ISSN: 2538-2152; ECOPERSIA. 2018;6(4):235-240

Temperature-Based Prediction of Sex Ratio in Hatchlings Green Sea Turtle (Chelonia mydas)

ARTICLE INFO

Article Type
Original Research

Authors
Sinaei M.*[1] *PhD*,
Bolouki M.[2] *PhD*,
Mirshekar D.[3] *PhD*

How to cite this article
Sinaei M, Bolouki M, Mirshekar D. Temperature-Based Prediction of Sex Ratio in Hatchlings Green Sea Turtle (Chelonia mydas). ECOPERSIA. 2018;6(4):235-240.

[1]Fisheries Department, Chabahar Branch, Islamic Azad University, Chabahar, Iran
[2]Marine Scince Department, Marine Science Faculty, Marine Science & Technology University of Khoramshahr, Khoramshahr, Iran
[3]Marine Scince Department, Marine Science & Technology Faculty, Science & Research Branch, Islamic Azad University, Tehran, Iran

***Correspondence**
Address: Fisheries Department, Chabahar Branch, Islamic Azad University, Chabahar, Iran. Postal Code: 9971774615
Phone: +98 (54) 35314308
Fax: +98 (54) 35314308
oceanography.sina@gmail.com

Article History
Received: April 15, 2018
Accepted: January 12, 2018
ePublished: September 11, 2018

ABSTRACT

Aims It has been shown that sea turtles have temperature-dependent sex determination. Therefore, their sex determination is useful in understanding their reproduction ecology and population status. The aims of the present study were to estimate the sex ratio and to study the effect of inundation on the sex ratio of the hatchling green sea turtle (*Chelonia mydas*).
Materials & Methods This experimental study was carried out on the 300km of Chabahar Beach on the northern coast of the Sea of Oman in July to December, 2015. Five areas which have the highest densities of nesting green sea turtles were chosen. The temperature of three different depths of green sea turtle clutches laid (50cm; above the egg hole, 85cm; center of egg hole and 120cm; below the egg hole) were recorded using automated intra-nest recording devices. Linear Regression Analysis and Pearson correlation coefficient were used. Statistical analyses of the data were conducted by SPSS 20 and Microsoft Office Excel 2010.
Findings The statistical mean temperature in thermosensitive period (TSP) of the nests at three depths of 50cm, 85cm, and 120cm at the chabahar beaches were recorded between 26.1±1.1 to 30.6±1.0. The storm had decreased the mean temperature in thermosensitive period of the nests.
Conclusion The storm decreases the mean temperature in thermosensitive period of the nests. The Nilofar storm stops the increasing feminization. It can be an important step in the implementation of conservation, rehabilitation, and reconstruction programmers.

Keywords *Chelonia Mydas*; Sea Of Oman; Sex Ratio; Feminization; Male-Biased

CITATION LINKS

[1] Past, Current and future thermal profiles of green turtle nesting grounds: Implications ... [2] Long-term conservation efforts contribute to positive Green Turtle Chelonia mydus nesting trend ... [3] Incubation temperature, energy expenditure and hatchling size in the Green Turtle (*Chelonia mydas*), a species with ... [4] Metals in blood and eggs of green sea turtles (Chelonia mydas) from Nesting Colonies of the ... [5] Nesting ecology and reproductive biology of the Hawksbill Turtle, Eretmochelys ... [6] The Olive Ridley Turtle, Lepidochelys olivacea, in the Persian Gulf: A review of the ... [7] Metals in blood and eggs of green sea turtles (Chelonia mydas) from nesting colonies of the northern ... [8] Identification and prioritizing important nesting sites of Green Turtle in Iranian beaches ... [9] Research and management techniques for the conservation ... [10] Blood values in free-ranging nesting Leatherback Sea Turtles (Dermochelys coriacea) on the coast of the ... [11] Maternal health status correlates with nest success of Leatherback Sea Turtles ... [12] The effects of nest environment on calcium mobilization by leatherback turtle embryos ... [13] Metabolic heating and the prediction of sex ratios for Green Turtles ... [14] The effects of sand temperature on pre-emergent Green Sea ... [15] Incubation temperatures and metabolic heating of relocated and in situ Loggerhead Sea Turtle (Caretta caretta) nests at a ... [16] Natural temperature regimes for Loggerhead and Green Turtle nests in the eastern ... [17] Incubation temperature, energy expenditure and hatchling size in the Green Turtle (Chelonia mydas), a species with ... [18] Swimming performance of hatchling green turtles is affected by ... [19] Sex ratios of two species of sea turtle nesting ... [20] Temperature dependent sex determination ... [21] Temperature dependent sex determination in the Green Turtle (Chelonia mydas): Effects on the ... [22] Estimating past and present sex ratios ... [23] Thermal tolerances and the timing of sea turtle ... [24] Pivotal temperature for Green Sea Turtle, Chelonia mydas, nesting ... [25] Endangered species: Pan-Atlantic Leatherback ... [26] Seasonal fluctuations in sand temperature ... [27] Effect of tidal overwash on the embryonic development of Leatherback Turtles ... [28] Effects of sand characteristics and inundation on the hatching success of Loggerhead Sea Turtle ...

Introduction

Climate change and global warming have the greatest effect on cold-blooded creatures, which, sea turtles are more vulnerable and sensitive because of an intensive dependence of egg-laying and hatching on temperature. Increasing temperatures cause an increase in the mortality and genetic defects in newborns. Ecological models show that climate change leads to rising temperatures on egg-laying beaches [1]. Considering the global warming trend and the effect of temperature on the sex of sea turtles, more feminization in turtle population will be observed in different areas in the future; this, in turn, will threaten their population and intensify the extinction process.

Sea turtles are migratory animals and can be encountered in most coastal areas with an average ambient temperature of about 20°C. Green sea turtles (*Chelonia mydas*) are widely distributed in the tropical and subtropical waters, and can be found near the continental coast and around the islands. The green sea turtle in the water temperature above 25°C is more abundant than the other species of sea turtles. Sex determination of the sea turtles is useful in understanding their reproduction ecology and behavior, as well as their population status, which greatly helps to implement the conservation and rehabilitation programs. Sex determination of the newborn sea turtles is not possible by examining their morphology due to the lack of external organs. A reliable solution for determining the gender is the analysis of the newborns' gonadal tissue, but this requires killing them; hence, it is not appropriate in terms of protection. There are several non-lethal methods to determine the sex, for example, estimation of the blood estrogen and progesterone levels, and the study of the chorioallantoic membrane by radioimmunoassay methods [2]. However, the gender prediction of the newborn turtles based on the temperature of the nesting habitat and the incubation period are commonly accepted [3]. The green sea turtle (*C. mydas*) in the Iranian coast of the Sea of Oman is facing low hatching success [4-8]. However, there is a lack of information on nesting ecology and also the effects of climate change on the sea turtle in this area. This is the first study to investigate the incubation temperature and the sex ratio of the regionally important green sea turtle (*C. mydas*) along the Iranian coast of the Sea of Oman. The aims of the present study were to estimate the sex ratio and to study the effect of inundation on the sex ratio of the hatchling green sea turtle.

Materials and Methods

This experimental study was carried out on the 300km of Chabahar Beach on the northern coast of the Sea of Oman in July to December, 2015. Five areas which have the highest densities of nesting green sea turtles, based on the earlier information available in the literature [8], were chosen (Figure 1).

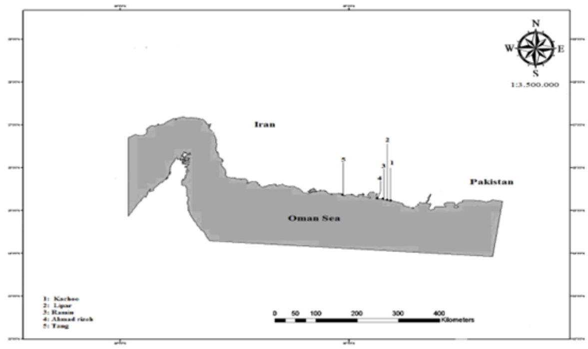

Figure 1) Location of all sampling sites

Monitoring program: The nesting was monitored using nightly foot patrols by two teams. Each team consisted of at least three trained observers that gathered the data according to the standard protocols of Eckert *et al.* [9]. Locations of the nests were recorded with Garmin Oregon 600 GPS (Garmin; USA). A wooden box was placed around each nest to prevent predators.

Biometric information: A complete visual examination of the female breeders was performed (n=18) and curved carapace length (CCL) was measured. Health status of turtles was rated based on nest-building behavior and general body condition [10-12].

Measuring the temperature of the nest: The temperature of the nests (Sand and egg clutch) were recorded with mini temperature data loggers model Swiss Quality MSR145 (Swiss; Switzerland) with a resolution of 0.1°C and an accuracy of 0.4°C. The temperature loggers, coated in three layers of plastic tool dip (Performix 11209 Plasti Dip Clear Multi-Purpose Rubber Coating Aerosol, 11 oz.), were calibrated according to the recommended procedures [13]. The temperature was recorded between 14:30 to 16:00hrs [14] from three depths: Above, center, and below the egg hole, at 50, 85, and 120cm, respectively, as the female green sea turtles oviposited. The temperature loggers were retrieved three days after hatchling emergence.

Linear Regression Analysis and Pearson correlation coefficient were used to analyze temperature of the nest and some ecological factors, respectively. Statistical analyses of the data were conducted by SPSS 20. Additionally, Microsoft Office Excel 2010 was applied to draw the diagrams and to estimate the linear regression coefficient between the temperature of the nest and some ecological factors. The p Values less than 0.01 had been considered significant.

Findings

The statistical mean temperature in thermosensitive period (TSP) of the nests at three depths of 50cm (A), 85cm (B), and 120cm (C) at the chabahar beaches were recorded between 26.1±1.1 to 30.6±1.0 (Tables 1 and 2).

Table 1) Characteristics of green sea turtle (*C. mydas*) nests monitored with temperature data loggers

Beach name/Nest Tag	Lay date	Emergence date	Hatching success (%)
Lipar			
L_2	13 July 2015	10 September 2015	37.2
L_5	22 August 2015	19 October 2015	44.3
Kacho			
L_{12}	20 September 2015	25 November 2015	30.3
K_5	19 October 2015	23 December 2015	32.1
K_2	3 August 2015	3 October 2015	37.8
K_4	10 September 2015	16 November 2015	34.3
Tang			
T_2	4 August 2015	27 September 2015	37.3
T_3	1 September 2015	4 November 2015	27.6

Table 2) Statistical mean incubation temperatures of green sea turtle (*C.Mydas*) nests monitored with temperature data Loggers

Nest tag/ Depth	July	August	September	October	November	December	TSP*
L2							
A	33.3±1.1	29.2±1.2	28.9±1.2	-	-	-	
B	32.9±1.2	30.8±1.2	30.5±1.1	-	-	-	30.6±1.0
C	30.8±1.2	28.6±1.1	27.8±1.2	-	-	-	
L5							
A	-	29.2±1.2	29.2±1.1	27.8±1.2	-	-	
B	-	31.4±1.1	30.7±1.2	29.3±1.3	-	-	29.5±1.1
C	-	28.9±1.1	28.0±1.2	26.8±1.3	-	-	
L12							
A	-	-	28.8±1.1	27.2±1.2	24.9±1.1	25.5±1.2	
B	-	-	29.8±1.1	28.2±1.0	26.9±1.1	26.5±1.0	26.1±1.1
C	-	-	27.9±1.1	26.2±1.1	26.1±1.0	26.0±1.1	
K5							
A	-	-	-	26.8±1.1	24.6±1.9	24.9±1.1	
B	-	-	-	28.4±1.1	27.1±1.0	26.8±1.0	26.5±1.1
C	-	-	-	26.1±1.1	26.0±1.0	26.0±1.1	
K2							
A	-	29.1±1.0	28.7±1.1	26.5±1.0	-	-	30.1±1.1
B	-	30.7±1.0	30.03±1.0	29.1±1.1	-	-	
C	-	28.08±1.1	28.6±1.1	26.0±1.1	-	-	
K4							
A	-	-	28.8±1.0	27.2±1.0	25.2±1.1	-	
B	-	-	29.8±1.1	28.2±1.0	26.2±1.1	-	26.4±1.0
C	-	-	27.8±1.0	26.1±1.0	26.0±1.1	-	
T2							
A	-	29.8±1.0	29.2±1.0	27.8±1.1	-	-	
B	-	31.3±1.2	30.7±1.1	29.3±1.0	-	-	30.3±1.0
C	-	29.2±1.0	28.5±1.1	26.9±1.0	-	-	
T3							
A	-	-	30.0±1.1	29.4±1.0	27.9±1.0	-	
B	-	-	31.1±1.1	30.5±1.1	29.0±1.2	-	30.4±1.0
C	-	-	29.3±1.1	28.7±1.0	27.0±1.1	-	

*Thermosensitive period; A: above the egg hole (50cm); B: Center of the egg hole (85cm); C: Below the egg hole (120cm); Statistics are given for the entire incubation duration of the clutch and for the thermosensitive period, the middle third of incubation when hatchling sex is determined

There was a significant correlation between the incubation period with temperature of the nest and a non-significant correlation between the distance of the latest high tide water and temperature of the nest. In addition, the correlation between the number of eggs and the temperature of the egg hole in different coasts was not significant (Table 3).

Diagram 1 shows the average temperature of the nests during the sex determination periods. In order to predict the sex ratio at different sites, the obtained average temperature was extended to all the nests (Diagram 2).

Table 3) Pearson rank correlation between temperature of the nest and some ecological factors

Ecological factors	1	2	3
1- Temperature of the nest			
2- Incubation period	0.766		
3- Distance with the latest high tide water	0.173	0.676	
4- Number of eggs	0.388	-0.156	0.156

R.values≥0.46 were significant (p<0.01)

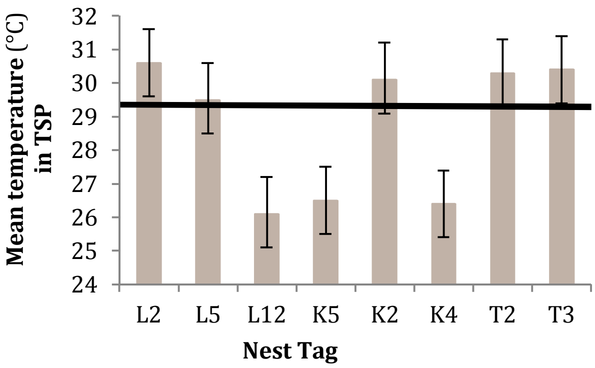

Diagram 1) Statistical mean of the temperatures during the middle third of incubation of green sea turtle (*C. mydas*) clutches; The horizontal line shows the nearest reported pivotal temperature (29.2°C) for green sea turtle

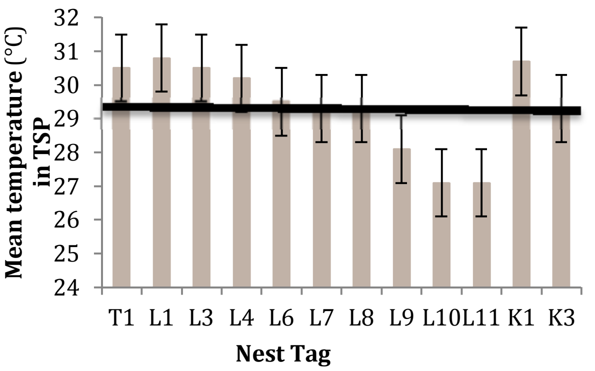

Diagram 2) Extended average temperatures to all the nests during the middle third of incubation of green sea turtle (*C. mydas*) clutches in different beach; The horizontal line shows the nearest reported pivotal temperature (29.2°C) for green sea turtle.

Discussion

The aims of the present study were to estimate the sex ratio and to study the effect of inundation on the sex ratio of the hatchling green sea turtle.

The eggs and the newborn of sea turtles live in a temperature range called transitional range of temperature (TRT) that can vary among different populations. Both 100% of male and 100% of female sex ratios are changed in this range. In general, a change of 3°C in the TRT can lead to the sea turtles producing either all female or all male newborns. Within this range, there is a temperature called the pivotal value (PV), at which the sex ratio of 1:1 is created. The type of beach, season, weather conditions, rainfall or storms, changes in the physical condition of the nest, human intervention, species, and even the intra-specific differences are several factors affecting the PV [15]. Several studies on different species of sea turtles have been done to determine the PV, most of which had reported it as a temperature between 29-30°C [14, 16, 17]. In this study, the PV temperature has been considered as 29.2°C according to previously published information regarding pivotal temperatures in this species [16, 18-24]. Clutches incubated below this temperature produce predominantly male hatchlings and clutches incubated above this temperature produce predominantly female hatchlings [15].

In the present study, the temperature of the egg hole was studied to predict the sex of newborns in different sites, the result of which showed no significant difference between the temperature at the three parts of above, center, and below the egg hole. The results also showed that ecological factor such as distance of the nest to the latest high tide and number of eggs had not effect on the mean temperature in TSP. It seems that the area around the egg hole acts as a buffer and prevents large fluctuations in temperature that modifies or amends the newborn's sex. Hays *et al.* [25] and Kaska *et al.* [16] also recorded low temperature fluctuations in the egg holes of the green sea turtles (*C. mydas*). Booth and Astill [17] and Matsuzawa *et al.* [26] stated that temperature of the egg hole is higher than its surroundings by about 1°C to 1.7°C; this increase in temperature is due to the metabolic activity of the egg and the embryos. A similar process appeared in the present study.

One of the factors affecting the hatchability of sea turtle eggs is the storm. During the egg-laying season, the Nilofar storm leads to a reduction in the hatching percentage of some nests. Inundation leads to sharp fluctuations in the temperature as well as its high reduction, which causes the destruction of eggs and a reduction of the hatching rate. The failure of development during inundation may be practically explained by the fluctuations of the three most important physical variables affecting the survival of sea turtle embryos: Water content, temperature, and respiratory gases [27]. Temperature is the most important factor in the development of the turtle embryo; its reduction to a lower value than normal or the optimal value can stop the development of the embryo and cause death [28]. Mortality during overwash greatly depends on the number and duration of overwash [27]. However, overwashed nests in the present study showed relatively high hatching rates, which suggests that sea turtle nests have a specific tolerance to immersion and can survive for a substantial period of time in saltwater [27, 28].

An interesting observation in this study was that in the nest where the sex determination period took place before the Nilofar storm, male-bias had occurred; meanwhile, the nest where the sex determination period took place after the Nilofar storm had also tended towards male-biased sex. This suggests that the tidal overwash caused the nests to incubate below the pivotal temperature, which meant that none of these nests were 100% female or female-biased.

These nests presumably experienced cooler incubation temperatures and, thus, may have created greater proportions of males [27]. This study only shows data from one nesting season; hatchling production can change from year to year, depending on meteorological factors such as rainfall and temperatures [27]. Future and long-term investigations into sex ratio production in this respect would be beneficial. In another part of this study, the results of the temperature in the nests were extended for other nests in different sites.

The study of the effects of global warming on the gender of newborn sea turtles can be an important step for the implementation of conservation, rehabilitation, and reconstruction programs. Studies show that with increase in temperature, the sex ratio in most beaches tends toward feminization. From this information, the green sea turtle (*C. mydas*) population in the Iranian coasts of the Sea of Oman would be expected to produce a higher proportion of females. However, the results of this study suggest that the Nilofar storm stopped the increasing feminization, which can be useful in this respect. Assuming that these female and male biases are present in this population, plans for conservation management must take these results into account.

Conclusion

The storm decreases the mean temperature in the thermosensitive period of the nests. The Nilofar storm stops the increasing feminization. It can be an important step in the implementation of conservation, rehabilitation, and reconstruction programs.

Acknowledgements: Special thanks to Mr. Hosseini, Mr. Arbabi, and Mr. Soltanpour, who were important in the data collection process. Thanks are due to Professor Sharon Wise (Associate Dean, Division of Natural Science and Mathematics Utica College) for critically going through the article and suggesting improvement.

Ethical permissions: Iranian department of environment issued scientific research Permit No. 93.860 that would authorize us to study green sea turtle. Turtles are measured, flipper-tagged, Passive Integrated Transponder (PIT) tagged, tissue sampled, and released. The permit is issued for a 3-year period with the potential to extend the permit duration for an additional year.

Conflicts of interests: There are no conflicts of interest with respect to the Islamic Azad University, Chabahar Branch.

Authors' Contribution: Sinaei M. (First author), Original researcher/ Statistical analyst (70%); Bolouki M. (Second author), Methodologist/ Discussion author (20%); Mirshekar D. (Third author), Introduction author (10%)

Funding/Support: The present study was supported by the Iranian Department of the Environment.

References

1- Fuentes MMPB, Hamann M, Limpus CJ. Past, Current and future thermal profiles of green turtle nesting grounds: Implications from climate change. J Exp Mar Biol Ecol. 2010;383(1):56-64.

2- Troëng S, Rankin E. Long-term conservation efforts contribute to positive Green Turtle Chelonia mydus nesting trend at Tortuguero, Costa Rica. Biol Conserv. 2005;121(1):111-6.

3- Booth DT, Astill K. Incubation temperature, energy expenditure and hatchling size in the Green Turtle (*Chelonia mydas*), a species with temperature-sensitive sex determination. Australian J Zoo. 2001; 49:389-96.

4- Sinaei M, Bolouki M. Metals in blood and eggs of green sea turtles (Chelonia mydas) from Nesting Colonies of the northern coast of the sea of Oman. Arch Environ Contam Toxicol. 2017;73(4):552-61

5- Askari Hesni M, Tabib M, Hadi Ramaki A. Nesting ecology and reproductive biology of the Hawksbill Turtle, Eretmochelys imbricata, at Kish Island, Persian Gulf. J Mar Biol Assoc UK. 2015;96(7):1373-8.

6- Tollab MA, Dakhteh MH, Ghorbanzadeh Zaferani Gh, Askari Hesni M, Ahmadi F, Shojaei Langari M, et al. The Olive Ridley Turtle, Lepidochelys olivacea, in the Persian Gulf: A review of the observations, including the first nesting of the species in the area. Chelonian Conserv Biol. 2015;14(2):192-6.

7- Sinaei M, Bolouki M. Metals in blood and eggs of green sea turtles (Chelonia mydas) from nesting colonies of the northern coast of the sea of Oman. Arch Environ Contam Toxicol. 2017;73(4):552-61.

8- Mohammadizadeh M, Soltanpour N. Identification and prioritizing important nesting sites of Green Turtle in Iranian beaches of Oman Sea during 2008-2010. Bull Environ Pharm Life Sci .2014;3(4):81-6.

9- Eckert KL, IUCN/SSC Marine Turtle Specialist Group, World Wildlife Fund (U.S.), Center for Marine Conservation, United States National Oceanic and Atmospheric Administration, International Union for Conservation of Nature and Natural Resources, Species Survival Commission. Research and management techniques for the conservation of sea turtles. Washington: IUCN/Species Survival Commission Marine Turtles Specialist Group Publication; 1999.

10- Deem SL, Dierenfeld ES, Sounguet GP, Alleman AR, Cray C, Poppenga RH, et al. Blood values in free-ranging nesting Leatherback Sea Turtles (Dermochelys coriacea) on the coast of the Republic of Gabon. J Zoo Wild Med. 2006;37(4):464-71.

11- Perrault JR, Miller DL, Eads E, Johnson C, Merrill A, Thompson LJ, et al. Maternal health status correlates with nest success of Leatherback Sea Turtles (Dermochelys coriacea) from Florida. PLoS One. 2012;7(2):e31841.

12- Bilinski JJ, Reina RD, Spotila JR, Paladino FV. The effects of nest environment on calcium mobilization by leatherback turtle embryos (Dermochelys coriacea) during development. Comp Biochem Physiol A: Mol Integr Physiol. 2001;130(1):151-62

13- Broderick AC, Godley BJ, Hays GC. Metabolic heating and the prediction of sex ratios for Green Turtles (Chelonia mydas). Physiol Biochem Zoo. 2001;74(2):161-70.

14- Segura LN, Cajade R. The effects of sand temperature on pre-emergent Green Sea Turtle hatchling. Herpetol Conserv Biol. 2010;5(2):196-206.

15- DeGregorio BA, Williard AS. Incubation temperatures and metabolic heating of relocated and in situ Loggerhead Sea Turtle (Caretta caretta) nests at a Northern Rookery. Chelonian Conserv Biol. 2011;10(1):54-61.

16- Kaska Y, Downie R, Tippett R, Furness RW. Natural temperature regimes for Loggerhead and Green Turtle nests in the eastern Mediterranean. Can J Zool. 1998;76(4):723-9.

17- Booth DT, Astill K. Incubation temperature, energy expenditure and hatchling size in the Green Turtle (Chelonia mydas), a species with temperature-sensitive sex determination. Aust J Zool. 2001;49(4):389-96.

18- Burgess EA, Booth DT, Lanyon JM. Swimming performance of hatchling green turtles is affected by incubation temperature. Coral Reefs. 2006;25(3):341-49.

19- Mrosovsky N, Dutton PH, Whitmore CP. Sex ratios of two species of sea turtle nesting in Suriname. Can J Zool. 1984;62(11):2227-39.

20- Standora EA, Spotila JR. Temperature dependent sex determination in sea turtles. Copeia. 1985;1985(3):711-22.

21- Spotila JR, Standora EA, Morreale SJ, Ruiz GJ. Temperature dependent sex determination in the Green Turtle (Chelonia mydas): Effects on the sex ration on a natural beach. Herpetologica. 1987;43(1):74-81.

22- Godfrey MH, Mrosovsky N, Barreto R. Estimating past and present sex ratios of sea turtles in Suriname. Can J Zool. 1996;74(2):267-77.

23- Drake DL, Spotila JR. Thermal tolerances and the timing of sea turtle hatchling emergence. J Thermal Biol. 2002;27(1):71-81.

24- Godfrey MH, Mrosovsky N. Pivotal temperature for Green Sea Turtle, Chelonia mydas, nesting in Suriname. Br Herpetol J. 2006;16(1):55-61.

25- Hays GC, Houghton JD, Myers AE. Endangered species: Pan-Atlantic Leatherback Turtle movements. Nature. 2004;429(6991):522.

26- Matsuzawa Y, Sato K, Sakamoto W, Bjorndal K. Seasonal fluctuations in sand temperature: Effects on the incubation period and mortality of Loggerhead Sea Turtle (Caretta caretta) pre-emergent hatchlings in Minabe, Japan. Mar Biol. 2002;140:639-46.

27- Caut S, Guirlet E, Girondot M. Effect of tidal overwash on the embryonic development of Leatherback Turtles in French Guiana. Mar Environ Res. 2010;69(4):254-61.

28- Foley AM, Peck SA, Harman GR. Effects of sand characteristics and inundation on the hatching success of Loggerhead Sea Turtle (Caretta caretta) clutches on low-relief mangrove islands in southwest Florida. Chelonian Conserv Biol. 2006;5(1):32-41.