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The role of temperature in *Chelonia mydas* nests in Tromelin: Influences and Predictions

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Abstract

On the island of Tromelin, in the South West Indian Ocean, the temperature of nine *C. mydas* nests were studied throughout the period of incubation. Weight and length characteristics of the eggs and the resultant hatchlings were taken, as well as the carapace length of the laying female. Clutch survival characteristics were obtained including number of turtles emerged, number alive and dead within the nest as well as the total number of unhatched eggs, divided into fertile and unfertile. Sand samples were taken at three depths for each nest and physical characteristics of individual nests were also recorded. Air temperature was correlated to mean nest temperature both overall and within the first third of incubation, but was more significantly correlated to control nest temperature. Results suggest that although air temperature does have an impact, this influence is reduced during periods of biologically induced temperature fluctuations. Rain had major impacts on nest temperature throughout duration, however the extent and timing of this impact was influenced by stage of incubation and mean grain particle diameter. Rain was seen to alter expected sex-ratios producing estimated male-dominant clutches. The impact of rain is also thought to have increased the influence of vegetation proximity on hatching success due to water absorption by the roots. Rain during final periods of incubation removed the ability of estimating emergence lag with a degree of accuracy. During the study, activity of the previous night's nesting turtles concluded that these females successfully nested 57% out of their beach mounts. The average internesting interval for this population was calculated as 13.69 days.

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1. INTRODUCTION

The green turtle, *C. mydas*, has been classified as endangered since 1982, predominately due to human disturbances and human predation. The assessment of their population trends are complicated due to marine turtle's high rates of , mortality and longevity, combined with the green turtles slow growth rates in comparison to other sea turtles and greater time required before sexual maturity is reached. Despite these complications, the number of nesting turtles increase on the protected nesting sites within the south-west Indian Ocean over the past two decades (Lauret-Stepler et al, 2007).

Green turtles lay a clutch of between 70-150 eggs and bury them approximately 75cm deep on sandy beaches. The temperature during the incubation phase not only plays a significant role in determining embryonic survival and hatchling fitness, but also controls the sex ratio of the brooding clutch as opposed to a genetic determinate. The pivotal incubation temperature is a specific constant temperature (Godfrey *et al.* 1997), occurring at some point during the second third of incubation, that produces an equal number of male and female embryos. Deviations above or below this temperature produce higher numbers of females or males, respectively, and dependent upon the incubation temperature's distance from the pivotal, may produce a clutch of only one sex (Mrosovsky, 1994). Temperature fluctuations within the nest over the course of incubation can be influenced by both biological and abiotic factors. This may result in unexpected success parameters, hatchling phenotypes or sex ratios of the clutch.

Conservation techniques and measures require detailed overviews in order to implement appropriate techniques. The exhibition of temperature-dependent sex determination within *C. mydas* ensures interest into this species prospect of survival when considering global change. As a consequence of planet warming, sex ratios may be skewed beyond natural patterns, potentially altering the ability of a population to survive. The endangerment of *C. mydas* emphasises the need for understanding, and hence research into its life-history and reproduction is necessary to ensure conservation techniques can support the population as environmental factors change.

This study focuses on the relationship between incubation temperature and the factors which may influence it. Data gathered during this research has also allowed the exploration of factors temperature may influence, as well as relationships between local nest parameters and both clutch success rates and phenotypes. This study will set a basis for population trend data, including nesting and fertility success of laying females, allowing population trend analyses and the revision of current and future conservation methods.

2. MATERIALS AND METHODS

2.1 Study site

Tromelin (Figure 1) is one of the five French Esparse islands located in the south West Indian Ocean north of Reunion Island (15° 33' S, 54° 31' E). It is the smallest of the five with a total land area of 1.2 km^2. Only the 1600 m of sandy beach situated northwest of the island is suitable for turtle nesting, the rest of the cost is covered with boulders. Vegetation found at the top of the sandy beach is exclusively *Tournefortia argentea.*

The island is managed by the TAAF (Terres Australes et Antarctiques Françaises) organisation. Only a few manmade structures stand on the small island and since 1954, four meteorologists have rotated monthly on and off the island to take weather observations. The population of turtles that 'home' (Carr, 1967) to the island for nesting remain largely undisturbed by human activity and are less familiar with human interaction than other green turtle populations. Meteorologists have been counting turtle tracks every morning since March 1986 in collaboration with Kelonia and Ifremer, establishing long term data to estimate frequentation and the evolution of the green turtle population visiting Tromelin.

This data was recently explored in a publication by Lauret-Stepler (2007). Green turtles come to nest on the shores of Tromelin throughout the year with the peak season of nesting during the wet season from November to February. Approximately 7178 ± 3053 (n = 19) turtle tracks are recorded annually on the entire nesting beach (Lauret-Stepler *et al.* 2007).
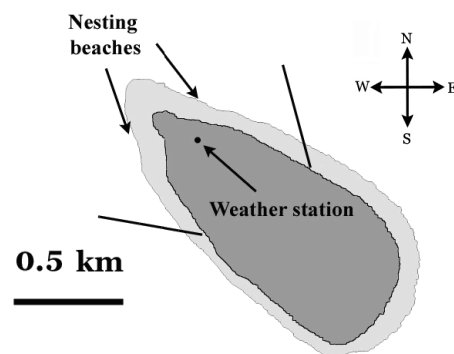
Figure 1: The Island of Tromelin 15° 33' S, 54° 31' E (Lauret-Stepler et al. 2007)

2.2 Study Protocol

Temperature within the Nest

During the first four days of the study period (19/04/08- 23/04/08) nine laying turtles were chosen haphazardly for the incubation study. Clutch temperatures were measured using VEMCO Minilog-T V3.09, programmed to record the temperature every hour throughout incubation. Thermometers were placed in the egg chamber after the 50th egg had dropped. After the female had covered the nest, a 70 cm diameter net was put around the nest at 40 cm deep to prevent nest destruction by other turtles and to trap hatchlings at emergence. A thermometer was buried at 75 cm depth on the laying beach to serve as a control nest. Temperature data was logged using Minilog software.

The nests were excavated 48 hours after emergence. The thermometers were removed, the number of dead and alive hatchlings, developed and undeveloped unhatched eggs recorded. As it occurs that some hatchlings can emerge up to a few days after the main emergence, hatchlings found alive in the neck were considered as emerged (Balazs & Ross, 1974), although hatchlings alive within the egg chamber were considered as dead. The difficulty to determine the fertility of an egg in the field resulted in two unhatched egg classifications: unhatched eggs with obvious embryo and unhatched with no obvious embryo (Miller, 1999).

Egg and Hatchling Parameters

The diameter and the weight of 10 eggs randomly chosen during oviposition for each nest were recorded. Egg length was taken from the furthest tip to the furthest tip using callipers accurate to 0.01cm. As much sand as possible was removed from egg shells before taken the weight. Eggs were placed on portable electronic scale (Professional Mini Pocket Scale, model EC-500, $\pm$0.01g) and measured to two decimal places. Eggs were immediately placed back in the egg chamber after measurement.

After 50 days of incubation, nests were checked from 6pm to 10pm and 3am to 7am for emergences. Once the emergence had occurred, 35 hatchlings randomly selected were weighed and the Straight Carapace Length (SCL) recorded. Hatchling SCL was taken from the anterior point at midline (nuchal scute) to the posterior notch at midline between the supracaudals (Bolten, 1999; Figure 2). Hatchling weights were taken to the second decimal place using electronic scales (Professional Mini Pocket Scale, model EC-500, $\pm$0.01g).

Figure 2: Hatchling Straight Carapace Length Measurement (Bolten, 1999).

Nests Characteristics

To estimate the daily amount of sun exposure nests were checked at hour intervals during day light and the percentage of shade within the area of the constructed net recorded (0.38m^2). A daily average of shading percentage was calculated using these figures.

The distances from nests to the High Tide Line and the vegetation line (defined as the point where dense vegetation starts) were recorded, as well as the distance to the closest bush trunk (Figure 3). Nests situated in the vegetation (above the vegetation line) were recorded with a negative value. As nests were excavated, top and bottom egg chamber depth were recorded and three sand samples of 200g were taken at the top of nest, 50cm deep and at the bottom of the egg chamber for grain size analysis.

Figure 3: Distance between nest centre and a) tree trunk, b) vegetation line, Tromelin 2008.

Grain Size Analysis

Sand samples were rinsed with fresh water and placed within an oven at 50°C for at least 24h for drying. Sieving of the particles was completed using Retsch Siev Analysis AS 200 for 10 minutes. Six sieves were used to separate the grains into the 7 main categories: gravel, very coarse sand, coarse sand, medium sand, fine sand, very fine sand and silts, according to grain diameters. Grain size data was analysed using Gradistat software. The Fork and Wald method was used to determine grain size.

Meteorological Data

Hourly rainfall and air temperature data were collected by the Tromelin Weather Station, run by MeteoFrance.

Capture-Recapture

In order to access the internesting interval within a season, (i.e. the period between consecutive successful nesting emergences within a season), 37 turtles were marked at the beginning of the study using epoxy glue on the carapace. Every night from 11pm to 7am the entire laying beach was checked for marked turtles. The Curved Carapace Length (CCL) of each marked turtle was recorded, as well as the turtle's behaviour when she was recaptured: layed or not layed. CCL was measured from the anterior point at midline (nuchal scute) to the posterior notch at midline between the supracaudals (Bolten, 1999).

Laying success

Every morning at 7am the entire laying beach was checked for turtle tracks and all tracks were followed to determine if the turtle had layed or not, and if there were no digging attempts. The laying success was calculated by the ratio: number of going up tracks resulting in laying/ number of going up tracks (total).

2.3 Statistical Analyses

Statistical analysis was preformed using the parametric tests of ANOVA and, where data did not meet parametric assumptions, the non parametric tests of Kruskal Wallis and Mann-Whitney U were used. Multiple comparison tests used to explore significant results further were Tukey tests. Due to this test's robustness against the assumption of normal distribution, it was also used to explore non-parametric data further for differences between groups.

Both linear regression and the non-parametric Spearman rho test were used to gain correlation coefficients and significance values of correlations. In some instances outliers were removed from data before correlations were made. All tests were preformed using the program SPSS V14.0.

3. RESULTS

3.1 General Biological and Nest parameters

Incubation Duration and Local Nest Parameters

Study nests had a mean incubation duration of 61.33 days (S.D.= $^\pm$4.4, range: 55-67 days, n=9) and a mean depth of 70.97cm (S.D.=$^\pm$7.3, range: 63-83cm, n=9) and 85.76cm (S.D.= $^\pm$9.52, range: 72-100cm, n=9) to top and bottom of the egg chamber, respectively.

The 9 studied nests were located at a mean distance of 63.93m to the sea (S.D.= $^\pm$10.74m, range: 49.50-78.50m, n=9), 3.27m to the closet bush trunk (S.D.= $^\pm$4.06, range: 1-13.70m, n=9) and -3.66m to the vegetation line (S.D.= $^\pm$8.33, range: -15.20-11.30). The mean average of shading per day around the 0.38m^2 area around the nest was 17.72% (S.D.= $^\pm$18.87, range: 0-42%)

Grain size analysis showed that for all the nests at the 3 different depths the sand was coarse, except for nest 3 where sand was very coarse at all depths. The mean grain size was 890.54μm (S.D.= $^\pm$113.26, range= 659.39-1085.92μm, n= 27) throughout the depth ranges. Sand at the surface of the nests, at 50cm deep and at bottom nest depth displayed a mean of 855.62μm (S.D.= $^\pm$122.63, range: 691.68-1025.37 μm, n=9), 858.39μm (S.D.= $^\pm$114.72, range: 659.39-1020.23 μm, n=9) and 964.66μm (S.D.= $^\pm$79.13, range: 884.33-1085.92 μm, n=9) respectively.

Nest by nest local parameters are presented in Appendix 1.

Laying Turtle, Egg & Hatchling Parameters

Table 1: C. mydas egg, hatchling and female descriptive statistics on Tromelin, 2008*

.	Mean	$^\pm$ S.D.	Range	N
Egg Length (cm)	4.36	0.11	4.09-4.58	90
Egg Weight (g)	45.64	2.73	40.58-51.77	90
Hatchling SCL (cm)	4.84	0.13	4.31-5.20	315
Hatchling Weight (g)	24.87	0.51	19.53-28.37	315
Female CCL (cm)	107.33	4.84	99-111	37

* For nest by nest data, see Appendix 2

Clutch Parameters

The following equations are global descriptive statistics for clutch parameters. Fertility rate, hatching, emergence, escape and nest success were calculated (Table 2) according to the following equations:

Fertility rate = (Number of fertile eggs/Number of eggs laid) x 100

.....Equation 1

Hatching Success= (Number of pipped eggs/Number of eggs laid) x 100

$$.....Equation\ 2$$

Emergence Success= (Number of juveniles that emerged/Number of eggs laid) x 100

$$.....Equation\ 3$$

Escape Success= (Number of juveniles that emerged/Number of pipped eggs) x 100

$$.....Equation\ 4$$

Nest success= mean number of juveniles that escaped from the nests

Table 2: Clutch parameters descriptive statistics for 9 *C. mydas* nests on Tromelin, 2008*

	Mean	**Range**
Clutch Size	133.33 ± 22.28	92-160
Fertility rate (%)	97.40 ± 2.40	92.73-100
Hatching Success (%)	96.65 ± 3.88	87.27-100
Emergence Success (%)	90.81 ± 6.57	80.92-98.91
Escape Success (%)	94.04 ± 6.99	82.81-100
Nest Success	119 ± 20.83	91-145

*Nest by nest data are presented in Appendix 3

3.2 Temperature Profiles

Table 3: Mean temperature recorded for each studied *C. mydas* nest in Tromelin 2008 using average daily temperatures.

	Incubation Duration (days)	Mean Temperature of Whole Incubation Period	Range	Mean Temperature 1st third of Incubation	Mean Temperature 2nd third of Incubation	Mean Temperature 3rd third o Incubation
Nest 1	58	29.92 ± 1.54	28-33.1	29.07 ± 0.18	29.04 ± 0.8	31.65 ± 1.41
Nest 2	55	29.47 ± 1.29	26.8-32.1	29.21 ± 0.19	28.22 ± 0.65	30.99 ± 0.76
Nest 3	56	30.35 ± 1.00	28.3-32.3	30.23 ± 0.17	29.64 ± 0.77	31.19 ± 1.07
Nest 4	61	29.58 ± 1.39	27.5-32.4	28.81 ± 0.24	28.62 ± 0.70	31.29 ± 0.90
Nest 5	66	28.14 ± 0.73	26.6-29.7	27.99 ± 0.38	27.66 ± 0.44	28.77 ± 0.78
Nest 6	61	29.52 ± 1.68	27.4-33.2	28.65 ± 0.37	28.57 ± 0.80	31.34 ± 1.66
Nest 7	62	29.12 ± 1.11	27.1-31.3	28.75 ± 0.58	28.29 ± 0.61	30.33 ± 0.83
Nest 8	67	27.73 ± 0.81	26.5-29.4	27.45 ± 0.44	27.08 ± 0.31	28.67 ± 0.56
Nest 9	66	28.01 ± 0.95	26.6-30.2	27.73 ± 0.57	27.18 ± 0.33	29.12 ± 0.33
Average Across all Nests (except control)		29.04 ± 1.48	26.5-32.2	28.60 ± 0.88	28.21 ± 1.01	30.31 ± 1.52
Control	-	27.67 ± 0.97	26.2-29.4	28.81 ± 0.54	27.58 ± 0.21	26.58 ± 0.35

N.B.: $\pm$ values correspond to standard deviations

N.B.: Control nest 'incubation' thirds align with nest average incubation thirds.

N.B.: Temperatures based on average daily temperature for each nest.

An ANOVA was used to test the daily mean temperatures between the three periods of incubation. This returned a significant result for all nests (Nest1 $F_{2,58}=49.896$, $p<0.001$; Nest2 $F_{2,55}=114.044$, $p<0.001$; Nest3 $F_{2,56}=17.619$, $p<0.001$; Nest4 $F_{2,62}=60.469$, $p<0.001$; Nest5 $F_{2,65}=25.181$, $p<0.001$; Nest6 $F_{2,63}=23.868$, $p<0.001$; Nest7 $F_{2,61}=19.364$, $p<0.001$; Nest8 $F_{2,67}=72.702$, $p<0.001$; Nest9 $F_{2,66}=97.834$, $p<0.001$). Through a post-hoc Tukey test, nests

were found to not significantly differ in temperatures between 1st and 2nd stage, except for nests 2, 3 and 9. For 8 of the 9 study nests, temperature within the first third was always higher than the second. The 3rd stage of incubation was of a significantly higher temperature than both the 1st and 2nd third of incubation within all nests.

A Kruskal Wallis on temperatures between different nests throughout the incubation period displayed a significant result (H=237.856, p<0.001). This finding was investigated through a Tukey test to determine where these differences occurred (Table 4).

Table 4: Tukey test outlining which *C. mydas* study nests in Tromelin, 2008, are significantly different based on daily temperature over the entire incubation period.

NestNumber	N	Subset for alpha = .05			
		1	2	3	4
8.00	68	27.7337			
9.00	67	28.0163			
5.00	66	28.1385			
7.00	63		29.1107		
2.00	56		29.4735	29.4735	
6.00	63		29.4958	29.4958	
4.00	63		29.5872	29.5872	
1.00	59			29.9195	29.9195
3.00	57				30.3355
Sig.		.628	.399	.494	.592

The same test was repeated using only the first third of the incubation period before the factor of metabolic heating peaks. Kruskal Wallis again returned a significant result (H=148.643, p<0.001) and this was explored through the use of a Tukey test (Table 5)

Table 5: Tukey test outlining which *C. mydas* study nests in Tromelin, 2008, are significantly different based on daily temperature within the first third of incubation only.

NestNumber	N	Subset for alpha = .05					
		1	2	3	4	5	6
8.00	23	27.4455					
9.00	18		27.9805				
5.00	22		27.9943				
6.00	21			28.6667			
7.00	21			28.7423	28.7423		
4.00	21			28.9054	28.9054	28.9054	
1.00	19				29.0702	29.0702	
2.00	19					29.2035	
3.00	19						30.2224
Sig.		1.000	1.000	.502	.113	.204	1.000

3.3 Embryonic Development

Although the nests and control graph show a relatively similar trend in the first third of the study period, there is major difference between the trends of the lines after this point as illustrated in Figure 3.
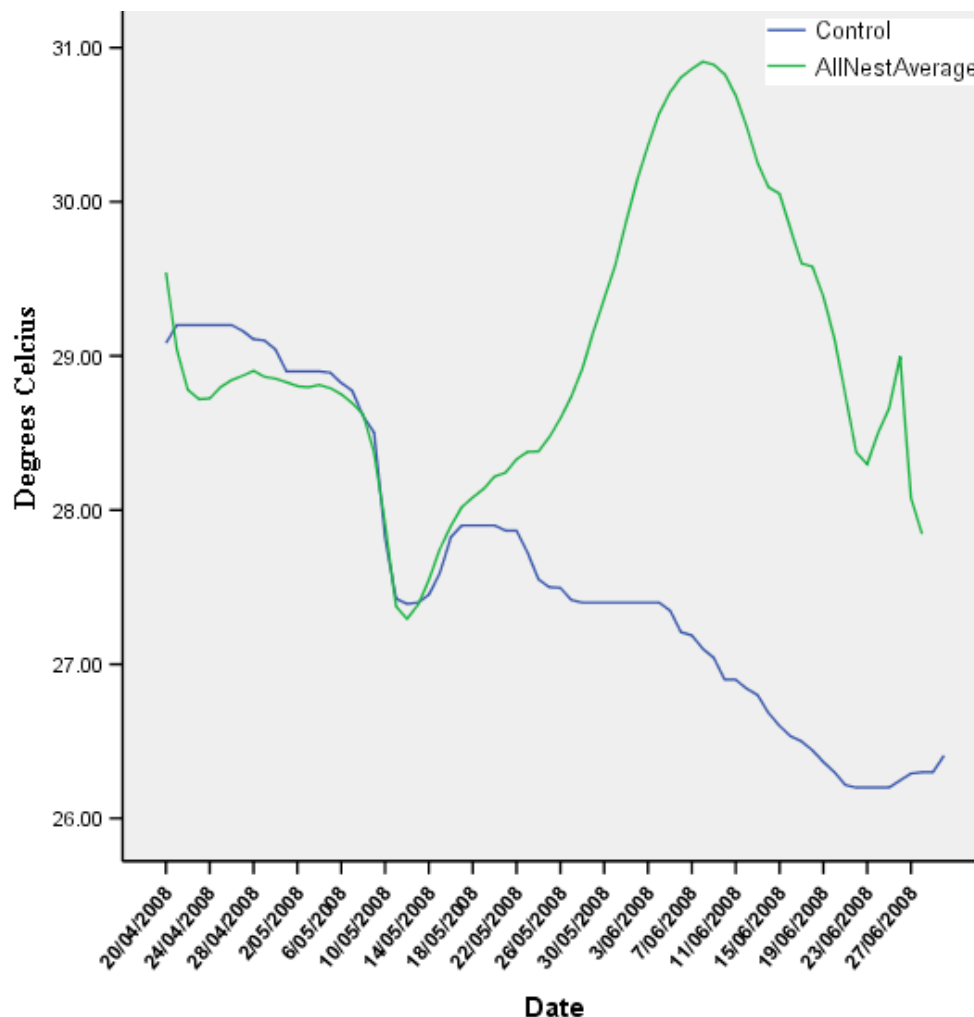
Figure 4: A composite of average daily temperatures during 2008 for *C. mydas* nests in Tromelin and the temperature within the control nest over the study period.

This change in association is presumed to be caused by metabolic heat produced within the clutches. The amount of this heat was calculated by the following formula and is displayed in Table 6.

Metabolic Heating = (Nest Temp – Control Temp)$_{\text{OVER ENTIRE INCUBATION PERIOD}}$
– the minimum observed value between the two
.....*Equation 5* (Broderick *et al.* 2000)

The increase in temperature directly after the large drop in temperature within the control nest (between the 4 – 10/05/08) was the last increase seen during nest incubation periods. Hence, it can be assumed that any increase in temperature within nests after this point is the consequence of metabolic heating. Therefore, a percentage increase in temperature caused by metabolic heating was calculated according to the following formula:

%(Metabolic Heating Increase) = (Highest Nest Temperature – Lowest Nest Temperature) OVER ENTIRE INCUBATION PERIOD / Highest Nest Temperature

.....Equation 6

As the effect of a consistently dropping air and control nest temperature is unknown on the temperature within the nest during this period, this calculated value is not absolute of the influence of metabolic heating, rather a relative value between nests.

Table 6: Descriptive Statistics of Metabolic Heating in *C. mydas* nests in Tromelin, 2008*.

	Over the Entire Incubation Period			During the 1st Third of Incubation		During the 2nd Third of Incubation		During the 3rd Third of Incubation	
Nest	**Mean**	**Range**	**% Increase**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
1	$2.49^{\pm}2.09$	0-6.38	15.40	$0.53^{\pm}0.31$	0-1.03	$1.71^{\pm}0.74$	0.98 – 3.40	$4.91^{\pm}1.22$	2.52-6.38
2	$1.79^{\pm}1.76$	-0.07-5.11	15.49	$0.48^{\pm}0.19$	0-0.66	$0.84^{\pm}0.69$	-0.07 – 2.21	$4.17^{\pm}0.75$	2.56-5.11
3	$1.54^{\pm}1.37$	0-4.06	12.15	$0.35^{\pm}0.18$	0-0.56	$1.09^{\pm}0.80$	0.12 – 2.80	$3.13^{\pm}0.90$	1.62-4.06
4	$2.18^{\pm}2.01$	0-5.86	14.70	$0.34^{\pm}0.39$	0-1.82	$1.58^{\pm}1.00$	0.60– 3.89	$4.86^{\pm}0.76$	3.58-5.86
5	$1.65^{\pm}1.31$	0-3.98	9.42	$0.40^{\pm}0.25$	0-0.92	$1.20^{\pm}0.51$	0.69-2.34	$3.24^{\pm}0.59$	2-3.98
6	$2.33^{\pm}2.21$	0-6.84	17.31	$0.41^{\pm}0.22$	0-0.67	$1.83^{\pm}1.20$	0.61-4.63	$5.06^{\pm}1.59$	2.27-6.84
7	$1.91^{\pm}1.64$	0-4.96	12.73	$0.50^{\pm}0.22$	0-0.73	$1.38^{\pm}1.02$	0.28-3.68	$4.05^{\pm}0.75$	2.19-4.96
8	$1.72^{\pm}1.53$	0-4.53	9.56	$0.38^{\pm}0.25$	0-0.82	$1.18^{\pm}0.57$	0.59-2.40	$3.79^{\pm}0.66$	2.48-4.53
9	$1.62^{\pm}1.62$	0-4.90	11.10	$0.30^{\pm}0.16$	0-0.55	$0.93^{\pm}0.62$	0.38-2.35	$3.93^{\pm}0.59$	2.64-4.90
Average	$1.91^{\pm}0.34$	-0.07-6.84	13.10	$0.41^{\pm}0.08$	0-1.82	$1.30^{\pm}0.34$	-0.07-4.63	$4.13^{\pm}0.70$	1.62-6.84

*All mean and range values are given in °C.
NB: $\pm$ is standard deviation around the mean.

A Kruskal Wallis was then used to test for difference between the amount of mean metabolic heating during each stage of incubation. All nests displayed a significant result (Nest1 H=50.463, p<0.001; Nest2 H=37.191, p<0.001; Nest3 H=41.154, p<0.001; Nest4 H=52.631, p<0.001; Nest5 H=56.039, p<0.001; Nest6 H=50.935, p<0.001; Nest7 H=42.266, p<0.001; Nest8 H=55.265, p<0.001; Nest9 H=52.858, p<0.001). Further exploration of the data through a Tukey test showed that metabolic heating in stage 3 was significantly higher than in stage 2 or 1 for all nests. Metabolic heating in 2nd third was significantly higher than stage 1 in all nests, excluding Nest 2. Although not significant, the 2nd third of incubation did have a higher level of metabolic heating than the 1st for Nest 2.

3.4 Effects on Temperature

3.4.1. Clutch Parameters

Five variables thought to influence the amount of metabolic heating within a clutch were selected to correlate against the calculated figures in the previous section, these were:
Clutch size, egg size, egg mass, fertile egg % and hatching success %.

The mean of metabolic heat throughout the incubation period was correlated using a Spearman rho to these variables, only the correlation with clutch size returned a significant result (r=0.0.817, p<0.05). All other attempted correlations produced a p-value>0.488.
This test was repeated using the mean of metabolic heat for each third of incubation.

The average metabolic heat within the 1st and 2nd thirds of incubation were not correlated to any of the outlined variables, however clutch size did return a very small p-value of 0.067 (r=0.633) when tested with the 2nd third of incubation. All other attempted correlations had significance values greater than 0.139. The last third of the incubation period did show a high statistical correlation with clutch size (r=0.867, p<0.01), however not with any other tested variables.

These tests were also repeated using the percentage of metabolic heating throughout the incubation period. This test also displayed a significant correlation between clutch size and relative percent of metabolic heating (r=0.667; p<0.05). The test also returned a significant correlation with mean egg mass within the clutch (r=-0.667; p<0.05), however due to the negative correlation coefficient, the raw data was explored further. The average egg mass of Nest 8 was identified as an outlier and the correlation was preformed again. The absence of this outlier removed the significant correlation between egg mass and metabolic heating percentage throughout incubation (r=-0.619; p>0.05).

Average temperatures, calculated using daily averages over the entire incubation period and within each third, were also tested against clutch size, egg size, egg mass, fertile egg % and hatching success %. Average egg length was correlated to both the mean temperature over the entire incubation period and to average temperature within the first third of incubation (r=-0.667, p=0.050; r=-0.733, p<0.025; respectively).

3.4.2 Meteorological Factors
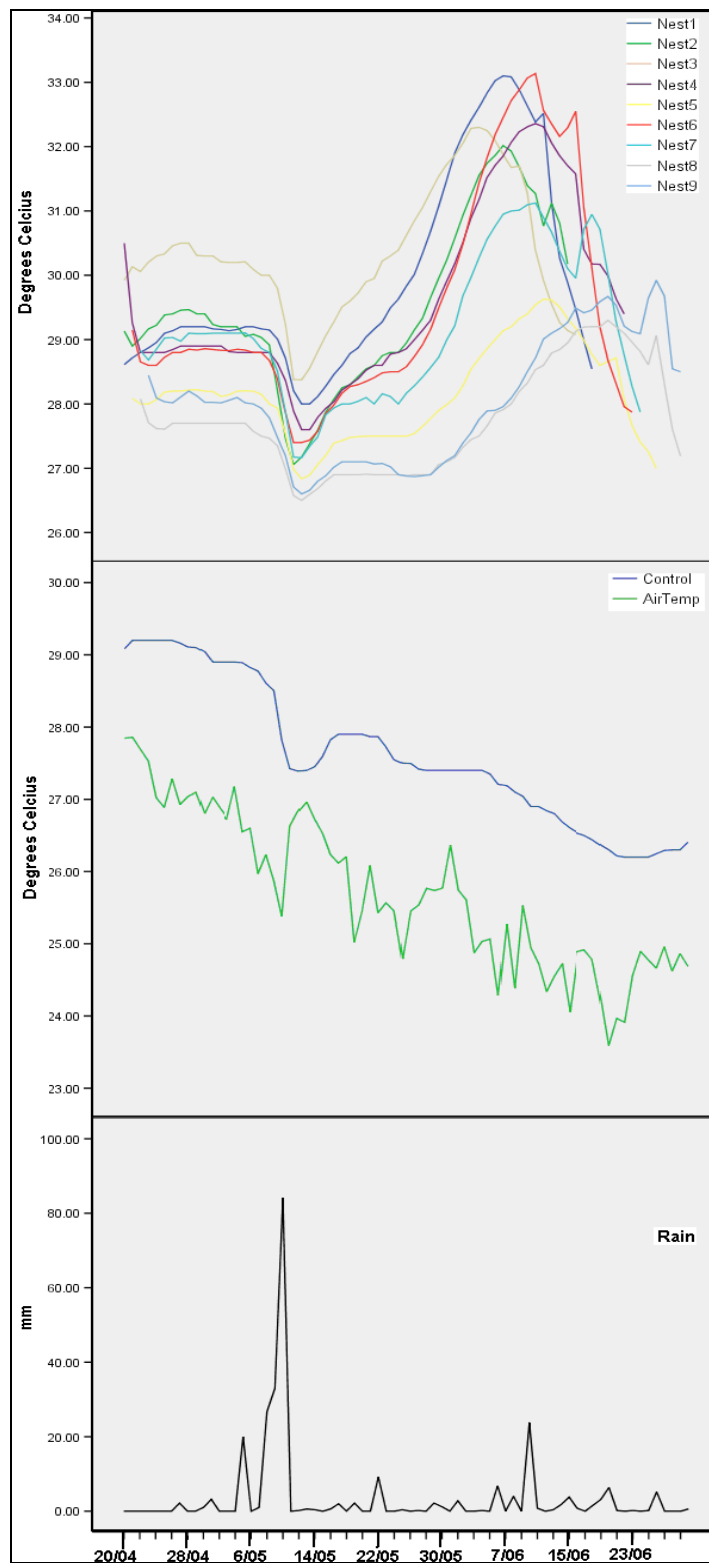
Rain

Figure 5: Rainfall, Air and Control Nest Temperatures and Study Nest Temperatures in Tromelin, 2008.

Graphic illustration indicates an effect on nest and control temperatures due to periods of rainfall (Figure 4). The first major rainfall event (55mm; 10/05-9am) during incubation

clearly demonstrates a decrease in temperatures for all the nests. All nest temperatures began to decrease within 4 hours of this rain event. Despite this, temperatures within nests were also seen to increase during periods of rainfall.

Rainfall was grouped into categories according to temperature fluctuation for the average daily nest temperature (Figure 5; Table 7). Periods of stability (change <0.25°C) or increase and periods of decrease within the nests and control were used to group rain data. These groups were analysed using a Mann-Whitney U Test to analyse for statistical difference.
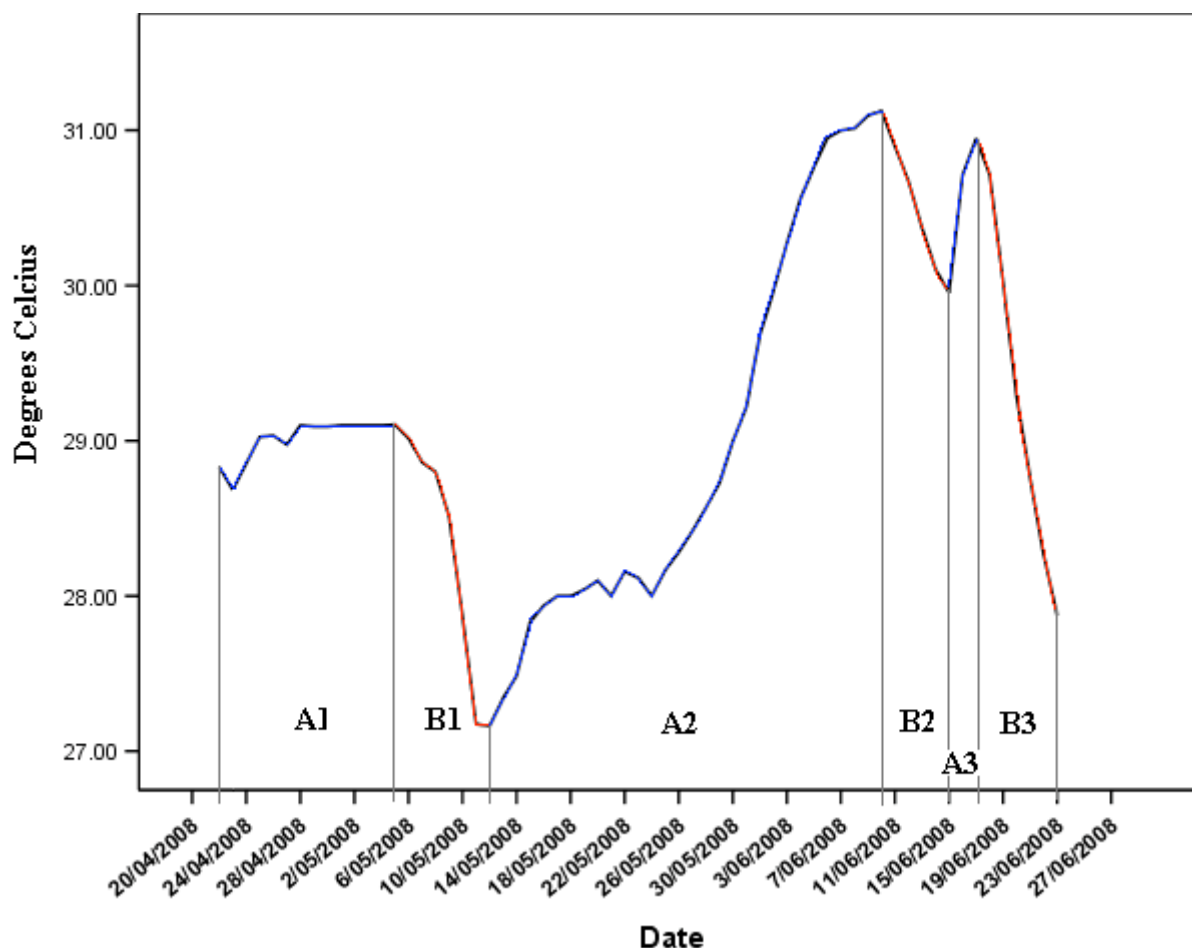
Figure 6: Daily temperature fluctuations in *C. mydas* Nest 7 on Tromelin used to group rain data.

Table 7: Rain Intervals according to *C. mydas* Nest 7 temperature increases & decreases on Tromelin.

	Increases in Nest 7 Temperature			Decreases in Nest 7 Temperatures		
Category	A_1	A_2	A_3	B_1	B_2	B_3
Interval	22/04-05/05	12/05-10/06	15-17/06	05-12/05	10-15/06	17-23/06
$\bar{x}$ Daily Rainfall (mm)	0.492	1.959	2.3	23.571	5.36	1.9

To decrease the confounding factors of time, only adjacent periods were tested against each other. This test was repeated using data from all nests with redefined rain groups. Rain groups were redefined as different nests displayed different dates for decreasing temperature after the 1st third of incubation. Using the categories outlined in Table 7 (above), the results are as follows:

1.	A1 to B1	Z= -2.306	p=0.030*
2.	A2 to B2	Z= -1.106	p=0.299
3.	A3 to B3	Z= -0.671	p=0.643
4.	A2 to B1	Z= -0.586	p=0.059
5.	A3 to B2	Z= -0.586	p=0.571

*statistically significant result.

The negative Z value indicates the second group within tests ranks higher more often than the first, i.e. Periods of decreasing temperature displayed higher rainfall per day more often.
All nests displayed significant results when increase category A1 was tested against decrease category B1. The majority of nests also displayed significant results when the adjacent categories of A2 and B1 were tested using Mann-Whitney U (Nests 2, 3, 6 $p<0.01$; Nests 4, 5 $p<0.05$; Nest 1 $p=0.051$; Nest 7 $p=0.059$, Nest 8 $p=0.080$) (Appendix 3).

Correspondingly, the results of the Mann-Whitney U regarding the control nest also returned a significant result for the first third (A1 Vs. B1: Z=-14.855, $p<0.001$). Unlike study nests, all periods examined outside the first third also displayed highly statistically significant results (A2 Vs. B2: Z=-8.793, $p<0.001$; A3 Vs. B3: Z=-18.198, $p<0.001$).

Air temperature

The mean daily sand temperature at the control sites was compared with mean daily air temperature since the first nest was laid until the occurrence of the last emergence.

Air temperature is consistently lower than temperature within the control nest with the latter devoid of any diurnal fluctuations. The normally distributed air and control nest temperatures were highly correlated by means of Linear Regression ($r^2 = 0.751$, $F_{1, 70} = 210.738$, $p<0.001$).

A combined mean daily nest temperature (transformed to meet parametric assumptions) was also correlated significantly with mean daily air temperature before any nests entered the second third of incubation period ($r^2 = 0.324$, $F_{1, 16} = 7.692$, $p<0.015$).

This was repeated for the entire incubation period (also transformed to meet parametric assumptions) and returned a significant result ($r^2 = 0.139$, $F_{1, 69} = 10.956$, $p=0.001$).
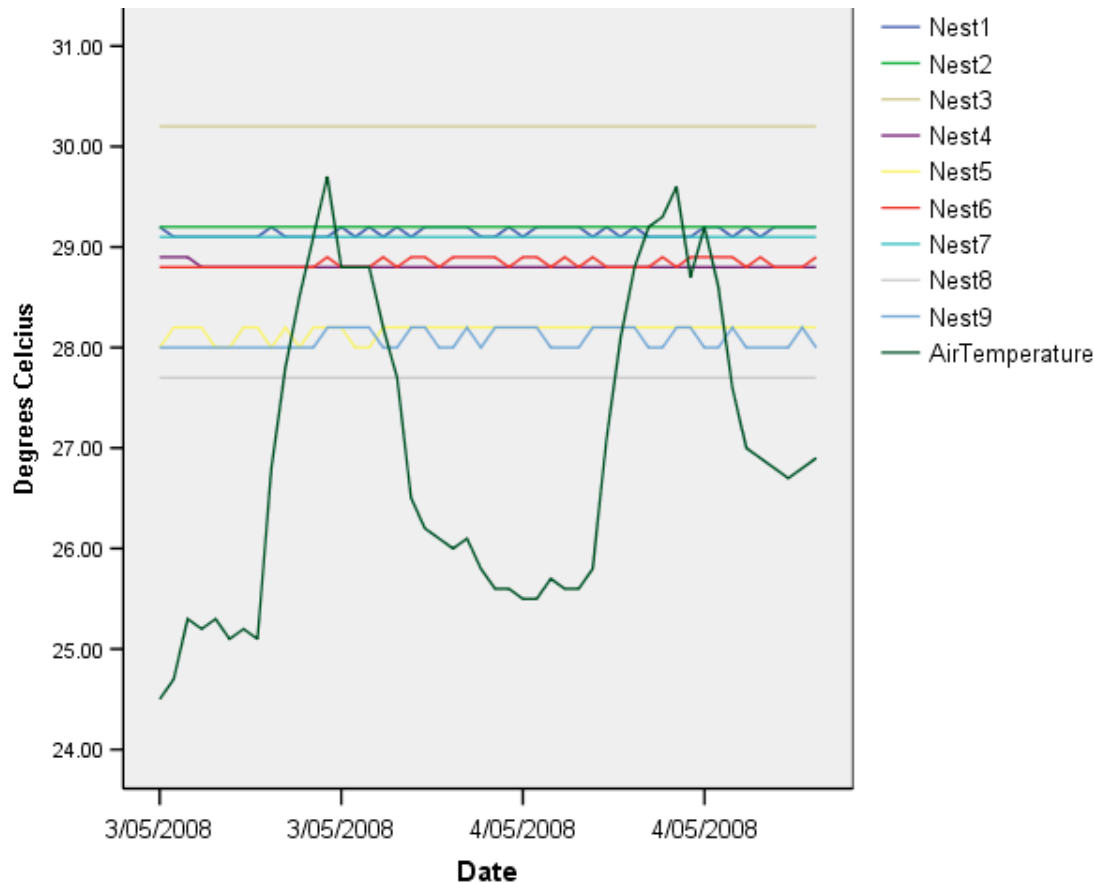
Figure 7: Hourly *C. mydas* Nest Temperatures with hourly Air Temperature over a two day period on Tromelin during 2008 showing nest fluctuations.

Some nests suffered from fluctuations (Figure 6) that appear unrelated to diurnal fluctuations of air temperature. The largest value for this variance was 0.2°C for both nests 5 & 9, with nests 1 & 6 showing variations of 0.1°C.

3.4.3 Local Parameters

Variables specific to each nest were tested to determine if these variables have an effect on nest temperature.

<u>Shade</u>

Shade percentage gathered at each nest site was tested against both average temperature over the entire incubation period, and average temperature within the 1st third of incubation (before the confounding factor metabolic heating increases). The correlations were tested using Spearman rho. Average temperature throughout incubation was not significantly correlated to shading ($r = 0.514$, $p > 0.05$). Although also not significantly correlated, temperature within the first third of incubation did display a very close relationship between the variables ($r = 0.627$, $p = 0.071$)

Nest Depth

The effect of nest depth was also tested against average temperature throughout incubation and average temperature over the first third of incubation. No significant results were returned from either correlation (AvTemp over IP: r =0.200, p>0.05; AvTemp 1st Third: r =-0.133, p>0.05). Nests suffering fluctuations (Figure 6) were tested against those not suffering fluctuations using a Mann-Whitney U. No significant difference was found in nest depth between these two groups (p>0.05).

Granulometrie

The mean diameter of sand particles taken from the surface, depth and middle of the nest column were tested by a Kruskal Wallis for each nest to determine if there was a significant change in sand size between depths. All nests returned an insignificant result (p>0.05).

A Kruskal Wallis was also used to determine if there was a signficant difference between sand samples taken at these depths between different nests. This also returned an insignificant results (p>0.05).

Mean particle diameter at each depth of a nest and overall was tested against the average temperature over the entire incubation period and per each third of incubation. No correlation returned a significant result (p>0.433 in all cases).

For the two largest rainfalls to occur within 3 hours (Reac1-8-9/05/06: 36mm; Reac2-10/05/06: 69.8mm), the time before nest temperatures dropped was examined. An 'average rain reaction time' was also calculated for all nests and a Spearman rho was used to examine the relationship between these times and mean particle diameter at different depths and on average. A significant result was found between mean particle diameter at nest depth and the reaction rate of temperature to the largest rainfall ($\bar{x}$ particle diameter at nest depth Vs. Reac2: r =0.672, p<0.05).

Distance to Sea

Distance to the sea was tested against average temperature over the entire incubation period and within each third of incubation using a Spearman rho test (r<0.611, p>0.05 in all cases). No significant correlation was found between the distance to the sea and mean temperature values.

Distance to Vegetation

Distance to vegetation was also tested against average temperature between different stages of incubation and average temperature over the entire incubation period. All returned negative correlation coefficients with no significant p values (p>0.05 in all cases).

The distance to the closest trunk to the nest was also tested against these temperature variables. Both the average temperature over the incubation period and the average temperature within the first third returned significant results through the Spearmans rho correlation test (r =-0.695, p=0.038; r =-0.763, p=0.017, respectively). Exploration of the raw data exhibited Nest 8 as an outlier in this category (Figure 7), hence the correlation test was

preformed again and returned insignificant results (r =-0.561, p=0.148; r =-0.659, p=0.076). Please note, however, the p value for the first third average temperature is still low.
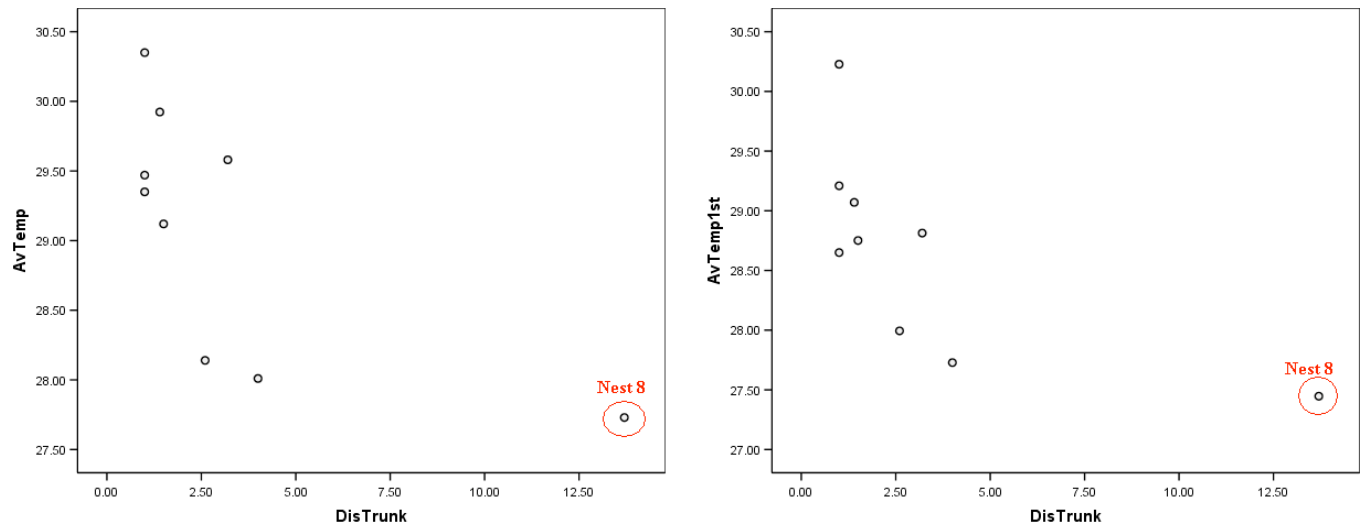
Figure 8: Scatterplots exhibiting outliers that were removed from correlation tests between temperature and distance to the closest trunk for *C.mydas*, Tromelin 2008.

3.5 Temperature as a Predictor

3.5.1. Incubation Period

The mean temperature over the entire incubation period and within the three periods of incubation (i.e. 1st, 2nd & Final Third) was correlated to the length of incubation for the nine study nests. Each correlation returned a significant result as listed in the table below (Table 8).

Table 8: Results of a correlation between temperature stages and incubation period for *C. mydas* nests in Tromelin, 2008.

	x Temp. during incubation	x Temp. during 1st ⅓ incubation	x Temp. during 2nd ⅓ incubation	X Temp. during 3rd ⅓ incubation
Spearmans Corr. Coeff.	-0.900	-0.967	-0.750	-0.833
Sig.	0.001	0.000	0.020	0.005

3.5.2 Hatchling Phenotype

Correlation between the temperature variables as above and hatchling length and mass was tested. Hatchling length returned no significant correlation (p>0.460 in all cases), however hatchling mass did. A highly statistically significant correlation was found with average temperature during the 2nd third of incubation (r=-0.733, p<0.01), and two statistical correlations with average temperature over the entire incubation period (r=-0.733, p<0.05) and average temperature in the 1st third (r=-0.667, p=0.050). No statistical correlation was found with the last third of incubation.

3.5.3 Clutch Success

The variables of temperature were also tested for correlations against hatching and emergence success to determine if these two variables could be predicted by temperature. Although some of these parameters were tested as predictors of metabolic heat, they may also be dependent variables temperature. Neither of the success rates produced a significant correlation with any of the temperature variables tested ($p > 0.125$ in all cases).

3.6 Phenotype Correlation and Impacts of Nest Parameters on Clutch Behaviour/Success

3.6.1 Laying Turtle Carapace Length & Biological Nest Parameters

The carapace length of the laying female was tested against several variables that may be influenced by this parameter. These were: Av Egg Mass per Nest; Av Egg diameter per Nest; Av Hatchling Mass per Nest; Av Hatchling Length per Nest; Fertility/Hatching/Emergence Success; Nest Depth & Clutch Size. None of these parameters returned a p value<0.139.
The clutch size for nests was also tested against egg and hatchling characteristics (mass and length), again, no significant results were returned.

3.6.2 Egg and Hatchling Mass & Length

Measured egg and hatchling mass and lengths were correlated using a Spearmans rho test. Egg mass and length were found to be correlated ($r=0.883$, $p<0.01$), however these parameters were not correlated to the hatchling characteristics of mass and length. Hatchling characteristics were uncorrelated to one another, yet did still return a small p value ($r=0.564$, $p=0.090$).

3.6.3 Local Parameter Impacts on Clutch Parameters

The different clutch success' calculated for this study were tested against hatchling phenotypes (mass & length) and local parameters for each nest (shade, nest depth, granulometrie, distance to sea/vegetation line/closest trunk). Hatching success within a clutch was correlated to both the distance of the nest to the vegetation line ($r= 0.728$, $p<0.05$) and to the closest trunk ($r=0.797$, $p=0.010$).

3.7 Internesting Intervals and Laying Success

An internesting interval within a season was calculated using the 37 turtles that were tagged during the study. The mean internesting interval was 13.69 days (S.D.: 1.8, range: 11-18 days). The calculated laying success for the tagged turtles was 57%.

4. Discussion

4.1 General Biological Parameters

No available data exists to compare general biological parameters to those previously found within this site. Hence, resultant biological parameters within this paper are compared to general findings for populations of *C. mydas*. Mean incubation duration for this site appears to be longer than the important nesting site of Ascension Island (55.8 days, Godley *et al*. 50-59 days, Glen *et al*. 2003). Ciccione *et al.* (2006) reported an incubation length of 53 days during the austral summer for a *C. mydas* nest on nearby La Reunion, an extent slightly shorter than seen in this study, yet this was the only nest found during this hotter period of the year. Incubation lengths for the austral winter as reported by Ciccione *et al*. (2006) were all longer than 80 days on La Reunion, hence all nests within this study had incubation lengths which lay between year-round duration range for *C. mydas* on close by La Reunion. All nest incubation lengths were also consistent with expected incubation durations for *C. mydas* of between 6 and 13 weeks (Miller, 1997).

The similarity of grain particle size between nests, and between nest depths indicates that gas transition between sand layers is the same for all nests. Nest 3 showed larger grain size within the nest chamber, categorised as 'very coarse' sand. However, grain size for this section of the nest was only 37.86µm larger than the maximum grain size from other nests within the previous sand category of 'coarse' sand.

Egg masses measured for each nest fall in the range of 35-55g for *C. mydas* eggs, as reported by Limpus *et al*. (1984) and Hirth (1988). Egg lengths are within ranges found in a study on Ascension Island by Glen *et al*. (2003) and in Costa Rica by Hirth (1988). Although hatchling length & mass are in accordance with previously found values for the geographically close La Reunion (Ciccione *et al*. 2008), the mean values of hatchling mass within this study are unexpected when compared to results from the Booth & Astill laboratory study in 2001. In their study, hatchlings produced at higher temperatures (30°C), than the mean within this study (29°C), produced larger hatchlings. It should be noted that Booth & Astill's study did, however, produce very similar sizes for temperatures separated by 4°C, suggesting other factors besides temperature may influence hatchling size in *C. mydas*.

No excavated nests showed the signs of predation on eggs or hatchlings. On the nesting site of Tromelin only ghost crabs have the ability to dig under the sand to predate turtle eggs or hatchlings, however this was not seen in any nests excavated within this study. This suggests that predation on turtle hatchlings is restricted to occurring as they make their way to the sea. However, as only 9 nests were excavated, this conclusion can not be strongly supported due to small sample size.

As expected, emergence success was lower than hatching success, due to some deaths of juveniles within the chamber or neck, as well as others becoming trapped within the egg chamber. Upon excavation of nests, some hatched juveniles were found stuck within shells due to dried yolk, decreasing their chances of emerging and reducing the percentage of emerged hatchlings. Despite this, emergence success was far greater than found by Glen *et al.* (2005) in Northern Cyprus, this may be attributed to the coarse found at the Tromelin site. Larger grains would fall more easily as hatchlings dig their way upward, resulting in greater emergence success. The percentage of viable hatchlings within this study was also of larger value than reported by Hirth (1988) in Costa Rica. Hirth (1988) experienced smaller clutches,

on average, than experienced during this research and attributed that larger number of variable hatchlings to this aspect of the study.

The fertility rate of green turtles is relatively high in comparison to other species (Horrocks & Scott, 1991; Whitmore & Dutton, 1985), however, fertility is difficult to classify in the field as early embryonic mortality may overestimate the infertility rate of a nest. Even providing this overestimation, and accounting for a difference in methodology, the number of fertile eggs during this study was lower than previously recorded by Whitmore & Dutton (1985) in Surinam. Their study found a percentage of <1% of infertile eggs within 74% of nests studied. The difference in results suggests that different populations may produce different amounts of infertile eggs. More likely, it suggests the methodology used during this study should be adapted to reduce the overestimation of infertile eggs, and a greater number of nests should be studied to gain a more accurate prediction of this, and other clutch parameter success rates. Whitmore & Dutton (1985) established infertility by the presence of a white circle on the outside of the eggshell, where shell membranes attach to the shell wall in very early stages of development. This methodology should be considered for use in further research studies.

4.2 Temperature Profiles

Study nests followed the same general pattern throughout incubation periods, with nests generally increasing in temperature as time progressed. Most nests dropped in temperature over the first days of incubation, with Nest 4 experiencing the largest of these drops. These preliminary drops were attributed to eggs establishing equilibrium to sand temperatures as they contain more heat than egg chamber sands when oviposition occurs. The general increase in temperatures beginning in the second third of incubation is attributed to metabolic heating, which caused a significant difference between temperatures within the last third of incubation compared to the first two stages. All nests suffered two drops in temperature during incubation greater than 1°C, the first of which occurred at the end of the first incubation stage. Due to this drop, temperature within the second stage was found to be lower and generally not significantly different to those within the first stage of incubation, contrary to expectations. However, temperatures did demonstrate a larger degree of increase within the second third, contrary to the first stage of incubation where daily average temperature remained comparatively stable.

The last temperature drop can be used to determine emergence lag of the juveniles, however previous studies have found this lag to last between 3 to 7 days (Balazs & Ross, 1974). The drop in temperature within all nests had a maximum of 12 days difference before the first emergence, indicating confounding factors resulting in this last major decrease and complicating the calculation of emergence lag. This confounding is illustrated through control nest temperatures during this period. Temperature within the control nest begins to decrease steadily before the majority of nests reach acceptable points of juvenile induced temperature reductions.

Mean temperatures of some nests within the 'sex determining period' of incubation were lower than previously reported pivotal temperatures of 28.8°C and 28.6°C (Godley *et al*. 2002; Reece *et al*. 2008). This, presumably, would result in a greater amount of male than female hatchlings being derived from these nests. However, pivotal temperatures have been found to change between populations in some sea turtle species (Chevalier et al. 1999), although conservative measures do apply, (Mrovosvky, 1994), hence, actual point of sex determination is unknown, allowing only an estimate of actual sex dominance within clutches.

Average temperature for each third was higher than 27°C for all nests, the minimum optimal sand temperature for embryonic development in sea turtles (Wibbles, 2003). Nests 2, 5, 8 and 9 did experience days with temperatures below this value, however, this is not reflected in individual nest successes such as hatching or emergence.

Nest temperatures appeared to be influenced by date of egg laying. Table 4 - Tukey test outlining which study nests are significantly different based on daily temperature over the entire incubation period, reveals that initial nest temperatures are grouped in general accordance to date of oviposition. Small differences in temperature may also be influenced by thermometer placement. Previous studies have shown that temperature does alter between the centre and peripheral zones of nests (Booth & Astill, 2001), clutches size differed between nests within this study, however all thermometers were placed after the 50th egg.

Surprisingly, tests between study nests on the different temperatures experienced during incubation displayed larger variance within the first third of incubation than based on mean temperature throughout the entire incubation period. As the first third is generally regarded as a stable period of temperature, influenced only by environmental pressures, this result suggests that biological pressures acting on nests throughout periods of incubation have the ability to, as is the case in this study, equilibrate temperatures between different nests. This being said the opposite may also be true, where biological factors may also increase temperature difference between nests, reducing the similarity of temperatures experienced by embryos throughout development. These biological pressures are explored within the two following sections.

4.3 Embryonic Development

Metabolic heating, a by-product of embryonic development within the nest, occurred throughout the entire incubation period, however it only became noticeable as the second stage of incubation commenced. This biological heating raised nest temperatures by between 3 and 5.5°C from their minimum to maximum temperature values, in agreement with previous studies on nest temperatures (Booth & Astill, 2001). Within the nine study nests, embryonic heat increased as incubation progressed with each third significantly different to the previous stage. Stage three showed the largest levels of metabolic heating, with the highest value being 6.84°C. Although the influence of metabolic heating on sex determination still remains unclear in green turtle nests (Broderick *et al.* 2000; Booth & Astill, 2001b), this effect was inconsequential within this study. Temperature rise caused by metabolic heating still failed to increase mean temperatures in the sex determination stage above pivotal temperature. Hence, if metabolic heating does influence sex differentiation, expected dominance would still remain the same within study nests.

4.4 Effects on Temperature

4.4.1. Clutch

Of all the clutch variables tested that may have influenced temperature within the nest over the course of incubation, only clutch size returned a significant result. Clutch size was found to be correlated to metabolic heating overall, and within the last third of incubation. These significant results support previous findings that the number of eggs within a clutch does influence nest temperature throughout the incubation period (Booth & Astill, 2001; Broderick *et al.* 2000) due to the biological by-product of heat during embryonic development. During the first third of incubation little metabolic heat is produced, removing the influence of clutch size on this period. Metabolic heating did raise temperatures dramatically during the second third, and although this did not return a significant result, the p value for this correlation was low (p=0.067). The lack of correlation between clutch size and metabolic heating during the 2nd third of incubation may be due to a reduced growth rate of embryos within this third, caused by significant amounts of rain (McGehee, 1990).

4.4.2 Meteorological Factors

Rain

During the study, nests all showed substantial drops in temperature, with the largest of these drops simultaneously occurring within the first third of incubation. This considerable reduction in nest temperature was accompanied by the largest rainfall event to occur during the incubation period of all nests. The significant results of the Mann-Whitney U test indicates that periods of decreasing temperature had significantly higher rainfall as opposed to periods of increasing or stable temperature. This results supports visual evidence that rain is the cause of the temperature drops within nests over the incubation period. Not surprisingly, only tests that included at least one group from the first third of incubation returned statistically significant results.

Once the second third of incubation is reached, the impact rain may have on nests is confounded due to the biological factor of metabolic heating. Further, at the end of incubation periods, the effect of rain is compacted alongside the natural drop of temperature within nests as juvenile turtles begin to hatch and emerge from their eggs and chamber. Despite insignificant results from tests ran using only categories within the last two thirds, all drops in temperature experienced within these periods are preceded by rainfall events (Figure 8). The amount of the 'temperature reaction', and also the time of reaction varied between nests after these rainfall events during these later stages of incubation. This difference is attributed to the nests being within different stages of embryonic development, whereby an amount of rainfall which may cause a temperature drop in Nest 1 would not illicit the same reaction in Nest 9 as the former is in a later stage of incubation, with different levels and types of confounding biological factors.
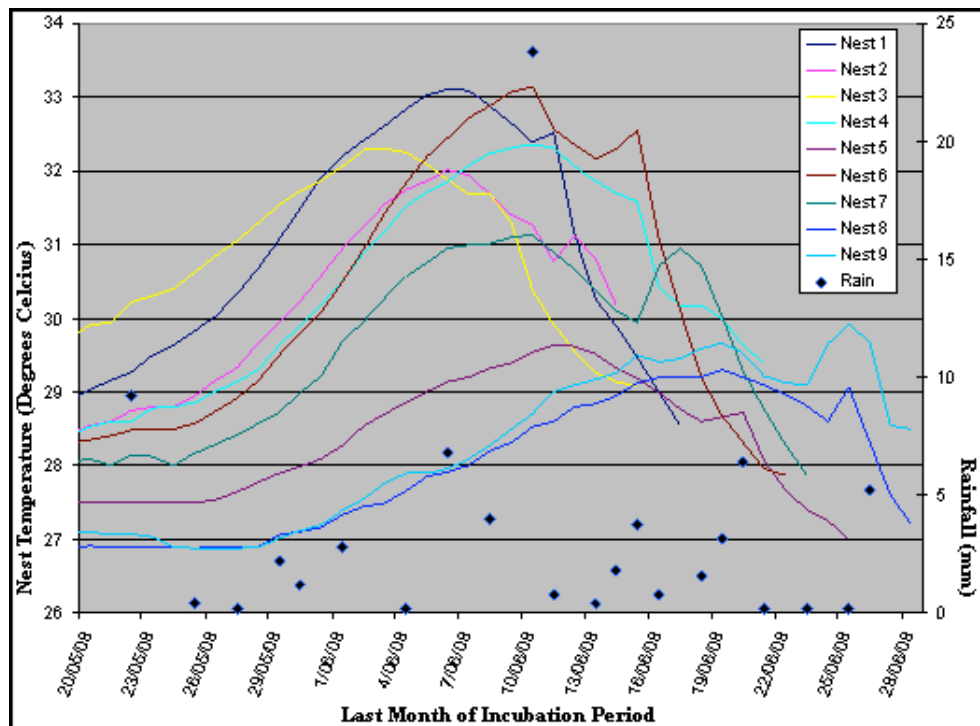
Figure 9: Daily nest temperature for *C.mydas* over the last third of incubation with rainfall events (>0.2mm), Tromelin 2008.

The Mann-Whitney U tests preformed on the control nest confirm that the insignificant results for nests returned after the commencement of the middle third of incubation (the first nest to begin this stage commenced on 9/05/08) is due to biological confoundment. Tests between all adjacent groups within the control nest returned highly significant results, proving a significant difference in the amount of rainfall occurring as the control nest temperature dropped and remained stable throughout the entire period of study nest's incubation. These results support different behaviour of temperature within the control nest between periods. This confirms the conclusion that non-significant results from nests are due to the aforementioned biological components of turtle nest fluctuations.

The temperature drop caused by rain within the first third of incubation for all nests had a carry on effect throughout incubation, altering the temperature that could be originally expected based simply on initial temperatures and expected nest fluctuations. Other studies have shown that temperature levels are significantly different between the first and second stage of incubation, with temperatures being higher in the latter stage (Broderick *et al*. 2000), however, findings from this study did not support this result. The first incidence of rain dropped temperature by at least 1°C in all cases. This resulted in no significant difference in temperature between the first and second stages of incubation, as well as temperature within the first stage being higher, on average, than within the second stage.

This reduction in temperature between the first two stages of incubation caused by rainfall also influenced expected sex-ratio outcomes from this study. As some temperatures during the second 'sex determination' stage were less than the pivotal incubation sex ratio values as given by previous studies (Godley *et al*. 2002; Reece *et al*. 2008), than the effect of rainfall in this instance may have reduced the amount of female hatchlings within study clutches. Just as McGehee (1990) found that higher amounts of 'rain' (laboratory experiment) increased incubation periods, the effect of rain within this study produced the same results (see 4.5.1).

McGehee found this also increased the size of hatchling, indicating dominantly male producing clutches and further supporting the conclusion drawn from temperatures found within nests during this research.

<u>Air Temperature</u>

Some nests demonstrated fluctuations over short-term periods that appear to be unrelated to air or rain effects. None of the nests that demonstrate these fluctuations over the diurnal period (1, 5, 6, 9) display characteristics that may account for this variation, additionally, nests deeper than 50cm have to been found to be unaffected by daily air fluctuations (van de Merwe, 2005). The variations are consistent with accuracy levels of the thermometers used (0.2°C), hence it is concluded that no nests experienced diurnal fluctuations in temperature caused by outside air temperatures.

Air temperature remained less than both nest and control temperatures throughout incubation. Control nest temperature throughout the study period was highly correlated with air temperature, both stably decreasing throughout the study period. These results propose that air temperature does have an impact on sand temperature, however, the extent of this influence was not great enough to cause diurnal fluctuations.

The composite nest temperature was significantly related over both the 1st third of incubation and the entire incubation duration to air temperature. Less of the variability in nest temperature was explained over the entire incubation period than within the first third ($r^2_{(first}$ $_{third)} > r^2_{(entire\ incubation\ period)}$) by changes in air temperature. The difference in r^2 values can be attributed to biological factors that result in nest temperature fluctuations within the 2nd and last third of incubation, such as metabolic heating. The significant result of the linear regression suggests that despite biological factors, air temperatures still impact nest temperatures over the latter stages of incubation. The reduced r^2 value indicates that during the last two periods of incubation, air temperature alone cannot be used to determine nest temperature over the final stages of incubation. The coarse grain sizes along the nesting beach may allow for easy gas and temperature transfer between strata from the atmosphere to the nest, but results suggest this exchange is limited.

4.4.3 Local Parameters

Shading was not found to be significantly correlated with temperature at any point during incubation. Percentages of shading were collected at the end of the final stages of incubation, and may have altered over the incubation duration. Consideration of the methodology may explain why 1st third temperatures where not correlated to temperature, but displayed a low p-value. Additionally, the generated correlation coefficient was a positive value, where one would expect a negative, i.e. higher shading percent results in lower temperatures. This may be due to the lack of data on light or shade intensity throughout the day or a very small sample size. Interestingly, although also uncorrelated to nest temperature, this illogical correlation coefficient value was also found by Booth & Astill (2001a).

Nest depth was not found to influence the temperature within the nest at any stage of the incubation period in accordance with results by van de Merwe *et al.* (2005).

Despite statistical analysis which found no significant difference between the mean diameters of sand grains within each nest, even where sand samples were defined under a different category, granulometry was found to affect nests differently. The reaction rate of nest temperatures after the occurrence of the largest rainfall was influenced by grain size despite a relatively small range of grain diameters. This small difference in grain size may have allowed quicker percolation of rainfall through the sand strata into the nest column. However, this percolation may also be influenced by permeability of the soil, or the extent of 'turtle patting' after oviposition has occurred. To further enforce the link between grain size and the reaction rate of nest temperature to rainfall events, permeability tests of sand are required as well as a larger sample size to ensure the link is not a Type 2 error, that is, a statistical significance is found where one does not exist.

Distance from the nest to both the sea, and to the vegetation line had no influence on temperature within the nest at any stage of incubation. Distance to the closest trunk did not have a significant correlation once outliers were removed from the data, however the p-value for this test was still low. Distance to the closest trunk from the nest is a variable yet to be explored in relation to any effect this variable may have on temperature within the nest. The negative correlation coefficient indicates that a decrease in distance to a trunk may result in an increase in nest temperature, this may be caused though the influence of metabolic heating by vegetation on temperatures within the nest. A reduction in moisture within the soil through the vegetations use would lower the amount of energy needed to heat the soil. As only 8 nests considered when analysing the effect of this variable, statistical analysis is not powerful. Nest sample size must be increased to ensure that distance between a trunk and nest can be investigated more reliably as an influential factor on temperature during incubation.

4.5 Temperature as a Predictor

4.5.1 Incubation Period

Temperature has been shown in previous studies to be a good predictor of incubation duration (Broderick et al, 2000; Godley et al, 2002). The mean temperatures of each third during incubation, as well as the mean temperature over the entire incubation period, were all significantly associated with incubation duration.

As sex ratio is often associated with incubation period (McGehee, 1990; Broderick *et al*. 2000) and this ratio is determined within the middle third of incubation, it is interesting to note that first third temperature had a closer relationship with incubation period than the middle third. This is also true for the mean temperature over the entire incubation period, reducing the chance the second third is not the best predictor of incubation duration due to unexpected drops in nest temperature.

Despite some nests having relatively high mean nest temperatures (max=32.35°C) incubation periods still had a minimum of 55 days, longer than expected at these temperatures based on previous studies (Broderick *et al*. 2000). This longer incubation period may be caused by rain events occurring during embryonic development which can slow development despite high average temperatures (McGehee, 1990). Primitive groupings of nests based on incubation periods align with results of post hoc tests on mean temperatures during the 1st third of incubation, concreting results that temperature within the first third of incubation is the best predictor of incubation period. The natural variation in nest temperature over the last two

stages of incubation caused by biological heating and cooling of the nest, as well as the influence of rain on temperature during these stages must have decreased linear association between these temperatures and incubation period. The more stable temperatures within the first third are therefore, better predictors of the nest's incubation period.

4.5.2 Hatchling Phenotype

Previous studies reported large temperature ranges during incubation reduced the influence of temperature on hatchling size (Booth & Astill, 2001; Broderick *et al*. 2001). Due to a drop in temperature over the end of the first third and beginning of the second period of incubation during this study, temperature range became more limited, increasing the influence of temperature on hatchling size. Results by Ackerman (1981) show that green turtle growth rates are almost exponential within the first two thirds of incubation however, become stable and even decrease at the 70th -80th percentile through incubation. This explanation of growth rates of embryos for green turtles clarifies conclusions from this study, indicating why only mean temperature during the final third of incubation was not related to hatchling mass.

4.6 Phenotype Correlation and Impacts of Nest Parameters on Clutch Behaviour/Success

The size of the female laying turtle cannot be used to predict clutch parameters or egg & hatchling weights. Congdon & Gibbons (1985) found that clutch size did increase with female size for some freshwater species, however only 1/3 of species included in their study showed this relationship in support of results from this study. There was no correlation between fertility rate and laying female size. Fertility rate may logically be more closely related to age of the reproducing female, or clutch number of the laying season, however the age of marine turtles is hard to define by size. The latter also requires long periods within the field before nest data can be collected to ensure clutch number is known which, for most colonies of green sea turtles, is impractical and costly.

The result of uncorrelated nest depth to female size can be explained from field observations where females where first seen to dig body pits of varying depths before beginning to excavate egg chambers. Further, time spent covering the nest also varied greatly between different females. These observations suggest that nest depth is a product of not only size, but body pit depth and covering extent.

Unlike temperature within the nest, local nest parameters were not seen to influence hatchling mass, or hatchling weight. Distances to the vegetation line and to the closest trunk were correlated to hatching success. As these factors were not statistically correlated with temperature, the actual influence of vegetation on hatching success is unknown for this study. Bustard & Greenham (1968) found that tree roots, hence closer distances to the vegetation line and tree trunk, indicated sufficient moisture levels within the soil to construct egg chambers. Previous studies have also suggested that moisture has an impact on the success of hatching for *Caretta caretta* (McGehee, 1990). Vegetation influences moisture levels within the soil which may affect hatching success, this may be through an increase of heat within the soil through biological activity within the tree roots, or the diminishment of moisture as it is employed by vegetation. Both correlation coefficients for distance to the vegetation line and

the closest trunk are positive, indicating that moisture levels within soils further from the vegetation may be too high for optimal hatching conditions of the green sea turtle. High levels of rain throughout the incubation period may have resulted in high strata moisture levels, generating a statistical influence of vegetation on hatching success.

3.8 Internesting Intervals and Laying Success

C.mydas nest all year round on the beaches of Tromelin, with a mark peak season during austral summer (Lauret-Stepler *et al.,* 2007). This research collected results for only three months out of peak season; hence results will not accurately reflect the mean beach frequentation in Tromelin.

The internesting interval calculated by the tagged turtles is within ranges found by other studies, specifically on the close island of Mayotte where Bourjea *et al.* (2007) found an internesting interval of 12 to 14 days. The standard deviation found from this study does suggest that 13.69 is precise, however, due to a lack of a reliable tagging method this precision could be improved. The Epoxy glue on the carapace of the tagged turtles was found to stay for up to several weeks on some turtles, however, in the majority of cases, observations of second sightings of the tagged turtles revealed indecipherable markings which had to be confirmed by measuring the carapace. Several turtles were only witnessed once upon the night of their tagging, suggesting that methodology was inadequate or tagged turtles were depositing their last clutch when tagged. Studies have found that between 25-50% of green turtles are 'one time nesters' (Carr *et al.* 1978; Hendrickson, 1958; Johnson & Erhart, 1996; Mortimer & Carr, 1987; Shulz, 1975), adding another possibility as to a low recapture success.

Laying success for turtle was calculated at 57%. The coarse grain sizes found at each of the nesting sites may have increased the difficultly of constructing egg chambers by increasing the incidence of 'cave-ins'. To accurately determine if this success is based on behaviour of the laying turtle, or on physicochemical properties of the beach, further research must take place. With efficient tagging and recapture of turtles, as well as broader studies into the properties of the beach, laying success can then be related to either the female or the beach characteristics.

5. CONCLUSION

Incubation temperature was found to be influenced by several factors during this study, both biological and abiotic. The biological influence of metabolic heating, caused by embryonic development, increases temperatures within nests significantly throughout incubation and occurs mainly in the middle and last stage of incubation. The number of eggs within the brooding clutch influences this heating, especially during the third stage where nest temperatures are significantly different to the premier stages. Air temperature does impact nest temperature, however the majority of this influence is seen within the first third of embryonic development, as when oviposition actually occurs the egg chamber is directly exposed to outside air temperatures. It is this initial influence by air temperature which created the larger variety of nest temperatures during the first stage of incubation.

Rain during incubation was a major environmental factor influencing temperature within the nest and resulted in several natural regimes to alter. Nest temperature was effected as well as the rate of development for embryos. The largest extent of this impact occurred during the first third of incubation where metabolic heating could not deter decreasing temperatures caused by rain. The reduced temperature in nests caused by rainfall events kept the mean temperature within the second third of six nests below previously reported pivotal temperatures (Godley *et al*. 2002; Reece *et al*. 2008) resulting in a male dominated clutch. As pivotal temperatures alter dependant upon nesting sites, where the warmer nesting sites have higher incubation periods (Miller, 1997), this suggestion can not be conclusively determined. However, as some mean temperatures for the sex-determination stage were over 1°C below previously reported pivotal temperatures adding credibility to this estimate.

As many green turtle nesting sites throughout the world already produce female bias clutches due to high temperatures, and with the onset of increasing global temperatures, the identification of male producing rookeries is becoming more important. Excluding the rains affect on nesting temperatures within this study, some nests may have stayed below pivotal temperature during the sex determining stage, increasing the need for further investigation into the nesting population of Tromelin. To limit animal ethic complications, dead hatchlings found within the nest or neck could be removed and histologically studied in later investigations to gain a greater idea of pivotal temperatures for this green turtle population. However, bias percentages of sexes may die within the egg chamber and neck of nests, this adaptation must be furthered explored.

The size of the laying female is not useful as a predictor of clutch, egg or hatchling parameters. Optimal egg size theories based on female size as suggested by Hays (2001) were not supported by this study. The amount of nests studied during this research is not of a great enough number to dispute this theory, more data is required to gain a sound result for the use of laying female characteristics as a predictor.

6. FUTURE DIRECTIONS

Results from this study have opened up avenues for further research to properly establish links between variables. The difference in reaction rates to rain between nests, despite the lack of significant difference in grain size, suggests two possible conclusions. Percolation of rain through the soil may be influenced greatly by slightly different grain particle diameter, or, other sediment characteristics also have an impact on rain influence. Despite significant results from the former, the latter would logically have a greater impact. Permeability of soil would have an effect on moisture percolation through the soil and can be easily tested within the field. Further to this, interesting correlations between hatching success and vegetation or trunk proximity has been attributed to moisture content within the soil, increased as a result of rainfall during the incubation duration. The measurement of moisture levels within the soil should be investigated in relation to the aforementioned variables to gain greater insight into the ability of individual nests to decrease the influence of rainfall during incubation on temperature and embryonic development.

Other sand characteristics have also been shown to have an impact on temperature within the nest and were not accounted for during this study. Sand albedo relates to the sands ability to reflect sunlight, ultimately influencing how quickly sand can heat. This has been shown to have an impact on nest temperatures (Hays et al, 1995; 2001) and can be easily determined within the field either through subjective evaluation, or using a lightmeter to measure the amount of reflected solar radiation (Hays et al, 2001).

Fertility rates within this study were obviously overestimated due to methodology being too basic. Other methods previously used (Whitmore & Dutton, 1985) established infertility by the presence of a white circle on the outside of the eggshell, where shell membranes attach to the shell wall in very early stages of development. This method, although still rudimentary, can be used in the field easily and would provide more accurate measures of infertility than the method used within this study. The recording of temperature within turtle nests that do not incur egg handling would also be a simple and effective method of exploring the difference this could make to temperature and embryonic development within the nest.

Results gained through the capture-recapture program were unreliable due to poor tagging methodology. As Tromelin is a small island with a small distance of nesting beach, further research using sufficient equipment would easily have the ability to recapture all marked turtles each night. This would increase mathematical accuracy on internesting intervals for this study site, long term tagging programs would improve the accuracy of this method as individual female turtles may not nest annually.. Tromelin, as it is small and relatively undisturbed by humans, also presents the opportunity to increase knowledge of the predation pressures suffered on beaches by emergent juveniles. The rate of juveniles to reach the sea from each nest could be easily calculated, allowing greater knowledge on survival rates for the Tromelin rookery.

The results of this research have provided some interesting insight into the influence of temperature within the nest of green turtles on Tromelin, as well as factors which may affect this temperature. The very small sample size used for these investigations (n=9) removed power from statistical analysis and could have resulted in inappropriate conclusions being drawn. A larger, more statistically satisfactory sample size (n>30) may reduce the incidence of type 1 or type 2 errors occurring during data analysis, as well as removing the detrimental effect of large ranged data. With an increased sample size, results drawn from a study would

allow for the removal of outliers without a large detrimental impact on statistical power, and could reinforce conclusions from this initial study.

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7. APPENDIX

Appendix 1: Local nest parameters data for *C.mydas* in Tromelin 2008

	Incubation Period (days)	Top nest Depth (cm)	Bottom nest depth (cm)	Distance to the sea (m)	Distance to the vegetation line (m)	Distance to the closest bush trunk (m)	Shading (average % per day)
Nest 1	58	71.8	98.8	75.5	-7.4	1.4	41
Nest 2	55	-	72	60	-10	1	42
Nest 3	56	-	82	60	-4.3	1	14.5
Nest 4	61	78	90	69.4	0	3.2	0
Nest 5	66	69	87	49.5	4	2.6	0
Nest 6	61	83	100	74.5	-11.3	1	38
Nest 7	62	63	76	78.5	-15.2	1.5	24
Nest 8	67	68	86	54.7	11.3	13.7	0
Nest 9	66	64	8	53.5	0	4	0

Table 9 – Mean particle size (µm) for sand samples taken from each nest (Falk & Ward Method) of *C.mydas*, 2008

	Nest 1	Nest 2	Nest 3	Nest 4	Nest 5	Nest 6	Nest 7	Nest 8	Nest 9
Surface	691.68	952.28	1025.37	841.62	851.04	695.92	804.51	952.96	821.73
Mid-Depth (50cm)	764.39	976.00	1020.23	795.33	834.44	659.39	851.78	968.95	854.96
Bottom Depth	1015.76	1030.26	1048.06	912.14	911.90	884.43	884.33	1085.92	909.11

Appendix 2: *C.mydas* **Eggs, Hatchlings and Female Turtle parameters nest by nest (mean, S.D. and range, respectively) in Tromelin, 2008.**

	Egg Length (cm)	Egg Weight (g)	Hatchling SCL (cm)	Hatchling Weight (g)	Female Size (cm)
Nest 1	**4,30 $^\pm$0,09** 4.14-4.45	**44,10 $^\pm$0,79** 42,81-45,26	**4.79 $^\pm$0.08** 4.62-4.93	**23.40 $^\pm$0.65** 22.41-24.86	**106**
Nest 2	**4.28 $^\pm$0.10** 4.09-4.39	**43.38 $^\pm$1.52** 40.58-45.38	**4.87 $^\pm$0.12** 4.62-5.20	**25.08 $^\pm$1.24** 22.79-27.37	**104**
Nest 3	**4.29 $^\pm$0.08** 4.15-4.36	**44.12 $^\pm$1.51** 42.72-46.75	**4.87 $^\pm$0.12** 4.31-5.09	**23.34 $^\pm$1.12** 19.53-25.36	**111**
Nest 4	**4.43 $^\pm$0.07** 4.32-4.58	**47.08 $^\pm$0.43** 46.69-48.05	**4.79 $^\pm$0.11** 4.57-5.05	**24.97 $^\pm$0.80** 23.13-26.41	**120.5**
Nest 5	**4.33 $^\pm$0.05** 4.25-4.40	**44.20 $^\pm$2.04** 42.66-48.64	**4.69 $^\pm$0.08** 4.53-4.85	**5.03 $^\pm$0.10** 4.80-5.20	**105.2**
Nest 6	**4.30 $^\pm$0.03** 4.26-4.34	**42.83 $^\pm$1.32** 40.98 44.80	**4.90 $^\pm$0.11** 4.64-5.13	**25.06 $^\pm$1.02** 22.94-27.40	**109.3**
Nest 7	**4.45$^\pm$0.05** 4.36-4.53	**47.59 $^\pm$1.38** 45.01-49.23	**4.88 $^\pm$0.11** 4.60-5.04	**24.08 $^\pm$1.06** 21.48-25.51	**109.2**
Nest 8	**4.52 $^\pm$0.04** 4.45-4.58	**50.65 $^\pm$0.92** 49.29-51.77	**4.90 $^\pm$0.11** 4.66-5.16	**27.34 $^\pm$0.66** 25.76-28.37	**111.5**
Nest 9	**4.33 $^\pm$0.06** 4.21-4.41	**45.50 $^\pm$0.61** 44.36-46.44	**4.84 $^\pm$0.12** 4.57-5.01	**26.02 $^\pm$0.94** 23.53-27.40	**110.6**

Appendix 3: Rain Grouping Results in Tromelin, 2008

Nest Number	Increase Classification	Increase Dates	Decrease Classification	Decrease Dates	Z-Value	U (Sig.)-Value
Nest 1	**A1**	20/04-8/05	**B1**	8/05-12/2005	-2.42	0.033
	A2	12/05-6/06	**B2**	6/06-10/2006	-2.42	0.016
	A3	5/06-10/06	**B3**	11/06-17/06	-1.025	0.429
	A3		B2		-0.939	0.533
	A2		B1		-2.057	0.051
Nest 2	**A1**	20/04-08/05	**B1**	08/05-11/05	-3.107	0.002
	A2	11/05-6/06	**B2**	6/06-11/-6	-1.37	0.214
	A3	5/05-11/06	**B3**	12/06-14/06	-1.225	0.221
	A3		B2		-0.297	1
	A2		B1		-2.93	0.001
Nest 3	**A1**	20/04-08/04	**B1**	08/04-11/05	-3.107	0.002
	A2	11/05-3/06	**B2**	3/06-7/06	-0.072	0.974
	A3	7/06-8/06	**B3**	8/06-15/06	-1.228	0.5
	A3		B2		-0.791	0.8
	A2		B1		-2.881	0.001
Nest 4	**A1**	22/04-08/05	**B1**	08/05-12/05	-2.294	0.039
	A2	12/05-11/06	**B2**	11/06-21/06	-1.518	0.148
	A2		B1		-2.069	0.048
Nest 5	**A1**	22/04-08/05	**B1**	08/06-12/05	-2.358	0.040
	A2	12/05-11/06	**B2**	11/06-18/06	-0.149	0.909
	A3	18/06-20/06	**B3**	20/06-25/06	-1.183	0.381
	A3		B2		-1.183	0.381
	A2		B1		-2.069	0.048
Nest 6	**A1**	20/04-7/05	**B1**	7/05-11/05	-3.127	0.002
	A2	11/05-10/06	**B2**	10/06-13/06	-0.965	0.382
	A3	13/06-15/06	**B3**	15/06-22/06	-0.293	0.889
	A3		B2		0	1
	A2		B1		-2.908	0.002
Nest 7	**A1**	22/04-05/05	**B1**	05/05-12/05	-2.416	0.030
	A2	12/05-10/06	**B2**	10/06-15/06	-1.106	0.299
	A3	15/06-17/06	**B3**	17/06-23/06	-0.671	0.643
	A3		B2		-0.586	0.571
	A2		B1		-1.983	0.059
Nest 8	**A1**	24/04-5/05	**B1**	5/05-12/05	-2.189	0.041
	A2	12/05-19/06	**B2**	19/06-24/06	-0.626	0.568
	A3	24/06-25/06	**B3**	25/06-27/06	-1.225	0.667
	A3		B2		-1.206	0.333
	A2		B1		-1.749	0.090
Nest 9	**A1**	23/04-07/05	**B1**	07/05-12/05	-2.320	0.034
	A2	27/05-15/06	**B2**	19/06-23/06	-0.67	0.557
	A3	23/06-25/06	**B3**	25/06-28/06	-1.225	0.333
		*no other comparisons as no other groups are directly adjacent.				
Control	**A1**	22/04-26/04	**B1**	08/05-12/05		
	A2	18/05-22/05	**B2**	22/05-24/05		
	A3	28/05-03/06	**B3**	05/06-20/06		