REDUCED GENERAL HEALTH AND REPRODUCTIVE SUCCESS IN GREEN TURTLES ACROSS EXTENDED NESTING PERIOD

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INTRODUCTION

Sea turtles are capital breeders who must accumulate energy reserves before proceeding with extended breeding periods. Post-oviposition, no maternal care is provided to the developing embryos. Embryo development is dependent on the limited nutrient reserve and growth factors the nesting turtle provides during vitellogenesis and egg production in the oviduct. This study was conducted at Chagar Hutang Turtle Sanctuary (CHTS), Redang Island, Malaysia, known as one of the nesting grounds with the highest density of endangered green turtles' nests in the country. In most clutches, the hatching success (number of eggs that successfully hatched out of the entire clutch) is between 70-80%. However, unpublished data from CHTS indicates some clutches demonstrate considerable numbers of unsuccessful eggs, potentially due to infertility of the eggs or death of the developing embryo. Although embryo mortality may happen due to environmental factors such as unsuitable incubation temperature and respiratory gas concentrations during incubation (Phillott & Parmenter, 2001; Booth & Dunstan, 2018; Booth *et al.*, 2020), maternal influence could also contribute to mortality (Tezak *et al.,* 2020). In this study, we aimed to measure the changes in maternal body reserves and reproductive parameters as the nesting season progressed. By collecting blood samples from females at the time of oviposition, we determined their physiological status and evaluated the general health of the nesting population.

METHODS

The nesting season at CHTS occurs from March to November each year, and peaks in July. This study was conducted towards the end of the nesting season, in August and September. We sampled 33 female individuals that were encountered nesting on the beach, which we classified as the first episode nesting group. We then specifically targeted the same individuals during subsequent nesting episodes (second episode nester group = 12 individuals, and third episode nester group = 5). For every individual sampled, we collected approximately 6.0mL of whole blood from the dorsal cervical sinus into a Lithium-heparin tube. Each blood sample was divided for preparation and analysis within 6-12hr of collection; blood biochemistry and analytes using a portable i-Stat clinical analyser (Lewbart *et al.*, 2014) suited with CG-4+ and Chem-8+ cartridges, erythrocytes count using a Hemacytometer counting chamber (Campbell, 2015), packed cell volume (PCV) using microhematocrit centrifugation (Nardini *et al.*, 2013), and leukocyte estimate and differential count analysed from thin blood smears stained with Liu solutions, respectively (Campbell, 2015; Flower *et al.*, 2015; Li *et al.*, 2015).

Clutches of eggs laid by females from which we collected blood samples were assessed throughout the incubation period for signs of successful development. During oviposition, we measured the mean egg mass and size from 10 randomly collected eggs. Within the first third of the incubation period (7-10 days post-oviposition), we carefully exposed eggs in the nest to look for white-spot formation (Figure 1), indicating the successful recommencement of embryo development in 20 randomly selected eggs. At the end of the incubation period, and after hatchlings had emerged, a routine nest excavation was performed to obtain information such as clutch size, number of unhatched and hatched eggs and to estimate hatching, and emergence success. We recorded

Figure 1. Egg development 10 days post-oviposition, chalk-white eggshell indicating a viable egg and successful development of the embryo (bold arrowhead) and yellowed appearance indicating a non-viable egg (open arrowhead). (Photo credit: Afif Aiman Azmi)

reproductive output data (clutch size, mean egg mass, and mean egg size) and reproductive success (proportion of eggs successfully recommencing embryonic development, and hatching and emergence success) for each nest. Every unsuccessful egg was opened (Figure 2), and the contents were inspected to categorise the stage of embryo development at the time of death based on Booth *et al.* (2021).

All methods were approved by the Animal Ethics Committee of Universiti Malaysia Terengganu, and the research was supported by the Department of Fisheries Malaysia.

FINDINGS TO DATE

We have analysed 16 suites of blood parameters including nutrients and metabolites (glucose, blood urea nitrogen, creatinine, and lactate), elements and electrolytes (sodium, potassium, chloride, calcium ion, bicarbonate ion, anion gap), and blood gases (partial pressure of carbon dioxide, partial pressure of oxygen, total carbon dioxide, oxygen saturation, blood pH, and base excess). At the population level, analysis of variance (ANOVA) revealed significant differences ($p<0.05$) in blood pH, carbon dioxide partial pressure, potassium, oxygen saturation, estimated count of leukocyte, absolute count of heterophils, and proportion of monocyte count among nesting groups. Some blood parameters for individual turtles (n=5) over three consecutive nesting episodes were statistically significant ($p<0.05$): pH, pCO_2, pO_2, sO_2, TCO_2, K, Cl, Ca^{2+}, PCV, and haemoglobin between nesting episodes, based on analysis of variance (ANOVA) with repeated measures.

Figure 2. Opening unsuccessful eggs to inspect contents. (Photo credit: Mohd Uzair Rusli)

None of the parameters of the reproductive output and success that were measured differed significantly ($p>0.05$) when compared among turtles that nested for one, two, and three episodes. The only measure of reproductive success of individuals (n=5) that successfully deposited three consecutive clutches that differed significantly ($p<0.05$) was hatching success.

RELEVANCE FOR SEA TURTLE CONSERVATION

The changing concentration in maternal reserves (blood biochemistry, gases, and haematology) and the reproductive output and success as the nesting season proceeds are common trends (Honarvar *et al.*, 2011; Perrault *et al.*, 2016; Flower *et al.*, 2018). In general, this nesting population can be described as clinically healthy. The values in this study are comparable to those of a previous study (Samsol *et al.*, 2020) from the same nesting population, thus conforming the ranges of normal blood parameters that can be utilised for future reference in health assessment of nesting sea turtles in the South China Sea region. We suggest further study into finding a possible association between maternal blood profiles with reproductive output/success to elucidate maternal provisioning as a potential contributing factor to the hatchling fitness in the first few days.

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BEACH PROFILE CHARACTERISTICS OF THE OLIVE RIDLEY MASS NESTING BEACH AT RUSHIKULYA, INDIA

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The dynamic ecosystem of sandy beaches is shaped by processes such as erosion, accretion, and sediment deposition. In many coastal regions around the world, these beaches also serve as nesting habitats for sea turtles and have a significant influence on their reproductive success and hatchling recruitment (Wood & Bjorndal, 2000). Beach characteristics such as slope, width and vegetation cover are known to influence sea turtle nest site selection (Jankie & Lawrence, 2013; Bladow, 2017). Olive ridley sea turtles nest *en masse*, in a phenomenon called an *arribada*, at a few known sites on the east coast of India and Mexico and Central America. These mass nesting beaches are often located near river-mouths and are highly dynamic in nature. To understand the characteristics of these nesting habitats, this study examined the variation in beach characteristics at the Rushikulya mass nesting beach in Odisha. Spatial and temporal variation in beach profiling data (Figure 1, 2), collected between 2012-2022, across different sections of the mass nesting beach were determined and the

Figure 1. People conducting beach profile surveys. (Photo credit: Chandana Pusapati)