Turk J Zool
32 (2008) 287-292

Embryonic Mortality in Loggerhead Turtle (*Caretta caretta*) Nests: A Comparative Study on Fethiye and Göksu Delta Beaches

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Received: 22.02.2007

Abstract: In total, 66 loggerhead turtle nests were examined and compared in terms of embryonic mortality during the 2004 and 2005 breeding seasons on Fethiye and Göksu Delta beaches. In all, 5012 eggs were counted, 2751 (54.90%) of which hatched successfully. Of the 2261 unhatched eggs, 68 (1.35%) were classified as unidentified eggs and 2193 (43.75%) were classified as containing dead embryos. Of these dead embryos, a high percentage were in the early stage (n = 1177, 23.48%) and late stage (n = 814, 16.24%,) of development. The lowest percentage of the eggs with dead embryos (n = 202, 4.03%) were in the mid-stage of development. The embryonic mortality rate was higher at Göksu Delta than at Fethiye Beach. Of the examined ecological variables, none accounted for the early stage embryonic mortality. Embryonic mortality in the middle and late stages of development increased with decreasing slope and dry sand depth of the nests; however, embryonic mortality during these stages decreased with declining moisture and wet sand depth of the nests.

Key Words: Embryonic mortality, *Caretta caretta*, Fethiye, Göksu, Abiotic factors

İri Başlı Deniz Kaplumbağası (*Caretta caretta*) Yuvalarında Embriyonik Mortalite: Fethiye ve Göksu Deltası Sahilleri Üzerine Karşılaştırmalı Bir Araştırma

Özet: Bu araştırmada, 2004 ve 2005 üreme sezonunda Fethiye ve Göksu sahillerine yuva yapan 66 *Caretta caretta* Deniz Kaplumbağası yuvasındaki embriyo ölümleri karşılaştırılarak incelenmiştir. 5012 adet yumurta sayılmış ve bunların 2751'inden (% 54,90) başarıyla yavru çıkışı olmuştur. Yavru çıkışı olmayan 2261 adet yumurtanın 68'i (% 1,35) tanımlanamayan yumurta, 2193 (% 43,75) ise ölü embriyo olarak sınıflandırılmıştır. Bu ölü embriyolar içinde yüksek yüzdeli olanlar, erken (% 23,48- 1177 yumurta) ve ileri gelişim safhalardır (% 16,24- 814). En düşük ise orta safha yüzdesidir (% 4,03- 202). Göksu sahilinin ölü embriyo oranı, Fethiye sahilinden daha yüksektir. Bu çalışmaya dahil edilen hiçbir ekolojik değişken erken safha embriyo ölümünü açıklamamaktadır. Yuvaların eğimi düştükçe ve kuru kum derinliği azaldıkça orta ve ileri safha embriyo ölümleri artmaktadır. Bununla birlikte, yuva nemi ve ıslak kum derinliği azaldıkça ise bu iki safha embriyo ölümleri azalmaktadır.

Anahtar Sözcükler: Embriyo ölümü, *Caretta caretta*, Fethiye, Göksu, Abiyotik faktörler

Introduction

The loggerhead turtle is the most abundant sea turtle species in the Mediterranean Sea (Broderick et al., 2002; Margaritoulis et al., 2003). It was listed as globally endangered according to IUCN Red List categories (IUCN, 2006). The average annual number of loggerhead nests throughout the Mediterranean is 5031, of which 27.2% (1366 nests per season) are in Turkey (Margaritoulis et al., 2003). According to nest numbers and nest densities among the Turkish nesting beaches, Fethiye Beach represents one of the most important nesting sites of loggerhead turtles (Türkozan, 2000; Canbolat, 2004). Its importance increases because of the relatively higher proportion of male-producing nests (35%-40% of the hatchlings were males during the period 2000-2002) than other Turkish beaches (Dalyan, Patara, and Kızılot) (Kaska et al., 2006). Fethiye Beach was designated a Specially Protected Area in 1988.

Sea turtles spend their lives in the sea, and females only leave it to lay their eggs on the beach (Bolten et al., 1998). Eggs incubate for approximately 2 months, after which time developed hatchlings emerge and trek across

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the sand and into the sea (Ackerman, 1997). It is accepted that there are 31 stages of embryological development for *Caretta caretta* (Miller, 1985). Sea turtle embryos grow a few cells at oviposition and become a self-sufficient organism at hatching some 50-80 days later (Ackerman, 1997). Incubation duration depends on several nest environment factors, which also may effect embryological development. The embryonic period of sea turtles is a critical period of their life cycle because they are exposed to a diversity of biotic and abiotic factors. Parameters considered important to the chance of survival include: salinity, moisture, gas exchange, temperature, rainfall, tidal inundation, erosion, and predation (Hendrickson, 1958; Bustard and Greenham, 1968; Prange and Ackerman, 1974; Limpus et al., 1979; Ackerman, 1980; Mrosovsky, 1980; Blanck and Sawyer, 1981; Yerli et al., 1997). The above-mentioned studies validate that not only maternal inheritance, like hereditary features and nutritional content of eggs, but also nest site factors can affect embryonic development and mortality.

There have been few studies on the embryonic development of sea turtles and how they are affected by nest site factors on Turkish beaches. Çıtak (1998) and Taşkın (1998) studied the effects of ecological conditions on embryonic development of loggerhead sea turtles at Dalyan and Patara beaches in Turkey. Kaska and Downie (1999) studied the embryologic development of sea turtles (*Chelonia mydas* and *Caretta caretta*) of Northern Cyprus and southwest Turkey (Fethiye, Dalyan, Patara, and Kızılot). The purpose of the present study was to determine the rate of loggerhead sea turtle embryonic mortality and the factors affecting the observed mortality as they are related to specific embryological stages at Fethiye and Göksu Delta beaches.

Materials and Methods

Eggs that failed to hatch were collected from randomly selected nests (predated, inundated, and relocated nests were not included) at Fethiye (n = 33) and Göksu Delta (n = 33) beaches in 2004 and 2005, respectively. The emergence of hatchlings lasts slightly longer at Fethiye Beach than at Göksu Delta (Özdemir et al., 2007). We therefore excavated the nests 7 days after the first emergence of hatchlings from the nests at Fethiye, while we applied the same procedure at Göksu Delta 3-4 days after the first hatchlings emerged. Dead embryos were classified into 3 groups: early (E), middle (M), and late (L) embryonic stages (Whitmore and Dutton, 1985). The classification was based on the following criteria: E: blood formation on yolk or extra-embryonic membranes, small (approximately ≤ 10 mm) white embryo, usually with eyes, without an obvious carapace; M: White embryo with a carapace, without dark scutes (approximately 10-30 mm); L: Large embryo (approximately ≥ 30 mm) with fully formed scutes (Figure 1); Unidentified egg: An egg without visible development of an embryo or an egg with a decaying yolk.

The nest environment was characterized by 6 parameters (Figure 2): DSDN: dry sand depth of nest, distance from the lowest point of dry sand to the highest point of the nest surface (cm); WSDN: wet sand depth of the nest, distance from the bottom of the egg chamber to the lowest point of dry sand in the nest (cm); NDiam: nest

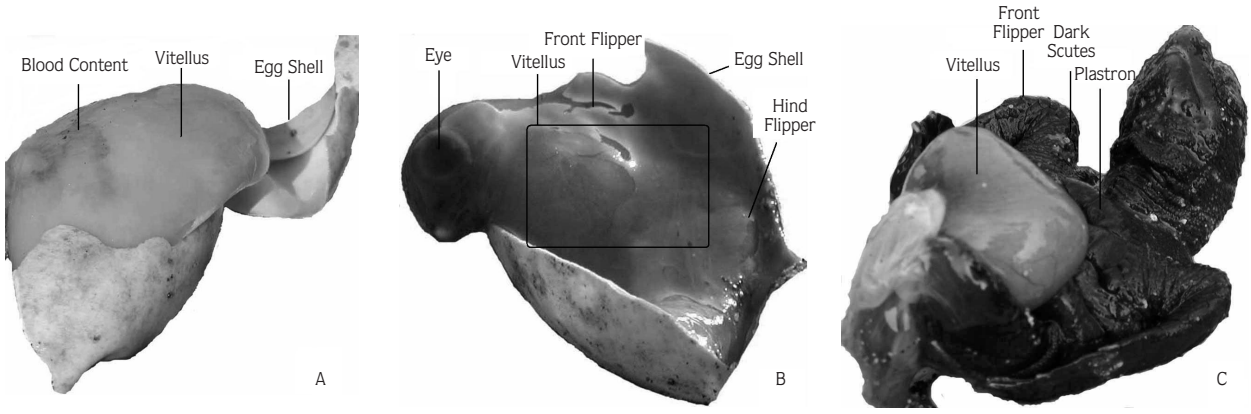

Figure 1. Early (A), middle (B), and late (C) stages of embryonic development (embryos from Fethiye Beach, Olympus Digital Camera-C700UZ, photos by Brigitte Baldrian).

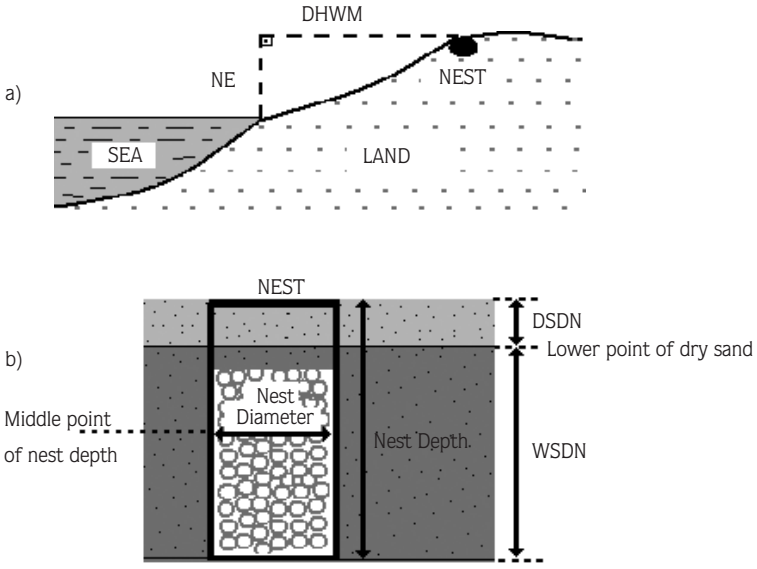

Figure 2. Geometric and schematic diagram of a hypothetical nest. a) External parameters of nest (DHWM: distance to high water mark; NE: nest elevation above high water mark). b) Internal parameters of nest (DSDN: dry sand depth of nest; WSDN: wet sand depth of nest).

diameter, measured at the middle point of total nest depth (cm); NE: nest elevation, measured at the high water mark (m); DHWM: distance to the high water mark (m); S: slope (degree), calculated using NE and DHWM; NM: nest moisture, measured at the middle point of nest depth at Göksu Delta. Moisture content was calculated by the ratio of water loss to dry mass multiplied by 100 (Head, 1992). Tape measures with an accuracy of 1 mm were used for the measurements.

Student's t-test was used to determine differences in the proportion of dead embryos and abiotic factors between the 2 beaches. Pearson's correlation coefficients were used to examine the relationships between embryonic mortality and abiotic factors. All P values were compared to an alpha level of 0.05. Statistical analyses were performed using SPSS v.11.0 (SPSS Inc., Chicago, 2001).

Results

Differences between the 2 beaches related to abiotic factors at the nest sites are presented in Table 1. The values for slope, nest elevation, and dry sand depth of the nests at Fethiye Beach were higher than those at Göksu Delta, and the wet sand depth and distance to the high water mark at Fethiye were lower than those at Göksu Delta. Mean moisture content was 7.39 (standard error of the mean: 0.80; range: 2.27-17.32) at Göksu Delta, while at Fethiye Beach this factor was 3.76 (SE: 0.16; range: 2.57-5.73) by Türkozan et al. (2003).

In all, 5012 eggs (Fethiye: n = 2578; Göksu: n = 2434) were deposited in the 66 nests examined and 2751 eggs (54.90%) hatched successfully. The 2261 unhatched eggs were opened to determine the stage of embryonic development. Of these eggs, 68 (1.35% of the total) were classified as unidentified and 2193 (43.75%) contained dead embryos. Embryonic mortality was higher at early (23.48%, n = 1177 eggs) and late (16.24%, n = 814 eggs) stages. The lowest percentage (4.03%, n = 202 eggs) of dead embryos were at the middle stage of development. At Fethiye Beach, embryonic mortality at the early stage was higher in comparison to middle and late stages; the situation was the same at Göksu Delta. When we compared the 2 beaches, Göksu Delta had a higher rate of embryonic mortality than Fethiye Beach (Table 1).

The relationships between physical nest parameters and embryonic mortality are presented in Table 2. Since the slopes of the nests were calculated using DHWM and NE, only the slope values were correlated with other

Table 1. Abiotic features of the nests and dead embryo ratios of loggerhead turtles at Fethiye and Göksu Delta beaches between 2004 and 2005, respectively (DSDN: dry sand depth of nest; WSDN: wet sand depth of nest; NDiam: nest diameter; DHWM: distance to high water mark; NE: nest elevation).

		DSDN (cm)	WSDN (cm)	NDiam (cm)	DHWM (m)	NE (m)	Slope (degree)	Early (%)	Middle (%)	Late (%)	Total Dead (%)
Fethiye	N	33	33	33	33	33	33	33	33	33	33
	Mean ± SE	22.88 ± 0.91	22.64 ± 0.78	21.00 ± 0.43	18.14 ± 1.39	1.36 ± 0.06	4.72 ± 0.19	22.41 ± 3.78	1.22 ± 0.41	5.79 ± 1.19	29.41 ± 4.38
	(min-max)	(10-35)	(9-33)	(16-27)	(7.30-52.30)	(0.72-2.17)	(1.21-6.66)	(0-100)	(0-10.14)	(0-23.08)	(0-100)
Göksu	N	27	33	33	33	33	33	33	33	33	33
	Mean ± SE	11.19 ± 1.14	35.84 ± 1.23	21.99 ± 0.31	24.61 ± 1.64	0.59 ± 0.03	1.70 ± 0.12	24.60 ± 4.47	7.01 ± 2.04	27.33 ± 5.29	58.94 ± 5.61
	(min-max)	(0-24)	(21-48)	(18-25.50)	(9.50-53.80)	(0.13-0.87)	(0.45-3.18)	(0-100)	(0-64.71)	(0-97.59)	(0-100)
t-test		t =8.126 df =58 P < 0.01	t = –9.134 df = 64 P < 0.01	t = –1.860 df = 64 P > 0.05	t = –3.016 df = 64 P < 0.01	t = 11.860 df = 64 P < 0.01	t = 13.669 df = 64 P < 0.01	t = –0.374 df = 64 P > 0.05	t = –2.782 df = 64 P < 0.01	t = –3.971 df = 64 P < 0.01	t = –4.146 df = 64 P < 0.01
Total	N	60	66	66	66	66	66	66	66	66	66
	Mean ± SE	17.62 ± 1.04	29.14 ± 1.09	21.49 ± 0.27	21.37 ± 1.14	0.98 ± 0.06	3.21 ± 0.22	23.48 ± 2.91	4.03 ± 1.09	16.24 ± 3.00	43.75 ± 3.99
	(min-max)	(0-35)	(9-48)	(16-27)	(7.30-53.80)	(0.13-2.17)	(0.45-6.66)	(0-100)	(0-64.71)	(0-97.59)	(0-100)

Table 2. The relationships between embryonic mortality and abiotic features.

	Slope	Moisture	NDiam	WSDN	DSDN
Moisture	r = –0.52 n = 33 P < 0.01				
nDiam	r = –0.11 n = 66 P > 0.05	r = 0.067 n = 33 P > 0.05			
WSDn	r = –0.70 n = 66 P < 0.01	r = 0.66 n = 33 P < 0.01	r = 0.29 n = 66 P < 0.05		
DSDn	r = 0.69 n = 60 P < 0.01	r = –0.52 n = 27 P < 0.01	r = –0.09 n = 60 P > 0.05	r = –0.78 n = 60 P < 0.01	
Early (%)	r = –0.13 n = 66 P > 0.05	r = 0.01 n = 33 P > 0.05	r = –0.08 n = 66 P > 0.05	r = 0.02 n = 66 P > 0.05	r = –0.07 n = 60 P > 0.05
Middle (%)	r = –0.37 n = 66 P < 0.01	r = 0.43 n = 33 P < 0.05	r = 0.09 n = 66 P > 0.05	r = 0.34 n = 66 P < 0.01	r = –0.42 n = 60 P < 0.01
Late (%)	r = –0.45 n = 66 P < 0.01	r = 0.58 n = 33 P < 0.01	r = 0.21 n = 66 P > 0.05	r = 0.54 n = 66 P < 0.01	r = –0.49 n = 60 P < 0.01
Total dead (%)	r = –0.53 n = 66 P < 0.01	r = 0.71 n = 33 P < 0.01	r = 0.12 n = 66 P > 0.05	r = 0.52 n = 66 P < 0.01	r = –0.47 n = 60 P < 0.01

factors and mortality. The middle and late developmental stages were both negatively correlated with slope and DSDN (Table 2); however, moisture and WSDN were positively correlated with middle and late stage embryonic mortality, and moisture was the most relevant parameter. None of the physical parameters were correlated with early stage embryonic mortality.

Discussion and Conclusion

Early and late embryonic stages were identified as the critical periods in the embryonic development of turtles (Çıtak, 1998; Taşkın, 1998). The present study confirmed and is in agreement with these results, since embryonic mortality was higher in both of these stages than during the middle stage. This study proves that

there were significant correlations between abiotic features of the nests and middle and late stage embryonic mortality; however, none of the variables included in this study accounted for the observed early stage mortality. The main cause of mortality at this stage may have been other abiotic factors (temperature, gas exchange, etc.), which this study did not examine.

Horrocks and Scott (1991) reported that nest elevation was positively correlated with hatching success for hawksbill sea turtles in Barbados. Wood and Bjorndal (2000) pointed out that the slope at cloacal positions of nest sites was the only factor that had a significant relationship with hatching success in loggerhead turtle nests. Özdemir and Türkozan (2006) found that moisture was associated with the hatching success in green turtle nests at Northern Cyprus. Taşkın (1998) claimed that nests closest to the sea had more dead embryos because of higher moisture and lower gas content in the nests. In contrast, the mean distance of the nests to the shore line at Göksu Delta was higher than at Fethiye, but embryonic mortality was also higher at Göksu Delta due to lower slopes of the nests.

The nests with lower slopes were closer to the water table than the nests with higher slopes; therefore, nests at the same distance to the shoreline, but with lower slopes contained more moisture, which caused higher mortality. For this reason embryonic mortality at Göksu Delta was higher than at Fethiye Beach. Since slope and moisture are negatively correlated they both have the greatest impact on embryonic mortality. Furthermore, of the internal nest parameters examined, DSDN and WSDN were negatively correlated (Table 2). This negative and high correlation coefficient suggests that the DSND to WSDN ratio may play an important role in nest site selection by females.

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