

Incubation environment affects phenotype of naturally incubated green turtle hatchlings

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A comparison of body size and flipper size was carried out on green turtle (*Chelonia mydas*) hatchlings produced from natural nests at two beaches on Ascension Island, South Atlantic and one beach in northern Cyprus in the Mediterranean (N=18 nests; N=180 hatchlings). Hatchlings from Ascension Island were significantly larger and heavier than hatchlings in Cyprus, a likely consequence of maternal size effects. Incubation temperature appeared to influence body size of hatchlings on Ascension Island with higher temperatures producing smaller hatchlings. Both hind and fore-flipper area scaled positively with body size. In proportion to body size, hind-flipper area appears relatively consistent among the Atlantic populations but is smaller than hatchlings measured in Hawaii.

INTRODUCTION

Marine turtles are ideal organisms in which to study intraspecific variation in phenotype. As a result of their wide distribution, and the fact that both males and females usually return to their natal coastline to breed, many discrete populations are found nesting at different locations (e.g. Bowen et al., 1989). Indeed, morphological studies on green turtles (*Chelonia mydas*) have shown variations in traits among regional populations, for example; variation in skull morphology (Kamezaki & Matsui, 1995), flipper size (Balazs et al., 1997; Wyneken et al., 1999) and body size (Carr & Goodman, 1970; Figueroa & Alvarado, 1990).

It has been suggested that in the eastern Pacific, *Chelonia mydas* constitutes two separate species; the black turtle (*Chelonia agassizi*) distinguished from the green turtle by its grey plastron, higher carapace, small size and a darker dorsal pigmentation (Pritchard, 1999). Comparison of the skull structure of green turtle populations lent support to this distinction (Kamezaki & Matsui, 1995; Pritchard, 1999), although DNA analysis has not revealed a unique lineage relative to other green turtle populations (Bowen & Karl, 1999).

A major problem in defining turtle populations using morphological traits is the presence of epigenetic effects. Hatchling phenotype, as well as being controlled by factors such as genetics and maternal effects such as egg size (e.g. Van Buskirk & Crowder, 1994; Hewavisenthi & Parmenter, 2001), is also determined by the incubation environment. Nest temperature and the hydric condition influence the metabolic rate of the embryo, and therefore control the length of incubation (see Miller, 1996 for review). Consequently, even though variation in hatchling morphology can be observed between and within populations, this is due to a combination of effects. Analyses of the contribution of parental influence or environment towards hatchling phenotype in marine turtles is as yet poorly understood, indeed in most reptiles, elucidation of

the proportion of phenotypic variation generated by temperature variation within and between nests is still a major problem (Shine, 1999).

The aim of this study was to describe the variation in hatchling morphology from nests incubated naturally under differing regimes. By comparing two different populations, i.e. Ascension Island, South Atlantic Ocean and northern Cyprus, eastern Mediterranean, we aimed to investigate the following: (1) do green hatchlings from the Atlantic have a different morphology to those in the Mediterranean; and (2) what effect does incubation regime have on phenotypic variation?

MATERIALS AND METHODS

Study sites

Data were collected on North East Bay and Long Beach, Ascension Island, South Atlantic (7°57'S 14°22'W), and Alagadi Beach (35°33'N 33°47'E), northern Cyprus, eastern Mediterranean.

Temperature logging and hatchling collection

Six clutches laid by different females on each beach were randomly selected. A temperature data logger (Tinytalk, Orion Components Ltd, Chichester, UK; precision of 0.3°C) was placed into the middle of the clutch during oviposition. Temperature loggers which had been previously calibrated (Hays et al., 1999), were set to record synchronously at hourly intervals. Following egg deposition, the curved carapace length (CCL) of the adult female was measured from the anterior of the precentral scute to the posterior of the postcentral scute using a flexible 1.5 m tape measure.

After 40 days of incubation, the area above each nest was enclosed by a low wire fence of a 2 m diameter. These corrals were closed at night, preventing hatchlings making their way to the sea, and opened again in the morning so

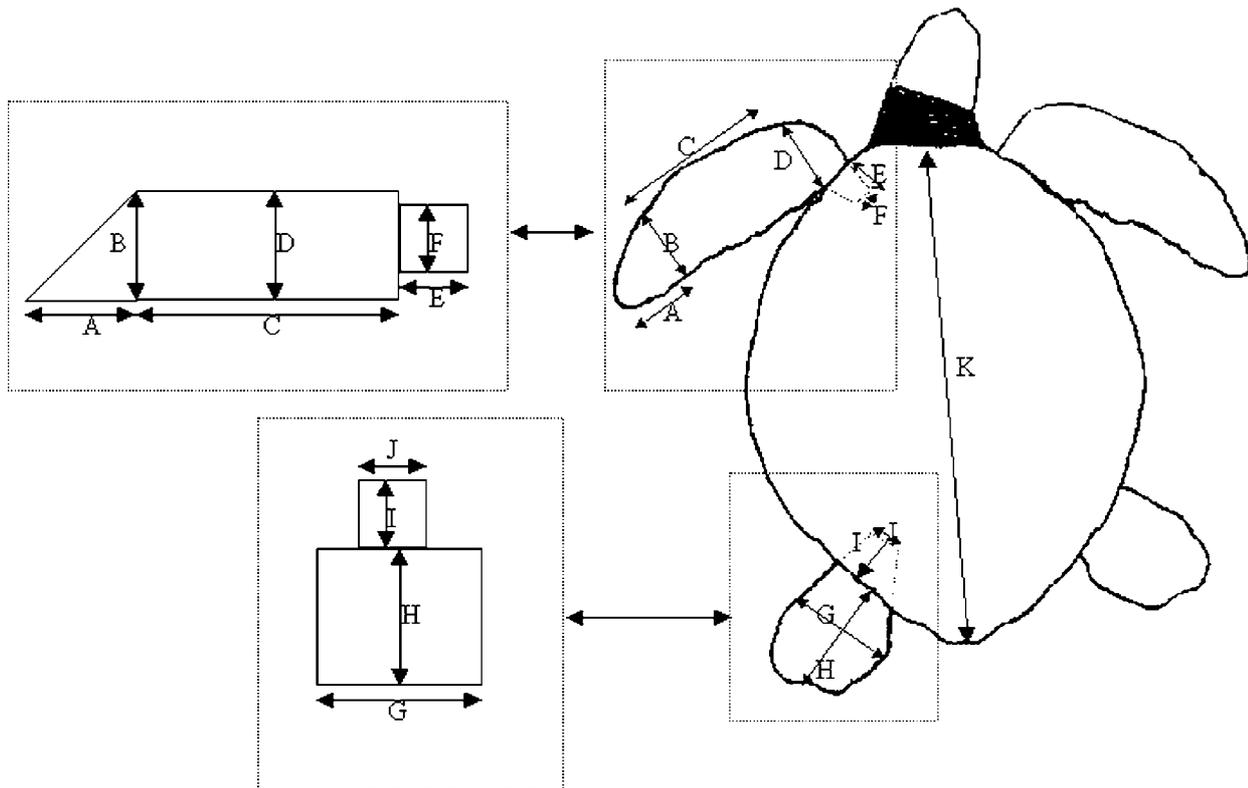


Figure 1. Diagram of the measurements taken. Straight carapace length (SCL)=K, hind flipper area (HFA)=(I×J) + (H×G), fore flipper area (FFA)=($\frac{1}{2}$ A×B) + (C×D) + (E×F).

hatchlings emerging during the heat of the day were able to move freely. Nests were checked throughout the night. Upon emergence of hatchlings, measurements were taken and the hatchlings were released. Nests were excavated 48 hours after the last observed emergence of hatchlings, the temperature data loggers were retrieved and data offloaded. Analyses of temperature data were confined to recordings taken from 24 h after egg deposition until 24 h prior to the first recorded emergence. Incubation period of the nests was determined as the number of days between the night on which the clutch was laid and the night on which the first group of hatchlings emerged.

Hatchling measurements

Ten individuals were chosen randomly from each clutch and 11 measurements were taken (see Figure 1) using dial callipers accurate to 0.1 mm (CAMLAB, Cambridge, UK). Hatchling weight was taken using electronic scales accurate to the nearest 0.1 g (Ohaus, LS200, Ohaus Europe Ltd, Cottenham, Cambridge). Using the flipper measurements, the area of the fore flipper (FFA) and hind flipper (HFA) were calculated for each hatchling using basic geometry (see Figure 1). Average values were obtained from each nest, thus we had six measures of body length, body weight, fore and hind flipper area for each beach. These mean values were used in subsequent data analysis.

In order to verify the calliper measurements, we randomly chose 20 dead hatchlings from our study nests on Ascension Island and subjected them to the same measurements as live hatchlings. In addition, flippers were

excised and using image analysis software (Image-Pro Plus Version 4.1, Media Cybernetics, Maryland 20910, USA) the flippers were scanned using a video camera (JVC Colour Video Camera Head, TK-1270, Victor Company of Japan Ltd). A paired *t*-test established that there was no significant difference between the areas calculated using the measurements taken by hand and those calculated by image analysis (FFA: $t_{1,19}=1.30$, $P>0.05$, HFA: $t_{1,19}=1.04$, $P>0.05$).

Egg measurements

In order to determine how egg size varied among the three sites, a random selection of nests (N=23 nests) from each of the three beaches were chosen. Once clutch deposition was complete, the curved carapace length of the adult female was measured and ten eggs randomly selected and measured. Maximum diameter of the eggs was measured using callipers accurate to 0.1 mm. Hatchlings from these nests were not measured. In order to prevent altering the natural regime of these nests, we chose not to measure the eggs from nests where temperature was recorded.

RESULTS

Analysis of variance (ANOVA) revealed a significant difference in adult CCL among the study sites (Table 1), where turtles nesting on Alagadi were significantly smaller than turtles from both sites at Ascension Island ($F_{2,22}=36.51$, $P<0.001$, post hoc Tukey test). Mean egg size was significantly smaller at Alagadi than at Long

Table 1. Synopsis of nest, adult and hatchling parameters. Figures in parentheses indicate the range of values.

	Alagadi Mean \pm SD	Long Beach Mean \pm SD	North East Bay Mean \pm SD
Adults			
Curved Carapace Length (cm)	96.2 \pm 5.4 (86.5–103.0) (N=7)	113.7 \pm 5.5 (109.3–122.5) (N=8)	117.9 \pm 4.6 (112.0–128.0) (N=8)
Egg Diameter (mm)	39.9 \pm 1.7 (37.7–42.6) (N=7 nests)	45.3 \pm 0.9 (44.1–46.5) (N=8 nests)	46.3 \pm 0.9 (45.1–47.7) (N=8 nests)
Nests			
Mean Incubation Duration (days)	49.5 \pm 2.9 (47.0–55.0) (N=6 nests)	57.0 \pm 1.4 (55.0–59.0) (N=6 nests)	51.3 \pm 1.5 (50.0–53.0) (N=6 nests)
Mean Temperature ($^{\circ}$ C)	31.0 \pm 1.1 (29.8–32.4) (N=4 nests)	29.7 \pm 0.5 (29.3–30.6) (N=6 nests)	32.2 \pm 0.5 (31.3–32.5) (N=6 nests)
Hatchlings			
Fore flipper area (mm ²)	335.3 \pm 19.0 (309.1–361.6) (N=6 nests)	399.7 \pm 13.9 (373.2–410.3) (N=6 nests)	354.0 \pm 10.5 (344.3–374.0) (N=6 nests)
Hind flipper area (mm ²)	251.7 \pm 8.1 (238.1–262.2) (N=6 nests)	304.3 \pm 16.5 (286.7–335.1) (N=6 nests)	280.0 \pm 14.3 (255.2–288.7) (N=6 nests)

Beach and North East Bay (ANOVA, $F_{2,22}=60.06$, $P<0.001$, post hoc Tukey test).

Hatchlings from Long Beach were longer ($F_{2,22}=8.33$, $P<0.005$, post hoc Tukey test), heavier ($F_{2,22}=19.42$, $P<0.001$, post hoc Tukey test) and had larger fore ($F_{2,22}=29.73$, $P<0.001$, post hoc Tukey test) and hind flippers ($F_{2,22}=22.88$, $P<0.001$, post hoc Tukey test) than hatchlings from North East Bay, which in turn were larger than hatchlings from Alagadi (Table 1).

Incubation temperature

A significant difference in incubation temperatures between the three sites was found with North East Bay nests being warmer than those on Alagadi, which in turn were warmer than on Long Beach (ANOVA $F_{2,17}=21.58$, $P<0.001$, post hoc Tukey test).

Flipper size

As hatchling length increased, so did fore and hind flipper area on all three beaches (Figure 2). A previous comparison of the relationship between HFA and straight carapace length (SCL) in green turtle hatchlings from Atlantic (Florida) and Pacific (Hawaii) populations was carried out using different but comparable measuring

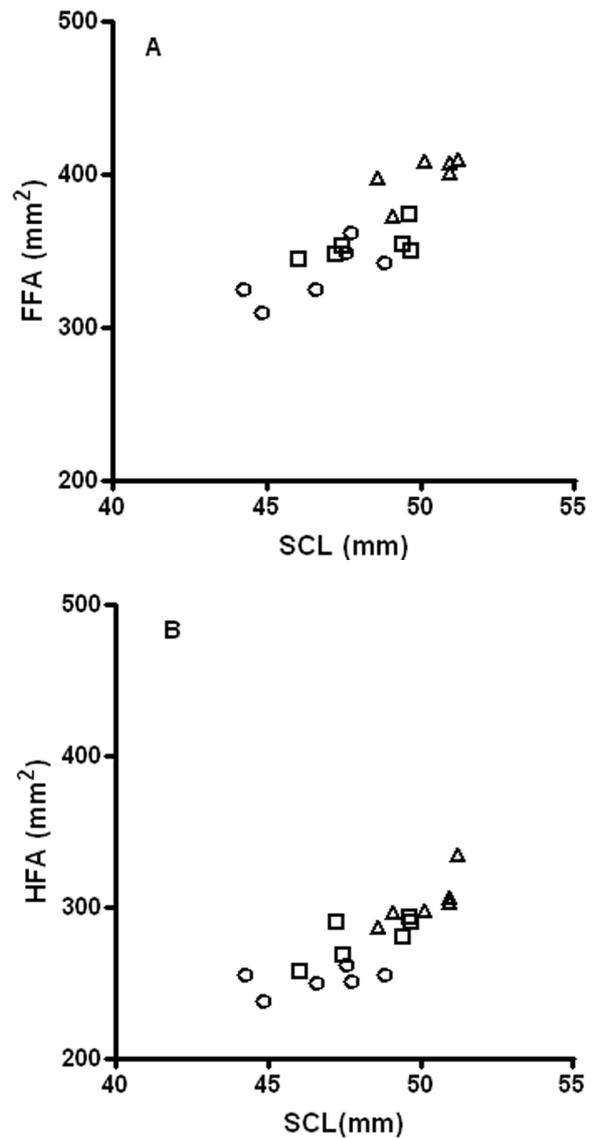


Figure 2. Straight carapace length of green turtle hatchlings plotted against: (A) front flipper area ($FFA = -264 + 13.0$ SCL, $F_{1,17}=40.38$, $P<0.001$, $r^2=0.70$), and (B) hind flipper area ($HFA = -227 + 10.4$ SCL, $F_{1,17}=37.14$, $P<0.001$, $r^2=0.68$). \circ , Alagadi beach; \square , North East Bay; \triangle , Long Beach.

techniques (Wyneken et al., 1999). In the Wyneken et al. (1999) study, green turtle hatchlings were held and an outline of their flippers were traced. The planar surface area of each of these tracings was measured using a digital scanning programme, and the area was calculated. This technique calculated a mean \pm standard error for the HFA area of $318 \text{ mm}^2 \pm 25 \text{ mm}$ in Florida hatchlings and $415 \text{ mm}^2 \pm 25 \text{ mm}$ in hatchlings from the Pacific. Using the average SCL measurement of hatchlings from Florida and Hawaii we calculated, using our regression equation ($HFA = -227 + 10.4$ SCL; Figure 3B), that hatchlings from Florida should have an average hind flipper area of $311 \text{ mm}^2 \pm 1.1$ and hatchlings from the Pacific, $313 \text{ mm}^2 \pm 0.2$. The allometric relationship of increasing hind flipper size from Cyprus, Ascension Island (two sites) and Florida is demonstrated in Figure 3 with the outlying nature of the value for Hawaiian green turtles clearly evident.

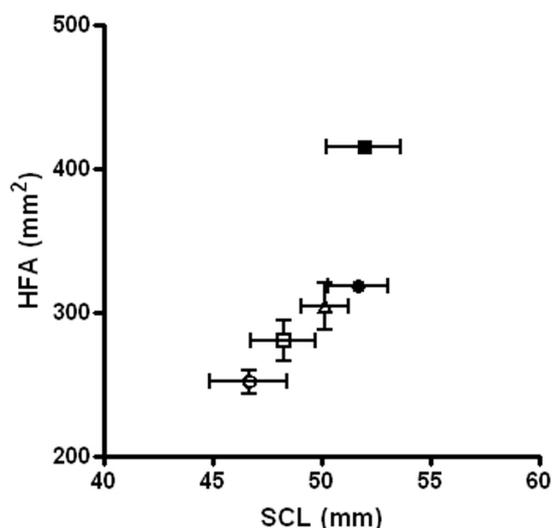


Figure 3. Relationship between the average straight carapace length and average hind flipper area. Error bars are \pm SE. y-error bars are smaller than plot symbols. ○, Alagadi beach; □, North East Bay; △, Long Beach; ●, Florida; ■, Hawaii.

DISCUSSION

As both the body size and weight of adult green turtles nesting in Ascension are greater than those nesting in northern Cyprus, due to a combination of genetic/maternal influences, most likely egg size, we expected Ascension hatchlings to be bigger than those from Cyprus. However, the differences in hatchling size observed between the two beaches on Ascension Island was not expected. As there was no significant difference in both adult size and egg size between North East Bay and Long Beach, we attribute some of the observed difference in phenotype to the profoundly differing incubation regimes experienced on the two beaches. Hatchlings on North East Bay experienced much warmer conditions during their incubation and were smaller. Unfortunately we were not able to measure other physical factors that might have affected hatchling phenotype, nevertheless we suggest that temperature may play an important part in determining hatchling size in naturally incubated nests. Size specific differences in mortality have been observed in reptiles (e.g. Janzen, 1993), and as hatchlings leaving North East Bay are smaller they may have a higher mortality.

For all green turtle study populations in the Atlantic, i.e. Cyprus, Ascension Island and Florida, as body size increased so did flipper size. The difference in relative hind-flipper size between Atlantic and Pacific hatchlings as found by Wyneken et al. (1999) stands after comparison with two additional Atlantic populations. Such disparity in flipper area between Pacific and Atlantic populations have been attributed to population specific differences in embryonic developmental rates (Wyneken et al., 1999). The life history of the Ascension green turtle involves long distance migrations from Brazil during their reproductive stage (Luschi et al., 1998) however, in relation to their body size, the Ascension green turtle's hind flippers are smaller than the Pacific green turtles, suggesting that migration distances are not the only explanation in the evolution of flipper size.

In conclusion, this study has shown that the range of nest temperatures experienced under natural conditions may contribute to the generation of meaningful levels of phenotypic variation in green turtle hatchlings.

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