Temperature Profiles And Sex Ratio Estimation For Green Turtle (*Chelonia mydas*) Hatchlings On Sugözü Beaches

Sugözü Kumsalları'ndaki Sıcaklık Profilleri ve Yeşil Kaplumbağa (*Chelonia mydas*) Yavrularında Eşey Oranı Tahmini

Research Article

Onur Candan[1*] and Dürdane Kolankaya[2]

[1]Ordu University, Faculty of Arts and Science, Biology Department, General Biology Section, Cumhuriyet Campus, Ordu, Turkey.
[2]Hacettepe University, Faculty of Science, Biology Department, Zoology Section, Beytepe, Ankara, Turkey.

ABSTRACT

In this study, the effect of nest temperature and surrounding sand temperature on sexual differentiation were examined for green turtle (*Chelonia mydas*) hatchlings on Sugözü Beaches (Adana, Turkey) in 2005 nesting season. Nest temperatures were found to be higher than the surrounding sand temperatures, especially during the middle and last third period of incubation (respectively, 0.6°C and 1.9°C). The nest temperature was mostly related to the incubation duration and this was followed by the distance of the nest from the sea and the clutch size. Any relationship between nest temperature and nest depth was not found. Nest temperatures (ranged between 29.4-31.6°C) and middle third temperatures (ranged between 29.5-31.3°C) were measured above 28.9°C, which is the pivotal temperature. The sex ratio in green turtle hatchlings in the area investigated according to the temperature profile was prominently biased toward females.

Key Words
Eastern Mediterranean, incubation, sea turtle, Sugözü Beaches, temperature dependent sex determination

ÖZET

Bu çalışmada, 2005 yuvalama sezonunda Sugözü Kumsalları'ndaki (Adana, Türkiye) yuva ve çevresindeki kumsal sıcaklıklarının yeşil kaplumbağa (*Chelonia mydas*) yavrularındaki eşeysel farklılşmaya olan etkisi incelenmiştir. Yuva sıcaklıkları, özellikle gelişimin orta ve son trimesterinde (sırasıyla 0.6°C ve 1.9°C) yuvayı çevreleyen kumsal sıcaklıklarından yüksek bulunmuştur. Yuva sıcaklıkları en yüksek olarak inkübasyon süresi ile ilişkilidir, bunu yuvanın denizden uzaklığı ve kuluçka büyüklüğü izlemektedir. Yuva sıcaklığı ve yuva derinliği arasında bir ilişki bulunamamıştır. Yuva (29.4-31.6°C arasında) ve orta trimester (29.5-31.3°C arasında) sıcaklıkları 28.9°C olan pivotal sıcaklık derecesinin üzerinde ölçülmüştür. Sıcaklık profillerine gore, çalışma alanındaki yeşil kaplumbağa yavrularındaki cinsiyet oranı belirgin şekilde dişi yönlüdür.

Anahtar Kelimeler
Doğu Akdeniz, inkübasyon, deniz kaplumbağası, Sugözü Kumsalları, sıcaklığa bağlı eşey belirlenmesi

Article History: Received: Oct 8, 2014; Revised: Nov 2, 2014; Accepted: Nov 15, 2014; Available Online: Dec 27, 2014.

Correspondence to: O. Candan, Ordu University, Faculty of Arts and Science, Biology Department General Biology Section, 52200, Cumhuriyet Campuss, Ordu, Turkey.

Tel: +90 452 234 50 10/1660 Fax: +90 452 233 91 49 E-Mail: onurcandan.phd@gmail.com

INTRODUCTION

Temperature-dependent sex determination (TSD), which results in female (ovary) development in high temperatures and male (testicle) development in low temperatures, is a common phenomenon for all sea turtle species [1,2]. The temperature which eggs are exposed to in the middle third of the incubation period is the thermo sensitive period (TSP) that the sex determination occurs [2, 3] and the last third term of incubation is the period wherein metabolic heat prevails [4,5].

Sex can be easily identified in adult sea turtles due to the sexual dimorphism, whereas it is difficult in juveniles (shorter than 30 cm curved carapace length) by gonadal morphology [6] and hatchlings to differentiate sex by morphologic examination [7].

Various invasive and non-invasive methods are used for sex determination in hatchlings. But the most accurate way is the histological examination [8,9]. On the other hand, when considering nesting beaches and the nest numbers, non-invasive methods which provide more accurate estimates should be developed instead of histological examination of the gonads [7].

Thermal data such as temperature profiles and their relations with each other should be obtained in order to apply this kind of methods linked to more exact estimates [10]. This results from the fact the temperature in the nest, that determines sex is directly affected by the sand temperature and has often a higher temperature value than the surrounding environment [5,11].

In this study, the relationship between nest temperatures, which is considered a critical and important environmental factor for green turtles, together with air and sand temperatures, were investigated. In addition, relationships between nest temperature values and sex ratio estimations, and four different nest parameters (distance from sea level, incubation duration, clutch size and nest depth) were evaluated.

MATERIALS AND METHODS

Study Area

The study was conducted in 2005 nesting season between June and September, on Sugözü (36°48.677'N-35°51.068'E, 36°52.795'N -35°56.017'E), which is a nesting beach for green turtle (*Chelonia mydas*) in the Mediterranean region. The study area consists of four subsections, respectively extending from west to east; Akkum (36°48.677'N-35°51.068'E,36°49.036'N-35°51.868'E), Sugözü (36°50.228'N-35°53.187'E, 36°50.352'N-35°53.802'E), Botaş (36°52.589'N-35°55.366'E,36°52.704'N-35°55.711'E) and Hollanda (36°52.737'N-35°55.778'E, 36°52.795'N-35°56.017'E).

Temperature Data

Air temperatures were obtained from the Turkish State Meteorological Service (TSMS). The average daily temperature of Yumurtalık-Adana (Station no.17979) and Dörtyol-Hatay (Station no.17962) stations were assessed in line with considering the location of the study area.

Sand temperatures were taken from 50 cm depth by means of a temperature data-loggers (Gemini Data Loggers-Tinytalk H -30°C/+50°C Part No: TK-0040) by 2-hours intervals. The mean value of the 12 measurements taken in one day was treated as daily sand temperature.

Temperature data loggers used in sand temperature measurement were also used for nest temperature measurements. The loggers were placed into the centre of nests during the nesting activity. The average value of 24 measurements taken by 1-hour intervals for each day was treated as daily nest temperature.

Nest Parameters

Relationships between temperature values taken during the incubation period and the four nest parameters (incubation duration, nest depth, clutch size and distance from sea level) were statistically evaluated (95% CI, Minitab® Statistical Software v.14). These parameters are; the time between the daily nesting was laid out and the first day hatchling emerged (day) as incubation duration; distance from the surface of the sand

Table 1. Air, Sand and Nest Temperatures with Estimated Sex Ratios.

Nest No	Mean Air Temperature (°C)		Mean Sand Temperature (°C)		Mean Nest Temperature (°C)		Sex ratio (% female)	
	whole period	middle third	whole period	middle third	whole period	middle third	Via whole period RE	Via middle third RE
1	27.4	27.6	29.6	29.8	30.8	30.7	83.1	76.4
2	28.0	28.4	30.3	30.5	31.2	31.2	91.8	86.5
3	27.6	27.9	29.5	29.8	31.1	31.3	89.2	87.4
4	28.0	28.2	30.2	30.2	29.9	29.7	66.3	57.9
5	27.7	28.4	30.1	30.5	30.2	30.4	71.8	71.2
6	27.5	28.4	30.0	30.5	30.7	30.8	82.8	78.8
7	27.4	27.6	29.1	29.4	29.4	29.5	57.5	54.3
8	27.7	27.9	29.4	29.6	30.5	30.5	79.2	72.4
9	28.1	28.3	29.8	29.9	31.1	30.6	90.0	75.5
10	28.0	28.3	29.8	30.1	31.6	31.0	98.9	82.0

to the bottom of the nest (cm) as nest depth; number of eggs in a nest (number) as clutch size; the distance from the high tide line to the nest (m) as distance from the sea level.

Sex Ratio Estimation of Hatchlings

Average nest temperature values were evaluated as male or female biased depending on the pivotal temperature (28.9°C), which is suggested for the beaches in Turkey by Kaska et al (1998). Regression equation (RE) figured in the same study was used for sex ratio estimation. For green turtle, the equations used were; "Sex ratio (% female) = -492 + 18.7*temperature" and "Sex ratio (% female) = -482 + 18.2*temperature" according to the whole incubation temperature and to middle third, respectively.

RESULTS

Air, Sand and Nest Temperatures

Sand and nest temperatures measured during the study and air temperatures obtained from TSMS were figured in Table 1. In June and July, temperatures of 10 nests laid in the beach and temperatures measured separately in subsections hosting the nests, were compared by taking into regard the air temperature of the area. As a result of this comparison, the sand temperature was found to be higher than the air temperature, and similarly the nest temperature was found to be higher than its surrounding sand.

The regression analysis performed to assess the relationship between nest temperatures and air and sand temperature showed to serve that that no relation between nest and air temperature ($N=10$ $R^2=0.18$ $p<0.5$), and between nest and sand temperature ($N=10$ $R^2=0.07$ $p<0.5$) did exist.

Temperature differences of the nests from surrounding sand in their first, middle and last third were increased and respectively found as 0°C, 0.6°C and 1.9°C (Table 2).

Nest Parameters and Nest Temperatures

Since one nest among the 10 measured nests was predated, only its distance from the sea level was measured. The data such as incubation duration, nest depth and clutch size could not be obtained. The nest temperatures measured in the Sugözü Beaches ranged between 29.4-31.6°C and temperatures measured within the TSP were measured as 29.5°C to 31.5°C. According to the data related to the nearly 10 nests figured in Table 3, it can be seen that there is not any relation between the nest temperature and the nest depth ($N=9$ $R^2=0.01$ $p<1$), and that there is not a strong correlation between the distance from the sea and the clutch size ($N=9$ $R^2=0.31$ $p<0.2$).

Considering the relationship between the last parameter and the nest temperature, we reached a conclusion that the incubation duration decreases by increasing temperature ($N=9$

Table 2. Nest - Sand Temperature Difference (°C).

Nest No	Whole Period			First Third			Middle Third			Last Third		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
1	0.0	2.7	1.2	0.0	0.5	0.3	0.4	1.5	0.9	1.6	2.7	2.2
2	0.0	2.0	0.9	0.0	0.4	0.1	0.1	1.5	0.8	1.2	2.0	1.7
3	0.8	2.0	1.5	0.8	1.7	1.3	1.3	1.6	1.5	1.5	2.0	1.7
4	-1.4	1.1	-0.3	-1.4	-1.1	-1.2	-1.1	0.1	-0.5	-0.1	1.1	0.7
5	-0.4	0.4	0.0	-0.4	0.1	0.0	-0.4	0.0	0.1	0.0	0.4	0.2
6	-0.7	2.4	0.7	-0.7	2.4	-0.4	-0.4	1.6	0.3	-0.1	2.4	1.8
7	-0.8	1.6	0.2	-0.8	-0.4	-0.6	-0.4	0.8	0.1	0.8	1.6	1.3
8	0.2	2.8	1.1	0.2	0.6	0.4	0.3	1.9	0.9	0.4	2.8	2.1
9	-0.5	4.5	1.4	-0.5	0.0	-0.3	0.0	2.1	0.7	2.2	4.5	3.5
10	0.4	4.1	1.8	0.4	0.7	0.6	0.4	1.8	0.9	2.0	4.1	3.5
Overall	-0.2	2.4	0.9	-0.2	0.5	0.0	0.0	1.3	0.6	1.0	2.4	1.9

Pearson=-0.743 P=0.022) and a correlation exists between the nest temperature and the incubation duration (*N=9* R^2*=0.55 p<0.03*).

Nest Temperature and Sex Ratio Estimation

Temperature measurements taken from a total of 10 green turtle nests in the 2005 nesting season (Table 1) were separately assessed as the whole incubation period and the middle third of incubation which involved TSP. Since these temperature values are above the pivotal temperature (28.9°C), the hatchlings emerged from these nests are biased toward females (Figure 1).

It is concluded that when the average temperature taken during whole incubation period was assessed depending on the RE, female ratio is calculated as 81.1%. However, this value was 74.2%, if average temperature was taken in the middle third of incubation which involved the TSP.

DISCUSSION

In this study, conducted on 10 nests in 2005 nesting season, any relation between nest temperatures and air and sand temperatures was not found. Shadow effect on the nests, seasonal climatic factors and particle structure of the sand could be considered among the reasons of this fact. This reality arises from the fact that the sand texture can affect hatchling success, nest

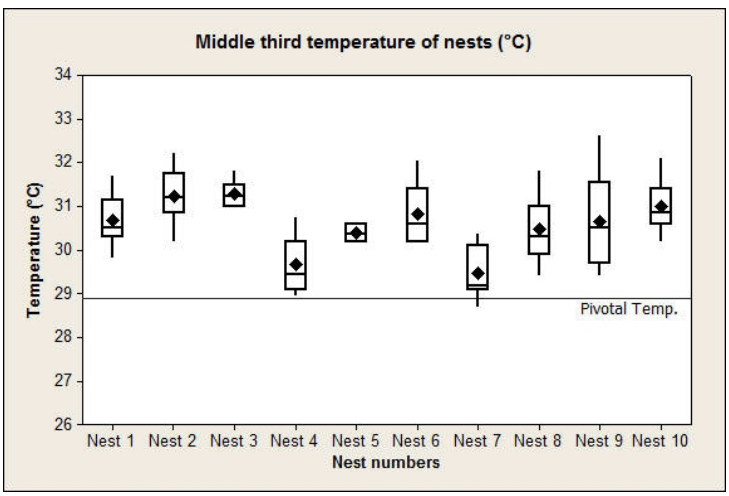

Figure 1. Middle third temperature (°C) of nests

Table 3. Nest Temperature and Some Nest Parameters.

Nest No	Mean nest temperature		Distance from sea level (m)	Incubation duration (day)	Clutch size	Nest depth (cm)
	Whole period (°C)	Middle third (°C)				
1	30.8	30.7	18.0	51	95	47
2	31.2	31.2	23.1	51	129	79
3	31.1	31.3	40.9	51	116	85
4	29.9	29.7	83.8	54	99	77
5	30.2	30.2	73.8	51	62	81
6	30.7	30.8	72.7	53	165	78
7	29.4	29.5	20.3	*	*	*
8	30.5	30.5	22.6	55	139	92
9	31.1	30.6	21.0	51	156	88
10	31.6	31.0	25.8	47	146	86

temperature, and therefore sex inversions [12].

On the other hand, the temperature of sand was found to be higher than the air temperature, and the temperature of nests was found to be higher than the sand temperature [5,11,13]. It has been reported that the nest temperature is 2-4 °C higher than the sand temperature particularly after the half of the incubation period [14]. The nest-sand temperature difference has also been reported in some green turtle studies as to be around 1°C [15-16]. In this study, similar to literature, temperature differences of the nests surrounded with sand had been increased during the first third period up to last third period of incubation, and the mean difference was found as 0.9°C during the whole incubation period.

The nest temperatures of whole incubation duration measured in the Sugözü Beaches were close to the range of temperatures measured within the TSP and both were higher than pivotal temperature. It was clear that the sex ratio of the hatchlings were female biased in the estimation made by using pivotal temperature in a very simple way and by just considering the nest temperatures.

It is known that incubation duration decreases when the temperature increases [2]. Besides, when considering the contribution of metabolic heating to the nest temperature, the more the number of eggs in the nest, the higher will be the metabolic heating produced by the embryos [5, 17]. Similar to these findings, the temperature difference between the last third of incubation and sand temperature was found to be 1.9 °C.

Among the parameters used, a relation between nest depth and nest temperature was found, and this result is quite similar with that of Booth and Freeman (2006) [14]. However, similar to the fact that the nest temperature decreases as approaching to the sea [17], there exits a correlation between the nest temperature and the distance from the sea. But, low correlation has been thought to be resulted from small number of sampling nests.

Depending on the nest temperatures measured, by reference to the Kaska et al., (1998) the sex ratios were found to be female biased [18] Thus, these values are in concordance with those estimated with those around the Mediterranean [15,17-21].

To determine the sex ratios of the hatchlings, after taking gonad samples from all of the nests and measuring all nest temperatures is really quite difficult. However, according to the results of this study, the clutch size and the distance from the sea should also be taken into consideration for sex ratio estimation dealing with the nest temperature and incubation duration. Using the parameters of the other nests in accordance with the data obtained from the sampled nests can allow more accurate estimations of sex ratio instead of measuring the temperature of only a

certain number of nests. By this means, a wider range of information about seasonal sex ratios can be obtained and new conservation strategies about sea turtles that have been faced with global warming risk can be developed.

ACKNOWLEDGMENTS

The data of this study have been excerpted from the 2006-YL-3997 coded postgraduate thesis of the author, which is as part of the Sea Turtle Expedition Project that is financially supported by the Baku–Tbilisi–Ceyhan Crude Oil Pipeline Company-Environmental Investment Program (BTC Co.-EIP). We would like to express our thanks to the supporting firm BTC Co. and the project coordinator, Ecological Research Society (EKAD). And also we thank to BOTAŞ Petroleum Pipeline Co. Provincial Management of Petroleum Operations and to BOTAŞ International Limited (BIL) for all their contribution and support.

References

1. J.J. Bull, Sex Determination in Reptiles. Q Rev Biol, (1980) 3.
2. C.L. Yntema, N. Mrosovsky, Sexual-Differentiation in Hatchling Loggerheads (Caretta-Caretta) Incubated at Different Controlled Temperatures. Herpetologica, 36,(1980) 33.
3. N. Mrosovsky, C. Pieau, Transitional range of temperature, pivotal temperatures and thermosensitive stages for sex determination in reptiles. Amphibia-Reptilia, 12 (1991) 169.
4. N. Mrosovsky, C.L. Yntema, Temperature-Dependence of Sexual-Differentiation in Sea Turtles - Implications for Conservation Practices. Biol Conserv 18, (1980) 271.
5. A.C. Broderick, B.J. Godley and G.C. Hays, Metabolic heating and the prediction of sex ratios for green turtles (Chelonia mydas). Physiol Biochem Zool, 74 (2001) 161.
6. B. Lazar, G. Lackovic, P. Casale, D. Freggi, N. Tvrtkovic, Histological validation of gonad gross morphology to sex juvenile loggerhead sea turtles (Caretta caretta). Herpetol J, 18 (2008) 137.
7. S.A. Ceriani and J. Wyneken, Comparative morphology and sex identification of the reproductive system in formalin-preserved sea turtle specimens. Zoology, 111 (2008) 179.
8. N. Mrosovsky, M. Benabib, An Assessment of 2 Methods of Sexing Hatchling Sea-Turtles. Copeia, 2 (1990) 589.
9. N. Mrosovsky, S.J. Kamel, C.E. Diez, R.P. van Dam, Methods of estimating natural sex ratios of sea turtles from incubation temperatures and laboratory data. Endanger Species Res, 8 (2009) 147.
10. E.A. Standora, J.R. Spotila, Temperature-Dependent Sex Determination in Sea Turtles. Copeia 3, (1985) 711.
11. N.T. Booth and K. Astill, Temperature variation within and between nests of the green sea turtle, Chelonia mydas (Chelonia :Cheloniidae) on Heron Island, Great Barrier Reef. Aust J Zool, 49 (2001) 71.
12. S.L. Milton, A.A. Schulman, P.L. Lutz, The effect of beach nourishment with aragonite versus silicate sand on beach temperature and loggerhead sea turtle nesting success. J Coastal Res, 13 (1997) 904.
13. B.J. Godley, A.C. Broderick, F. Glen and G.C. Hays, Temperature-dependent sex determination of Ascension Island green turtles. Mar Ecol Prog Ser, 226,(2002) 115.
14. D. T. Booth, C. Freeman, Sand and nest temperatures and an estimate of hatchling sex ratio from the Heron Island green turtle (Chelonia mydas) rookery, Southern Great Barrier Reef. Coral Reefs, 25 (2006) 629.
15. P. Casale, G. Gerosa and S.V. Yerli, Female-biased Primary Sex Ratio of the Green Turtle, Chelonia mydas, Estimated Through Sand Temperatures at Akyatan, Turkey. Zool Middle East, 20 (2000) 33.
16. M.H. Godfrey, Sex ratios of sea turtle hatchlings: direct and indirect estimates. Ph.D. Thesis, University of Toronto, Ottawa, 1997.
17. A.H. Ucar, Y. Kaska, S. Ergene, C. Aymak, Y. Kacar, A. Kaska, P. Ili, Sex Ratio Estimation of the Most Eastern Main Loggerhead Sea Turtle Nesting Site: Anamur Beach, Mersin, Turkey. Isr J Ecol Evol, 58 (2012) 87.
18. Y. Kaska, R. Downie, R. Tippett, R.W. Furness, Natural temperature regimes for loggerhead and green turtle nests in the eastern Mediterranean. Can J Zool, 76 (1998) 723.
19. A.C. Broderick, B.J. Godley, S. Reece and J.R. Downie, Incubation periods and sex ratios of green turtles: highly female biased hatchling production in the eastern Mediterranean. Mar Ecol Prog Ser, 202 (2000) 273.
20. M. Oz, A. Erdogan, Y. Kaska, S. Dusen, A. Aslan, H. Sert, M. Yavuz, M.R. Tunc, Nest temperatures and sex-ratio estimates of loggerhead turtles at Patara beach on the southwestern coast of Turkey. Can J Zool, 82 (2004) 94.
21. Y. Kaska, C. Ilgaz, A. Ozdemir, E. Baskale, O. Turkozan, I. Baran, M. Stachowitsch, Sex ratio estimations of loggerhead sea turtle hatchlings by histological examination and nest temperatures at Fethiye beach, Turkey. Naturwissenschaften, 93 (2006) 338.