BEACH TEMPERATURES, INCUBATION DURATIONS AND ESTIMATED HATCHLING SEX RATIO FOR LOGGERHEAD SEA TURTLE NESTS IN SOUTHERN KYPARISSIA BAY, GREECE

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INTRODUCTION

It is well established that loggerhead sea turtles (*Caretta caretta*) exhibit temperature-determined sexual differentiation (TSD) that depends on the proportion of time at temperature during the thermosensitive period (TSP), which is the middle third of the incubation duration (Mrosovsky & Pieau, 1991). A theoretical constant pivotal temperature produces a 50% male:female ratio of hatchlings and within a small transitional range of temperatures (TRT), cooler temperatures produce more males and warmer temperatures more females. TSD facilitates significant variation from the sex ratio of 1:1 that is suggested to be normal for species with equal cost of producing male and female offspring (Fisher, 1930).

In the field, incubation temperature and hence sex ratio may vary with a diversity of factors such as latitude, beach orientation, sand albedo and composition and due to inter- and intra-seasonal environmental changes.

Where pivotal temperatures are known, incubation temperatures can be used to predict hatchling sex ratios (Mrosovsky, 1988; Marcovaldi *et al*, 1997). Likewise, differences in nest temperatures manifest themselves as extended or shortened incubation durations (ID), for cooler and warmer conditions respectively, and thus ID can be used to some extent to infer sex hatchling ratios (Mrosovsky *et al*, 1999).

Use of either incubation temperature or duration is preferred for determining sex ratios for long-term studies, as they do not require sacrifice of hatchlings for sex determination analysis.

Pivotal temperatures and estimates of hatchling sex ratio have been elucidated for several loggerhead populations (Australia, Brazil, Cyprus, Greece, Japan, Turkey and the USA [for references see Mrosovsky *et al*, 2002]) and have shown a conservative range of pivotal temperatures (within about 1 degree of 29°C) with often highly skewed primary sex ratios (Mrosovsky, 1988, Marcovaldi *et al*, 1997; Kaska *et al*, 1998; Godley *et al*, 2001a&b;). The skewed sex ratio has been

proposed to be either a result of sampling bias or real. If real, then it is possible, but unlikely, that hatchlings of opposite sex to those predominating from studied populations hatch elsewhere, thus balancing the overall ratio in the population (Godley *et al*, 2001a).

Within Greece, Mrosovsky *et al* (2002) calculated the pivotal nest temperature for loggerheads nesting in southern Kyparissia Bay at 29.7°C. Using this and related data (Godfrey & Mrosovsky, 1997) the pivotal incubation duration was estimated at 56.6 days.
It should be noted that some authors quote temperatures found within eggs for pivotal temperatures (Mrosovsky, 1988; Marcovaldi *et al*, 1997; Mrosovsky *et al*, 2002), which are 0.4°C or 0.5°C lower than the corresponding incubation temperatures, but as this article presents beach temperatures the incubation temperatures are used.

Better knowledge of beach temperatures and records of incubation duration will provide increased understanding of natural sex ratios for sea turtle populations that have implications for conservation practices (Mrosovsky & Yntema, 1980) such as the possible effects that nest relocation to hatcheries, may have.

In this article we describe the natural beach temperature regimes and incubation durations for nests in southern Kyparissia bay from the May to October 2003 nesting season and from these make some predictions of primary sex ratio of hatchlings.

MATERIALS AND METHODS
Study site
Kyparissia Bay (37°22′N, 21°41′E) is an open bay comprising a wide sandy beach for the majority of its 40km. Human development is restricted to a few villages that encroach upon the shore most notably at the southern tip of the bay where a

Kyparissia Bay
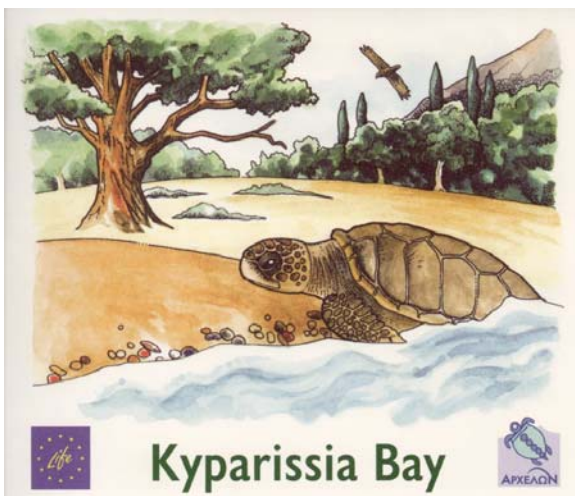
1km stretch of the beach is backed by an asphalt road, municipal street lights, bars and taverns. The bay hosts the second largest nesting population of loggerheads in the Mediterranean, with on average 620 nests per season (Margaritoulis & Rees, 2001). The most prevalent threats to sea turtle nest in this area are predation by red foxes (*Vulpes vulpes*) and feral dogs and severe inundation from the sea during the common periods of high surf (Margaritoulis, 1988; Rees *et al*, 2002). Field actions were carried out on the southernmost 9.2km of beach in the core nesting area (Margaritoulis & Rees, 2001). There is no natural shading of the beach and beach umbrellas etc. were kept at a distance from incubating nests.

In order to better understand the variability of beach conditions existing in southern Kyparissia Bay and hence predict hatchling sex ratio, the beach was divided along its length into ten sections. Sections were defined in distances from the southernmost origin of the beach. The first 2,200m of beach is narrowest and exhibits varying conditions hence section lengths were 600, 900 and 700m, stations (where temperature measurements were taken) at 420, 800 and 1,700m respectively, and from thereon sections were 1km in length, centered on stations from 2,700m to 8,700m.

Nest data collection

To identify variation in ID, nesting and hatching activity was observed over the ten beach sections, in the morning on a daily basis, as part of the long-term monitoring project undertaken by ARCHELON since 1984 (Margaritoulis & Rees, 2001). Each nest was dug to precisely locate the clutch, so that protective screens could be placed over them to deter predators. If a nest was considered "doomed", as it was made too close to the sea, it was relocated further up the beach to a safe spot. Nest distance from the sea was recorded from the nest location to the sea-line, measured on calm days. For the purpose of this article, ID is defined as the period in days between observation of the newly made nest and the first record of hatching, from observation of hatchlings or their tracks on the beach.

Temperature data collection

Temperature was recorded by deploying loggers (Tinytalk and Tinytag varieties, Gemini Data Loggers, UK.) at the predetermined stations.
Sand surface and air temperatures were recorded together with along-the-beach temperatures at mid-nest depth. Additionally, temperatures were recorded at different depths in the sand and at differing distances from the sea.

Sand surface temperature (logger buried 2cm in sand) was recorded at 26m from the sea, to avoid cooling from inundation, at 3,700m. Air temperature was recorded in an area of permanent shade, 1m above the ground, approximately 100m behind the beach at 3,700m.

Mid-nest depth temperatures were recorded at 40cm depth at a distance of 26m from the sea, at stations 2,700m to 8,700m, and at a distance of 21m from the sea at stations 420m, 800m and 1,700m. The depth of 40cm was chosen, as it is the average mid-nest depth (top of nest=29cm, bottom of nest=49cm, unpublished data) for nests in southern Kyparissia Bay. Loggers at 5,700m and 7,700m were removed on 22 July to be used for the other investigations.

Loggers used for sand surface, air and mid-nest depth temperatures were programmed to record at 90-minute intervals.Sand temperature variation with depth and distance to sea were recorded between 23 and 25 July. The sea was calm and there was no cloud cover during this period.
To record variation of temperature with depth, loggers were programmed to record every 20 minutes and were placed at 1, 10, 25, 40 and 55cm for 48 hours, 30m from the sea at 6,500m. To record the variation of temperature with distance from the sea, loggers were programmed to record every 5 minutes and were placed at 10, 20, 30 and 40 m from the sea, 40cm depth for 24 hours at 6,800m. In all cases, initial temperatures recorded by the loggers were excluded until they had reached equilibrium, which was determined by visual examination of data.

RESULTS
Nesting season and nest numbers
The nesting season lasted from 25 May to 6 Aug, in which time 451 nests were recorded of which 106 were relocated to avoid destruction by storm waves. Distribution of nests in time and space are shown in Figures 1 & 2. Nests that were inundated or depredated (N=78) were excluded from calculations of ID and mean distance from the sea.
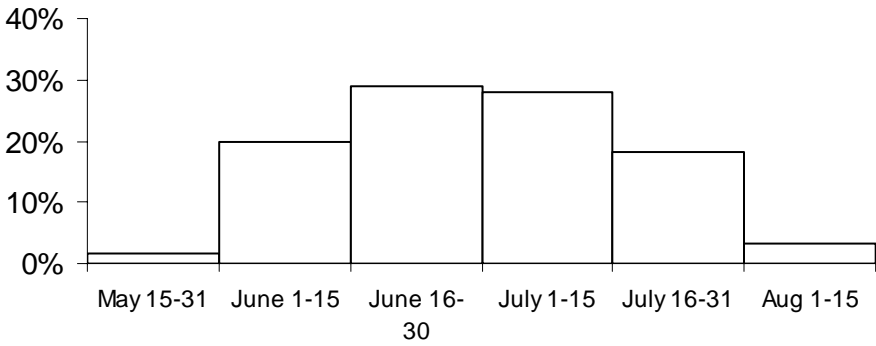
Figure 1. Nest distribution in southern Kyparissia Bay divided into half-month bins defined by laying date.
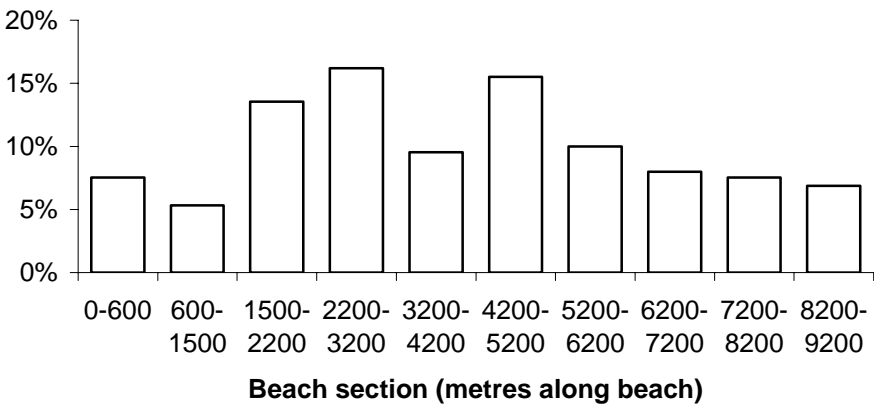

Figure 2. Nest distribution in southern Kyparissia Bay divided into 10 sections defined by topological changes and temperature logger stations that were generally located near the centre of each section.

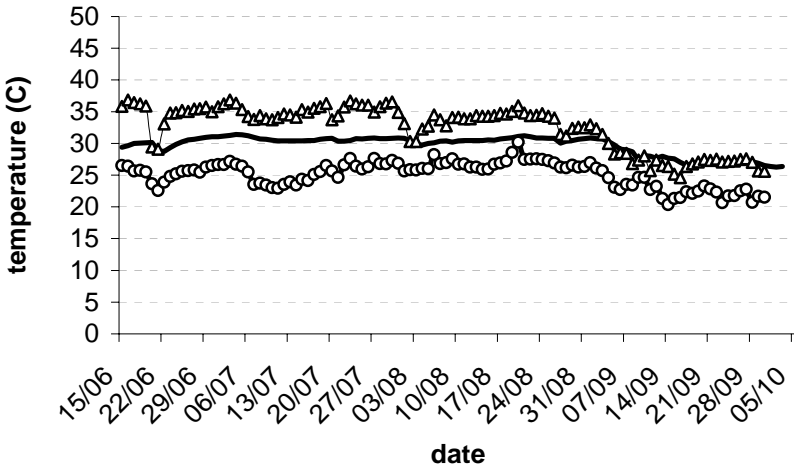# Correlation between air, sand-surface and mid-nest depth temperatures

Figure 3. Averaged daily temperatures recorded over the season in southern Kyparissia Bay (at station 3,700m). ○= air temperature, Δ= sand-surface temperature and — = sand temperature at mid-nest depth.

Comparing air, sand-surface and mid-nest depth temperatures at the same station (Figure 3), we get best correlation between the sand-surface and mid-nest depth (R^2=0.88) and slightly less between air and either sand-surface (R^2=0.78) or mid-nest depth (R^2=0.80). It should be noted that sand-surface temperature sometimes exceeded 51.9°C, which is the maximum in the loggers' temperature range and hence the value reported is biased to a lower than actual temperature.

Mid-nest depth temperatures
A delay in logger placement, due to logistical and personnel restrictions, meant that temperature recording commenced 13 June (when approximately 70 of the 451 nests made at the study site had already been deposited) and continued until 3 October when most of the nests had hatched. This time frame managed to encompass the entire important middle-third, thermosensitive period for all but 7 nests. However, the logger at 1,700m was never retrieved and hence beach temperature is missing for that section which held 21 nests.

The mid-nest depth temperature, as recorded by loggers stationed along the beach, was generally high until the start of September when it decreased rapidly. Several periods of inclement weather (overcast skies, rain and heavy seas) that caused notable temperature changes included 19-21 June, 20 July, 31 July-3 Aug,

26 Aug, 1-15 September (when rough seas and overcast skies prevailed) and 29 Sept. More rain was reported for 7-8 October and hence beach temperature levels were not expected to have subsequently recovered (over about 26°C) after the period of data collection by the loggers.

The mid-nest depth temperatures along the beach correlated well between stations (mean R^2=0.95, range 0.90 to 0.99) for the first 38 days, when all loggers were present at the nine 9 stations from which loggers were retrieved. Diel variation of mid-nest depth temperatures had a mean that ranged from 0.6 to 1.1°C (S.D. ≤0.3) from the different stations. The maximum variation recorded was 2.3°C for a single day at one station.

Temperature variation with depth and distance from the sea
As to be expected, beach temperatures were less variable and decreased with increasing depth (Table 1). The mean daily temperature was shown to differ by 1.2°C between the average top and bottom nest depths.

Logger depth (cm)	Mean (°C)	S.D.	Min	Max	N
2	36.2	9.9	23.7	51.9	144
10	34.1	3.2	29.8	39.4	144
25	32.7	0.9	31.4	33.9	144
40	31.6	0.3	31.0	32.2	144
55	30.8	0.2	30.6	31.0	144

Table 1. Temperature variation with depth in beach. Loggers placed for 48 hours on 23 July at 30m from the sea and 6,500m along the beach.

Beach temperature was shown to increase with distance from the sea in a near linear fashion (R^2=0.99). However a quadratic equation (Equation 1) was used to adjust beach temperatures for distance from the sea as it matched the data points even more closely (R^2=1.0).

$$\text{Beach temperature} = -0.0019X^2 + 0.261X + 24.98$$
where X is distance from the sea in metres

Equation 1. Temperature variation with distance from sea. Loggers placed at 40cm depth, for 24 hours on 24 July, at 10, 20, 30 and 40 m from the sea and 6,800m along the beach.

Mid-nest depth temperatures during the TSP
Temperatures for middle third of incubation period, based on individual-nest
laying date, were calculated per beach section and were adjusted for mean nest
distance to the sea (using Equation 1) as nest distance to the sea differed
significantly in certain sections of beach (ANOVA, P=0.00, normality checked
with the Kolmogorov-Smirnov test). These temperatures were compiled into
half-month bins for later analysis (Table 2). Figure 4 shows the temperature
limits of the TRT and the zones differing sex ratio that are used in later analysis
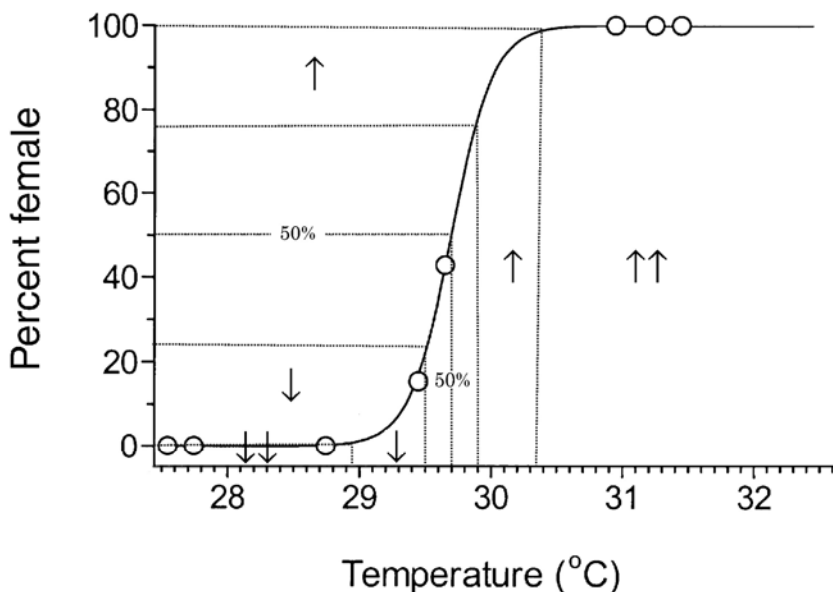and as indicators in Table 2.

Figure 4. Percent female hatchlings as a function of temperature (adapted from
Mrosovsky *et al*, 2002). ↓= 0.2°C less than pivotal temperature to lower TRT
boundary. ↓↓= below TRT. ↑= 0.2°C more than pivotal temperature to upper TRT
boundary. ↑↑=above TRT.

	420	800	1700	2700	Station 3700	4700	5700*	6700	7700*	8700
May 16-31	28.4↓↓	27.9↓↓	X	30.2↑	30.4↑↑	31.3↑↑	29.6	30.4↑	30.8↑↑	29.3↓
June 1-15	29.1↓	28.3↓↓	X	30.7↑↑	31.1↑↑	32.1↑↑	30.6↑↑	31.1↑↑	31.7↑↑	30.1↑
June 16-30	29.4↓	28.2↓↓	X	30.6↑↑	31.0↑↑	32.3↑↑	30.4↑	30.8↑↑	31.4↑↑	29.8
July 1-15	29.4↓	28.3↓↓	X	30.6↑↑	30.9↑↑	32.3↑↑	30.4↑	30.4↑	31.3↑↑	29.5
July 16-31	29.5	28.5↓↓	X	30.8↑↑	31.0↑↑	32.6↑↑	30.6↑↑	30.5↑↑	31.5↑↑	29.7
Aug 1-15	29.6	28.6↓↓	X	30.7↑↑	31.0↑↑	32.7↑↑	30.6↑↑	30.6↑↑	31.5↑↑	30.0↑

Table 2. Average temperature (°C) at mid-nest depth per station, in half-month bins, during TSP defined by individual-nest laying date. Arrows indicate the sex ratio zone in which the temperatures occur, as defined in Figure 4. *Values from 7 July are extrapolations from data obtained over the first 38 days of data acquisition. Temperatures were averaged for the period when data was collected for the seven May nests for which temperatures of the full TSP are not known. X indicates no data obtained as the temperature logger was not retrieved.

ID per beach section

All ten beach sections displayed an average ID that was below the theoretical 56.6 days derived by Mrosovsky *et al* (2002), thus by this measure, the majority of nests produced mainly or exclusively female hatchlings. The range of incubation periods indicated that only four beach sections had any significant number of nests with ID greater than pivotal (Figure 4).
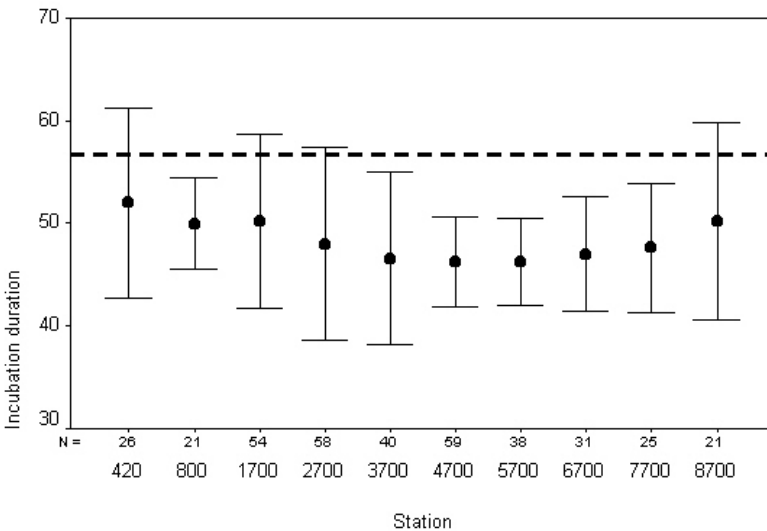

Figure 5. Incubation periods for non-inundated and non-predated nests in southern Kyparissia Bay. Data are plotted in days as means ±2SD. The horizontal bar is the theoretical pivotal ID (56.6 days) from Mrosovsky *et al* (2002).

Correlation of mean ID with beach temperature (temporal & sectional)

Spearman's Rank correlation indicates a significant negative association between mean beach temperature for middle third of ID (derived from sectional and temporal division of data) and ID (r_s=-0.516, P<0.01). Likewise, station-mean temperature has a significant negative association with ID (r_s=-0.678, P<0.05). However, these associations explain respectively only 24% and 55% of variation in ID and hence beach temperature cannot be used to accurately predict ID at this level. Approximately kilometer-long sections contain conditions too variable to accurately relate ID with beach temperature from a single station, highlighted most notably in the beach section containing station 800m where ID indicates all-female hatchling production and the mid-nest depth temperature indicates all-male hatchling production.

Estimation of primary hatchling sex ratio

Using the temporal and spatial distribution of nesting and beach temperatures presented above (Figures 1 and 2 and Table 2) and information derived from Mrosovsky *et al** (2002), it was calculated that 70% of hatchlings produced in southern Kyparissia Bay were female. Sex of 17% of the hatchlings was undetermined using this method, due to loss of the logger at 1700m. If we take into account the ID for nests in this section (Figure 5) then the vast majority of these hatchlings were also female, thus the primary hatchling sex ratio in southern Kyparissia Bay was appreciably female skewed.

*The temperature zone above TRT (↑↑) was treated as producing 100% females, the zone between the higher TRT limit and 0.2°C more than the pivotal temperature (↑) was treated as producing 75% females, the zone within ±0.2°C of pivotal temperature was treated as producing 50% females, the zone between the lower TRT limit and 0.2°C less than the pivotal temperature (↓) was treated as producing 25% females and the zone lower than the TRT (↓↓) was treated as producing 0% females.

DISCUSSION

The results of both beach temperatures and incubation duration indicate that the primary sex ratio of hatchlings in 2003 was strongly female-biased in southern Kyparissia Bay. This skewing has been shown for other nesting colonies and is generally accepted to be a widespread phenomenon (Mrosovsky *et al*, 2002 and references therein). However it seems that factors such as latitude (Mrosovsky, 1988) and sand albedo (Hays *et al*, 2001) may provide conditions for balanced localised sex ratios.

Beach temperatures were shown to broadly correlate with ID when whole beach sections were taken into account, as would be expected because incubation temperature influences ID (Miller, 1985). Some of the variation in the relationship found here might be due to placement of temperature loggers in atypical sites within the beach or that there is high intra-sectional variation in beach conditions.

Having stated the primary sex ratio of hatchling production was female biased, various factors must be taken into account that might alter this ratio, even before the hatchlings have left the beach, and bring the actual sex ratio of hatchlings in the open sea nearer to 50%. The three most important factors that must be considered are: The effects of nest inundation, the effects of extended hatching periods and the effects of nest predation.

Nest inundation has been shown to increase ID by approximately 3 days in southern Kyparissia Bay and is suggested to possibly play a significant role in masculinisation of turtle hatchlings (Margaritoulis & Rees, in press). Data analysed here did not take ID of inundated nests into account. ARCHELON's nest management activities aim to reduce the number of nests inundated by the sea, as this can be an important cause of nest mortality. Borderline nests that are relocated away from the dangerous surf zone are moved to safer spots further up the beach, which are thermally inclined to the production of female turtles. Thus the number of male hatchlings may be reduced slightly but the overall number of hatchlings is greatly increased.

Nest hatching can extend over several nights and upwards of a week in Greece (Houghton & Hays, 2001; Rees, in press) and this extended period results from intra-nest thermal variation (Houghton & Hays, 2001). Analysis of ID in the present study uses only the first date of hatching which was shown to produce only about 40% of the total hatchlings per nest in southern Kyparissia Bay (Rees, in press). Thus, estimates of hatchling sex ratios will be biased to indicate higher levels of females, as hatchlings emerging on subsequent nights have increased chances to be male if we follow that ID is inversely related to incubation temperature.

Nest predation is also said to have effects on the sex ratio of hatchlings that emerge from the nest and reach the sea. Kaska (2000) states that loggerhead nests in Turkey were depredated before or during hatching. Kaska *et al* (1998) and Houghton & Hays (2001) also showed that loggerhead nest temperatures were higher at the top. A combination of the above factors would imply an

increased chance of predation of female hatchlings that would be the first ready to leave the nest and be derived from the uppermost and hence more accessible eggs. Nest predation, as stated, is an important factor affecting the loggerheads in Kyparissia Bay and therefore the resulting sex ratio may naturally be more balanced than predicted here. But, as for nest inundation, ARCHELON's nest protection (Margaritoulis *et al*, 1996) activities increase absolute number of hatchlings with most significant effect on the survival of female hatchlings.

In conclusion, although a female-biased primary sex ratio of hatchling production is indicated in this study, many natural factors may play a role in altering the calculated ratio of hatchlings that successfully hatch from the nest and add to the at-sea turtle population. Accordingly, nest management activities applied in southern Kyparissia Bay are believed to provide an absolute increase in numbers of hatchlings produced with a tendency for these extra hatchlings to be female. Therefore the resulting hatchling sex ratio is further biased towards females but not at the expense of the number of naturally produced males.

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