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Dipteran infestation of loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtle nests in northern Cyprus

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This paper describes dipteran infestation of loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtle nests in northern Cyprus in 1996 and 1997. Insect larvae were collected from infested nests, reared to adulthood and identified to species. A minimum of 13.4% (1996) and 17.4% (1997) of loggerhead turtle nests and 3.3% and 20.7% of green turtle nests were infested in 1996 and 1997, respectively. Eleven dipteran species were found infesting turtle nests with one species (*Sarcotachina aegyptiaca*) being dominant. Gross estimates, utilizing the speeds of development for three species of sarcophagid showed that infestation was taking place differentially between the two turtle species. Various aspects of the effect of dipteran infestation on marine turtle nests in northern Cyprus and recommendations for future investigations are discussed.

KEYWORDS: Diptera, Sarcophagidae, Phoridae, nest infestation, marine turtle, *Chelonia mydas*, *Caretta caretta*, Cyprus, Mediterranean.

Introduction

Globally, there have been several studies reporting the presence of larvae from two dipteran families (Phoridae and Sarcophagidae) in marine turtle nests. Fowler (1979) recorded larvae of the phorid, *Megaselia scalaris* (Loëw), in 50 green turtle (*Chelonia mydas*) clutches at Tortuguero, Costa Rica, and suggested that they were

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feeding on dead and weakened hatchlings. Also at Tortuguero, *M. scalaris* larvae were recorded in nests of hawksbill turtles (*Eretmochelys imbricata*) (Bjorndal *et al.*, 1985). The larvae of the sarcophagid, *Eumacronychia sternalis*, were reported in 90% of transplanted green turtle clutches on the pacific coast of Mexico (Lopes, 1982) and the results indicated that the larvae were reducing hatching success by 30%. *E. sternalis* larvae have also been reported in green turtle nests in Michoacán, Mexico (López Barbosa, 1989). Andrade *et al.* (1992) recorded sarcophagid larvae of the genera *Phrosinella* and *Eusenotainia* in nests of leatherback turtles (*Dermochelys coriacea*) and olive ridley turtles (*Lepidochelys olivacea*) in Mexico and concluded that the sarcophagid larvae were not seriously affecting the survival of either turtle species. Another study carried out on the nests of leatherback turtles at Michoacán (Vásquez, 1994), revealed that larvae of *Eusenotaina rufiventris* were living on live, as well as dead, hatchlings. Species from these two dipteran families are also known to infest the nests of freshwater turtles. Vogt (1981) recorded larvae of *Metosarcophaga* sp. in nests of false map turtles (*Graptemys pseudogeographica*) and Acuña-Mesen and Hanson (1990) reported *M. scalaris* larvae in the eggs of painted wood turtles (*Rhinochemmys pulcherrima*). Additionally, Phoridae larvae were reported on the eggs and egg sacs of hatchlings of the common slider turtle (*Trachemys* (*Pseudemys*) *scripta*) (Moll and Legler, 1971). These infested hatchlings were subsequently attacked and died. Iverson and Perry (1994), who also report *E. sternalis* larvae in nests of eastern box turtles (*Terrapenne carolina*), review infestation in freshwater turtles.

Previously in the Mediterranean there have only been two reports describing the occurrence of insect infestation of marine turtle nests. Baran and Türkozan (1996) recorded coleopteran larvae infesting loggerhead turtle (*Caretta caretta*) eggs in Turkey. They estimated that larvae were responsible for the destruction of 73 eggs in nine clutches. The only other study of this phenomenon in the Mediterranean was in northern Cyprus (Broderick and Hancock, 1997). Here Broderick and Hancock (1997) estimated that 23% of loggerhead and 9% of green turtle nests were infested during the 1995 season. Four species of flies from two families (Sarcophagidae and Phoridae) were identified as well as a single species of wasp, *Brachymeria podagarica*, a parasitoid of one of the dipterans.

In this paper we record the insect species infesting loggerhead and green turtle nests in northern Cyprus in 1996 and 1997. In addition, we present the development times of dipteran larvae, the stage at which infestation is occurring and the effects on the clutches.

Materials and methods

The research was undertaken on the marine turtle nesting beaches of northern Cyprus between June and October in 1996 and 1997. For a full description of study sites refer to Broderick and Godley (1996). The major part of this study was conducted at Alagadi Beach (35°33′N, 33°47′E), which is the main nesting site for marine turtles in northern Cyprus and was surveyed daily. Additional data were collected from 38 other beaches, which were surveyed every 1–4 days.

At Alagadi beach all data collection on turtle nesting behaviour and hatching was carried out as described by Broderick and Godley (1996). Turtle nests were excavated following hatchling emergence. In 1996, this was carried out the morning following the first wave of hatchling emergence. In 1997, nests were excavated 48 h after the last hatchling had emerged. It was during the process of excavating nests that insect larvae were encountered. In order to quantify any damage caused, the number of affected eggs were recorded at this point. Larvae were placed in jars with a supply of decaying tissue matter and the contents covered with sand. Jars were then covered using muslin and a lid with aeration holes which prevented further infestation whilst allowing the passage of air and moisture. The jars containing larvae were returned to the field laboratory and placed in muslin-covered boxes for incubation. A small tray of water was placed inside the box to reduce moisture loss. All larvae from 1996, and those collected from beaches other than Alagadi in 1997, were kept in jars until all adult flies had emerged and thus were only utilized for identification of species infesting nests.

In 1997, larvae collected from infested nests at Alagadi were checked daily by emptying the contents into a metal tray. Any puparia found were removed, placed in individual vials already containing a single sheet of tissue paper to soak up excess moisture, and covered with muslin. The vials were then placed in an identical box to the one that housed the jars and were incubated at ambient temperature. Temperature was recorded using a temperature data logger ('Tiny Talk' Orion Components Ltd, UK) and ranged between 21 and 30°C with an average temperature of 26°C (SE $\pm$ 0.03, $n = 3463$). Checking and removal of puparia continued until 30 puparia were collected from each infested nest. These vials were monitored on a daily basis for adult emergence. Adult flies were left alive for 24 h for the cuticle to harden, before being killed using ethyl acetate, and preserved for identification. Samples of all the insects collected were deposited at the National Museum and Gallery, Cardiff, and the Hunterian Museum and Art Gallery (Zoology Museum), University of Glasgow.

Results

The levels of infestation for loggerhead and green turtle nests are shown in table 1. The number of infested loggerhead turtle nests represent a minimum of 13.4% (1996) and 17.4% (1997) of the total. Clutches which were partially destroyed by other means such as excavation by feral dogs or inundation, in addition to being infested, were not included in the data set. In 1996, the number of infested loggerhead turtle eggs represented 0.5% of the estimated total loggerhead turtle eggs laid (mean clutch size 1996 = 75.4; total number of clutches laid 1996 = 313; Godley and Kelly, 1996), while 0.8% were infested in 1997 (mean clutch size 1997 = 76.1; total number of clutches laid 1997 = 315; Broderick *et al.*, 1997).

In 1996 and 1997, 3.3% and 20.7% of green turtle nests were infested (table 1). However, as a percentage of the overall number of eggs laid in 1996 (mean clutch size 1996 = 117.2; number of nests laid 1996 = 86; Godley and Kelly, 1996) 0.01%

Table 1. Levels of insect infestation in loggerhead and green turtle nests in northern Cyprus in 1996 and 1997.

	Loggerhead turtle		Green turtle	
	1996	1997	1996	1997
Number of infested nests	21	33	1	11
Total number of infested eggs	117	185	1	49
Mean number of infested eggs ± SE	5.6 ± 1.01, $n = 21$ (1–12)	5.6 ± 1.23, $n = 33$ (1–32)	–	4.5 ± 1.11, $n = 11$ (1–12)

were infested, while 0.2% were infested in 1997 (mean clutch size 1997 = 129.6; number of nests laid 1997 = 156; Broderick *et al.*, 1997).

Adult flies did not emerge successfully from all samples collected and therefore were only identified from 21 loggerhead turtle nests in 1996 and 27 in 1997. Flies were identified from one green turtle nest in 1996 and 10 nests of this species in 1997.

A total of 11 species of Diptera were recorded in loggerhead turtle nests during the course of this study (table 2). All dipteran species recorded in green turtle nests were also present in the nests of loggerhead turtles in both years. It is clear that *Sarcotachina aegyptiaca* (Villeneuve) is the most prevalent insect species found infesting nests of both turtle species in both years. Additionally, four other dipteran species were present in nests of both turtle species in both years. In comparison to *Sarcotachina aegyptiaca*, the other insect species were present in fewer nests. There is a trend in both years for a greater diversity of dipteran species to infest loggerhead turtle nests rather than green turtle nests.

The majority of infested loggerhead turtle nests (29), and green turtle nests (9), contained only a single species of Diptera and the pattern was similar in both years (table 3). However, over the course of this study two dipteran species were recorded in eight loggerhead turtle nests and two green turtle nests. Only five loggerhead turtle and no green turtle nests had greater than two dipteran species present in the years that the studied was conducted. Additionally, only loggerhead turtle nests contained coleopteran larvae.

Data were collected regarding the duration of development of dipteran larvae reared from infested nests laid on Alagadi beach. A mean duration was calculated for each period of development for each species, thus giving an estimated number of days from collection to formation of puparia and then on to adult emergence. These data were pooled to give a mean of means for these periods of development. The results are given in tables 4 and 5 for loggerhead and green turtle nests, respectively.

Of the three dipteran species examined (*S. aegyptiaca*, *P. argyrostoma*, *W. nuba*), all developmental periods were shorter for larvae collected from loggerhead turtle

Table 2. Frequency table of the species recorded in loggerhead and green turtle infested nests in northern Cyprus in 1996 and 1997.

			Loggerhead		Green	
Order	Family	Species	1996	1997	1996	1997
Diptera	Sarcophagidae	*Sarcotachina aegyptiaca*	20	16	1	6
		Parasarcophaga argyrostoma	5	3	–	1
		Sarcophaga tibialis	1	1	–	–
		Sarcophaga sp.	1	–	–	–
		Wohlfahrtia nuba	1	2	–	2
		Phyllotelles pictipennis	1	1	–	1
	Milichiidae	*Desmometopa m-nigrum*	3	–	–	–
	Muscidae	*Atherigona orientalis*	–	1	–	–
	Chloropidae	*Eutropha fulvifrons*	–	1	–	–
	Ephydridae	*Hecamede albicans*	–	2	–	–
	Phoridae	*Megaselia scalaris*	1	3	–	2
Coleoptera	Elateridae	*Cardiophorine* sp.	2	2	–	–
		Agriotine sp.	1	1	–	–

Table 3. The number of infested loggerhead and green turtle nests in northern Cyprus in 1996 and 1997 from which adult flies were obtained and the number of different species of insect that they contained.

	Loggerhead		Green	
	1996	1997	1996	1997
1 species Diptera present	10	19	1	8
2 species Diptera present	4	4	–	2
3 species Diptera present	3	1	–	–
4 species Diptera present	–	–	–	–
5 species Diptera present	1	–	–	–
Coleoptera present	1	2	–	–
Coleoptera and Diptera present	2	1	–	–
Total	21	27	1	10

Table 4. Duration of development periods of dipteran larvae found in loggerhead turtle nests at Alagadi in 1997.

	Number of days between loggerhead nest hatching and puparium formation				Number of days between puparium formation and imago emergence			
Dipteran species	Mean of means ± SE	Range of indi-viduals	No. of indi-viduals	No. of nests	Mean of means ± SE	Range of indi-viduals	No. of indi-viduals	No. of nests
S. aegyptiaca	6.5 ± 0.74	3–11	142	10	13.5 ± 0.60	5–18	142	10
P. argyrostoma	8.7 ± 2.3	6–11	8	2	12.0 ± 1.0	10–13	8	2
W. nuba	9	9–9	15	1	9.5 ± 0.19	9–11	15	1

Table 5. Duration of development periods of dipteran larvae found in green turtle nests at Alagadi in 1997.

	Number of days between green nest hatching and puparium formation				Number of days between puparium formation and imago emergence			
Dipteran species	Mean of means ± SE	Range of indi-viduals	No. of indi-viduals	No. of nests	Mean of means ± SE	Range of indi-viduals	No. of indi-viduals	No. of nests
S. aegyptiaca	8.9 ± 0.83	6–11	38	4	15.8 ± 0.64	10–19	38	4
P. argyrostoma	13.4 ± 0.16	13–14	10	1	12.4 ± 0.22	12–14	10	1
W. nuba	10.4 ± 0.4	9–11	12	2	15.6 ± 0.4	14–16	12	2

nests than from green turtle nests. There was a significant difference between the development times of *S. aegyptiaca* larvae that were collected from loggerhead turtle nests than conspecific larvae collected from green turtle nests (Mann-Whitney U test, $W = 60.0$, $p = 0.04$).

From the time of a loggerhead turtle nest hatching until larval pupation *S. aegyptiaca* larvae had the shortest development time of the three species reared and paradoxically the longest development time from pupation until adult emergence (table 4). This pattern was also reflected for *S. aegyptiaca* larvae that were collected from green turtle nests.

For all three species, however, the total time of development from the point of the nest hatching until adult emergence took on average 18.5–20 days for larvae collected from loggerhead turtle nests and 24–26 days for larvae from green turtle nests.

Discussion

The higher levels of nest infestation in 1997 particularly in green turtle nests, in comparison to 1996, is likely to be attributable to a change in the methodological protocol regarding nest excavation. In 1997, turtle nests at Alagadi were not excavated until 48 h after the last hatchling had emerged and green turtle nests hatched over a greater number of days than loggerhead turtle nests. Therefore, in 1997, green turtle nests at Alagadi were left for a longer period of time before excavation commenced, thereby increasing the opportunity of infestation. This evidence supports Vasquez (1994) who reported that a longer delay between hatchling emergence and excavation of leatherback turtle nests increased the likelihood of nests being infested.

As a percentage of the estimated eggs laid, infestation is calculated at 0.5% and 0.8% for eggs of the loggerhead turtle and 0.01% and 0.2% of eggs of the green turtle in 1996 and 1997, respectively. All of these levels of infestation in northern Cyprus are lower than the 2.2% for leatherback turtle, and 1.1% for olive ridley turtle eggs found to be infested in the study at Michoacán, Mexico (Andrade *et al.*, 1992). This may be a result of the fact that a vast majority of infested nests at Michoacán were transplanted, suggesting a common hatchery may elevate levels of infestation. The low levels of egg infestation in northern Cyprus seem to suggest that infestation only occurs in eggs that have failed to hatch and contain decaying tissue matter, although a single moribund hatchling was found infested in a green turtle nest. If dipteran larvae were actively attacking viable turtle eggs then it would be expected that the percentage of eggs infested would be much higher than that recorded in this study. However, Phoridae larvae are known to cause the death of common slider turtle hatchlings (Moll and Legler, 1971). Therefore, if dipteran larvae are a cause of hatchling mortality in these endangered species then preventative measures must be installed.

There were 11 species of Diptera, from six different families, found in marine turtle nests in northern Cyprus during 1996 and 1997 (table 2). Seven of these species had not previously been recorded infesting marine turtle nests. In comparison, the study by Broderick and Hancock (1997) found five species of Sarcophagidae, two of which (*P. argyrostoma* and *W. nuba*) were recorded in this study, as well as the phorid species (*M. scalaris*), also seen in both years. This suggests that the dipteran species responsible for infesting marine turtle nests may vary annually, possibly dependent on environmental conditions.

The presence of *Phylloteles pictipennis* (Loëw) is most unusual, as this species is known as a sphecid wasp kleptoparasite. It is possible that *P. pictipennis* is capable of breeding in turtle nests although the presence of this species could also be due to sphecid wasp nests being present in the sand and that infestation is due to sand disturbance during excavation.

A general pattern that is present in both studies is that members of the Sarcophagidae family were the most prevalent dipterans found infesting marine turtle nests. *S. aegyptiaca* was the most common species found in both loggerhead and green turtle nests (table 2). The majority of turtle nests were found containing only this single dipteran species. However, nests that contained more than one species of Diptera always contained *S. aegyptiaca* in addition to other species. It is possible that *S. aegyptiaca* larvae may out-compete the larvae of other species for available resources and thereby appear as the most prevalent species. However, further research concerning the competitive ability of larvae is required before this idea can be confirmed or rejected.

The three species that were reared from infested turtle nests at Alagadi in 1997 were sarcophagids. The Sarcophagidae are known colloquially as flesh flies, and are a large family distributed world-wide although there are no true arctic representatives (Pape, 1996). The flesh flies are all viviparous and commonly breed on invertebrate and vertebrate carrion (Busvine, 1980; Pape, 1996). Sarcophagids deposit first instar larvae just under the surface of the sand and the larvae burrow down through the sand column to the clutch of eggs (Vasquez, 1994, personal observation). There are limited data on times of development for individual flesh fly species (refer to Smith, 1986, for a full description), but the complete life-cycle is cited as: 25 days at 21°C; 17 days at 25°C; and 13 days at 29°C for sarcophaga species (Busvine, 1980). The average daily temperature of selected *in situ* turtle nests in northern Cyprus has been established (Broderick, 1997). The temperature of loggerhead turtle nests has been shown to increase throughout the incubation period from 28.1 to 33°C with a diel variation of less than 1°C. Similarly the temperature of green turtle nests increases from 28.5 to 33°C throughout incubation with a maximum diel variation of less than 0.5°C (Broderick, 1997). The temperature of turtle nests in northern Cyprus at the end of the incubation period is higher than that given in Busvine (1980). Therefore, one would expect the development of sarcophagid larvae in turtle nests to be less than 13 days due to the higher temperature regime. However, the temperature of the field laboratory ranged from 21°C to 30°C, with a mean temperature of 26°C, and this was the temperature regime under which larvae carried out the majority of their development. Therefore, an overall *a priori* gross estimate of the speed of development for the larvae of Sarcophagidae that were reared during the course of this study would be approximately 13–17 days.

The overall mean number of days between a loggerhead turtle nest hatching and a *S. aegyptiaca* adult emerging was 20 days and ranged from 10 to 27 days. The same parameter for *S. aegyptiaca* found in green turtle nests was found to be 24.7 days ranging from 17 to 28 days (tables 4, 5). There was a significant difference in development times of *S. aegyptiaca* larvae collected from loggerhead and green turtle nests and this may be due to the greater depth at which green turtle nests are found within the sand column. However, more studies with increased sample sizes are required before any definitive answer can be provided for this difference. These results include the number of days over which hatchlings emerged and green turtles, on average, took longer to complete emergence which may have some, as of yet, unknown effect. Nevertheless, if it is assumed that a gross estimate for speed of development of 17 days is reasonable then it is apparent that infestation is taking place both prior to and after emergence begins in loggerhead turtle nests but only post emergence in green turtle nests. These findings are reinforced by similar results for *P. argyrostoma* and *W. nuba* larvae lending further support to the theory that infestation is taking place differentially between the two turtle species.

This study has shown that a wide range of dipteran species infest marine turtle nests in northern Cyprus with one species being dominant. It seems that insect infestation is not having any adverse effects on loggerhead and green turtle clutches due to the low percentage of eggs that are affected and are probably those that have failed late in their development. In fact infestation appears to have no effect on the hatching success of nests (McGowan, 1997). However, dipteran larvae were found infesting one moribund hatchling which may be evidence of a threat to hatchlings remaining within the sand column or egg chamber. Therefore, it is recommended, due to the threatened/endangered status of sea turtles in the Mediterranean, that further studies of this nature be undertaken to fully elucidate the effects of insect infestation on marine turtle clutches. In addition, more detailed information is needed on the development times of dipteran larvae under controlled conditions which would enable a more accurate assessment of the timing of infestations. Conservation projects might then be able to adopt suitable preventative measures where necessary.

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