

Metabolic Heating and the Prediction of Sex Ratios for Green Turtles (*Chelonia mydas*)

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ABSTRACT

We compared incubation temperatures in nests ($n = 32$) of the green turtle (*Chelonia mydas*) on Ascension Island in relation to sand temperatures of control sites at nest depth. Intrabeach thermal variation was low, whereas interbeach thermal variation was high in both control and nest sites. A marked rise in temperature was recorded in nests from 30% to 40% of the way through the incubation period and attributed to metabolic heating. Over the entire incubation period, metabolic heating accounted for a mean rise in temperature of between 0.07° and 2.86°C within nests. During the middle third of incubation, when sex is thought to be determined, this rise in temperature ranged between 0.07° and 2.61°C . Metabolic heating was related to both the number of eggs laid and the total number of hatchlings/embryos produced in a clutch. For 32 clutches in which temperature was recorded, we estimate that metabolic heating accounted for a rise of up to 30% in the proportion of females produced within different clutches. Previous studies have dismissed any effect of metabolic heating on the sex ratio of marine turtle hatchlings. Our results imply that metabolic heating needs to be considered when estimating green turtle hatchling sex ratios.

Introduction

In all nest-building species the placement, both temporally and spatially, of a clutch or brood is crucial to the survival of the offspring (Resetasar 1996; Downes and Shine 1999). This is perhaps more apparent in oviparous species and where no parental care is found. In addition to the success and survival of

the offspring, the position of the nest site can also affect their sexual development as a result of the incubating temperature (e.g., Standora and Spotila 1985). This phenomenon has been recorded in many oviparous reptilian species and is termed "temperature-dependent sex determination" (TSD; Shine 1999). Not only is the temperature of turtle nests critical for sex determination but it also affects the rate of embryonic development (Ackerman 1994) and phenotype of the offspring (Packard and Packard 1988; McGehee 1990). The site that a female chooses for her clutch may have profound consequences for the successful development and subsequent survival of her offspring (Mortimer 1990) and consequently may affect her own fitness (Madsen and Shine 1999).

In marine turtles, females generally emerge onto the beach at night and dig a nest into which they lay their eggs. They then return to the sea and play no future role in the survival of their offspring (Miller 1997). Over the past 2 decades TSD in marine turtles has been the subject of a number of studies (Mrosovsky 1988; Mrosovsky and Provancha 1992; Godfrey et al. 1996). Many of these studies have been based upon laboratory research and the incubation of eggs at constant temperatures (Mrosovsky and Yntema 1980; Miller and Limpus 1981; Billett et al. 1992; Georges et al. 1994). Such studies have indicated that nests that incubate at high temperatures ($>29^{\circ}\text{C}$) produce a larger proportion of females, with cooler nests ($<29^{\circ}\text{C}$) 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).

Field-based studies have been conducted, but commonly these involve extrapolation of nest conditions through the monitoring of sand temperatures (Limpus et al. 1983; Mrosovsky et al. 1984; Godfrey et al. 1996). Very few studies have examined the variations that occur within incubating nests as a result of seasonal and diel fluctuations in environmental temperatures (Spotila et al. 1987; Kaska et al. 1998). The temperature rise within an incubating clutch caused by metabolic heat produced by developing embryos and emerging hatchlings has been recorded in nests of green (*Chelonia mydas*; Hendrickson 1958; Carr and Hirth 1961; Bustard 1972; Morreale et al. 1982; Kaska et al. 1998), hawksbill (*Eretmochelys imbricata*; Raj 1976), loggerhead (*Caretta caretta*; Maxwell et al. 1988; Neville et al. 1988; Maloney et al. 1990), and leatherback (*Dermochelys coriacea*; Godfrey et al. 1997) turtles. Few studies have taken into account the effect that metabolic heating may have upon both the rate

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Table 1: Mean temperatures (°C) recorded in control sites on each of the three beaches and the mean intrabeach range

	Beach 1			Beach 12			Beach 27		
	<i>n</i>	Temperature	Range	<i>n</i>	Temperature	Range	<i>n</i>	Temperature	Range
Control A	762	27.6 ± .99	26.3–29.2	1,082	28.2 ± .62	27.0–29.2	957	30.8 ± .95	29.2–33.3
Control B	1,051	28.1 ± .80	26.4–29.6	940	27.7 ± 1.04	26.3–29.6	1,079	30.4 ± .75	29.2–31.8
Control C	931	28.2 ± .87	26.6–29.6	940	27.7 ± 1.06	24.5–29.6	959	30.4 ± .85	29.2–31.8
Beach mean	1,079	28.0 ± .86	26.4–29.5	1,082	27.9 ± .83	26.1–29.3	1,088	30.6 ± .74	29.2–32.5
Intrabeach range	931	.4 ± .29	0–1.1	940	.7 ± .55	0–2.9	963	.8 ± .68	0–2.7

Note. ± standard deviations.

of development and the sex of the offspring (Maxwell et al. 1988; Godfrey et al. 1997). Godfrey et al. (1997) recorded the temperature in clutches of the leatherback turtle in addition to recording the sand temperature to the side of the clutch. Nest temperatures were found to vary, on average, from control temperatures by 0.82°C during the thermosensitive period, suggesting that metabolic heating may play some role in influencing hatchling sex ratios.

It has been suggested (Mrosovsky and Yntema 1980) that metabolic heating can only be important if it elevates the nest temperature by >1°C during the middle third of incubation. Since most previous studies have shown that such a rise in temperature only occurs >50% of the way through incubation, it has been suggested that metabolic heating is not important with respect to TSD because it occurs after the critical thermosensitive period (Mrosovsky and Yntema 1980).

The green turtles that migrate to Ascension Island to nest use 32 beaches, upon which they deposit their clutches (Mortimer and Carr 1987). Previous studies on Ascension have shown that interbeach thermal variation is large, resulting from the high variation in sand albedo, whereas the lack of vegetation in the coastal zone results in little intrabeach thermal variation (Hays et al. 1995, 1999). These previous studies have, however, only described the temperature of the sand at nest depth and not within nests throughout incubation.

The fundamental objectives of this study were, therefore, to assess the extent of metabolic heating and its possible effects on incubation temperature and therefore the sex of the incubating hatchlings. In addition, we attempt to identify key factors that affect the timing and extent of the metabolic heating process.

Material and Methods

This study was conducted during the 1998/1999 nesting season on the three major nesting beaches of Ascension Island (7°57'S, 14°22'W): South West Bay (beach 1); Long Beach (beach 12); and North East Bay (beach 27; beach numbers per Mortimer and Carr 1987). These three beaches have been shown to hold approximately 50% of all nesting on the island (Mortimer and Carr 1987; Godley et al., in press).

Information on sand and nest temperature was gathered us-

ing Tinytalk data loggers (Orion Components, Chichester) that recorded synchronously at 4-h intervals. These data loggers were calibrated with a mercury thermometer of known accuracy (NAMAS certified to read ± 0.1°C of absolute temperature; Hays et al. 1999). Data loggers were placed in control sites from December 8, 1998, until June 7, 1999 (encompassing >95% of all nesting).

At the onset of the nesting season, temperature loggers were placed in three randomly selected control sites within the nesting zone on each of the study beaches. Loggers were placed at a depth of 77 cm, the mean depth of green turtle nests on Ascension Island (Hays et al. 1993). Data loggers took <4 h to equilibrate with the surrounding sand, and thus initial readings within this period were not included in our analysis. Loggers were retrieved and offloaded every 2 mo throughout the season and replaced with different data loggers to reduce the chance of logger or data loss.

In addition, between January 3 and June 4, 1999, temperature loggers were placed in the centre of clutches of eggs as they were being laid ($n = 13$ clutches for each beach). We did not actively select nest sites but placed data loggers among the eggs of the first laying female that we observed in a night. Using a rigid tape measure, we recorded the depth of the data loggers in relation to the surface of the sand (Hays et al. 1993). The female covered up the nest herself. Data loggers measured $5 \times 5 \times 3$ cm (volume = 75 cm³), representing some 1% of an average clutch as calculated conservatively by assessing the volume of eggs (mean clutch volume = 7,278 cm³, mean number of eggs per clutch = 127.5 eggs, mean egg diameter = 4.55 cm, egg volume = $4/3\pi r^3$; Hays et al. 1993).

Due to the risk of disturbance, as a result of the high density of nesting on these beaches, we protected study nests by hammering four stakes (>1 m long) into the sand at the corners of a 4 m² area centred over the nest/control site. At least 30–50 cm of each stake was left above the surface of the sand. Through this method we were able to monitor all but one of our study nests to completion; that one, despite our precautions, was disturbed by another female.

After 45 d, each nest was checked daily at dawn. After the initial wave of hatchling emergence, nest contents were excavated and data loggers were retrieved. Through a count of

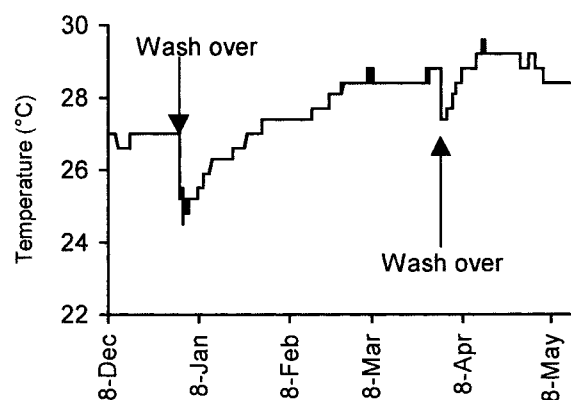


Figure 1. Control site C of beach 12, illustrating periods of temperature reduction due to wash over.

unhatched eggs and hatched shell fragments, clutch size was calculated. Unhatched eggs were classified as nonviable (containing no yolk), dead in shell (containing dead embryos), and yolked with no gross sign of development (no embryo visible to the naked eye). Incubation periods were calculated as the number of days between the night of laying and the night the first hatchlings emerged.

Results

Control Sites

The intrabeach thermal variation recorded by the three control site data loggers was, on average, less than 1°C for each study beach and ranged from 0° to 2.9°C (Table 1). Larger variations (>1°C) were rare and were attributed to occasional waves washing over the sand at a control site during stormy weather (Fig. 1). Data from the three control sites for each beach were pooled to compare interbeach thermal variation (Fig. 2). It is clear from the data presented in both Table 1 and Figure 2 that beach 27 demonstrates the highest sand temperatures of the three beaches, on average 2.6°C higher than beach 12. The thermal conditions recorded at beaches 1 and 12 were remarkably similar, varying on average by 0.1°C. When all data were pooled, temperatures in the control sites ranged from 24.5° to 33.3°C.

Nests

Excluding one nest that was disturbed by the digging of another female, all 38 of the remaining study nests hatched successfully. However, due to high winds or heavy seas destroying hatchling tracks, we were not always able to determine the exact date of the first hatchling emergence of some nests; these were excluded from our analysis ($n = 6$). In total, 32 nests (beach 1, $n = 9$; beach 12, $n = 11$; beach 27, $n = 12$) of known incubation periods were included in our analysis. Within-nest temperatures

demonstrated a similar pattern of interbeach variation as controls, with those recorded in nests on beach 12 being marginally cooler than beach 1 and both recording significantly cooler temperatures than beach 27 (Table 2). Mean temperatures ranged more widely for nests than for control sites on the same beach (3.1°C on beach 1, 0.9°C on beach 12, 2.1°C on beach 27).

Data are also presented (Table 2) for the middle third of incubation, since this period contains the critical period during which sex determination occurs. The overall range in temperatures during the middle third was 27°C to 35.7°C, with mean temperatures ranging from 28.1° to 33.5°C.

Control Sites versus Nests

Illustrations of the typical relationship between control site and within-nest temperatures are given in Figure 3 for each of the three beaches throughout the incubation period. Note the lack of regular diel fluctuation and the steady increase in temperatures toward maxima a few days before hatchling emergence. For all nests, the range in differences between control sites and nest temperatures was -1.9° to 7.3°C , with the three beaches showing similar patterns (beach 1, 1.55°C; beach 12, 1.29°C; beach 27, 1.34°C; Table 3). During the middle third of incubation, nests on average always demonstrated higher temperatures than control sites on the same beach (beach 1, 1.05°C; beach 12, 0.68°C; beach 27, 1.27°C). However, every nest was found to be $>1^{\circ}\text{C}$ above control sites at some point during the middle third of incubation, with some being as much as 5°C above control site temperature.

For each nest we converted the incubation period in days into the proportion of incubation period so that a clearer among-nests comparison could be made of the temperature

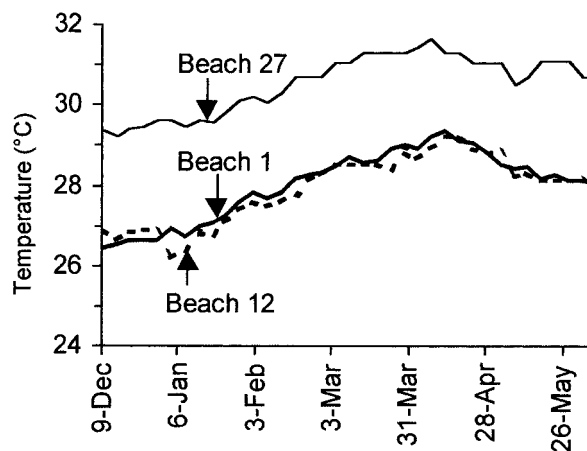


Figure 2. Interbeach thermal variation: mean temperature of three control sites recorded at 4-h intervals throughout the nesting season on each of the three study beaches on Ascension Island, 1998/1999.

Table 2: Nest temperatures (°C) recorded on each of the three study beaches for the entire incubation period and middle third of the incubation period

	Entire Incubation Period				Middle Third of Incubation Period			
	Mini- mum	Maxi- mum	Range of Means	Mean of Means $\pm$ SEM	Mini- mum	Maxi- mum	Range of Means	Mean of Means $\pm$ SEM
Beach 1 ($n = 9$)	26.6	35.7	28.5–31.6	29.9 $\pm$.34	27.0	32.6	28.7–31.3	29.5 $\pm$.34
Beach 12 ($n = 11$)	25.9	34.9	29.0–29.9	29.5 $\pm$.10	27.0	31.6	28.1–29.6	28.9 $\pm$.13
Beach 27 ($n = 12$)	28.4	36.5	31.5–33.6	32.2 $\pm$.18	29.6	35.7	31.0–33.5	32.1 $\pm$.23

Note. $\pm$ standard error of mean (SEM).

profiles. Figure 4 gives the mean difference between nests and control site temperatures for each of the three beaches for each percentile of the incubation period. For each beach the mean temperatures recorded in nests are slightly lower than those of the control sites at the onset of incubation (ranging from -0.4° to -0.8°C).

We examined the depth of nests in relation to the difference in the temperatures recorded at control and nest site on day 5 of incubation for each nest. Day 5 was chosen because it represented a point during incubation at which no evidence of metabolic heating was present. No significant relationship was found between nest depth and the mean temperature difference within any of the three beaches or collectively (linear regression analysis, $P > 0.05$). In addition, there were no significant differences among the mean depths of nests on each of the three beaches (one-way ANOVA: $F_{2,31} = 0.57$, $P = 0.572$; mean depth 77.7 cm, SE = 2.33, $n = 31$). Thus, interbeach thermal variation in nests was not a result of depth of nests.

We defined metabolic heating as the difference between nest temperature and mean control temperature for that beach at any point during incubation, minus the minimum observed difference between the two values. The amount of metabolic heat recorded did not vary significantly among beaches during the middle third (one-way ANOVA: $F_{2,31} = 2.04$, $P = 0.148$) or final third of incubation (one-way ANOVA: $F_{2,31} = 1.28$, $P = 0.294$). In addition, there was no relationship between the day of the season on which a nest was laid (day 1 = December 1, 1998) and the mean metabolic heating recorded during the middle third of incubation for all nests collectively or for individual beaches when either linear or curvilinear regression was applied ($P > 0.05$). No relationship was recorded between mean control site temperature during the middle third of incubation and mean metabolic heating during this period ($P > 0.05$; Fig. 5a). When we examined the difference in metabolic heat between the final and middle thirds of incubation we found that this value was significantly lower on nests on beach 27 than beaches 1 and 12 (one-way ANOVA: $F_{2,31} = 4.58$, $P = 0.019$). The relationship between this difference and

the mean control temperature during the middle third of incubation is illustrated in Figure 5b.

The amount of metabolic heating within nests was found to be related to clutch size for all beaches collectively (Fig. 6; $n = 31$; in one instance we were not able to analyse nest contents). Larger clutches produced a greater amount of metabolic heating (total metabolic heat = $0.0317 \text{ clutch size} + 1.1926$, $r^2 = 0.30$, $F_{2,31} = 12.58$, $P < 0.001$). A higher-degree model did not improve the curve fit. Clutch size did not vary significantly among beaches (one-way ANOVA: $F_{2,31} = 2.3$, $P = 0.119$). Hatching success (total number of eggs hatched/clutch size) was significantly lower on beach 27 (mean hatch success, beach 27: 57%, SD = 0.23; mean hatch success, beach 1: 82%, SD = 0.25; mean hatch success, beach 12: 85%, SD = 0.16; Kruskal-Wallis test $H = 9.11$, $P = 0.011$). Of the eggs that failed to hatch on beach 27, the majority were found to contain dead embryos. For this reason we also examined the number of hatchlings produced in a clutch, although no relationship was found with the degree of metabolic heating ($F_{2,31} = 2.16$, $P < 0.153$, $r^2 = 0.07$). When we examined the number of hatchlings plus the number of dead in shell of a clutch (x) and metabolic heating (y), we found a significant relationship ($F_{2,31} = 11.18$, $P < 0.002$, $r^2 = 0.28$, $y = 2.02 + 0.0256x$). In addition, on beach 27 hatchling sizes (straight carapace length) were significantly smaller than on beaches 1 and 12 (one-way ANOVA: $F_{2,25} = 18.09$, $P < 0.001$).

Effect of Metabolic Heating on the Sex Ratio of a Clutch

Utilising data from previous studies (Ackerman 1997) where incubation temperature and sex ratios were known, we calculated the expected female sex ratio of green turtle hatchlings on Ascension Island for each of the three study beaches using three different methods.

1. For each nest we calculated the expected ratio using the mean sand temperature during the middle third of incubation. This gave us an expected female sex ratio of 40% on beach 1 (SD = 6.62, range = 24.7–43.2), 40% on beach 12 (SD =

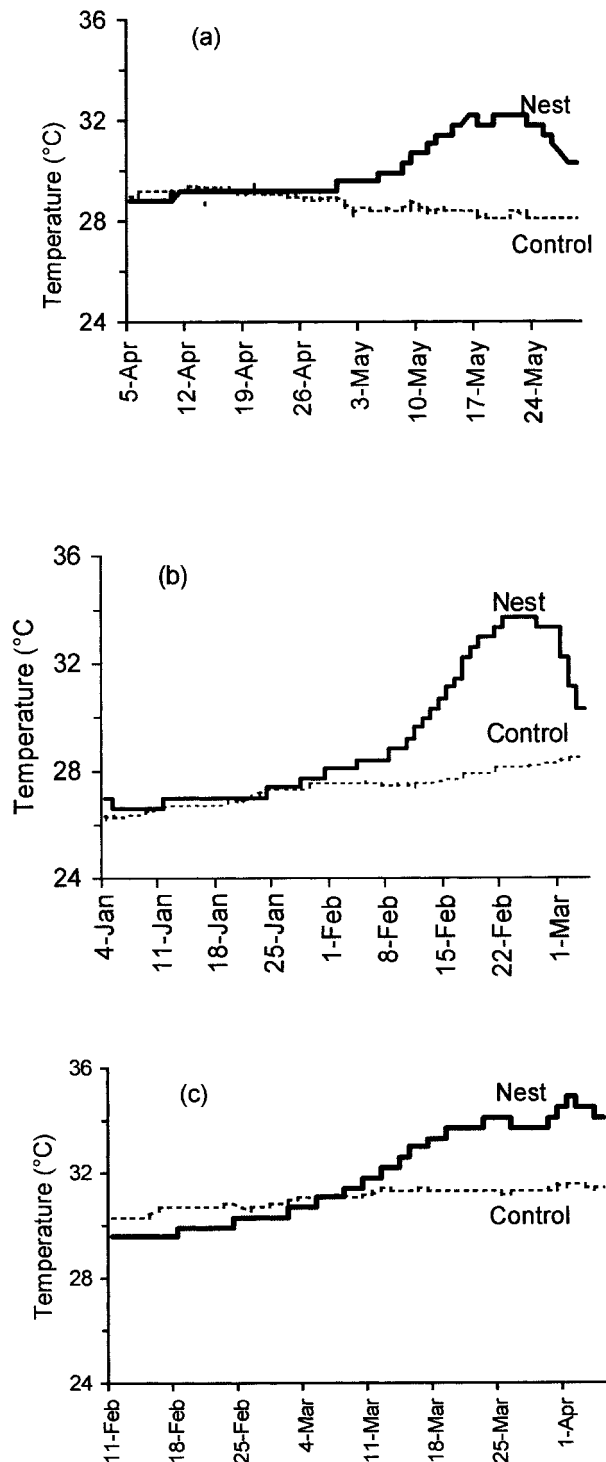


Figure 3. Comparison of control site and nest temperatures recorded for one nest on (a) beach 1, (b) beach 12, (c) beach 27. Broken lines denote temperatures within control sites; solid lines denote temperatures within nests.

74.8, range = 24.7–43.2), and 90.5% on beach 27 (SD = 7.67, range = 74.1–93.8).

2. Mean sand temperature (as in item 1) plus actual mean metabolic heating recorded during the middle third of incubation in each clutch. This gave us an expected female sex ratio of 63% on beach 1 (SD = 18.28, range = 43.2–93.8), 53.4% on beach 12 (SD = 10.24, range = 33.3–65.4), and 99.2% on beach 27 (SD = 1.77, range = 93.8–100).

3. Mean sand temperature (as in item 1) plus mean metabolic heating recorded during the middle third of incubation for each beach (Table 3). This gave us an expected female sex ratio of 62.7% on beach 1 (SD = 8.84, range = 43.2–74.1), 52.5% on beach 12 (SD = 10.45, range = 33.3–65.4), and 99.8% on beach 27 (SD = 0.47, range = 98.8–100).

Thus, metabolic heating accounted for an estimated mean rise in female sex ratio of 23% on beach 1, 13% on beach 12, and 8% on beach 27 and ranged from 6% to 30% within individual nests. It is not known exactly when the eggs hatch for this population, but there will be a lag of the order of several days between hatching and emergence. Work by Godfrey and Mrosovsky (1997) suggested that for loggerhead turtles, the mean lag from hatching to emergence is 4.1 d. If we assume this is the case in the Ascension Island green turtle population, and the incubation period in all nests is 4.1 d shorter, the results of the calculations above change little. There is no change in the predicted sex ratios based on control site temperatures only. As a result of slight reduction in metabolic heating of the order of 20%–30% (beach 1, -0.22°C ; beach 12, -0.25°C ; beach 31, -0.31°C), the estimated mean rise in female sex ratio resulting from metabolic heat is reduced to 21%, 10%, and 7% on beaches 1, 12, and 27, respectively.

Discussion

The sex ratio of offspring is a fundamental component of demographic studies that attempt to explain how populations remain self-sustaining. Most theoretical models predict balanced sex ratios (see Bull and Charnov 1988). As such, the occurrence of TSD in reptiles has provoked considerable interest because this phenomenon potentially can produce highly skewed sex ratios under certain environmental conditions (Miller and Limpus 1981; Mrosovsky et al. 1992). For example, in Florida, warm nest temperatures for loggerhead turtles produce predominantly female hatchlings (Mrosovsky and Provanha 1989, 1992). In such circumstances, TSD would appear to be maladaptive and potentially lead to population extinction. While some theoretical arguments have recently been put forward to suggest a functional explanation for the occurrence of TSD, it remains an enigmatic phenomenon (Shine 1999).

The sex ratios being produced by animals exhibiting TSD are, therefore, of scientific and conservation interest. However, the assessment of sex ratios in some groups, such as marine turtles, is not straightforward. While adult male turtles have

Table 3: Differences recorded between the mean of the control sites for each beach and nest temperature (°C) for the entire incubation period and middle third of the incubation period

	Entire Incubation Period				Middle Third of Incubation Period			
	Mini- mum	Maxi- mum	Range of Means	Mean of Means $\pm$ SEM	Mini- mum	Maxi- mum	Range of Means	Mean of Means $\pm$ SEM
Beach 1 (<i>n</i> = 9)	-1.3	7.3	.07–2.86	1.55 $\pm$.31	-.4	4.6	.27–2.69	1.05 $\pm$.31
Beach 12 (<i>n</i> = 11)	-1.7	6.0	.72–2.44	1.29 $\pm$.16	-1.4	3.5	.07–1.48	.68 $\pm$.12
Beach 27 (<i>n</i> = 12)	-1.9	5.9	.55–2.53	1.34 $\pm$.18	-.8	5.0	.16–2.61	1.27 $\pm$.24

Note. $\pm$ standard error of mean (SEM).

an extended tail that allows them to be easily differentiated from females, in hatchlings and juveniles, males and females are morphologically identical, and the only routine method of ascertaining the sex of hatchlings is to sacrifice individuals and then to examine their gonads microscopically (Yntema and Mrosovsky 1980; Mrosovsky et al. 1984). For both logistic and ethical (most sea turtle populations are endangered) reasons, it is impossible to sacrifice large numbers of hatchlings to determine sex ratios directly. Instead, many studies employ indirect methods, most notably using sand temperature, to predict sex ratios (Mrosovsky et al. 1984; Godfrey et al. 1996). Consequently, the ability to accurately define the nest temperature during the critical middle third of incubation is crucial when attempting to calculate sex ratios being produced for different populations. In theory, it should be possible to calculate sex ratios from the sand temperature measured at nest depths if the factors that cause variation between these control temperatures and those within nests are known. First, there may be spatial patterns in sand temperature on beaches. For example, for nesting beaches in Costa Rica, the incubation temperature is influenced by whether the nest site is shaded or unshaded, and hence the positioning of nests needs to be considered when predicting sex ratios from control site temperatures (Morreale et al. 1982). Second, nest temperatures may differ from control site temperatures due to metabolic heating of the nest by the eggs themselves (Carr and Hirth 1961; Bustard 1972; Maxwell et al. 1988). Therefore, before sex ratios can be predicted from control site sand temperatures with reliability, the extent of metabolic heating needs to be assessed.

The first requirement to quantify metabolic heating is to record the temperatures within nests. In the past, this was difficult to achieve because of the expense and unreliability of temperature loggers, which result in small sample sizes (Carr and Hirth 1961; Limpus et al. 1983; Maxwell et al. 1988) and unknown accuracy of measurements. However, the increased miniaturisation and capacity of loggers now make accurate measurement of nest temperatures feasible. It is still important to determine the accuracy and precision of such instruments,

and hence we cross-calibrated our loggers against absolute standards, as has recently been done in other studies (Hanson et al. 1998). This will allow our measurements to be compared reliably with those made elsewhere.

It is important to describe both the extent of metabolic heating in nests within a beach and the variation that exists between beaches utilised by the same population. With this aim, we deployed data loggers at random into nests on the three main nesting beaches of Ascension Island. These beaches were chosen because in previous studies (Hays et al. 1995) they were shown to span the spectrum of sand colours and, hence, sand temperatures on the island. Our results supported these findings and illustrate the significantly higher temperatures recorded on beach 27 in comparison to beaches 1 and 12 (Fig. 2).

To maximise sample size of study nests, we deployed only one temperature logger in the middle of each nest. It is possible that metabolic heating may vary according to thermal contours between the centre and the periphery of the clutch. Until it is possible to have a detailed description of how these thermal conditions vary in three dimensions, the levels of metabolic heating generated should be considered maximal.

Variation within beaches was low. Any large temperature fluctuations that were observed between control sites and nests within individual beaches were a result of the cooling of the sand due to occasional wash over from the sea. Such events are not uncommon on Ascension Island, owing to the huge waves that break along its coast, and although some clutches are lost due to erosion, clutches that suffer a degree of occasional wash over do hatch successfully. This factor must be accounted for when preparing a temperature profile for a beach.

The general pattern of metabolic heating was similar to that found in previous studies, with metabolic heating recorded mainly during the second half of incubation (Hendrickson 1958; Carr and Hirth 1961; Morreale et al. 1982; Maxwell et al. 1988; Neville et al. 1988; Maloney et al. 1990; Godfrey et al. 1997; Kaska et al. 1998), with a peak followed by a gradual decline in nest temperature toward the end of incubation. It has been suggested that this drop in temperature indicates the

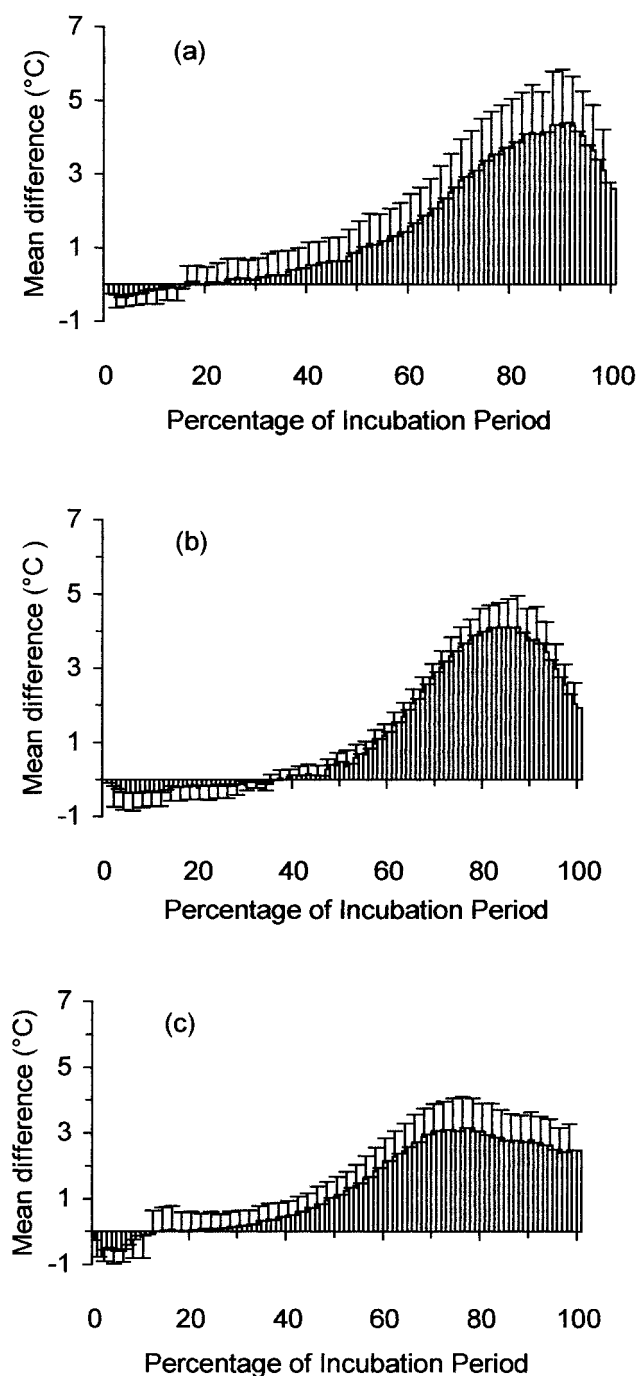


Figure 4. Mean difference between control sites and nest temperatures throughout the incubation period on (a) beach 1, (b) beach 12, (c) beach 27. Bars denote upper 95% confidence limit.

point at which hatchlings leave the nest mass and begin to move through the sand column to the surface (Bustard 1972; Raj 1976; Neville et al. 1988). While there is a clear rise and fall in temperature when the composite temperature profiles

of nests on beaches 1 and 12 are examined, it was not possible to clearly ascertain the point of hatching on a nest by nest basis.

Because it was not possible to ascertain the point at which nests in this study hatched, thermal parameters for the thermosensitive period were calculated during the middle third of the period from laying to hatchling emergence. Godfrey and Mrosovsky (1997) have calculated that for loggerhead turtles, the lag from hatching to emergence will be approximately 4 d. Our results suggest that if this was the case for green turtles on Ascension Island, mean temperatures in the revised middle third of incubation would only be reduced slightly on each beach, largely as result of a mean reduction of the amount of attributable metabolic heating ranging from 0.2° to 0.3°C on the three study beaches.

Mean temperatures in this study ranged from 28.5° to 33.6°C, similar to those recorded at other sites for this species worldwide (Spotila et al. 1987; Kaska et al. 1998) and falling within the thermal tolerance range, described by Ackerman (1997), of 25°–35°C. Within individual nests on beach 27 ($n = 6$), temperatures frequently rose to greater than 35°C (one nest reaching 36.5°C). On this beach temperatures may be exceeding the maximum for successful embryonic development, leading to heat stress and embryo mortality. The low hatching success as a result of high embryo mortality recorded on this beach supports this suggestion. In addition, our results indicate that both live and decomposing embryos may contribute to metabolic heating within a clutch, possibly explaining why nests on beach 27, although showing similar overall levels of metabolic heating (Table 3), did not show the same patterns in time (Fig. 4c). Although on average, levels of metabolic heating did not reach the same levels as on the other two beaches, they stayed relatively high throughout the last third of incubation, possibly as a result of heat generated by the process of embryonic decay.

While the effect of incubation temperature on hatchling phenotype has been recorded previously in fresh water turtles (O'Steen 1998; Packard et al. 1999), we are not aware of this relationship having been noted in marine turtles prior to this study. One suggested functional explanation for TSD is that there is differential fitness of males and females at the same temperatures (Shine 1999). Our preliminary results show that in green turtles, a high temperature will produce predominantly small females, while cooler temperatures may produce larger hatchlings in addition to a higher proportion of males. The survival of small females may be compromised, with their fitness with respect to digging, crawling, and swimming likely to be reduced. Indeed, it is difficult to imagine a benefit of this smaller phenotype. Both the longevity and the at-sea habitat of these species make it difficult to examine further the consequences of such dimorphism on the survival and subsequent fitness of individuals.

The statistical relationship between clutch size and metabolic heating (Fig. 6) may be driven by a few data points and should not be overinterpreted without further similar investigations.

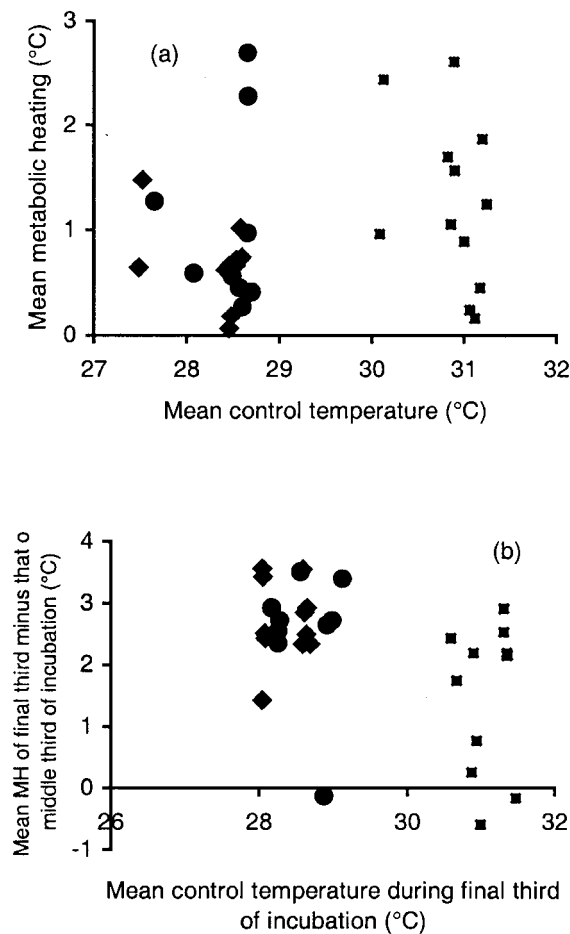


Figure 5. *a*, Relationship between mean control temperature and mean metabolic heating recorded during the middle third of incubation for all study nests. *b*, Relationship between mean control temperature during final third of incubation and the difference between the mean metabolic heat recorded in the middle and final thirds of incubation. Circles represent beach 1; diamonds, beach 12; and squares, beach 27.

In addition, we have only explained 30% of the variation in metabolic heating between nests as a result of clutch size, suggesting that exogenous factors relating to the physical properties of the sand, for example, conductivity (Speakman et al. 1998), may also be important. These other factors presumably vary randomly between beaches, since there was no significant interbeach variation in metabolic heating by the middle third of incubation. The key finding of this study was that when metabolic heating was taken into account, the prediction for hatchling sex ratios always changed, increasing female production by up to 30% in individual nests. In the past, metabolic heating has largely been ignored as a factor that could influence sex ratios in TSD reptiles. Our data on Ascension Island indicate that metabolic heating occurs in green turtle nests and suggest

that such heating could alter the process of sex determination in natural nests.

In practical terms, it is not possible to directly measure metabolic heating in all nests on the island since the population size is several thousand nesting females (Mortimer and Carr 1987). Our results demonstrate that simply applying a mean metabolic heating value for nests on individual beaches produced very similar values for the estimated hatchling sex ratio compared to when we examined the actual known metabolic heating within a clutch. A fundamental goal for long-lived iteroparous breeders, such as marine turtles, is to assess their overall lifetime production of male and female offspring. For sea turtle nesting beaches it is therefore important to derive estimates of hatchling sex ratios over periods of decades. One approach to solving this question is to reconstruct past sex ratios from routinely recorded meteorological data (Godfrey et al. 1996). Indeed, on Ascension Island we have shown that control site sand temperatures are closely linked to air temperatures (Hays et al. 1999). As such, there is the potential for predicting control site temperatures throughout the nesting season for most of the past century, since such long-term meteorological data exist. In order to derive hatchling sex ratios from the long-term prediction of control site temperatures, we clearly need to incorporate metabolic heating into any predictive model. At present, our estimates of the effect of metabolic heating on sex ratios are also based on past studies of temperature versus sex ratio, and we aim to validate them through the sexing of hatchlings from Ascension Island. With this knowledge we will be able to predict hatchling sex ratios of green turtles of Ascension Island for the twentieth century.

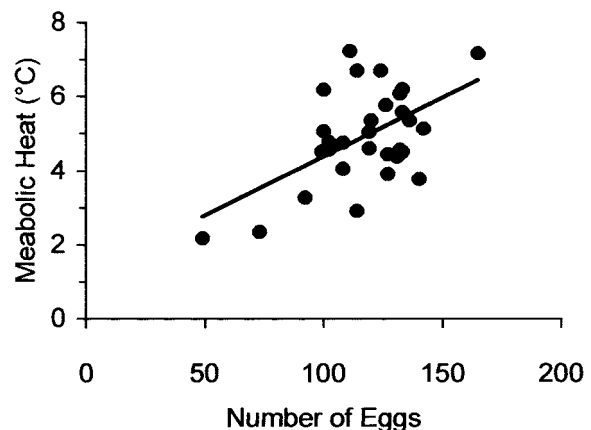


Figure 6. Relationship between clutch size and total amount of metabolic heat recorded in all nests on beaches 1, 12, and 27.

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