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Does Euphrates softshell turtle nest in unfavourable substratum? Description of nests from Euphrates River, Türkiye

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Abstract: Nest site selection differs among species based on a characteristic crucial to the embryo's survival. The identified nest-site characteristics of the Euphrates softshell turtle highlight that these turtles, like their other soft-shelled relatives, highly prefer the sandy substrate, even pure sand, to nest. However, alteration and loss of natural habitats for many decades limited the suitable oviposition sites along the Euphrates River. This study presents the uncharacteristic nest-site choice and the nesting time of Euphrates softshell turtle for the first time. I discovered four nests, as well as six unhatched eggs in a nest on 20 August 2017. This discovery extends the known nesting season-end from early June to at least mid-August for the Euphrates River. The eggs were spherical, and their size was 28.38 (±0.2, 28.1–28.6) mm on average. The nesting substrate, consisting of only 51.8% sand, was substantially dissimilar to what was previously reported. Apart from the soil texture, the nest and its site characteristics concerning nest dimension, clutch size, egg size, and distance to the shoreline concord with what was formerly reported. I conclude that many anthropogenic alterations, such as sand mining and damming and hence the scarcity of suitable oviposition sites, may have caused location and time shift of breeding and forced the turtles to nest in these uncommon soil textures.

Key words: *Rafetus euphraticus*, nest-site characteristics, soil composition, grain size

1. Introduction

Turtles are a diverse group of reptiles that inhabit a variety of aquatic and terrestrial habitats worldwide. All species of turtles are oviparous; they bury their eggs in the ground, and like many other species, turtles require suitable nesting sites for reproduction. Nest site selection is a crucial aspect of a turtle's life history, as it can greatly influence the survival and success of its offspring. The process of nest site selection involves a complex interplay between environmental variables, maternal behaviour, and individual traits of the female. Environmental variables such as temperature, humidity, substrate type, and vegetation cover can influence the suitability of a nesting site (e.g., Refsnider and Janzen, 2010). Among turtles, sex is determined in two different ways: most turtles have temperature-dependent sex determination, whereas soft-shelled and some other species have sex chromosomes that determine the sex of the offspring (Lovich and Gibbons, 2021).

The range of the family Trionychidae is generally confined to the northern hemisphere, including Africa, Asia, and North America. All species of Trionychidae are highly aquatic, leaving the water only to bask or nest. They live in rivers, tributaries, lakes, marshes, and other primarily permanent wetlands. Two soft-shelled turtle species are found in Türkiye: *Trionyx triunguis* (Forskål, 1775) and *Rafetus euphraticus* (Daudin, 1802).

Nesting patterns related to environmental variables have been widely documented for Nile soft-shell turtle *T. triunguis* in comparison with *R. euphraticus*. Türkozan et al. (2006) stated that *T. triunguis* generally preferred to nest on the slopes of the vegetated sand dunes. The nesting season starts in May and ends in July; thereafter, the emergence of hatchlings takes place from July to September (Gidiş and Kaska, 2004). Information about the distance of nests to the shoreline, depth of nests, nest temperature, clutch size, and hatching success were also reported from many populations in Türkiye (e.g., Candan, 2018; Yılmaz et al., 2020).

The genus *Rafetus* includes two endangered species. One of the largest freshwater turtles, Swinhoe's softshell turtle (*Rafetus swinhoei*), is probably the most threatened turtle species in the world (Stanford et al., 2018). *R. swinhoei* is known historically to inhabit China and Vietnam, but the population size currently contains only one wild individual and a single captive male (Stanford

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et al., 2018). The other species, Euphrates softshell turtle (*Rafetus euphraticus*), is a medium-sized freshwater turtle endemic to Euphrates and Tigris rivers and their tributaries in Türkiye, Iraq, Iran, and Syria. Currently, the Euphrates softshell turtle is classified as an "Endangered" species by the IUCN[1]. Populations of Euphrates softshell turtles have been threatened for several decades by continuing fragmentation, alteration, and loss of habitat, especially by the construction of dams throughout its range (Taskavak et al., 2016; Bayrakcı et al., 2021).

Life history traits of riverine turtles occupied large and dynamic floodplain ecosystems and have developed some adaptations which have evolved in response to natural flow regimes dealing with variable streamflow, drought, and indeterminate floods (Moll and Moll, 2004). These characteristics may facilitate their survival rate against natural disturbances such as floods and droughts (Welcomme, 1979; Bunn and Arthington, 2002). In oviparous species, a female's choice about where to lay eggs can have crucial results for her reproductive fitness. For example, the nest site determines the survival of the embryo, the performance of the juvenile, the phenotype of the offspring, and potentially the survival of the breeding female (Refsnider and Janzen, 2010).

The reproductive biology of the Euphrates softshell turtle is poorly known, and knowledge on this issue is based on just a few studies (e.g., Biricik and Turğa, 2011; Ghaffari et al., 2013). This study aims to provide information on nest characteristics and nest site preferences of the Euphrates softshell turtle from the Euphrates River, Türkiye.

2. Materials and methods

2.1. Study area and field surveys

The field surveys were conducted in the Euphrates Basin, Gaziantep, Türkiye (Figure 1). The Euphrates River, which constitutes almost the northernmost border of the distribution of Euphrates softshell turtle, is the largest in southwestern Asia.

I conducted field surveys along the Euphrates River between 2015 and 2017. I focused on potential nesting sites along the river and its tributaries during the breeding season (May–September). I carried out visual surveys of potential nesting areas to look for signs of turtle nesting activity which can include looking for tracks or other signs of disturbance in the sand or vegetation, as well as searching for actual nests or hatchlings. Among the four nests detected, one of them had unhatched eggs (eggs with visible embryos or blood formation), two of them had hatched/predated eggs (eggs with broken shells), while the last one had neither eggs nor eggshells. Considering the average egg diameter, the surface area of the eggshell can be calculated for spherical eggs ($A = 4\pi r^2$). This corresponds to an area of approximately 25 cm^2. Eggshells that filled a 5 cm × 5 cm square area were counted as one egg. Considering that there may be pieces of eggshell that cannot be found/are lost, actual clutch size should be higher than detected. In this case it would be more appropriate to accept the determined number of eggs as "minimum".

The sand was carefully cleared by hand until the egg chamber appeared. The eggs were removed carefully for counting, measuring, and photographing. The distance of the nests to the shoreline and the nest dimensions (width and depth) were measured using a ruler. Egg measurements were taken using a digital calliper with an accuracy of 0.01 mm.

2.2. Soil grain size

One kilogram of soil sample was collected from the closest point that was close enough to represent the nest soil but far enough not to damage the nest chamber. The sample was collected from a depth range of 0–40 cm. Therefore, soil sample was left to dry in the sun for several weeks before grain size analysis. A series of metal sieves (16.8 mm, 2 mm, 1.3 mm, 0.6 mm, 0.2 mm) were used from largest to smallest, with the sand sample placed into the top sieve. The sieves were then placed onto an electronic shaker for 10 min to sort the soil samples into different grain sizes. The soil volume was measured in a graduated cylinder to the nearest 0.5 mL after vibrating it for 10 min on a shaker.

According to the MIT soil classification system developed by Prof. G. Gilboy at Massachusetts Institute of Technology, soils may be classified as clays (particle size less than 0.002 mm), silts (particle size ranging from 0.002 mm to 0.06 mm), sands (particle size ranging from 0.06 mm to 2 mm), gravels (particle size greater than 2 mm), cobbles (particle size between 60 mm to 200 mm), or boulders (particle size greater than 200 mm).

3. Results

Four nests of *R. euphraticus* were discovered in Gaziantep, Kumla, in August 2017 (Figure 1). In the nest site, the main riverbed is approximately 300 m wide and has a strong current in the North-South direction. The location where the nests were found is a filled soil embankment area just next to the main riverbed for transportation purposes, especially for fish farms through seasonal floodplain sites. The filling materials of the embankment were composed of gravel and stones, and there was no sand patch in the vicinity. Apart from the river, on the other side of this embankment, swamps and reed-covered marshy areas formed by floods occurred, and turtles frequently use

[1] Ghaffari H, Taskavak E, Turkozan O, Mobaraki A (2017). *Rafetus euphraticus*. The IUCN Red List of Threatened Species 2017: e.T19070A1956551. Website https://dx.doi.org/10.2305/IUCN.UK.2017-3.RLTS.T19070A1956551.en. [Accessed 27 February 2023].

this habitat. The nesting site is dominated by the giant reed *Arundo donax*, and the other common species of vegetation are *Epilobium* sp., *Mentha* sp., and *Platanus orientalis*.

All four nests were located 50–60 cm above the water level and facing south with a slope of 10–15 degrees. Nests were exposed to direct sunlight except for nest number four which was partly shaded by vegetation. The mean depth of the nests is 20.5 (SD: ± 4.1, min–max: 16–24) cm, and the mean diameter is 24.25 (±3.86, 20–28) cm. The mean distance to the shoreline is 142.5 (±51.9, 80–190) cm. Six unhatched and nine hatched/predated eggs were found in nest number three on 20 August 2017. The eggs were spherical, and their mean size was 28.38 (±0.2, 28.1–28.6) mm. Nest characteristics are given in Table.

The nesting soil of nest sites was composed of 30.7% fine sand, silt and clay (<0.2 mm), 6.4% medium sand (0.2–0.6 mm), 4.9% coarse sand (0.6–1.3 mm), 9.8% very coarse sand (1.3–2 mm), 27% gravel (2–16.8 mm), and 21.2% large gravel (>16.8 mm). According to the MIT soil classification system, 51.8% of the soil sample analysed was classified as "sand".

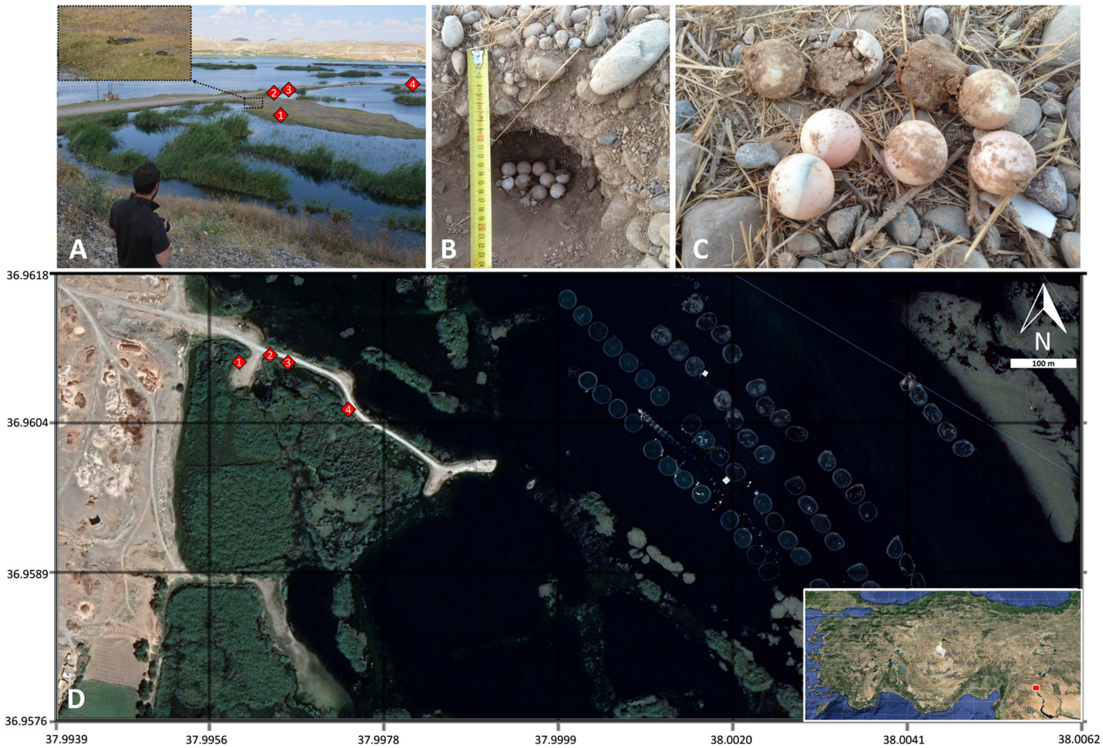

Figure 1. Location of the nests in the study site. Note the soil texture especially in B, C, and D. **A**: Photographical view of the nest site when the water level rose in late June 2015. **B**: Photograph of nest-3, **C**: A closer view of the eggs from nest-3. **D**: Location of the nests in Google Earth view.

Table. Nest characteristics of *R. euphraticus*.

	Nest 1	Nest 2	Nest 3	Nest 4
Clutch size	8 predated	3 predated	9 predated, 6 unhatched	None
Nest chamber size (cm)	22	20	27	28
Nest depth (cm)	18	16	24	24
Distance to shoreline (cm)	190	120	180	80
Vegetative ground cover	None	Scarce	Scarce	Sparse
Sunlight	Exposed	Exposed	Exposed	Partly Shaded

4. Discussion

The reproductive biology of *R. euphraticus* is poorly known, and we have knowledge based on just a few studies up to date. Ghaffari et al. (2013) reported a nest with a clutch size of 37 eggs. Fazaa et al. (2015) found 34 eggs in five nests, but they did not count all the eggs per clutch to avoid disturbing the embryos. The first description of a nest from Türkiye was made by Biricik and Turğa (2011) from the Tigris River, and they found a nest with a clutch size of 32 eggs. I found eggs and eggshells in all nests, except for nest number four. Egg shells may be the residuals of the previous breeding seasons, but six unhatched eggs indicate that the nesting site was being used by turtles actively. Considering the small clutch size and the shifting of the typical breeding season, this clutch is probably the second or subsequent clutch of the same female in a year. My findings are the first description of a nest from the Euphrates River and the second for Türkiye.

The nesting season of Euphrates softshell turtle extends from late April to early June (Ghaffari et al., 2008; Biricik and Turğa, 2011; Ghaffari et al. 2013; Taskavak et al., 2016). Although Taskavak et al. (2016) claimed that the nesting season may extend into the second half of September in southern Türkiye, there is no record of this. The eggs that I found on 20 August 2017 were several days old (M. V. Plummer per. com.), which indicates that the eggs must have been laid in the second half of August. This finding corroborates the extension of the nesting season claimed by Taskavak et al. (2016). This extension may be related to the unstable flow regime of the Euphrates River, indeed in June 2015, I observed that the river level was quite high, and the water almost reached the nesting area (Figure 1B).

Nest site preference of females differs among species based on the sort of attributes that are crucial to the survival of embryos: sunlight and moisture levels are required for incubation; soil texture affects the gas exchange of embryo; and distance from the shoreline and elevation above the water level may determine the fate of the nest against flooding. Nests closer to water are at maximum risk of inundation, on the contrary, eggs that are far from the water face the risk of desiccation (Kamel and Mrosovsky, 2004). For example, when predation pressure on turtles occurs, the females tend to nest closer to the shoreline at the expense of reduced offspring fitness due to the increased inundation risk to the nest (Spencer, 2002; Refsnider and Janzen, 2010).

The identified nest-site characteristics of the Euphrates softshell turtle highlight that these turtles, like their other soft-shelled relatives, highly prefer the sandy substrate. For example, the nesting soil was composed of silt/sand, and the grain size of all soil samples was smaller than 0.6 mm (100% < 0.6 mm) in Iran (Ghaffari et al., 2013) and the soil composition of the nest described from Tigris River was pure sand (98.2% < 0.6 mm; Biricik and Turğa, 2011). In addition, Fazaa et al. (2015) reported that nest site soil is composed of 100% sand, silt, and clay. Similar preferences of sandy substratum by another soft-shelled turtle *Apalone mutica* and *Trionyx triunguis* were reported by other studies (e.g., Plummer, 1976; Riley et al., 2005; Türkozan et al., 2006, Yılmaz et al., 2020). Bayrakcı et al. (2021) pointed out potential nesting sites with similar substratum especially in Saray and Kelekli alongside the Euphrates River. Even supposing that 51.8% of the soil sample analysed was classified as "sand" according to the MIT soil classification system, only 37.1% of the grain size was smaller than 0.6 mm. The soil composition of the nesting site at Euphrates River is far below the reported sand content and points out an unusual nest site preference.

The coarse-grained soil texture of the nest site can reduce the hatching success due to the moisture loss, especially in warmer areas. For example, Mortimer (1990) reported that the mortality rate of *Chelonia mydas* is positively correlated with the mean particle diameter of the sand, suggesting physiological stress from desiccation or difficulties of digging out from the nest in coarser sands. However, Foley et al. (2006) detected no effect of extraordinarily rough and porous sands on hatching success. Nevertheless, it should be considered that these results were based on only the "sand" type of soil texture. We still do not know the hatching success in nests that have coarser grained soil such as gravel grained.

R. euphraticus depends on the riverine habitat and does not prefer to nest or bask far from water. Certain turtle species need a stream flow level below a certain threshold for nesting. Lotic species depend more on changes in hydrologic regime than lentic species that live in ponds or lakes (Lenhart et al., 2013). Artificial regulation of dammed rivers can cause long-term flooding or shrinkage of nesting areas. Lacking an appropriate nesting habitat, the nest-site choice of females may not be the optimal preference for eggs, hatchlings, or themselves (Kolbe and Janzen, 2002; Refsnider and Janzen, 2010; Bárcenas-García et al., 2022).

Regulation of river flow and fragmentation of habitats in connection with dams lead to the loss of river connectivity and freshwater biodiversity (Grill et al., 2019; Harper et al., 2021). For example, Jian et al. (2013) evaluated that planned hydropower dams may permanently flood 73% of the potential nesting site of *R. swinhoei*. According to Taskavak et al. (2016), damming for many years has caused many nesting sites of *R. euphraticus* to be submerged. Besides, they stated that even if suitable habitats are found for nesting in a recently composed dam reservoir environment, water level fluctuations may lead to extremely low survivorship of embryos.

Sandy substrates are essential for softshell turtles to bask and nest; thereby, the destruction of these favorable habitats may be a critical threat to the survival of *R. euphraticus* populations. Bayrakcı et al. (2021) classified the natural system modifications like dam building and sand mining as a significant threat with high scope to Euphrates softshell turtle due to the loss of potential breeding sites. The ongoing pressures like habitat fragmentation, alteration, destruction, and human impact for many decades may force Euphrates softshell turtles to oviposit in unfavorable substratum in anthropogenic areas where recently inhabited. My conclusion is parallel with that of Walde et al. (2007), who indicated that anthropogenic degradation of habitats leads to a decrease in the availability of high-quality nest sites, which could cause turtles to postpone nesting and/or to nest in an unfavourable habitat. Furthermore, when natural nesting habitat is restricted, turtles may nest in anthropogenic sites such as roadsides or agricultural fields, which can negatively affect hatch success (Kolbe and Janzen, 2002; Mui et al., 2016).

Freshwater turtles are particularly vulnerable to the effects of anthropogenic development of terrestrial habitats (Steen et al., 2012). Creating artificial nesting habitats near wetlands for freshwater turtles can be a useful tool in turtle conservation efforts (Buhlmann and Osborn, 2011). Turtles face many threats in the wild, including habitat loss, fragmentation, and degradation, as well as predation, poaching, and accidental mortality. By providing suitable nesting sites, conservationists can help increase turtle populations and ensure the survival of turtles. One potential benefit of creating artificial nesting habitats is that they can be placed in locations that are more protected from predators or human disturbance, such as along the edges of ponds or wetlands. Additionally, artificial nesting habitats can be designed to provide optimal conditions for turtle egg incubation and hatchling emergence, which can increase the survival rates of young turtles. However, it is important to note that creating artificial nesting habitats is not a substitute for protecting and preserving natural turtle habitat. For example, we suggested constructing artificial nesting sites specific to *R. euphraticus* in the report on "conservation action plan" submitted to Ministry of Agriculture and Forestry Directorate of Nature Conservation and National Parks of Türkiye. Although we have determined the characteristics of the artificial nests that have been planned to construct near the potential nesting sites, unfortunately, there is no work in progress up to date. Artificial nesting habitats are most probably more effective if they would be part of a larger conservation strategy that includes habitat protection, management, and restoration.

5. Conclusion

Some nest-site characteristics and nesting ecology of the Euphrates softshell turtle were presented in this study. In contrast with the former studies, the results of present research suggest that the nest-site choice of Euphrates softshell turtles is not limited to the pure "sandy" substratum. Females may prefer to nest in substrates with coarser grain sizes, and the proportion of sand in these substrates is nearly half (51.8%) of what was reported previously. Besides, the egg-laying period may extend to August.

In conclusion, the nest and nest-site characteristics are in concordance with those formerly reported concerning nest dimension, clutch size, egg size, and distance to shoreline, except soil composition. The uncharacteristic nest-site choice and the nesting time of the Euphrates softshell turtle were presented for the first time. I conclude that many anthropogenic alterations, such as sand mining and damming and hence the scarcity of suitable oviposition sites, may have caused location and time shift of breeding and forced the turtles to nest in these uncommon soil textures.

These findings on Euphrates softshell turtle's atypic nest-site choice and nesting time may indicate a wider range of ecological niches than we have known until now. These results should be considered when making projections about future scenarios such as environmental niche modelling.

Further studies would seek answers for new research questions: How likely Euphrates softshell turtle can shift its oviposition-site/time preferences to compensate for environmental changes? What effects do similar types of soil compositions have on hatching success? We do not yet have any information about this issue for Euphrates softshell turtle. However, further studies may reveal successful breeding in this type of soil composition; otherwise, the severity of the threat due to breeding in sites with low hatching success would be demonstrated.

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