Faculty of Biomedical & Life Sciences

The Influence of Incubation Temperature on Hatchling Leatherback (*Dermochelys coriacea*) Morphology: Effects on Locomotion Performance and Fitness.

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1. Abstract

Incubation temperature profiles were obtained from sixteen leatherback turtle (*Dermochelys coriacea*) nests in Tobago, W.I. from March to June 2008. There was significant variation observed between nest incubation temperatures from monitored nests, and nest incubation temperature had a significant influence on hatchling morphology traits. Hatchlings with reduced carapace width and increased flipper reach had a significant advantage in terrestrial locomotion performance, and hatchlings with greater measures of density had a significant advantage in aquatic locomotion performance. Lower mean incubation temperatures produced hatchlings with these advantageous morphological traits. Nest incubation temperature is an important factor in determining hatchling fitness, as it has a significant influence on hatchling morphology and locomotor capabilities.

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3. Acknowledgements

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4. Introduction

Sea turtles belong to a group of reptiles that spend the majority of their life in the ocean, and are well adapted to marine life (Clovis 2005). There are seven species of sea turtles that are found in two distinct families: family Cheloniidae and family Dermochelyidae. The green turtle (*Chelonia mydas*), flatback turtle (*Chelonia depressa*), hawksbill turtle (*Eretmochelys imbricata*), loggerhead turtle (*Caretta caretta*), olive ridley turtle (*Lepidochelys olivacea*) and kemp ridley turtle (*Lepidochelys kempi*) are members of the family Cheloniidae. The leatherback turtle (*Dermochelys coriacea*) is the sole member of the family Dermochelyidae (Davenport, 1997).

Sea turtles in the family Cheloniidae are found in tropical waters (within the 20°C isotherm) where they feed and breed, whereas leatherback turtles (*D. coriacea*) have a wider global distribution (Dutton *et al.* 1999; Pilcher *et al.* 2000). Leatherback turtles (*D. coriacea*) breed in tropical waters (as do all members of the family Cheloniidae) but they migrate into cooler waters to forage for food outside of the breeding season (Davenport 1997).

The life history strategy of sea turtles is one of high fecundity and high mortality (Davenport 1997). Sea turtles must return to land to lay their eggs, and large numbers of soft-shelled eggs are oviposited into nests that they dig on sandy beaches (Hendrickson 1980; Binckley *et al.* 1998; Wallace *et al.* 2004; Kamel *et al.* 2005). After a female lays her clutch of eggs, she will cover the nest back up and subsequently return to sea where she will remain until her next clutch is ready (Binckley *et al.* 1998; Wallace *et al.* 2006).

A simple diagram of general sea turtle life history was described in the literature by Davenport (1997), and is shown in Fig. A.

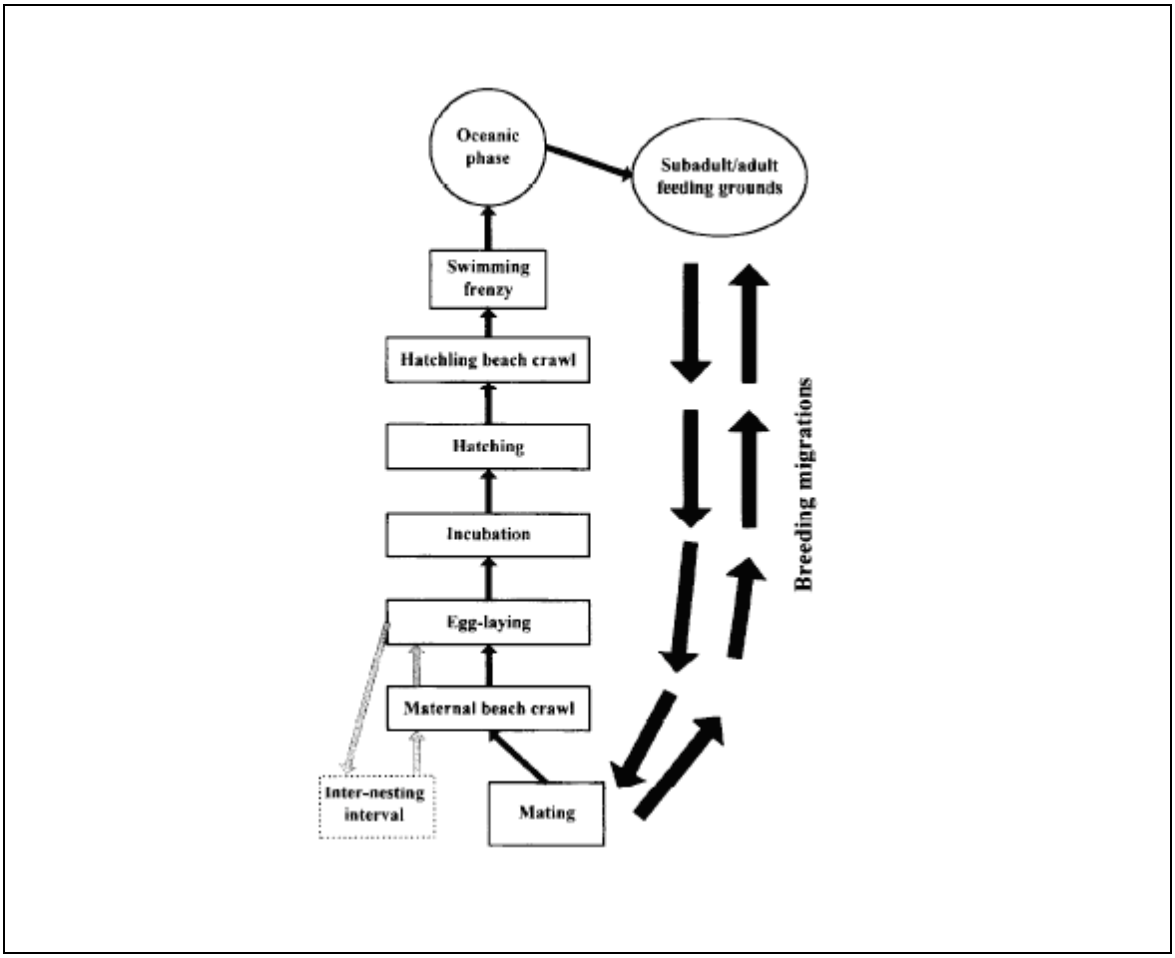


Figure A: A schematic diagram of the general life history of sea turtles (Davenport 1997)

Leatherback turtles (*D. coriacea*) show the greatest reproductive investments of all reptiles (Wallace *et al.* 2008), with females laying between 60-80 eggs per clutch and approximately seven clutches are laid during a single nesting season (Clovis 2005). Furthermore, leatherback turtles (*D. coriacea*) have the lowest hatching success rates of all species of sea turtles, but reasons for this are unknown (Wallace *et al.* 2003).

The sex of sea turtle hatchlings is determined by nest incubation temperatures (Davenport 1997; Binckley *et al.* 1998; Booth 2006; Zbinden *et al.* 2006), and this mechanism is referred to as temperature-dependent sex determination (TSD). Female hatchlings are produced at higher temperatures and males are produced at lower temperatures (Fig. B1) (Davenport 1997; Booth 2006). Hatchling sex is determined during the middle third of the incubation period, and a pivotal temperature (that produces a 1:1 sex ratio of males : females) determines the sex ratio of hatchlings (Davenport 1997).

Pivotal temperatures have been determined for all species of sea turtles, and is generally close to 29°C (Davenport 1997). The pivotal temperature for leatherback hatchlings (*D. coriacea*) is 29.4°C and the critical threshold temperature that produces 100% female hatchlings is 29.75°C (Houghton *et al.* 2007). Fig. B2 illustrates a simplified diagram of the biochemistry involved in temperature-dependent sex determination (TSD) in sea turtle hatchlings.

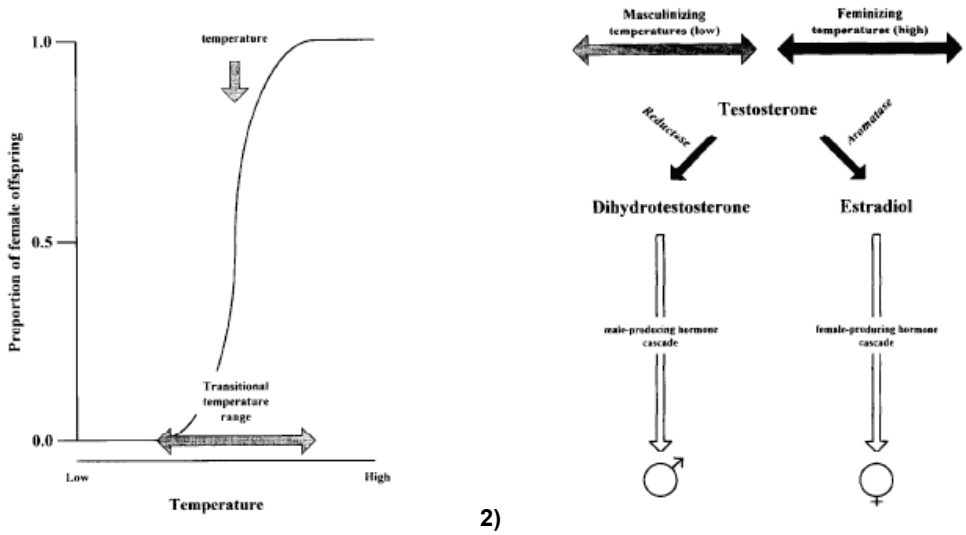


Figure B: Schematic diagrams that illustrate a) the basic principal of temperature-dependant sex determination (TSD) and b) the simplified biochemistry mechanisms of TSD (Davenport 1997).

In addition to determining hatchling sex, nest incubation temperature has been shown to influence other factors of reptile development such as size, (Ashmore *et al.* 2003; Do *et al.* 2003; Wallace *et al.* 2006; Janzen *et al.* 2007), morphology (Brana *et al.* 2000; Parker *et al.* 2007; Hare *et al.* 2008) and locomotion performance (Shine *et al.* 1997; Brana *et al.* 2000; Do *et al.* 2003; Glen *et al.* 2003; Booth *et al.* 2005; Booth 2006; Elnitsky *et al.* 2006; Watkins *et al.* 2006; Hare *et al.* 2008). For example, Glen *et al.* (2003) found that higher incubation temperatures produced smaller hatchling size in green turtles (*Chelonia mydas*), and Downes *et al.* (1999) observed a significant difference in lizard tail lengths at different incubation temperatures.

Sea turtles possess unique morphological traits that are conducive to in-water migrations over great distances (Wyneken 2000; Ji 2002). Physical adaptations include a streamlined carapace, large flippers (formed through hypertrophy of phalanges), reduced head size, and highly elastic lungs that are capable of rapid air exchange (Wyneken, 2000). As mentioned previously, the first stage of a sea turtle hatchlings life is the crawl from the nest to the sea, therefore hatchling morphology must allow for successful both terrestrial and aquatic locomotion (Wyneken *et al.* 1992; Wyneken, 2000). Relationships between species morphology, locomotion performance, and ecology have been widely acknowledged (Garland *et. al.* 1994), which provides evidence that most species are adapted to their surrounding environments.

Effective locomotion across a range of environmental conditions is extremely important for the survival and/or reproduction of many species (Elnitsky *et al.* 2006). Sea turtle hatchlings are vulnerable to predators such as crabs, birds, and dogs during the initial crawl to sea, and are vulnerable to numerous marine predators during the swimming phase away from the shore (Salmon 1987; Wyneken, J. 1992; Burgess *et al.* 2006).

Several biological and environmental factors can have an influence on the incubation temperatures of reptile nests (Wallace *et al.* 2004; Wallace *et al.* 2006). These factors can include: nest depth (Webb *et al.* 2001; Zbinden *et al.* 2006), clutch size (Reece *et al.* 2002; Ashmore *et al.* 2003; Zbinden *et al.* 2006), egg size (Wallace *et al.* 2006), seasonal rainfall (Houghton *et al.* 2007), location, external ambient temperature, sand temperature, and metabolic heating (Reece *et al.* 2002; Ashmore *et al.* 2003; Wallace *et al.* 2004; Zbinden *et al.* 2006).

Embryo development is an important stage in the life history of a species (Shine *et al.* 1997). The potential influence of environmental factors on embryo development is greatest in oviparous species, such as reptiles, where a large portion of development occurs within the nest (Shine *et al*. 1997). Therefore, determining the influence of nest incubation temperatures on leatherback hatchling morphology, and the subsequent effect on locomotion performance may provide insight towards the ecological and evolutionary significance of nest incubation temperature on hatchling fitness.

Aims of project:

- Establish incubation temperature profiles for leatherback turtle (*Dermochelys coriacea*) nests from three 'index-beaches' in Tobago, W.I., and estimate sex-ratio of hatchlings from monitored nests.

- Determine if nest incubation temperature has a significant influence on leatherback hatchling morphology traits.

- Using principal components analysis (PCA), verify which morphology traits explain the most variation in leatherback hatchlings.

- Investigate the relationship between hatchling morphology traits and terrestrial locomotion performance.

- Investigate the relationship between hatchling morphology traits and aquatic locomotion performance; and from this information, develop a model to predict hatchling aquatic locomotion performance.

5. Materials and Methods

5.1. Determining Incubation Temperature and Nest Success

5.1.1. *Location of study sites*

Incubation temperatures were obtained from 16 leatherback turtle nests (*Dermochelys coriacea*) in April and May 2008 from three beach sites on the Caribbean Coast of Tobago, West Indes (Fig. 1)

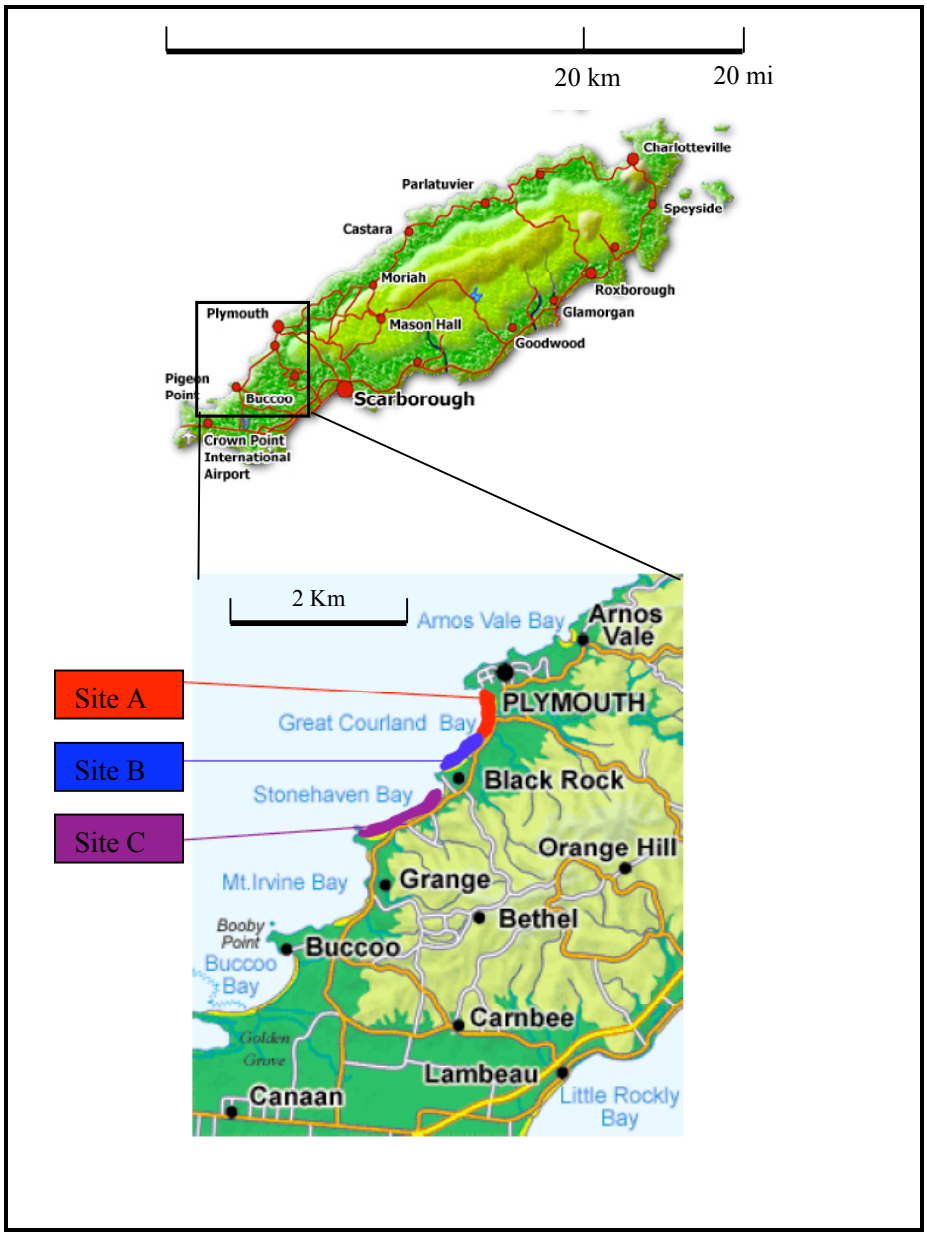


Figure 1: Map of study sites A, B, and C, located on two beaches (Great Courland Bay and Stonehaven Bay) on the Caribbean Coast, Tobago, WI, where temperature data-loggers (TDLs) were placed into 16 leatherback turtle (*Dermochelys coriacea*) nests to determine incubation temperature regimes during the 2008 nesting season.

5.1.2. *Temperature Data-Logger (TDL) preparation*

For this study, 'TinyTalk' temperature data-loggers (TDL's) were used to record incubation temperatures from 16 leatherback (*Dermochelys coriacea*) sea turtle nests. Before use, the TDL's 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 TDL's were pre-programmed with Gemini 'Tinytag' software (as needed), and were set-up to record ambient temperature every hour (for up to 75 days). Detailed information for each TDL was saved electronically and entered into a database. After initial programming set-up, the TDL's were housed in a 35mm 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 TDL's, a 2m-length nylon twine was fastened around the TDL case and secured with duct tape. Each TDL was labelled and serial numbers recorded.

5.1.3. *Field methods*

To locate laying leatherback turtles, two index beaches (Fig. 1) were patrolled nightly between the hours of 7:00pm and 4:00 am (in coordination with SOS Tobago's patrol schedules) from March 31st, 2008 to April 25th, 2008. When a leatherback turtle was encountered (and had successfully started the laying process) a 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 length measuring tape to identify the location of the nest. Spray paint and a permanent marker were used to mark and number the trees/landmarks used during triangulation. A detailed description of the location was recorded along with the date, time of oviposition, temperature (°C), and humidity levels (%).

The carapace length (cm), carapace width (cm), and flipper tag numbers (if any) were measured/recorded from the adult leatherback during the laying process. If the turtle was not tagged, both rear flippers were fitted with metal flipper tags, and numbers recorded.

5.1.4. *Temperature controls / Intra-nest temperature range*

At each study site (Fig. 1), a TDL was buried in a 'mock' leatherback nest chamber (approximately 1m depth) and left to monitor 'sand-only' temperatures for 20 days in May 2008. In addition, one nest at each beach (Great Courland Bay and Stonehaven Bay) was 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.

5.1.5. *Nest maintenance and monitoring*

Nests were monitored throughout the entire incubation period for any signs of disturbance (predation, exposed TDL twine 'tags'), and triangulation marks were maintained / re-marked as necessary. At day 55 of incubation, nests were monitored nightly for signs of hatchling emergence (see section 5.2.1).

5.1.6. *TDL recovery and nest excavation*

TDLs were recovered after hatchling emergence (see below). Within two days of hatching, each nest was excavated and contents were divided into the following categories: Hatched = hatched shells, Unhatched = dead in shell/bacteria, Inert = undeveloped, SAGs = shelled albumin globs. Unsuccessful nests that did not hatch were excavated between days 65 – 70. Once TDLs were recovered from each nest, data were downloaded onto a laptop for future analysis.

5.2. Hatchling Morphology and "Size" Index

5.2.1. *Emergence and collection of hatchlings*

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/as required), and was checked for signs of hatchling emergence every hour from 5:00pm – 5:00am. A morning patrol was also made between 6:00 and 9:00 am to check for any overnight hatching activity. Once hatchlings emerged from monitored nests, 10-20 hatchlings were randomly selected and placed in a clean 10-litre plastic bucket to undergo locomotion performance trials (discussed in section 5.3). The remaining hatchlings were allowed to proceed to the sea.

5.2.2. *Morphological measurements of hatchlings*

Each selected hatchling was weighed using a 100-gram spring balance that had been properly calibrated after being fitted with a polythene bag (large enough for a hatchling). A caliper was used to measure hatchling carapace length/width, flipper length/width (both sides), and head width (Fig. 2) to an accuracy of 0.1 mm. Latex gloves were worn when handling hatchlings.

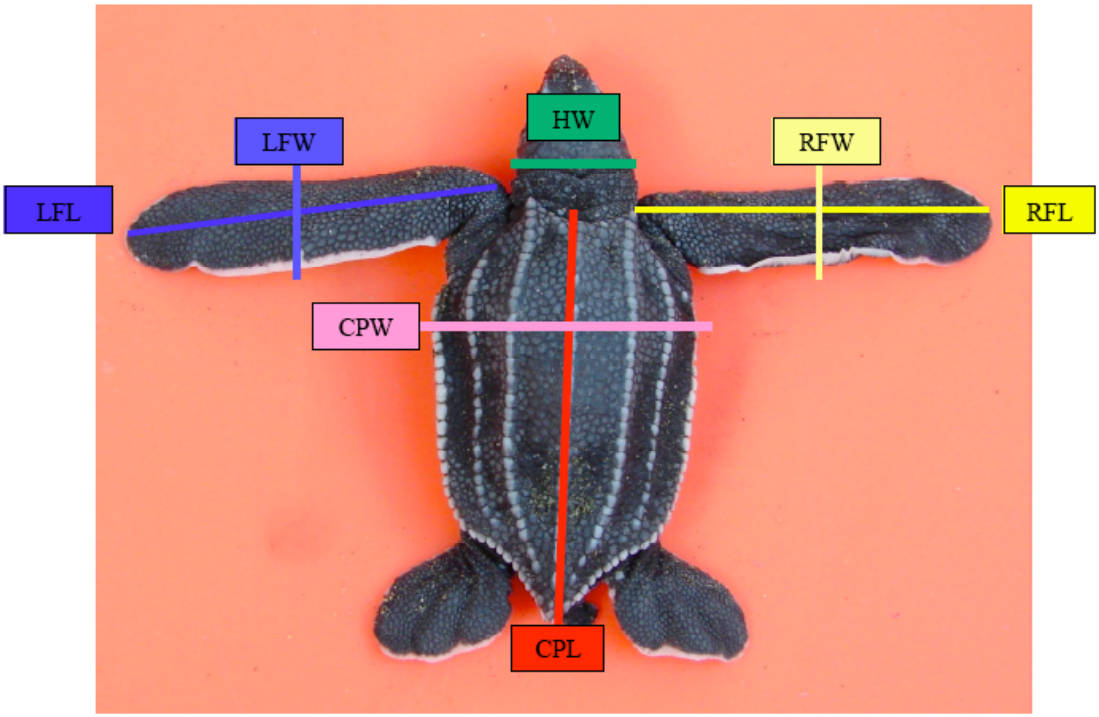


Figure 2: Photo of a leatherback (*Dermochelys coriacea*) turtle hatchling that shows standard measurements used for morphology/phenotype data. HW = head width, CPL = carapace length, CPW = carapace width, LFL/RFL = left/right flipper length, LFW/RFW = left/right flipper width. Photo: Mickelson, L. (2008).

5.2.3. *Hatchling 'size' index*

To deal with the covariance of the eight hatchling morphology measurements taken (shown in Fig. 2), a multivariate method called Principal Components Analysis (PCA) was carried out using Minitab statistical software. The morphology variables from all hatchlings were used to generate a correlation matrix that produced six 'size-index' scoring systems (PC1-PC6). The first three Principal Component scores (PC1, PC2, PC3) explained 74% of the total variance, and were used to represent hatchling 'size' and morphology for this study.

5.3. Hatchling Locomotion Performance - Terrestrial

5.3.1. *Initial set-up*

A 'locomotion-track' was set up to measure locomotion performance of hatchlings after emergence (Fig. 3). Two pre-cut lengths of wood were placed on the sand to create a 2.0-metre x 0.5-metre track. The track was set up perpendicular to the sea and was positioned approximately 2-3 metres away from the current high-tide mark, on a slightly downward slope. The track sand was cleared of any debris and was raked smooth. A "start" and "finish" line were drawn in the sand at each end of the track, with the start line furthest away from the sea.

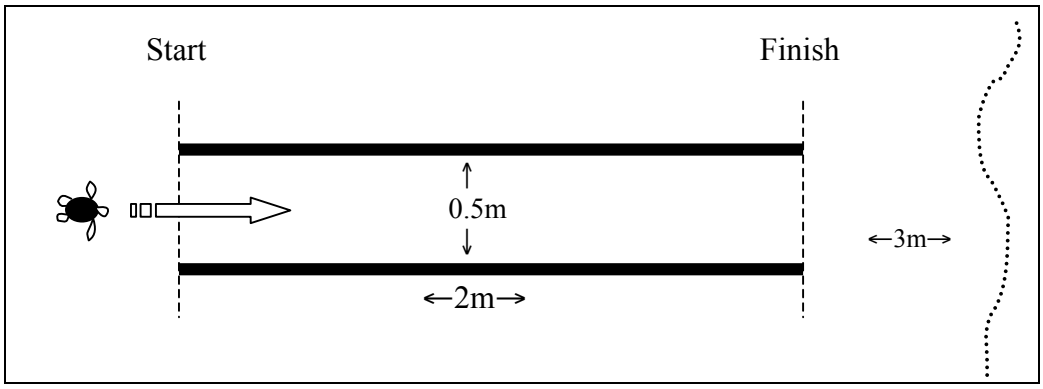


Figure 3: Schematic diagram and dimensions (m) of 'locomotion-track' used to measure terrestrial locomotion performance of leatherback (*Dermochelys coriacea*) hatchlings in Tobago, W.I. in May and June 2008.

5.3.2. *Field Methods*

Each hatchling was weighed and its physical measurements taken/recorded (as described in section 5.2.2.) prior to terrestrial locomotion performance trials. On an individual basis, hatchlings were placed approximately 10cm behind the "start" line of the 'locomotion-track' and were allowed to progress forward. A dim headlamp was held approximately 20cm 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" = continuous time measurement (s) from 'start' to 'finish', and "Movement Only Time" = stopwatch was paused during any hatchling stops (s) to obtain hatchling locomotion speed (m_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 locomotion trial. Times were recorded and measurements of speed were calculated for each hatchling.

5.4. Hatchling Locomotion Performance – Aquatic

Hatchlings from 'unmonitored' nests were used to develop an 'aquatic fitness model' to predict aquatic locomotion performance in hatchlings from monitored nests in the present study, based on hatchling 'size index' scores.

5.4.1. *Initial set-up*

Fifteen hatchlings from five separate 'unmonitored' nests were selectively chosen to undergo aquatic locomotion performance trials. When hatchlings emerged from a nest that was not involved in the present study, 3-5 hatchlings of various sizes were selected, placed in a 20-litre plastic bucket (H35cm x W22cm), and subsequently taken to the SOS Tobago craft shop that was located at Site B (Fig. 1). An additional 20-litre plastic bucket (H35cm x W22cm), was filled 3/4 full with clean seawater, which was used for the aquatic performance trials.

5.4.2. *Field methods*

Each hatchling was weighed and its physical measurements taken/recorded (as described in section 5.2.2.) prior to aquatic locomotion performance trials. On an individual basis, hatchlings were placed in a 20-litre bucket of seawater to swim. After five swim-strokes, each subsequent stroke was recorded with a hand-held counter for a time-period of two minutes, which was determined by a stopwatch. Total strokes were recorded and stroke rate (strokes_s^{-1}) calculated as a measure of swimming fitness.

5.4.3. *Aquatic Locomotion Performance model*

Principle Components Analysis (PCA) was carried out with Minitab statistical software and a 'size-index' score was produced (as previously described in section 5.2.3).

Regression analysis was used to generate an equation to predict aquatic fitness performance for all hatchlings (strokes_s^{-1}) - based on their 'size' index scores.

6. Results

6.1. Nest Success

A total of 16 leatherback nests were monitored from April to June 2008. Temperature datasets were recovered for 14 nests, and 10 of those nests successfully hatched. Six nests were unsuccessful; two nests did not successfully hatch/emerge, one nest was infertile, one nest was predated by dogs, and two nests were not successfully relocated. Temperatures for three sand-only 'control' nests (one at each study site) were also monitored for a time period of 20 days, and were successfully recovered. With regards to nest success at each beach site; site A and B each had five successful hatched nests, whereas site C had no successful hatched nests.

Table 1: Descriptive Information and hatching success of leatherback (*Dermochelys coriacea*) nests that were monitored with temperature data loggers from 3 beach sites in Tobago, W.I. in April/May 2008. Three sand-only 'control' loggers are also included.

Nest #	*Beach Site*	*Oviposition Date*	*Hatching Success*	*# Hatchlings Measured*	*Temperature Logger Retrieved*
1	B	01/04/08	Hatched	7	Y
2	A	01/04/08	Hatched	15	Y
3	A	05/04/08	Hatched	10	Y
4	B	05/04/08	Hatched	8	Y
5	A	06/04/08	Unsuccessful	-	Y
6	A	06/04/08	Hatched	10	Y
7	C	06/04/08	Infertile	-	Y (x3)
8	B	07/04/08	Hatched	16	Y
9	A	07/04/08	Hatched	10	Y (x3)
10	B	14/04/08	Hatched	12	Y
11	B	11/04/08	Unsuccessful	-	Y
12	A	12/04/08	Hatched	10	Y
13	B	12/04/08	Predated by dogs	-	Y
14	B	15/04/08	Hatched	10	Y
15	A	15/04/08	Not relocated	-	N
16	C	16/04/08	Not relocated	-	N
C1	A	19/05/08	Control	-	Y
C2	B	15/05/08	Control	-	Y
C3	C	23/05/08	Control	-	Y

6.2. Incubation Temperature Regimes

6.2.1. *Summary Statistics*

Leatherback nest temperatures were recorded every hour for the entire incubation period (60 days). Summary statistics are presented in Table 2.

Table 2: Mean temperature (°C) values (± Standard Deviation) of total incubation period, plus temperature minimum, maximum, and range are shown for 10 leatherback (*Dermochelys coriacea*) nests that were monitored with temperature data loggers in Tobago, W.I. in April/May 2008. Mean temperature (°C) values (± Standard Deviation) are also shown for three developmental periods: A= days 1-20, B= 21-40, and C= 41-60. Values for nest 9* (which had 3 TDLs) are taken from the centrally positioned TDL.

Nest	Mean Temp	Min Temp	Max Temp	Range	Mean T Day 1-20 "Dev Period A"	Mean T Day 21-40 "Dev Period B"	Mean T Day 41-60 "Dev Period C"
1	31.52 ± 2.19	28.1	34.9	6.8	28.95 ± 0.68	31.97 ± 1.02	34.16 ± 0.39
2	31.30 ± 2.36	27.0	34.9	7.9	28.39 ± 0.65	31.17 ± 0.78	34.03 ± 0.55
3	31.21 ± 2.09	27.7	34.5	6.8	28.72 ± 0.71	31.05 ± 0.64	33.56 ± 0.42
4	31.80 ± 1.88	27.7	34.5	6.8	29.59 ± 1.21	32.2 ± 0.51	33.26 ± 0.47
6	31.87 ± 1.87	27.7	34.5	6.8	29.59 ± 1.09	32.27 ± 0.65	33.87 ± 0.24
8	31.80 ± 2.22	28.1	34.9	6.8	29.16 ± 0.86	31.91 ± 1.02	34.42 ± 0.49
9 *	31.77 ± 1.79	28.4	34.1	5.7	29.65 ± 0.87	32.11 ± 0.74	33.64 ± 0.27
10	31.96 ± 1.77	28.1	34.5	6.4	29.9 ± 0.91	31.81 ± 0.68	34.09 ± 0.37
12	31.97 ± 1.85	28.1	34.1	6.0	29.75 ± 0.97	31.99 ± 0.81	33.8 ± 0.34
14	31.81 ± 1.69	28.1	34.1	6.0	29.94 ± 1.0	31.74 ± 0.48	33.64 ± 0.36

Fig. 4a presents results from a One-way Analysis of Variance (ANOVA) that was applied to the overall mean temperature data to determine if nest temperatures for the total incubation period were significantly different from one another ($F_{9,14579} = 26.51$, $P < 0.001$). A Tukey test (Fig 4B) was subsequently carried out to identify specific nests that had significantly different overall mean incubation temperatures.

a)

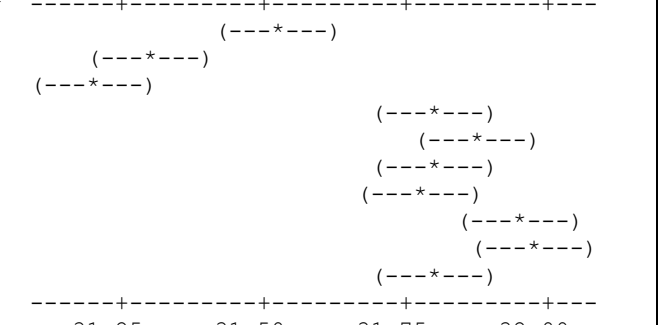
Source	DF	SS	MS	F	P
Nest	9	937.98	104.22	26.51	0.000
Error	14570	57272.89	3.93		
Total	14579	58210.86			

S = 1.983 R-Sq = 1.61% R-Sq(adj) = 1.55%

b)

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev
1	1464	31.524	2.194
2	1445	31.295	2.361
3	1464	31.207	2.093
4	1511	31.801	1.884
6	1441	31.871	1.871
8	1437	31.796	2.217
9	1439	31.774	1.790
10	1481	31.958	1.770
12	1458	31.971	1.847
14	1440	31.806	1.688



Pooled StDev = 1.983

Figure 4: **a)** Results from a One-way ANOVA of overall mean incubation temperatures (°C) from successfully hatched leatherback (*Dermochelys coriacea*) nests, and **b)** results from a subsequent Tukey test.

One way ANOVA tests were also applied to overall mean incubation temperature data from three developmental periods (A, B, C) to determine if mean nest temperatures were significantly different between nests within each developmental period. Table 3 demonstrates that mean nest incubation temperatures were significantly different between nests for all three developmental periods.

Table 3: Results from One-way Analysis of Variance (ANOVA) of mean incubation temperatures for 10 leatherback (*Dermochelys coriacea*) nests for three incubation stages (A = days 1-20, B = days 21-40, and C = days 41-60).

Incubation Stage	*Degrees of freedom*	*F-Statistic*	*P-value*
A – days 1-20	9, 4758	170.52	<0.001
B – days 21-40	9, 4790	147.05	<0.001
C – days 41-60+	9, 4931	489.51	<0.001

Incubation temperature profiles for 10 leatherback nests that hatched successfully during this study are shown in Fig. 5. Nest temperatures were taken every hour for the duration of the entire incubation period, and gradually increased over time from a minimum temperature of 27.0°C – 28.4°C to a maximum temperature of 34.1°C – 34.9°C. Temperature data loggers (TDLs) that were buried in sand-only 'control' nests stayed at a steady temperature throughout incubation and did not exceed nest temperatures of 30.0°C (Fig. 5). This demonstrates that there is a significant metabolic heating process during incubation. Fig. 7 illustrates the temperature profile of an unsuccessful/infertile nest, and when compared to a successful nest (Fig. 6) it is clear that metabolic heating, and further development, stopped at ~ day 35.

6.2.2. *Within-nest temperature variation*

Due to uncontrollable factors (such as nest shape, clutch size, egg size) the temperature data loggers (TDLs) used to monitor nest incubation temperatures could not be placed in identical positions within each nest. To investigate within-nest temperature variation, two nests were monitored with three TDLs placed in different locations within the egg chamber (bottom, middle, top) (Figs. 6 and 7). It was determined that mean incubation temperatures between TDL's within the same nest were not significantly different from one another (ANOVA, $F_{2,4314}=2.48$, $P>0.05$), therefore different positioning of TDLs within different nests was not a confounding factor.

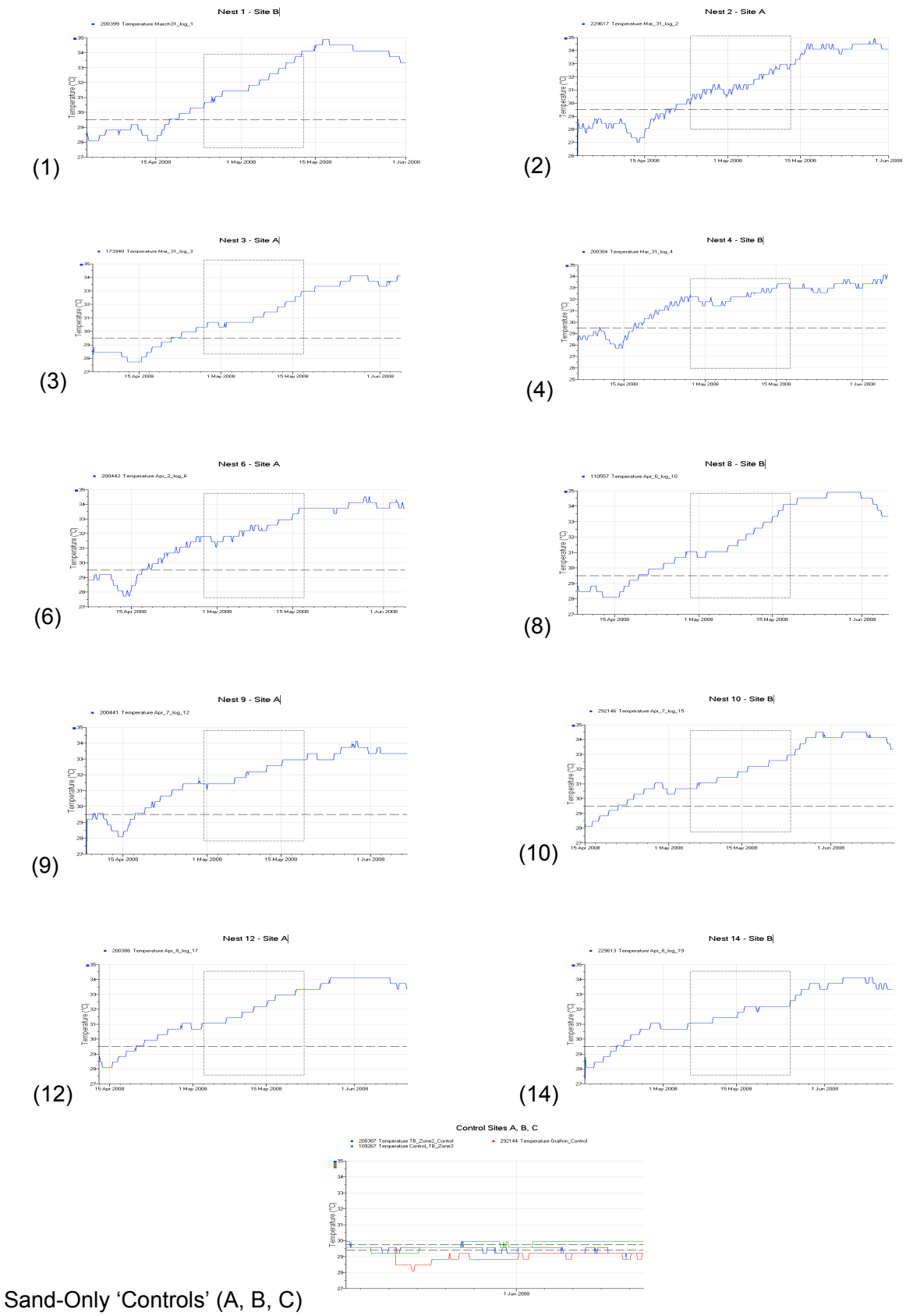


Figure 5: Temperature profiles from 10 successfully hatched leatherback (*Dermochelys coriacea*) nests that were monitored every hour for their entire incubation period between April and June 2008, in Tobago, WI. The dashed horizontal lines mark the pivotal temperature of 29.4°C that produces a sex ratio of 50% females/50% males. The dotted square represents Incubation Stage B (days 21-40) where temperature sex determination (TSD) for hatchlings occurs. Temperature profiles for sand-only 'Control' nests (sites A, B, C) for a 20 day time period are also shown.

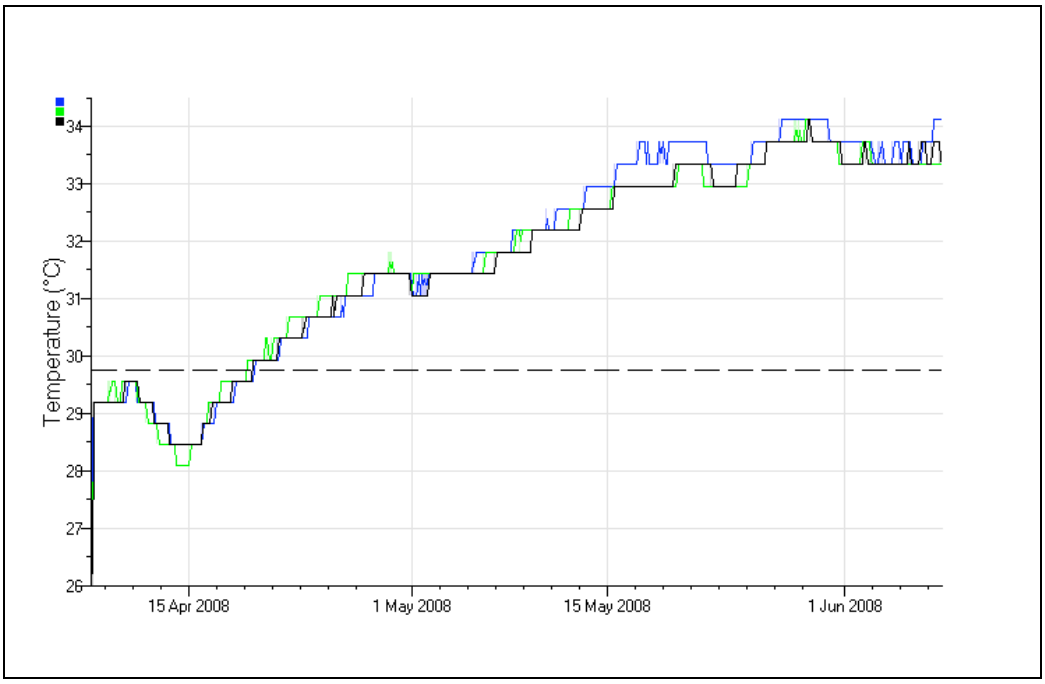


Figure 6: 60 day incubation temperature profile for leatherback (*Dermochelys coriacea*) nest 9, which was monitored with three TDLs placed in different positions within the egg chamber (top, middle, bottom) to determine within-nest temperature variation. Note: nest 9 hatched successfully.

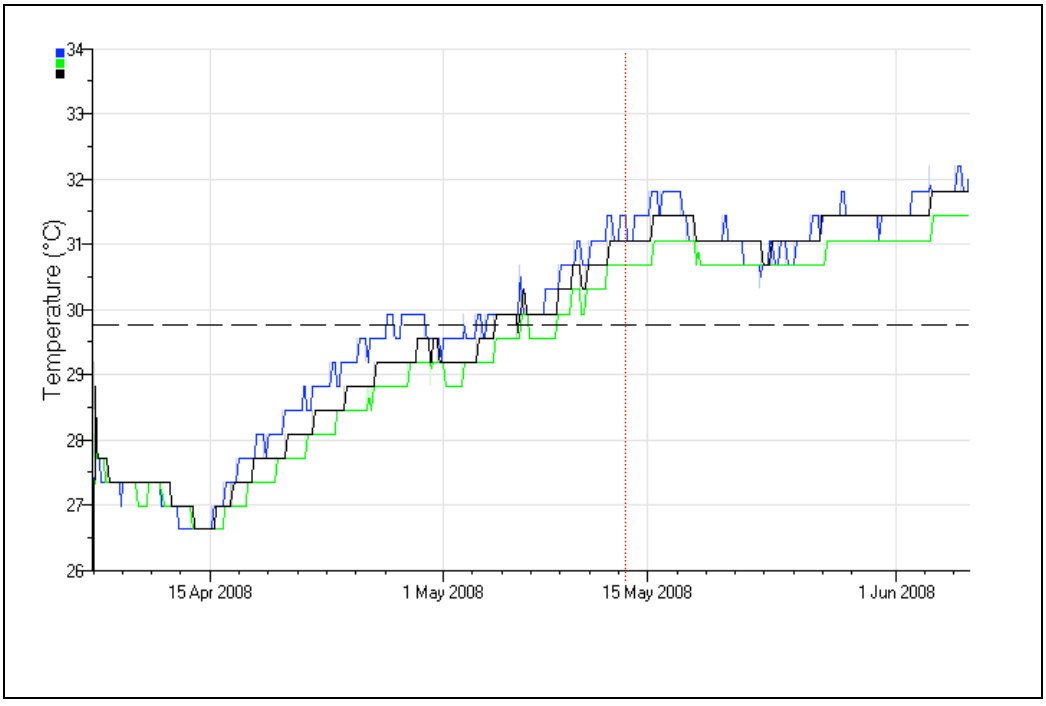


Figure 7: 60 day incubation temperature profile for leatherback (*Dermochelys coriacea*) nest 7, which was monitored with three TDLs placed in different positions within the egg chamber (top, middle, bottom) to determine within-nest temperature variation. Note: nest 7 was unsuccessful and any development is estimated to have ceased ~ day 35 (represented by red dotted line).

6.3. Hatchling Morphology and Phenotype

6.3.1. *Sex ratio estimation*

Mean nest incubation temperatures monitored during this study were well above the pivotal temperature for sex determination (29.4°C) for the majority of the incubation period (as previously shown in Fig. 5). All nests had a minimum temperature of only 1.0°C – 2.4°C below the pivotal temperature (29.4°C) during incubation stage A (days 1-20).

Fig. 5 clearly demonstrates that nest temperatures exceeded the pivotal temperature of 29.4°C during incubation period B (days 21-40), when sex is determined (Davenport 1997; Houghton 2007). In addition, incubation temperature profiles from the 10 successfully hatched nests exceeded the critical threshold temperature of 29.75°C, (example shown in Fig. 8), where 100% of the hatchlings that develop will be female (Davenport 1997; Houghton 2007) Therefore, it is estimated that 100% of the hatchlings measured were female, and that hatchling sex was not likely to be a confounding factor in this study.

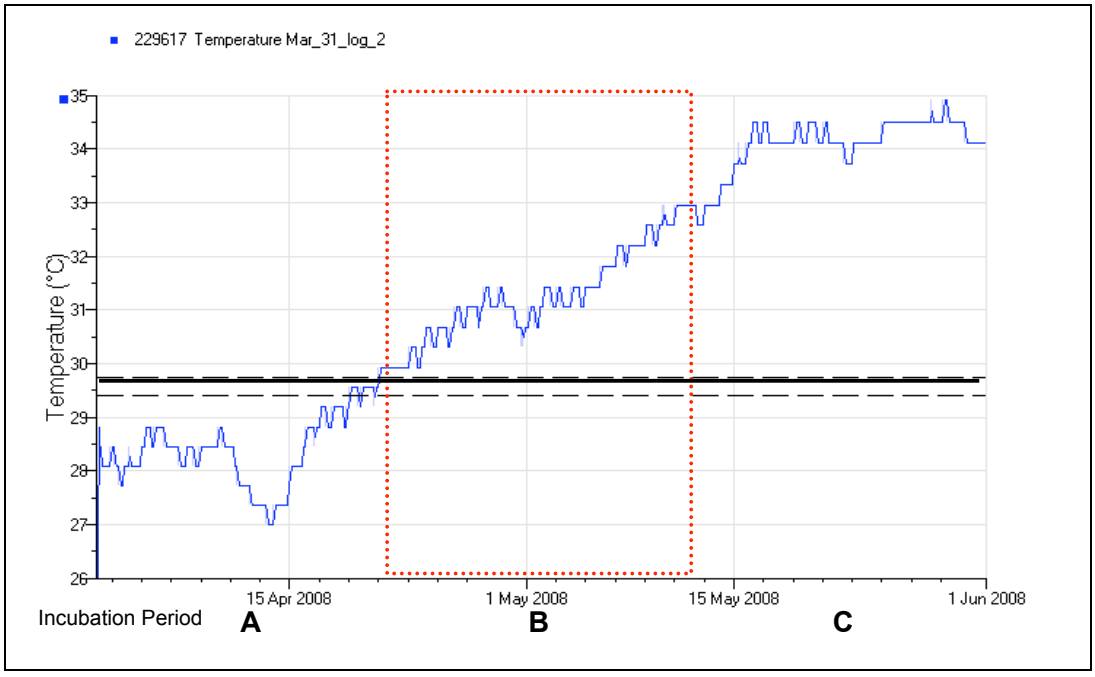


Figure 8: Leatherback (*Dermochelys coriacea*) nest temperature profile over three incubation periods (A, B, C). The dotted red box represents Incubation period B (days 21-40) where temperature sex determination (TSD) of hatchlings occurs. The black dashed line represents the pivotal temperature of 29.4°C where 50% female hatchlings are produced, and the black solid line represents the threshold temperature of 29.75°C, where 100% female hatchlings are produced.

6.3.2. *Summary statistics*

Eight biometric measurements (previously shown in Fig. 2) were obtained from 107 hatchlings from 10 nests in June 2008. Summary statistics of those measurements are presented in Table 4.

Table 4: Descriptive Information (Mean ± Standard Deviation) of morphology measurements taken from 107 leatherback turtle (*Dermochelys coriacea*) hatchlings in Tobago, W.I. in June 2008. Minimum, maximum, and range values for each measurement are also shown.

Measurement	Mean ± S.D.	Minimum	Maximum	Range
Weight (g)	40.39 ± 3.02	31.0	47.0	16.0
Carapace Length (mm)	59.23 ± 3.14	51.0	67.6	16.6
Carapace Width (mm)	37.90 ± 2.25	31.9	42.2	10.3
Right Flipper Length (mm)	51.11 ± 2.81	51.4	64.5	13.1
Right Flipper Width (mm)	17.92 ± 1.10	15.1	20.6	5.5
Left Flipper Length (mm)	58.59 ± 3.07	51.0	65.4	14.4
Left Flipper Width (mm)	18.19 ± 1.18	14.8	21.5	6.7
Head Width (mm)	17.75 ± 0.91	15.0	20.4	5.4

6.3.3. *Principal Component Analysis (PCA)*

Principal Component Analysis (PCA) was carried out on the following hatchling measurements: weight (g), carapace length (mm), carapace width (mm), left flipper length (mm), left flipper width (mm), and head width (mm). Six principle component 'scoring systems' were calculated.

Eigenanalysis results from the PCA and loadings for the six principal components are presented in Table 5, and a scree plot for these results is shown in Fig.9. Results indicate that variation of hatchling morphology and 'size' is concentrated in the first three Principal Components (PC1, PC2, PC3). These three components describe 74% of the morphology and 'size' variation between individuals.

Table 5: Eigenanalysis results produced from the Principal Components Analysis of leatherback (*Dermochelys coriacea*) hatchling morphology measurements. Eigenvalues, proportion of variance, cumulative proportion of variance, and variable loading scores for each Principal Component (PC1-PC6) are shown. The dominant coefficients for PC1, PC2, and PC3 scoring systems are underlined. CPL=carapace length, CPW=carapace width, LFL=left flipper length, LFW=left flipper width, HW=head width.

Eigenanalysis of the Correlation Matrix

Eigenvalue	2.4676	1.2348	0.7385	0.6657	0.5251	0.3684
Proportion	0.411	0.206	0.123	0.111	0.088	0.061
Cumulative	0.411	0.617	0.740	0.851	0.939	1.000

Variable	**PC1**	**PC2**	**PC3**	PC4	PC5	PC6
Weight	0.511	0.272	0.116	0.006	0.315	-0.743
CPL	0.480	0.312	-0.115	0.317	0.431	0.611
CPW	0.402	-0.130	-0.798	-0.299	-0.307	-0.028
LFL	0.461	0.161	0.471	0.018	-0.720	0.144
LFW	0.199	-0.686	-0.021	0.685	-0.048	-0.132
HW	0.308	-0.561	0.338	-0.584	0.316	0.189

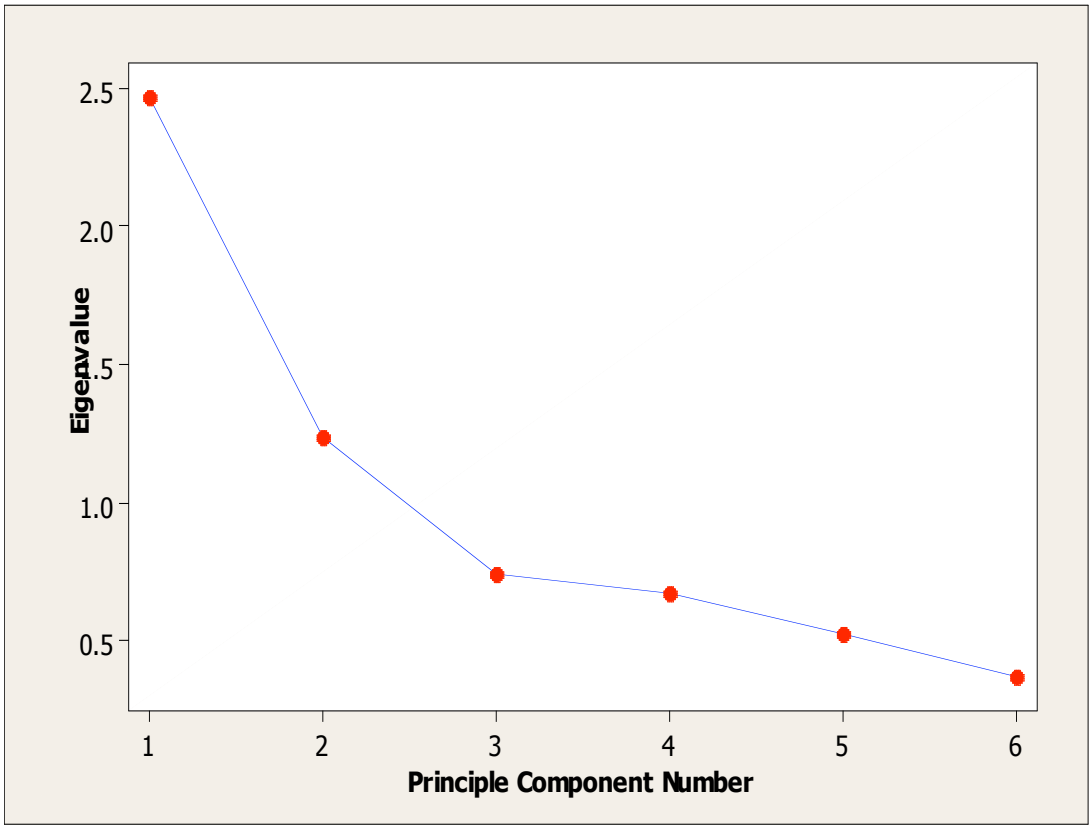


Figure 9: Scree plot of eigenvalues for the six principal components generated by the Principal Component Analysis (PCA) applied to leatherback hatchling morphology measurements (*Dermochelys coriacea*).

6.3.3.1. *The first principal component* (*PC1*) – '*density'*

The first principal component (PC1) scoring system is dominated by the following loadings: weight, carapace length (CPL), carapace width (CPW), and flipper length (LFL). The scoring system equation for PC1 is approximately:

0.511 [weight] + 0.480 [CPL] + 0.402 [CPW] + 0.461 [LFL]

The loadings have positive coefficients in the PC1 scoring system (Table 5), which indicates that hatchlings with larger measurements of weight, carapace length, carapace width, and flipper length 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, 116.16, 66.02, 110.88, $P<0.001$). For this study, PC1 scores are considered to be a measure of hatchling 'density'.

6.3.3.2. *The second principal component* (*PC2*) – *'appendage width'*

The second principal component (PC2) scoring system is dominated by the following loadings: flipper width (LFW) and head width (HW). The scoring system equation for PC2 is approximately:

-0.686 [LFW] – 0.561 [HW]

The loadings have negative coefficients in the PC2 scoring system (Table 5), which indicates that hatchlings with smaller flipper width measurements and smaller head width measurements will have higher PC2 scores. A General Linear Model (GLM) was used to confirm the interpretation of the PC2 scoring system (GLM, $F_{2,104}$ = 138.75, 103.98, $P<0.001$). For this study, PC2 scores are considered to be a measure of hatchling 'appendage width'.

6.3.3.3. *The third principal component* (*PC3) – 'narrowness & flipper reach'*

The third principal component (PC3) scoring system is dominated by the following loadings: carapace width (CPW) and flipper length (LFL). The scoring system equation for PC3 is approximately:

-0.798 [CPW] + 0.471 [LFL]

The loading for carapace width (CPW) has a negative coefficient, and the loading for flipper length (LFL) has a positive coefficient in the PC3 scoring system (Table 5). This indicates that hatchlings with narrower carapace width measurements and longer flipper length measurements will have higher PC3 scores. A GLM was used to confirm the interpretation of the PC3 scoring system (GLM, $F_{2,104}$ = 355.27, 36.03, P<0.001). For this study, PC3 scores are considered to be a measure of hatchling 'narrowness and flipper reach'.

6.4. The Relationship between Hatchling Morphology and Incubation Temperature

Pearson's product moment correlation was used to investigate relationships between Principal Component scores (PC1, PC2, PC3) and nest incubation temperatures, and results are presented in Table 6. Overall mean nest incubation temperature had a significant negative correlation with hatchling PC1 score 'density' (P < 0.001), as well as hatchling PC3 score 'narrowness & flipper reach' (P = 0.008). Overall mean nest incubation temperature had a significant positive correlation with hatchling PC2 score 'appendage width' (P = 0.015). Mean temperatures for Incubation period A (days 1-20) had significant negative correlations with hatchling PC1 score 'density' (P = 0.025), as well as hatchling PC3 score 'narrowness & flipper reach' (P = 0.002), and a significant positive correlation with hatchling PC2 score 'appendage width' (P < 0.001). Mean temperatures for Incubation Period B (days 21-40) had the same positive/negative correlations with hatchling 'density' (P < 0.001), 'appendage width' (P = 0.003), and 'narrowness/flipper reach' (P = 0.005) as overall mean nest incubation temperatures did, but to a stronger degree. Mean temperatures for Incubation period C (days 41-60) had a significant positive correlation with hatchling PC2 score 'appendage width' (P = 0.003), but showed lack of a relationship with hatchling PC1 'density' (P = n.s.) and hatchling PC3 'narrowness & flipper reach' (P = n.s.) scores.

Table 6: Pearson correlation coefficients (*r*) between nest incubation temperatures and hatchling morphology principal component scores of leatherback hatchlings (*Dermochelys coriacea*).

Mean Nest Incubation Temperature	*PC1 - hatchling 'density'*	*PC2 - hatchling 'appendage width'*	*PC3 - hatchling 'narrowness/flipper reach'*
Overall (days 1-60+)	r = -0.375 P = 0.000	r = 0.235 P = 0.015	r = -0.256 P = 0.008
Incubation Period A (days 1-20)	r = -0.217 P = 0.025	r = 0.367 P = 0.000	r = -0.295 P = 0.002
Incubation Period B (days 21-40)	r = -0.507 P = 0.000	r = 0.269 P = 0.003	r = -0.272 P = 0.005
Incubation Period C (days 41-60)	r = -0.010 P = n.s.	r = 0.284 P = 0.003	r = -0.375 P = n.s.

In general, hatchling PC1 'density' measurement scores were negatively correlated with overall mean nest incubation temperature (P<0.001). These results suggest that lower mean incubation temperatures produce larger hatchlings, and higher mean incubation temperature produce smaller hatchlings. Fig. 10 illustrates the linear relationship between overall mean nest incubation temperature and hatchling PC1 'density' scores.

Hatchling PC2 scores 'appendage width', were positively correlated with overall mean nest incubation temperature (P = 0.015). These results suggest that lower mean incubation temperatures produce hatchlings with greater flipper and head width, and higher mean incubation temperatures produce hatchlings with lesser flipper and head width. Fig. 11 illustrates the linear relationship between overall mean nest incubation temperature and hatchling PC2 'appendage width' scores.

Hatchling PC3 scores 'body narrowness/flipper reach' were negatively correlated with overall mean nest incubation temperature (P = 0.008). These results suggest that lower mean incubation temperatures produce hatchlings with narrower carapace width and longer flipper length, and higher mean incubation temperatures produce hatchlings with greater carapace width and shorter flipper length. Fig. 12 illustrates the linear relationship between overall mean nest incubation temperature and hatchling PC3 'narrowness/flipper reach' scores.

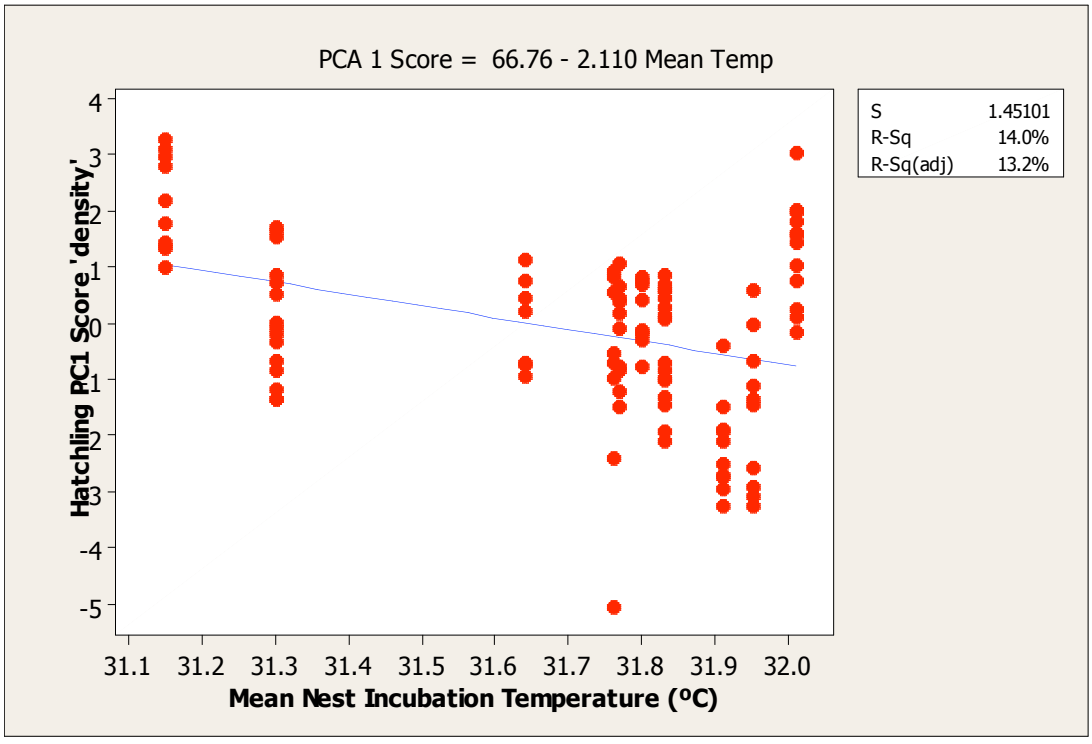


Figure 10: The relationship between leatherback (*Dermochelys coriacea*) hatchling 'density' (PC1 score) and mean nest incubation temperature (°C) (y = 66.76 – 2.110x, r^2 = 0.14, P < 0.001).

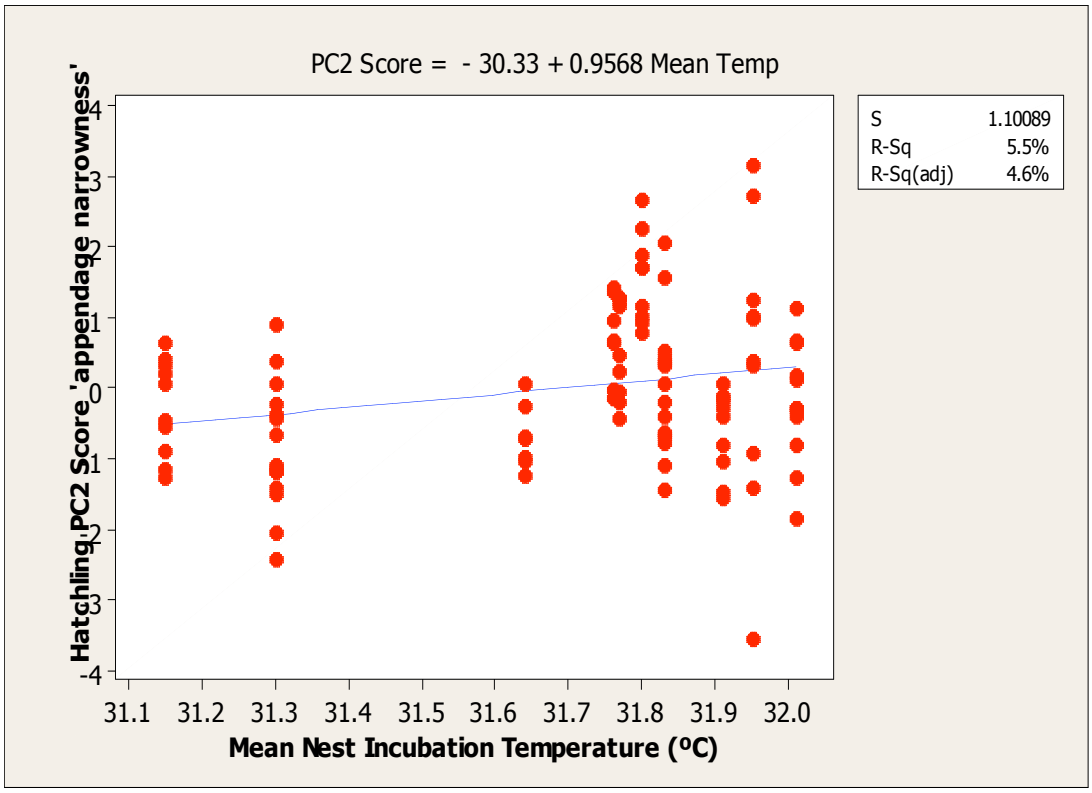


Figure 11: The relationship between leatherback (*Dermochelys coriacea*) hatchling 'appendage narrowness' (PC2 score) and mean nest incubation temperature (°C) (y = -30.33 + 0.957x, r^2 = 0.06, P < 0.05).

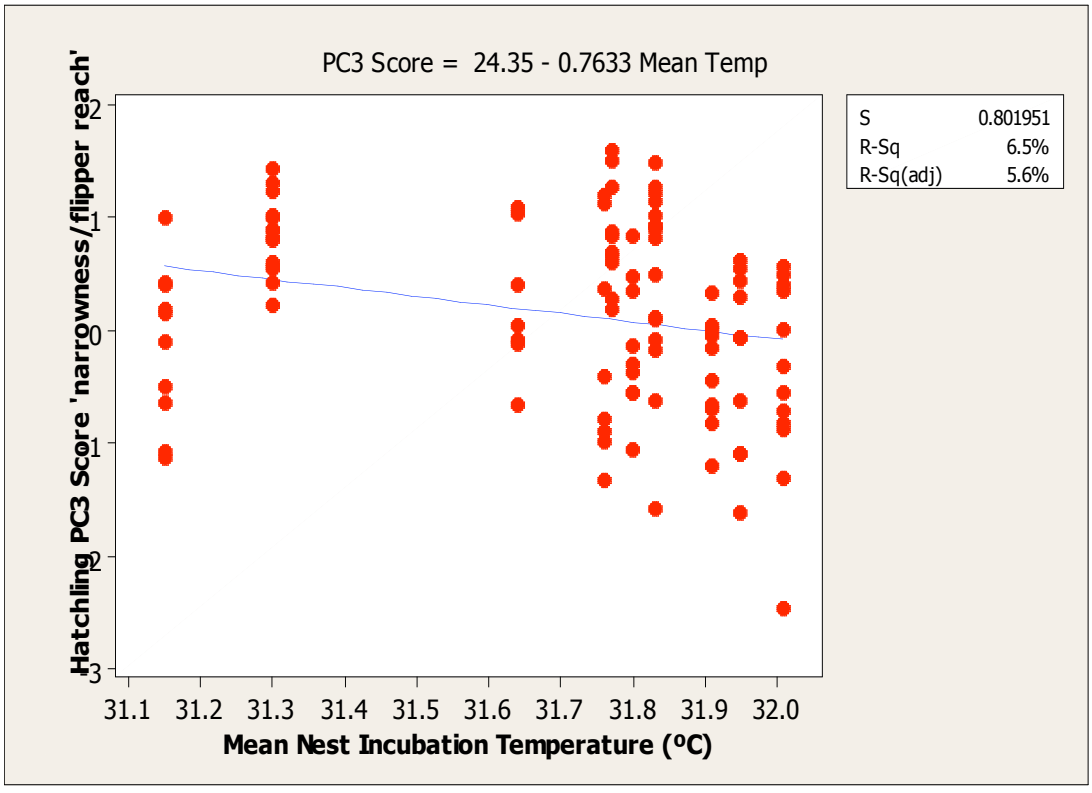


Figure 12: The relationship between leatherback (*Dermochelys coriacea*) hatchling 'narrowness/flipper reach' (PC3 score) and mean nest incubation temperature (°C) (y = 24.35 – 0.763x, r^2 = 0.07, P < 0.05).

As mentioned in the introduction, leatherback hatchling sex is determined during the middle third of incubation. Interestingly, the mean nest temperatures for Incubation Period B (days 21-40) had the same positive/negative correlations with hatchling morphology scores as overall mean nest incubation temperatures did, but to a stronger degree: hatchling PC1 scores 'density' ($P < 0.001$), hatchling PC2 scores 'appendage width' ($P = 0.003$), hatchling PC3 scores 'body narrowness/flipper reach' ($P = 0.005$).

Fig. 13 illustrates the linear relationship between mean nest incubation temperatures during incubation period B (days 21-40) and hatchling morphology scores (PC1, PC2, PC3).

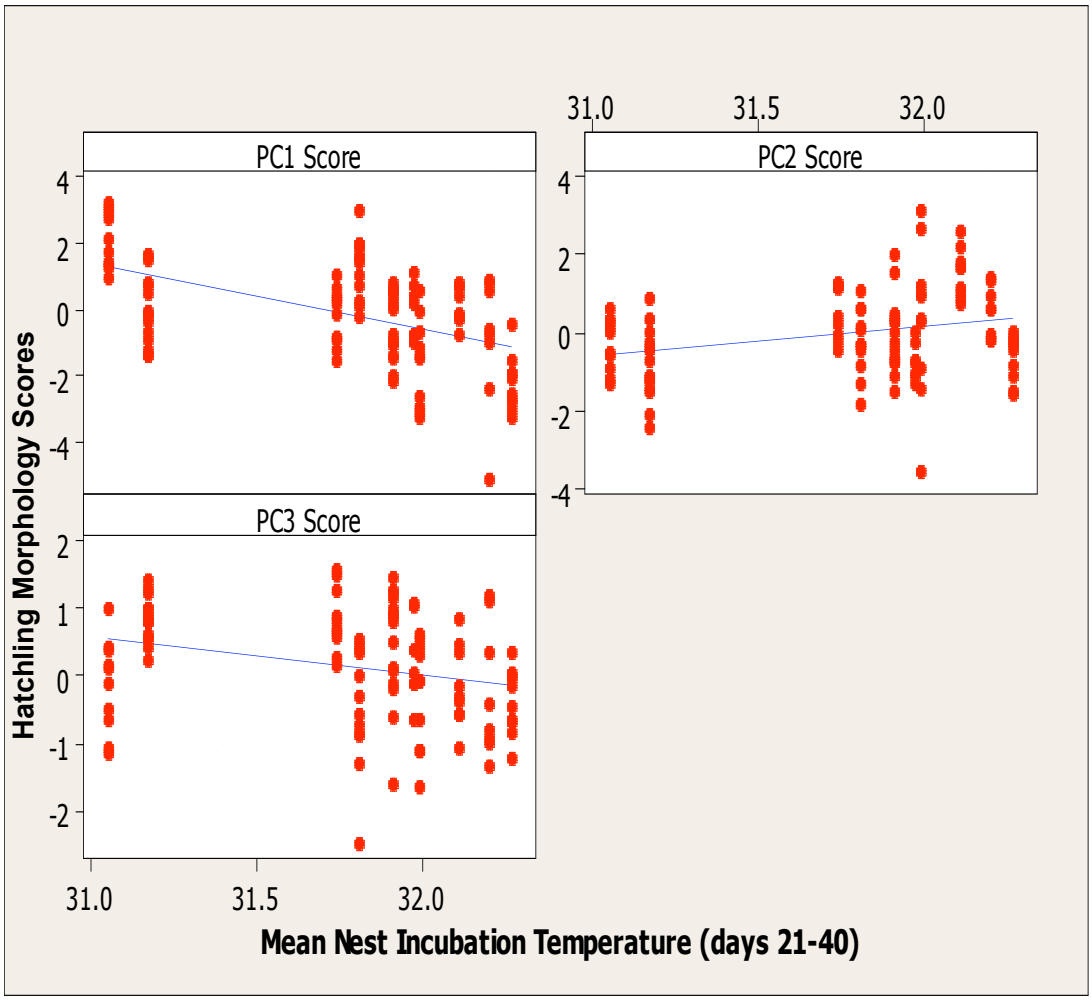


Figure 13: The relationship between leatherback (*Dermochelys coriacea*) hatchling morphology scores (PC1, PC2, PC3) and mean nest incubation temperature (°C). PC1= hatchling 'density' ($y = 63.1 - 1.99$ x, $r^2 = 0.26$, $P < 0.001$), PC2= hatchling 'appendage width' ($y = -24.3 + 0.763$ x, $r^2 = 0.05$, $P = 0.005$), PC3= hatchling 'narrowness/flipper reach' ($y = 18.1 - 0.565$ x, $r^2 = 0.07$, $P = 0.005$).

6.5. Hatchling Locomotion Performance - Terrestrial

6.5.1. Summary statistics

In June 2008, 80 leatherback hatchlings (*Dermochelys coriacea*) underwent terrestrial locomotion performance trials, where two measures of locomotion performance were recorded: total run time (s) = total time from 'start' to 'finish' line, and locomotion speed (m_s^{-1}) = 'movement only' time. Table 7 presents the summary statistics. There was a wide range of terrestrial locomotion performance scores, with the fastest hatchlings performing 12 - 13 times as fast as the slowest hatchlings.

Table 7: Descriptive statistics for leatherback (*Dermochelys coriacea*) hatchlings that underwent terrestrial locomotion trials in Tobago W.I. in June 2008. 'Total run time (s)' is hatchling terrestrial locomotion over a distance of 2m. 'Speed (m_s^{-1})' is 'movement only' time over a distance of 2m.

	Mean ± SD	*Min*	*Max*	*(n)*
Total run time (s)	123.29 ± 93.63	29.0	348.0	80
Speed (m_s^{-1})	0.026 ± 0.016	0.005	0.064	80

6.5.2. Relationship between hatchling morphology and terrestrial locomotion

There is a significant relationship between terrestrial locomotion performance of hatchlings and PC3 morphology scores, which represent hatchling 'body narrowness and flipper reach'. Results from a Pearson's product-moment correlation show there is a strong positive correlation between hatchling PC3 morphology scores and hatchling terrestrial locomotion 'speed' (m_s^{-1}) ($r = 0.625$, $P < 0.001$). Not surprisingly, there is a strong negative correlation between hatchling PC3 morphology scores and hatchling terrestrial locomotion 'total run time' (s) ($r = -0.616$, $P < 0.001$).

General Linear Models (GLM) were used to investigate the linear relationships between hatchling 'narrowness and flipper reach' morphology scores (PC3) and hatchling terrestrial locomotion performance: 'speed' (m_s^{-1}) (GLM, $F_{1,87} = 55.62$, $r^2 = 0.39$, $P < 0.001$) and 'total run time' (s) (GLM, $F_{1,87} = 53.17$, $r^2 = 0.38$, $P < 0.001$). Hatchlings with a narrower carapace width (mm) and longer flipper length (mm) had significantly faster terrestrial locomotion 'speed' (m_s^{-1}), which is illustrated in Fig. 14, and significantly faster terrestrial locomotion 'total run time' (s), which is illustrated in Fig. 15.

There was no significant correlation found between hatchling terrestrial locomotion speed (m_s^{-1}) and PC1 (P = 0.27) or PC2 (0.58) hatchling morphology scores.

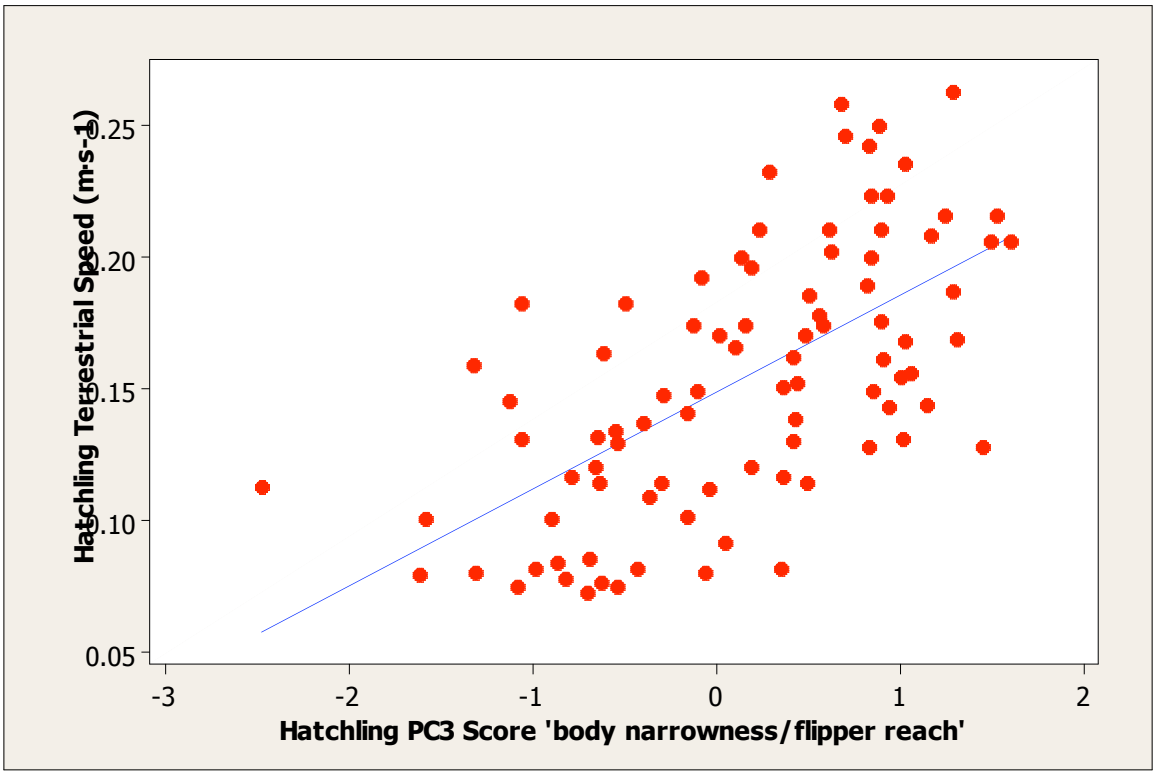


Figure 14: The relationship between leatherback hatchling (*Dermochelys coriacea*) terrestrial locomotion - 'speed' (m_s^{-1}) vs. vs. hatchling PC3 scores 'narrowness and flipper reach' (y = 0.148 + 0.0368 x, r^2= 0.39, P<0.001)

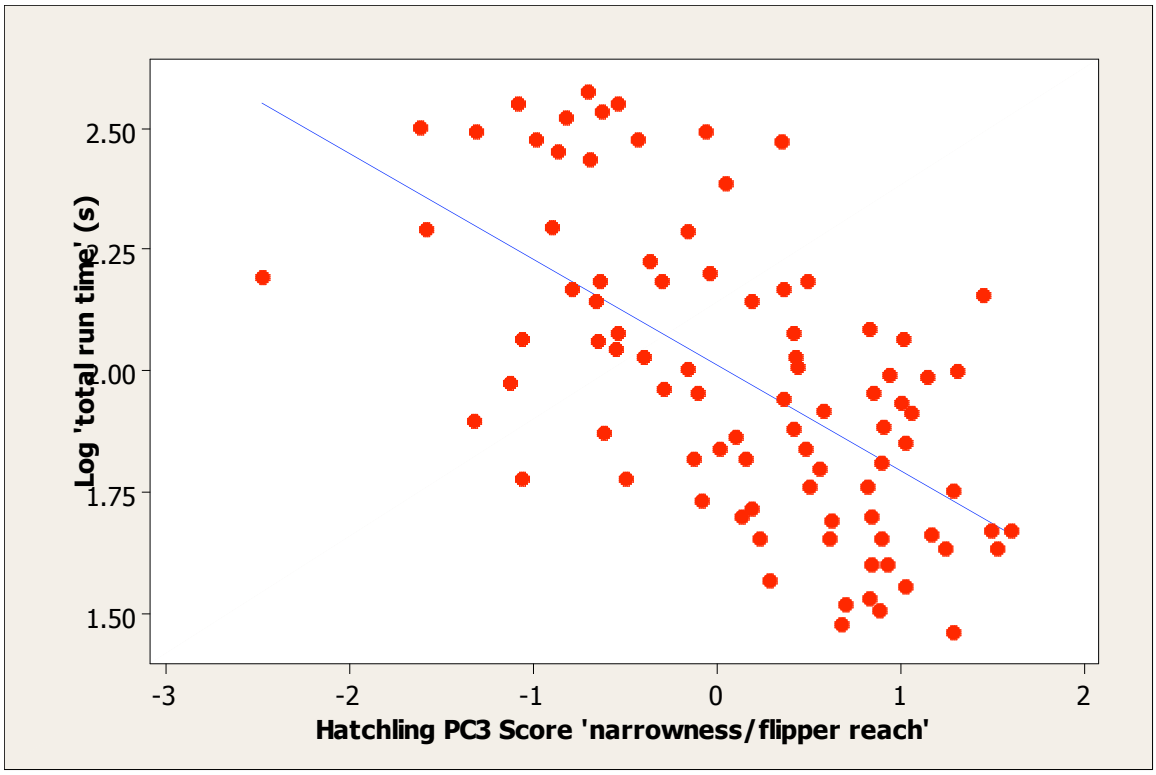


Figure 15: The relationship between leatherback hatchling (*Dermochelys coriacea*) terrestrial locomotion - 'total run time' (s) vs. hatchling PC3 scores 'narrowness and flipper reach' (y = 4.64 - 0.504 x, r^2 =0.38 ,P<0.001). Data for 'total run time' are log transformed.

6.6. Hatchling Locomotion Performance – Aquatic

6.6.1. *Summary statistics*

In June 2008, 15 leatherback hatchlings (*Dermochelys coriacea*) underwent aquatic locomotion performance trials, where stroke rates (strokes_s^{-1}) were determined as a measure of aquatic fitness, and results are presented in Table 8.

Table 8: Descriptive statistics for leatherback (*Dermochelys coriacea*) hatchlings that underwent aquatic locomotion trials in Tobago W.I. in June 2008.

	Mean ± SD	*Min*	*Max*	*(n)*
Total strokes	134.7 ± 42.5	48.0	229.0	15
Stroke rate (strokes_s^{-1})	1.08 ± 0.28	0.40	1.37	15

6.6.2. *The relationship between hatchling morphology and aquatic locomotion*

There is a significant relationship between hatchling stroke rate (strokes_s^{-1}) and PC1 morphology scores, which represent hatchling 'density' (Fig. 16) (r = 0.709, P = 0.003). There was no significant correlation found between hatchling stroke rate (strokes_s^{-1}) and PC2 (P >0.05) or PC3 (P>0.05) hatchling morphology scores.

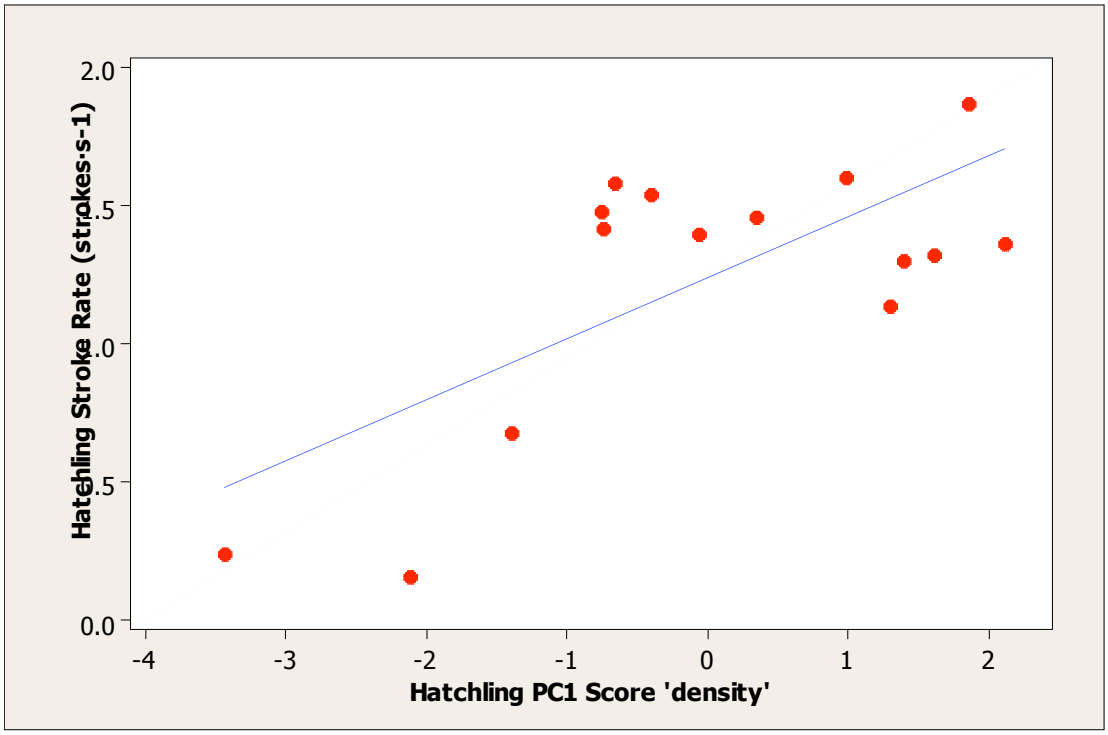


Figure 16: The relationship between leatherback (*Dermochelys coriacea*) hatchling stroke rate (strokes_s^{-1}) vs. hatchling PC1 scores 'density' (y = 1.24 + 0.220 x, r^2 =0.53, P=0.003).

6.6.3. *Aquatic locomotion performance model*

Regression analysis was applied to the aquatic locomotion performance data (summarised in section 6.6.1) and the resulting regression equation (y = 1.24 + 0.220 x, r^2 = 0.53, P=0.003) was used to predict stroke rates (strokes_s^{-1}) for the hatchlings that underwent terrestrial locomotion performance trials (n = 108) (summarised in section 6.3.2). Hatchling 'density' scores (PC1) from hatchlings that underwent aquatic locomotion performance trials (n=15) had similar distributions of PC1 scores to the hatchlings that underwent terrestrial locomotion performance trials (n=107) (Fig. 17).

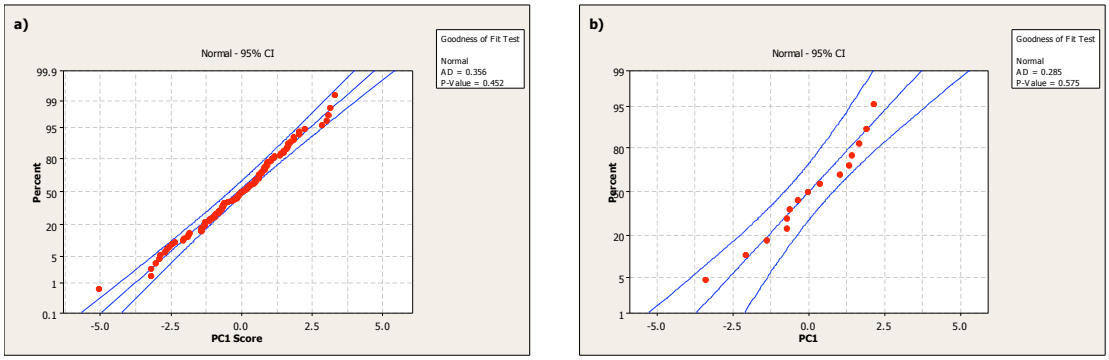


Figure 17: Probability plots of morphology PC1 scores 'density' for hatchlings (*Dermochelys coriacea*) measured in a) terrestrial locomotion performance trials and b) aquatic locomotion performance trials.

Fig. 18 illustrates the linear model used to predict hatchling stroke rate (strokes_s^{-1}) based on their 'density' scores (PC1). The distribution and spread of points along the regression line is similar to actual results presented in Fig. 16.

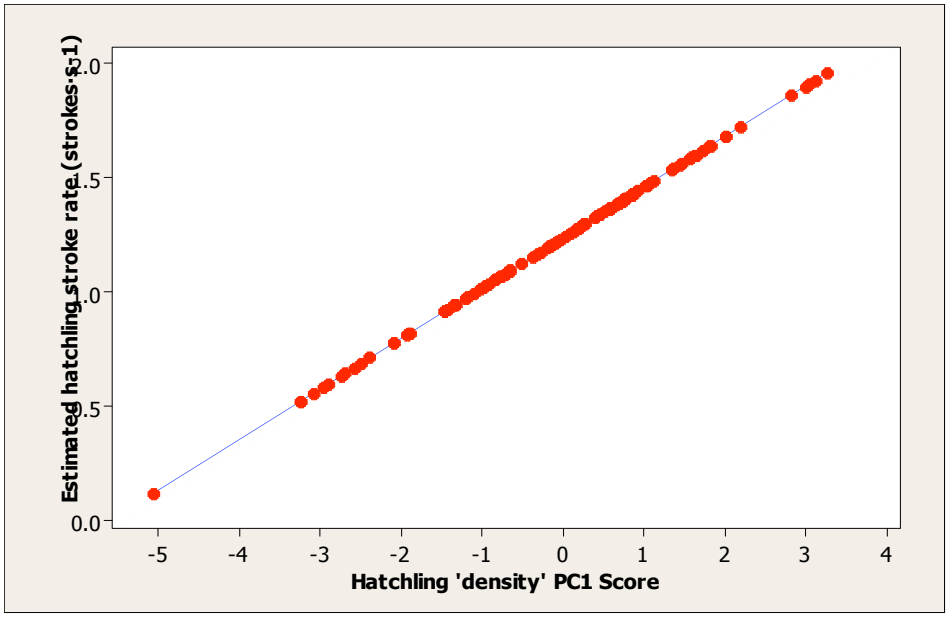


Figure 18: The estimated stroke rate (strokes_s^{-1}) of leatherback turtle (*Dermochelys coriacea*) hatchlings, based on hatchling 'density' (PC1 morphology scores).

7. **Discussion**

7.1. Nest Success and Incubation Temperature Profiles

In the present study, sixteen leatherback turtle (*Dermochelys coriacea*) nests were monitored from April to June 2008, and ten of those clutches successfully hatched. Most marine turtle species have a hatch success rate for clutches that is approximately 80%, however leatherback turtles have a hatching success rate for clutches that is approximately 50% (the lowest hatch success rate of all marine turtles) (Wallace *et al.* 2004). The overall hatching success of clutches for the current study was approximately 62% (successfully hatched). The above average clutch hatch success rate observed in the current study may be attributed to the following factors: 1) the location of nests selected for this study were chosen rather selectively to prevent nest washout (ideal nests were distanced from tidal inundation and river mouths), 2) nests were monitored regularly to deter poaching and to maintain identification of nest triangulation, and 3) nests/hatchlings were somewhat protected from dogs and human disturbance through hourly monitoring and nest marking around the hatchling emergence date.

Hatching success and Temperature Data Logger (TDL) recovery results from a similar study conducted by Houghton *et al.* (2007), instilled confidence in the chosen methodology used in the current study to determine nest incubation temperature regimes, and further validated the decision to avoid monitoring nests that may be washed away. Natural nest incubation temperatures were monitored in fifteen leatherback turtle nests by Houghton *et al.* (2007) during the 2003 nesting season in Grenada, W.I. (March – June), and results show that two nests were poached, three nests were washed out to sea (as well as five 'control' nests), and two nests were not relocated. Temperature Data Loggers (TDLs) were recovered from a total of eight nests, and from those nests, three hatched successfully (Houghton *et al.* 2007).

The methodology used in the current study (to obtain information about nest incubation temperature regimes) was utilized to collect data from as many nests as possible. Therefore, the hatching success rate of clutches observed in the present study should not be considered an estimate of overall hatching success rates for the 2008 nesting season in Tobago, WI.

Nest temperatures observed from naturally occurring leatherback nests in the present study ranged between 27.0°C – 34.9°C. These results are similar to results presented by Houghton *et al.* (2007), where observed incubation temperatures of leatherback nests in a natural environment ranged between 26.30°C – 36.0°C. The overall mean incubation temperatures observed in the current study ranged between 29.12°C and 33.82°C, and were significantly different between monitored nests. Mean nest temperatures for each third of the incubation period (days 1-20, 21-40, 41-60) were also significantly different. Not surprisingly, there was a significant difference observed between 'sand-only' control nests and monitored nests in the current study. Nests that were monitored in the present study had mean temperatures that were approximately four to five degrees (°C) higher than the surrounding sand temperature, at the time of hatchling emergence. These results accord with several field studies that observed significant differences between incubation temperature profiles in reptile nests, which were similarly monitored in natural environments (Shine *et al.* 1997; Binckley *et al.* 1998; Glen *et al.* 2003; Zbinden *et al.* 2006; Houghton *et al.* 2007).

The increase in temperature during incubation can be attributed to metabolic activity within the nest's egg chamber, as high metabolic activity during incubation occurs in developing reptile clutches (Zbinden *et al.* 2006). Evidence of metabolic heating during incubation has been presented in several studies investigating incubation temperature profiles of developing reptile nests (Wallace *et al.* 2004; Zbinden *et al.* 2006; Wallace *et al.* 2008). Wallace *et al.* (2004) investigated the effect of metabolic heating on the

development of leatherback embryos and observed that the large egg mass, characteristic in leatherback clutches, generates significant metabolic heating. Wallace *et al.* (2004) also suggested that developing embryos collectively affect their own nest environment through the process of metabolic heating. It is interesting to note that the 'sand-only' temperatures observed in control nests from the current study were clustered around the pivotal and critical threshold temperatures that determine hatchling sex (which is discussed in detail further on), and that metabolic heating within monitored nests in the current study raised the mean nest incubation temperatures well past both.

It is well documented that temperature sex determination (TSD) occurs in marine turtles, and is determined during the middle third of the incubation period (Davenport 1997; Binckley *et al.* 1998; Booth 2006). In the dataset presented here, results show that metabolic heating raised mean nest incubation temperatures above the pivotal sex determination temperature of 29.4°C (which produces a 50:50 sex ratio of leatherback hatchlings) during the first third of the incubation period (days 1-20). Furthermore, metabolic heating raised mean nest incubation temperatures above the critical threshold temperature of 29.75°C (which produces 100% female leatherback hatchlings) during the middle third of the incubation period (days 21-40), which is the period when the sex of hatchlings is determined.

Incubation temperature profiles from natural leatherback nests monitored in the current study lead to an estimate of a 100% female: 0% male sex-ratio of hatchlings. This estimation follows the worldwide trend that there is a female sex-ratio bias of leatherback turtle hatchlings being produced (Binckley *et al.* 1998; Houghton *et al.* 2007). However, it should be noted that the nests monitored in this study completed their incubation period during the dry season in Tobago, W.I. (March – June), and it has been suggested that increased rainfall may have a cooling effect on nest incubation temperatures (Houghton *et al.* 2007).

Therefore, additional data from natural leatherback nests that complete their incubation during the rainy season (July – September) would be a useful comparison of incubation temperature regimes, and would provide more information about the influence of rainfall on mean incubation temperature and the resulting sex-ratio of hatchlings.

7.2. The Influence of Incubation Temperature on Hatchling Morphology

The present study has demonstrated that nest incubation temperature has a significant influence on leatherback hatchling (*Dermochelys coriacea*) morphology. Several studies that have investigated the influence of incubation temperatures on hatchling morphology in other reptiles accord with these results (Brana *et al.* 2000; Webb *et al.* 2001; Ji X 2002; Reece *et al.* 2002; Ashmore *et al.* 2003; Do *et al.* 2003; Glen *et al.* 2003; Willingham 2005; Burgess *et al.* 2006). In the literature, most studies that have investigated the influence of incubation temperature on reptile morphology have focused mainly on lizards, for example Brana (2000) and Ji *et al.* (2002) observed a positive correlation between hatchling body size and incubation temperature in wall lizards (*Podarcis muralis*) and oriental garden lizards (*Calotes versicolor*), respectively.

Higher mean nest incubation temperatures observed in the present study produced hatchlings of lower 'density'. Studies investigating the influence of incubation temperature on sea turtle hatchling morphology are limited, but there are a few recent studies that accord with the results from the current study. Glen *et al.* (2003) investigated the influence of incubation temperature on the morphology of green turtle hatchlings (*Chelonia mydas*), and found a negative correlation between incubation temperatures and hatchling size, as higher incubation temperatures produced smaller hatchlings. Booth *et al.* (2004) also found that green turtle hatchlings (*C. mydas*) from higher incubation temperatures were smaller than hatchlings from lower incubation temperatures.

In a recent study by Burgess *et al.* (2006), principal components analysis (PCA) was applied to morphology measurement data from green turtle hatchlings (*C. mydas*). A significant negative correlation was observed between incubation temperature and hatchling 'size-index' scores (where higher principal component scores represent larger hatchling size). Therefore, hatchlings from higher incubation temperatures were smaller than hatchlings from lower incubation temperatures (Burgess *et al.* 2006).

The increased 'density' of turtle hatchlings observed at lower incubation temperatures in this study could be attributed to larger amounts of residual yolk present, as hatchlings with slower metabolism of yolk reserves may be produced at lower temperatures (Willingham 2005). Conversely, increased 'density' of hatchlings at lower incubation temperatures could also be attributed to larger amounts of yolk being converted to hatchling tissue (Booth *et al.* 2005; Burgess *et al.* 2006). Booth *et al.* (2006) noted that hatchlings incubated at lower temperatures had heavier 'yolk-free' mass and lighter yolk reserve mass than hatchlings incubated at higher temperatures. Wallace *et al.* (2006) investigated the relationship between egg size/components and hatchling size in leatherbacks (*D. coriacea*). They found that hatchling mass increased with egg size, and that up to 50% of hatchling mass was derived from albumen components and/or from water absorption in the nest (Wallace *et al.* 2006). However, Wallace *et al.* (2006) did not take into consideration the influence of incubation temperature (which was carried out in natural nests) on egg size or components. Therefore, the influence of incubation temperature on egg size and egg components in leatherback nests would be an interesting future study.

In addition to hatchling 'density', nest incubation temperatures observed in the present study had a significant influence on other hatchling morphology traits (such as appendage width, carapace width, and flipper reach). Hatchlings from lower incubation temperatures had larger flipper width and head width, as well as narrower carapace

width and longer flipper length. These results are similar to those presented by Glen *et al.* (2003) and Booth *et al.* (2004), where an increase in front flipper area (mm^2) in green turtle hatchlings (*C. mydas*) was observed at lower incubation temperatures.

Several other studies have observed significant effects of incubation temperature on specific hatchling morphology traits in other reptiles, such as lizards and snakes (Shine *et al.* 1997; Downes *et al.* 1999; Brana *et al.* 2000; Do *et al.* 2003; Glen *et al.* 2003; Booth *et al.* 2005; Booth 2006; Burgess *et al.* 2006). Brana *et al.* (2000) and Ji *et al.* (2002) both observed a negative correlation between appendage length and incubation temperature in lizards, where increased temperatures produced hatchlings that had shorter limb lengths. However, higher incubation temperatures do not automatically lead to a reduced appendage length in all reptiles: for an example, higher incubation temperatures have been shown to increase tail length in lizards (Shine *et al.* 1997; Andrews *et al.* 2000).

The influence and effect of incubation temperature on hatchling morphology in reptiles varies between species. Downes *et al.* (1999) observed different effects of incubation temperature on the morphology traits of three species of lizards (*Lampropholis delicata*, *Saproscincus mustelina, Nannoscincus maccoyi*). 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. mustelina*) (Downes *et al.* 1999). Snout-vent lengths increased with higher incubation temperatures in *L. delicata*, decreased with higher incubation temperatures in *S. mustelina*, and were not significantly different from lengths at higher and lower incubation temperatures in *N. maccoyi* (Downes *et al.* 1999).

Morphological traits that are influenced by incubation temperature vary in magnitude and direction between reptile species (Downes *et al.* 1999), and are likely linked to qualities that will affect their locomotion performance.

7.3. *The Influence of Hatchling Morphology on Locomotion Performance*

7.3.1. *Terrestrial locomotion*

Hatchling morphology had a significant influence on terrestrial locomotion performance in the present study. Hatchlings with higher PC3 morphology scores, which represent hatchling 'narrowness and flipper reach', showed a significant advantage in terrestrial locomotion. Hatchlings with a narrower carapace width and longer flipper reach, covered a distance of two metres in shorter time periods, and had faster terrestrial speed (m_s^{-1}) than hatchlings with wider carapace width and shorter flipper reach. Therefore, hatchlings with these morphology traits (narrow carapace width and greater flipper length) showed an advantage in terrestrial locomotion performance. This advantage can be explained by the unique locomotion mechanism that leatherback hatchlings (*D. coriacea*) use to crawl on land.

Sea turtle hatchlings in the family Cheloniidae crawl with synchronous movements of diagonal appendages (front and rear flippers), whereas sea turtle hatchlings in the family Dermochelyidae (leatherbacks) crawl using a "swing and stance" or "rowing" movement (Davenport 1987; Wyneken *et al.* 1997). During terrestrial locomotion of leatherback hatchlings, the front flippers are simultaneously brought forward and then repositioned once they touch the substrate (Wyneken *et al.* 1997). The entire body is then lifted up as the flippers are "swept" back, which moves the body in a forward direction (Wyneken *et al.* 1997). Fig 19. illustrates the synchronous diagonal mechanism used by hatchlings from the family Cheloniidae during terrestrial locomotion, and Fig. 20 illustrates the unique "rowing" mechanism used by leatherback hatchlings during terrestrial locomotion (Wyneken *et al.* 1997).

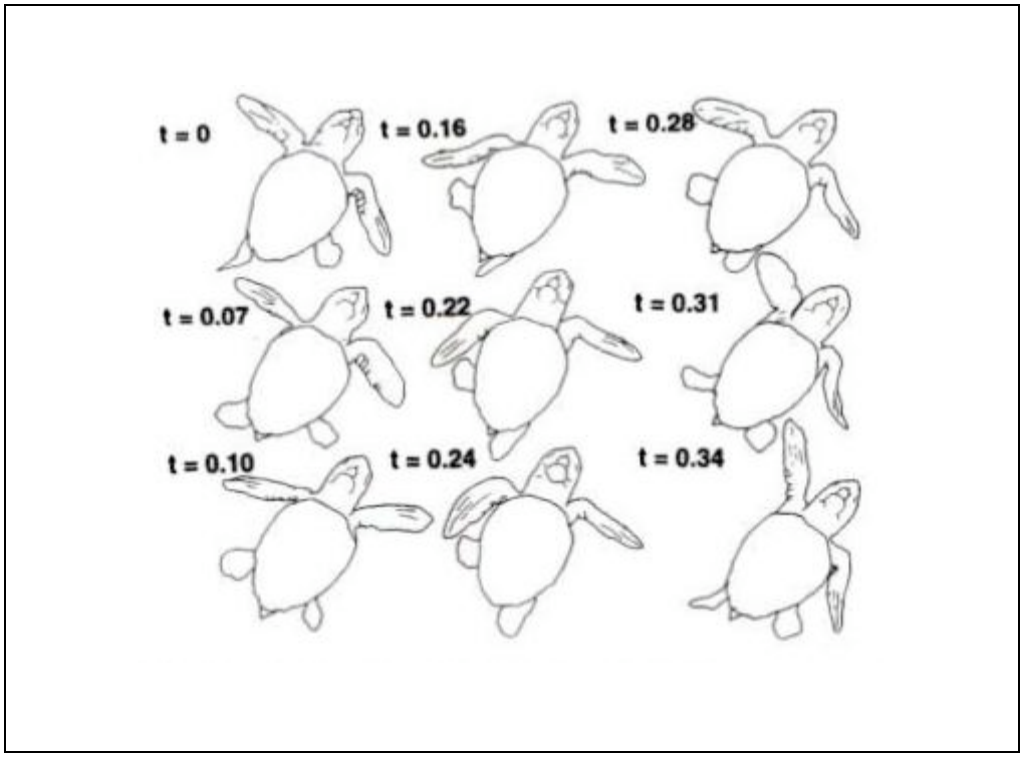


Figure 19: Illustration from Wyneken *et al.* (1997) that demonstrates the crawling pattern of green turtle hatchlings (*Chelonia mydas*) during terrestrial locomotion.

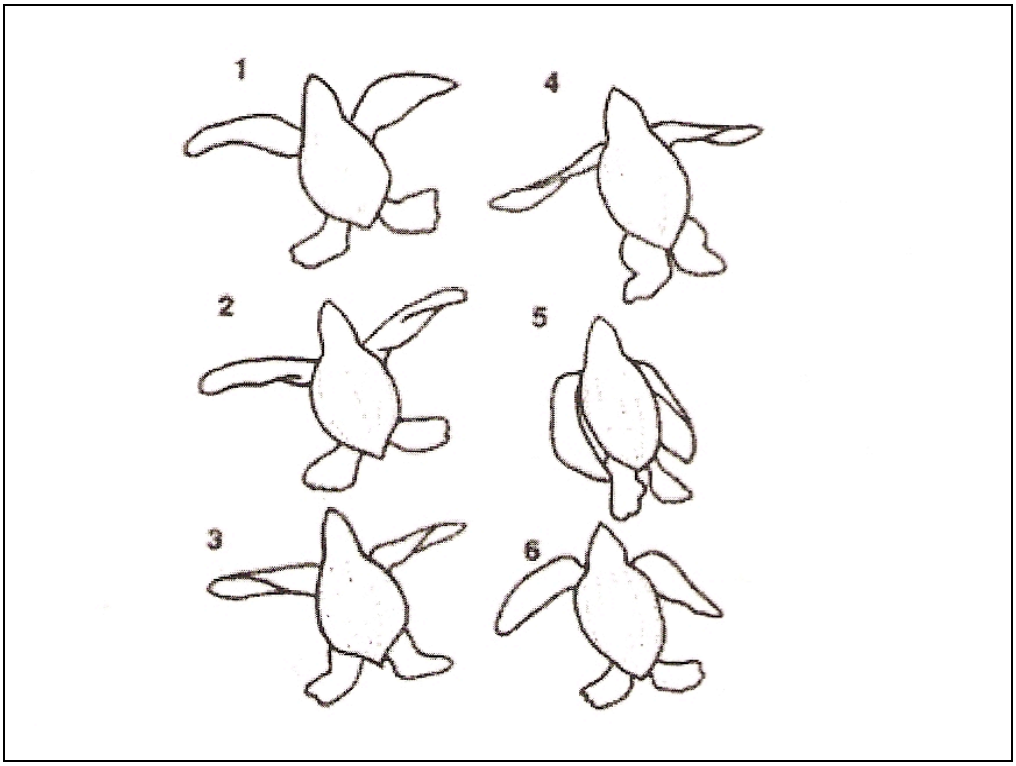


Figure 20: Illustration from Wyneken *et al.* (1997) that demonstrates the crawling pattern of leatherback turtle hatchlings (*Dermochelys coriacea*) during terrestrial locomotion.

It is easy to see how longer flipper length, coupled with narrow carapace width would be advantageous to hatchling speed (m_s^{-1}) during terrestrial locomotion. Longer flipper length would allow for greater distances of forward movement, and narrower carapace width would create less 'drag' and allow for a more streamlined effect during forward propulsion.

Hatchling terrestrial speed (m_s^{-1}) is probably a more useful indication of hatchling locomotion performance than the 'total time' it takes to cross a certain distance, as it is not known if (or how) stopping during the crawl to the beach affects predation rates of hatchlings. Therefore, it would be valuable to examine the behaviour of leatherback hatchlings during their terrestrial migration to the sea, to determine if predation rates are different between hatchlings with different morphology traits and different locomotion performance/behaviour.

7.3.2. *Aquatic locomotion*

Hatchling morphology had a significant influence on aquatic locomotion performance in the present study, where hatchlings with higher PC1 morphology scores showed a significant advantage in aquatic locomotion performance. Hatchlings with larger measures of 'density' (PC1) had higher stroke rates ($strokes\_s^{-1}$) than hatchlings with smaller measures of 'density'.

Other studies have also observed significant relationships between hatchling morphology traits and aquatic locomotion performance (Ashmore 2003; Burgess *et al.* 2006; Myers *et al.* 2007). For example, Burgess *et al.* (2006) found that green turtle hatchlings (*Chelonia mydas*) with greater 'size-index' scores (generated by principle components analysis) exerted greater mean force during powerstroking bouts in observed aquatic locomotion trails (Fig. 21). Ashmore and Janzen (2003) found that swimming speed (cm_s^{-1}) in smooth softshell turtles (*Apalone mutica*) was

positively correlated with body size, and more recently Myers *et al*. (2007) observed a significant positive correlation between hatchling mass and swimming speed in slider turtle hatchlings (*Trachemys scripta elegans*).

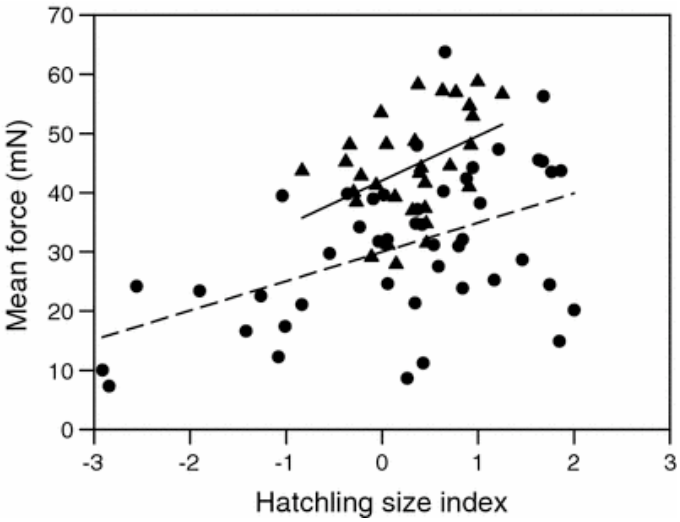


Figure 21: Results from Burgess *et al.* (2006) that illustrates the relationship of hatchling 'size-index' vs. mean force (mN) of swimming strokes in green turtle hatchlings (*Chelonia mydas*).

A limitation of the current study was the inability to determine the swimming speed of hatchlings. This provided difficulties when trying to compare aquatic performance results from the current study with results from other studies, due to the different measures of aquatic performance (stroke rate vs. swimming speed or swimming distance). There were no studies found in the literature that discussed the relationship between stroke rate (strokes_s^{-1}) and swimming speed (m_s^{-1}) in leatherback hatchlings, which would be useful in assessing aquatic locomotion performance.

Although the majority of studies in the literature obtained measurements of hatchling aquatic fitness in the form of swimming speed (m_s^{-1}) or distance travelled (m) in the sea (Webb *et al.* 2001; Ashmore *et al.* 2003; Myers *et al.* 2007), it seems probable that a higher stroke rate (strokes_s^{-1}) would increase swimming speed in leatherback hatchlings, as a higher number of strokes would likely increase forward propulsion.

7.4. *The Relationship between Incubation Temperature and Hatchling Fitness*

In the current study, natural nest incubation temperature affected leatherback hatchling (*Dermochelys coriacea*) morphology, which subsequently influenced terrestrial and aquatic locomotion performance of hatchlings. These results naturally lead to the conclusion that a relationship exists between nest incubation temperature and fitness in leatherback hatchlings.

Sea turtle hatchlings are exposed to predators on land and in sea, and it would make sense that the 'fittest' hatchlings would possess traits that would allow for success during both modes of locomotion (terrestrial and aquatic). Hatchlings are vulnerable to predators during their initial migration from the nest to the sea, and during their offshore migration 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 may be advantageous for leatherback hatchlings as it decreases the duration of time exposed to predators such as crabs, birds, and dogs (Downes *et al.* 1999). In addition, lower incubation temperatures produced hatchlings of a greater 'density' than those from higher incubation temperatures in the current study. Greater hatchling 'density' had a significant influence on hatchling stroke rate (strokes _ s^{-1}), which may increase swimming speed. Greater hatchling 'density' may also allow hatchlings to generate a greater mean force with each powerstroke, which may also enhance swimming performance (Burgess *et al.* 2006). In addition, hatchlings with greater 'density' may also elude aquatic predators that are gape-limited (Downes *et al.* 1999). Therefore, greater 'density' and faster swimming speed may reduce predation rates of hatchlings, which would increase their overall fitness.

This study demonstrates that lower incubation temperatures produce hatchlings with morphological traits that are advantageous to terrestrial and aquatic locomotion performance. Therefore, nest incubation temperature influences leatherback hatchling fitness, as it has a significant influence on hatchling morphology and subsequent locomotion performance.

Future research that investigates the influence of leatherback hatchling morphology on hatchling survival during terrestrial locomotion to the sea is needed, as it is significantly influenced by incubation temperature.

8. Conclusions

- The estimated sex-ratio of leatherback hatchlings (*Dermochelys coriacea*), that emerged from natural nests studied in Tobago WI from April – June 2008, was 100% females: 0% males

- Lower nest incubation temperatures produced hatchlings with narrower carapace width and longer flipper length. This hatchling phenotype had a significant advantage in terrestrial locomotion performance.

- Lower nest incubation temperatures produced hatchlings with higher measures of 'density'. This hatchling phenotype had a significant advantage in aquatic locomotion performance.

- Nest incubation temperature may be an important factor in determining hatchling fitness, as it has a significant influence on hatchling morphology and locomotor capabilities.

- In the face of global warming, overall fitness of sea turtle hatchlings is uncertain. A global increase of nest incubation temperatures will most likely produce a female biased sex-ratio and influence hatchling morphology traits which affect locomotor performance. If increasing temperatures compromise hatchling fitness, the existing population of this critically endangered species will be further pressed.

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