

Pipping dynamics in marine turtle *Lepidochelys olivacea* nests

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ABSTRACT

In marine turtles, incubation begins when females lay eggs on sandy beaches. Emergence of newborns from the sand marks the conclusion of a much longer process. At the end of incubation, the amnion ruptures inside the egg, and the chorioallantois moves posterior to the embryo to reveal the head and forelimbs, thus freeing the embryo to pip the shell. The juveniles then slowly move to the surface of the sand. The interval between egg pipping and emergence from the nest is not firmly established. We therefore used a motion detector and temperature dataloggers in five Olive Ridley sea turtle nests to evaluate the time between pipping and emergence. The peak of movement is detected between 2 and 3.7 days before emergence, but the first signs of movements are detected as early as 6.5 days prior to this. No thermal signature of pipping was detected. The proximal and ultimate mechanisms relating to emergence synchrony are discussed in the light of these results.

KEYWORDS: Olive Ridley, pipping time, incubation, movement, embryo, *Lepidochelys olivacea*.

INTRODUCTION

The spectacle of marine turtle hatchlings emerging from tropical or temperate beaches is

an unforgettable time for any visitor of nesting beaches. However, emergence from the sand marks the conclusion of a much longer process that ends incubation. In turtles, oxygen consumption increases to a peak several days before hatching [1]. The amnion then ruptures inside the egg, and the chorioallantois moves posterior to the embryo to reveal the head and forelimbs, thus freeing the embryo to pip the shell [2]. This is an important stage for hatchlings, as it gives them time to close and straighten the plastron. Indeed, the residual yolk is metabolized by hatchlings while still in the nest [3]. At this time, the first breaths are taken, and lung secretions are maximal [4]. The hatchlings then emerge from the shell and move toward the surface. The interval between egg pipping and emergence from the nest is not firmly established [5]. The variability of this time between species, beaches, nests, or individuals is also unknown.

Understanding the interval between hatching and emergence has conservation and management implications. For instance, in some types of sand, sea turtles may have more difficulty in digging to the surface after pipping. In the case of beach nourishment, a common technique used to stem beach erosion, the introduced sand sometimes differs from the naturally occurring sand, as it may be more compacted [6]. This would lead to an increased emergence time, not only because the hatchlings must work harder to reach the surface, but also because the greater exertion produces

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higher amounts of lactate in the body. Consequently, the high lactate levels would require a longer resting period for hatchlings just beneath the surface to allow the degradation of lactate [7].

Previous studies estimating the hatching-emergence interval often manipulated the nest: for example, digging into the nests prior to emergence to see if the eggs had hatched, or placing a glass pane on one side of the nests [5]. An alternative indirect method compared the sex ratio measured at constant temperature and the sex ratio from natural nests [5]. Here, the estimated hatching-emergence interval is estimated by calculating the interval between egg hatching in the laboratory and hatchling emergence in the field. The development rate was standardized using similar sex ratios, since the sex ratio and development rate are correlated to incubation temperature [8, 9]. However, this method necessitates killing the embryos, as sex can only be identified after dissection, and thus it cannot be recommended as a general practice. It was previously proposed that pipping commenced when nest temperatures did not react predictably to changes in ambient soil temperatures at similar depths [10]. However, this method has not been validated experimentally.

In this paper, we attempt to evidence the time interval between egg pipping and crawling up to the surface from the nest. A total of five Olive Ridley sea turtle nests were monitored using temperature and accelerometer dataloggers. When plotting the temperature and movement series, it was found that temperatures do not abruptly increase with the pipping event, as assumed by some authors in the past. Furthermore, the movement dynamics detected in the nest showed a period of increased movement lasting approximately 2-3 days before a peak of movement.

MATERIALS AND METHODS

Field data

Night patrols searching for nesting *Lepidochelys olivacea* were made over 8 km of the “Área de Usos Múltiples Hawaíi” beach located in the eastern Pacific coast of Guatemala. After finding a nest, the eggs were collected, and the original shape of the nests was measured. Given the very

high levels of legal human removals of this species, natural incubation is impossible to monitor. The eggs were relocated to the hatchery within 2 hours of egg deposition to minimize the potential disturbance of the developing embryos. The hatchery nests were built to respect the same depth and width of the original nests. A total of five nests were monitored between August 11 and October 2 during the 2019 nesting season. Two dataloggers were placed in the center of the clutch. First, HOBO Pendant G dataloggers (Onset Computer) were set to record every half hour to measure movement inside the nest. The HOBO Pendant G Acceleration datalogger is a three-channel logger with 8-bit resolution that uses an internal three-axis accelerometer with a range of ± 3 g. The software displayed acceleration in units of *g*-force, with the returned value being dependent on the spatial position of the datalogger. Second, temperatures were measured hourly using HOBO Temp Pro dataloggers (Onset Computer), which had a resolution of 0.01 °C. The temperature dataloggers were calibrated prior to use and were guaranteed to fall within an accuracy range of ± 0.18 °C.

Nests were monitored nightly after the 40th day of incubation to check for hatchling emergence. Between 4 and 6 days after the last emergence, each nest was exhumed to characterize the remaining eggs and calculate the hatching success and number of emerging hatchlings.

Data analysis

For each nest, both temperature and movement data series were truncated so as to retain only records between the beginning of incubation and the emergence from the sand that occurred 45 to 46 days later (Figure 1). Acceleration data were converted into their first-order derivative using an R script to measure the displacement of the datalogger within the nest. The total movement is the sum of movement on the x, y, and z axes. During the first 30 days, the embryos are too small to generate movements that can be recorded. The standard deviations of the recorded movement during these first 30 days were calculated. When recorded movement deviated from mean $\pm k$ SD, it was counted as a period of movement in the nest.

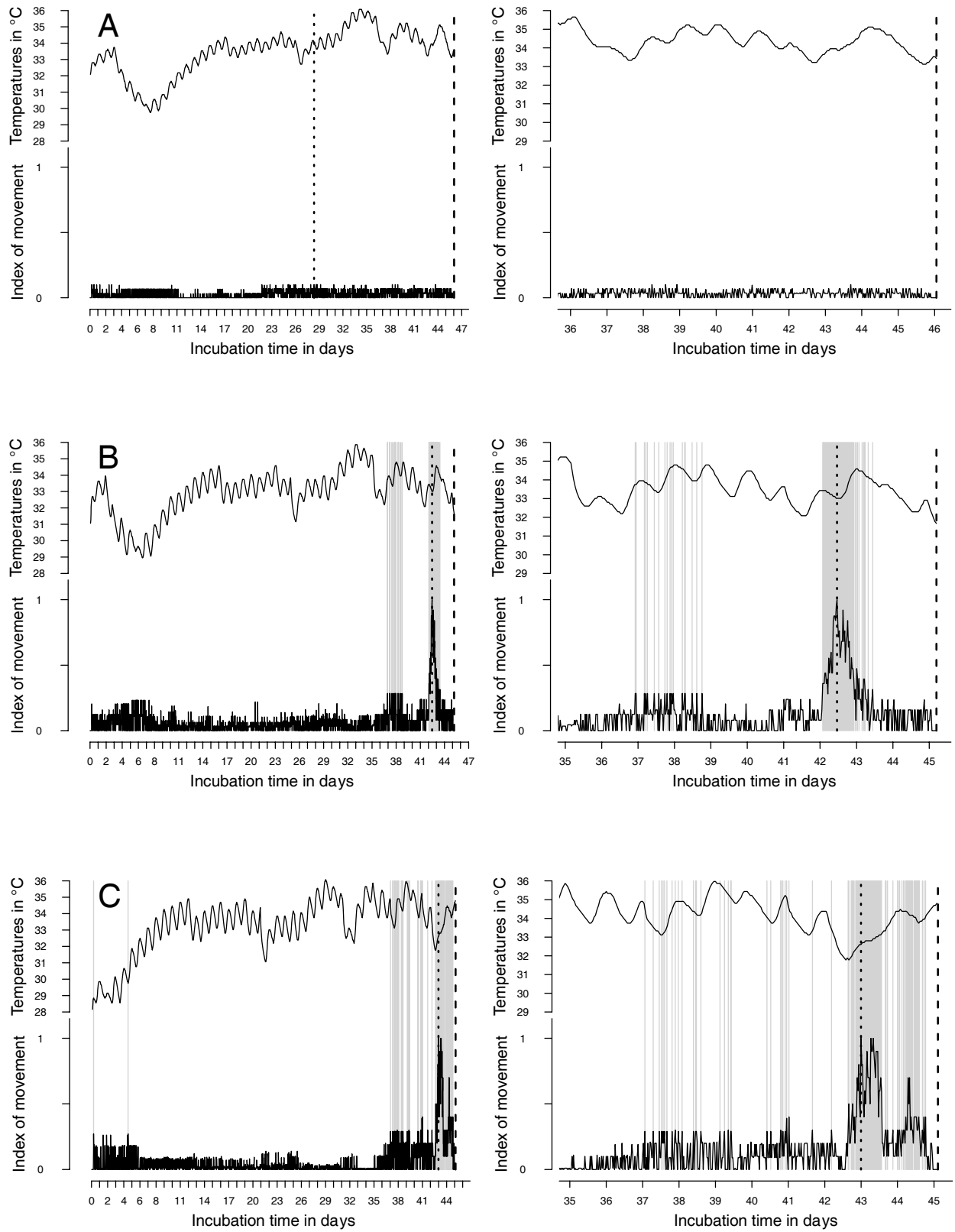


Figure 1

Figure 1 continued..

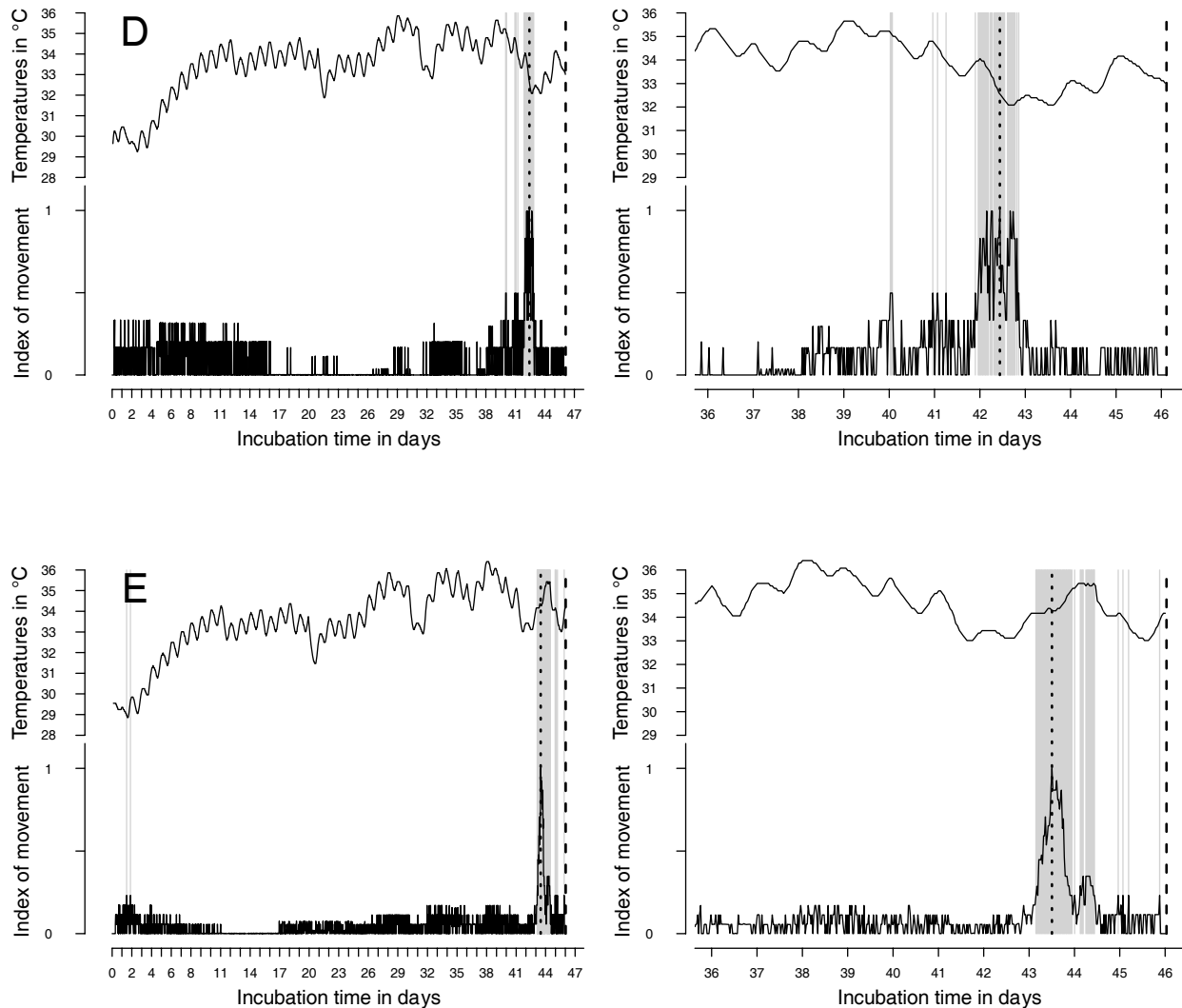


Figure 1. Temperatures (top graph) and index of movements (bottom graph) for the five nests (one per row, A-E) monitored from egg laying (day 0) to emergence from the sand (dashed line at the right of the graphs). The left panels include the entire incubation period, while the right panels focus on the last 10 days. The periods with significant movements are shown as gray rectangles. The time with the highest detected movement is marked with a dotted line. The acceleration datalogger deployed in nest A supposedly failed.

Then we searched for the k factors (one per nest) that ensured that no false positive movement was detected during the early incubation. An R function, `movement()`, is provided to analyze the recorded data using the HOBO Pendant G Acceleration datalogger in the nest. It is available in the R package `embryogrowth` version 7.7 and higher (<https://CRAN.R-project.org/package=embryogrowth>).

Temperatures in the five nests were also plotted using their calendar date as a reference to check whether pipping events could be detected based on temperature records.

Hatching success was tested using the depth and number of eggs as cofactors based on a generalized linear model with binomial distribution and logit link.

RESULTS

The laying date and time for each nest is shown in Table 1, along with the total depth of the nest, the number of eggs laid in each nest, the date and time of emergence, the number of emerged hatchlings, and the hatching success. Hatching success is linked to both the depth and number of eggs in the nest ($\Delta AIC = 39$, Akaike weight = 0), but this result should be used cautiously, because only five nests were analyzed. For the five nests, all juveniles emerged at the same time.

All dataloggers emitted the expected signals except the HOBO Pendant G datalogger deployed in nest A (Figure 1A). This nest was not further analyzed for this characteristic. Movement was detectable at the end of incubation for the remaining four nests (Figure 1B-E). The peak of

movement was observed 2.73, 2.11, 3.67, and 2.52 days before emergence from the sand. When a standard deviation $k = 4$ was chosen, only four false positive movements were detected (two for nest B and two for nest D) (Figure 1) for a total of around 8,000 measurements (excluding nest A). The rate of false positive is therefore around 5.10^{-4} . The first signs of movement detected in the nests were between 6.5 and 0.5 days before the peak of movement (Table 2). No relationship can be detected between the start of movements and the interval between the peak of movement and emergence.

Temperatures and movements during the incubation period until hatchling emergence are plotted in Figure 1 for each nest. To facilitate the comparison, temperatures are plotted alongside the calendar dates in Figure 2. No distinct thermal pattern

Table 1. Data for the five nests collected in natural conditions and relocated to the hatchery. Dates are shown as DD-MM-YYYY format and times as HH:MM format.

Nest	Date/time of female nesting	Total nest depth (cm)	Number of eggs	Date/time of emergence	Number of emerged hatchlings	Nest hatching success
A	11-08-2019 / 20:40	26	83	26-09-2019 / 22:00	73	89.2%
B	12-08-2019 / 21:20	29	78	27-09-2019 / 23:00	74	93.6%
C	16-08-2019 / 20:36	35	112	30-09-2019 / 23:00	72	64.3%
D	16-08-2019 / 21:55	34	95	02-10-2019 / 00:00	60	66.7%
E	17-08-2019 / 21:53	33	112	02-10-2019 / 23:00	67	59.8%

Table 2. Incubation timing events. The acceleration datalogger deployed in nest A supposedly failed.

Nest	Total incubation period (until emergence)	Time from the first movement to the peak of movement	Time from the peak of movement to emergence
A	46 days	na	na
B	46 days	5 days	2.73 days
C	45 days	6 days	2.11 days
D	46 days	2.5 days	3.67 days
E	46 days	0.5 days	2.52 days

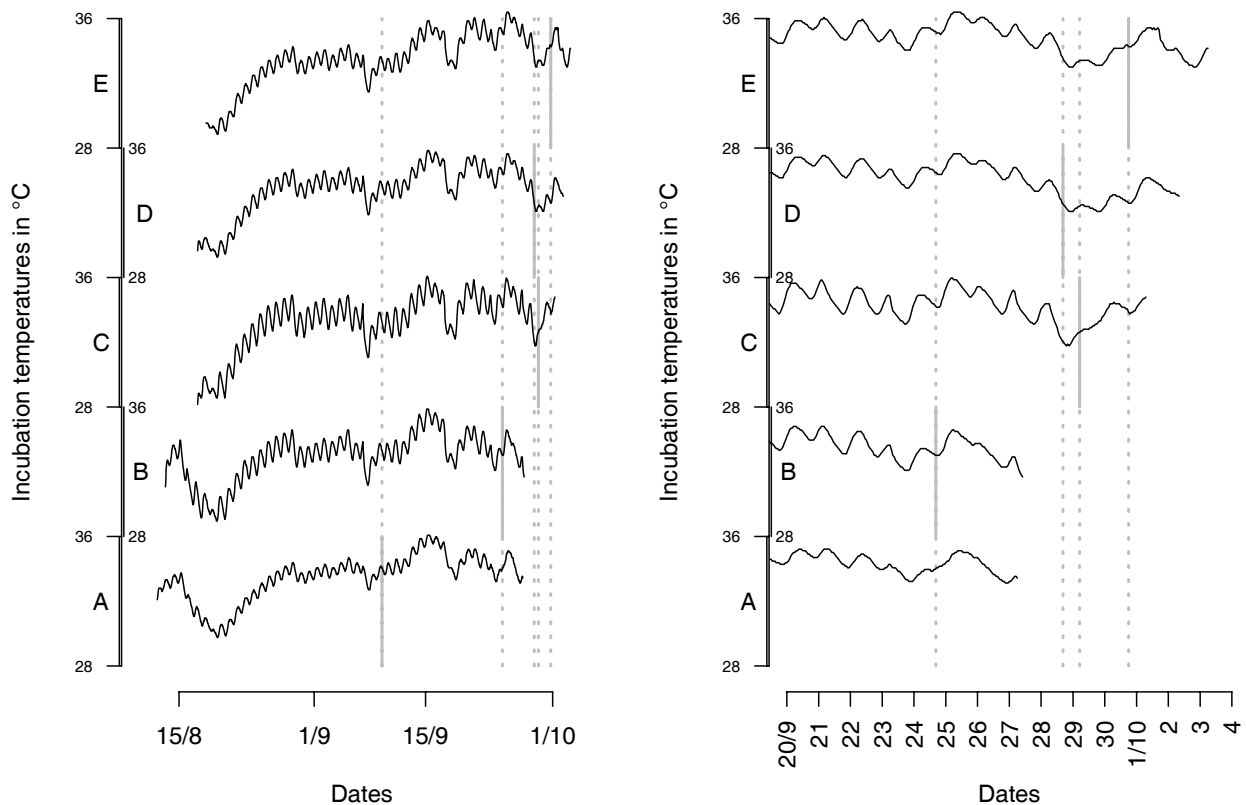


Figure 2. Temperatures recorded in the five nests (A-E) during the entire incubation period (left) and at the end of incubation (right). The dotted vertical lines indicate the periods with the highest movement for at least one of the nests. The solid gray vertical line shows the periods with the highest movement for each nest. The acceleration datalogger deployed in nest A supposedly failed.

emerged for the incubation period, pipping, hatching, or emergence.

DISCUSSION

Incubation duration is not clearly defined in the scientific literature. Indeed, opinions differ regarding the end of the incubation period: pipping, when the hatchling is half or completely out of the shell, or when it emerges from the sand. A clear definition and understanding of incubation duration are important for biological and ecological purposes in order to model embryonic growth. It is also important in terms of the management of nests in natural or hatchery conditions. Anticipating the incubation duration is crucial for hatcheries where eggs are incubated for conservation purposes [11], because the knowledge of emergence timing can help these programs be prepared to collect the hatchlings and avoid predation. The pattern of

high intra-nest synchronicity in emergence was shown to be beneficial as an anti-predator strategy for sea turtles [12]. Synchronous hatching may therefore be an adaptive “bet-hedging” strategy, with different mechanisms used across turtle species [12-15].

Synchrony is a common phenomenon in marine turtle embryos. First, embryos are maintained in a pre-ovipositional embryonic arrest in the oviduct (developmental arrest), so that they all synchronize and continue their growth simultaneously after being laid by nesting females [16]. The rate of embryonic growth depends on temperature [11]. Although the intra-nest temperature variations experienced by embryos can be as large as 6 °C, synchronous emergence generally occurs. Synchrony can occur because embryos delay hatching until stimulated by an environmental cue [14, 17, 18], or because less developed embryos either adjust

their developmental rate through metabolic compensation [15] or hatch at an earlier developmental stage [13, 19].

Our results show that the first movements are detected from 6 to 0.5 days before the peak of movement (Table 2). The precise dynamics of pipping in different eggs are not well understood, but some wait up to 6 days in the egg chamber before moving toward the surface and emerging. The time between the peak of movement and emergence is also variable, ranging from 4 to 2 days. One factor to take into account is the respiration process of hatchlings. On their way to the surface, their respiration process is essentially composed of a single breath followed by a long respiratory pause with a slow frequency and high metabolic rate, which is similar to diving adults [20]. Nevertheless, it was found that the timing of emergence may be influenced by non-physiological factors, including thermoregulatory constraints [12].

CONCLUSION

Embryo growth and temperature modeling is a valuable tool in studies aiming to develop embryo behavior and related models. For instance, the embryo growth model was based on the period of incubation from the beginning of incubation until emergence depending on the size of the hatchling. Knowing when embryos pipped may lead to more exact calculations in embryo growth, considering that hatchlings may still grow between pipping and emergence. Indeed, during this period, they not only improve behavioral synchrony but also close and straighten their plastron and absorb the remnants of the yolk sac [5]. In terms of energetic cost, the ascension to the surface represents between 11 and 68% of the energy contained in the residual yolk at hatching [21], which means that the timing may depend on the type of sand, the energetic component of each individual, or even the respiration process. We demonstrate here that movement can be detected within the nest using 3D accelerometer datalogger located within the nest and pipping time can be estimated.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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