



Effects of moisture during incubation on green sea turtle (*Chelonia mydas*) development, morphology and performance

Bill L. Matthews[#], Christopher R. Gatto^{*,#}, Richard D. Reina

School of Biological Sciences, Monash University, Clayton, VIC 3800, Australia

ABSTRACT: While the effect of temperature on embryonic development in sea turtles has been well studied over recent years, our understanding of the effect of substrate moisture, another important environmental variable, is limited. High sand moisture decreases nest temperature through evaporative and direct cooling during rainfall, but its direct effect on hatchling development, morphology and performance is unclear. To address this knowledge gap, we incubated 40 green sea turtle *Chelonia mydas* clutches in a beach hatchery under either high (~8% v/v) or low (~5% v/v) sand moisture concentrations for the duration of embryonic development. In half of the clutches, temperature sensors were deployed to measure any effect of sand moisture on nest temperature. As hatchlings emerged, we measured body size and locomotory performance during the first 24 h, an important period of frenzied activity for sea turtles. We excavated clutches post-emergence to determine hatching success, emergence success and to determine the stage of embryonic death for unsuccessful eggs. High moisture concentrations increased incubation duration, decreased nest temperature and had marginal effects on hatchling morphology, but no effect on hatching success, stage of embryonic death, crawling speed or initial swimming performance. However, after 24 h of swimming, hatchlings from high-moisture clutches produced less mean swim thrust and spent less time powerstroking than hatchlings from low-moisture clutches, suggesting reduced swimming endurance and potentially impacting the ability of hatchlings to successfully disperse. The effect of moisture on nest temperature and hatchling endurance highlights the importance of considering rainfall patterns when predicting future impacts of climate change on sea turtle populations.

KEY WORDS: Swimming performance · Hatchlings · Incubation conditions · Dispersal · Crawling

1. INTRODUCTION

During the evolution of amniotes, a continuum of different reproductive modes evolved, ranging from oviparity (egg laying) to placental viviparity (live bearing) (Pyron & Burbrink 2014, Shine 2015). As the eggs of oviparous species develop externally, environmental conditions, mainly temperature, moisture and oxygen, act as key determinants of developmental success, hatchling phenotype, performance and post-hatching survival (Blackburn 1999, Radder et al. 2008). Oviparous species such as sea turtles exhibit a

range of adaptations to reduce the impact of environmental variation, mainly in the plasticity of the timing of nesting and active nest site selection (Mortimer 1990, Rafferty & Reina 2012, Rafferty et al. 2017). Nevertheless, maternal moderation is limited in extent and duration, and it is therefore important to understand how environmental conditions affect offspring development, morphology and performance, as indicators of overall fitness.

The effect of temperature on embryonic development has been well studied in sea turtles (Booth 2017). Sea turtle eggs develop within a thermal

*Corresponding author: christopher.r.gatto@gmail.com

[#]These authors contributed equally

range of approximately 10°C (25–35°C), but the specific temperature at which eggs incubate heavily influences embryonic development (Ackerman 1997, Hays et al. 2017). All species of sea turtle exhibit temperature-dependent sex determination, with warmer conditions producing female hatchlings and cooler conditions producing males (Standora & Spotila 1985, Spotila et al. 1987). Moreover, incubation temperatures are directly linked to egg and hatchling mortality rates, with survival decreasing significantly as sea turtle clutches reach their upper thermal limit for successful development (Hays et al. 2017, Laloë et al. 2017, Santos et al. 2017).

Along with sex ratios and mortality, incubation temperatures affect the rate of embryonic development (Bernardo 1996). Embryos that develop faster, at warmer temperatures, convert less egg yolk into hatchling mass and therefore hatch at smaller body size but with larger yolk reserves (Braña & Ji 2000). These smaller hatchlings produce less thrust when swimming, but their larger energy reserves mean they can sustain their swimming effort for longer than larger hatchlings (Booth et al. 2004, Cavallo et al. 2015). Incubation temperatures may also influence muscle development, directly affecting locomotory performance (Booth 2017). Swimming performance is critical for sea turtle hatchlings, particularly during the first 24–36 h of swimming, called the frenzy period, when hatchlings swim continuously and rapidly to disperse offshore as quickly as possible (Wyneken & Salmon 1992). High rates of predation occur in shallow inshore waters, and hatchling dispersal is significantly influenced by surface and oceanic currents that direct them offshore (Hoover et al. 2020), making swimming performance during this period important for hatchling survival (Gyuris 1994, Booth 2017). It is unclear whether a larger–stronger or smaller–endurance dispersal phenotype results in higher hatchling survival, but this will likely fluctuate with changing environmental conditions such as weather, currents and predator density (Massot et al. 2002). Therefore, it is likely that the existence of different dispersal phenotypes results in more resilient sea turtle populations.

Temperature has been clearly linked to phenotypic variation in sea turtles, but most studies have excluded potential interactions between temperature and other environmental variables. For instance, sand moisture, hereafter ‘moisture’, influences nest temperatures through evaporative and direct cooling during rainfall (Houghton et al. 2007, Sifuentes-Romero et al. 2018). By decreasing nest temperature, high-moisture concentrations can indirectly alter

hatchling sex ratios (Lolavar & Wyneken 2015, 2017). Moisture concentration during incubation has also been shown to influence hatchling locomotor performance in freshwater turtles (Miller et al. 1987, Miller 1993, Finkler 1999), possibly by altering lactate accumulation, desiccation resistance, aerobic capacity or body size (Miller et al. 1987, Finkler 1999). However, the effects of moisture during incubation on locomotor performance in sea turtle hatchlings are not consistent. Gatto & Reina (2020b) found that moisture concentration had no effect on swimming performance in 3 sea turtle species and had differing effects on crawling speed, although higher moisture concentrations generally increased crawling speed. Recent research has focused on identifying the effects of nest moisture by controlling other environmental variables (Lolavar & Wyneken 2017, Gatto & Reina 2020b). However, developing embryos in natural nests experience dynamic and interacting environmental variables. Thus, understanding how sand moisture influences hatchling traits, both directly and indirectly, in natural nests is critical for management and conservation.

The movement of water in and out of the egg depends largely on the relative water potential of the egg and the environment (Ackerman 1991). Substrate water potential is largely determined by substrate composition and how saturated the substrate is (Ackerman 1991). Water potentials below that of the egg, typically –900 kPa (Ackerman 1997), draw water from the egg while those above result in net movement of water into the egg (Ackerman 1991). Thus, moisture may have significant impacts on incubation success and hatchling morphology and performance. Because these factors ultimately affect post-hatching survival, there is an urgent need to understand their ecological consequences. This is important for natural nests as well as those in hatcheries, where clutches of eggs are relocated to protected areas of the beach to control incubation conditions, protect clutches from threats (i.e. predators, poachers) and to maximise incubation success. However, much of the existing research on sea turtle embryonic development has been conducted under laboratory conditions. While this research is important for isolating specific relationships between environmental variables and embryonic development, more field studies are required to observe if the same results occur in natural and semi-natural (i.e. hatcheries) settings. This data is of particular importance when monitoring moisture and temperature because nest depth, beach sand characteristics and clutch size affect water exchange and nest temperature (Mortimer 1990, Lolavar & Wyne-

ken 2015, Rusli et al. 2015, Rusli & Booth 2016). To make reliable predictions about the future impacts of climate change and better inform hatchery management, a greater understanding of how moisture affects the embryonic development and hatchling performance is needed.

The aims of our study were to determine the effect that moisture concentration has on (1) the relationship between incubation period and nest temperature and (2) hatchling development, morphology and performance. We interpreted these results in the context of dispersal ability, and therefore hatchling survival, and as an indication of the potential effects of watering nests—a management technique that has been suggested to combat the effects of anthropogenic climate change (Smith et al. 2021).

2. MATERIALS AND METHODS

2.1. Study site and experimental clutches

In total, 40 clutches of *Chelonia mydas* eggs were collected on Kijal beach, Dungun, Terengganu, Malaysia (4° 22' 45.64" N, 103° 27' 41.49" E) during 2 periods in 2018: 6–16 May and 1–9 June. Nesting females were unmarked, so it was unknown if any of the same individuals were sampled during the 2 different periods. However, with up to 40 females nesting per night, this is unlikely. During oviposition, eggs were collected in a bucket, covered with sand and transported to the hatchery by motorbike. Experiments were conducted at the shaded hatchery of Lang Tengah Turtle Watch, located on Kuala Abang beach (4° 48' 53.56" N, 103° 25' 25.97" E), approximately 48 km north of Kijal beach (straight line distance). The hatchery was built with a palm frond roof and a wooden lattice fence that provided shade for most of the day (Fig. 1). All clutches were reburied in the hatchery within 6 h of oviposition, before arrested embryonic development recommenced (Miller et al. 2003, Williamson et al. 2017).

2.2. Experimental design

In the hatchery, 40 artificial nests were constructed with 1 × 1 m grid spacing (Fig. 1), to the depth (70 cm) and shape of natural *C. mydas* nests. All eggs from each clutch were randomly deposited in the same artificial nest. The number of eggs in each clutch (i.e. clutch size) was counted, and every third egg was brushed clean of sand and weighed (± 0.5 g) prior to

being added to the nest. In every second artificial nest ($n = 20$), an 8 bit Thermochron ibutton (Templog Australia, DS1921G#F50) was placed into the centre of the clutch to record the temperature ($\pm 0.5^\circ\text{C}$) every 3 h for the duration of incubation (see Table 1). Once the eggs were placed in the nest, they were covered to ground level with the sand initially removed from the artificial nest.

A Pasco ECH₂O EC-5 soil moisture probe (resolution: 0.1%, Pasco Scientific) was used to record the sand moisture concentration of each nest during incubation. Prior to use in the study, we calibrated the probe using 5 sand samples of known sand volumes and differing water volumes. The probe was then used to record the sensor voltage output in millivolts (mV) of these samples before they were weighed and placed in an oven to dry for 24 h at 105°C. The samples were then reweighed to record the change in mass (i.e. water loss), which allow us to calculate the volumetric water content (VWC) of the sample. VWC is the ratio of the volume of water to the volume of sand. The sand VWC was then converted to moisture concentration and plotted against sensor voltage output (mV) to determine the calibration curve and equation used for subsequent measurements.

Each clutch in the beach hatchery was randomly assigned to either a high- (8% volume of water/volume of sand) or low-moisture (5% v/v) treatment (Fig. 1), such that half of the clutches in each treatment contained a temperature datalogger (10 temperature-logging clutches treatment⁻¹). We selected these 2 treatments, as they represent high- and low-moisture concentrations found on natural nesting beaches, without compromising hatching success (Patino-Martinez et al. 2014). Moisture concentrations above ~8% v/v can limit oxygen supply to developing embryos, and in early-stage embryos can interfere with water exchange (Limpus et al. 2020). Additionally, clutches in our study were not monitored during incubation. Thus, we selected a high-moisture treatment that represented high-moisture concentrations in *in situ* nests and that we were confident would not reduce hatching success. Although sea turtle eggs are resilient to dry nest conditions, moisture concentrations below 1–2% v/v generally result in reduced hatching success compared to intermediate moisture concentrations of 4–8% v/v (McGehee 1990). Thus, we selected a low-moisture treatment of 4% v/v that represented low-moisture concentrations *in situ* without compromising hatching success. We allocated 10 clutches to both high- and low-moisture nests per collection round. Prior to

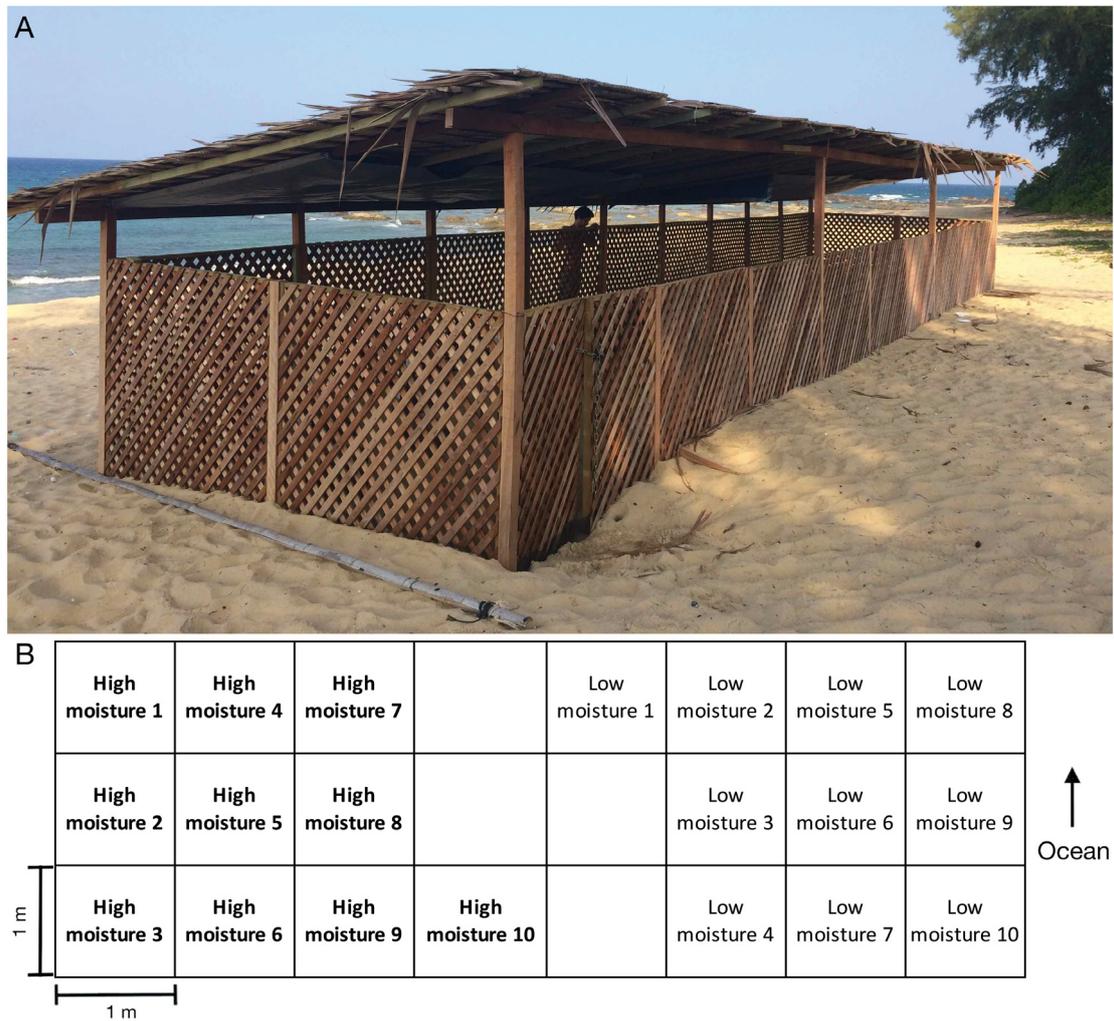


Fig. 1. The (A) shaded Lang Tengah Turtle Watch hatchery on Kuala Abung beach, Dungun, Terengganu, Malaysia, and (B) layout of green sea turtle nests in the hatchery. Each clutch was buried in a 1 m² plot, with wet and dry nests separated by at least one empty plot. Photo and diagram from Gatto et al. (2021)

collecting the first clutch of eggs, we undertook a pilot study to establish an appropriate watering regime to maintain nests at 8 and 4% v/v moisture concentration. Once eggs were placed in the hatchery, we measured the moisture concentration of each nest daily and added the necessary volume of fresh water to maintain the predetermined moisture concentration at nest depth. To measure moisture concentration, we dug down to nest depth in one corner of each plot and inserted the moisture probe into the sand at that depth. We rotated corners of each plot daily to ensure that the sand at nest depth had time to equilibrate with the surrounding sand before being tested again. The required volume of water, as determined from our pilot study, was evenly applied to nests using a watering can. In low-moisture nests, sand moisture concentration naturally stayed between

4.4 and 5.4%, so no water was added to those nests. Nests were generally watered in the morning.

At 40 d following egg deposition, mesh corrals (40 cm diameter) were placed around the top of the nest chamber to retain hatchlings in order to be able to measure their morphology and performance immediately following emergence (see Section 2.3). We determined incubation period (days) as the number of days between each clutch being buried in the hatchery and when the majority of hatchlings emerged from the nest. However, this calculation also includes the number of days that hatchlings spent digging out of the nest, usually 3–4 d (Rusli et al. 2016). Two nights after the main hatchling emergence, nest contents were excavated and inventoried. Excavations were used to determine hatching success (%; hatched eggs/clutch size) and emergence success (%; emerged

hatchlings/clutch size), using standard methods described in (Matsuzawa et al. 2002). The contents of the nest were spread out on the sand, where unhatched eggs were opened and separated into different developmental stages. We used Leslie et al. (1996) field staging methods: undeveloped = no observable development; stage 1 = formation of a blood spot; stage 2 = embryo with length of 10–20 mm and pigmented eye; stage 3 = fully pigmented embryo, >20 mm length; unhatched term = fully developed hatchling that has not broken the egg; and dead pipped = hatchling that has partially broken out of the egg. During nest excavation, iButtons were retrieved to subsequently download temperature data recorded throughout incubation. Depredated eggs were excluded from the calculations of success because they may have hatched or emerged in the absence of predators and their developmental stage could not be determined. Eggs were only depredated by crabs and were identified by the characteristic ‘snips’ on the eggshell.

2.3. Hatchling morphology and performance

Immediately following hatchling emergence, one-third of the hatchlings from each clutch were randomly selected to be weighed (± 0.5 g) and measured. We used electronic callipers to measure their straight carapace length (SCL; ± 0.01 mm), straight carapace width (SCW; ± 0.01 mm) and left and right front flipper length (± 0.01 mm). The first 10 randomly selected hatchlings from each clutch that were measured for body size were kept for further testing as described below, while all other hatchlings were released on the night of emergence.

Crawling performance was tested before swimming performance, as this is the natural sequence in which hatchlings perform these behaviours after

emerging from the nest. A total of 10 hatchlings from each clutch were individually timed crawling down a 3 m runway covered with moist sand and angled 10° below the horizontal, as per the methods described by Rusli et al. (2015). A light source was placed at the end of the runway to encourage unidirectional crawling. Hatchlings were given 2 scores: time(s) taken to crawl the length of the runway and completion of the crawl test (yes = 1, no = 0). If a hatchling did not complete the crawl test within 1 min, they failed the test and were given a score of 60 s.

Following crawl testing, each hatchling was placed in a harness and tethered to a Pasco PS-2201 load cell (range: -5 to 5 N). Load cells were calibrated using an object of known mass and were calibrated before each swimming trial. Hatchlings were then placed in tanks partially filled with seawater (approx. 26°C) to record their swimming performance (Fig. 2) as described by Gatto & Reina (2020a). A light source was placed at the opposite end of the tank to ensure unidirectional swimming away from the load cell. The amount of thrust generated by each swimming hatchling was recorded continuously for 10 min to calculate mean swim thrust (N).

During the first 24 h of continuous swimming (frenzy period), hatchlings intermittently perform powerstroking bouts, defined as sequences of 3 or more strokes where the hatchling generates more than 0.03 N thrust (Booth 2017). For each hatchling, the proportion of time spent powerstroking (%) was calculated from the cumulative length of all powerstroking bouts performed between minutes 4 and 5 of the 10 min trial. The first powerstroking bout of minute 4 was used to calculate (1) mean maximum thrust (N), calculated as the mean peak thrust generated by each stroke in the powerstroking bout; (2) powerstroking bout duration (s), calculated as the difference between the time of last peak and the time of the first peak; (3) number of strokes, defined as the

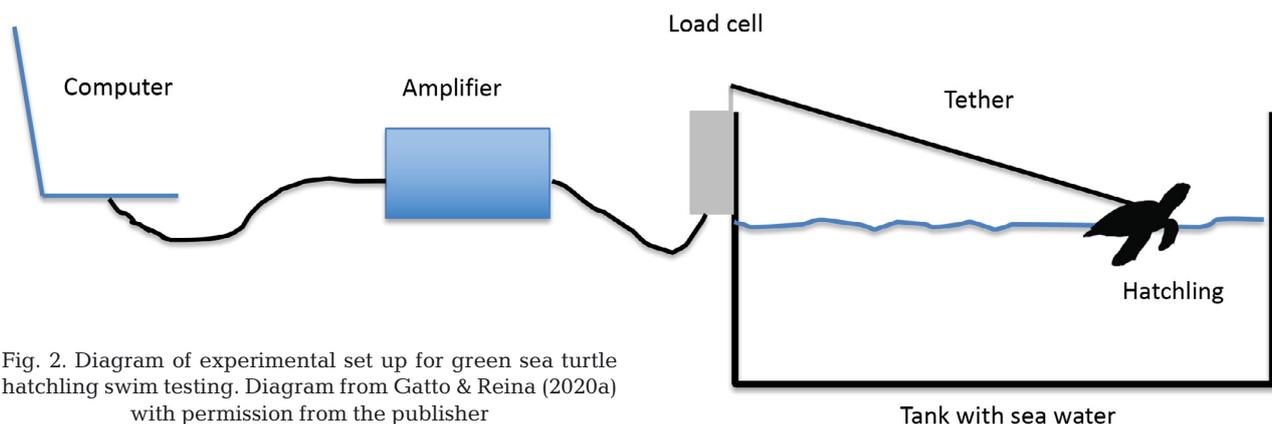


Fig. 2. Diagram of experimental set up for green sea turtle hatchling swim testing. Diagram from Gatto & Reina (2020a) with permission from the publisher

total number of strokes in powerstroking bout; and (4) powerstroke frequency (per min), calculated from:

$$\frac{\text{Number of strokes}}{\text{Length of powerstroking bout (s)}} \times 60 \quad (1)$$

Identical swim tests were repeated for all hatchlings 12 and 24 h after the initial test to record the changes in hatchling swimming performance at the start, middle and approximate end of the frenzy period. Between swimming tests, when hatchlings were not being tested, they were kept tethered, in separate tanks, with a light source placed at one end. This ensured that hatchlings continuously swam for 24 h to replicate a natural frenzy period and so that endurance could be accurately recorded. After the 24 h trial, hatchlings were released to the ocean.

2.4. Data analysis

Although clutches were randomly allocated to moisture treatment, egg mass and clutch size were compared to ensure there was no initial difference between clutches in the high- and low-moisture treatments, as these variables have been known to affect hatchling morphology and performance (Rusli & Booth 2016). For this comparison, egg mass and clutch size were analysed using linear mixed effect (LME) models, with moisture treatment as a fixed effect and clutch ID as a random effect. LME models were also used to analyse hatching success, emergence success and incubation period, with moisture treatment as the fixed effect and clutch ID as a random effect. MANOVA was used to identify any differences in stages of embryonic development (undeveloped, stage 1, stage 2, stage 3, unhatched full-term, dead pipped) between clutches in high- and low-moisture treatments.

Nest temperature was recorded and compared throughout incubation, starting from midday on the first day of incubation and finishing at midday on the day of emergence. This ensured that fluctuations resulting from the mixing of sand during relocation and from the emerging hatchlings did not influence our measure of incubation temperature. An LME was used to compare temperature between moisture treatments, interacting with elapsed time (i.e. each individual measurement taken at 3 h intervals) and clutch ID as a random effect. To estimate the hatchling sex-ratio of each clutch, we calculated constant temperature equivalents (CTE) for each clutch with temperature data. Constant temperature incubation

studies for the Malaysian population of green turtles are lacking, so we calculated CTE following Booth & Freeman (2006). First, we calculated the fractional stage of development for each 24 h period, then multiplied each fractional stage of development by that day's average nest temperature. Lastly, we summed all daily CTE contributions for days between 0.33 and 0.67 of development and divided this value by 0.34 (the fractional development that occurred during this period) (Booth & Freeman 2006). The CTE during the middle third of incubation was compared between moisture treatments using 1-way ANOVA.

A structural equation model (SEM) (Hoyle 2012) was used to compare the different morphological features (mass, SCL, SCW and average flipper length) of hatchlings. Percent moisture, incubation period and mean egg mass for each clutch were used as explanatory variables. The ibuttons were only included in half of the nests in each treatment; thus, incubation period was used as a proxy for temperature in the SEM, a reasonable assumption given that incubation period is highly correlated with temperature (Noble et al. 2018).

To investigate the effect of moisture concentrations on crawling performance of hatchlings, their crawl tests were analysed using a non-parametric Cox proportional hazards survival analysis. This analysis takes into account 2 variables: (1) whether or not the crawling trial was successfully completed (yes = 1; no = 0) and (2) the time elapsed for successful completion to occur, creating a plot of survival probability over time. Average flipper length was included as a continuous, fixed effect to determine if differences in crawling performance were the result of morphological variation. Clutch ID was included as a random effect.

We first used an SEM to analyse swimming performance during all 3 time periods (0, 12 and 24 h) together, with time, moisture concentration, incubation period and average flipper length as the explanatory variables. Average flipper length was included as a continuous variable to determine if differences in swimming performance were the result of morphological variation. Hatchlings that did not powerstroke were excluded from the SEM analysis. We then compared each indicator of swimming performance for each moisture treatment and time period using LME models. This analysis included hatchlings that did and did not powerstroke during their swimming trials. Time and moisture concentration were the fixed effects, and hatchling ID nested in clutch were the random effects. Including hatchling ID as a random effect accounted for our repeated

measures. We constructed pairwise comparisons using Tukey’s HSD in the package ‘emmeans’ to explore each fixed effect separately.

All data were analysed using Microsoft Excel v.16.17 and Rstudio v.1.1.423 (R v.3.4.3) (R Core Team 2020). LME models were created using the package ‘lme4’ (Bates et al. 2015), Tukey’s HSD tests were run using the package ‘emmeans’ (Lenth et al. 2018) and SEM were created in the package ‘sem’ (Fox 2006). All data met the assumptions of normality and homogeneity of variances (based on visual examination). We used a significance level of $\alpha = 0.05$.

3. RESULTS

In total, out of 3910 *Chelonia mydas* eggs buried in the 40 artificial nests, 3131 hatchlings emerged. Of these, 1114 hatchlings were weighed and measured, while 386 were tested for crawling and swimming performance (Table 1). A total of 162 eggs were depredated by crabs (4.1%) but no other predation occurred. Throughout incubation, the high-moisture nests (n = 20) were maintained at a mean moisture

concentration of $7.9 \pm 0.4\%$ v/v, and the low-moisture nests (n = 20) at a mean moisture concentration of $4.9 \pm 0.3\%$ v/v during incubation.

3.1. Clutch and incubation characteristics

There was no significant difference in mean egg mass ($F_{38,1276} = 0.327$, $p = 0.571$) or mean clutch size ($F_{37,37} = 0.106$, $p = 0.746$) between high- and low-moisture treatments at time of burial or in their subsequent hatching ($z = 1.210$, $p = 0.226$) and emergence success ($z = 0.984$, $p = 0.325$; Table S1 in the Supplement at www.int-res.com/articles/suppl/n046p253_supp.pdf). Two clutches (13 and 31, both high moisture) had one-tenth the hatching success of other clutches in the study. These results suggested a maternal or human handling issue rather than a difference associated with moisture concentration, and therefore these clutches were excluded from all data analysis. Moisture treatment also did not affect the embryonic developmental stages at which failed eggs died ($F_{6,32} = 1.355$, $p = 0.262$; Table S2), with most failed eggs showing no visible development.

Table 1. Sample sizes in our initial design and final comparisons of green sea turtle *Chelonia mydas* nests in high and low moisture conditions (differed as a result of technical issues and low hatching success). Straight carapace length (SCL), straight carapace width (SCW), average flipper length (FL) are morphological measures; mean swim thrust (MST), mean maximum thrust (MMT), number of strokes (STR), powerstroke frequency (PSF), powerstroking bout duration (DPB) and proportion of time spent powerstroking (TSP) are swimming performance indicators; (-) no data

Metric	Initial design				Final comparisons			
	Clutches		Hatchlings		Clutches		Hatchlings	
	High	Low	High	Low	High	Low	High	Low
Egg mass	20	20	-	-	20	20	-	-
Clutch size	20	20	-	-	20	20	-	-
Hatching success	20	20	-	-	18	20	-	-
Emergence success	20	20	-	-	18	20	-	-
Developmental stages	20	20	-	-	18	20	-	-
Temperature	10	10	-	-	6	6	-	-
Hatchling mass	20	20	656 ^a	647 ^a	18	20	545	569
SCL	20	20	656 ^a	647 ^a	18	20	545	569
SCW	20	20	656 ^a	647 ^a	18	20	545	569
FL	20	20	656 ^a	647 ^a	18	20	545	569
Crawling speed	20	20	200	200	18	20	180	200
Crawling success	20	20	200	200	18	20	180	200
MST	20	20	200	200	18	19 ^b	180–180–180	185–189–179 ^c
MMT	20	20	200	200	18	19 ^b	178–133–94	185–170–128 ^c
STR	20	20	200	200	18	19 ^b	178–133–94	185–170–128 ^c
PSF	20	20	200	200	18	19 ^b	178–133–94	185–170–128 ^c
DPB	20	20	200	200	18	19 ^b	178–133–94	185–170–128 ^c
TSP	20	20	200	200	18	19 ^b	180–180–180	185–189–179 ^c

^aApproximately one-third of the mean clutch size multiplied by the mean emergence success for clutches in this population
^bAll swimming trials were excluded from Clutch 2 (low moisture) because of technical issues during the initial swim test
^cThe 24 h swim test was excluded from Clutch 4 (low moisture) because of technical issues

Of the 20 ibuttons deployed to record temperature throughout incubation, 8 failed, meaning that temperature data were recorded in only 12 nests: 6 high- and 6 low-moisture clutches. Both high- and low-moisture clutches displayed similar thermal profiles, increasing in temperature around the midpoint of incubation before decreasing prior to hatchling emergence (Fig. 3). Over the entire incubation period, high-moisture clutches had an average temperature of $29.3 \pm 0.8^\circ\text{C}$, which was significantly cooler than low-moisture clutches with an average temperature of $30.1 \pm 0.9^\circ\text{C}$ ($F_{1,10} = 15.84$, $p = 0.003$). Nest temperature increased with elapsed time of development ($F_{1,5302} = 4378.21$, $p < 0.001$), and the interaction between elapsed time and moisture was significant ($F_{1,5302} = 49.01$, $p < 0.001$), highlighting that moisture treatment influenced how nest temperature changed over time. High-moisture clutches had an average incubation period of 58.33 ± 2.34 d, which was significantly longer than low-moisture clutches with an average incubation period of 54.17 ± 1.60 d ($F_{1,35} = 22.27$, $p < 0.001$).

Similar to the overall trend, during the sex-determination period (approximately Days 18–37), high-moisture clutches had a CTE of $30.2 \pm 0.9^\circ\text{C}$ but did not differ from low-moisture clutches which had a CTE of $31.0 \pm 1.0^\circ\text{C}$ ($F_{1,10} = 2.196$, $p = 0.169$). Due to

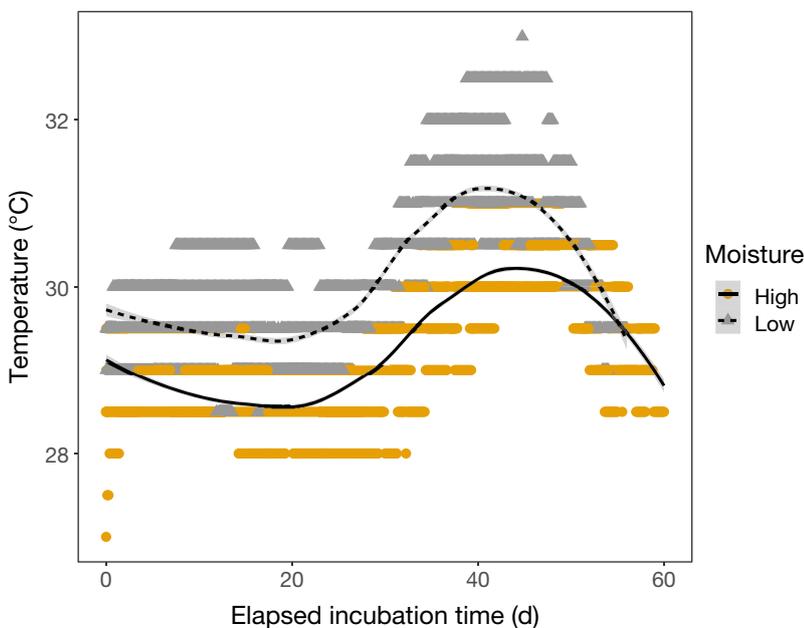


Fig. 3. Temperature of high- and low-moisture green sea turtle clutches throughout incubation. Orange: raw temperature data for the low-moisture clutches ($n = 6$); grey: raw temperature data for the high-moisture clutches ($n = 6$). A line of best fit has been overlaid over the raw data for each of the treatment groups, showing the average temperature of the clutches throughout incubation

the small sample size ($n = 12$), the interactive effect of clutch ID could not be determined and was therefore not included in the analysis. The average CTEs of high- and low-moisture clutches were above the pivotal temperature of 29.2°C previously recorded for this population of *C. mydas* (Abdullah & Ismail 2004). These temperatures would produce ~70% and ~90% female hatchlings in high- and low-moisture clutches, respectively.

3.2. Hatchling morphology and performance

Hatchlings incubated in high-moisture nests were heavier than hatchlings from low-moisture nests but had smaller SCL and SCW (Tables 2 & 3). Incubation period had no effect on hatchling mass, but longer incubation periods produced hatchlings that were wider, longer and had shorter flippers. Heavier eggs produced hatchlings that were longer and wider but had smaller flippers (Table 3). The model explained 45% of the variation in incubation period and 21.6% of the variation in SCW. Less than 5% of the variation in hatchling mass, SCL and flipper length was explained by the model (Table 3).

There was no significant difference in the time for hatchlings to complete the crawl test between the high-moisture clutches ($n = 180$) and the low-moisture clutches ($n = 200$; Table 2). Flipper length did not influence crawling performance ($z = 0.57$, $p = 0.570$). Four hatchlings, all from low-moisture clutches, did not complete the crawling trial within 60 s.

Swimming performance data from hatchlings from one clutch (Clutch 2, low-moisture) were all excluded from analysis because of technical issues with the load cells during the initial swim test. In addition, the 24 h swim test for Clutch 4 (low moisture) was excluded from the analysis because of technical issues, but other swim trials for this clutch were run successfully. Additionally, some hatchlings did not powerstroke during their trial and therefore we were unable to obtain measurements of mean maximum thrust, powerstroking bout duration, the number of strokes in the powerstroking bout or powerstroke frequency from these hatchlings. Hatchlings that did not powerstroke were

Table 2. Mean (\pm SD) Green sea turtle hatchling mass, morphology and crawling performance from high- and low-moisture concentrations. SCL: straight carapace length; SCW: straight carapace width. The p-values for mass and morphology were obtained from structural equation models; p-value for crawling performance was obtained from a non-parametric Cox proportional hazard survival analysis that incorporated both the time taken to complete the trial and whether the trial was completed successfully. *p < 0.001

Moisture treatment	Mass (g)	SCL (mm)	SCW (mm)	Flipper length (mm)	Crawling trial time (s)	Crawling trial success (%)
High-moisture (~8% v/v)	21.58 \pm 1.73	46.11 \pm 1.61	36.24 \pm 1.83	22.36 \pm 1.00	19.06 \pm 5.6	100% (n = 180)
Low-moisture (~5% v/v)	21.08 \pm 1.95	46.26 \pm 1.70	35.52 \pm 2.01	21.95 \pm 1.04	18.49 \pm 7.16	98% (n = 196/200)
z-value	4.95*	-4.52*	-4.11*	-0.60	0.28	

Table 3. Structural equation model (SEM) results describing moisture (MST), initial egg mass (EM) and incubation period (INC) effects on hatchling mass (HM), straight carapace length (SCL), straight carapace width (SCW) and average flipper length (FL) of green sea turtle hatchlings. SEM uses multiple regression to test the strength of the relationship between predictor and response variables. Values on the right: standardised coefficients between -1 and 1 for each of the parameters indicating the relationship strength; values along the diagonal (grey): random error estimated and removed from the structural equation model; p-values are reported on the left (significance level: $\alpha = 0.05$). We report the R² explaining how much variation in the dependent variable was accounted for by the entire model. Blank squares indicate comparisons not made by the model

	MST	EM	INC	HM	SCL	SCW	FL	R ²
MST	1.00	0.017	0.671	0.182	-0.178	-0.148	-0.024	
EM	0.574	1.00		0.585	0.267	-0.018	-0.176	<0.001
INC	<0.001		0.550	-0.021	0.228	0.550	-0.177	0.450
HM	<0.001	<0.001	0.524	0.972				0.028
SCL	<0.001	<0.001	<0.001		0.971			0.029
SCW	<0.001	0.372	<0.001			0.784		0.216
FL	0.547	<0.001	<0.001				0.963	0.037

excluded from the SEM analysis. In total, 205 out of 1093 total recordings of swim thrust did not contain any powerstroking. Two hatchlings (0.6%) did not powerstroke at 0 h, 66 (17.9%) did not powerstroke at 12 h and 137 (38.2%) hatchlings did not powerstroke at 24 h (Table 1). These hatchlings were only excluded from the SEM analysis for the time period(s) that they did not powerstroke. Only 1 hatchling did not powerstroke during all 3 time periods, 46 did not powerstroke during 2 time periods and 110 hatchlings did not powerstroke during 1 time period. Of the hatchlings that did not powerstroke during 2 time periods, all except 1 (initial and 24 h swim test) did not powerstroke during the 12 and 24 h swim tests. The mean number of hatchlings that did not powerstroke per clutch over all 3 swim tests was 5.54 \pm 4.19 (18.5%, range: 0–15).

When comparing the swimming performance of all hatchlings (both low- and high-moisture clutches)

tested at each time period (0, 12 and 24 h) using SEM, hatchlings produced less mean swim thrust, mean maximum thrust, powerstroked at lower frequencies and spent less time powerstroking the longer they swam. However, hatchlings had longer powerstroking bout durations as the swim trial continued (Fig. 4, Table 4). Over all 3 swim tests, hatchlings from high-moisture treatments produced less mean swim thrust, less mean maximum thrust and spent less time powerstroking than low-moisture hatchlings. Longer incubation periods produced hatchlings with greater mean swim thrust, mean maximum thrust, which spent more time powerstroking and had longer flippers than hatchlings with shorter incubation periods. Longer incubation periods also resulted in

hatchlings having shorter powerstroking bout durations and completing fewer strokes per bout. When holding incubation period and moisture constant, hatchlings with longer flippers produced greater mean swim thrust, mean maximum thrust and had higher powerstroking frequencies. Longer flippers led to hatchlings completing less strokes per powerstroking bout and having shorter powerstroking bout durations. The SEM explained a high amount of the variation in incubation period, mean swim thrust, mean maximum thrust and time spent powerstroking, but explained less than 10% of the variation in powerstroke frequency, the duration of powerstroking bouts and the number of strokes per powerstroking bout (Table 4).

We evaluated the effect of moisture treatment on the change in swimming performance over time using LME. Hatchlings incubated at high- and low-moisture concentrations both experienced similar

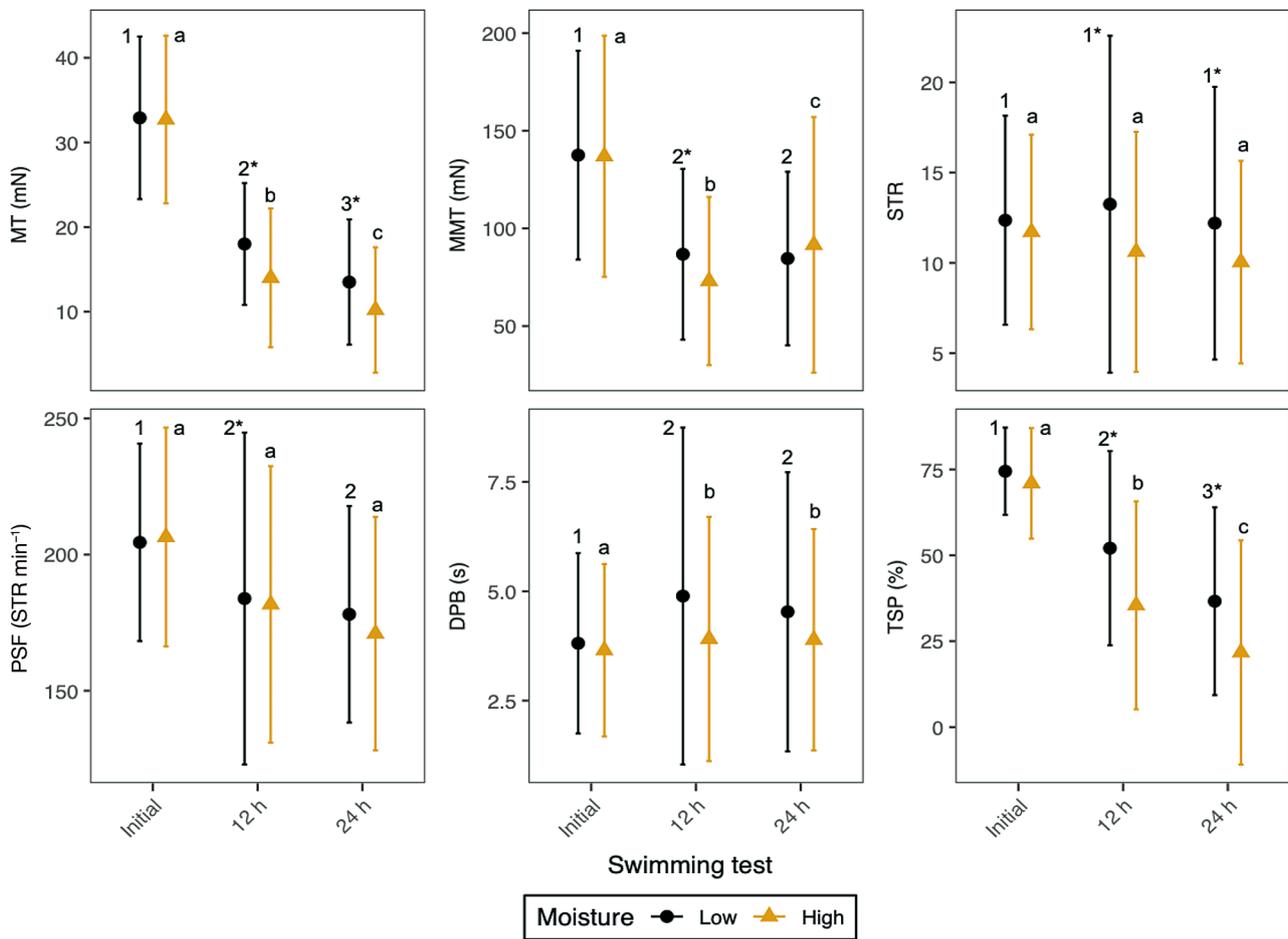


Fig. 4. Mean ± SD swimming performance attributes of green sea turtle *Chelonia mydas* hatchlings from high- (~8% v/v) and low-moisture (~5% v/v) clutches for the initial swim test, 12 and 24 h swim test. Swimming performance indicators are mean swim thrust (MT, in millinewtons), mean maximum thrust (MMT), number of strokes (STR), powerstroke frequency (PSF), powerstroking bout duration (DPB) and proportion of time spent powerstroking (TSP). Asterisk indicates statistical differences ($p < 0.05$) between moisture treatments (within time periods). Statistical differences among the initial, 12 and 24 h swim tests are marked with letters for high-moisture clutches and numbers for low-moisture clutches. Refer to Tables S3 and S4 for further details on statistical differences among moisture treatments and swim tests

Table 4. Structural equation model (SEM) results from all green sea turtle swim tests (0, 12 and 24 h) describing time, moisture (MST, % v/v), incubation period (INC, days) and average flipper length (FL, mm) effects on mean swim thrust (MT, N), mean maximum thrust (MMT, N), number of strokes (STR), powerstroke frequency (PSF, STR min⁻¹), powerstroking bout duration (DPB, s) and proportion of time spent powerstroking (TSP, %). See Table 3 for further details

	Time	MST	INC	FL	MT	MMT	STR	PSF	DPB	TSP	R ²
Time	1.00		0.004	0.010	-0.646	-0.381	-0.057	-0.276	0.076	-0.467	
MST		1.00	0.583	0.137	-0.191	-0.134	-0.046	-0.046	-0.024	-0.192	
INC	0.876	<0.001	0.66	0.127	0.177	0.126	-0.082	0.045	-0.085	0.082	0.341
FL	0.756	<0.001	0.002	0.94	0.088	0.098	-0.090	0.092	-0.090	0.017	0.055
MT	<0.001	<0.001	<0.001	<0.001	0.55						0.450
MMT	<0.001	<0.001	0.001	0.002		0.83					0.168
STR	0.094	0.274	0.049	0.010			0.97				0.030
PSF	<0.001	0.262	0.278	0.006				0.91			0.086
DPB	0.024	0.569	0.043	0.009					0.97		0.028
TSP	<0.001	<0.001	0.026	0.574						0.76	0.242

changes in their mean swim thrust, number of strokes per powerstroking bout, powerstroking bout duration and proportion of time spent powerstroking as they swam. However, the change in mean maximum thrust and powerstroke frequency as hatchlings swam differed between high- and low-moisture treatments (Fig. 4 & Table S3). During the initial swim test, high- and low-moisture hatchlings did not differ in any swimming performance indicators. However, after 12 h, mean swim thrust, mean maximum thrust, number of strokes per powerstroking bout, powerstroke frequency and proportion of time spent powerstroking were all higher in hatchlings incubated in low-moisture clutches. After 24 h of swimming, hatchlings from low-moisture clutches had higher mean swim thrust, completed more strokes per powerstroking bout and spent a greater proportion of time powerstroking (Fig. 4 & Table S4).

Mean values (\pm SD) for all swimming performance attributes are shown in Fig. 4. We report statistical differences between treatment groups and time periods based on Tukey's tests of LME. Full results from Tukey's test of LME can be found in Tables S3 & S4.

4. DISCUSSION

4.1. Moisture effects on nest temperature and development

Approximately 3% higher moisture decreased green sea turtle nest temperature by 0.8°C and increased incubation period by more than 4 d relative to the low-moisture clutches. Generally, the cooling effect of watering is greater in nests exposed to the sun than in the shade (Hill et al. 2015, Jourdan & Fuentes 2015). Thus, it is likely that the amount of cooling that we observed would have been greater if our hatchery was not shaded. Watering of nests simulates rainfall, which has been directly linked to reductions in nest temperature through evaporative and direct cooling (Houghton et al. 2007, Lolavar & Wyneken 2015, Staines et al. 2020). As expected, the decrease in nest temperature increased the incubation period because of slower embryonic development at lower temperatures (Miller 1997, Matsuzawa et al. 2002). Our selected moisture treatments represented high and low values within the range of moisture concentrations that still result in high hatching success (Patino-Martinez et al. 2014). Thus, it is not surprising that we did not detect a difference in hatching success between our 2 treatments. If we had selected 2 extreme moisture concentrations then

it is likely that we would have observed lower hatching success in one or both treatment groups (McGehee 1990, Patino-Martinez et al. 2014, Laloë et al. 2017).

Given that the pivotal temperature for this population of *Chelonia mydas* is 29.2°C (Abdullah & Ismail 2004), the drop in CTE observed in high-moisture clutches compared to low-moisture nests was not enough to create balanced primary sex ratios. However, high-moisture clutches produced 20% more males (~70% females) than low-moisture clutches (~90% females). Many natural sea turtle populations are female-biased, ranging from ~50–96% female hatchling production (Mrosovsky 1994, Broderick et al. 2000). Feminised populations provide an advantage for sea turtles because egg production is increased, allowing faster population growth through greater reproductive capacity (Miller 1997, Hays et al. 2017, Santidrián Tomillo et al. 2021). However, in time, highly feminised populations are likely to experience a decrease in reproductive output because of a lack of males (Hawkes et al. 2009, Booth et al. 2020). Our results show that moisture concentration is an important environmental variable to consider when evaluating the potential for over-feminisation of sea turtle populations as a result of rising temperature and changing rainfall patterns (Laloë et al. 2021).

4.2. Moisture effects on hatchling morphology and performance

Overall, moisture treatment, initial egg mass and incubation duration explained less than 4% of the variation in each measure of morphology except SCW, of which 21.6% was explained by incubation duration. Higher incubation temperatures have regularly been shown to reduce the duration of incubation, thereby minimising the period in which yolk can be converted into hatchling tissue, resulting in smaller hatchlings with larger residual yolk masses (Hewavisenthi et al. 2001, Pan & Ji 2001, Booth 2006, Burgess et al. 2006). However, studies that have observed thermal effects on morphology usually create temperature differences of 2–4°C between treatments—much greater than the difference between treatments in our study (Booth et al. 2004, Booth 2017), potentially explaining the lack of a clear correlation between incubation duration and morphology in our study. Initial egg mass had the strongest relationship with hatchling mass and SCL, highlighting the importance of resource provisioning by female sea turtles (Wallace et al. 2006, Warner et al. 2010).

Overall, differences in moisture, incubation duration and initial egg mass in our study only had marginal and inconsistent effects on hatchling morphology.

Moisture concentration had no effect on crawling speed or swimming performance during the initial swim test, but in the 24 h swim test, higher moisture concentrations during incubation resulted in hatchlings that had lower or less swimming endurance compared to low-moisture clutches. The cause of these results is difficult to identify due to the confounding effect of temperature. While temperature could not be directly measured for all of the clutches in our study, the strong correlation between temperature and incubation period means that incubation period should be acceptable as an appropriate proxy explanatory variable for temperature. Incubation period was correlated with multiple measures of swimming performance and thus was also likely an important influence on embryonic development in our study. High moisture concentrations have a cooling effect within nests (Staines et al. 2020), and incubation temperature has been shown to strongly influence multiple hatchling traits (Booth 2017, Noble et al. 2018). Low incubation temperatures, in high-moisture clutches, generally produce hatchlings that are larger and that emerge from the nest with smaller yolk reserves (Van Damme et al. 1992). Despite only observing marginal differences in size between moisture treatments, hatchlings from high-moisture clutches in our study may have emerged with smaller yolk reserves. As hatchlings rely solely on their yolk reserves during the frenzy period to fuel swimming behaviours, hatchlings with smaller yolk reserves are anticipated to fatigue more quickly despite being larger (Wyneken & Salmon 1992).

Generally, sea turtle hatchling swimming performance is optimised between temperatures of 27 and 32°C (Booth 2017). Both high- and low-moisture nests in our study were comfortably within this range (29.3 and 30.1°C, respectively), yet longer incubation periods still led to hatchlings spending a greater proportion of their swimming trial powerstroking. Temperature studies have also detected thermal effects on hatchling morphology (Booth et al. 2004, Weber et al. 2012, Booth 2017), crawling performance (Ischer et al. 2009, Sim et al. 2015, Rivas et al. 2019) and initial swimming performance (Burgess et al. 2006), which were not observed in this study. Some of these inconsistencies may be explained by the direct effect of moisture on embryonic development. Higher moisture concentrations in our study may have resulted in greater conversion of yolk mass into hatchling mass (Packard et al. 1988), leaving smaller yolk

reserves to power hatchling swimming behaviours post-emergence (Ischer et al. 2009, Booth 2017). Thus, the effects of moisture on yolk utilisation may have overridden the effects of temperature. Eggs in both moisture treatments are likely to have been in environments with high substrate water potentials that facilitated the easy absorption of water (Ackerman 1991, 1997) and therefore would not have experienced water loss.

An alternative explanation for the influence of moisture on swimming endurance may be that hatchlings from high-moisture clutches experience greater energetic costs of nest escape. *C. mydas* hatchlings have been found to use from 11 to 68% of their yolk reserves during nest escape as they dig upwards through the sand from nest depth to the surface (Rusli 2016). This energetic expenditure varies between clutches and is affected by the characteristics of the nest. For example, hatchlings from larger clutches use less energy during nest escape due to shared digging effort (Rusli & Booth 2016). Similarly, substrate characteristics have been found to affect the energetic costs of digging in river turtles (Rusli & Booth 2018). It is possible that high-moisture concentrations make digging more difficult because moist sand is heavier and stickier than dry sand, requiring hatchlings to expend greater energy during nest escape. Water concentrations above 5% have been shown to increase friction between particles in sand, requiring more force to displace it (Fall et al. 2014). We used hatchling emergence as an estimate of incubation period; thus, variation in the time it takes a hatchling to escape the nest would also alter our measure of incubation period. Overall, our results suggest that developing embryos are influenced by both moisture and temperature, as well as by important interactions between the 2 environmental factors.

Sea turtles rely on active swimming as well as surface currents for dispersal (Putman & Mansfield 2015, Hoover et al. 2020), so swimming performance likely affects dispersal patterns and survival (Kobayashi et al. 2018). Hatchlings that disperse slowly away from the coastline likely have decreased chances of survival. Compared with the low-moisture group, the lower endurance observed in high-moisture hatchlings is likely to increase predation rates and affect hatchling recruitment. While predation rates are generally highest within the first 1–2 h of dispersal (Gyuris 1994, Pilcher et al. 2000), during which time we did not observe differences between moisture treatments in our study, the reduced endurance of hatchlings from high-moisture clutches is

likely to result in those hatchlings taking longer to reach foraging grounds and therefore being at greater risk of death (Kraemer & Bennett 1981).

4.3. Management implications

Current climate change predictions highlight increased temperature as a major threat to sea turtles (Santidrián Tomillo et al. 2015, Laloë et al. 2017, Santos et al. 2017). It is predicted that increased temperature will result in over-feminised populations and increased embryonic mortality as temperatures exceed the upper thermal limit for successful embryonic development (Hays et al. 2010, Laloë et al. 2017, 2021, Booth et al. 2020). Rainfall is an important source of environmental cooling and should be included in predictions of climatic effects on sea turtle populations (Rafferty et al. 2017, Laloë et al. 2021). If rainfall is high and consistent but not extreme (Rivas et al. 2018), high-moisture concentrations may at least partially offset increased environmental temperature, giving sea turtles more resilience to climate change than currently predicted. Conversely, if rainfall is predicted to decrease or become more sporadic, the potential for moisture concentrations to offset temperature increases will be reduced.

Many conservation projects around the world relocate eggs into hatcheries to protect eggs from predators and poachers and control incubation conditions. Changing the incubation environment alters primary sex ratios, hatching success and other important hatchling traits including locomotor performance (Booth & Astill 2001, Matsuzawa et al. 2002, Booth & Evans 2011, Gatto et al. 2021). While the implementation of hatcheries may be a good management strategy for climate change, their design needs to be carefully planned. The benefit of a hatchery is that we can control the environmental conditions to produce a female-biased sex ratio without clutches reaching their upper thermal limit. However, we do not fully understand how different incubation environments influence long-term survival; thus, it is important to allow some climatic fluctuations to produce a variety of hatchling phenotypes, creating a more resilient sea turtle population.

Our study is the first to conduct performance tests on sea turtle hatchlings incubated in a field nest environment under altered moisture concentrations. Moisture concentrations did not have consistent effects on hatchling development, possibly because the stable hatchery environment decreased variation in moisture concentrations and temperature between

treatment groups. The hatchery shading limited solar heating, evaporation and airflow, presumably slowing the rate of water loss within nests and reducing temperature variation among nests. In future studies, sea turtle clutches could be incubated at moisture concentrations of 3 and 14 %, closer to the upper and lower limit of embryonic survival (McGehee 1990) and where differences have been observed in other studies (Lolavar & Wyneken 2020). We also suggest that future studies use varying levels of shading in combination with different moisture treatments. This should provide more variation between treatment groups and lead to a greater understanding of the relationship between moisture concentrations and temperature, enabling the identification of optimum conditions for maximising hatching success while allowing for seasonal variation.

Acknowledgements. Thanks to Monash University, Faculty of Science for supporting this project with a cross-campus initiative grant and to Dr. Mohd Uzair Rusli from Universiti Malaysia Terengganu for helping secure a research permit to study in Malaysia. C.G. was supported by an Australian Government Research Training Program (RTP) scholarship. Thank you also to Lang Tengah Turtle Watch for providing the hatchery space, nests, hardworking staff and passion to this study. Research was conducted under animal ethics approval BSCI2018/08 from Monash University and an approval to carry out research work from the Director of Terengganu State Fisheries Office, Malaysia. We thank David Booth and 2 anonymous reviewers for insightful and useful comments on a draft of the manuscript.

LITERATURE CITED

- Abdullah S, Ismail M (2004) Temperature dependent sex determination and hatching performance of green turtle (*Chelonia mydas*) at Chendor Rookery on the east coast of Peninsular Malaysia. In: Arai N (ed) Proc Int Symp SEASTAR2000 and bio-logging science (The 5th SEASTAR2000 workshop), 5–6 March 2009, Bangkok. Graduate School of Informatics, Kyoto University, Kyoto, p 11–15
- Ackerman RA (1991) Physical factors affecting the water exchange of buried reptile eggs. In: Deeming DC, Ferguson MW (eds) Egg incubation: its effects on embryonic development in birds and reptiles. Cambridge University Press, Cambridge, p 193–212
- Ackerman RA (1997) The nest environment and the embryonic development of sea turtles. In: Lutz PL, Musick J (eds) The biology of sea turtles, Vol 1. CRC Press, Boca Raton, FL, p 83–106
- ✦ Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1–48
- ✦ Bernardo J (1996) Maternal effects in animal ecology. *Am Zool* 36:83–105
- Blackburn DG (1999) Viviparity and oviparity: evolution and reproductive strategies. In: Knobil TE, Neill JD (eds) *Encyclopedia of reproduction*, Vol 4. Academic Press,

- New York, NY, p 994–1003
- Booth DT (2006) Influence of incubation temperature on hatchling phenotype in reptiles. *Physiol Biochem Zool* 79:274–281
- Booth DT (2017) The influence of incubation temperature on sea turtle hatchling quality. *Integr Zool* 12:352–360
- Booth DT, Astill K (2001) Incubation temperature, energy expenditure and hatchling size in the green turtle (*Chelonia mydas*), a species with temperature-sensitive sex determination. *Aust J Zool* 49:389–396
- Booth DT, Evans A (2011) Warm water and cool nests are best. How global warming might influence hatchling green turtle swimming performance. *PLOS ONE* 6:e23162
- Booth DT, Freeman C (2006) Sand and nest temperatures and an estimate of hatchling sex ratio from the Heron Island green turtle (*Chelonia mydas*) rookery, southern Great Barrier Reef. *Coral Reefs* 25:629–633
- Booth DT, Burgess E, McCosker J, Lanyon JM (2004) The influence of incubation temperature on post-hatching fitness characteristics of turtles. *Int Congr Ser* 1275: 226–233
- Booth DT, Dunstan A, Bell I, Reina R, Tedeschi J (2020) Low male production at the world's largest green turtle rookery. *Mar Ecol Prog Ser* 653:181–190
- Braña F, Ji X (2000) Influence of incubation temperature on morphology, locomotor performance, and early growth of hatchling wall lizards (*Podarcis muralis*). *J Exp Zool* 286:422–433
- Broderick AC, Godley BJ, Reece S, Downie JR (2000) Incubation periods and sex ratios of green turtles: highly female biased hatchling production in the eastern Mediterranean. *Mar Ecol Prog Ser* 202:273–281
- Burgess EA, Booth DT, Lanyon JM (2006) Swimming performance of hatchling green turtles is affected by incubation temperature. *Coral Reefs* 25:341–349
- Cavallo C, Dempster T, Kearney MR, Kelly E, Booth D, Hadden KM, Jessop TS (2015) Predicting climate warming effects on green turtle hatchling viability and dispersal performance. *Funct Ecol* 29:768–778
- Fall A, Weber B, Pakpour M, Lenoir N and others (2014) Sliding friction on wet and dry sand. *Phys Rev Lett* 112: 175502
- Finkler MS (1999) Influence of water availability during incubation on hatchling size, body composition, desiccation tolerance, and terrestrial locomotor performance in the snapping turtle *Chelydra serpentina*. *Physiol Biochem Zool* 72:714–722
- Fox J (2006) Teacher's corner: structural equation modeling with the sem package in R. *Struct Equ Modeling* 13: 465–486
- Gatto CR, Reina RD (2020a) The ontogeny of sea turtle hatchling swimming performance. *Biol J Linn Soc* 131:172–182
- Gatto CR, Reina RD (2020b) Sea turtle hatchling locomotor performance: incubation moisture effect, ontogeny and species-specific patterns. *J Comp Physiol B* 190:779–793
- Gatto CR, Matthews B, Reina RD (2021) Role of incubation environment in determining thermal tolerance of sea turtle hatchlings. *Endang Species Res* 44:397–408
- Gyuris E (1994) The rate of predation by fishes on hatchlings of the green turtle (*Chelonia mydas*). *Coral Reefs* 13: 137–144
- Hawkes LA, Broderick AC, Godfrey MH, Godley BJ (2009) Climate change and marine turtles. *Endang Species Res* 7:137–154
- Hays GC, Fossette S, Katselidis KA, Schofield G, Gravenor MB (2010) Breeding periodicity for male sea turtles, operational sex ratios, and implications in the face of climate change. *Conserv Biol* 24:1636–1643
- Hays GC, Mazaris AD, Schofield G, Laloë JO (2017) Population viability at extreme sex-ratio skews produced by temperature-dependent sex determination. *Proc R Soc B* 284:20162576
- Hewavisenth S, Parmenter CJ, Gatten R Jr (2001) Influence of incubation environment on the development of the flatback turtle (*Natator depressus*). *Copeia* 2001: 668–682
- Hill JE, Paladino FV, Spotila JR, Santidrián Tomillo P (2015) Shading and watering as a tool to mitigate the impacts of climate change in sea turtle nests. *PLOS ONE* 10: e0129528
- Hoover AL, Shillinger GL, Williamson SA, Reina RD, Bailey H (2020) Nearshore neonate dispersal of Atlantic leatherback turtles (*Dermochelys coriacea*) from a non-recovering subpopulation. *Sci Rep* 10:18748
- Houghton JDR, Myers AE, Lloyd C, King RS, Isaacs C, Hays GC (2007) Protracted rainfall decreases temperature within leatherback turtle (*Dermochelys coriacea*) clutches in Grenada, West Indies: ecological implications for a species displaying temperature dependent sex determination. *J Exp Mar Biol Ecol* 345:71–77
- Hoyle RH (2012) Handbook of structural equation modeling. Guilford Press, New York, NY
- Ischer T, Ireland K, Booth DT (2009) Locomotion performance of green turtle hatchlings from the Heron Island Rookery, Great Barrier Reef. *Mar Biol* 156:1399–1409
- Jourdan J, Fuentes M (2015) Effectiveness of strategies at reducing sand temperature to mitigate potential impacts from changes in environmental temperature on sea turtle reproductive output. *Mitig Adapt Strategies Glob Change* 20:121–133
- Kobayashi S, Aokura N, Fujimoto R, Mori K and others (2018) Incubation and water temperatures influence the performances of loggerhead sea turtle hatchlings during the dispersal phase. *Sci Rep* 8:11911
- Kraemer JE, Bennett SH (1981) Utilization of posthatching yolk in loggerhead sea turtles, *Caretta caretta*. *Copeia* 1981:406–411
- Laloë JO, Cozens J, Renom B, Taxonera A, Hays GC (2017) Climate change and temperature-linked hatchling mortality at a globally important sea turtle nesting site. *Glob Change Biol* 23:4922–4931
- Laloë JO, Tedeschi JN, Booth DT, Bell I, Dunstan A, Reina RD, Hays GC (2021) Extreme rainfall events and cooling of sea turtle clutches: implications in the face of climate warming. *Ecol Evol* 11:560–565
- Lenth R, Singmann H, Love J (2018) emmeans: estimated marginal means, aka least-squares means. <https://CRAN.R-project.org/package=emmeans> (accessed 7 August 2021)
- Leslie A, Penick D, Spotila JR, Paladino FV (1996) Leatherback turtle, *Dermochelys coriacea*, nesting and nest success at Tortuguero, Costa Rica, in 1990–1991. *Chelonian Conserv Biol* 2:159–168
- Limpus CJ, Miller JD, Pfaller JB (2020) Flooding-induced mortality of loggerhead sea turtle eggs. *Wildl Res* 48: 142–151
- Lolavar A, Wyneken J (2015) Effect of rainfall on loggerhead turtle nest temperatures, sand temperatures and hatchling sex. *Endang Species Res* 28:235–247
- Lolavar A, Wyneken J (2017) Experimental assessment of

- the effects of moisture on loggerhead sea turtle hatchling sex ratios. *Zoology* 123:64–70
- ✦ Lolavar A, Wyneken J (2020) The impact of sand moisture on the temperature-sex ratio responses of developing loggerhead (*Caretta caretta*) sea turtles. *Zoology* 138: 125739
- ✦ Massot M, Clobert J, Lorenzon P, Rossi JM (2002) Condition-dependent dispersal and ontogeny of the dispersal behaviour: an experimental approach. *J Anim Ecol* 71: 253–261
- ✦ Matsuzawa Y, Sato K, Sakamoto W, Bjørndal K (2002) Seasonal fluctuations in sand temperature: effects on the incubation period and mortality of loggerhead sea turtle (*Caretta caretta*) pre-emergent hatchlings in Minabe, Japan. *Mar Biol* 140:639–646
- McGehee MA (1990) Effects of moisture on eggs and hatchlings of loggerhead sea turtles (*Caretta caretta*). *Herpetologica* 46:251–258
- ✦ Miller K (1993) The improved performance of snapping turtles (*Chelydra serpentina*) hatched from eggs incubated on a wet substrate persists through the neonatal period. *J Herpetol* 27:228–233
- Miller JD (1997) Reproduction in sea turtles. In: Lutz PL, Musick JA (eds) *The biology of sea turtles*, Vol 1. CRC Press, Boca Raton, FL, p 51–79
- ✦ Miller K, Packard GC, Packard MJ (1987) Hydric conditions during incubation influence locomotor performance of hatchling snapping turtles. *J Exp Biol* 127:401–412
- Miller JD, Limpus CJ, Godfrey MH (2003) Nest site selection, oviposition, eggs, development, hatching, and emergence of loggerhead turtles. In: Bolten AB, Witherington BE (eds) *Loggerhead sea turtles*. Smithsonian Books, Washington, DC, p 125–143
- ✦ Mortimer JA (1990) The influence of beach sand characteristics on the nesting behavior and clutch survival of green turtles (*Chelonia mydas*). *Copeia* 1990:802–817
- ✦ Mrosovsky N (1994) Sex ratios of sea turtles. *J Exp Zool* 270: 16–27
- ✦ Noble DW, Stenhouse V, Schwanz LE (2018) Developmental temperatures and phenotypic plasticity in reptiles: a systematic review and meta-analysis. *Biol Rev Camb Philos Soc* 93:72–97
- ✦ Packard GC, Packard MJ, Miller K, Boardman TJ (1988) Effects of temperature and moisture during incubation on carcass composition of hatchling snapping turtles (*Chelydra serpentina*). *J Comp Physiol B* 158:117–125
- Pan Z, Ji X (2001) The influence of incubation temperature on size, morphology, and locomotor performance of hatchling grass lizards (*Takydromus wolteri*). *Acta Ecol Sin* 21:2031–2038
- ✦ Patino-Martinez J, Marco A, Quiñones L, Hawkes LA (2014) The potential future influence of sea level rise on leatherback turtle nests. *J Exp Mar Biol Ecol* 461:116–123
- Pilcher N, Enderby S, Stringell T, Bateman L (2000) Near-shore turtle hatchling distribution and predation. In: Pilcher NJ, Ismai MG (eds) *Sea turtles of the Indo-Pacific: research, management and conservation: Proc 2nd ASEAN Symposium and Workshop on sea turtle biology and conservation*, Kota Kinabalu, 15–17 July 1999. ASEAN Academic Press, London, p 151–166
- ✦ Putman NF, Mansfield KL (2015) Direct evidence of swimming demonstrates active dispersal in the sea turtle 'lost years'. *Curr Biol* 25:1221–1227
- ✦ Pyron RA, Burbrink FT (2014) Early origin of viviparity and multiple reversions to oviparity in squamate reptiles. *Ecol Lett* 17:13–21
- R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- ✦ Radder RS, Elphick MJ, Warner DA, Pike DA, Shine R (2008) Reproductive modes in lizards: measuring fitness consequences of the duration of uterine retention of eggs. *Funct Ecol* 22:332–339
- ✦ Rafferty AR, Reina RD (2012) Arrested embryonic development: a review of strategies to delay hatching in egg-laying reptiles. *Proc R Soc B* 279:2299–2308
- ✦ Rafferty AR, Johnstone CP, Garner JA, Reina RD (2017) A 20-year investigation of declining leatherback hatching success: implications of climate variation. *R Soc Open Sci* 4:170196
- ✦ Rivas M, Spínola M, Arrieta H, Faife-Cabrera M (2018) Effect of extreme climatic events resulting in prolonged precipitation on the reproductive output of sea turtles. *Anim Conserv* 21:387–395
- ✦ Rivas ML, Esteban N, Marco A (2019) Potential male leatherback hatchlings exhibit higher fitness which might balance sea turtle sex ratios in the face of climate change. *Clim Change* 156:1–14
- ✦ Rusli MUB (2016) The energetics of nest escaping by turtle hatchlings. PhD dissertation, The University of Queensland, Brisbane
- Rusli MUB, Booth DT (2016) Bigger clutch sizes save offspring energy during nest escapes. *Behav Ecol Sociobiol* 70:607–616
- ✦ Rusli MUB, Booth DT (2018) Sand type influences the energetics of nest escape in Brisbane river turtle hatchlings. *Aust J Zool* 66:27–33
- ✦ Rusli MUB, Joseph J, Liew HC, Bachok Z (2015) Effects of egg incubation methods on locomotor performances of green turtle (*Chelonia mydas*) hatchlings. *Sains Malays* 44:49–55
- ✦ Rusli MU, Booth DT, Joseph J (2016) Synchronous activity lowers the energetic cost of nest escape for sea turtle hatchlings. *J Exp Biol* 219:1505–1513
- ✦ Santidrián Tomillo P, Genovart M, Paladino FV, Spotila JR, Oro D (2015) Climate change overruns resilience conferred by temperature-dependent sex determination in sea turtles and threatens their survival. *Glob Change Biol* 21:2980–2988
- ✦ Santidrián Tomillo P, Wallace BP, Paladino FV, Spotila JR, Genovart M (2021) Short-term gain, long-term loss: how a widely-used conservation tool could further threaten sea turtles. *Biol Conserv* 261:109260
- ✦ Santos KC, Livesey M, Fish M, Lorences AC (2017) Climate change implications for the nest site selection process and subsequent hatching success of a green turtle population. *Mitig Adapt Strategies Glob Change* 22:121–135
- ✦ Shine R (2015) The evolution of oviparity in squamate reptiles: an adaptationist perspective. *J Exp Zool B Mol Dev Evol* 324:487–492
- ✦ Sifuentes-Romero I, Tezak BM, Milton SL, Wyneken J (2018) Hydric environmental effects on turtle development and sex ratio. *Zoology* 126:89–97
- ✦ Sim EL, Booth DT, Limpus CJ (2015) Incubation temperature, morphology and performance in loggerhead (*Caretta caretta*) turtle hatchlings from Mon Repos, Queensland, Australia. *Biol Open* 4:685–692
- ✦ Smith CE, Booth DT, Crosby A, Miller JD, Staines MN, Versace H, Madden-Hof CA (2021) Trialling seawater irrigation to combat the high nest temperature feminisation of

- green turtle *Chelonia mydas* hatchlings. Mar Ecol Prog Ser 667:177–190
- Spotila JR, Standora EA, Morreale SJ, Ruiz GJ (1987) Temperature dependent sex determination in the green turtle (*Chelonia mydas*): effects on the sex ratio on a natural nesting beach. Herpetologica 43:74–81
- ✦ Staines MN, Booth DT, Hof CAM, Hays GC (2020) Impact of heavy rainfall events and shading on the temperature of sea turtle nests. Mar Biol 167:190
- ✦ Standora EA, Spotila JR (1985) Temperature dependent sex determination in sea turtles. Copeia 1985:711–722
- Van Damme R, Bauwens D, Braña F, Verheyen RF (1992) Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. Herpetologica 48:220–228
- ✦ Wallace BP, Sotherland PR, Tomillo PS, Bouchard SS, Reina RD, Spotila JR, Paladino FV (2006) Egg components, egg size, and hatchling size in leatherback turtles. Comp Biochem Physiol A Mol Integr Physiol 145: 524–532
- ✦ Warner DA, Jorgensen CF, Janzen FJ (2010) Maternal and abiotic effects on egg mortality and hatchling size of turtles: temporal variation in selection over seven years. Funct Ecol 24:857–866
- ✦ Weber SB, Broderick AC, Groothuis TG, Ellick J, Godley BJ, Blount JD (2012) Fine-scale thermal adaptation in a green turtle nesting population. Proc R Soc B 279: 1077–1084
- ✦ Williamson SA, Evans RG, Reina RD (2017) When is embryonic arrest broken in turtle eggs? Physiol Biochem Zool 90:523–532
- ✦ Wyneken J, Salmon M (1992) Frenzy and postfrenzy swimming activity in loggerhead, green, and leatherback hatchling sea turtles. Copeia 1992:478–484

Editorial responsibility: Mark Hamann,
Townsville, Queensland, Australia
Reviewed by: D. T. Booth, J. B Pfaller and
1 anonymous referee

Submitted: April 1, 2021
Accepted: October 8, 2021
Proofs received from author(s): November 20, 2021