

Nest Factors Predisposing Loggerhead Sea Turtle (*Caretta caretta*) Clutches to Infestation by Dipteran Larvae on Northern Cyprus

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This paper reports the physical and biological nest parameters of loggerhead turtle (*Caretta caretta*) nests on northern Cyprus that predispose individual nests to infestation. All data were collected between June and September 1997, and data were analyzed using generalized linear models. The final model explained 66.5% of the total deviance, and the most significant factor was the depth to the egg chamber (deviance = 63.86, $P < 0.0001$). The implications of these results are discussed, and recommendations for future work are proposed.

THE choice of nesting site by a female marine turtle is likely to strongly influence offspring sex, phenotype, and hatching success (Ackerman, 1997) as well as affecting likelihood of nest predation (Blamires and Guinea, 1998). Nest site factors that have been considered include location with respect to vegetation and high water mark, nest depth and humidity (Hays and Speakman, 1993; Hays et al., 1995; C. K. Dodd Jr., 1988, unpubl.), temperature (Stoneburner and Richardson, 1981), sand type and compactness (Cardinal et al., 1998; Foote and Sprinkel, 1994; Mortimer, 1995), and for a review, see Ackerman (1997). To date, few studies have considered which nest parameters might predispose sea turtle nests to insect infestation.

The larvae of two families of diptera (Phoridae and Sarcophagidae) are known to infest the clutches of both freshwater (Iverson and Perry, 1994), and marine turtles (Bjørndal et al., 1985). It has been suggested that these larvae feed on weakened or already dead hatchlings (Fowler, 1979) and therefore pose no real threat to the reproductive success of turtles. However, larvae have been reported to attack viable hatchlings (Moll and Legler, 1971) and reduce hatching success by at least 30% (Lopes, 1982). In a more recent study, Andrade et al. (1992) concluded that fly larvae did not seriously affect the survival of leatherback (*Dermochelys coriacea*) or olive ridley (*Lepidochelys olivacea*) sea turtles at Michoacán, Mexico. Despite the wealth of sea turtle research undertaken, it is still uncertain whether fly larvae have detrimental effects on turtle populations.

A common feature of all the aforementioned studies is that most have failed to ascertain why only certain nests become infested while others remain free of larvae. Prior to this investigation, only a single study by Vásquez (1994) attempted to identify nest parameters that might be related to observed levels of infestation. Vásquez

(1994) considered the location of hatcheries with respect to high water mark, the number of nests in a hatchery, and the density of nests and concluded that these had no effect on the levels of infestation of leatherback turtle clutches. However, the major drawback with the Vásquez study is that it was carried out at the hatchery level and did not consider nests individually. In the Mediterranean, only two studies have described dipteran infestation of marine turtle nests (Broderick and Hancock, 1997; McGowan et al., 2001). Both of these studies were conducted in northern Cyprus and estimated infestation levels of loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) turtle clutches as well as documenting the dipteran species involved. In this paper, we report the physical and biological parameters of loggerhead sea turtle nests in northern Cyprus that predispose individual nests to infestation and suggest recommendations for future work.

MATERIALS AND METHODS

All data were collected at Alagadi Beach (35°33'N, 33°47'E), northern Cyprus between June and October 1997. This is a major nesting site for loggerhead and green turtles in northern Cyprus (Broderick and Godley, 1996).

During the breeding season, all nesting behaviors were timed and classified as follows: ascent of beach; digging body pit; digging egg chamber; oviposition; covering; camouflaging; descent of beach (Broderick and Godley, 1999; Johnson et al., 1996). The data on the duration of covering and camouflaging were combined to produce the variable "total cover-up time." Additionally, data on the position of nests were recorded, and the distance to the high water mark was the distance between the nest and the highest point of wet sand. In some cases, females nested below the high water mark, and these nests were transplanted to a safer area

above the high water mark directly inland from the original nest within one hour of the female returning to the sea. Transplantation involved the excavation of the original nest by hand and measuring the depth to the top and bottom of the egg chamber using a meter stick. Eggs were then transferred to a newly prepared nest, with similar egg chamber measurements, and were placed in the new nest in the same order. Clutch sizes of all transplanted nests were recorded at this time.

Nests were deemed to have hatched when signs of hatchling emergence were present (for protocol, see Broderick and Godley, 1996). Nests were excavated by hand after 48 h had elapsed with no additional hatchling emergence. When the first egg shell fragments were uncovered, the depth to the top of the egg chamber was measured three times and an average depth recorded. The nest contents were then removed, and an average depth to the bottom of the egg chamber was calculated in the same manner. Because of the correlation ($r = 0.63$, $df = 48$, $P < 0.001$) between the two egg chamber depths, only the average depth to the top of the egg chamber was included in analyses. From the nest contents, the number of egg-shell fragments and unhatched eggs were counted to ascertain clutch size and hatching success. Unhatched eggs were opened and their contents categorized as "yolked" if there were no gross signs of embryonic development or "dead embryo" if eggs contained developing embryos. It was during the process of nest excavation that fly larvae were encountered, and to quantify any damage caused, the number of affected eggs (hereafter referred to as number of infested eggs) was recorded. The data on the numbers of dead embryos and dead hatchlings were combined to produce the variable "number of dead" (hatchlings + embryos) per nest. The number of days over which a clutch hatched (hereafter referred to as hatchling emergence duration) was also recorded, and this included the period of 48 h prior to excavation in which no hatchlings had emerged. Nests that exceeded a 60-day incubation period were classified as having failed. These nests were then excavated by hand, and all the nest parameters and remains were recorded as described above.

The data were analyzed using Generalized Linear Models with negative binomial error distributions. Significance levels were calculated, using chi-squared tests, from the deviance explained by each variable following stepwise deletion (Crawley, 1993). The number of infested eggs was used as the response variable. Variables included in the starting model were julian date,

total cover-up time, distance to the high water mark, depth of the egg chamber, clutch size, number of hatched eggs, number of yolked eggs, number of dead, hatchling emergence duration, and whether the nest was transplanted. All variables were included as covariates in the analyses except nest transplantation, which was included as a categorical variable. The starting model also included all the biologically relevant interaction terms that could be constructed from the variables under consideration.

RESULTS

Data were collected from 39 nests, which were left in situ (nine were infested), and 16 nests, which were transplanted (three were infested). The number of infested eggs followed an aggregated pattern that was not significantly different from a negative binomial distribution [arithmetic mean = 1.64, positive exponent (k) = 0.09, chi-squared test; $\chi^2 = 2.45$, $df = 3$, $P = 0.29$].

Variables that were left in the final model were hatching duration, number of dead, depth of the egg chamber, distance to the high water mark, and whether the nest was transplanted (Table 1). Although nonsignificant, the predictor variable, number of dead, was left in the final model because the interaction between this predictor variable and the depth of the egg chamber was found to be significant (Table 1). The final model explained 66.52% of the total deviance.

The depth of the egg chamber was found to be the most important variable measured in determining whether a nest was infested by dipteran species (Table 1). In fact, the depth of the egg chamber accounted for 21.56% of the total deviance with clutches laid at shallower depths more likely to be infested than those laid at greater depths. However, the interaction between the number of dead and the depth of the egg chamber was also found to be significant (Table 1). The relationship between the number of infested eggs in a nest and the nest depth also varies with the number of dead. Shallow nests were found to have a high number of infested eggs if they contained a high number of dead, but the total number of dead in a nest was not found to have an effect on deeper nests. In short, the number of infested eggs in a nest decreases with increasing nest depth, and in shallow nests the number of infested eggs increases with increasing number of dead.

The distance from the high water mark to the nest was also found to have a significant effect on the number of infested eggs in a nest (Table

TABLE 1. THE FACTORS IN THE FINAL MODEL, WHICH EXPLAIN THE NUMBER OF INFESTED EGGS IN A LOGGERHEAD TURTLE NEST ON NORTHERN CYPRUS, THE DEVIANCE EXPLAINED AND THE ASSOCIATED *P*VALUE. The null deviance was 296.13 and the deviance explained by the final model 197.00 (* represents an interaction between two variables). All factors have *df* = 1.

Factor	Number of nests	Deviance explained	% of total deviance	<i>P</i> value (χ^2)
Depth of the egg chamber	51	63.86	21.56	<0.0001
Distance to the high water mark	53	52.29	17.66	<0.0001
Number of dead * Depth of the egg chamber	50	36.49	12.32	<0.0001
Hatchling emergence duration	47	35.98	12.15	<0.0001
Transplanted	16	5.41	1.83	0.020
Number of dead	55	2.96	1.00	>0.05
Total	55	197.00	66.52	

1), with infestation decreasing with increasing distance from the high water mark. Hatchling emergence duration was also found to be significant (Table 1), with the number of infested eggs increasing as hatchling emergence duration increased. Finally, whether or not the nest was transplanted was found to explain a significant amount of the deviance in the model (Table 1), with transplanted nests having fewer infested eggs. However, this may be explained by the fact that transplanted nests tended to be located at a greater depth in the sand column when compared to natural nests ($t = -2.1$, *df* = 46, $P = 0.04$). Transplanted nests did not differ from natural nests in any of the other variables that were measured.

DISCUSSION

The nest parameter that accounted for almost a third of the total deviance explained by the final model was the average depth to the top of the egg chamber. Shallower nests appear to be more prone to fly infestation, and at first this would seem to be intuitive with shallower nest being easier to detect. However, some of the fly species that infest loggerhead clutches in northern Cyprus also infest the clutches of green turtles (McGowan et al., 2001), which are laid at depths exceeding those of loggerhead clutches (Broderick and Godley, 1996). The fact that shallower nests are more prone to infestation could possibly reflect a high degree of variation in the burrowing ability of the larvae of the different fly species with deeper nests being more difficult to reach. However, shallow nests were found to have a higher number of infested eggs if the nest contained a high number of dead although the total number of dead did not have an effect when nests were deeper. This interaction between nest depth and the total number of dead suggests that there may possibly be dif-

ferences in the ability of dipteran species to detect decaying tissue matter within the sand column. Furthermore, these results may suggest that chemical odors from decaying tissue may lose potency as they permeate up through the sand column. Nevertheless, this does not rule out variation in larval burrowing ability as a possible explanation as to why nest depth plays such an important role in turtle clutch infestation. Studies to determine the burrowing ability of larvae and the ability of the female flies to detect decaying tissue would be useful in resolving some of these issues.

The distance to the high water mark was also found to have a significant effect on the number of eggs in a clutch that were infested (Table 1), with nests further from the high water mark less prone to infestation. These findings contrast with those of Vásquez (1994) who reported that distance to the high water mark had no effect of the levels of infestation experienced in hatcheries containing leatherback turtle clutches. It is possible that female flies in northern Cyprus are concentrated in the area surrounding the high water mark to exploit food sources that may be washed ashore, or because of their own moisture requirements, and that the observed differences are a result of the species-specific behaviors of the flies involved.

As hatchling emergence duration increased, the number of infested eggs in a clutch also increased. This evidence supports that of Vásquez (1994) who reported that the longer the delay between hatchling emergence and excavation of leatherback turtle nests the higher the likelihood of clutches being infested. Additionally, in northern Cyprus higher levels of infestation were experienced in the 1997 breeding season compared to 1996, and this could possibly be a result of changes in nest excavation protocol (McGowan et al., 2001). It is entirely possible that hatchling emergence advertizes the loca-

tion of nests to fly species by the release of chemical cues and that the longer this process continues, the more likely infestation becomes. However, there is some evidence to suggest that infestation occurs prior to as well as after hatching emergence in this species (McGowan et al., 2001). Alternatively, it may be that chemical odors from decaying tissue build up over time making nests easier to locate. An experimental approach manipulating nest contents and the length of time nest contents spend in the sand would be useful in gaining a better understanding of infestation.

The clutches that were transplanted in northern Cyprus had fewer infested eggs than those that were left in situ and transplantation explained 1.83% of the total deviance (Table 1). However, transplanted nests tended to be located at a greater depth when compared to natural nests, and it may be that depth is the main factor involved and not transplantation per se. Nevertheless, nests that were transplanted to a common hatchery in Michoacán, Mexico, experienced elevated levels of insect infestation (Andrade et al., 1992), although Vásquez (1994) reported that the number of nests and density of nests had no effect on infestation levels in Michoacán. In northern Cyprus, nests are not transplanted to a communal hatchery, and the transplantation process does not appear to increase the chances of a clutch becoming infested. Communal hatcheries could possibly retain residual odors from previous years, and if the use of a communal hatchery does elevate the infestation level, then adoption of the transplantation protocol practiced in northern Cyprus could be viewed as a preventative measure.

Dipteran infestation of turtle clutches is a common phenomenon, in pliable egg-shelled species throughout the world, although whether dipteran infestation is detrimental to the lifetime reproductive success of turtles is still unknown. However, under conditions of intensive management of small populations, dipterans may pose a threat as in the increased infestation in hatcheries. Therefore, a more detailed understanding of all aspects of infestation would seem appropriate. Our results have identified some of the key nest parameters that predispose sea turtle nests to insect infestation, but this is only the first step. Further detailed studies of the behavior and ecology of the flies themselves, incorporating some of the suggestions made here, would be useful in gaining a better understanding of this still poorly understood system.

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LITERATURE CITED

- ACKERMAN, R. A. 1997. The nest environment and the embryonic development of sea turtles, p. 83–106. *In: The biology of sea turtles*. P. L. Lutz and J. A. Musick (eds.). CRC Marine Science Series, CRC Press, Inc., Boca Raton, FL.
- ANDRADE, R. M., R. L. FLORES, S. R. FRAGOSA, C. S. LÓPEZ, L. M. SARTI, M. L. TORRES, AND L. G. B. VÁSQUEZ. 1992. Efecto de las larvas de diptero sobre el huevo y las crías de tortuga marina en el playon de Mexiquillo, Michoacán, p. 27–37. *In: Memorias Del VI Encuentro Interuniversitario Sobre Tortugas Marinas en México*. N. M. Benabib and L. M. Sarti (eds.). Publicaciones de la Sociedad Herpetologica Mexicana, Mexico.
- BJORNDAAL, K. A., A. CARR, A. B. MEYLAN, AND J. A. MORTIMER. 1985. Reproductive biology of the hawksbill, *Eretmochelys imbricata*, at Tortuguero, Costa Rica, with notes on the ecology of the species in the caribbean. *Biol. Conserv.* 34:353–368.
- BLAMIRE, S. J., AND M. L. GUINEA. 1998. Implications of nest site selection on egg predation at the sea turtle rookery at Fog Bay, p. 20–24. *In: Marine turtle conservation and management in Northern Australia*. R. Kennet, A. Webb, G. Duff, M. L. Guinea, and G. Hill (eds.). Centre for Indigenous Natural and Cultural Resource Management and Centre for Tropical Wetlands Management, Northern Territory Univ., Darwin, Northern Territory, Australia.
- BRODERICK, A. C., AND B. J. GODLEY. 1996. Population and nesting ecology of the green turtle, *Chelonia mydas*, and the loggerhead turtle, *Caretta caretta*, in northern Cyprus. *Zool. Middle East* 13:27–46.
- , AND ———. 1999. Effect of tagging marine turtles on nesting behaviour and reproductive success. *Anim. Behav.* 58:587–591.
- , AND E. G. HANCOCK. 1997. Insect infestation of Mediterranean marine turtle eggs. *Herpetol. Rev.* 28:190–191.
- CARDINAL, J. L., B. WILLIS, B. WEAVER, AND E. T. KOEPFLER. 1998. Influence of meteorological and beach sand physical characteristics upon nest location of the loggerhead sea turtle (*Caretta caretta*), p. 30. *In: Proceedings of the 16th annual symposium on sea turtle biology and conservation*. R. Byles and Y. Fer-

- andez (eds.). NOAA Tech. Memo. NMFS-SEFSC-412, Miami, FL.
- CRAWLEY, M. J. 1993. GLIM for ecologists. Blackwell Scientific Publications, London.
- FOOTE, J., AND J. SPRINKEL. 1994. Beach compactness as a factor affecting turtle nesting on the west coast of Florida, p. 217–220. *In: Proceedings of the 14th annual symposium on sea turtle biology and conservation*. K. A. Bjorndal, A. B. Bolten, D. A. Johnson, P. J. Eliazar. (eds.). NOAA Tech. Memo. NMFS-SEFSC-351, Miami, FL.
- FOWLER, L. E. 1979. Hatching success and nest predation in the green sea turtle, *Chelonia mydas*, at Tortuguero, Costa Rica. *Ecology* 60:946–955.
- HAYS, G. C., AND J. R. SPEAKMAN. 1993. Nest placement by loggerhead turtles, *Caretta caretta*. *Anim. Behav.* 45:47–53.
- , A. MACKAY, C. R. ADAMS, J. A. MORTIMER, J. R. SPEAKMAN, AND M. BOEREMA. 1995. Nest site selection by sea turtles. *J. Mar. Biol. Assoc. U.K.* 75: 667–674.
- IVERSON, J. B., AND R. E. PERRY. 1994. Sarcophagid fly parasitoidism on developing turtle eggs. *Herpetol. Rev.* 25:50–51.
- JOHNSON, S. A., K. A. BJORNDAL, AND A. B. BOLTEN. 1996. Effects of organized turtle watches on loggerhead (*Caretta caretta*) nesting behaviour and hatching production in Florida. *Conserv. Biol.* 10:570–577.
- LOPES, H. S. 1982. On *Eumacronychia sternalis* Allen (Diptera, Sarcophagidae) with larvae living on eggs and hatchlings of the east Pacific green turtle. *Rev. Brasil. Biol.* 42:425–429.
- MCGOWAN, A., A. C. BRODERICK, J. DEEMING, B. J. GODLEY, AND E. G. HANCOCK. 2001. Dipteran infestation of loggerhead (*Caretta caretta*), and green (*Chelonia mydas*), sea turtle nests in northern Cyprus. *J. Nat. His.* 35:573–586.
- MOLL, E. O., AND J. M. LEGLER. 1971. The life history of a neotropical slider turtle, *Pseudemys scripta* (Schoepff), in Panama. *Sci. Bull. Los Angeles Co. Mus.* 11:1–102.
- MORTIMER, J. A. 1995. Factors influencing beach selection by nesting sea turtles, p. 45–51. *In: Biology and conservation of sea turtles*. K. A. Bjorndal (ed.). Smithsonian Institution Press, Washington, DC.
- STONEBURNER, D. L., AND J. I. RICHARDSON. 1981. Observations on the role of temperature in loggerhead turtle nest site selection. *Copeia* 1981:238–231.
- VÁSQUEZ, L. G. B. 1994. Dípteros de la familia Sarcophagidae que actúan como depredadores de crías de tortuga laúd (*Dermochelys coriacea*) en el playón de Mexiquillo, Michoacán, p. 1–64. Tesis Facultad de Ciencias Universidad Nacional Autónoma de México, México.
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