min - Max
June	26.59 ± 1.61	23.6 - 29.0	26.95 ± 1.37	24.3 - 29.0
July	29.47 ± 0.57	28.4 - 30.4	29.03 ± 0.95	27.3 - 30.4
August	30.15 ± 0.58	28.4 - 31.5	29.55 ± 0.57	28.4 - 30.8
September	27.28 ± 2.42	22.1 - 30.3	26.10 ± 2.51	20.9 - 29.4
Overall	28.40 ± 2.10	22.1 - 31.5	27.93 ± 2.08	20.9 - 30.8

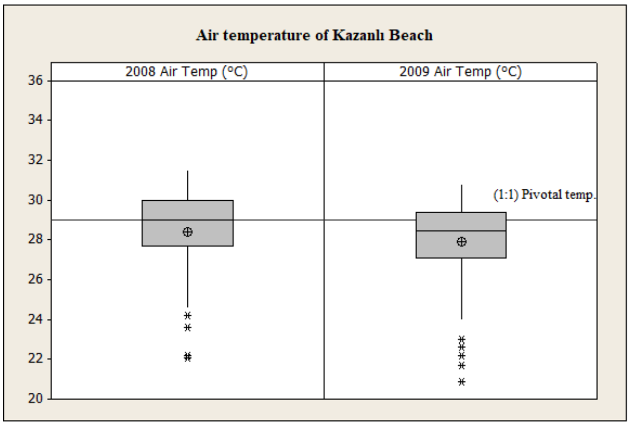

Figure 2. Air temperature of Kazanlı Beach in 2008 and 2009 nesting season.

Similar to the air temperatures, sand temperatures were also higher in the 2008 nesting season than 2009 nesting season. The difference between seasonal sand temperatures was significant (T-Value = 18,49 P< 0,005 DF = 3212). The mean sand temperatures in the K1 and K2 sub-sections of Kazanlı Beach were 30.1°C in the 2008 nesting season and 29.1°C in the 2009 nesting season. The average sand temperature was higher in the 2008 nesting season, as was the air temperature. The mean temperature values measured during the nesting season were above 29°C in both years (Table 3, Figure 3).

Table 3. Sand temperature (50 cm depth) of Kazanlı Beach at 2008 and 2009 nesting season

Subsection	2008 nesting season		2009 nesting season	
	Mean ± SD	Min - Max	Mean ± SD	Min - Max
K1	29.08 ± 0.76	27.2 - 30.6	29.41 ± 0.78	27.2 - 30.2
K2	31.15 ± 0.99	28.7 - 33.0	29.63 ± 0.68	27.9 - 30.2
Overall	30.11 ± 1.36	27.2 - 33.0	29.52 ± 0.74	27.2 - 30.2

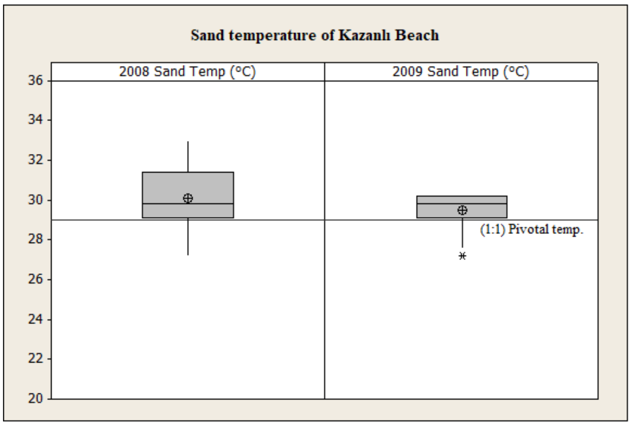

Figure 3. Sand temperature of Kazanlı Beach in 2008 and 2009 nesting season.

A total of six nests were equipped with temperature data loggers in both seasons. In the 2008 nesting season, the mean nest temperature was 31.6 °C and in the 2009 nesting season, the mean nest temperature was 30.2 °C. The difference of nest temperatures was significant between 2008 and 2009 nesting seasons (T-Value = 40,50 P< 0,005 DF = 3740). As with air and sand temperatures, the mean nest temperature in the 2008 nesting season was 1.4 °C higher than the mean nest temperature in the 2009 nesting season. The temperature values obtained in both seasons were between 29.6 - 32.2 °C. As in air and sand temperature, nest temperatures were also above 29 °C (Figure 4).

The differences between air, sand and nest temperatures for 2008 and 2009 nesting season were; (Tempnest - Tempair) 3.18 °C and 2.31 °C respectively, (Tempnest - Tempair) 1.47 °C and 0.72 °C respectively. Seasonal mean nest temperatures were higher than the surrounding sand and air temperatures (Table 1-3).

According to the histological examination, it was determined that the ratio of female:male was 2:1 (67% ovary, 33% testis) in 75 gonad samples in 2008 (n=29) and 2009 (n=46) (Table 4). In the 2008 nesting season at Kazanlı Beach, ovaries were observed in all 4 samples (100%) from nests with 45 - 49 days incubation duration, five ovaries and five testes were observed in 10 samples from nests with 50-54 days incubation duration. Testes (100%) were found in all 4 gonads that could be examined in the samples taken from the nest with 55-59 days incubation duration. In the nest with 60-64 days incubation duration, one of the six gonads examined had ovaries (16.7%) and rest of six had testes (83.3%). In the 2009 nesting season, there were fluctuations in the ratio of males to females depending on the incubation duration. During this season, only one hatchling was sampled from the nest with 45-49 days incubation duration, which was female. In 13 samples examined from nests with 50-54 days incubation duration, 10 ovaries (76.9%) and 3 testes (23.1%) were found, and in 8 samples collected from nests with 55-59 days incubation duration, 5 ovaries (62.5%) and 3 testes (37.5%) were found. No samples were collected from nests with 60-64 days incubation duration. In two samples collected from nests with incubation duration over 65 days, ovaries (100%) were detected. Of the 22 specimens with unknown incubation duration, 19 specimens had ovaries (86.4%) and 3 specimens had testes (13.6%). Although there is a difference between the sex ratios in percentage of the two seasons, this difference is not significant (T-Value = 1,37 P>0,05 DF = 10).

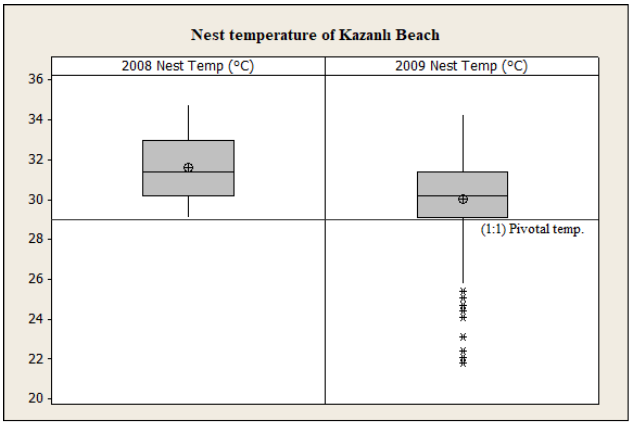

Figure 4. Nest temperature of Kazanlı Beach in 2008 and 2009 nesting season.

Table 4. Histological examination results of gonad samples

Sex		Nesting season 2008	2009	Overall
Female	n	13	37	50
	%	44.8	80.4	66.7
Male	n	16	9	25
	%	55.2	19.6	33.3

The sand grain size is coarser in the K-2 sub-section compared to K-1 sub-section. In sub-section K-1, sand is predominantly composed of 0.125 mm (56.93%) and 0.25 mm (37.25%) sized grains. In the other sub-section K-2, the proportion of 0.5 mm grains increased (16%) while the proportion of 0.125 mm grains (37.4%) decreased (Table 5).

Table 5. Histological examination results of gonad samples

	Grain size (mm)				
Subsection	1.00	0.50	0.25	0.125	<0.125
K1	0.40	2.40	37.25	56.93	3.02
K2	3.24	16.05	41.63	37.36	1.72

There are various conservation practices to ensure the survival of endangered species. Knowing the primary sex ratio of the species makes these conservation practices more effective in sea turtles, where sex is determined by hatching temperature [22]. Kazanlı Beach, which is the largest green turtle nesting area for both Türkiye and the entire Mediterranean basin, has been monitored for many years [23]. However, this is the first assessment of the sex ratio of green turtle hatchlings on this important beach has been reported here.

The sex ratio of green turtle hatchlings is between 67-100% [24]. According to the results of this study, the sex ratio of hatchlings was within the range of other studies. Kazanlı Beach, which is one of the areas producing high numbers of hatchling for the Mediterranean population, contributes to the population with a female bias. According to the results of histological examination of gonad samples for two nesting seasons, there was a difference between the seasons but not significant. Sex ratios show differences between seasons and beaches [25], [26]. However, the difference in this study is due to the limitation in sample numbers. When both seasons are evaluated together, the results of histological examination are calculated as 67% female.

It has been reported that the core region of a nest is hotter than the peripheral regions [27]. Effects such as non-uniformly distributed metabolic heating and sand grain size are likely to alter the temperature inside the nest. This temperature difference can affect sex determination when it occurs during the critical period. For this reason, the nest with 65 days incubation period, which is a limited sample, was completely female.

The most accurate way to determine sex of hatchling is histological examination [28]. However, this practice requires the sacrifice of hatchling, or leads to sample size limitations [29]. Therefore, it is necessary to develop accurate methods to estimate sex ratio. It has been reported that incubation duration is more accurate than other estimation methods [15]. In this study, predictions based on incubation duration showed close results between seasons.

Air and sand temperatures interact with nest temperatures. According to ecological niche modeling, temperature-related parameters (especially the mean temperature of warmest quarter) are among the factors affecting sea turtle nesting beach suitability [30]. An increase in air temperatures directly increases sand temperatures [31]. Nest temperatures are mostly higher than air and sand temperatures [18]. The fact that sand and nest temperatures are above 29 °C, which is the pivotal temperature for green turtles, clearly shows that Kazanlı Beach produces female biased hatchlings. By the end of the 21st century, air temperatures are projected to increase by 2 °C [32]. This global temperature increase is predicted to further increase female biased production. Moreover, it may also exceed the maximum temperature at which embryos can develop.

Considering the air, sand, and nest temperatures, 2008 nesting season was warmer than 2009 nesting season. There is a clear relation between air, sand, and nest temperatures [33], [34]. However, the highest values among these temperatures are observed in nest temperature. Because nest temperatures are not only affected by environmental temperature. Especially considering the contribution of metabolic heating produced by the developing embryos, nest temperatures are expected to be above the surrounding sand temperatures. It has been reported that a healthy developing embryo in green turtle nests increases the nest temperature by approximately 0.02°C [35].

Sand grain size is also among the factors affecting the temperature. When the sand grain size and temperature values in the two sub-sections are analyzed, the sand grain size in sub-section K2 is larger than K1 and the sand temperatures in K2 are higher than K1. As the sand grain size increases, its temperature increases. Thus, different temperatures can be observed on the same beach due to the effect of sand grain size.

When the relationship between nest temperature and incubation duration is analyzed, it is observed that the incubation duration shortens as the incubation temperature increases in both seasons. In laboratory-based studies, it was found that a 1°C temperature increase shortened the incubation period by 4.5 days [36]. However, there may be differences between laboratory-based results and results in the natural environment. The results obtained here are close to the literature, but the small sample size should also be taken into consideration.

4. Conclusion and Suggestions

There are many threats to wildlife. Global warming is among the most serious threats. All species will be affected by the global temperature increase. However, it can be assumed that the effects of global warming will occur in the shorter period on endangered species especially with TSD. Although it is thought that species will adapt to this temperature increase, as they have done in previous climatic changes, the rate of change may prevent this adaptation [37].

Climatic changes were considered to have dramatic impacts not only on hatchlings sex ratios but also nesting site suitability [38]. Practical applications such as measuring nest and sand temperatures and histological examination of dead hatchlings can be used to assess the effects of global warming on sex ratios in sea turtles. The results obtained at the local scale can be used to make global assessments. Therefore, it is of great importance to present data on temperature profiles and sex ratios in all nesting sites, especially in major ones.

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The study is complied with research and publication ethics

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