Received: 28 November 2021 | Revised: 29 January 2022 | Accepted: 8 February 2022

DOI: 10.1002/jez.2585

RESEARCH ARTICLE

JEZ-A ECOLOGICAL AND INTEGRATIVE PHYSIOLOGY WILEY

# How well do embryo development rate models derived from laboratory data predict embryo development in sea turtle nests?

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**Funding information**

Koala Furniture Company through the World Wildlife Fund, Australia

## Abstract

Development rate of ectothermic animals varies with temperature. Here we use data derived from laboratory constant temperature incubation experiments to formulate development rate models that can be used to model embryonic development rate in sea turtle nests. We then use a novel method for detecting the time of hatching to measure the in situ incubation period of sea turtle clutches to test the accuracy of our models in predicting the incubation period from nest temperature traces. We found that all our models overestimated the incubation period. We hypothesize three possible explanations which are not mutually exclusive for the mismatch between our modeling and empirically measured in situ incubation period: (1) a difference in the way the incubation period is calculated in laboratory data and in our field nests, (2) inaccuracies in the assumptions made by our models at high incubation temperatures where there is no empirical laboratory data, and (3) a tendency for development rate in laboratory experiments to be progressively slower as temperature decreases compared with in situ incubation.

**KEYWORDS**

development, embryo, incubation, marine turtles, temperature

## 1 | INTRODUCTION

Modeling the effect of temperature on the growth and development of ectotherms has received a great deal of attention, particularly with insects because of their economic impact on agricultural crops (e.g., Damos, 2012; Davidowitz & Nijhout, 2004; Girondot & Kaska, 2014; Ikemoto et al., 2013; Jarosik et al., 2004; Schoolfield et al., 1981; Sharpe & DeMichele, 1977). In general, the development rate increases with increased temperature in a nonlinear fashion because of the Arrhenius effect on chemical reactions, chemical reactions speed up exponentially with an increase in temperature (Atkinson, 1994). Hence, within living systems, biochemical reactions, and consequently the rate of physiological processes such as cellular differentiation and growth increase with an increase in temperature. These processes are dependent on the intricate structure of large molecules, principally proteins. The intricate structure of proteins is directly affected by temperature, such that at too lower and too higher temperatures the structure is disrupted, which in turn deactivates its function. As a consequence, living systems can only operate within a limited range of temperatures. These concepts are well developed from the chemical reaction rate theory. For example, Sharpe and DeMichele (1977) introduced a six-parameter model based on

Arrhenius's empirical relationship and Eyring's theoretical equation assuming that there can be multiple, temperature-dependent, states of control enzymes what are key to cellular development. The model was later adjusted by Schoolfield et al. (1981) to make the solving of the six unknown parameters mathematically easier. This model assumes that the development rate is controlled by a single control enzyme (a simplification that satisfactorily explains most empirical development rate–temperature data from biological systems) (Sharpe & DeMichele, 1977). This control enzyme has two states, active and inactive, and the probability of being in one state or the other depends on temperature (Sharpe & DeMichele, 1977). The thermal reaction-norm of this development rate–temperature model is approximately linear in the middle of the organism's viable temperature range, where all, or almost all, of the control enzyme is in its active state. But the reaction-norm becomes nonlinear at the lower and higher end of the viable temperature range indicating that a significant proportion of the key control enzyme has entered an inactive state (Sharpe & DeMichele, 1977).

In ectotherms, the terms growth and development are frequently used interchangeably, which is a mistake, because they are different entities. Development is the process of ontogenetic progression, where cells progressively differentiate from stem cells into specialist cells which then form different tissue types that go on to form the organs and organ systems of the body. Whereas growth is the process of increase in an organism's size as measured by an increase in body mass or physical dimensions. Obviously, both of these processes are closely linked, and both are affected by temperature, but the temperature reaction-norm usually has a different shape for each of these processes. For example, it is well known that ectotherms reared at lower temperatures are physically larger and have greater mass at the same developmental stage compared with siblings reared at a higher temperature, a phenomenon termed the "temperature-size rule" (Atkinson, 1994; Davidowitz & Nijhout, 2004; van der Have & de Jong, 1996). The proximate explanation for this phenomenon is that at lower temperatures development rate slows at a relatively faster rate than the growth rate, so that growth occurs at a faster rate relative to development (Atkinson, 1994). In reptile embryonic development incubation at lower temperature results in hatchlings that have a larger body size in terms of physical dimensions, but they have a smaller residual yolk (Booth, 2018).

Modeling the effect of temperature on embryonic development can take two forms: (1) formulating a model using first principles based on thermodynamic theory, such as the Sharpe and DeMichele (1977) model which has six parameters, or (2) by fitting mathematical functions that approximate the pattern of the data. Both approaches statistically fit the algorithm of interest to empirically derived temperature–development rate data, and both approaches have been used to model development rate in turtle embryos. The second approach has been more commonly used because there are fewer parameters that need to be resolved to fit the models (Bentley et al., 2020; Booth & Freeman, 2006; Chu et al., 2008; Georges et al., 2005; Reboul et al., 2021).

Sea turtle embryos are good experimental organisms for modeling the influence of temperature on developmental rate because temperature affects development rate (Miller et al., 2017) and because there are good quality data on the relationship between incubation temperature and incubation period derived from constant temperature incubation experiments between the temperatures of 26°C and 33°C. In these data, the inverse of the relationship between incubation temperature and incubation period reflects the relationship between incubation temperature and development rate. This approach may not be suitable for shallow nests that experience relatively large daily fluctuations, a situation where near-lethal temperatures may be experienced for a short period of a few minutes to a few hours on a daily basis. In such cases, it is better to use relationships developed using instantaneous developmental rates estimated from temperature fluctuation experiments, as has been demonstrated for the shallow nesting pig-nosed turtle (*Carettochelys Insculpta*) (Georges et al., 2005). However, in the deeper green and loggerhead turtle nests that we examine, regular daily temperature fluctuations are absent, but nest temperatures typically steadily increase as incubation proceeds because of the increase in heat production of the developing embryos (Booth & Astill, 2001b; Broderick et al., 2001). Hence, sea turtle nests can experience high temperatures, in some cases high enough to approach fatal temperatures for days even weeks. In these circumstances, using the inverse of incubation period as a proxy for developmental rate is appropriate. Because prolonged incubation at temperatures above 33°C and below 25°C is fatal to early-stage sea turtle embryos (Ackerman, 1997; Howard et al., 2014; Miller, 1985), there is little data on embryo development rate at temperatures above 33°C, despite the fact that later-stage sea turtle embryos can continue to develop at temperatures to at least 36°C (Booth, 2017). Hence, the extrapolation of model functions to temperatures above 33°C is speculative. Extrapolation of development rates above 33°C is necessary because temperatures in natural sea turtle nests frequently exceed 33°C late in incubation.

Modeling development rate of sea turtle embryos can be used to predict important milestones of development such as the time when the gonads are differentiating into either testis or ovaries or the time of hatching. Because sea turtles exhibit temperature-dependent sex determination (TSD) where the incubation temperature during gonad differentiation determines the sex of hatchlings (Limpus et al., 1985; Wibbels, 2003; Yntema & Mrosovsky, 1980), knowing the time of gonad differentiation and the temperature during this time, can be used to predict the hatchling sex-ratio in natural nests (e.g., Booth & Freeman, 2006; Chu et al., 2008; Reboul et al., 2021).

In this study, we first generate temperature–development reaction-norm algorithms by statistically fitting mathematical functions to data derived from constant temperature laboratory experiments and then apply these algorithms to temperatures recorded every hour within a nest throughout incubation (hereafter termed "temperature traces") from in situ sea turtle nests to predict the time of clutch hatching. At the same time, we describe a novel method to determine the hatching time of hatchlings in these nests and use these data to test the accuracy of the predictions from the temperature–development reaction-norm algorithms.

## 2 | MATERIALS AND METHODS

We evaluated our models using two nesting populations of sea turtles, the southwest Pacific population (swPac) of loggerhead turtles (*caretta caretta*) nesting at Mon Repos beach (24°47′S, 152°26′E), and the southern Great Barrier Reef (sGBR) population of green turtles (*Chelonia mydas*) nesting on Heron Island (23°26′S, 151°51′E). These populations were chosen because data relating incubation period to incubation temperature derived from constant temperature incubation experiments have been published for these populations, and both sites were easily accessible to obtain nest temperature trace data.

### 2.1 | Formulating temperature–development reaction-norms

Data reporting the incubation period at constant temperature were obtained from the literature for swPac loggerhead turtles (Limpus et al., 1985) and sGBR green turtles (Booth & Astill, 2001a; Booth et al., 2005; Burgess et al., 2006; Bustard & Greenham, 1968; Miller, 1985; Miller & Limpus, 1981; Porter et al., 2021). The incubation period data (units, day) was converted into development-rate data, units of % per day, where 0% represents the beginning of incubation and 100% represents the point of hatching, by taking the inverse of incubation period (days) and multiplying it by 100. Development-rate was plotted against incubation temperature and linear ($y = ax + b$), quadratic ($y = c + ax + bx^2$), and rise to maximum exponential ($y = c + a(1 - b^x)$) functions were fitted to these data by the least-squares method using the global fit wizard function in SigmaPlot Ver 14 (Systat Software Inc.). Additionally, the four-parameter DEVAR model (Dallwitz & Higgins, 1992), and the six-parameter Sharpe and DeMichele (1977) thermodynamic model as modified by Schoolfield et al. (1981) were fitted to these data. Sharpe and DeMichele (1977) recommend using an anchoring reference temperature of 25°C because in many ectothermic organisms this temperature is typically in the midrange of viable temperatures so that all of the key enzymes are likely to be in their active state. However, we used a reference temperature of 28°C because this temperature is in the midrange of viable sea turtle egg incubation temperatures, whereas 25°C is at the very bottom of the viable temperature range. Both the DEVAR and Sharpe and DeMichele (1977) models have been used previously when examining turtle embryonic development (Bentley et al., 2020; Georges et al., 2005).

### 2.2 | Using temperature–development reaction-norms to predict hatching time

The temperature traces throughout incubation from each nest were used to predict when a clutch should hatch, that is, when development = 100%. To do this, the temperature–development reaction-norm algorithm for each of the fitted models was used to calculate the amount of development that occurred per hour using the nest temperature recorded during that hour. These hourly development increments were then summed sequentially from the beginning of incubation until development reached 100%, which is the theoretical point of hatching. This summation approach has been used to predict the development of insects (reviewed in Hagstrum & Milliken, 1991), and to predict embryonic development in sea turtle embryos (Booth & Freeman, 2006; Chu et al., 2008; Georges et al., 1994; Reboul et al., 2021).

### 2.3 | Field measurements of nest temperature, incubation period, and the hatch-to-emerge time

The field procedures were similar for loggerhead and green turtle clutches, but the field locations and times were different. Experiments with loggerhead turtle clutches were conducted at Mon Repos Beach between December 6, 2019 and February 15, 2020, and experiments with green turtle clutches were conducted at Heron Island between December 13, 2020 and February 9, 2021.

A nesting female was located, and immediately after oviposition had finished, her eggs were collected into a bucket and carried by hand to an area of the nesting beach which was corralled to prevent subsequent nesting turtles from disturbing monitored nests. An artificial nest hole was dug by hand to a depth of 60 cm (loggerhead turtles) or 70 cm (green turtles) and the collected eggs placed into this hole. When approximately 50 eggs had been placed in the hole, an iButton temperature data logger (iButton™ Maxim, Model DS1922L, resolution of 0.06°C, accuracy ± 0.2°C) programmed to log temperature every hour was placed in the nest and then the remainder of the eggs were placed on top of the logger in the nest. The hole was then backfilled with sand and the nest site marked with a wooden stake. The clutch collection and relocation procedures were completed within 1 h of the end of oviposition. The clutch was then left to incubate naturally on the beach. Two weeks before the clutch was expected to hatch, the nest was excavated by hand until the top layer of eggs was exposed, and a "hatching detector" was installed. The hatching detector consisted of a ~2-mm-wide strip of aluminum foil 15 cm long that was placed either beneath the top layer of eggs (loggerhead turtles) or on top of the incubating eggs (green turtles), and the ends of the foil were connected via alligator clips to wires that lead to the surface. The nest was then back-filled with sand and left to continue incubation. Thereafter, between six and eight times per day the ends of the wires at the surface were connected to a 9-volt transistor battery in a series circuit with a 1000 ohm resistor. A voltmeter was then used to measure the electrical potential (emf) (V) across the resistor. When the aluminum foil strip was intact, the emf was always ~9 V, but when it was broken (by hatchlings as they emerged from their eggs) the emf fell dramatically to between 2 and 5 V. The emf did not fall to zero when the aluminum strip was broken because electricity continued to be conducted via ions dissolved in water within the sand in the nest between the broken ends of the aluminum strip. The time when the emf fell from 9 V was recorded as the hatch date and time.

After the emf fell below 9 V, a plastic mesh corral was placed on top of the nest between 17:00 and 6:00 each day and visited every 30 min (loggerhead turtles) or at least once every 2 h (green turtles) until the first hatchling appeared on the sand's surface. This was recorded as the nest emergence time. Hence, the time between when the clutch was laid and the time that the emf of the hatch detector fell below 9 V was calculated as the incubation period (days). The time between the emf falling below 9 V and the time the first hatchling was observed at the surface was calculated as the hatch-to-emerge period (days).

Two days after the first appearance of a hatchling on the surface, the nest was dug out and the data logger recovered and downloaded and the number of hatched eggshells and unhatched eggs were counted. The number of hatched eggshells was used as a measure of the number of hatchlings produced from the clutch. The sum of hatched eggshells and unhatched eggs was the clutch size.

### 2.4 | Statistical analysis

Repeat measures one-way ANOVA followed by a Tukey multiple comparison test was used to compare measured incubation period with the incubation period predicted from the various algorithms derived from constant incubation temperature–incubation period data. Student's *t* test was used to compare loggerhead and green turtle hatch-to emergence periods. All statistical analyses were performed using Statistica ver. 14.0 (Dell Corporation) and results are reported as means ± SE.

### 2.5 | Animal ethics approval

The work with loggerhead turtle clutches was conducted under the University of Queensland NEWMA animal ethic certificate number SBS/518/19/DES. The work with green turtle clutches was conducted under the University of Queensland NEWMA animal ethic certificate number SBS/237/20, and Queensland Government Department of Environmental Science scientific purposes permit number PTU19-002377.

## 3 | RESULTS

All of the models trialled had excellent fits to the data (all $R^2 > 0.96$, Table 1) and were virtually indistinguishable from each other over the data range, but diverged from each other at temperatures above the data range (32°C for loggerhead turtle eggs, 33°C for green turtle eggs) (Figure 1). The development rate–temperature reaction-norms reported in Figure 1 were then used with the temperature trace data from monitored nests (Figure 2) to predict the incubation period for each of our monitored nests (Table 1).

We recorded nest temperatures inside 14 loggerhead turtle and 15 green turtle nests. Because of the different seasons, study locations, and nest depths, the nest temperature profiles of the two species were different. Loggerhead turtle nests were on average 1°C warmer than green turtle nests. Loggerhead turtle nests started incubation at 28–30°C and increased by ~ 4°C during the last third of incubation to 32–34°C (Figure 2a), while green turtle nests started incubation at 27–28°C and increased by ~ 7°C during the last third of incubation to 34–35°C (Figure 2b).

Using the hatching detectors, we successfully measured incubation period in all 29 study nests. Because both the time of laying and the time of hatching were known, we were able to record incubation period to within 4 h. For both loggerhead and green turtle nests, the observed incubation period was shorter than that predicted by any of the development rate algorithms by between 5.5 and 7.8 days for loggerhead turtle eggs, and between 1.6 and 8.6 days for green turtle eggs (Table 1, Figure 3). Repeat measures one-way ANOVA followed by a Tukey post-hoc analysis indicated that for loggerhead turtle eggs, all models significantly ($p < 0.05$) overestimated the incubation period, and the Sharpe and DeMichele (1977) +1.5°C model had the smallest difference between actual and predicted incubation periods (Figure 3a). Repeat measures one-way ANOVA followed by a Tukey post-hoc analysis indicated that for green turtle eggs, only the DEVAR and Sharpe and DeMichele (1977) models overestimated the incubation period ($p < 0.05$), with the DEVAR model giving the largest overestimate. The Sharpe and DeMichele (1977) +1.5°C model had the smallest difference between actual and predicted incubation periods (Figure 3b). In three loggerhead turtle nests (4, 6, and 8) and two green turtle nests (10 and 15) where nest temperature stayed at or below 32°C throughout incubation, the quadratic model was the closest predictor of the incubation period (Table 1). When the difference between the actual and modeled incubation periods was analyzed with respect to mean nest temperature, there was a clear relationship between these two variables (Figure 4). For loggerhead turtle nests, the overestimate (~10 days) was greatest at the lowest nest temperatures, and least for the highest nest temperatures (~1 day) (Figure 4a). For green turtle nests, the overestimate (~5 days) was greatest at the lowest nest temperatures, and there was a tendency to underestimate incubation temperature (~2 days) at the highest nest temperatures (Figure 4b).

The hatch-to-emergence period was considerably longer ($t = 3.266$, $p = 0.003$, $n = 27$) for loggerhead turtle hatchlings ranging between 3.5 and 13.7 days and averaging 8.3 days compared with green turtles, which ranged between 1.1 and 8.8 days and averaged 4.3 days (Table 1). There was a significant relationship between the hatch-to-emergence period and mean nest temperature for both loggerhead turtle nests (Figure 5a) and green turtle nests (Figure 5b). In both cases, the hatch-to-emergence period increased as mean nest temperature decreased (Figure 5).

## 4 | DISCUSSION

All models were an excellent fit to constant temperature laboratory incubation data. However, all of the models grossly overestimated the in situ incubation period of loggerhead turtle eggs and

**TABLE 1** Clutch size, number of hatchlings emerged from the clutch, mean nest temperature over the entire incubation period, empirical observed incubation period, hatch-to-emergence time, and incubation periods predicted from embryo development models for green and loggerhead turtle clutches of eggs incubated in situ on the beach

| Nest ID | Clutch size | Number of hatchlings | Mean nest temperature (°C) | Observed incubation period (days) | Linear predicted incubation period (days) | Quadratic predicted incubation period (days) | Rise to maximum exponential predicted incubation period (days) | DEVAR predicted incubation period (days) | Sharpe and DeMichele (1977) predicted incubation period (days | Sharpe and DeMichele (1977) predicted incubation period (days) +1.5°C | Hatch-to-emerge time (days) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Loggerhead* | | | | | $R^2 = 0.96$ | $R^2 = 0.97$ | $R^2 = 0.97$ | $R^2 = 0.97$ | $R^2 = 0.96$ | $R^2 = 0.94$ | |
| 1 | 100 | 87 | 31.6 | 46.19 | 47.62 | 48.42 | 48.71 | 50.92 | 48.58 | 45.25 | 3.97 |
| 2 | 95 | 49 | 30.9 | 46.12 | 49.75 | 49.79 | 50.25 | 50.04 | 49.62 | 47.37 | 7.82 |
| 3 | 145 | 132 | 29.8 | 45.28 | 52.96 | 52.63 | 53.08 | 53.04 | 53.21 | 51.25 | 12.45 |
| 4 | 149 | 116 | 29.5 | 45.36 | 53.62 | 53.17 | 53.62 | 53.33 | 53.75 | 52.04 | 9.51 |
| 5 | 135 | 103 | 31.6 | 45.33 | 47.58 | 48.46 | 48.71 | 51.75 | 48.87 | 45.33 | 4.90 |
| 6 | 147 | 125 | 29.6 | 44.83 | 53.42 | 53.08 | 53.5 | 53.62 | 53.75 | 51.87 | 12.90 |
| 7 | 163 | 46 | 31.4 | 45.53 | 47.92 | 48.54 | 48.83 | 58.2 | 48.62 | 45.46 | 5.57 |
| 8 | 142 | 128 | 29.6 | 44.49 | 53.21 | 52.96 | 53.37 | 53.83 | 53.71 | 51.71 | 13.24 |
| 9 | 143 | 89 | 31.7 | 44.47 | 47.08 | 48.12 | 48.33 | 55 | 48.67 | 44.83 | 3.60 |
| 10 | 129 | 90 | 29.2 | 43.53 | 54.83 | 54.12 | 54.62 | 54.08 | 54.87 | 53.37 | 13.69 |
| 11 | 141 | 91 | 29.6 | 42.97 | 53.17 | 52.79 | 53.21 | 53.37 | 53.37 | 51.58 | 12.90 |
| 12 | 154 | 127 | 31.3 | 46.39 | 47.83 | 48.67 | 48.92 | 52.33 | 49 | 45.58 | 3.51 |
| 13 | 140 | 95 | 30.8 | 46.13 | 49.96 | 50.04 | 50.46 | 51.08 | 50.08 | 47.79 | 4.96 |
| 15 | 155 | 90 | 30.8 | 45.79 | 49.96 | 50.08 | 50.46 | 51.33 | 50.15 | 47.79 | 7.31 |
| Mean ± SE | 138 ± 5 | 98 ± 7 | 30.5 ± 0.3 | 45.17 ± 0.27 | 50.64 ± 0.74 | 50.78 ± 0.59 | 51.15 ± 0.61 | 52.99 ± 0.55 | 51.16 ± 0.65 | 48.66 ± 0.84 | 8.31 ± 1.07 |
| *Green* | | | | | $R^2 = 0.96$ | $R^2 = 0.99$ | $R^2 = 0.99$ | $R^2 = 0.97$ | $R^2 = 0.96$ | $R^2 = 0.93$ | |
| 1 | 121 | 78 | 29.9 | 56.42 | 56.21 | 56.87 | 56.96 | 75 | 61 | 55.83 | 2.31 |
| 2 | 113 | 92 | 29.3 | 56.08 | 57.96 | 57.58 | 58 | 61.75 | 59.75 | 56.46 | 1.70 |
| 3 | 135 | 89 | 29.7 | 54.71 | 56.46 | 56.17 | 56.62 | 60.04 | 58.29 | 54.79 | 3.20 |
| 6 | 128 | 89 | 29.5 | 55.67 | 57.33 | 56.79 | 57.25 | 62.25 | 59 | 55.75 | 1.11 |
| 7 | 123 | 108 | 29.3 | 56 | 57.75 | 57.62 | 58 | 78 | 60.5 | 56.58 | 3.00 |
| 8 | 135 | 79 | 29.6 | 55.79 | 57.79 | 56.96 | 57.54 | 58.58 | 58.62 | 55.96 | 5.05 |
| 9 | 135 | 94 | 29.4 | 58.25 | 58.33 | 57.96 | 58.33 | 65.12 | 60.37 | 57.00 | 2.49 |
| 10 | 108 | 83 | 28.4 | 57.12 | 61.67 | 60.12 | 60.79 | 61 | 61.58 | 59.83 | 7.58 |

**TABLE 1** (Continued)

| Nest ID | Clutch size | Number of hatchlings | Mean nest temperature (°C) | Observed incubation period (days) | Linear predicted incubation period (days) | Quadratic predicted incubation period (days) | Rise to maximum exponential predicted incubation period (days) | DEVAR predicted incubation period (days) | Sharpe and DeMichele (1977) predicted incubation period (days | Sharpe and DeMichele (1977) predicted incubation period (days) +1.5°C | Hatch-to-emerge time (days) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 11 | 120 | 117 | 29.2 | 57.71 | 58.83 | 58.79 | 59.08 | 73.88 | 61.42 | 57.75 | 4.21 |
| 12 | 102 | 35 | 28.1 | 57 | 63.42 | 61.46 | 62.17 | 62.42 | 63.12 | 61.71 | 8.82 |
| 13 | 95 | 81 | 29.1 | 56.17 | 58.54 | 57.58 | 58.12 | 59.75 | 59.42 | 56.79 | 5.73 |
| 14 | 80 | 73 | 28.7 | 59.54 | 61.12 | 60.04 | 60.58 | 61.33 | 61.67 | 59.58 | 4.21 |
| 15 | 129 | 86 | 28.5 | 55.87 | 61.25 | 59.58 | 60.21 | 60.42 | 61.08 | 59.33 | 8.00 |
| 16 | 119 | 100 | 29.7 | 56.37 | 56.92 | 57.37 | 57.46 | 79.5 | 62.71 | 56.46 | 3.58 |
| 17 | 80 | 70 | 29.8 | 54.33 | 56.12 | 55.37 | 55.87 | 57.42 | 57.21 | 54.17 | 4.53 |
| Mean ± SE | 115 ± 5 | 85 ± 5 | 29.4 ± 0.1 | 56.47 ± 0.34 | 58.65 ± 0.57 | 58.02 ± 0.43 | 58.47 ± 0.45 | 65.10 ± 1.93 | 60.38 ± 0.43 | 57.20 ± 0.53 | 4.37 ± 0.60 |

*Note*: The Sharpe and DeMichele (1977) +1.5°C had the temperature at which the enzyme is half active and half high temperature inactive (TH) increased by 1.5°C above the test fit value. See text and supplementary material for details of the different embryo development models used.

moderately overestimated the in situ incubation period of green turtle eggs. Assuming that temperature affects the development rate of embryos incubating in the laboratory and the field in an identical manner, we can think of two possible hypotheses for why the fitted models overestimate the incubation period in field nests.

The first hypothesis is that during the last half of incubation, almost all of our naturally incubating clutches experienced considerable periods of time when nest temperatures exceeded 32–33°C (Figure 2), temperatures that were above the range of viable constant incubation temperatures reported for laboratory experiments. Consequently, our predictions relied on the models accurately extrapolating the relationship between development rate and temperature in this untested temperature range. Hence, it is possible, even probable, that the temperature–development rate functions derived from lower incubation temperatures do not extrapolate well to higher temperatures. For example, the Sharpe and DeMichele (1977) model assumes that only one key temperature-sensitive enzyme controls development rate over the entire viable temperature range, an assumption that is almost certainly incorrect. If more than one key controlling enzyme is involved, and each of these enzymes becomes controlling over a different range of temperatures, then the development rate–temperature relationship will change as the temperature range changes (Sharpe & DeMichele, 1977). If this is the case, in order for our models to predict the observed incubation periods, embryonic development rate would have to increase at temperatures above 32°C at a faster rate than predicted by our fitted models. The fact that the DEVAR and Sharpe and DeMichele (1977) models predict a decrease in development rate above 32°C, and that these models were the poorest predictor of the incubation period is consistent with this hypothesis. If the high temperature at which the enzyme activity is reduced by half ($H_H$) in the Sharpe and DeMichele (1977) model is increased by 1.5°C above the best fit value (the net effect of which is to increase the development rate at high temperatures), the model goodness of fit decreases slightly ($R^2$ decreases from 0.96 to 0.93 and 0.94, Table 1). In this scenario, the predicted incubation periods for green turtle eggs are similar to the observed incubation periods (Table 1) ($t = 1.153$, $df = 28$, $n = 15$, $p = 0.105$), and although still significantly overestimating loggerhead turtle incubation periods ($t = 3.943$, $df = 26$, $n = 14$, $p = 0.005$), the overestimation is smaller (Table 1). This modeling exercise suggests that the development rate continues to increase considerably at incubation temperatures above 32–33°C. Indeed, a recent study found that the development rate does continue to increase in green turtle embryos incubated continuously at 34°C, but all hatchlings were inviable (Yao et al., 2022). Clearly, experiments that measure embryonic development rates at incubation temperatures above 32°C are needed to test the hypothesis that the rate of sea turtle embryonic development increases considerably above 32°C. This hypothesis could be tested in an experiment where eggs are incubated at 32°C for two-thirds of the incubation period, at which time embryos have matured and become tolerant to incubation at higher temperatures. At this point eggs would then be split into different groups, one group would continue to be incubated at 32°C, but others would be incubated at

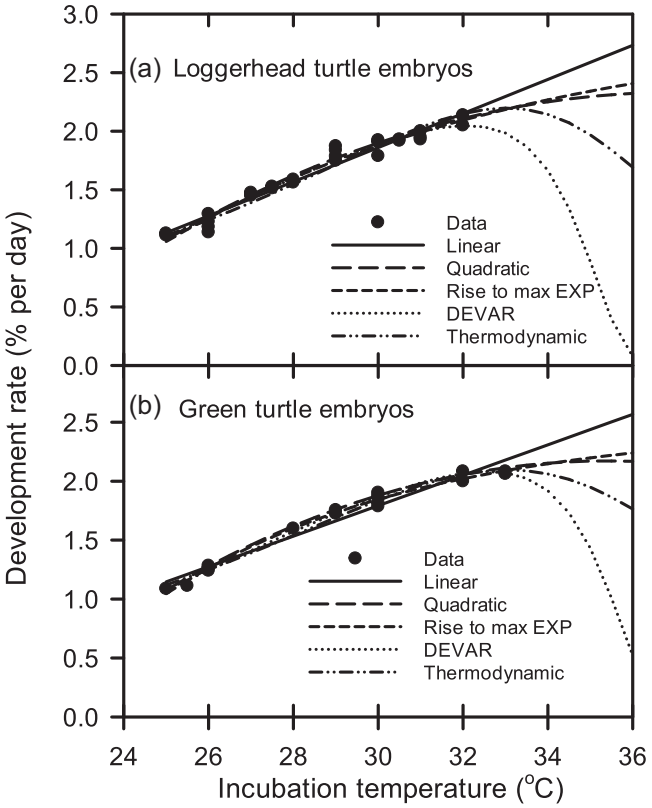


**FIGURE 1** Plots of temperature-development rate data from constant temperature laboratory experiments and fitted reaction-norms for (a) loggerhead and (b) green turtle embryos, where 0% represents the embryo at laying and 100% represents a mature embryo at hatching. See supplementary material for the parameters that describe the function fits

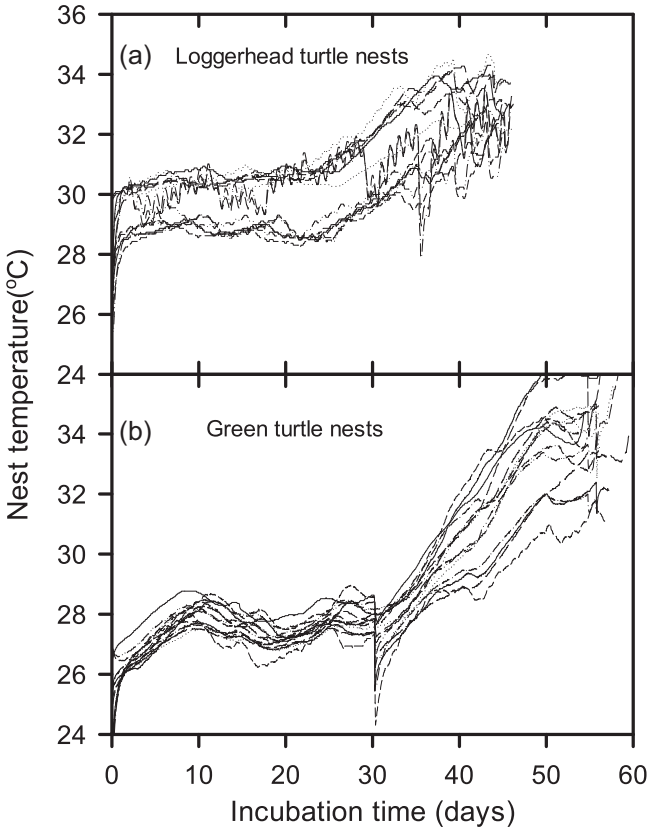


**FIGURE 2** Hourly plots of natural nest temperatures throughout incubation for (a) loggerhead turtle clutches incubated on Mon repos beach, and (b) green turtle clutches incubated on Heron Island

33°C, 34°C, 35°C, and 36°C, and the difference in the incubation periods of embryos incubated under these different regimes is used to calculate the embryo development rates at higher incubation temperatures.

The second hypothesis to explain the overestimation of in situ incubation period by the fitted models could be that embryos incubated in an entire clutch clumped together in an in situ nest simply hatch earlier than eggs incubated under laboratory conditions. Like bird eggs, turtle eggs "pip," a process in which the eggshell is first pierced by a carbuncle on the end of the nose and lung ventilation begins before they "hatch," the process whereby the hatching removes itself from the eggshell completely (Colbert et al., 2010). For sea turtle eggs incubated in laboratory experiments, the pip to hatch period takes 1–2 days. In natural turtle nests there is often a thermal gradient from the top to the bottom of a nest, with the top typically beginning warmer than the bottom (Colbert et al., 2010). This results in embryos developing faster at the top of the nest compared with embryos at the bottom of nests, and leads to asynchronous pipping and hatching, with eggs at the top of the nest pipping and hatching earlier than at the bottom of the nest. However, the embryos in the first pipped eggs appear to stimulate the other eggs in the clutch to hatch, so this asynchrony is thought to be minimal in natural nests (Colbert et al., 2010; Spencer et al., 2001). Hence, in our monitored nests, because the hatching detector was installed at the top of the nest, our detected time of hatching was for the very first eggs to hatch within the clutch. In eggs incubated artificially under controlled temperature conditions in the laboratory, temperature gradients are minimal, but there is still some natural variation in pip and hatch times, such that eggs incubated under the same conditions hatch over a period of several days (Spencer et al., 2001). Hence, in constant temperature laboratory experiments, the published incubating period is the average of all eggs incubated at that temperature, a longer period than if the first eggs to hatch were recorded as the incubation period. Consequently, laboratory incubation periods that were used to calculate our temperature–development algorithms may be consistently longer than natural nest incubation periods as determined by our hatching detector method. This hypothesis could be tested in an experiment, where several clutches of eggs are incubated at a constant temperature in the laboratory, each egg separated from each other buried in sand as is common practice. Then, a week before egg pipping is expected, half of each clutch of eggs would be combined together, touching each other in a cluster in a similar way to eggs incubating in a natural nest. The remainder of the clutch would be left to incubate as single eggs. The time to pip and hatch of these two treatments would then be compared, with the prediction that the eggs in the cluster would have shorter pip and

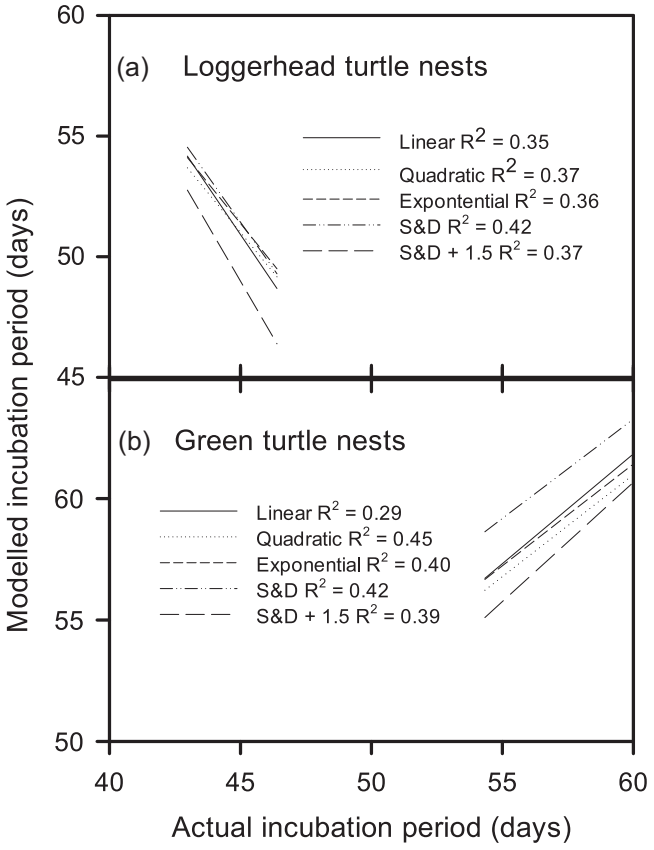


**FIGURE 3** Relationships between actual incubation period and the incubation period predicted by the various models. The DEVAR model results are not reported because the correlation was poor for both loggerhead ($R^2$ = 0.18) and green ($R^2$ = 0.03) turtle nests. (a) Loggerhead turtle nests ($n$ = 14 nests). (b) Green turtle nests ($n$ = 15 nests). Linear, linear model; Quadratic, quadratic model; exponential, rise to maximum exponential model; S&D, Sharpe and DeMichele (1977) thermodynamic model; S&D +1.5, Sharpe and DeMichele (1977) thermodynamic model with the high temperature enzyme half activity temperature increased by 1.5°C

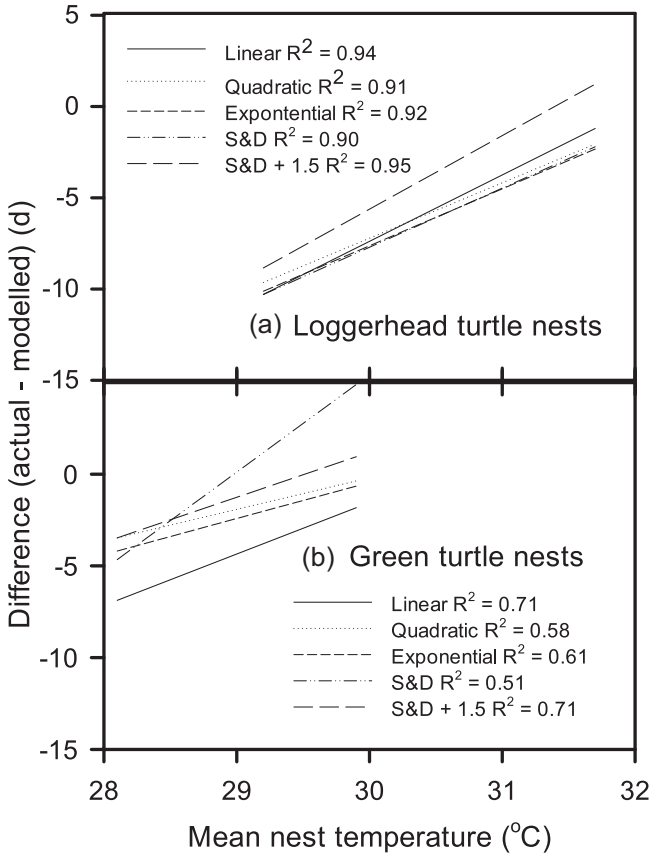


**FIGURE 4** Relationships between the difference between actual incubation period and the incubation period predicted by the various models and the mean incubation temperature for each nest. The DEVAR model results are not reported because the correlation was poor for both loggerhead ($R^2$ = 0.10) and green ($R^2$ = 0.14) turtle nests. The correlations were stronger for loggerhead turtle nests ($R^2$ range, 0.90–0.95) than for green turtle nests ($R^2$ range, 0.51–0.71). (a) Loggerhead turtle nests ($n$ = 14 nests). (b) Green turtle nests ($n$ = 15 nests). Linear, linear model; Quadratic, quadratic model; Exponential, rise to maximum exponential model; S&D, Sharpe and DeMichele (1977) thermodynamic model; S&D +1.5, Sharpe and DeMichele (1977) thermodynamic model with the high temperature enzyme half activity temperature increased by 1.5°C

incubation times compared with the eggs incubated as isolated individuals.

There is a third possible explanation for the difference between modeled and measured incubation periods: that for reasons unknown, the development rate in the laboratory is inherently slower than in natural nests. Our observation that the difference between modeled incubation periods and measured incubations period increases at lower incubation temperatures in both loggerhead and green turtles strongly suggests that, particularly at lower temperatures embryos developing in situ do so at a faster rate than in the laboratory. The explanation as to how this might occur remains obscure, but there may be some form of inter-embryo communication in clutches that develop in situ that speeds embryonic development up. This hypothesis could be tested by incubating eggs from the same clutch at constant temperature in the laboratory. A clutch of 100 eggs could have 20 eggs incubated as isolated individuals surrounded by sand, and the remaining 80 eggs incubated as a group in contact with each other as occurs in nature. This group would need to have a temperature sensor placed in the middle of it and the temperature of the incubator adjusted during the last half of incubation to counter the metabolic heat production of the clutch and thus ensure that the clutch temperature remained constant throughout incubation. In this way, the incubation temperature of the isolated and group incubation eggs would be identical, but inter-embryo communication would not be possible in the isolated eggs. This hypothesis would be supported if the incubation period of the group incubated eggs was shorter than the isolated eggs.

Although our temperature–development models overestimated the incubation period in natural nests, when nest temperature exceeded 32°C for substantial periods of time, they were considerably better at predicting incubation period when nest temperature

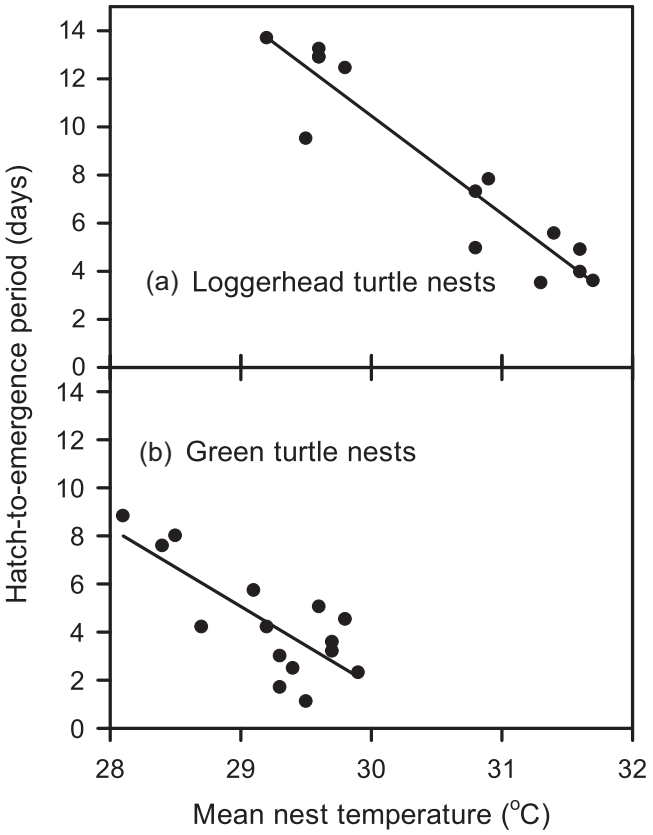


**FIGURE 5** Regression of the hatch-to-emergence period against mean nest temperature for (a) loggerhead turtle nests ($y = 132.54 - 4.0694x$, $R^2 = 0.89$, $t = -9.78$, $p < 0.001$, $n = 14$) and (b) green turtle nests ($y = 99.55 - 3.2583x$, $R^2 = 0.60$, $t = -4.43$, $p = 0.001$, $n = 15$)

remained below 32°C for the entire incubation period. The DEVAR model was the poorest fitting model and this was because it predicts a relatively large decrease in embryo development rate when incubation temperature exceeds 34°C, when it is most likely that embryo development rate continues to increase above this temperature. When nest temperature remained below 32°C, the quadratic model and the Sharpe and DeMichele (1977) models were the best predictors of the incubation period. For this reason, and because the Sharpe and DeMichele (1977) model is based on thermodynamic theory, and not just a statistical fit, we recommend using the Sharpe and DiMichele (1977) model for predicting embryo development rate from in situ nest temperature traces. This model will become more refined once data on embryo development rate at temperatures above 32°C become available.

Previous work has modeled the influence that variable incubation temperature has on sea turtle embryo growth rate (Georges et al., 1994, 2005, Girondot and Kaska, 2014). However, using embryo growth data to predict embryo development rate is not as useful as modeling embryo development rate directly, because as previously underlined, although growth rate and development are intimately related to each other, their reaction-norms with temperature are different. Modeling embryo growth also requires information about embryo growth rate, information which is not as easy to obtain for different populations of sea turtles and requires killing a time-series of embryos, compared with incubation periods which are more commonly published and does not require the killing of embryos.

The hatch-to-emergence period we observed for our loggerhead turtle clutches (8.3 days) was considerably longer than that reported previously for loggerhead turtle clutches incubating at Mon Repos beach (3.9 days, range: 0.8–6.9 days; Chu et al., 2008). This difference is probably due to the very dry year and the consequent very low water content of the beach sand during our study. Hatchlings dig as a group during the nest emergence process (Carr & Hirth, 1961) which decreases the time needed and lowers the energy required per hatchling to escape the nest (Rusli et al., 2016). Typically, hatchlings at the top of the clutch create an air chamber above them and scrap sand from the surface of the chamber which is trampled to the bottom of the emerging cohort of hatchlings (Carr & Hirth, 1961), and thus the cohort slowly rises through the sand like an elevator. This process does not happen in very dry sand, as the sand collapses preventing the formation of a digging chamber. Hence, individual hatchlings are forced to "swim" through sand to reach the surface, and become more separated from each other than is usual. Consequently, the emergence process is prolonged, and hatchlings tend to emerge through a series of several holes spread relatively widely at the sand's surface compared with the one emergence hole typically observed in the sand with greater water content.

We also observed that the hatch-to-emergence period was longer in nests with lower incubation temperatures in both loggerhead and green turtles. This observation could be explained if the general digging activity of hatchlings was inherently lower as a result of experiencing lower incubation temperatures, and/or the temperature experienced during digging out was lower. Nest that experienced lower mean temperature also experienced lower temperatures at hatching. As a general rule, locomotion performance increases with body temperature in ectotherms, as long as the temperature does not increase to sub-lethal levels. Hence, higher temperatures at hatching could lead to greater digging activity in hatchlings which would result in a decrease in the hatch-to-emergence period.

In summary, we used nest temperature traces and models derived from laboratory experiments to predict incubation periods of in situ nests, and describe a method for measuring the time of hatching in in situ nests. We found these models overestimated natural nest incubation periods, probably because our hatching detector detects the very first eggs to hatch, as opposed to the average hatching time across the entire clutch, and because the models become less accurate when nest temperatures exceed 32–33°C for prolonged periods.

## ACKNOWLEDGMENTS

We thank staff and volunteers, at Mon Repos Conservation and the staff at Heron Island research station for facilitating this project. The authors thank the Koala Furniture Company through the World Wildlife Fund, Australia and The University of Queensland for funding support. Open access publishing facilitated by The University of Queensland, as part of the Wiley - The University of Queensland agreement via the Council of Australian University Librarians.

## CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

## AUTHOR CONTRIBUTIONS

*Conceptualization*: David T. Booth. *Methodology*: David T. Booth, Alysabeth G. Turner, Colin J. Limpus. *Formal analysis*: David T. Booth, Jacques-Olivier Laloë. *Investigation*: David T. Booth, Alysabeth G. Turner. *Resources*: David T. Booth, Colin J. Limpus. *Data curation*: David T. Booth, Alysabeth G. Turner. *Writing—original draft*: David T. Booth. *Writing—review & editing*: David T. Booth, Alysabeth G. Turner, Jacques-Olivier Laloë, Colin J. Limpus. *Visualization*: David T. Booth. *Supervision*: David T. Booth, Colin J. Limpus. *Project administration*: David T. Booth. *Funding acquisition*: David T. Booth.

## DATA AVAILABILITY STATEMENT

All data used in this manuscript are available from the Dryad repository.

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**How to cite this article:** Booth, D. T., Turner, A. G., Laloë, J.-O, & Limpus, C. J. (2022). How well do embryo development rate models derived from laboratory data predict embryo development in sea turtle nests? *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, 337, 516–526. https://doi.org/10.1002/jez.2585