



The effects of nest incubation temperature on embryos and hatchlings of the loggerhead sea turtle: Implications of sex difference for survival rates during early life stages



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ABSTRACT

In sea turtles, higher hatch and emergence successes, larger body size of hatchlings, and superior terrestrial and swimming locomotor performances are important to maximize survival rates. Here, the hatch and emergence successes, embryonic mortality rates, hatchling morphology, and terrestrial and swimming locomotor performances of the loggerhead sea turtle (*Caretta caretta*) from male and female producing nests were investigated. Mortality rates during the early embryonic stage in the male producing nests were significantly lower than those in the female producing nests. The hatchlings from male producing nests showed higher terrestrial locomotor performances than those from female producing nests. Furthermore, while the other factors did not show differences between the two nesting groups, the high incubation temperatures significantly affected the mortality rate of embryos and the swimming performance of hatchlings. The present study indicates that male hatchlings are likely to have higher survival rates than female hatchlings during the critical terrestrial period. In addition, the present study suggests that as global warming proceeds, female-fate embryos and/or female hatchlings are likely to face lower survival rates than males, owing to high mortality rate of embryos and low swimming performance of hatchlings.

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1. Introduction

The life history of sea turtle species commences when a mature female nests on her natal beach and lays approximately 50–150 eggs. Hatchlings emerge from the nest at night after approximately six to thirteen weeks of incubation (Miller, 1997). The hatchlings then crawl along the beach sand to the shoreline, and vigorously swim (known as “frenzy swimming”) through the neritic zone for several days until they reach the sea current flowing towards their foraging ground (Davenport, 1997; Lohmann et al., 1997; Wyneken, 1997). After development in the foraging ground, the turtles reenter the natal area to participate in reproduction (Davenport, 1997; Ishihara et al., 2011). Thus, in the life history of sea turtles, first, better hatch and emergence successes are important for sustaining the species. Second, having a bigger body size is advantageous since hatchlings with bigger body sizes can avoid predation from gape-limited predators during the period when they are defenseless from emergence until arrival at the foraging grounds

(Gyuris, 2000; Spotila, 2004). Third, hatchlings with high terrestrial and swimming locomotor performance can move through this critical period quicker. Therefore, individuals possessing these characteristics have greater advantage in their fitness.

Recent studies have showed that incubation temperature affects hatch and emergence successes, hatchling morphology, and terrestrial and swimming locomotor performances. For example, Howard et al. (2014) reviewed the thermal tolerance of sea turtle embryos, and discovered that incubation temperature of 35 °C or higher substantially reduced the hatch success. Other studies have shown that when the mean three days maximum incubation temperature (T3dm) exceeded 34 °C, the emergence success and terrestrial locomotor performance declined significantly (Maulany et al., 2012a, 2012b; Sim et al., 2015). Furthermore, the incubation temperature have been discovered to be negatively correlated with the incubation period (e.g., Kaska et al., 1998) and shorter incubation periods convert less yolk material to the tissue of the hatchling (Booth et al., 2004); therefore, warmer incubation temperatures produce relatively small hatchlings (e.g., Wood et al., 2014). Warmer incubation temperatures result in the reduction of hatchling swimming performance (Booth and Evans, 2011; Booth et al., 2013) as

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well as terrestrial locomotor performances such as crawling speed and self-righting ability (Booth et al., 2013; Ischer et al., 2009; Maulany et al., 2012a; Wood et al., 2014), although the opposite results have also been reported (Booth et al., 2004; Burgess et al., 2006; Fisher et al., 2014). This indicates that, incubation temperature possibly affects the survival rate of sea turtles during their early life stages (embryo to post-hatchling).

Sea turtle species possess a temperature dependent sex determination system, with warmer incubation temperatures producing females and cooler incubation temperatures producing males (Matsuzawa, 2012). Hence, it has been hypothesized that there is a sex difference in survival rate during early life stage. Tomillo et al. (2014) revealed that the sex ratio of the total number of emerged hatchlings of the leatherback sea turtle (*Dermochelys coriacea*) in the eastern Pacific were less female-biased than the primary sex ratio due to the death of the female hatchlings in the nest by high incubation temperatures. This is also supported by the fact that sex ratios at latter life stages were not fully translated from the primary sex ratios (Braun-McNeill et al., 2007; Delgado et al., 2010; Hawkes et al., 2013). To date, there is no comprehensive field research to reveal whether temperatures that produce male and female affect hatch and emergence successes, hatchling morphology, and terrestrial and swimming locomotor performances of hatchlings. Answering these questions is essential for assessing the effect of global warming on sea turtle species and for establishing conservation strategies.

In the present study, hatch and emergence successes, embryonic mortality rates, hatchling morphology, and terrestrial and swimming locomotor performances of the loggerhead sea turtle (*Caretta caretta*) from male and female producing nests were investigated. Generally, the incubation temperature was strongly correlated with the air temperature and showed seasonal changes, increasing towards the end of the nesting season. Correspondingly, the primary sex ratio should have a seasonal change, from a male-biased sex ratio to a female-biased sex ratio. To achieve the sampling of both male and female producing nests, the nests from both the early nesting and late nesting seasons were collected.

2. Material and methods

2.1. Study area

The present study was conducted at Kochi Beach (33°28' N, 133°30' E), which is located south of Kochi and Tosa cities, Kochi prefecture, southwestern Japan. Kochi Beach is approximately 10 km in length and is known to be one of the most important nesting grounds for loggerhead turtles in the North Pacific. From early May to late July during the 2015 nesting seasons, Kochi Beach was patrolled every early morning. When a nesting track was observed, the nest was excavated and the clutch size was counted. The clutch was then transported to an open-air hatchery at Kochi Beach, with relocation occurring within 1.5 h of discovery. The sand temperature at 40 cm depth (mean depth at the center of the loggerhead sea turtle clutch) in the hatchery was recorded using a temperature data logger (TidbiT v2, Onset, MA, USA), with every 30 min. The capture of loggerhead sea turtles eggs for experimental use was approved by the Department of Forestry and Environment, Kochi prefecture government.

2.2. Experimental nests and sampling hatchlings

Three clutches from the early nesting season (end of May to beginning of June: E1–E3) and the late nesting season (July: L1–L3) were successfully collected. From the total number of eggs in each clutch, one half was used in the present study, with three to twenty eggs from each clutch weighed before being relocated.

The relocated nest was 50 cm depth and 16–18 cm diameter. Nest temperatures were measured every 30 min using a temperature data

logger (TidbiT v2, Onset, MA, USA), positioned in the center of the clutch. The emergence period was defined as the period from the nest relocation to emergence of the first hatchling. Since average period from hatch to emergence has been previously reported for the loggerhead sea turtle as being 4.1 days (Godfrey and Mrosovsky, 1997), the incubation period was calculated by subtracting four days from the emergence period.

Several days before the predicted emergence, a plastic mesh fence (40 cm diameter × 20 cm high) was positioned above the nest at night to retain emerging hatchlings. Signs of emergence such as a hole or crevice on the upper surface of the nest were monitored twice a day (morning and evening). Once signs were spotted, the nest was continuously monitored from 6:00 PM to midnight to record the time of emergence and collect the hatchlings. This observational series was continued for a few days from the first emergence to collect all emerged hatchlings for measurement of their morphologies.

Hatchlings that emerged from the nest within 5 min after the first emergence of hatchling were collected for measurements of their morphology, as well as their terrestrial and swimming locomotor performances. The collected hatchlings were immediately transferred to the laboratory of Usa Marine Biological Institute, Kochi University, approximately 7 km away from the hatchery by car. Each hatchling was weighed using an electronic balance (± 0.1 g) and their straight carapace length (SCL, mm) and width (SCW, mm) were measured using a set of calipers (± 0.1 mm). These measurements were then used to calculate the carapace size index ($SCL \times SCW$).

2.3. Quantifying crawling speed

Three to five hatchlings were randomly selected to test their crawling speed in the laboratory. Following the method described by Ischer et al. (2009), the crawling speed was calculated using a stopwatch and was measured as the time taken (cm/s) to transverse a 14 cm wide × 3 m long raceway made of cardboard, with a 10° slope that was covered with moist beach sand. The laboratory was maintained in darkness, with only an incandescent lamp of 60 W (quantum approximately 97) positioned to guide the hatchlings towards the end of the raceway. A hatchling was released onto the raceway at the top of the slope so that let it ran down the slope towards the lamp. During the experiment, the air temperature in the laboratory was maintained between 27 and 29 °C (mean 28 °C) using an air conditioner.

2.4. Quantifying self-righting performance

Immediately after measuring the crawling speed of the hatchlings, their self-righting ability was tested following the method described by Booth et al. (2013). A hatchling was placed upside down on its carapace on a moist and smooth sand surface in a tray, and then the time taken to right itself was measured using a stopwatch. Regardless of whether the hatchling succeeded to self-right itself or not within 10 s, it was placed right side up for 30 s to rest, and the test continued until the hatchling righted itself 3 times, for up to 6 trials. Self-righting ability (or “self-righting propensity”) was quantified using a score from 0 to 6, where 0 = no self-rightings in six attempts, 1 = one self-rightings in six attempts, 2 = two self-rightings in six attempts, 3 = three self-rightings in six attempts, 4 = three self-rightings in five attempts, 5 = three self-rightings in four attempts, and 6 = three self-rightings in three attempts.

2.5. Quantifying swimming performance

After the self-righting test, three to four hatchlings were selected to analyze their swimming performance following the methods described by Burgess et al. (2006) and Booth et al. (2013). The hatchlings swam individually in a plastic tank (70 cm length × 40 cm width × 30 cm height) filled to 20 cm depth with sea water. Both seawater and air

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