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Factors influencing variations of oxygen content in nests of green sea turtles during egg incubation with a comparison of two nesting environments

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ABSTRACT

Several biotic and abiotic factors can influence nest oxygen content during embryogenesis of sea turtle eggs. These factors were evaluated during each stage of egg development on Lanyu Island, Taitung County, Taiwan. We measured oxygen content of 14 nests in 2010. Oxygen in the adjacent sand, total and viable clutch sizes, air, sand and nest temperatures and sand characters of each nest were also determined. The study found that the oxygen available to an egg differs within the clutch, resulted in this value being highest in the upper layer and decreased with depth. Metabolic heat increased with incubation and reached the peak value in the middle or late stage of incubation. This result in the oxygen content was highest in the early incubation stage and decreased afterwards. Clutch size is the major factor that influences the nest oxygen content during the incubation. Hatching success was found to decrease with decrease of average oxygen partial pressure in the nest. Comparison between Wan-an Island of Penghu Archipelago and Lanyu Island of Taitung County showed that the incubation microenvironment and the local weather pattern are the major factors responsible for differences in oxygen content during embryo development. It is possible that the nesting beach loyalty result in different adaptive morphological characters of hatchlings is produced from each island.

1. Introduction

Embryos develop in the environments of nests where the eggs are deposited (Shine, 1991). Sea turtle embryos, for example, incubate in beach sand. The nest environment has a profound effect on the survivorship of the embryos (Ackerman, 1981b; Ischer et al., 2009; Maloney et al., 1990; Vladimirova et al., 2005). Habitat factors, temperature, gas partial pressures and water content in the nest, can influence embryonic growth rate (Ackerman, 1981a; Maloney et al., 1990), incubation duration (Ackerman, 1981b; Deeming and Ferguson, 1991; Miller and Limpus, 1980) and hatching as well as emergence success (Glen et al., 2005; Matsuzawa et al., 2002; Mortimer, 1999).

Among various factors, oxygen availability is of particular importance. Oxygen influences the metabolism of embryo, thus the development rate of embryo. However, the changes in both temperature and moisture can influence the oxygen available to the nest. Higher temperature e.g., in the dry weather, can speed up the metabolism and increase the oxygen demand (Ackerman et al., 1985; Booth, 1998; Reid et al., 2009; Vleck and Hoyt, 1991). When oxygen consumption exceeds the diffusive supply from the surrounding medium, it will lower the oxygen partial pressure in the nest (e.g., Maloney et al., 1990) and result in hypoxia during incubation. This will consequently result in the early hatching, smaller body size and cardiac hypertrophy and decrease the fitness of the hatchling (Packard and Packard, 2002). Higher moisture content, on the other hand, tends to decrease the incubation temperature, slow down the metabolism and decrease the oxygen demand. This will lengthen the incubation, in turn, produce larger hatchling.

Development of sea turtle embryos can be influenced by the interaction of environmental and biotic factors (Garnett et al., 2010; Wallace et al., 2004). The environmental factors include geographic location (Packard and Packard, 2002), weather (Tomillo et al., 2009), sand characteristics (e.g., mean grain size, inclusive graphic standard deviation; Foley et al., 2006; Garnett et al., 2010), sand and nest temperatures (Ischer et al., 2009), gas partial pressures (Garnett et al., 2010), precipitation (Frinker, 2006; Houghton et al., 2007), nest depth, water content in the sands (Gettinger et al., 1984), vegetation coverage (Conrad et al., 2011) and tidal pumping (Maloney et al., 1990). Temperature is an important environmental factor (Booth, 2000). Development is faster at higher incubation temperature and result in higher gas exchange rate (e.g., Chen et al., 2010). Sea turtles also have TSD and so incubation temperature drives hatchling sex resulting in important climate change impacts on sex ratios (e.g., Laloê et al., 2014). Sand characters such as grain size, mineral type and sorting coefficient can influence gas and water circulation in the clutch (Ackerman, 1977, 1980; Foley et al.,

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2006; Garnett et al., 2010), heat conductivity (Speakman et al., 1998) and clutch viability (Mortimer, 1990). Precipitation can promote circulation and increase gas exchange in the clutch (Finkler, 2006; Hillel, 1998). Precipitation can also cause temperature variation during embryogenesis (Booth, 1998; Houghton et al., 2007; Trullas and Paladino, 2007), decreased nest temperature and increasing incubation duration (Houghton et al., 2007). Excess rainfall (protracted rain) can suffocate the embryos and increase the mortality rate (Finkler, 2006; Kam, 1993; Kraemer and Bell, 1980).

The biotic factors include total and viable clutch sizes (Honarvar et al., 2008), position of eggs within the nest (Ackerman, 1991, 1997) and incubation duration (Ackermann, 1997; Booth and Astill, 2001; Chen et al., 2010; Kraemer and Bell, 1980). These factors can influence the oxygen demand and the embryonic development. Spatial variation of oxygen consumption occurs within the clutch during development. Ackerman (1977) proposed a radial steady-state diffusion model to explain that variation. Oxygen partial pressure in the nests of green and loggerhead turtles increases from the center toward the periphery of the clutch (Houghton and Hays, 2001; Ralph et al., 2005; Wallace et al., 2004). Total and viable clutch sizes are also the important factors (Williams, 1996). Wallace et al. (2004) found that, when the number and weight of leatherback embryos was greater, the minimum oxygen partial pressure in the nest was lower, while the maximum nest temperature was greater. Chen et al. (2010) found similar results in green turtles. In the case of insufficient gas exchange, the incubation will be longer, jeopardizing normal development and increasing mortality (Ackerman, 1981b).

There are three nesting sites for green turtles in Taiwan; namely Wan-an Island of Penghu Archipelago, Lanyu Island of Taitung County, and LiuChiu Island of Pentung County (King et al., 2013). Chen et al. (2010) determined the change of oxygen content during embryogenesis of green turtles on Wan-an and identified the major influential factors. However, Cheng et al. (2008) found that, both the nesting environment and nesting characters were different between Wan-an Island and Lanyu Island. The aim of this study was to investigate temporal and spatial changes to O_2 in green turtle nests and understand the abiotic and biotic factors that are most related to these changes in O_2 on Lanyu Island and determine the differences between two islands.

2. Material and methods

2.1. Brief description of the study site

Lanyu Island of Taitung County, Taiwan (22°00′–22°08′N, 121°50′–121°60′E), is situated in the Pacific Ocean approximately 76 km off Taiwan's southeastern coast and has an area of about 45.7 km^2 (Fig. 1). Of the six beaches on Lanyu, the majority of nesting occurs on three: Badai, Big Badai, and Donchin beaches (Cheng et al., 2009, Fig. 1). The respective lengths of these nesting beaches are 200, 1500, and 800 m. These beaches are dissipative, with less than a 5° slope, although the slope increases sharply (to >20°) above the primary vegetation line. The sand characteristics range from moderately sorted to well sorted, very coarse sand. The annual precipitation exceeds 3000 mm, and average temperature is about 23 °C with relative humidity about 90% (Cheng, unpublished data). The vegetation on the upper beach is mainly composed of seashore-vine morning glory (*Ipomocea pes-caprae* (L)), and screw pine (*Pandanus odoratissimus sinensis* (Warburg)). Because of the overcrowding of nests and the narrow width of the Badai beach, all nests were relocated to Donchin beach.

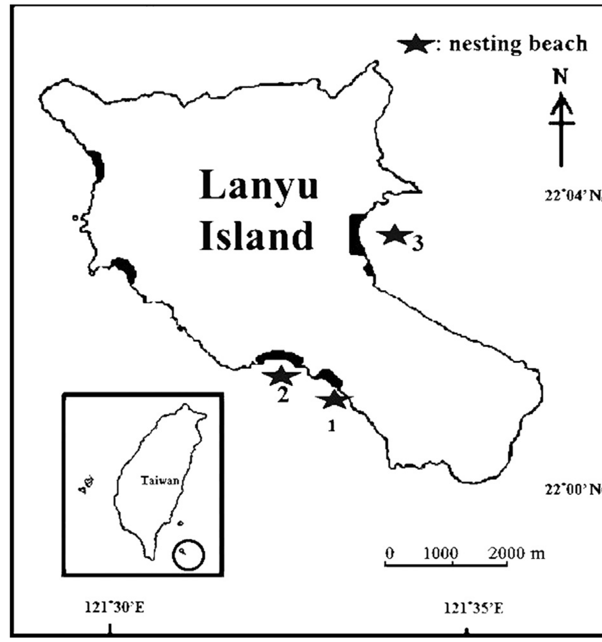

Fig. 1. Map of Lanyu Island with nesting beaches denoted by stars. 1, Badai beach; 2, Big Badai beach; 3, Donchin beach.

2.2. Experimental procedures

2.2.1. Nest relocation and temperature and oxygen measurements

The experiments were carried out from July 8 until September 18, 2010. Sixteen nests were relocated to the experimental site where the chance of flooding is minimal. The nests that mimic the natural shape were buried at a nest-bottom depth of 65 cm, approximately the in situ nest depth on this island (Cheng et al., 2009). Nest relocation was done within 1.5 h after oviposition. Clutch sizes were determined by counting all the eggs before relocation. Mean egg size and weight for each nest were determined by measuring 30 randomly chosen eggs. Egg diameter was measured with a Vernier slide caliper (±0.5 mm) and weight was measured with an electronic balance (Kang–Jen Model KH, ±0.01 g).

To determine the change in oxygen content during embryogenesis, both oxygen and temperature were measured. Temperature was recorded by a temperature logger (Model U22 Water Temp Pro V2, Onset Computer Corporation) that can record from −40 to 70 °C and was set to measure the temperature every 30 min. Loggers were calibrated in °C against a traceable mercury-in-glass thermometer in the laboratory. Temperature was recorded throughout the incubation period by loggers in the nests, in the control sites and in artificial nests with simulated eggs (see below). In order to determine the changes in temperature caused by embryonic development, the differential in nest temperature during incubation was calculated by subtracting the background sand temperatures from the daily nest temperatures.

Oxygen was measured with an Absolute Oxygen analyzer (Qubit System Inc., Model S108), with oxygen partial pressure ranges 0–25% and minimum detection of 0.01%. The actual units are oxygen content in kPa, very close to the percentage of a standard atmosphere, hence also %. A gas cylinder containing standard air of 19.9% oxygen content (±1%). Due to logistic limitations, the instrument was only calibrated twice a month. All data were corrected to the standard air pressure and temperature. During the measurement, gas was extracted by air pump for 30 s (150 ml/min). Because the sampling tube contains 40 ml gas, measurements from about 35 ml were used as data. The extracted air flows through a magnesium perchlorate column to remove water vapor and carbon dioxide prior to measurement. Atmospheric air was sampled before conducting the next measurement and before machine calibration. In order to decrease the impact of oxygen extraction on the gas environment in the nests, the oxygen contents were determined in the same vertical layer in every nest and other sites, and then proceeding through the layers in sequence.

Air in nests was sampled using aeration stones (available from aquarium stores). Each stone was connected to the beach surface by