The Nile Softshell Turtle (*Trionyx triunguis*): Nest Parameters and A New Nesting Site

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Abstract

There are no studies carried out on nest parameters of the Nile softshell turtle (*Trionyx triunguis*) in Turkey except for few studies on the Western Mediterranean coast of Turkey. In this study, 12 nests of the Nile softshell turtle were examined in terms of nest parameters during 2016 and 2017 nesting seasons on Belek, Göksu, and Burnaz beaches located on the Central and Eastern Mediterranean coast of Turkey. Nesting period started in May (17%) but intensified in June (83%). The distance of nests to the shore, depth of nests, clutch temperature, clutch size, and hatching success in the nests were recorded and calculated. Among three beaches, the lowest hatching success rate was on Göksu Beach (0.18 ± 0.26), whereas the values were similar on Belek (0.75 ± 0.22) and Burnaz (0.72 ± 0.36) beaches. Although clutch temperatures were measured in a range (29.5–33.0 °C) that is optimal for embryo development, most of the eggs inside nests were dead at the late embryonic period (5.1 ± 9.6) which was followed by early (1.8 ± 3.1) and middle stage embryos (0.3 ± 0,6). The highest number of unfertilized eggs was recorded on Burnaz Beach (14.5 ± 19.1) and the lowest was on Belek Beach (1.0 ± 1.5). Regarding the relationship between the nest parameters, it was detected that only the clutch temperature has a significant relationship with the clutch size. In this study, a new nesting site for the softshell Nile turtle on Burnaz Beach was discovered and nest parameters in this species were indicated on three beaches.

Keywords**:** *Trionyx triunguis*, nest parameters, temperature, Burnaz Beach, Mediterranean Sea

Yumuşak Kabuklu Nil Kaplumbağası (*Trionyx triunguis*): Yuva parametreleri ve yeni bir yuvalama alanı

Özet

Türkiye'de bulunan *Trionyx triunguis* (Yumuşak Kabuklu Nil Kaplumbağası) yuva parametreleri ile ilgili batı Akdeniz sahillerinde yapılan az sayıda çalışma dışında bir çalışma bulunmamaktadır. Bu çalışmada, Türkiye'nin orta ve doğu Akdeniz sahillerinde bulunan Belek, Göksu ve Burnaz kumsallarında 12 adet Yumuşak Kabuklu Nil Kaplumbağası yuvasına ait 2016 ve 2017 sezonlarındaki yuva parametreleri değerlendirilmiştir. Yuvalamaların mayıs ayında (%17) başladığı ancak haziran ayında (%83) yoğunlaştığı görülmüştür. Yuvaların kıyı çizgisine uzaklığı, derinliği, sıcaklığı, kuluçka büyüklüğü ve yavru çıkışı başarıları hesaplanmıştır. Çalışmanın gerçekleştirildiği üç kumsalda en düşük yavru çıkışı başarısı Göksu Kumsalı'nda (0,18 ± 0,26) iken Belek (0,75 ± 0,22) ve Burnaz (0,72 ± 0,36) birbirine yakın değerdedir. Kuluçka sıcaklıkları, embriyoların sağlıklı bir şekilde gelişebileceği aralıkta (29,5–33,0 °C) ölçülmesine karşın, yuva içerisindeki yumurtaların en fazla geç embriyonik dönemde öldüğü (5,1 ± 9,6) bunu erken (1,8 ± 3,1) ve orta dönem (0,3 ± 0,6) embriyoların izlediği tespit edilmiştir. Döllenmemiş yumurta sayıları en yüksek Burnaz Kumsalı'nda (14,5 ± 19,1) ve en düşük Belek Kumsalında (1,0 ± 1,5) kaydedilmiştir. Yuva parametrelerinin birbirleri arasındaki ilişkileri incelendiğinde yalnızca yuva içi sıcaklığının, kuluçka büyüklüğü ile istatistiksel olarak anlamlı bir ilişki gösterdiği tespit edilmiştir. Bu çalışma ile, Burnaz kumsalı Yumuşak Kabuklu Nil Kaplumbağası için yeni bir yuvalama alanı olarak tespit edilerek, bu türün üç kumsala ait yuvalarındaki yuva parametreleri ortaya konmuştur.

Anahtar kelimeler: *Trionyx triunguis,* yuva parametreleri, sıcaklık, Burnaz Kumsalı, Akdeniz

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INTRODUCTION

In Turkey, there are only two species in family Trionychidae which are, namely, *Trionyx triunguis* (the Nile softshell turtle) and *Rafetus euphraticus* (the Euphrates softshell turtle) (Kinzelbach, 1986). Among these two species, the Nile softshell turtles are commonly found in brackish water, as well as in freshwater reaching seas and sometimes in the sea. This species is distributed across Turkey, Egypt, Syria, Lebanon, Israel and Greece (van Dijk et al., 2017). There is only one record of this species on Kos Island, Greece and the biggest population can be found in Turkey (Taşkavak, Reimann, and Polder, 1999).

According to an action plan for the Nile softshell turtle status in Turkey, 15 subpopulations were identified (Kasparek, 2001). These subpopulations are divided into 3 main groups, namely, large populations with the highest conservation priority (Category I), relatively large populations with an urgent protection priority (Category II), and relatively small populations where protection is required (Category III) (Kasparek, 2001).

There is no temperature-dependent sex determination in the Nile softshell turtle although it is common in most of the turtles. However, it has been reported that the clutch temperature values between 24–33 °C provide the highest embryonic success (Leshem, Ar, and Ackerman, 1991).

Recent studies conducted on this species include population size and structure (Akçınar and Taşkavak, 2017), molecular genetics (Güçlü et al., 2009; Güçlü, et al., 2011; Shanas et al., 2012), review of marine records (Taşkavak and Akçınar, 2009), and assessment of mite infestations in nests (Katılmış and Urhan, 2007). There are also some studies on the International Union for Conservation of Nature (IUCN) assessment of the Mediterranean population of the Nile softshell turtle which is threatened at international and national scales (van Dijk et al., 2017). However, there are few studies about the reproductive biology of this species (Atatür, 1979; Baran et al., 1994; Gidiş & Kaska, 2004; Kaska, et al., 2017; Türkozan et al., 2006). These studies focused on the Western Mediterranean coast of Turkey (for further details see van Dijk et al., 2017)

Sufficient amount of data on the reproduction biology is necessary for the protection of an endangered species. The purpose of this study is to examine the relationship between nest parameters in the Nile softshell turtle nests which were detected in the Central and Eastern Mediterranean beaches of Turkey (i.e., Belek, Göksu and Burnaz) and contribute to the existing data on the reproductive biology of the species.

MATERIALS and METHODS

Study site

This study was conducted from the middle of May to the end of September on three different beaches in 2016 and 2017 nesting seasons. These beaches were Belek Beach covering Köprüçay/Acısu in Category III, Göksu and Burnaz beaches in Category II (Kasparek, 2001) (Figure 1).

Belek and Göksu beaches are the largest nesting sites for *Caretta caretta* (the loggerhead sea turtle) in the Mediterranean Sea. Belek Beach, that is 30 km long, is

also used by *Chelonia mydas* (the green sea turtle) and the Nile softshell turtle for nesting apart from the loggerhead sea turtle. Göksu Beach is also a nesting site with a length of 34 km where nesting of the loggerhead sea turtle is more often than that of the green sea turtle as it is in Belek Beach. Burnaz Beach is the most eastern nesting site of the Nile softshell turtle in Turkey that is a 13 km long beach between the Ceyhan and Asi rivers. Although Belek and Göksu Beach are classified as nesting beaches, Burnaz Beach is officially not in the status of a nesting site.

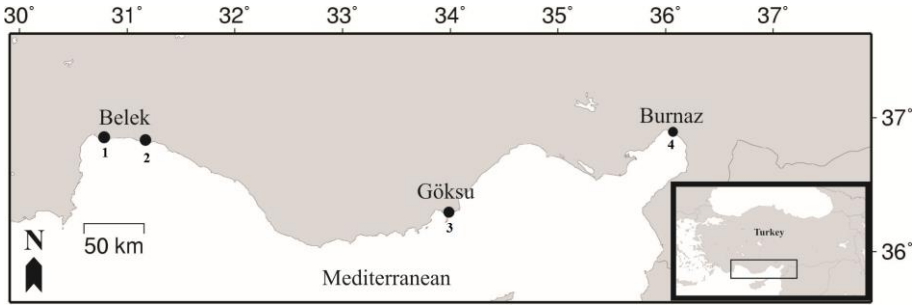

Figure 1. Locations of the detected nests in this study: 1) Aksu-Beşgöz in Belek, 2) Köprüçay-Niğit in Belek, 3) Göksu Delta, and 4) Burnaz Beach

Fieldwork and measurements

The nests were detected during the daytime fieldwork using the traces on beaches left by nesting females. Detected nests were protected against predation using cages (1×1 m in size and with 9 cm mesh size). Temperature data loggers (Gemini Data Loggers, TinyTalk Range H, TK-0040, UK) were placed in the middle of each nest within 12 hours after nesting to determine the clutch temperature inside nests. Data loggers are programmed with original software (Tinytag Explorer 4.8) to take 10 measurements per hour.

Nest parameters (distance to the shore, clutch size, hatching success, and nest depth) of the detected nests were recorded. Distances to the shore were detected using a tape measure (in meters). After the completion of the hatching period, all nests were opened and the eggs inside were counted to determine the clutch size. The eggs in the nest were separated into two main groups, namely, hatched and unhatched. Each failed egg were opened and classified in either unfertilized, early, middle, or late embryonic stage according to the criteria of Whitmore and Dutton (1985). Hatching success was calculated by dividing the number of hatched eggs to clutch size (Hatching success = Hatched egg number / Total egg number). During the opening of nests for control, after collecting data loggers and counting all eggs, nest depth was measured (in cm) from the surface of the sand to the nest bottom using a tape measure.

Data analyses

Data collected by data loggers during the clutch period were transferred to a computer using the original software. All measurements were calculated as daily averages. Descriptive statistics of all parameters (including temperature data) were prepared. Normal distributions of all data were assessed by the Shapiro-Wilk test during the determination of relationships between parameters. All data with a normal

distribution were tested using the Pearson correlation coefficient. MiniTab v.17 (Minitab Inc., PA, USA) statistical software package was used for all analyses.

RESULTS

In total, 12 nests were identified on Belek, Göksu, and Burnaz beaches (n= 7, 3, and 2, respectively) during the two nesting seasons (Table 1). Although Belek and Göksu beaches are known to be nesting sites for *T. triunguis*, the nesting has been detected for the first time on Burnaz beach. The earliest nesting was detected on 20th May on Belek Beach and the latest nesting was on 25th June on Göksu Beach. Regarding the temporal distribution of the nests, there were 2, 5, and 5 nests in May, the first half, and the second half of June, respectively. Therefore, 17% and 83% of nesting occurred in May and June, respectively. There was no nesting in July.

Table 1. Data collected from nests on Belek, Göksu and Burnaz beaches.

					Dead embryo numbers						
Nesting site	**Nest no**	**Clutch size**	**Hatched egg number**	**Unfertilized egg number**	**Early embryonic stage**	**Middle embryonic stage**	**Late embryonic stage**	**Hatching success**	**Clutch temperature (°C)**	**Nest depth (cm)**	**Nest distance (m)**
Belek	1	15	4	0	11	0	0	0.27		55.1	7.8
	2	26	23	0	0	1	2	0.88	29.5	19	9.5
	3	33	28	1	1	0	3	0.85		34	29
	4	47	35	0	1	0	11	0.74		31	2
	5	25	22	0	2	0	1	0.88	30.4	37	1.2
	6	32	23	4	3	0	2	0.72	30.6	31	6
	7	47	41	2	1	0	3	0.87	32.4	51	4.5
Göksu	8	29	14	13	2	0	0	0.48		35	5.5
	9	15	1	9	0	0	5	0.07		32	8.4
	10	44	0	8	0	2	34	0		37	13.1
Burnaz	11	52	24	28	0	0	0	0.46	33.0	35	21.3
	12	43	42	1	0	0	0	0.98	32.3	36	17.6
Overall mean±SD		**34.0± 12.6**	**21.4 ± 14.4**	**5.5 ± 8.3**	**1.8 ± 3.1**	**0.3 ± 0.6**	**5.1 ± 9.6**	**0.60 ± 0.34**	**31.4 ± 1.4**	**36.1 ± 9.3**	**10.5 ± 8.4**

The mean distances of the nests to the nearest shore were calculated as 10.5 m on average (8.6, 9.0, and 19.5 m on Belek, Göksu, and Burnaz beaches, respectively). The mean clutch size was determined to be 34 eggs per nest. Hatching success of 12 nests ranged from 0 to 98%. The average hatching success was 75% on Belek Beach, 72% on Burnaz Beach, and 18% on Göksu Beach. The total numbers of eggs detected in 12

nests were 408. The total hatched eggs were 257 (63%), total unfertilized eggs were 66 (16.2%) and dead in shell embryos were 85 (20.8%). Majority of the dead in shell embryos were at the late stage (n=61) and the early stage (n=21) and only 3 eggs were found at the middle stage.

A total of 6 temperature data loggers (4 on Belek Beach and 2 on Burnaz Beach) were installed to determine clutch temperatures (Figure 2). The average clutch temperatures were 32.7 °C on Burnaz Beach, whereas on Belek Beach it was 2 °C lower (no data from Göksu Beach). The mean depth of the nests was measured as 36.1 cm.

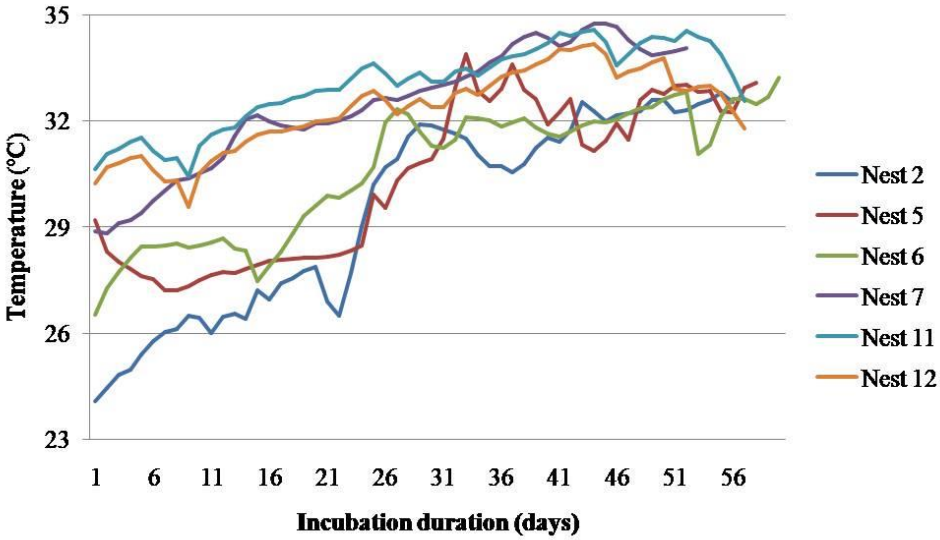

Figure 2. Clutch temperatures of *Trionyx triunguis* nests (Nest 2-7 on Belek Beach and Nest 11-12 on Burnaz Beach).

In data analyses of the nest parameters (i.e., clutch temperature, clutch size, hatching success, embryonic mortality, nest depth and distance to the sea), a very strong positive correlation was found between clutch temperature and clutch size (Pearson value: 0.967, $p<0.005$). However, no relationship was found between the parameters ($p>0.05$ for all parameters).

DISCUSSION

Even though more studies carried out on the Nile softshell turtle in Turkey than in other countries, these studies are still insufficient (van Dijk et al., 2017). Especially in regions near the Syrian border, there is no information about the status of this species (Kasparek, 2001). Therefore, the nests found on Burnaz Beach provide a significant contribution to the known nesting sites on the Eastern Mediterranean coast of Turkey. Presence of only two nests on Burnaz Beach suggests that this site should be regarded as a small nesting site as in Patara, Fethiye, Köprüçay, Bozyazı, and Asi River and it should be monitored (Gramentz, 2005).

During this study, the first nesting was detected on 20th May and the last was on 25th June. Similarly, nesting events have been reported between May and July (Gidiş and

Kaska, 2004). Moreover, in this study, more nesting was observed in June than in May, whereas nesting occurred more frequently in July than in June on Dalyan Beach (Türkozan et al., 2006) because nesting densities may vary across nesting seasons and beaches (Frazer and Richardson, 1985).

The nest distances to the shore may differ across beaches. In previous studies, the nest distance to the shore varied from 2 to 18 m. The mean values were calculated as 10.5 m (Atatür, 1979), and 7.8 m (Gidiş and Kaska, 2004) on Dalaman Beach, whereas it was 6.9 m on Dalyan Beach (Türkozan et al., 2006). In this study, the nest distances to the shore on three different beaches varied between 1–29 m and the mean value was 10.5 m. Moreover, the nest distances increased from west to the east direction.

Several studies have been carried out on the Western Mediterranean beaches regarding reproductive ecology of the Nile softshell turtle. In these studies, the mean clutch size per nest was 23 (Türkozan et al., 2006) and 23.7 eggs (Baran et al., 1994) on Dalyan Beach and 17.7 (Atatür, 1979) and 31 eggs (Gidiş and Kaska, 2004) on Dalaman Beach. In the present study, the mean clutch size was 34 eggs (ranged between 15 and 52). Therefore, the clutch size can vary across beaches as well as across seasons on the same beach. In a study with the same genus (*Trionyx muticus*), the clutch size indicated significant differences between seasons on the same beach which was related to the size of the nesting female (Plummer, 1977).

Low hatching success is one of the threatening factors for this species (Kasparek, 1994). Hatching success values in studies conducted in Turkey were 45.7% and 49.6% on Dalyan and Dalaman beaches, respectively (Kaska et al., 2017). In a three-year study conducted in Nahal Alexander (Israel), the mean hatching success was 60% (ranged from 29 to 75%) (Rozner and Shaines, 2010). In our study, the number of unfertilized eggs and deaths in the embryonic period were quite high. The ratio of unfertilized eggs in 13 nests on Dalaman Beach was calculated as 3% and death in the embryonic period was 7% (Gidiş and Kaska, 2004). Although the mean hatching success in this study was 63%, this value was very low on Göksu Beach compared to the other two beaches. Göksu Beach is a nesting site with a high embryonic mortality not only for the Nile softshell turtle but also for the loggerhead sea turtle and the green sea turtle (Akçınar, 2006; Candan, 2018; Özdemir, Türkozan, and Güçlü, 2008). This situation could be caused by groundwater inundation and tidal inundation of nests.

One of the most serious threats to nests of the Nile softshell turtle is predation (van Dijk et al., 2017). The predation rates calculated using the egg number were 91% on Dalyan (Türkozan et al., 2006) and 20% on Dalaman beaches (Gidiş and Kaska, 2004). The predation rates based on the nest number was %50 (Baran et al., 1994) and 60.8% (Kaska et al., 2017) on Dalyan and 37.9% on Dalaman beaches (Kaska et al., 2017). In this study, the nests were caged for protection against predation and 100% success was achieved. Use of cage is an adequate protection measure for the Nile softshell on Belek, Göksu, and Burnaz beaches.

The optimal temperature range for a healthy embryogenesis is between 25 and 33 °C in sea turtles. A high morphological anomaly and low hatchling success can be observed in temperatures outside this range (Miller, 1985). Although sex of the Nile softshell turtle is not determined by the temperature, the clutch temperature is important for a healthy embryo development. High embryonic success in the Nile softshell turtle is between 24 and 33 °C, as in sea turtles (Leshem et al., 1991). The mean clutch temperature was 31.2 °C (with a minimum of 29.2 °C and a maximum of

33.3 °C) on Dalyan Beach (Gidiş and Kaska, 2004). Manually measured clutch temperatures on Dalaman Beach were 28–32 °C (Atatür, 1979). In this study, the clutch temperatures varied between 29.5 and 33.0 °C and the mean temperature was 31.6 °C. According to these results, the Nile softshell turtle clutch temperature in Turkey is in the range where a high embryonic success occurs.

There are many factors that affect the clutch temperature in sea turtles. Metabolic heating caused by developing embryos inside nests is one of these factors (Carr and Hirth, 1961; Mrosovsky and Yntema, 1980). The presence of a few embryos produces less heat, whereas many embryos would produce more heat. Metabolic heating in the green sea turtle increased the mean temperature inside nests by 0.8 °C during the clutch period (Önder and Candan, 2016). In the present study, the relationship between clutch temperature and clutch size may be caused by the effect of metabolic warming.

In conclusion, a new nesting site was discovered on Burnaz Beach for the Nile softshell turtle, a significant relationship between clutch temperature and clutch size was detected, and a detailed data set about the nest parameters was provided. Therefore, this study makes a valuable contribution to the literature and future studies in the reproductive biology of the Nile softshell turtle.

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