

Thermal conditions in nests of loggerhead turtles: further evidence suggesting female skewed sex ratios of hatchling production in the Mediterranean

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Abstract

Temperature was recorded in 23 nests of the loggerhead turtle (*Caretta caretta*) and control sites of nest depth at Alagadi (35°33'N, 33°47'E), Northern Cyprus, eastern Mediterranean. Control site sand temperature was found to be highly correlated with mean daily air temperature and mean nest temperature. Mean temperature in nests ranged from 29.5°C to 33.2°C, with mean temperature in the middle third of incubation ranging from 29.3°C to 33.7°C. Hatching success was significantly correlated with incubation temperature, with nests experiencing very high temperatures exhibiting low hatching success. All nests demonstrated regular diel variation in temperature with mean daily fluctuations ranging from 0.3°C to 1.4°C. Increase in temperature above that of the prevailing sand temperature attributed to metabolic heating was clearly demonstrated in 14 of 15 clutches, with the mean level of metabolic heating of all nests being 0.4°C. However, the level of metabolic heating varied markedly throughout the incubation period with levels being significantly higher in the final third of incubation. Incubation duration was found to be significantly correlated to both the mean temperature of nests throughout the incubation period and during the middle third of incubation. The relationship between incubation duration and mean incubation temperature was used to estimate mean incubation temperatures at

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most major nesting sites throughout the Mediterranean from available data on incubation durations, showing that mean incubation temperature is likely to be above 29.0°C at most sites in most seasons. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

In marine turtles, as in many oviparous reptiles, the direction of sexual differentiation depends on the temperature prevailing during incubation; a phenomenon termed temperature-dependent sex determination (TSD; recently reviewed by Shine, 1999). In addition, the temperature of turtle nests also affects the rate of embryonic development (Ackerman, 1994, 1997), and phenotype of the offspring (Packard and Packard, 1988; Packard et al., 1999; McGehee, 1990). Recent studies to estimate sex ratio of hatchling production in marine turtles have employed several different techniques including: laboratory incubation of eggs (Mrosovsky and Yntema, 1980), inferences from long-term control site temperature monitoring at nest depth (Godfrey et al., 1996), intra-nest temperature logging (Spotila et al., 1987), and inferences from incubation durations (Marcovaldi et al., 1997).

Studies have indicated that nests which incubate at high temperatures (> 29.0°C) produce a larger proportion of females, with cooler nests (< 29.0°C) producing a greater proportion of males (Mrosovsky, 1994; Ackerman, 1997). The thermosensitive period when the sex is determined has been shown to occur during the middle third of incubation (Yntema and Mrosovsky, 1980; Mrosovsky and Pieau, 1991). The temperature at which a 1:1 sex ratio is produced is termed the pivotal temperature (Yntema and Mrosovsky, 1980; Miller and Limpus, 1981; Mrosovsky and Pieau, 1991). Although the possibility for some degree of inter-population variation in patterns of TSD in at least some sea turtle species exist (Chevalier et al., 1999), pivotal temperatures have been shown to be relatively conservative (Mrosovsky, 1994).

Of the marine turtles, the first to be intensively scrutinised concerning TSD was the loggerhead turtle (*Caretta caretta*) in which some populations have extremely female-biased hatchling production (Mrosovsky and Provancha, 1989, 1992; Marcovaldi et al., 1997; Hanson et al., 1998; Provancha and Corsello, 1998). The pivotal temperatures so far described in this species in the Atlantic are close to 29.0°C (Mrosovsky, 1994; Ackerman, 1997), with values of 29.2°C for Brazil (Marcovaldi et al., 1997), 29.0°C for the USA (Mrosovsky, 1994) and just below 29.0°C in the Mediterranean (Kaska et al., 1998). Although some estimates are present from the Pacific, they are more variable (28.9°C and 28.7°C in Australia: Georges et al. (1994); Limpus et al., 1983; and 29.7°C in Japan: Matsuzawa et al., 1998). Studies have involved laboratory incubations (Georges et al., 1994; Billett et al., 1992; Limpus et al., 1983; Mrosovsky and Yntema, 1980; Mrosovsky, 1988), extrapolation from control site temperatures (Baptistotte et al., 1999; Limpus et al., 1983; Mrosovsky and Provancha, 1989, 1992) or monitoring of a

relatively small number of nests in situ (Kaska et al., 1998). Few studies have actually looked at the thermal conditions in a large sample of nests (Maxwell et al., 1988; Hanson et al., 1998).

Field studies involving extrapolation of nest conditions through the monitoring of sand temperatures, have shown that several factors influence sand temperatures on nesting beaches including: latitudinal variation, seasonal temperature changes, shading by vegetation, sand colour, depth of the eggs and episodic events such as rainfall (Morreale et al., 1982; Mrosovsky et al., 1984a,b; Mrosovsky, 1988; Godfrey et al., 1996; Hays et al., 1999, 2001). In addition, an increase in temperature attributed to metabolic heat produced by developing embryos has been recorded in green (*Chelonia mydas*) (Hendrickson, 1958; Carr and Hirth, 1961; Bustard, 1972; Morreale et al., 1982; Kaska et al., 1998; Broderick et al., 2001), hawksbill (*Eretmochelys imbricata*) (Raj, 1976), loggerhead (Maxwell et al., 1988; Neville et al., 1988; Maloney et al., 1990; Hanson et al., 1998), and leatherback (*Dermochelys coriacea*) (Godfrey et al., 1997) turtles.

The loggerhead turtle is widely distributed and extends into the Mediterranean with major nesting sites in Cyprus, Greece, Libya and Turkey (Groombridge, 1990; Laurent et al., 1998; Margaritoulis et al., in press). The regional annual nesting population, excluding a large, and as yet unquantified population in Libya (Laurent et al., 1997), is estimated as 2000 females and is considered regionally endangered (Groombridge, 1990). Analysis of mitochondrial (mt) DNA suggests that loggerhead turtles nesting in different parts of the world are genetically distinct (Encalada et al., 1998; Laurent et al., 1998). Given that the population should be considered as a management unit at the regional or individual rookery scale (Laurent et al., 1998), it is of fundamental interest to ascertain the sex ratio of hatchling production.

Sex ratios of loggerhead turtles in the Mediterranean have, as yet, been little studied. Kaska et al. (1998) found a mean sex ratio of 81.6% female in a small sample ($n = 8$) of loggerhead clutches at four sites in Cyprus and Turkey. These preliminary data suggested that the pivotal temperature was in the region of 29.0°C. Using another approach, Godley et al. (2001) analysed data regarding incubation durations of nests at Alagadi, Northern Cyprus, over six seasons (1993–1998). Because incubation duration depends on temperature, using inferences from other studies (Godfrey and Mrosovsky, 1997; Godfrey et al., 1999; Marcovaldi et al., 1997, 1999), it was possible to convert data on durations to hatchling sex ratios, which resulted in an estimation that 89–99% of the hatchlings produced at this site were females. In addition, on beaches other than Alagadi throughout Northern Cyprus, mean incubation duration of 70 nests in 1998 was 48.8 days, suggesting a similar female-biased sex ratio was typical.

In this study, our aim was to examine the thermal environment in the nests of loggerhead turtles in the Mediterranean. (1) We investigate the seasonal pattern of temperatures in both control sites and in nests and investigate the link with air temperature. (2) We describe temperature regimes experienced in 23 loggerhead turtle nests utilising intra-nest temperature recording devices, detailing incubation temperatures, diel temperature fluctuations and metabolic heating. (3) We describe the relationship between temperature and incubation duration. (4) Using this empirical relationship, we estimate the incubation temperature in nests of loggerhead turtle rookeries through-

out the Mediterranean and draw wider inferences as to the likely sex ratio of hatchlings produced in the region.

2. Materials and methods

2.1. Study site

Fieldwork took place at Alagadi (35°33'N, 33°47'E) situated in Northern Cyprus in the eastern Mediterranean. This is one of the main nesting sites in Cyprus for loggerhead turtles (range in seasonal total of nests: 38–95 per season, 1993–1999). The beach consists of two short coves, 0.8 and 1.2 km long, separated by a rocky headland and backed by extensive dunes and low scrub. There is no natural shading of the beach and although there is a high level of human usage, beach umbrellas and shades are only used at the water's edge, a region in which no clutches are laid. The climate is typical of the eastern Mediterranean, with virtually no rain from May to October and mean temperature for each 24 h during this period is generally in the region of 20–30°C.

2.2. Data collection

Nesting was recorded during nightly beach monitoring according to a previously described protocol (Broderick and Godley, 1996). Information on sand and nest temperature was gathered using 'Tinytalk' dataloggers (Orion Components, Chichester, UK) that recorded synchronously at 1-h intervals in 1999 and at 48-min intervals in 1996–1998. These dataloggers were calibrated with a mercury thermometer of known accuracy (NAMAS certified to read $\pm 0.1^\circ\text{C}$ of absolute temperature; Hays et al., 1999). Dataloggers were placed in two control sites in the nesting zone from 30 May 1999 until 30 September 1999 (encompassing the incubation of loggerhead turtle clutches). Dataloggers were placed at a depth of 45 cm, the mean depth of the middle of loggerhead clutches at this site (Broderick, 1997). In each of the seasons 1996–1999, temperature dataloggers were placed in the centre of clutches of eggs as they were being laid (n : 2 in 1996; 4 in 1997; 2 in 1998; 15 in 1999). The female covered and camouflaged the nest herself. Deployment date of dataloggers was spread throughout the season to give as wide a temporal coverage as possible. On chosen deployment dates, study nests were chosen randomly, with the first clutch on a deployment date being used. Dataloggers measured $5 \times 5 \times 3$ cm (volume: 75 cm³), representing some 3.3% of an average clutch as calculated conservatively by assessing the volume of eggs (mean clutch volume = 2306 cm³; mean number of eggs per clutch = 84.2 eggs; mean egg diameter = 3.74 cm; egg volume = $4/3\pi r^3$; Broderick and Godley, 1996; Margaritoulis et al., in press). Dataloggers started recording data at 6 am on the morning following deployment.

After 40 days, each nest was checked periodically throughout the night and again at dawn for signs of hatchling emergence. Incubation durations were calculated as the number of days between the night of laying and the night the first group of hatchlings emerged. Nest contents were excavated, nest chamber depth measured and dataloggers retrieved. Through a count of unhatched eggs and hatched shell fragments, clutch size (total number of eggs) and hatch success (number of eggs hatched/clutch size) were

calculated. In addition, the number of eggs with macroscopic dead embryos was enumerated.

Dataloggers were downloaded to a personal computer. For data manipulation purposes, each day was delineated at midnight, with the end of incubation being considered midnight on the night of the initial wave of hatchling emergence. Additional temperature data in the form of air temperature readings taken every 3 h at the Meteorological Centre at Kyrenia Harbour (16 km west of the study site) were made available by the North Cyprus Meteorological Service for May–August, 1999 from which a daily mean temperature was calculated.

3. Results

3.1. Basic nest parameters

Study nests had a mean incubation duration of 47.5 days (S.D.: 2.5, range: 42–52; Table 1) and a mean depth of 31 cm (S.D.: 7.8, range: 14–45 cm) and 48.5 cm (S.D.: 5.1, range: 38–57 cm) to top and bottom of the egg chamber, respectively. The mean

Table 1

Basic study nest information and descriptive statistics of temperature recorded throughout incubation, during the middle third of incubation and regarding mean daily range recorded

Nest	Lay date	ID (days)	Whole IP				Middle third IP				Daily range			
			Mean	±S.D.	Min	Max	Mean	±S.D.	Min	Max	Mean	±S.D.	Min	Max
1	13 June 1996	49	30.8	1.3	28.1	32.5	30.8	0.7	29.8	32.1	0.5	0.2	0.0	1.2
2	17 June 1996	48	30.8	1.5	27.7	33.0	30.8	0.5	29.8	32.1	0.4	0.2	0.0	0.9
3	10 June 1997	48	31.8	1.8	27.0	34.1	32.2	0.6	31.1	33.3	0.8	0.3	0.3	1.5
4	14 June 1997	42	31.8	1.3	27.0	34.1	32.1	0.5	30.6	32.9	0.9	0.7	0.3	4.9
5	27 June 1997	43	32.9	0.8	30.6	34.1	32.8	0.6	31.8	34.1	0.7	0.3	0.0	1.2
6	04 July 1997	44	32.9	0.5	31.6	34.0	32.9	0.2	32.5	33.5	0.3	0.3	0.0	1.0
7	09 June 1998	48	31.8	1.3	28.1	34.0	32.3	0.6	30.7	33.5	1.0	0.4	0.4	1.8
8	20 June 1998	50	32.2	1.6	28.8	34.9	31.8	0.9	30.3	33.7	0.7	0.2	0.3	1.2
9	30 May 1999	46	30.7	2.2	27.4	34.9	30.4	0.6	28.8	31.1	0.4	0.2	0.0	0.8
10	01 June 1999	50	29.5	1.6	26.3	32.2	29.3	0.5	28.1	30.3	0.4	0.3	0.0	1.4
11	02 June 1999	51	29.7	1.9	26.3	33.0	29.4	0.5	28.4	30.7	0.3	0.3	0.0	1.4
12	03 June 1999	46	32.0	1.9	28.1	35.7	31.5	0.9	29.2	33.0	1.4	0.5	0.4	3.8
13	05 June 1999	52	30.1	2.0	26.6	33.3	29.7	0.8	28.1	31.4	0.4	0.3	0.0	1.5
14	06 June 1999	52	30.8	1.8	26.6	33.3	30.4	1.0	28.4	32.2	0.6	0.3	0.3	1.5
15	13 June 1999	47	32.0	1.7	27.7	34.5	31.9	0.9	29.9	34.1	0.5	0.2	0.0	1.1
16	15 June 1999	48	31.3	1.4	28.1	33.7	31.4	0.9	29.9	33.3	0.9	0.3	0.4	2.6
17	15 June 1999	48	31.9	1.4	29.2	34.1	32.0	0.8	30.7	33.3	0.3	0.2	0.0	0.7
18	20 June 1999	46	31.8	1.2	29.0	33.5	32.2	0.5	31.0	33.0	0.4	0.2	0.0	1.1
19	23 June 1999	45	32.2	1.5	28.7	34.2	32.4	0.7	31.2	33.8	0.4	0.2	0.2	1.1
20	23 June 1999	47	32.8	1.2	29.0	34.5	33.7	0.5	32.6	34.5	1.0	0.2	0.5	1.6
21	27 June 1999	48	32.0	1.1	27.7	33.8	32.3	0.4	31.4	33.0	1.0	0.3	0.7	2.0
22	01 July 1999	46	33.2	0.8	29.9	34.5	33.5	0.4	32.6	34.1	0.9	0.3	0.4	1.9
23	10 July 1999	48	32.1	1.0	30.3	34.2	31.6	0.5	31.0	32.7	0.3	0.1	0.1	0.8

ID = incubation duration, IP = incubation period.

depth of study nests was, therefore, 39.8 cm. The mean clutch size was 84.7 eggs (S.D.: 16.0, range: 54–108 eggs) and clutches hatched with a mean hatching success of 0.77 (S.D.: 0.2, range: 0.27–0.98).

3.2. Seasonal profile in temperature at nest depth

The temperature recorded at the two control sites differed throughout the 1999 season (mean difference: 2.1°C, S.D.: 0.90; Fig. 1a). However, the hourly temperatures at these two sites followed similar seasonal patterns and were highly correlated during the period of study nest incubation (31st May–27th August: $r^2 = 0.82$, $F_{1,2128} = 9740$, $p < 0.001$) and the mean temperature of these two sites was used to illustrate the seasonal changes in sand temperature. When mean hourly temperature in nests throughout the 1999 season (range of n : 1–15; nest numbers: 9–23; Table 1) was compared with mean hourly control temperature (Fig. 1b), it was clear that although the two parameters were highly correlated ($R^2 = 0.99$, $F_{1,2128} = 187544$, $p < 0.001$; mean hourly nest temperature = 1.19 mean hourly control temperature -5.27), that after the first few weeks of monitoring, mean nest temperature was consistently higher than that of mean control temperature. In addition, the line of 29.0°C helps to illustrate that most nests will have been incubating above this threshold.

The mean daily sand temperature at the control sites was compared with mean daily air temperature during the same period (see Fig. 1c). Although air temperature is both lower than sand temperature and prone to greater short-term fluctuations, these parameters were highly correlated ($r^2 = 0.78$, $F_{1,87} = 311$, $p < 0.001$: mean daily sand temperature = 0.811 mean daily air temperature + 8.16). Additionally, composite mean daily nest temperature correlated significantly with mean daily air temperature ($r^2 = 0.77$, $F_{1,87} = 294$, $p < 0.001$: mean daily nest temperature = 0.962 mean daily air temperature + 4.44).

3.3. Intra-nest temperatures

Lay dates, summary incubation data and incubation temperatures for all 23 nests from four seasons are presented in Table 1. Temperatures ranged from 26.3°C to 35.7°C with mean temperatures of individual nests ranging from 29.5°C to 33.2°C (annual means: 1996, 30.8°C ($n = 2$); 1997, 32.4°C ($n = 4$); 1998, 32.0°C ($n = 2$); 1999, 31.5°C ($n = 15$); Table 1). During the middle third of incubation, temperatures ranged from 28.1°C to 34.5°C, with mean temperatures of individual nests during this period ranging from 29.3°C to 33.7°C (annual means: 1996, 30.8°C ($n = 2$); 1997, 32.5°C ($n = 4$); 1998, 32.1°C ($n = 2$); 1999, 31.4°C ($n = 15$); Table 1). Hatching success was not normally distributed (Anderson Darling, $p < 0.05$) so was subject to arcsine transformation. Arcsine hatching success of nests declined significantly with increasing temperature ($r^2 = 0.63$, $F_{2,20} = 17.4$, $p = 0.001$; Fig. 2). All nests experienced regular diel variation in temperature (see Fig. 3a and b) with mean values ranging from 0.3°C to 1.4°C in individual nests (annual means: 1996, 0.5°C ($n = 2$); 1997, 0.7°C ($n = 4$); 1998, 0.9°C ($n = 2$); 1999, 0.6°C ($n = 15$); Table 1). There was no correlation between the mean level of diel temperature change and either distance to the top ($r^2 = 0.01$,

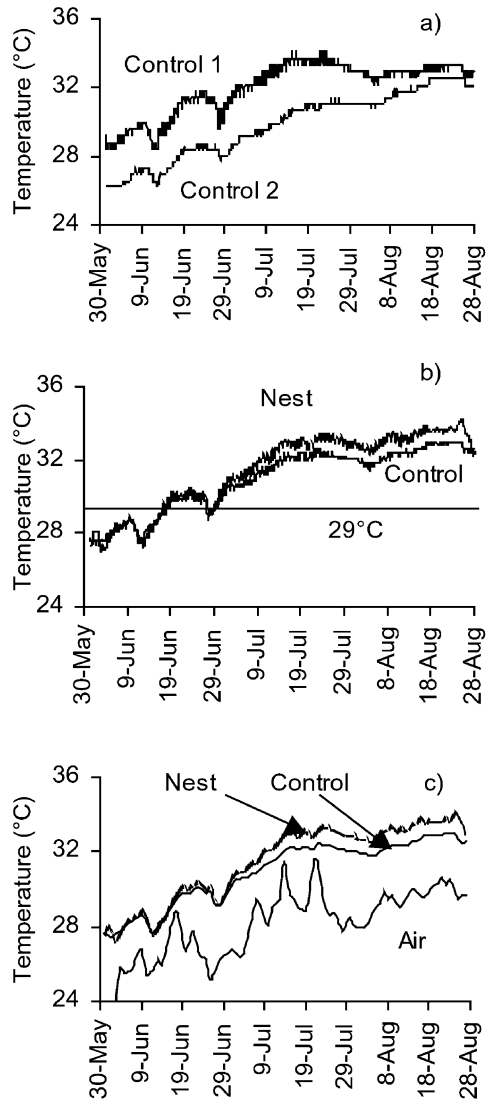


Fig. 1. Seasonal pattern in sand and nest temperatures at Alagadi on 31st May–27th August 1999 when the final study nest hatched. (a) Hourly temperature recorded at two control sites. (b) Mean hourly temperature at control sites and mean hourly nest temperature. (c) Mean daily nest temperature, mean daily control temperature and mean daily air temperature recorded at the Meteorological Centre at Kyrenia.

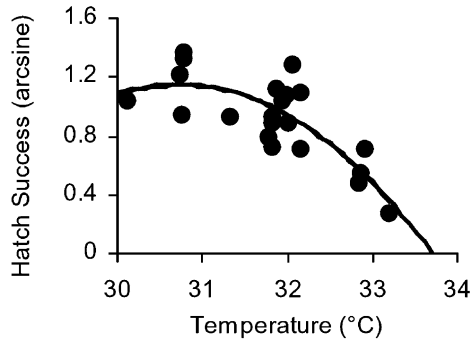


Fig. 2. Arcsine hatch success compared with mean nest temperature recorded throughout the incubation period. (Arcsine hatching success = $7.87 \text{ mean temperature} - 0.13 \text{ mean temperature}^2 - 120$.)

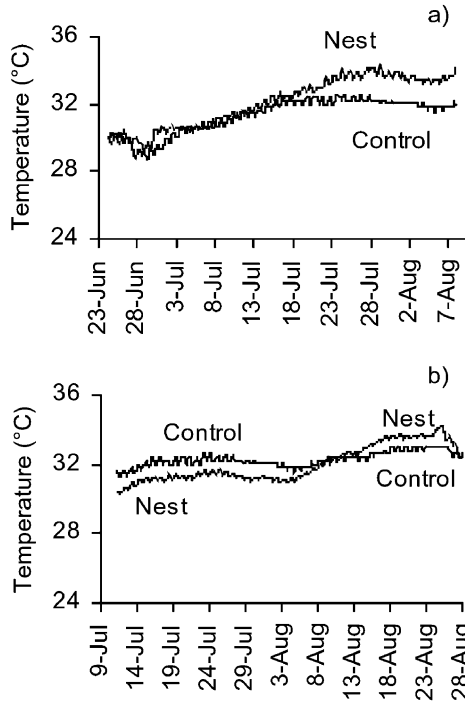


Fig. 3. Nest temperature profiles in comparison with mean control temperature. (a) Temperature recorded in nest 19. Clutch was laid on 23rd June and hatchlings emerged on 7th August 1999. (b) Temperature recorded in nest 23. Clutch was laid on 10th July and hatchlings emerged on 27th August 1999.

Table 2

Descriptive statistics of the level of metabolic heating recorded throughout incubation and during each third of incubation in study nests in 1999

Nest	Metabolic heating throughout IP				First third IP				Middle third IP				Final third IP			
	Mean	± S.D.	Min	Max	Mean	± S.D.	Min	Max	Mean	± S.D.	Min	Max	Mean	± S.D.	Min	Max
9	0.7	1.0	-0.6	2.3	-0.1	0.4	-0.6	0.8	0.2	0.6	-0.6	1.3	1.8	0.5	0.5	2.3
10	0.3	0.3	-0.6	0.7	0.3	0.2	-0.2	0.5	0.1	0.3	-0.6	0.6	0.5	0.2	0.2	0.7
11	0.7	0.5	-0.1	1.6	0.4	0.2	-0.1	0.7	0.5	0.2	0.0	0.7	1.3	0.2	0.9	1.6
12	0.2	0.6	-2.0	1.2	0.0	0.5	-1.4	-0.1	-0.2	0.6	-2.0	0.4	0.7	0.3	0.2	1.2
13	0.4	0.6	-0.1	1.5	0.0	0.3	-0.6	0.3	0.0	0.3	-0.8	0.4	1.1	0.3	0.5	1.5
14	0.1	0.5	-1.4	0.8	-0.1	0.4	-1.1	0.3	-0.2	0.4	-1.4	0.3	0.6	0.2	0.2	0.8
15	0.6	0.5	-0.9	1.5	0.2	0.4	-0.9	0.7	0.5	0.3	0.1	1.1	1.1	0.3	0.5	1.5
16	-0.4	0.4	-1.8	0.3	-0.6	0.5	-1.8	0.0	-0.5	0.3	-0.9	0.0	0.0	0.2	-0.3	0.3
17	0.0	0.3	-0.7	0.6	-0.3	0.3	-0.7	0.0	-0.1	0.2	-0.4	0.3	0.4	0.2	0.0	0.6
18	0.0	0.3	-0.9	0.4	-0.2	0.4	-0.9	0.2	0.0	0.1	-0.3	0.2	0.2	0.1	0.0	0.4
19	0.9	0.7	-0.5	2.0	0.3	0.4	-0.5	1.0	0.6	0.3	0.2	1.4	1.7	0.2	1.4	2.0
20	1.0	0.4	-0.7	1.6	0.8	0.6	-0.7	1.5	1.3	0.2	0.9	1.6	0.9	0.2	0.5	1.2
21	0.5	0.4	-0.5	1.1	0.0	0.3	-0.3	0.5	-0.2	0.2	-0.5	0.1	0.6	0.3	0.1	1.1
22	0.7	0.3	-0.1	1.1	0.7	0.4	-0.1	1.1	0.6	0.2	0.4	0.9	0.7	0.2	0.4	1.0
23	0.6	0.7	-0.2	2.0	0.0	0.1	-0.2	0.2	0.3	0.4	0.0	1.1	1.5	0.3	0.9	2.0

IP = incubation period.

$F_{1,21} = 0.17, p = 0.686$) or the bottom ($R^2 = 0.08, F_{1,21} = 1.75, p = 0.20$) of the egg chamber.

3.4. Metabolic heating

Illustrations of the typical relationship between the mean control site temperatures and within nest temperatures are given in Fig. 3a and b for two nests throughout the incubation period. Note the regular diel fluctuation in both the nest and control site temperatures and that although nest temperatures generally track the pattern of control site temperatures, there is a marked progressive increase in temperature in mid incubation. This increase was attributed to metabolic heating.

There are two main processes that can drive changes in nest temperature over extended time scales: (1) seasonal changes and (2) metabolic heating. Control loggers described the seasonal change in sand temperature. After 1 day of incubation, nest temperatures had equilibrated with the surrounding sand. To calculate the extent of metabolic heating, we simply determined the change in nest temperature from day 2 to day x of incubation and subtracted the seasonal change in sand temperature over the corresponding period.

Table 2 details descriptive statistics of estimated levels of metabolic heating for all 15 nests that were studied in 1999 for which 14 of the nests show demonstrable levels. Over the whole incubation period, mean levels of our index of metabolic heating ranged from -0.4°C to $+1.0^{\circ}\text{C}$ (mean: 0.4°C , S.D.: 0.4). However, the level of metabolic heating varied markedly throughout the incubation period with levels being significantly higher in the final third of incubation period (ANOVA: $F_{2,42} = 13, p < 0.001$; mean first third: 0.1°C , S.D.: 0.4; mean middle third: 0.2°C , S.D.: 0.5; mean final third: 0.9°C , S.D.: 0.5).

A multiple stepwise regression approach was used to investigate if there were any effects of arcsine hatch success, clutch size, number of hatchlings, or total number of embryos, i.e. hatchlings and partially developed embryos on overall mean metabolic

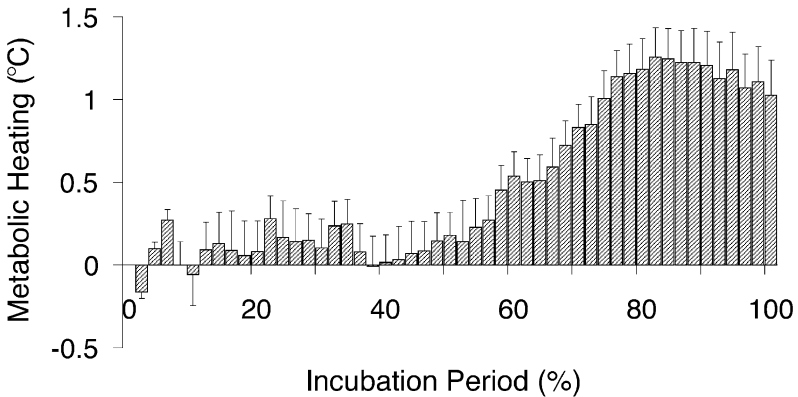


Fig. 4. Mean metabolic heating in each two percentiles of incubation in clutches ($n = 15$) studied in 1999. Error bars denote 1 S.D.

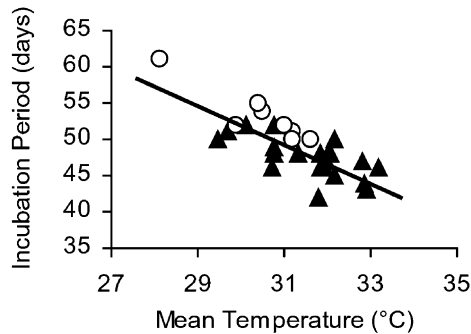


Fig. 5. Relationship between mean incubation temperature and incubation period in 23 clutches from this study (triangles) and 8 clutches (circles) from the study by Kaska et al. (1998). (Incubation period = -2.66 mean temperature + 132.11.)

heating or the mean measure in either the first, middle or final third of incubation. None of the variables were found to exert a significant effect (regression analysis; $p > 0.05$).

To more clearly visualise the pattern of metabolic heating, we converted the incubation period in days into the proportion of incubation period. Fig. 4 shows the mean amount of metabolic heating in each two percentiles of incubation. Although there appears to be some level of metabolic heating from very early in incubation, it is variable and generally below 0.1°C until about 40% of the way through incubation. At this point, a steady rise in the level of heating is demonstrated to a peak of 1.0°C , 84% of the way through incubation, at which point there is a slow decline, with levels of heating staying above 0.8°C until hatching.

3.5. Relationship between nest temperature and incubation period

Incubation durations (Table 1) were found to be significantly correlated to both mean temperature throughout the incubation period ($R^2 = 0.43$, $F_{1,21} = 15.87$, $p = 0.001$) and during the middle third of incubation ($R^2 = 0.44$, $F_{1,21} = 16.39$, $p = 0.001$). When we augment this data set with the eight nests studied by Kaska et al. (1998), we obtain similar relationships of higher significance, with incubation durations being correlated to both mean incubation temperature ($r^2 = 0.62$, $F_{1,29} = 48.09$, $p < 0.001$) and that during the middle third ($r^2 = 0.51$, $F_{1,29} = 29.97$, $p < 0.001$). The relationship between incubation period and mean incubation temperature is shown in Fig. 5.

4. Discussion

4.1. Basic nest parameters

The basic nest parameters in the study nests are comparable with those described previously for this site (Broderick and Godley, 1996; Godley et al., 2001; Margaritoulis et al., in press). Females nesting in the Mediterranean are smaller than most populations

(Dodd, 1988) and those nesting in Cyprus are the smallest in the Mediterranean (Broderick and Godley, 1996; Margaritoulis et al., in press). It is likely that small body size is a factor leading to the small clutches deposited at this site that are at the extreme low end of the global range of 85–105 reviewed by Dodd (1988). Although we inferred clutch size from a posthatching egg count, any associated error is unlikely to exert a systematic bias to the results of this study. Equally, although this sample of nests had a mean depth slightly shallower than that of the control dataloggers, it appears that the control sites, were on average, adequate to describe the prevailing seasonal change in sand temperature.

4.2. Seasonal profile in temperature at nest depth

It is clear from our results (Fig. 1b and c) that there is a close linkage between prevailing air temperature and sand temperature at nest depth and temperature within nests as has been found in other studies (Maloney et al., 1990; Hays et al., 1999). These relationships can be useful for predictive purposes. However, it is likely that sand colour and relative reflectance will mean that any such relationship is beach-specific, even when meteorological data are comparable (Hays et al., 1995, 2001). However, the difference between the two control sites demonstrated here (Fig. 1a) highlights the need for multiple control sites to account for any intra-beach variability when control site temperatures are used in a predictive manner. The difference in control site temperatures in this study was not important as these data were only used to illustrate seasonal changes and both sites showed very similar seasonal profiles. In the calculation of metabolic heating, differences between absolute values of temperature in the nest and at control sites were controlled for.

4.3. Intra-nest temperatures

Mean temperatures throughout incubation and during the thermosensitive period are above 29.0°C (approximate pivotal temperature) in all nests, often approaching the upper critical limit for incubating eggs of 35°C (Ackerman, 1997). Indeed, it is likely that the decline in hatch success with increasing mean incubation temperature demonstrated here (Fig. 2) is a manifestation of critical effects. From a TSD perspective, these data are suggestive of nests that would produce an extremely high proportion of females, consistent with the estimates generated by Godley et al. (2001).

Although few nests were studied 1996–1998, these earlier data appear similar to those of 1999. Nests were selected at random on given study nights but deployment dates were selected so as to optimise resources and monitor nests through as much of the season as possible. In 1999, the main season of study, this was attained with monitoring being carried out from the first clutch to be laid (nest 9), with constant monitoring of between 1 and 15 nests until nest 23 hatched on 27th August, with only 4 of 49 nests hatching after this date, all within 14 days. The composite nest profile (Fig. 1b and c) can, therefore be relied upon as representative.

Diel temperature fluctuations are expected in such shallow nests in contrast to the deeper nests of the green turtle (*C. mydas*) at the same site (Broderick et al., 2000) that

show no regular diel temperature variation. The levels and patterns of diel variation are comparable with nests of the same species studied in Australia (Maloney et al., 1990), South Africa (Maxwell et al., 1988), Cyprus and Turkey (Kaska et al., 1998), and the USA (Hanson et al., 1998).

Direct measurement of temperature in a large sample of nests is a step forward from inferential studies based solely on control site temperatures. However, one criticism that could be levelled at studies such as this is that by recording temperatures from only one datalogger, we may not be collecting data that are typical for the clutch. This criticism has been highlighted by the work of two studies to date by the placement of dataloggers at the top, middle and bottom of the clutch. Hanson et al. (1998) studied three such nests of the loggerhead turtle in the USA, finding a mean of 0.9°C within the nest range, with mean temperatures in the middle of the clutch during the middle third of incubation being 0.4°C and 0.9°C above the top and bottom of the clutch, respectively. Hanson et al. (1998) suggested, therefore, that the middle dataloggers seemed reasonable for predicting temperature of most eggs but that it might slightly overestimate temperature of some at the periphery of the clutch, a factor that is only likely to be of significance for nests incubating within the transitional range of temperatures. Comparable results were found in eight loggerhead turtle nests in the Mediterranean studied by Kaska et al. (1998), which also showed a mean intra-nest range of 0.9°C. In addition, although not yet expressly investigated, metabolic heating is likely to be greatest in the centre of the clutch, so estimates herein should be considered maximal values.

4.4. Metabolic heating

Metabolic heating was described in all but one of the 1999 study nests, and was in general confined to the latter part of incubation as has been found in previous studies of the same species in South Africa (Maxwell et al., 1988). However, in the South African study, the magnitude of metabolic heating was greater, possibly as much as 3°C. It may be that the small clutch volume of Mediterranean loggerhead turtles leads to less metabolic heating. It has been suggested that metabolic heating will be insignificant concerning TSD unless greater than 1°C (Mrosovsky and Yntema, 1980). If prevailing sand temperatures used for predicting nest temperatures are close to the pivotal temperature, then the metabolic heating may significantly affect calculations. Within the population under study, the mean difference of 0.2°C is not likely to be significant. A far more important source of potential error of such predictions would be the intra-nest thermal range.

In this study, we cite field incubation durations, i.e. duration from laying to emergence, which is the period it takes for hatchlings to complete the hatching process and ascend through the sand to the surface. This will in fact differ from the true incubation duration, i.e. laying to hatching, by the order of several days. Although Godfrey and Mrosovsky (1997) estimate this lag as some 4.1 days for loggerhead turtles in the USA, we do not know what the duration is in Mediterranean loggerhead turtles. Assuming it is comparable, although slight changes in the estimated timings of the thermosensitive period will result, the total difference to mean metabolic heating calculations will be negligible.

The systematic positive difference between mean nest temperature and mean control temperature (Fig. 1b) is undoubtedly indicative of metabolic heating. The difference only evolves after the first clutches have reached mid to late incubation, the period when, as we have demonstrated, metabolic heating begins to rise (Fig. 4).

4.5. Relationship between nest temperature and incubation period

Although incubation period is a crude measure of developmental rate (Georges et al., 1994), it is a parameter for which data are readily collected in a low-tech manner giving meaningful integrated information regarding the nest environment. It is clear that incubation period is largely influenced by temperature as has been previously described (Ackerman, 1997), and incorporating the data from Kaska et al. (1998) has allowed us to describe this relationship for Mediterranean loggerhead turtles in a more rigorous manner than using our data alone. In the 23 study nests at Alagadi, there was little difference in the amount of variance in incubation period accounted for by the significant relationships with mean incubation temperature and mean temperature during the middle third of incubation. Worthy of note, however, is the fact that there is a closer co-relationship between the overall mean incubation temperature than that of the middle third (r^2 of 0.62 versus 0.51). Although the thermal environment in the middle third is that which will dictate the sex of the offspring, the incubation duration is an integration of the speed of development throughout incubation. These results mirror those for green turtles nesting at Alagadi (Broderick et al., 2000).

4.6. Estimating nest temperatures throughout the Mediterranean

As yet, no overall estimation of loggerhead hatchling sex ratio has been undertaken in the Mediterranean region, although Godley et al. (2001) have suggested that based on data regarding incubation durations in Cyprus and available published data on incubation durations, sex ratio of hatchling production is likely to be female-biased. The biology of loggerhead turtles in the Mediterranean has recently been reviewed (Margaritoulis et al., in press) and these authors presented many data on mean incubation durations over many seasons at several key sites subject to monitoring in the region. The incubation durations cited in Margaritoulis et al. (in press) and additional published and unpublished data are given in Table 3. On available nesting figures, these beaches constitute a large proportion of nesting activity in the region, representing approximately 10%, 85% and 50% of totals for Cyprus, Greece and Turkey, respectively. It should be noted that data from Egypt and Israel are from clutches transplanted to hatcheries and are the only data available.

Given that we have demonstrated a clear link between incubation temperature and incubation duration in this regional population (Fig. 5), it is possible to use this relationship to estimate mean incubation temperatures for the studies cited in Table 3. Estimated mean incubation temperatures for these studies are plotted in Fig. 6.

At the present, no pivotal incubation period for Mediterranean loggerheads has been generated from laboratory work, with the only study carried out in the region (Kaska et

Table 3

Mean incubation durations for sites subjected to monitoring around the Mediterranean

Country, area	Mean number of nests per season	Number of seasons monitored	Range of annual mean incubation durations	Literature source
<i>Cyprus</i>				
Alagadi	60	7	47.3–48.7	Margaritoulis et al., in press
<i>Greece</i>				
Bay of Chania	115	2	53.3–54.3	Margaritoulis et al., in press
Kefalonia	29	1	54.9	Margaritoulis et al., in press
Kyparissia Bay	581	3	48.2–55.5	Margaritoulis et al., in press
Lakonikos Bay	192	5	52.1–59.3	Margaritoulis et al., in press
Rethymno	387	3	51.7–55.2	Margaritoulis et al., in press
Rhodes	11	2	49.0–55.0	Margaritoulis et al., in press
Zakynthos	1286	4	57.4–61.9	Margaritoulis et al., in press
<i>Libya</i>				
	Large but not quantified	1	55.0	Margaritoulis et al., in press
<i>Turkey</i>				
Akyatan	15	1	52.0	Margaritoulis et al., in press
Anamur	191	1	51.3	Margaritoulis et al., in press
Dalyan	165	1	59.3	Margaritoulis et al., in press
Fethiye	124	2	55.0–56.9	Margaritoulis et al., in press
Göксу	65	2	54.8–57.0	Van Pigglen, 1993; Peters and Verhoeven, 1992
Kizilot	107	1	59.6	Kaska, 1993
Patara	53	1	60.0	Kaska, 1993
<i>Egypt</i>				
Sinai Peninsula ^a	Small but not quantified	1	49.3	Clarke et al., 2000; Clarke, pers. comm.
<i>Israel</i>				
Whole coastline ^a	30	6	52.7	Kuller, 1999; Kuller, pers. comm.

^aRepresents data from hatcheries where no other data are available.

al., 1998) suggesting that the pivotal temperature was just below 29°C. This is similar to the values of 29.2°C for Brazil (Marcovaldi et al., 1997) and 29.0°C for the USA (Mrosovsky, 1994). As a point of reference, if we assume that the pivotal temperature is 29.0°C, it can be seen in Fig. 6, that because the mean temperature experienced by nests incubating throughout the Mediterranean is likely to be well above 29.0°C at most sites in most years, that female-biased sex ratios must be common. However, it would appear that at least in some years and some sites (Zakynthos and Lakonikos Bay (Greece), Dalyan, Kizilot and Patara (Turkey)), mean temperatures may be just below 29°C; suggestive of balanced or even slightly male-biased hatchling sex ratios.

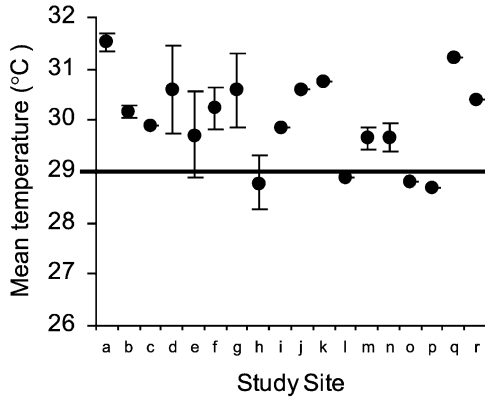


Fig. 6. Estimated mean nest temperatures throughout the Mediterranean as calculated using the equation derived in Fig. 5 and the data in Table 3. Bars represent range of values when data were present for several seasons. (Key: Cyprus: (a) Alagadi; Greece: (b) Bay of Chania; (c) Kefalonia; (d) Kyparissia Bay; (e) Lakonikos Bay; (f) Rethymno; (g) Rhodes; (h) Zakynthos; Libya: (i) not specified; Turkey: (j) Akyatan; (k) Anamur; (l) Dalyan; (m) Fethiye; (n) Göksu; (o) Kizilot; (p) Patara; Egypt: (q) Sinai Peninsula; Israel: (r) whole coastline.)

This may be analogous to the situation in Brazil where, although most sites are largely female-producing, some key sites may have conditions that are biased toward the production of male hatchlings (Baptistotte et al., 1999). Although subject to limited geographical sampling and possible artefact as a result of transplantation (see Table 3), it would appear that sites such as Cyprus, Israel, Egypt and Libya are likely to produce hatchlings with highly female-biased sex ratios. The data are not yet available to make a detailed comparison of expected sex ratios among sites or among years but it would appear that with data already collected by workers in the region, a plausible estimate of hatchling sex ratio for the Mediterranean may be attainable once a clearly defined pivotal temperature for the Mediterranean population is generated by laboratory and/or field-based studies.

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