Influence of incubation temperature on morphology and locomotion performance of Leatherback (*Dermochelys coriacea*) hatchlings

L.E. Mickelson and J.R. Downie

Abstract: The journey of Leatherback (*Dermochelys coriacea* (Vandelli, 1761)) hatchlings from nest to the sea is a vulnerable life-history stage. Studies have shown that nest incubation temperatures influence hatchling morphology and locomotor performance, which may affect hatchling fitness. We obtained incubation temperature profiles from 16 Leatherback nests in Tobago, West Indies, during the 2008 nesting season (March–June). There was significant variation among mean nest incubation temperatures, which had a significant influence on hatchling morphology. Using principal components analysis, we determined the morphological traits that explained the most variation among hatchlings, which allowed investigation of the relationship between hatchling morphology and terrestrial locomotion speed. Hatchlings with a narrower carapace width and longer flipper reach (produced at lower incubation temperatures) had significantly faster terrestrial speed and total run time than those with opposite characteristics (produced at higher incubation temperatures). Our results demonstrate that lower incubation temperatures produce hatchlings with traits that are significantly advantageous to terrestrial locomotion. These findings suggest that nest incubation temperature is important in determining hatchling fitness, as nest incubation temperature significantly influences hatchling morphology and locomotor capabilities. This study supplements related findings in Green Turtles (*Chelonia mydas* (L., 1758)), but also illustrates some unique features in Leatherbacks.

Résumé : Le trajet que font les tortues-luths (*Dermochelys coriacea* (Vandelli, 1761)) nouveau-nées de leur nid à la mer constitue une étape vulnérable de leur cycle biologique. Des études ont démontré que la température d'incubation des nids influence la morphologie et la performance locomotrice des nouveau-nés, ce qui peut affecter la fitness de ces nouveau-nés. Nous avons obtenu les profils des températures d'incubation de 16 nids de tortues-luths à Tobago, Antilles, durant la saison de nidification de 2008 (mars–juin). Il y a une variation significative entre les températures moyennes d'incubation au nid, qui a une influence significative sur la morphologie des nouveau-nés. Une analyse des composantes principales nous a servi à déterminer quels traits morphologiques expliquent le maximum de variation entre les nouveau-nés, ce qui nous a permis d'étudier la relation entre la morphologie des nouveau-nés et leur vitesse de déplacement au sol. Les nouveau-nés possédant une carapace plus étroite et une portée plus grande des nageoires (produits aux températures d'incubation plus basses) ont une vitesse au sol plus rapide et une « durée totale du trajet » plus courte que ceux qui ont les caractéristiques opposées (produits aux températures d'incubation plus élevées). Nos résultats démontrent que les températures d'incubation plus basses produisent des nouveau-nés qui possèdent des traits qui favorisent significativement la locomotion terrestre. Ces observations indiquent que la température d'incubation au nid est importante pour la détermination de la fitness des nouveau-nés, puisqu'elle influence significativement la morphologie des tortues néonates et leurs capacités de locomotion. Notre étude confirme des observations semblables faites chez la tortue verte (*Chelonia mydas* (L., 1758)) tout en soulignant des caractéristiques particulières aux tortues-luths.

[Traduit par la Rédaction]

Introduction

Sea turtles start their existence on land but spend the majority of their life at sea, with only females returning to land during the nesting season. Leatherbacks (*Dermochelys coriacea* (Vandelli, 1761); Dermochelyidae) show the greatest reproductive investment of all reptiles, with females laying between 60 and 80 eggs per clutch and approximately seven clutches during a single nesting season (Wallace et al. 2006). Leatherback eggs undergo an incubation period of approximately 60 days before they hatch, and nest incubation temperature influences the phenotype of developing hatchlings (Booth 2006). Nest incubation temperature determines the sex of hatchlings during the middle third of the incubation period (Binckley et al. 1998), where females are produced at higher temperatures and males at lower temperatures (Booth 2006). The pivotal temperature that produces a 50:50 sex ratio in Leatherback hatchlings is 29.4 °C, and the critical

Received 26 October 2009. Accepted 21 January 2010. Published on the NRC Research Press Web site at cjz.nrc.ca on 5 March 2010.

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threshold temperature above which a clutch contains 100% females is 29.75 °C (Houghton et al. 2007). In addition to determining the sex of reptile hatchlings, nest incubation temperature has also been shown to influence factors such as size (Elphick and Shine 1998; Ashmore and Janzen 2003; Wallace et al. 2006; Janzen et al. 2007), morphology (Braña and Ji 2000; Parker and Andrews 2007; Hare et al. 2008), and locomotion performance (Shine et al. 1997; Elphick and Shine 1998; Braña and Ji 2000; Glen et al. 2003; Booth 2006; Elnitsky and Claussen 2006; Hare et al. 2008). Although there have been previous studies on the effects of incubation temperature on the morphology (Glen et al. 2003) and locomotion of sea turtles (Burgess et al. 2006), there is a gap in the literature on the link between hatchling morphological traits (influenced by incubation temperature) and subsequent locomotion performance in hatchlings. Hatchling morphology and locomotion performance are phenotypic factors that will likely affect the overall survival and fitness of Leatherback hatchlings.

Effective locomotion in a range of environmental conditions is important for the survival and (or) reproduction of many species (Elnitsky and Claussen 2006). Sea turtles possess unique morphological traits that are conducive to in-water migrations over great distances (Hendrickson 1980; Wyneken 2000). Physical adaptations include large and powerful front flippers (formed through hypertrophy of phalanges), a streamlined carapace, reduced head size, and highly elastic lungs that are capable of rapid air exchange (Wyneken 2000). However, because the first stage of a sea turtle hatchling's life is terrestrial, hatchling morphology must also be suited to successful locomotion on land (Wyneken and Salmon 1992; Wyneken 2000). In their terrestrial environment, sea turtle hatchlings are relatively defenceless against predators and obstacles, and are therefore highly vulnerable during their initial crawl to sea (Salmon and Wyneken 1987). Therefore, it seems that faster terrestrial locomotion performance of hatchlings would likely benefit their survival rates. The purpose of this study was to determine whether nest incubation temperatures influence Leatherback hatchling morphological traits and their subsequent terrestrial locomotor performance, which may provide insight on the ecological and evolutionary significance of nest incubation temperature on hatchling fitness.

Materials and methods

Study area and temperature recording

Incubation temperatures were obtained from 16 Leatherback nests from April to June 2008 located at two index beaches in the village of Black Rock (10°3′N, 62°1′W) on the Caribbean coast of Tobago, West Indies. For this study, Tinytag Talk II (Gemini Data Loggers, Chichester, West Sussex, UK) temperature data-loggers (TDLs) were used to record incubation temperatures. Before use, the TDLs were tested and calibrated by the Faculty of Biomedical and Life Sciences (FBLS) Electronics Department at the University of Glasgow. Each TDL was tested for accuracy using a mercury thermometer and was fitted with a new lithium battery. The TDLs were preprogrammed with Tinytag Explorer software (Gemini Data Loggers, Chichester, West Sussex, UK) and were setup to record ambient temperature every hour (for up to 75 days). After initial programming setup, the TDLs were housed in a 35 mm film roll case with a silica gel moisture absorption packet, and the cap of the film roll was sealed with petroleum jelly and firmly secured with duct tape. To facilitate future retrieval of TDLs, a 2 m long nylon twine was fastened around the TDL case and secured with duct tape.

Field methods

To locate laying Leatherbacks, two beaches (Turtle Beach and Stonehaven Beach) were patrolled nightly between the hours of 2000 and 0400 from 31 March 2008 to 25 April 2008. When a Leatherback was encountered (and had successfully started the laying process), a single TDL was placed into the centre of the egg chamber, approximately halfway through the laying process. The nylon twine attached to the TDL was held out and away from the egg chamber during the remainder of the laying process until the covering-up stage, where the turtle subsequently buried the exposed twine tag. Triangulation of the nest was carried out using a 30 m long measuring tape to identify the location of the nest.

Temperature controls and intranest temperature range

To test for the occurrence of metabolic heating in nests, individual TDLs were buried in three "mock" Leatherback nests (at approximately 1 m chamber depth) on Turtle Beach (i.e., mock nest sites A and B) and Stonehaven Beach (mock nest site C) to monitor "sand-only" temperatures. In addition, two nests in the current study were monitored with three TDLs (placed in the bottom, middle, and top of the egg chamber) to determine the temperature variation within the nest, as all TDLs would not be positioned identically in each monitored nest.

TDL recovery and nest excavation

Nests were checked throughout the entire incubation period for any signs of disturbance and triangulation marks were maintained. TDLs were recovered after hatchling emergence (see below). Within 2 days of hatching, each nest was excavated and contents were divided into the following categories: "hatched" refers to hatched shells; "unhatched" refers to dead in shell or bacteria infected; "inert" refers to undeveloped; and "SAGs" refers to shelled albumin globs. Unsuccessful nests that did not hatch were excavated between days 65 and 70.

Hatchling morphology and size index

On day 55 of incubation, each nest was relocated by triangulation and marked with a nest-post made from PVC pipe and a waterproof label. The sand area in front of the nest-post was levelled and smoothed (daily or as required), and was checked for signs of hatchling emergence every hour from 1700 to 0500. Once hatchlings emerged from monitored nests, 7–15 hatchlings were randomly selected to undergo locomotion performance trials. Each selected hatchling was weighed immediately prior to trial using a 100 g spring balance. Callipers were used to measure hatchling carapace length and width, flipper length and width (both sides), and head width (Fig. 1, Table 1) to an accuracy of 0.1 mm.

Fig. 1. Photograph of a Leatherback (*Dermochelys coriacea*) hatchling showing standard measurements used for morphological data. HW, head width; CPL, carapace length; CPW, carapace width; RFL, right flipper length; RFW, right flipper width.

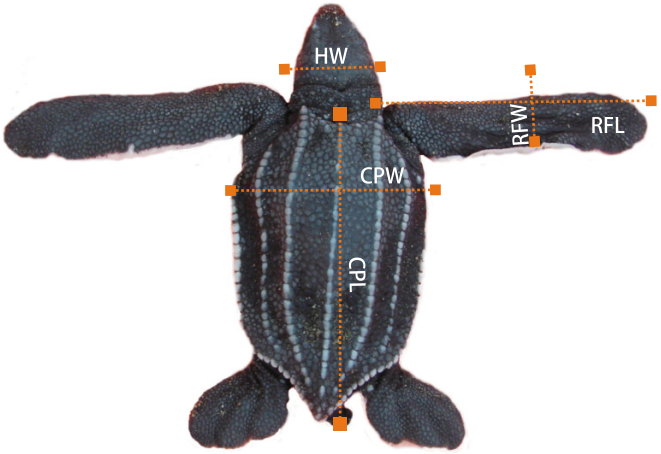


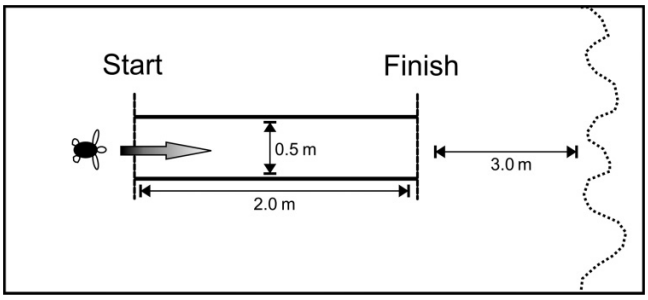
Table 1. Descriptive statistics for Leatherback (*Dermochelys coriacea*) hatchlings (n = 107).

Measurement	Mean ± SD	Minimum	Maximum
Mass (g)	40.39±3.02	31.0	47.0
Carapace			
Length (mm)	59.23±3.14	51.0	67.6
Width (mm)	37.90±2.25	31.9	42.2
Right flipper			
Length (mm)	51.11±2.81	51.4	64.5
Width (mm)	17.92±1.10	15.1	20.6
Left flipper			
Length (mm)	58.59±3.07	51.0	65.4
Width (mm)	18.19±1.18	14.8	21.5
Head width (mm)	17.75±0.91	15.0	20.4

Hatchling locomotion performance

A track was set up to measure locomotion performance of hatchlings after emergence (Fig. 2). Two precut lengths of wood were placed on the sand to create a 2.0 m × 0.5 m track. The track was set up perpendicular to the sea and was positioned approximately 2–3 m away from the current high-tide mark on a slightly downward slope. Start and finish lines were drawn in the sand at each end of the track, with the start line farthest from the sea. Sand on the track was cleared of any debris and subsequently raked smooth. In turn, each hatchling was placed approximately 10 cm behind the start line of the track and allowed to progress forward. A dim headlamp was held approximately 20 cm in front of the hatchling to encourage unidirectional movement down the track. At the moment when the hatchling's nose crossed the start line, a stopwatch was used to record total time and movement-only time. Total time refers to a continuous time measurement (s) from start to finish, while movement-only time refers to when the stopwatch was paused during any hatchling stops (s) to obtain hatchling locomotion speed ($m \cdot s^{-1}$). When the hatchling's nose touched the finish line, timing was stopped and the hatchling was allowed to proceed to the sea. The track was raked clear after each individual hatchling trial.

Fig. 2. Schematic diagram and dimensions of locomotion track used to measure terrestrial locomotion performance of Leatherback (*Dermochelys coriacea*) hatchlings.



Statistical analyses

Data were checked for normal distributions with the Anderson–Darling test for normality, and for homoscedasticity with the analysis of variance (ANOVA) residual plots. If necessary, data were transformed to meet the above assumptions for parametric statistical tests. Data analysis and transformations were carried out using Minitab version 15 (Minitab Ltd., Coventry, UK). To deal with the covariance of the hatchling morphological measurements taken (shown in Fig. 1), principal components analysis (PCA) was used to generate a correlation matrix that produced six "size-index" scoring systems (PC1–PC6). The first three principal component (PC1, PC2, PC3) scores explained 74% of the total variance, and were used to represent hatchling size and phenotype.

Results

Nest success and incubation temperature profiles

Temperature data sets were recovered for 14 nests, and 10 of those nests successfully hatched (Table 2, Fig. 3). All 10 nests were located on Turtle Beach, therefore beach site was not a confounding factor in this study. Incubation temperatures recorded were from 27.0 to 34.9 °C (Table 2). There was significant variation in nest incubation temperatures between nests for the overall incubation period (ANOVA, $F_{[9,14579]} = 26.51$, $P < 0.001$), and also for incubation period A (days 1–20; ANOVA, $F_{[9,4758]} = 170.52$, $P < 0.001$), period B (days 21–40; ANOVA, $F_{[9,4790]} = 147.05$, $P < 0.001$), and period C (days 41–60+; ANOVA, $F_{[9,4931]} = 489.51$, $P < 0.001$). TDLs that were buried as sand-only controls stayed at a relatively steady temperature throughout incubation and did not exceed 30.0 °C (Fig. 3). Mean nest temperatures for the 10 successful nests were significantly different from the control nests during incubation period B (ANOVA, $F_{[1,6238]} = 11404.0$, $P < 0.001$) and period C (ANOVA, $F_{[1,6379]} = 85284$, $P < 0.001$) but were not significantly different during incubation period A (ANOVA, $F_{[1,6206]} = 0.04$, $P > 0.05$). Mean incubation temperatures recorded by TDLs within the same nest (three TDLs each, two nests) were not significantly different from one another (ANOVA, $F_{[2,4314]} = 2.48$, $P > 0.05$), therefore slightly different positioning of TDLs within different nests was unlikely to be a confounding factor.

Table 2. Summary of temperature profiles obtained from 10 Leatherback (*Dermochelys coriacea*) nests in Tobago, West Indies, during the 2008 nesting season.

		Temperature (°C)						
Nest No.	Hatchlings masured (*n*)	Overall	Minimum	Maximum	Range	Period A	Period B	Period C
1	7	31.52±2.19	28.1	34.9	6.8	28.95±0.68	31.97±1.02	34.16±0.39
2	15	31.30±2.36	27.0	34.9	7.9	28.39±0.65	31.17±0.78	34.03±0.55
3	10	31.21±2.09	27.7	34.5	6.8	28.72±0.71	31.05±0.64	33.56±0.42
4	8	31.80±1.88	27.7	34.5	6.8	29.59±1.21	32.2±0.51	33.26±0.47
6	10	31.87±1.87	27.7	34.5	6.8	29.59±1.09	32.27±0.65	33.87±0.24
8	15	31.80±2.22	28.1	34.9	6.8	29.16±0.86	31.91±1.02	34.42±0.49
9*	10	31.77±1.79	28.4	34.1	5.7	29.65±0.87	32.11±0.74	33.64±0.27
10	12	31.96±1.77	28.1	34.5	6.4	29.9±0.91	31.81±0.68	34.09±0.37
12	10	31.97±1.85	28.1	34.1	6.0	29.75±0.97	31.99±0.81	33.8±0.34
14	10	31.81±1.69	28.1	34.1	6.0	29.94±1.0	31.74±0.48	33.64±0.36

Note: Values are mean ± SD for overall temperature (entire incubation period), as well as developmental periods A (days 1–20), B (days 21–40), and C (days 41–60). Values for nest 9* (which had three temperature data loggers (TDLs)) are taken from the centrally positioned TDL.

Hatchling morphology and phenotype

Eight biometric measurements (Fig. 1) were obtained from 107 hatchlings from 10 nests (Table 1). Of the 107 hatchlings that were measured, 89 were live and 18 were dead. The 18 dead hatchlings that were measured had successfully hatched and were in good body condition. PC1, PC2, and PC3 (see below) for the dead hatchlings were not significantly different from those of the live hatchlings (PC1: ANOVA, $F_{[1,105]} = 0.26$, $P > 0.05$; PC2: ANOVA, $F_{[1,105]} = 0.12$, $P > 0.05$; PC3: ANOVA, $F_{[1,105]} = 0.08$, $P > 0.05$), and were included to increase the sample size for the PCA. Mean nest incubation temperatures monitored during this study were well above the critical threshold temperature for Leatherback hatchling sex determination (29.75 °C) for the majority of the incubation period (Fig. 3). Therefore, it is estimated that 100% of the hatchlings measured were female, and that hatchling sex was not a confounding factor in this study.

PCA

PCA was carried out on the hatchling morphology data and six PC scoring systems were calculated. Results indicated that variation of hatchling morphology and size was concentrated in PC1, PC2, and PC3. These three components describe 74% of the morphology and size variation between individuals (Table 3) and are described below.

PC1: density

The first principal component (PC1) scoring system is dominated by the following loadings: mass, carapace length (CPL), carapace width (CPW), and right flipper length (RFL). The scoring system equation for PC1 is 0.511(mass) + 0.480(CPL) + 0.402(CPW) + 0.461(RFL). The loadings have positive coefficients in the PC1 scoring system (Table 3), which indicates that hatchlings with larger measurements of mass, CPL, CPW, and RFL will have higher PC1 scores. A general linear model (GLM) was used to confirm the interpretation of the PC1 scoring system (GLM, $F_{[4,102]} = 693.03_{mass}$, 116.16_{CPL}, 66.02_{CPW}, 110.88_{RFL}, $P < 0.001$). For this study, PC1 scores are considered to be a measure of hatchling density.

PC2: appendage width

The second principal component (PC2) scoring system is dominated by the following loadings: right flipper width (RFW) and head width (HW). The scoring system equation for PC2 is –0.686(RFW) – 0.561(HW). The loadings have negative coefficients in the PC2 scoring system (Table 3), which indicates that hatchlings with smaller RFW measurements and smaller HW measurements will have higher PC2 scores (GLM, $F_{[2,104]} = 138.75_{RFW}$, 103.98_{HW}, $P < 0.001$). For this study, PC2 scores are considered to be a measure of hatchling appendage width.

PC3: narrowness and flipper reach

The third principal component (PC3) scoring system is dominated by the following loadings: carapace width (CPW) and right flipper length (RFL). The scoring system equation for PC3 is –0.798(CPW) + 0.471(RFL). The loading for CPW has a negative coefficient and the loading for RFL has a positive coefficient in the PC3 scoring system (Table 3). This indicates that hatchlings with narrower CW measurements and longer RFL measurements will have higher PC3 scores (GLM, $F_{[2,104]} = 355.27_{CW}$, 36.03_{RFL}, $P < 0.001$). For this study, PC3 scores are considered to be a measure of hatchling narrowness and flipper reach.

Relationship between incubation temperature and hatchling morphology

Mean nest incubation temperature had a significant influence on hatchling size and biometric measurements based on PCA scores (Table 4, Fig. 4). Lower mean incubation temperatures produced hatchlings that had a greater measure of density, greater appendage width, narrower carapace width, and longer flipper length, whereas higher nest incubation temperatures produced hatchlings with smaller measures of density, smaller appendage width, wider carapace width, and shorter flipper length.

Hatchling locomotion performance

The live hatchlings that were measured ($n = 89$) underwent terrestrial locomotion performance trials and results are presented in Table 5. There is a strong positive correlation

Fig. 3. Temperature profiles from 10 Leatherback (*Dermochelys coriacea*) nests that were monitored between April and June 2008, in Tobago, West Indies. Broken horizontal lines mark the pivotal temperature of 29.4 °C that produces a sex ratio of 50% females to 50% males. The shaded squares represent incubation period B (days 21–40) where temperature sex determination occurs for Leatherback hatchlings. Temperature profiles for sand-only controls (sites A, B, C) for a 20-day time period are also shown.

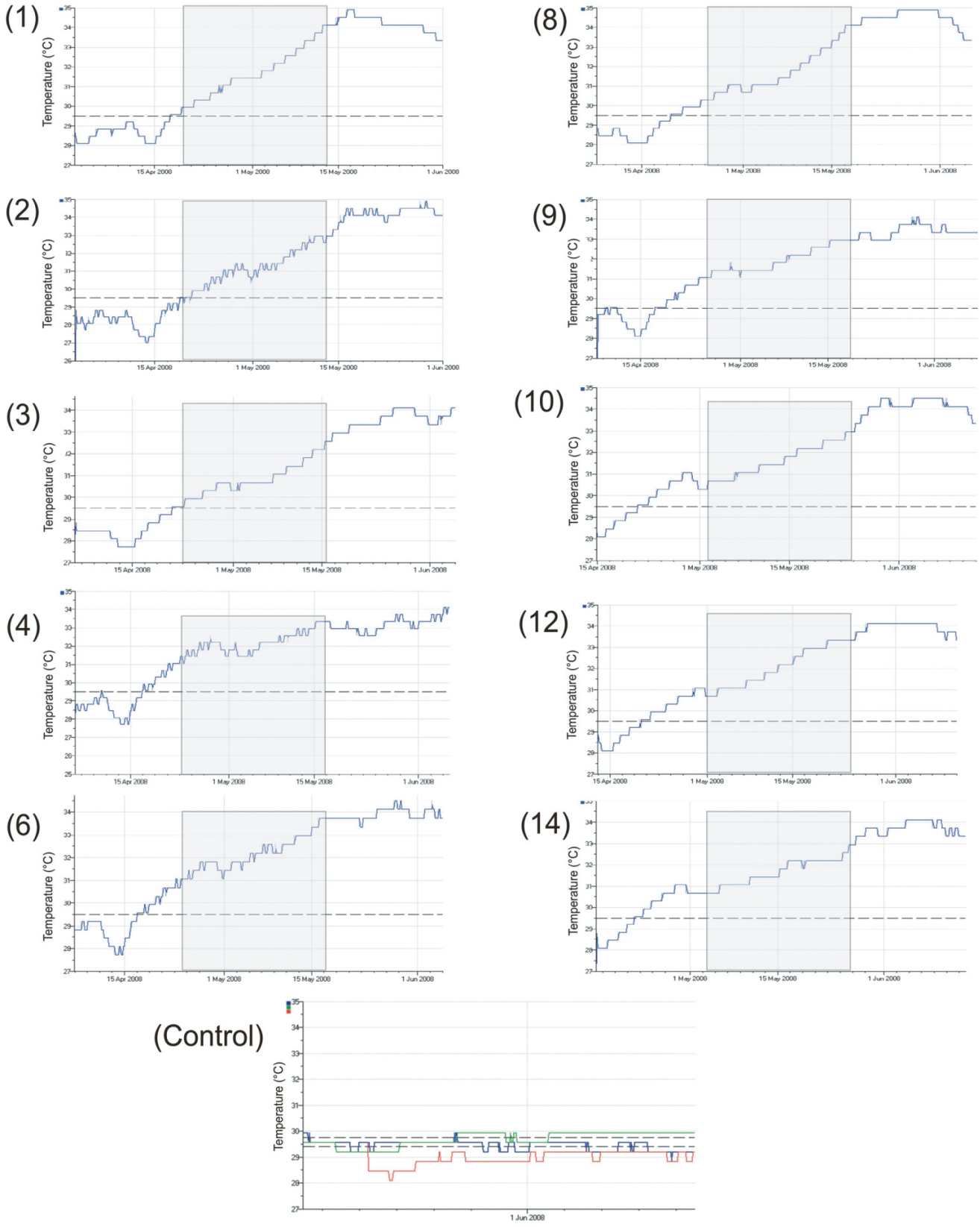


Table 3. Results of the principal components analysis (eigenvalues and coefficient loading scores) applied to Leatherback (*Dermochelys coriacea*) hatchling morphology measurements.

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	2.468	1.235	0.739	0.667	0.525	0.368
Proportion of variance	0.411	0.206	0.123	0.111	0.088	0.061
Cumulative proportion of variance	0.411	0.617	0.740	0.851	0.939	1.000
Variable						
Mass	**0.511**	0.272	0.116	0.006	0.315	–0.743
Carapace						
Length	**0.480**	0.312	–0.115	0.317	0.431	0.611
Width	**0.402**	–0.130	**–0.798**	–0.299	–0.307	–0.028
Right flipper						
Length	**0.461**	0.161	**0.471**	0.018	–0.720	0.144
Width	0.199	**–0.686**	–0.021	0.685	–0.048	–0.132
Head width	0.308	**–0.561**	0.338	–0.584	0.316	0.189

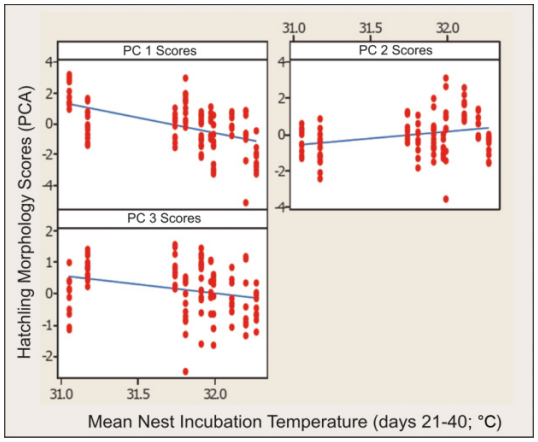
Note: Values are loading scores for each principal component (PC1–PC6), with the dominant variable coefficients for the PC1, PC2, and PC3 scoring systems in boldface type.

Table 4. Pearson's correlation coefficients (r) between nest incubation temperatures and principal component analysis morphology scores of Leatherback (*Dermochelys coriacea*) hatchlings.

	PC1: hatchling density		PC2: hatchling appendage width		PC3: hatchling narrowness and flipper reach	
Nest incubation temperature	r	P	r	P	r	P
Overall (days 1–60+)	–0.375	0.000	0.235	0.015	–0.256	0.008
Period A (days 1–20)	–0.217	0.025	0.367	0.000	–0.295	0.002
Period B (days 21–40)	–0.507	0.000	0.269	0.003	–0.272	0.005
Period C (days 41–60)	–0.010	ns	0.284	0.003	–0.375	ns

Note: ns, not significant.

Fig. 4. Relationship between Leatherback (*Dermochelys coriacea*) hatchling morphology scores (PC1, PC2, PC3) and mean nest incubation temperature (°C). PC1 represents hatchling density (y = 63.1 – 1.99x; r^2 = 0.26, P < 0.001), PC2 represents hatchling appendage width (y = –24.3 + 0.763x; r^2 = 0.05, P = 0.005), PC3 represents hatchling narrowness and flipper reach (y = 18.1 – 0.565x; r^2 = 0.07, P = 0.005).



between hatchling terrestrial locomotion speed (m·s^{-1}) and PC3 morphology scores, which represent hatchling body narrowness and flipper reach (r = 0.625, P < 0.001). Furthermore, there is a strong negative correlation between hatchling terrestrial locomotion total run time (s) and PC3 morphology scores (r = –0.616, P < 0.001). Hatchlings with higher PC3 morphology scores had narrower CW and longer RFL, and performed significantly faster in locomotion trials than hatchlings with lower PC3 morphology scores, which had wider CW and shorter RFL. GLMs were used to determine the linear relationships between hatchling PC3 morphology scores and hatchling terrestrial locomotion performance of speed (m·s^{-1}) (GLM, $F_{[1,87]}$ = 55.62, r^2 = 0.39, P < 0.001) (Fig. 5) and total run time (s) (GLM, $F_{[1,87]}$ = 53.17, r^2 = 0.38, P < 0.001) (Fig. 6). There was no significant correlation found between hatchling terrestrial locomotion speed (m·s^{-1}) and PC1 (P = 0.27) or PC2 (P = 0.58) hatchling morphology scores.

Discussion

Nest success and incubation temperature profiles

Our results are similar to those presented by Houghton et al. (2007) from Grenada, West Indies, where incubation temperatures of Leatherback nests in a natural environment ranged between 26.3 and 36.0 °C. Our finding that mean nest incubation temperatures (overall and for each third of the incubation period) varied significantly agrees with several field studies that observed significant differences between incubation temperature profiles in reptile nests (Shine et al. 1997; Binckley et al. 1998; Glen et al. 2003; Zbinden et al. 2006; Houghton et al. 2007). Monitored nests in the current study were 4–5 °C higher than sand-only controls by the time of hatchling emergence, with increased temperatures attributed to metabolic activity within the nest cham-

Table 5. Descriptive statistics for Leatherback (*Dermochelys coriacea*) hatchlings that underwent terrestrial locomotion trials.

	Mean ± SD	Minimum	Maximum	*n*
Total run time (s)*	123.29±93.63	29.0	348.0	89
Speed ($m \cdot s^{-1}$)†	0.026±0.016	0.005	0.064	89

*Total run time (s) is hatchling terrestrial locomotion over a set distance of 2 m.
†Speed ($m \cdot s^{-1}$) is movement-only time over a set distance of 2 m.

Fig. 5. Relationship between Leatherback (*Dermochelys coriacea*) hatchling terrestrial locomotion speed ($m \cdot s^{-1}$) and hatchling PC3 score narrowness and flipper reach ($y = 0.148 + 0.0368x$; $r^2 = 0.39$, $P < 0.001$).

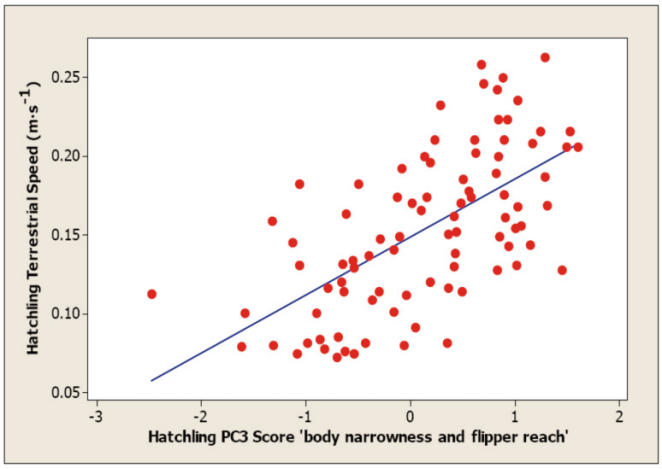
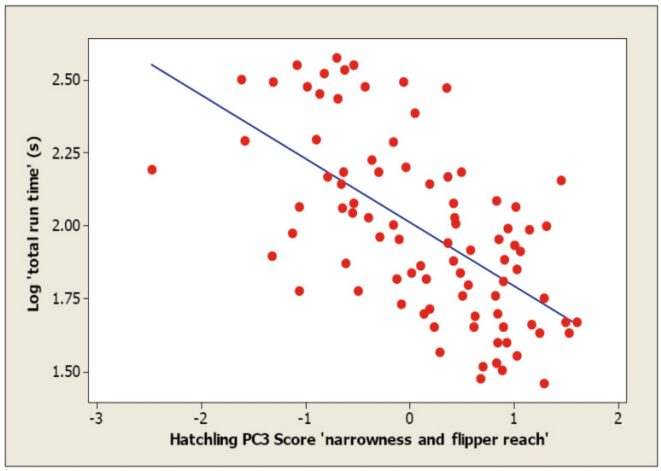


Fig. 6. Relationship between Leatherback (*Dermochelys coriacea*) hatchling terrestrial locomotion total run time (s) and hatchling PC3 score narrowness and flipper reach ($y = 4.64 - 0.504x$; $r^2 = 0.38$, $P < 0.001$). Data for total run time are log-transformed.



ber. We found that metabolic heating increased nest temperatures before the middle third of the incubation period, which is an important finding because it is sometimes assumed that metabolic heating only raises the nest temperature after the middle third of incubation. Analogous results have been reported in previous studies for other sea turtle species (Broderick et al. 2001; Zbinden et al. 2006).

It is well documented that temperature sex determination occurs in marine turtles, and is determined during the middle third of the incubation period (Davenport 1997; Binckley et al. 1998; Booth 2006). We found that a combination of environmental temperature and metabolic heating raised mean nest incubation temperatures above the pivotal sex determination temperature of 29.4 °C and the critical threshold temperature of 29.75 °C (which produces 100% female Leatherback hatchlings) well before the middle third of the incubation period. Incubation temperature profiles from natural Leatherback nests monitored in the current study lead to an estimate of a 100% female to 0% male sex ratio of hatchlings, which follows the worldwide trend that there is a female sex-ratio bias of Leatherback hatchlings (Binckley et al. 1998; Houghton et al. 2007).

Influence of incubation temperature on hatchling morphology

Several studies have investigated the influence of incubation temperatures on hatchling morphology in reptiles (Elphick and Shine 1998; Braña and Ji 2000; Webb et al. 2001; Ji et al. 2002; Reece 2002; Du and Ji 2003; Willingham 2005; Burgess et al. 2006). Most studies that have investigated the influence of incubation temperature on reptile morphology have focused on lizards. Studies investigating the influence of incubation temperature on sea turtle hatchling morphology are limited, but there are a few studies that agree with our results. The present study has demonstrated that nest incubation temperature has a significant influence on Leatherback hatchling morphology, with higher incubation temperatures producing hatchlings with smaller measures of density or size. Glen et al. (2003) investigated the influence of incubation temperature on the morphology of Green Turtle (*Chelonia mydas* (L., 1758); Cheloniidae) hatchlings and found a negative correlation between incubation temperature and hatchling size, as higher incubation temperatures produced smaller hatchlings. Booth et al. (2004) also found that Green Tturtle hatchlings from higher incubation temperatures were smaller than hatchlings from lower incubation temperatures. Burgess et al. (2006) applied PCA to morphology measurement data from Green Turtle hatchlings and observed a significant negative correlation between incubation temperature and hatchling size-index scores.

In addition to Leatherback hatchling density, nest incubation temperatures observed in the present study had a significant influence on other hatchling morphological traits. Higher incubation temperatures produced hatchlings with narrower flipper, head, and carapace widths, as well as longer flipper length. Our results are similar to those presented by Glen et al. (2003) and Booth et al. (2004), where a decrease

in front flipper area (mm^2) in Green Turtle hatchlings was observed at higher incubation temperatures. Several other studies have observed significant effects of incubation temperature on hatchling morphological traits in turtles (Du and Ji 2003; Glen et al. 2003; Booth 2006; Burgess et al. 2006) and in other reptiles such as lizards and snakes (Shine et al. 1997; Downes and Shine 1999). Braña and Ji (2000) and Ji et al. (2002) both observed a negative correlation between incubation temperature and appendage length in lizards, where increased temperatures produced hatchlings that had shorter limb lengths. Higher incubation temperatures have also been shown to increase tail length in lizards (Shine et al. 1997; Andrews et al. 2000). Downes and Shine (1999) observed different effects of incubation temperature on the morphological traits of the Garden Skink (*Lampropholis delicata* (De Vis, 1888)), Southern Weasel Skink (*Saproscincus mustelinus* (O'Shaughnessy, 1874)), and McCoy's Skink (*Nannoscincus maccoyi* (Lucas and Frost, 1894)). Higher incubation temperatures produced hatchlings with smaller mass measurements for all three species of lizard. However, tail lengths increased with temperature in only two of the lizard species (*L. delicata* and *S. mustelinus*). Snout–vent lengths increased with higher incubation temperatures in *L. delicata*, decreased with higher incubation temperatures in *S. mustelinus*, and were not significantly different at higher and lower incubation temperatures in *N. maccoyi*.

Morphological traits that are influenced by incubation temperature vary in magnitude and direction between reptile species and are likely linked to qualities that will affect their locomotion performance (Downes and Shine 1999). As the influence of incubation temperature on hatchling morphology has been shown to vary between species, it is important to investigate and compare effects between species. The majority of studies that have investigated the influence of incubation temperature on sea turtle hatchling morphology have focussed on Green Turtle hatchlings (Booth 2006; Burgess et al. 2006; Glen et al. 2003). The results presented in the current study fill a gap in the research on the influence of incubation temperature on the morphology of Leatherback hatchlings from natural nests.

Influence of hatchling morphology on locomotion performance

We found that hatchling morphology had a significant influence on terrestrial locomotion performance in the current study, with narrow carapace width and longer flipper length affording hatchlings an advantage in overall terrestrial locomotion performance. This advantage can be explained by the unique locomotion mechanism that Leatherback hatchlings use to crawl on land. Cheloniid sea turtle hatchlings crawl with synchronous movements of diagonal appendages (front and rear flippers), whereas dermochelyid sea turtle hatchlings crawl using a "swing and stance" or "rowing" movement (Davenport 1987; Wyneken 1997). During terrestrial locomotion of Leatherback hatchlings, the front flippers are simultaneously brought forward and then repositioned once they touch the substrate (Wyneken 1997). The entire body is then lifted up as the flippers are "swept" back, which moves the body in a forward direction (Wyneken 1997). Longer flipper length, coupled with narrow carapace width, would be advantageous to Leatherback hatchling speed ($m \cdot s^{-1}$) during terrestrial locomotion. Longer flipper length allows a greater distance to be covered during each forward stroke on land, while narrower carapace width creates less drag and a more streamlined effect during forward propulsion.

Ecological implications and future research

Faced with global warming, there is concern for reptile populations with temperature-dependant sex determination, which may be adversely affected by increased nest temperatures (Booth 2006). For the past 100 000 years, reptiles with temperature sex determination have adapted through considerable climate changes, but earlier temperature shifts have occurred slowly enough to allow species to adapt by adjusting their habitat range, nesting period, nest microenvironment, or pivotal temperature range (Booth 2006). The increase of nest incubation temperatures as a result of global warming has the potential to affect hatchling fitness, which may put further pressure on the existing population of these critically endangered species. In the current study, natural nest incubation temperature affected Leatherback hatchling morphology, which subsequently influenced their terrestrial locomotion performance. These results naturally lead to a conclusion that a relationship exists between nest incubation temperature and fitness in Leatherback hatchlings.

Hatchlings are vulnerable to predators during their initial migration from the nest to the sea, and it would make sense that fitness would increase with a decrease in the length of time a hatchling is exposed to predators. Faster terrestrial locomotion speed should be advantageous for Leatherback hatchlings, as faster speed decreases the duration of time exposed to predators such as crabs, birds, and dogs (Downes and Shine 1999). Lower nest incubation temperatures produce Leatherback hatchlings with advantageous morphological traits for terrestrial locomotion performance, which most likely benefits their overall fitness. Future research that investigates the influence of Leatherback hatchling morphology and locomotion performance on hatchling survival during terrestrial locomotion to the sea is needed, as overall hatchling fitness may be significantly reduced by higher nest incubation temperatures. It is not known if (or how) stopping during the crawl to the beach affects predation rates of Leatherback hatchlings, and it would be valuable to investigate survival rates of Leatherback hatchlings during their terrestrial migration to the sea to determine if predation rates are significantly lower for hatchlings that possess the advantageous morphological traits (with regards to terrestrial locomotion performance) described in this study. Research that further investigates the influence of incubation temperature on Leatherback hatchling morphology and subsequent aquatic locomotion performance would be beneficial, as hatchlings that are successful during their terrestrial journey spend the majority (or all) of their life at sea. Our experimental design did not account for the possible effect of parental influence on hatchling traits, so it would be useful to investigate the influence of incubation temperature on Leatherback hatchling morphology and locomotion performance in a controlled laboratory setting where eggs from a single clutch could be incubated at a range of temperatures. Furthermore, as 100% of the Leatherback hatchlings in the current study were female, there is a need for future research

that investigates and compares the effects of incubation temperature on morphology and locomotion performance of male Leatherback hatchlings.

Acknowledgements

We thank Tanya Clovis and all members of the SOS Tobago turtle patrol team (Save Our Sea Turtles) for support and assistance throughout the field season. Special thanks go to Grant Walker, Mark McCulley, Neil Bertram, and members of the 2008 University of Glasgow undergraduate expedition for field assistance during hatchling emergence. We are also grateful to Graeme Ruxton, Ruedi Nager, and Deborah McNeill for their contributions to the study. Comments from Graeme Hays and an anonymous reviewer are greatly appreciated.

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