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-RESEARCH ARTICLE-

An Assessment of Egg Size in The Green Turtle (*Chelonia mydas*) on Samandağ Beach, Turkey

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Abstract

Many environmental factors such as temperature and moisture are known to influence the eggs of sea turtles. Egg diameter, egg circumference and egg weight were compared in the three stages on Samandağ beach, Turkey. Also, distance from sea, total nest depth, total eggs (clutch size), number of unhatched eggs were measured. In total, 284 eggs from 44 nests were measured. There were significant differences among the stages in term of egg diameter and egg weight. However; there are no significant differences among the stages in term of egg circumference. The egg diameter and egg weight were positively correlated with distance from the sea. Total nest depth was positively correlated with egg circumference and egg weight. The nests further from the sea and deeper from the surface have large egg size. If larger females produce large eggs, large females are attempting to nesting further from the sea and deeper from the surface. The morphometric characters of female turtles not measure in this study. Similar research can be done including female measurements in the future. These results would be useful for conservation workers or nest site managers.

Keywords:

Egg size, Reproduction, Green turtle, *Chelonia mydas*, Samandağ

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Introduction

The green turtle (*Chelonia mydas* L. 1758) regularly occurs in the Mediterranean, nesting along the coasts of Greece, Northern Cyprus and Turkey (Kasparek et al. 2001).

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Previous studies indicate that Samandağ Beach is an important nesting site for green turtles (Yalçın Özdilek, 2007). The green turtle is globally categorized as Endangered using criteria A2bd (ver 3.1), but the Mediterranean subpopulation is listed as critically endangered (Hilton-Taylor, 2000) based on criteria A1a, B1+2ce, E. Moreover, the green turtle is listed on Appendix 1 of CITES and, are protected by the Bern Convention.

Sea turtle eggs are deposited in a large clutch in a nest excavated by the female on sandy beaches. The incubation period of the eggs is about two month process to become a complete organism of the embryo. As well as genetic factor, physical properties such as moisture, temperature, gas flow are important to complete the embryonic development, too. (Glen et al., 2003; Wallace et al., 2006). All sea turtles have flexible-shelled eggs which exchange water with the surrounding substrate (Packard & Packard, 1988), and successful development of them dependent on uptake of moisture from the environment. (Packard & Packard, 1988; Ackerman, 1996). To the development of the embryo, O_2, CO_2, H_2O and heat exchange plays an important role. Gas exchange should be sufficient for the development of the embryo. If gas exchange is insufficient, the embryo either develops slowly or dies (Ackerman, 1996). Cheng et al. (2009) stated that the eggs, which has a larger surface area make more water and gas exchange rate than smaller. Successful development of the embryo in sea turtles is influenced by climatic factors, salinity, pH, environmental factors such as temperature and moisture, bacterial and fungal diseases (Miller, 1985; Garcia et al., 2003). Especially, the climate is geting higher in this region (Turan et al., 2016). Therefore, the information on the size of sea turtle eggs and the location of the nest are important. It's also known that female turtles have effects on their hatchlings and eggs (Özdemir et al., 2007; Cheng et al., 2009). Most hypotheses addressing the significance of intra specific variation in size of reptilian eggs have focused on the potential benefits accruing to the large hatchlings that usually emerge from large eggs (Gutzke & Packard, 1985; Van Burskirk & Crowder, 1994) and large female that usually produce large eggs (Bjorndal & Carr, 1989). At this point, egg size is important for survival of their own hatchlings, because larger size may allow hatchlings to escape gape-limited predators, to swim faster, and to handle successfully larger prey items, i.e. the "bigger is better hypothesis" (Booth et al., 2004). Janzen et al. (2000) found that larger hatchlings of *Cheldra serpentina* (Linnaeus, l758) exhibited significantly greater survivorship than smaller individuals. Given the importance of morphological data on egg size for survival of hatchlings, research on egg size would be useful for conservation workers or nest site managers. This research addresses the following questions; (1), are there any significant differences in the egg diameter, egg circumference and egg weight among the different stages on the beach? (2) Are there any correlation between eggs size and distance from sea, total nest depth, total number of eggs, total number of unhatched eggs? Which parameters are important in term of affecting on egg size among stages?

Material and Methods

Data were collected on Samandag Beach (36''07'N, 35''55'E) located in the eastern Mediterranean in Turkey during the 2010 nesting season. Samandağ Beach is about 14 km in length and it extends from the Çevlik Harbour on the North to Sabca Promontory on the south. The study area corresponded to a 9-km stretch of beach between Çevlik Harbor to the north and the mouth of the Asi River to the south (Figure 1), because this section has the highest nesting activity (Yalçın-Özdilek & Sönmez, 2011).

According to the distance from the sea, the nests were divided into three stages, where is the first stage (0 - 20 meter) and second stage (21 - 40 meter) and third stage (41 meter and more). Distance from sea (DFS) of each nest was measured with a flexible tape measure to the nearest distance when the nest was first found as a straight beach surface distance from the egg chamber to the sea (Sönmez et al., 2011). The nests were excavated and the remains examined about one week after the first hatchlings emerged from the nest (Whitmore & Dutton, 1985). Total eggs (TE, Clutch size) of each nest was determined by counting the number of unhatched eggs (UHE) and empty egg shells. Concurrently, total nest depth (TND) of each nest was measured with a flexible tape measure to the nearest depth as a straight vertical distance from the sand surface to the deepest point of the nest (Sönmez et al., 2011). During the nest excavation, unhatched eggs were collected for measurement, and transferred to laboratory in the research center.

In total, 361 eggs from 49 nests were measured, and then all unhatched eggs were checked for the embryos. Unhatched eggs were divided into 3 groups, they are (1) early embryonic stage (< 10 mm), (2) middle embryonic stage (10 mm - 30 mm) and (3) late embryonic stage (>30 mm) (Whitmore & Dutton, 1985). Unfertilized (without embryo) and abnormal (unusual shape, bigger or smaller) unhatched eggs were removed from the data of measurement. In addition, middle and late stages were removed from the data, because all egg size measurement is needed to be homogeneous and close to original size. Finally, 284 eggs from 44 nests were evaluated after the elimination. The all unhatched eggs were cleaned before the measurements, and taken into account that they no have decomposition in original shape. Egg diameter (ED) was measured with calipers to the accurate to 0.1 mm (Bjorndal & Carr, 1989). Egg weight (EW) was taken using an electronic scale accurate to 0.1 g (AND, EK 3000i) (Wallace et al., 2006; Cheng et al., 2009). Egg circumference (EC) was measured with a flexible tape measure. All measurements were measured independently by two researchers and average values were calculated when measurements have different values.

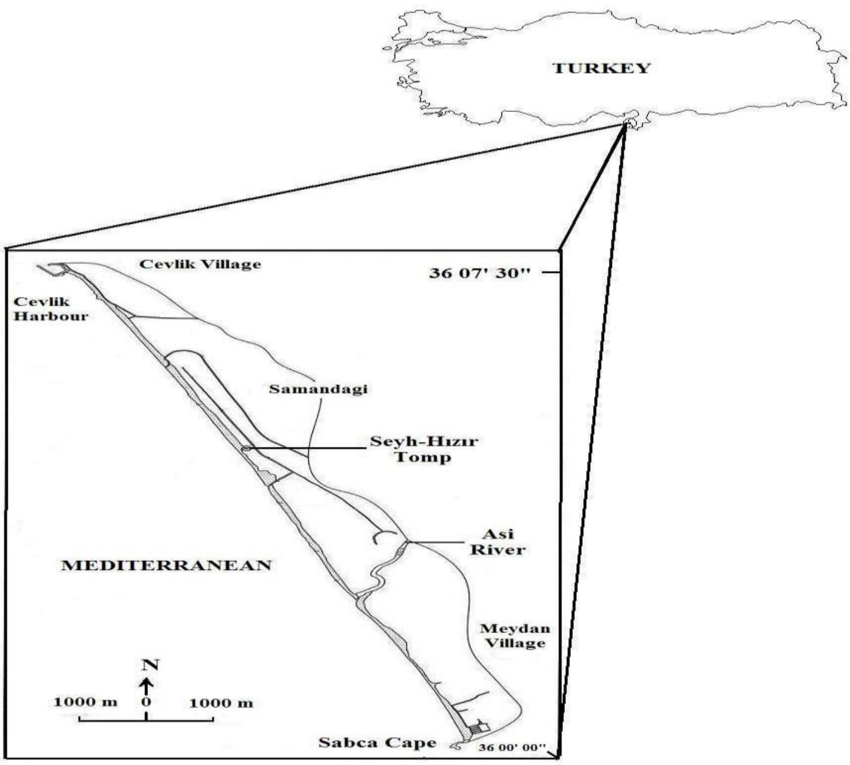

Figure 1. The map of study area

Normality and homogeneity of tests were determined before any comparison of data groups. The Levene statistic and Kolmogorov-Smirnov tests were used to test for homogeneity of variances. The differences in three or more groups were compared by means of Kruskal - Wallis H-test. The correlations between parameters (egg diameter, egg circumference, egg weight, distance from sea, total nest depth, total eggs and total unhatched eggs) were tested with "Spearman's rho" correlation coefficient (two tailed). The parameters were standardized and submitted to principal component analysis (PCA) to which were important of stages (Sönmez et al., 2013). All P-values were compared to an α level of 0.05, and all analyses were conducted with the Statistical Package for Social Sciences (SPSS) version 17.0 statistical package. All means are presented with ± S.D. and range (min - max).

Results

Data on the nests taken from different stages are given in Table 1. There were statistically significant differences among nests for distances of nests to the sea (DFS) (χ^2 =37.561, df=2, P=0.001) and total unhatched eggs (UHE) (χ^2 =7.578, df=2, P=0.023). However; there were no statistically significant differences for total eggs (TE) (χ^2 =2.093, df=2, P=0.351) and total nest depth (TND) (χ^2 =2.419, df=2, P=0.298).

Table 1. Descriptive statistics of the nests by stages

	Stages	**N**	**Mean**	**SD**	**Min-Max**
DFS (m)	1	10	18.1	1.72	15-20
	2	16	29.8	5.31	23-38
	3	18	52.4	9.66	40-74
	Total	44	36.4	15.77	15-74
TND (cm)	1	7	68.0	19.24	32-86
	2	16	75.8	9.62	55-93
	3	13	79.7	7.58	69-89
	Total	36	75.7	11.86	32-93
TE (numbers)	1	6	131.6	33.46	93-189
	2	16	112.1	26.36	80-163
	3	13	118.0	26.14	91-192
	Total	35	117.6	27.59	80-192
UHE (numbers)	1	7	81.1	31.62	39-140
	2	15	72.0	43.13	5-163
	3	13	34.0	31.31	3-102
	Total	35	59.7	41.26	3-163

The descriptive statistics of the measurements of egg size are shown in the Table 2. There were significant differences among the stages in term of egg diameter (ED) (χ^2 =38.174, df=2, P=0.000) and egg weight (EW) (χ^2 =11.191, df=2, P=0.004). However; there are no significant differences among the stages in term of egg circumference (χ^2 =3.212, df=2, P=0.201). The statistical correlations were computed among egg diameter, egg circumference, egg weight, distance from sea, total nest depth, total eggs and total unhatched eggs of nests in the stages. The egg diameter was positively correlated with distance from the sea (r=0.42, P=0.004) and the egg circumference was positively correlated with total nest depth (r=0.40, P=0.014). The egg weight was positively correlated with distance from the sea (r=0.32, P=0.30) and total nest depth (r=0.37, P=0.023). Moreover, total unhatched eggs were negatively correlated with distance from the sea (r= -0.53, P=0.001). However; total eggs were not correlated with any parameters. Egg size parameters (egg diameter, weight and circumference) showed a positive correlation with each other. The bigger egg diameter has bigger egg weight and egg circumference.

Table 2. The descriptive statistics of the measurements of egg size by stages

	Stages	N	Mean	SD	Min-Max
ED (cm)	1	80	4.13	0.17	3.80-4.50
	2	121	4.06	0.16	3.70-4.40
	3	83	4.21	0.13	3.90-4.50
	Total	284	4.12	0.17	3.70-4.50
EC (cm)	1	80	13.28	0.49	12.20-14.30
	2	121	13.35	0.54	11.50-14.50
	3	83	13.42	0.47	12.40-14.60
	Total	284	13.35	0.51	11.50-14.60
EW (g)	1	80	32.37	5.57	16.90-44.80
	2	121	32.46	7.45	12.50-46.20
	3	83	35.24	6.52	18.00-47.00
	Total	284	33.25	6.79	12.50-47.00

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 36.5 % and the second principal component accounted for 23.6 % of the shape variation among the stages and the first two PC factors accounted for 60.1 % of the variability in the original data. Examination of the contribution of each parameter to the PCA showed that the first component was dominated by the effects of the DFS and TND. The second component was dominated mostly by the contributions of the TE, UHE, ED, EC and EW. The vector representation of the contribution of the physical properties of nesting beaches on the first two PCs is shown in Figure 2.

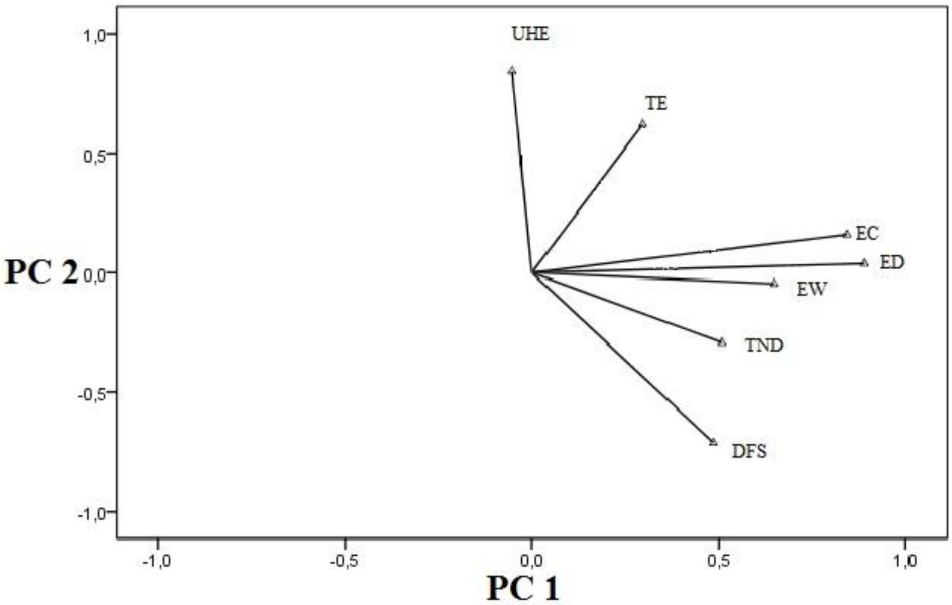

Figure 2. Contribution of parameters to the principal components. Vectors indicate the loadings of the scores for each variable on the first two principal components (DFS: Distance from sea, TND: Nest depth, EW: Egg wight, ED: Egg diameter, EC: Egg circumference, TE: total eggs, UHE: total unhatched eggs).

Discussion

The mean of egg diameter is 4.12 cm and egg weight is 33.25 g and egg circumference is 13.35 cm on Samandağ beach. In general, the egg size of sea turtles is variable among species, and the egg diameter is range 3.5 and 5.5 cm (Limpus & Miller, 1993). Kaska & Downie (1999) stated that the mean egg diameter of green turtle is 4.2 cm and the mean egg weight 43.3 g on the Cyprus. Similarly, the egg diameter is range 3.7 cm and 4.3 cm (mean 4.2 cm) and egg weight is range 40 g and 47.6 g (mean 43.3 g) for the green turtle on the Orchid Island, Taiwan (Cheng et al., 2009). In addition of these, Bjorndal & Carr (1989) found that the mean egg diameter is 4.4 cm that range 3.9 cm and 4.8 cm for the green turtle on the Tortuguero beach, Costa Rica. The result of this research is supported with those conclusions in term of egg diameter. However, egg weight showed differences from other nesting beaches and it is obvious that the eggs were lighter than of other researches. The reason of differences may be due to examined after hatchling emergence. The eggs can lose weight, but its diameter and circumference are not. Hirth (1980) stated that the clutch size differences much more among populations of green turtles than does egg size, and optimal egg diameter is 4.5 cm.

It was specified that there may be several reasons for the differences of egg size among the different nests for both freshwater and marine turtles. The first of these; the size of the eggs may be related to body size of females (Roosenburg, 1996), and it was specified that it may be larger females produce larger eggs (Van Buskirk & Crowder, 1994). Furthermore, Roosenburg (1996) stated that size of eggs in the same nest is showing very low variations. For all that, larger females tend to deposit large clutches and large eggs (Hays, 2001; Cheng et al., 2009). The second reason for differences of egg size among the nests, the total number of eggs may be effected on egg size (Roosenburg, 1996). However; the egg size was no correlation with total number of eggs on the Samandağ beach. A similar result was found by

Bjorndal & Carr (1989) on the Tortuguero beach, Costa Rica. They stated that the correlation of egg size and clutch size is not significant. It should not be forgotten that environmental factors such as temperature, metabolic heat, moisture, salinity may be effected on an egg and hatchling size during the incubation (Wallace et al., 2006; Sönmez et al., 2013; Kılıç & Candan, 2014; Önder & Candan, 2016).

The egg size showed positive correlation with distance from the sea and total nest depth on Samandağ beach. The nests closer to the sea have smaller egg size on Samandağ beach. Some researcher claims that large female tends to deposit large clutches and large eggs (Bjorndal & Carr, 1989; Hays, 2001; Cheng et al., 2009). If so, probably, more experienced females tend to deposit bigger eggs, making deeper nests and more distant stages from the sea than less experienced females. However; it suggest to this idea, there were no morphological data of female green turtle in this research on Samandağ beach. It can suggest that morphological data of females and their hatchlings may be investigated in future, and relationship of them may be revealed at Samandağ beach. On the contrary, the number of total unhatched eggs showed negative correlation with distance from the sea. Nests closer to sea have a higher risk of tidal inundation. Sönmez & Yalçın Özdilek (2013) stated that nests which are in the distance from 20 m to sea have a high risk under the flooding. When the nests were exposed at least once tidal inundation, water exchange of eggs for embryonic growth was negatively affected on the Samandağ beach (Sönmez & Yalçın Özdilek, 2013). On the contrary, a large egg surface area is increased in oxygen consumption and metabolic heating in the nests which have a larger egg size in the further from to the sea during the incubation. Cheng et al. (2009) stated that both factors may result in increased hatching mortality. The nests, which have a smaller egg size in the close to sea are might not directly affected by oxygen consumption and metabolic heating. However, they are affected by water exchange during incubation.

Examination of the distance of the variables from the origin revealed that the observed differences were mainly derived from DFS and TND. Indication of these parameters is important in the description of the parameters of the stages on the beach, which shows that DFS and TND is more important than TE, UHE and egg size (ED, EW and EC) discriminating among stages

Altogether, egg size is different among the stages on Samandağ beach. These differences may be due to causes distant to sea and nest depth. It should be not ignored that female morphology might effect to the this difference. If larger females produce large eggs, large females are attempting to their nest further from the sea and deeper from the surface. It suggests that the relationship among these parameters should be taken into consideration for future researches.

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