Core temperatures of nesting female hawksbill turtles (*Eretmochelys imbricata*) in the north-east of Tobago

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Introduction

All marine turtle body temperatures are influenced by external temperatures. The extent of this influence varies between species. Leatherbacks (*Dermochelys coriacea*) exhibit physiological and behavioural adaptations to maintain body temperature allowing them to inhabit a broad range of water temperatures (Burns et al. 2015). The six other marine turtles, including hawksbills (*Eretmochelys imbricata*), can be characterized as ectothermic. This means that the regulation of their body temperature is largely dictated by the surrounding environment, and that they are bound by physical limits of temperature (Hawkes et al. 2007). Temperature plays a key role in every stage of their life history, including sex determination of embryos, incubation time, emergence from the nest, growth, survival, activity, distribution and inter-nesting interval (Mrosovsky & Pritchard 1971; Davenport 1997).

Physiological studies of sea turtles are notoriously difficult due their life history. Upon entering the water as hatchlings, their life cycles are spent entirely at sea, with the exception of adult females returning to shore to nest. Therefore, physiological studies without major disturbance are limited to the demographic of breeding females in the terrestrial nesting phase (Burns et al. 2015; Byrne et al. 2018). The IUCN classifies all sea turtle species as vulnerable or endangered with hawksbills critically endangered (IUCN 2020; Mortimer & Donnelly 2008). For this reason invasive physiological studies are rare. This, combined with the fact that females are slow to reach sexual maturity, with two to three years between breeding cycles, has resulted in a gap in the knowledge of many physiological features of sea turtles, including their thermal biology (Mrosovsky 1980; Davenport 1997).

Studies have attempted to measure the core temperature of nesting marine turtles by measuring the cloacal temperature with a probe, and inserting a probe to the body cavity through an incision between the plastral scutes (Mrosovsky & Pritchard 1971; Mrosovsky 1980). These methods are highly invasive and are likely to cause significant stress and some disturbance to the

turtle that might affect their body temperatures. Mrosovsky and Pritchard (1971) found that the temperatures of freshly laid eggs measured with a fast reading mercury thermometer are a good reflection and a valid way of assessing sea turtles' core body temperatures. Burns et al. (2015) more recently measured the temperatures of freshly laid turtle eggs using non-contact infrared thermometry. They used adjusted surface temperatures of freshly laid leatherback and hawksbill eggs as a non-contact proxy for core body temperature.

Marine turtles, especially those with restricted thermal tolerances such as hawksbills, may be particularly vulnerable to climate change due to the influence of environmental temperatures on their basic physiological functions (Deutsch et al. 2008). Hawksbill turtles have a global tropical and subtropical distribution, and their primary habitat is situated within a water temperature range from 22°C to 30°C (Pilcher et al. 2014). There is a paucity of data and studies in understanding hawksbill turtle thermal biology (Burns et al. 2015). Extant species of turtles have lived on the planet for over 150 million years and survived as species through numerous climate fluctuations. However, the relatively rapid rate of current anthropogenic climate change may pose severe risks, for one because of the long generation cycles of marine turtles (Poloczanska et al. 2009). There has been criticism of the lack of timely reaction of environmental conservation planners to the real time challenges faced by many species from anthropogenic climate change (Keith et al. 2014). Development of species' climate adaptability and susceptibility assessment practices paves the way to a deeper understanding of species' biology and responses to climate change. This alongside close environmental monitoring can foster better conservation planning and efforts, not just in the protection of marine turtles but other marine reptiles and the complex ecosystems within which they exist (Hamann et al. 2007). The adoption of the technique outlined in this study and that of Burns et al. (2015) into longer-term monitoring projects may allow for a baseline core-temperature estimate to be generated for marine turtle species. This could be used as a comparator against which deviations can be monitored, in the context of climate change.

This field report presents the findings of an investigation into the core temperatures of nesting hawksbill turtles using non-contact infrared thermometry. The data were collected over four weeks on two index beaches in north-east Tobago in July 2019.



Fig. 1. Map of study sites within Tobago and the Caribbean including Hermitage Bay and Cambleton Bay.

Methods Study sites

Tobago (11.248338°N, 60.654797°W) is the smaller of the two islands which make up Trinidad and Tobago and is located in the southernmost extent of the Caribbean. It is an important nesting site for hawksbill turtles. A wide distribution of nesting hawksbill sites have been described in Tobago by Walker et al. (2014), with a significant concentration of activity in the northeast of the island (Walker & Gibson 2015).

The study sites were Cambleton Bay (11.317259°N, 60.560034°W) and Hermitage Bay (11.316507°N, 60.570934°W) (Fig.1). Both are located on the Caribbean side of the north-east of Tobago. Cambleton is a sandy bay which is only accessible by rainforest trail or by boat. There is no nearby light pollution or human settlement. Hermitage is a mixed sand and rocky beach which can be accessed by vehicle via a dirt track off Windward Road or by boat. For this reason, Hermitage has more human activity than Cambleton. The beaches are known for high numbers of hawksbill turtles (Walker et al. 2015), and the research team have a long-standing relationship with the local sea turtle conservation non-governmental organisation NEST (North East Sea Turtles).

Patrol protocols

Egg temperatures were collected from nesting female hawksbills over a period of four weeks. Patrols were carried out on 16 nights, four nights per week, from 30th June to 27th July 2019. Two researchers patrolled the beaches at Cambleton and Hermitage on alternate nights. Due to higher human activity and a number of recent potential poaching attempts, patrols on Hermitage Bay were conducted alongside a volunteer member of NEST. Foot patrols were conducted every 30 minutes between 19:30 and 01:00. A red light was used where necessary to reduce light disturbance to turtles. Each patrol surveyed the entire length of the beach. Turtles were detected visually by their tracks on the shore, or upon hearing them scattering sand.

Temperature recordings

Methods were adapted from Burns et al. (2015). A Fluke 62 Max+ portable laser-guided infrared thermometer (spectral range = $8-14 \mu m$, accuracy = $1^{\circ}C$ or 1%, response time <0.3 seconds, resolution 0.1°C) was used to obtain temperatures without contact. The thermometer was calibrated against water a week before the project and the emissivity was set to 0.98 (which is the emissivity for water) for all recordings. This value is within the range used previously for emissivity of living tissues, and particularly applies here given that the surface of newly emerged eggs is covered in a thin watery mucus laver (McCafferty et al. 2013: Burns et al. 2015). Turtles were confirmed as laying once the recorder saw that she was depositing eggs into the nesting chamber. The recorder positioned themselves on the ground behind the turtle with a clear view into the chamber. Immediately after an egg was dropped, the recorder positioned the infrared laser onto the egg and called out the temperature reading to the scribe. Egg temperatures were recorded within 1-2 seconds of leaving the cloaca. To successfully record the temperature of an egg, the thermometer was held within 20cm of the egg, with both guide laser beams from the thermometer positioned on the egg. If an egg became covered in sand, it was disregarded.

A correction factor was added to the temperature recordings to account for any inaccuracies in emissivity setting and/or post-laying cooling of the eggs. We adopted the correction factor used by Burns et al. (2015) on hawksbill egg temperature recordings from the same beaches. This was calculated using a linear regression of mean internal temperature to mean surface temperature of sterile 'yolkless eggs' of 20 different leatherback turtles. All results are displayed with this correction applied.

Sea surface temperature (SST)

To obtain an estimation of SST we analysed data for Trinidad and Tobago from the National Oceanic and Atmospheric Association (NOAA) Coral Reef

Watch's (2020) daily 5km virtual stations. We obtained an estimate of SSTs for our research period as well as the same period in 2013, and 2014 to allow comparison with the results from Burns et al. (2015). This was done by taking the median of the daily 5km maximum and minimum SST from the Tobago virtual station and obtaining a mean SST for the region for each time period.

Data analysis

RStudio version 1.2.1335 was used for statistical analysis and to produce graphs. Project weeks were numbered from 1 to 4. Week 1 included from 30th June – 6th July, week 2 included from 7th - 13th July, week 3 included from 14th- 20th July, and week 4 included from 21st – 27th July. A one-way analysis of variance (ANOVA) was conducted on a General Linear Model (GLM) with week as a categorical explanatory variable to assess the effect of week number on average egg temperature per nest. A Tukey's Honestly Significant Test (HSD) was conducted post-hoc to confirm the results. Difference in mean SST recorded during the study periods of 2019, 2014 and 2013 were analysed to determine if temperatures varied significantly between the years. An ANOVA test was run on a GLM with year as a categorical explanatory variable. A Tukey's HSD was run on the results to confirm them.

Results

Nests recorded

A total of 17 female hawksbills were recorded over the two collection sites. Of this 17, three individuals false crawled and egg temperature data were not collected for two nesting individuals. From the remaining 12 nesting females, 353 egg temperatures were collected. Of these nesting females seven were recorded on Cambleton and five on Hermitage. The highest number of temperatures recorded for one turtle was 57 and the lowest was nine. The large range in number of recordings per female was due to factors such as detecting a turtle when she had already laid most of her eggs or having multiple turtles laying simultaneously. This means that these data do not show total numbers of eggs per nest but only number of temperature recordings made per nest. The average temperatures were recorded from three nests in week 1, three nests in week 2, four nests in week 3 and two nests in week 4 (n=12).

Egg temperatures

The mean egg temperature was $30.67^{\circ}C \pm 0.38$ (mean \pm standard deviation; n=353). Temperatures ranged from 29.73°C to $31.45^{\circ}C$ (Figs 2 & 3).

Effect of week on egg temperatures

This model was run to analyse whether there was a change in egg



Fig. 2. Average egg temperatures per nest (n=12).

temperatures over the course of the study. The results of an analysis of variance (ANOVA) on the effect of week number on average egg temperature per nest yielded significant variation among weeks (p=0.028) (Fig. 3). A Tukey's HSD post hoc test showed that week numbers 2 and 4 differed significantly at a 95% confidence interval (p<0.05), with temperatures in week 4 being significantly higher than in week 2.

The greatest intraweek variation was seen in week 2, with temperatures ranging from 29.99°C to 31.19°C.

Sea surface temperature (SST)

Mean SST was significantly lower during July 2014 (p<0.05) than July 2019. It was also significantly lower in July 2014 (p<0.05) than July 2013. There was no significant difference between mean SST in July 2013 and July 2019 (Table 1).



Fig. 3. Boxplot showing average temperature versus week number. 'X' represents mean of average egg temperatures per nest.

Table 1	. Showing	mean SST	and egg	temperatures	in °C fo	or the year	s 2013,	2014 & 2019.
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Year	Mean SST	Mean Egg Temperature
2013	28.40	30.30
2014	27.55	29.45
2019	28.11	30.67

Discussion

In this study the egg temperatures of nesting female hawksbills were measured by non-contact infra-red thermometry as a proxy for core temperature. Burns et al. (2015) reported egg temperatures of nesting hawksbills recorded in 2013 and 2014 at the same sites in Tobago. They reported a mean egg temperature of 30.0 ± 0.13 °C, which is 0.67°C lower than our recorded 30.67 ± 0.38 °C. The temperatures they recorded ranged over a 1.38°C wider distribution of 29-32°C when compared to our range of 29.73-31.45°C. Our study took place over a shorter time frame which may provide an explanation for the narrower range. This variation in temperatures between years aligns with the findings of Burns et al. (2015). They reported significant differences between both hawksbill and leatherback egg temperature recordings between 2014 and 2013, with lower temperatures recorded for both species in 2014.

Burns et al. (2015) did not find any significant correlation between core temperature and any environmental variable. They did report a significant drop in satellite-derived SST between the years of their study, which coincided with a drop in their recordings of hawksbill core body temperature. However, they noted an insufficient amount of information to infer a causal relationship between SST and core body temperature. Our analysis of SST found an increase in temperature in 2019 compared to 2013 and 2014. This increase was only statistically significant between 2014 and 2019. This appears to align with the higher recorded mean core body temperature in our study compared with that of Burns et al. (2015). This adds weight to the hypothesis put forward by Burns et al. (2015), that variation in core temperature may be partially determined by the sea temperature conditions experienced by the turtle. Our small sample size and cross sectional study design did not produce enough data to formally analyse this, but it may warrant further research.

This hypothesis is plausible as ectothermic sea turtles', including hawksbills', core temperatures are largely regulated by the surrounding environment (Deutsch et al. 2008; Pilcher et al. 2014). Our field study was limited by the small size of its research group, which meant that we were unable to record environmental variables such as sea temperature alongside the egg temperatures, in real time, so we had to use simple estimations from regional satellite data. This would have allowed a more in-depth analysis of factors affecting the egg temperatures recorded. We acknowledge, however, that one month of recordings would not produce sufficient data to infer a causal relationship. Obtaining large enough datasets to investigate aspects of sea turtle behaviour and biology is hindered by aspects of their life history, in particular their long lifespans, late sexual maturity and two to three year gaps in female breeding seasons (Byrne et al. 2018). The most reliable method of monitoring and investigating trends in sea turtles is by long-term

population assessments which are conducted at nesting beaches (Bjorndal 1995). Thus, a larger dataset of egg temperatures collected over many years and paired with climatic and environmental variables would be necessary to determine whether an association exists between the core temperatures of nesting females and the sea temperature of their environment.

The significant changes in average temperature per week were particularly interesting. There was an upward trend seen in the egg temperatures over the four week study period with a significant increase (p=<0.05) between week 2 and week 4. These may be an artefact of our small sample size and the intrinsic variability between turtles. In future, longer-term studies of this nature, more reliable SST data and the inclusion of other environmental variables could add weight to the analysis. This may provide more information on the mechanisms underlying the temporal differences recorded in sea turtles' core temperatures.

Ectotherms may be disproportionately affected by global warming due their high dependence on environmental temperature for basic physiological functions (Deutsch et al. 2008). To the best of our knowledge, extensive data do not exist on hawksbill temperature tolerance limits, nor their ability to thermoregulate under increasing ambient temperatures, aside from one study by Pilcher et al. (2014). They document a potential adaptive behavioural measure to increased sea surface temperatures, with hawksbills moving to cooler waters for the warmest months of the year. Long-term studies into changes and adaptations in hawksbill thermal biology and activity in response to rising global temperatures would be of merit and may become increasingly relevant in the coming years. This field report, and the study by Burns et al. (2015), show that minimal disturbance methods of infra-red thermometry to record egg temperatures can be used as a proxy for sea turtle core temperature estimations. Long-term studies could incorporate these methods to gather a baseline core temperature for nesting female sea turtles, against which future changes can be observed.

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