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RESEARCH ARTICLE

Head and plastron scalation patterns of the green turtle, *Chelonia mydas*, hatchlings in natural and relocated nests on Samandağ Beach

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Abstract

An effective protection and management plan for endangered species such as sea turtles is important to secure the existence of their future generations. Positive and negative effects of nest relocation, a conservation strategy, on the morphology and ecology of sea turtles have been previously discussed. However, the effect of this strategy on the plastron scute and head scalation pattern is unknown. Therefore, this study aims to provide further information on plastron scutes and head scalation patterns of the green turtle, *Chelonia mydas*, hatchlings in relocated and natural nests on Samandağ Beach, Turkey. A total of 195 hatchlings from two nest types were examined during the 2018 nesting season. Significant differences were determined in plastral scutes, while no differences were observed in head scalation between natural and relocated nests. These differences may be related to the nest location in the vertical position because the distance of natural nests to vegetation is significantly less than that of relocated nests (t=-3.612, P=0.004). Considering that the morphology of the green turtle hatchlings (e.g., normal modal scalation) influences their survival, *in situ* conservation of nests can help us to ensure that healthy individuals will be recruited to the sea turtle population.

Keywords: Green turtle, relocated nest, plastron scute, head scale, Samandağ

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Introduction

Green turtle (*Chelonia mydas*) is one of two nesting sea turtle species in the Mediterranean. Their nesting activity has a distribution limited to the eastern part of the basin (Turkey and Cyprus). According to the IUCN (International Union for Conservation of Nature) Red List criteria, the Mediterranean subpopulation of the green turtle is categorized as Endangered (EN) (Seminoff 2004).

Scalation patterns (the presence of scutes on the plastron and scales on the head) are used for the classification of sea turtle species (Margaritoulis and Chiras 2011). Keratinous plates on shells are called scute, and thickened areas of epidermis and keratin that cover skin and head are called scales (Wyneken 2003). Scutes are identified by their position and number, and they are species-specific. A typical chelonian carapacial scutellation consists of a median longitudinal series of unpaired elements (vertebral scutes). Also, it consists flanked on each side by a series of bilaterally paired scutes (costals), which are exteriorly bordered by another series of bilaterally paired scutes (marginals). Situated anteriorly between the first pair of marginals is a single nuchal. Situated posteriorly between the last pair of marginals is a pair of supracaudals. The scutes connecting plastron and carapace are called inframarginals (Mast and Carr 1989; Wyneken 2003). The position and number of plastral scutation from anterior to posterior are intergular (which are single and the closest to the throat), paired gular, humeral, pectoral, abdominal, femoral and anal scutes (Wyneken 2003). The scale of head pattern is species-specific in terms of number. The number of scales can differ, but their positions are specific. The head scales include prefrontal, subraocular, postocular, frontal, frontoparietal, parietal, interparietal, temporal and tympanic scales (Wyneken 2003).

Although the number and arrangement of scutellation in turtles are highly conserved, individual differences can be observed in almost all species that possess scales. While there are many studies on the carapacial scute variation of sea turtles (Mast and Carr 1989; Türkozan *et al.* 2001; Ergene *et al.* 2011; Margaritoulis and Chiras 2011; Sönmez *et al.* 2011; Sim *et al.* 2014), studies on the head scale and plastron scute are limited (Margaritoulis and Chiras 2011; Phillott and Parmenter 2014; Chew *et al.* 2015; Carpentier *et al.* 2016). Individual differences in scutellation may arise from various genetic and environmental factors such as “orthogenetic variation” (Gadow 1899) and “atavistic reappearance” (Newman 1906), and “accidents or disturbances during ontogenetic development” (Hildebrend 1938). Moreover, many different environmental factors may affect scute variation during the incubation period. For example, differences in available oxygen supply during the incubation causes scute abnormalities in the diamondback terrapins (*Malaclemys terrapin*) (Hildebrend 1938). Similarly, scute abnormalities may arise from temperature variations (Yntema and Mrosovsky 1980) and moisture content of nests during the incubation (Sönmez *et al.* 2011). Kamezaki (1989) found that long incubation periods (or low incubation temperature) are associated with an increase in the number of scute anomalies.

Identification of threats in life cycles of sea turtles is crucial to maintain the healthy population status as it will be help elaborate an effective protection and management plan. Threats have been identified under two habitat categories as terrestrial (tourism or agriculture, lighting, pollution, beach erosion, predation) and marine habitats (bycatch, marine debris, intentional killing) (Sönmez 2016).

Many conservation strategies such as nest relocation or hatcheries have been proposed against these threats (Türkozan and Yılmaz 2007; Sönmez *et al.* 2011). However, nest relocation or hatcheries on the beaches to reduce threats to eggs and hatchlings may cause scute abnormalities (Mast and Carr 1989; Suganuma *et al.* 1994; Türkozan and Yılmaz 2007; Sönmez *et al.* 2011). Although there is a standard procedure for nest relocations (Boulon 1999) such as choosing a new location with similar features and a new egg chamber with the same depth as the natural nest, microenvironmental factors including temperature and moisture may vary between natural and relocated nests (McGehee 1990; Foley *et al.* 2000; Sönmez *et al.* 2011). As a result of these differences in environmental factors, it can be expected that hatchlings emerge from two nest types may have scalation differences.

The scalation abnormalities might have a negative effect on the survival of hatchlings. Adult turtles tend to have fewer scute abnormalities than their hatchlings or sub-adults (Suganuma *et al.* 1994; Türkozan *et al.* 2001; Sönmez 2018). Hatchlings with smaller size and scute abnormalities can be related to reduced fitness (Türkozan and Yılmaz 2007) as they have smaller survival rates (Sönmez *et al.* 2011). Besides on hatchlings size and weight, plastron and head scalation, reflecting genetic or teratogenic factors (Velo-Anton *et al.* 2011), can also be used to examine the fitness of hatchlings. The presence of abnormal scutes and scales may indicate physical or physiological defects that affect fitness and reflect mutations or developmental abnormalities (Phillott and Parmenter 2014). Sim *et al.* (2014) found that hatchlings with the normal scute patterns were better swimmers than those with abnormality. This feature may decrease the predation risk, and improve their survival rates (Türkozan and Yılmaz 2007; Sönmez *et al.* 2011; Sim *et al.* 2014). It is important to obtain further information on how the nest relocation as a protection strategy affects the plastron scute and head scale (proxies for the survival rate). Therefore, this study aims to provide detailed information on the number and arrangement of plastron scutes and head scales of the green turtle, *C. mydas*, hatchlings in relocated and natural nests on Samandağ nesting beach.

Materials and Methods

Scutellation and nest data were collected on Samandağ Beach (36°07′N, 35°55′E) (Figure 1) on the eastern Mediterranean coast of Turkey during the 2018 nesting season. The beach is approximately 14 km in length, extending from the Çevlik Port in the north to Sabca Promontory in the south.

Only eggs under the risk of tidal inundation were transferred to an upper part of the beach. The relocation of eggs was completed within the first 6 hours after the egg deposition. A plastic bucket filled with 5 cm thick sand at the bottom was used to transfer the eggs to minimize possible damages to eggs. The eggs from each clutch were carefully transferred to new nests constructed according to the original nest dimensions. Nesting data regarding clutch size, egg laying and relocation dates, distance from sea to the nest and to the nearest vegetation were noted for each nest (Boulon 1999). Nests were checked for hatchling emergence every hour at night. Upon the emergence of hatchlings, scutellation data (plastron scute and head scale) were collected, and the hatchlings were released.

A total of 14 nests (seven natural and seven relocated) were studied and 14 individuals were randomly chosen from each clutch. In total, plastron scute and head scale patterns of 195 hatchlings were examined. Hatchlings on Samandağ Beach predominantly emerge from their nests within 1-2 days. Therefore, all examined hatchlings were randomly collected on the first day emerge. The examined plastron scutes from anterior to posterior are intergular, gular, humeral, pectoral, abdominal, femoral, anal and inframarginal (Figure 2). The examined head scales are prefrontal, frontal, frontoparietal, supraocular and postocular (Figure 2).

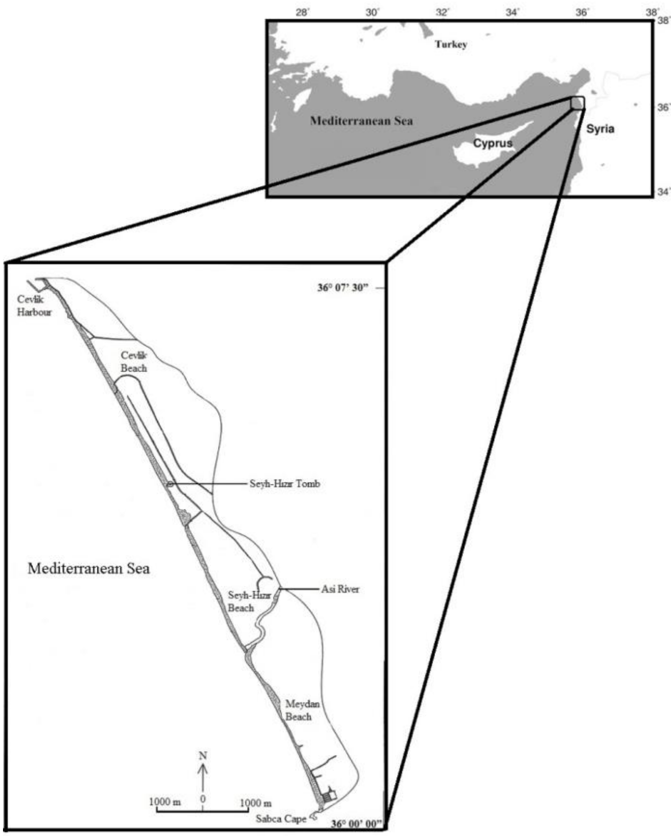

Figure 1. Map of the study area (shadowed area shows survey area)

All nests were selected within 10-13 days to make sure that these nests belong to different females (Broderick *et al.* 2002). Eight to ten days after the emergence of hatchlings, nests were opened to measure the number of remaining hatchlings, empty eggshells, undeveloped eggs and dead embryos. The distance of the nest from the sea (DFS) and vegetation (DFV) were measured using a tape measure (accuracy ±1cm). The incubation duration (ID) of nests was calculated as the time between the day the eggs were laid and the day the first hatchlings emerged. The clutch size (CS) was determined by counting the number of unhatched eggs and hatched eggs. Nest depth (ND) was measured using a tape measure as a straight vertical distance from the sand surface to the deepest point of the nest.

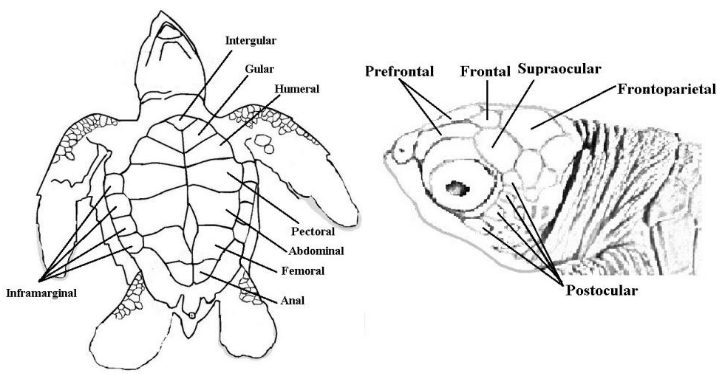

Figure 2. The number and arrangement of plastron scutes and head scales patterns in green turtle hatchlings

The normality analysis of data was carried out using the Levene's test (P>0.05). The independent sample t-test was used to compare features of relocated and natural nests which are namely, DFS, DFV, ID, CS and ND. The number of each scute and scale on plastron and head were considered as a countable variable (Mast and Carr 1989). Scute and scale deviations were determined by monitoring each individual's normal patterns. Depending on the presence or absence of scute and scale deviations, the trait was classified as either 1 or 0, respectively. Differences in the frequency distribution of scute deviations were analysed using the chi-squared Fisher's exact test because the expected count was less than five. Statistical analyses were conducted using SPSS v. 17.0. (SPSS Inc., Chicago, United States). All means are presented with standard deviation (± SD) and min-max values.

Results

The descriptive statistics of nest features for natural and relocated nests are presented in Table 1. There were no significant differences in DFS, ID, ND and

CS between relocated and natural nests (t-test, P>0.05). However, DFV significantly differed between the two nest types (t-test, t = -3.612, df = 12, P=0.004), suggesting that natural nests were significantly closer to the vegetation.

Table 1. The descriptive statistics of nest features for the natural and relocation nests (DFS: distance from sea, DFV: distance from vegetation, ND: nest depth, ID: incubation duration, CS: clutch size)

		n	Mean	SD	min-max
DFS (cm)	**Natural**	7	42.7	10.75	29-60
	Relocated	7	35.0	4.69	26-39
	Total	14	38.8	8.91	26-60
DFV (cm)	**Natural**	7	3.8	7.08	0-18
	Relocated	7	25	13.77	6-51
	Total	14	14.4	15.20	0-51
ND (cm)	**Natural**	7	75.5	5.06	67-82
	Relocated	7	79.4	2.69	77-85
	Total	14	77.5	4.38	67-85
ID (days)	**Natural**	7	49.2	1.60	47-52
	Relocated	7	51.2	2.75	47-56
	Total	14	50.2	2.40	47-56
CS	**Natural**	7	106.7	16.59	84-128
	Relocated	7	109.5	16.16	88-132
	Total	14	108.1	15.80	84-132

The number of intergular scutes varied from 1 to 5 in both nest types. The most common pattern was those with 1 intergular with a frequency of 91.8% and 75.3% in natural and relocated nests, respectively (Table 2). Two combinations of gular were noted. The most common individuals were those with a 1-1 pattern in natural (100%) and relocated (94.9%) nests (Table 2). The number of humeral varied from 1 to 2 on either side. Four combinations of humeral were noted. The most common individuals were those with a 1-1 humeral with a frequency of 100% and 88.6% in natural and relocated nests, respectively (Table 2). Numbers of pectoral scutes were stable and they were 1-1 in natural and relocated nests (Table 2).

Four combinations of abdominal were detected in natural and relocated nest types. The most common individuals were those with the 1-1 pattern for natural and relocated nests with a frequency of 100% and 94.8%, respectively (Table 2). Two combinations of femoral were noted in both nest types. The most common individuals were those with a 1-1 femoral with a frequency of 100% and 99% in natural and relocated nests, respectively (Table 2). The numbers of anal scutes were stable, and they were 1-1 in natural and relocated nests (Table 2).

The number of inframarginals varied from 3 to 7 on either side. Nine combinations of inframarginal were identified from natural and relocated nests.

The most common individuals were those with 4-4 inframarginal with a frequency of 98% and 82.4% in natural and relocated nests, respectively (Table 2).

Table 2. The number and arrangements of plastron scutes and head scales patterns in both nests (# there is no variation values in the present group)

		Left- Right	Natural Nests		Relocated Nests	
			n	**%**	**n**	**%**
Plastron Scutes	**Intergular**	1	90	91.8	73	75.3
		2	8	8.2	22	22.7
		4	#	#	1	1
		5	#	#	1	1
	Gular	1-1	98	100	93	94.9
		2-1	#	#	4	4.1
	Humeral	1-1	98	100	86	88.6
		1-2	#	#	4	4.1
		2-1	#	#	6	6.1
		2-2	#	#	1	1.2
	Pectoral	1-1	98	100	97	100
	Abdominal	1-1	98	100	92	94.8
		1-2	#	#	2	2.1
		2-2	#	#	1	1
		2-1	#	#	2	2.1
	Femoral	1-1	98	100	96	99
		2-2	#	#	1	1
	Anal	1-1	98	100	97	100
	Inframarginal	4-3	1	1	2	2
		4-4	96	98	80	82.4
		4-5	1	1	6	6.2
		4-7	#	#	1	1
		5-3	#	#	1	1
		5-4	#	#	3	3.1
		5-5	#	#	1	1
		5-6	#	#	2	2
		7-8	#	#	1	1
Head Scales	**Prefrontal**	1-1	98	100	97	100
	Frontal	1	98	100	97	100
	Frontoparietal	1	98	100	97	100
	Supraocular	1-1	98	100	93	95.8
	Postocular	2-1	#	#	4	4.2
		2-3	1	1	#	#
		3-3	1	1	6	6.2
		3-4	5	5.1	5	5.1
		4-3	4	4.1	16	16.5
		4-4	76	77.5	63	64.9
		4-5	9	9.2	6	6.2
		5-4	1	1	1	1
		5-5	1	1	#	#

The number of prefrontal scales was stable and 1-1 in natural and relocated nests (Table 2). Similarly, the number of frontal and frontoparietal scales was stable and they were 1 for both nest types (Table 2). Two combinations of supraocular were detected in natural and relocated nests. The most common individuals were those with a 1-1 pattern in natural (100%) and relocated (95.8%) nests (Table 2). However, the number of postocular scales varied from 2 to 5 on both sides. Eight combinations of those were noted in natural and relocated nests. The most common individuals were those with 4-4 postocular with a frequency of 77.5% and 64.9% in natural and relocated nests, respectively (Table 2).

Table 3. The descriptive statistics of the plastron scute and head scale deviation rates within natural and relocated nests and Fisher's Exact test results (0 = no scute deviations; 1 = scute deviations, * cannot be computed because this is a constant variable, # there is no variation values in the present group)

	Scute	Deviant	Natural	Relocation	Total	Fisher's Exact
Plastron Scutes	**Intergular**	0	90	73	163	**0.02**
		1	8	24	32	
	Gular	0	98	93	191	0.059
		1	#	4	4	
	Humeral	0	98	86	184	**0.001**
		1	#	11	11	
	Pectoral	0	98	97	195	*
		1	#	#	#	
	Abdominal	0	98	92	190	**0.029**
		1	#	5	5	
	Femoral	0	98	96	194	0.497
		1	#	1	1	
	Anal	0	98	97	195	*
		1	#	#	#	
	Inframarginal	0	96	80	176	**0.001**
		1	2	17	19	
Head Scales	**Prefrontal**	0	98	97	195	*
		1	#	#	#	
	Frontal	0	98	97	195	*
		1	#	#	#	
	Frontopariatal	0	98	97	195	*
		1	#	#	#	
	Supraocular	0	98	93	191	0.059
		1	#	4	4	
	Postocular	0	76	63	139	0.059
		1	22	34	56	

Variation rates of both plastral scutes and head scales for two nest types are shown in Table 3. There were statistically significant differences in the number of intergular, humeral, abdominal and inframarginal scutes (see Table 3 for further information). Head scales and gular, pectoral, femoral, anal scutes did not indicate any significant difference between two nest types (P>0.05, Table 3).

Discussion

Previous studies on the green turtle hatchlings were limited to taxonomic information (Pritchard and Mortimer 1999; Wyneken 2003), photographic identification (Chew *et al.* 2015; Carpentier *et al.* 2016), and the effect of fungal colonization (Phillott and Parmenter 2014). Existing literature data on the plastron scute and head scale in hatchlings are limited. The most common plastron scute number and arrangement in this study were compatible with previous studies. Intergular scute was 1, and the others were a pair that gular, humeral, pectoral, abdominal, femoral and anal scutes. Although the number of inframarginal scutes, which is important for the classification of sea turtle species, indicated a high variation. The most common combination was detected as 4-4. Similarly, the postocular scale on head displayed great variation, and most common was found as 4-4. Interestingly, the postocular scale conveyed high frequency deviations in two nest types. Prefrontal and supraocular scales were found as a pair, and also frontal and frontoparietal scales were found as 1.

Intergular, humeral, abdominal and inframarginal scutes in hatchlings from relocated nests indicated more deviations than those from natural nests. However, head scales of green turtle hatchlings in relocated nests showed no differences compared to those from natural nests. Scalation anomalies can be caused by thermal and hydric micro-climatic conditions of nests (Phillott and Parmenter 2014). Although the mechanisms behind it have not been described, experimental studies indicated that scalation abnormalities can originate from accompanying environmental conditions such as available oxygen (Hildebrend 1938), temperature (Yntema and Mrosovsky 1980), moisture content (Sönmez *et al*. 2011) and incubation duration (Kamezaki 1989) during the incubation of eggs.

Many scientists stated that the relocation of nests has a negative effect on the hatchling morphology (including scute abnormalities) of the green turtle (Suganuma *et al.* 1994; Özdemir and Türkozan 2006; Sönmez *et al.* 2011). Considering that there may be differences in microenvironmental factors between relocated and natural nests (McGehee 1990; Foley *et al.* 2000; Sönmez *et al.* 2011), the relocation of nests may cause scalation deviation in the green turtle hatchlings. However, in the present study, these environmental factors such as temperature, moisture content and available oxygen were not tested.

There were no significant differences between two nest types in terms of DFS, ID, ND, and CS on Samandağ Beach. Whereas, DFV showed a significant difference between nest types and natural nests were closer to the vegetation.

The moisture content of nests showed differences in the vertical line on the Samandağ Beach (Sönmez and Yalçın Özdilek 2011). These differences may have caused plastron scute deviations because nests closer to the vegetation area have lower moisture content (Sönmez and Yalçın Özdilek 2011). Similarly, Sönmez *et al.* (2011) suggested that moisture content can have a negative effect on carapacial scute deviations of the green turtle hatchlings on Samandağ Beach. It should be noticed that plastral and head scalation abnormalities may depend on more complex conditions, even in the absence of additional factors such as microenvironmental of nests (Phillott and Parmenter 2014). Moreover, the level of DNA methylation, an epigenetic mechanism during embryonic development, may be related to scalation deviations. DNA methylation levels correlate with environmental conditions (Varriale 2014). Environmental parameters can affect the DNA methylation and consequently, cause the activation or suppression of certain genes (Carappa *et al.* 2016).

Hatchlings with scute deviations have narrower carapace and lighter body mass (Sim *et al.* 2014). Hatchlings size is positively correlated with crawling speed (Janzen *et al.* 2000) and swimming performance (Ischer *et al.* 2009). A lighter body mass may mean a smaller yolk to provide energy, thus decreasing the probability of survival (Booth *et al.* 2004). Smaller hatchlings may have the disadvantage of evading gape-limited predators, swimming faster and handling of large prey items (Janzen *et al.* 2000; Booth *et al.* 2004). Hatchlings with plastron scute abnormalities that emerged from relocated nests probably die before they mature and tend to be removed from the population (Suganuma *et al.* 1994; Türkozan *et al.* 2001). Stranding data showed that adult green turtles tend to have less carapace scute deviations than their sub-adult and oceanic stage on Samandağ Beach (Sönmez 2018).

Artificial incubation of sea turtle eggs (i.e., relocation of nests, hatchery safe area and handling of eggs) can increase population size owing to nest protection (i.e., raising hatching success or reducing mortality by saving eggs from inundation) (Dutton *et al.* 2005). However; these efforts may also introduce negative effects on scute number and arrangement. This could have a negative impact on the survival of the population in the future. The presence of healthy individuals ensuring the future of populations is more important factor. Therefore, more research is needed on the effect of relocation and artificial incubation on the sea turtle eggs. *In situ* protection of nests is recommended not only to prevent high percentages of scute deviations but also to reduce high percentages of early deaths in the sea turtles (Suganuma *et al.* 1994; Türkozan *et al.* 2001). Once the effect of relocation is well-understood, it will help conservationists to better handle the sea turtle eggs.

In conclusion, the number and arrangement of the green turtle's plastron scute can be adversely affected by the relocation of nests. Therefore, the choice of a new nest location is important for future generations as it can determine the survival of hatchlings. Since the distance of new nest to vegetation can change the nest moisture content, the choice of the new nest location is of paramount significance for the development of an effective protection and management plan.

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Samandağ kumsalında doğal ve yeri değiştirilen yuvalarda yeşil kaplumbağa yavrularının, *Chelonia mydas*, kafa ve plastron skalasyon desenleri

Öz

Deniz kaplumbağaları gibi nesli tükenmekte olan türler için etkili bir koruma ve yönetim planı, gelecek nesillerinin varlığını güvence altına almak için önemlidir. Daha önce literatürde bir koruma stratejisi olarak yuva yeri değiştirmesinin, deniz kaplumbağalarının morfolojisi ve ekolojisi üzerindeki olumlu ve olumsuz etkileri tartışılmıştır. Bununla birlikte, bu stratejinin plastron plak ve kafa skalası modeli üzerindeki etkisi bilinmemektedir. Bu nedenle, bu çalışma, Türkiye'nin Samandağ sahilinde, yeşil kaplumbağanın, *Chelonia mydas*'ın, yeri değiştirilmiş ve doğal yuvalardaki yavrularının plastron plakları ve kafa skalasyon desenleri hakkında daha fazla bilgi vermeyi amaçlamaktadır. 2018 yuvalama mevsimi boyunca iki yuva tipinden toplam 195 yavru incelendi. Plastral plaklarda önemli farklılıklar tespit edilirken, doğal ve yeri değiştirilmiş yuvalar arasında kafa skalasyonunda bir farklılık gözlenmedi. Bu farklılıklar yuvaların dikey konumdaki konumu ile ilişkili olabilir, çünkü doğal yuvaların bitki örtüsüne olan uzaklığı, taşınan yuvalarınkinden önemli ölçüde daha yakındı ($t = -3.612$, $P = 0.004$). Yeşil kaplumbağa yavrularının morfolojisinin (örneğin normal model skalasyon) hayatta kalmalarını etkilediğini göz önüne alarak yuvaların yerinde korunmaları, sağlıklı bireylerin deniz kaplumbağası popülasyonuna katılmasını sağlamamıza yardımcı olabilir.

Anahtar kelimeler: Yeşil kaplumbağa, yuva yeri değiştirme, plastron plak, kafa skalası, Samandağ

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