The incubation environment where sea turtles lay their eggs is predicted to rapidly warm with climate change over the next 80 years. Sea turtles have long generation times and innate breeding behaviours, so their ability to thermally adapt to rapidly changing incubation environments might be apprehended by temperature-dependent sex determination (TSD). There could be potentially zero male recruitment into the adult population if only females are produced at high temperatures associated with climate change.

We investigated two under-studied management strategies, seawater-irrigation and palm-frond-shade, to reduce the temperature of sea turtle nests and produce male-biased clutches. Sex-ratios were predicted based on nest temperature during sex-determination using logistic models and validated by the sex-ratios observed through gonad histology of embryos.

Following rain and cloudy weather, palm-frond shade and artificial shade was predicted to produce male-biased clutches (> 90 % male). Seawater treatment also produced mostly males during this rainfall event with the volume of seawater applied having an additional mild evaporative-cooling effect on nests. The seawater treatment also reduced incubation success and produced clutches with poorer hatchling quality, although this effect was not replicated in the smaller study in Papua New Guinea with green turtle eggs. Palm-frond-shade however, had the highest incubation success and produced nests with more active hatchlings, increasing their chances of surviving the first 48 hours of life and their journey to the open ocean.

In future research, we want to determine the best method for cooling nests with irrigation. With some design improvements, elements of these sand-cooling strategies could assist with increasing male hatchling production and improving hatching success at rookeries in the South Pacific and northern Great Barrier Reef, which are at the highest risk to adverse incubation effects of climate change.
THE RESEARCH DETAILS

INTRODUCTION

The Intergovernmental Panel on Climate Change (IPCC) confirmed recently in a special report that there would be an irreversible loss of ecosystems with the predicted 2 – 4 °C increase in global atmospheric temperature by the year 2100 (IPCC, 2018). Important research has focused on modelling the effects of rapid climate change on population demographics of species with thermal sensitivities. This is particularly relevant for large reptiles like crocodilians and turtles that have temperature-dependent sex determination (TSD) (Janzan and Paukstis 1991; Valenzuela and Lance 2004; Refsnider and Janzen 2015).

For TSD organisms, the sex of the embryo is not determined by chromosomes, but instead by the incubation temperature during the middle third of embryonic development when the gonads are differentiating (Yntema and Mrosovsky 1980; Janzen and Paukstis 1991). A theoretical 1:1 ratio of males to females is produced at a pivotal temperature, P (Ackerman 1997). For all sea turtle species, nest temperatures above P will produce predominately females, whereas cooler nest temperatures produce predominately males (Yntema and Mrosovsky 1980; Lang and Andrews 1994). If rapid climate change were to increase temperatures of the incubation environment, this would skew the sex-ratio of hatchlings produced and lead to low (potentially zero) male recruitment into future breeding populations of sea turtles (Jensen et al. 2018).

‘Feminisation’ has already been documented in sea turtle populations within Australia. Of the northern Great Barrier Reef (nGBR) population of green turtles (Chelonia mydas), 337 individuals were sampled from the feeding grounds surrounding the Howick Island group in Far North Queensland during July and August of 2014 and 2015. From this study, 99% of juveniles and sub-adults, and 87% of adults captured where female, indicating a strong female-biased population (Jensen et al. 2018).

The temperature of a sea turtle nest is determined by two main factors; (1) exterior solar heating which is absorbed by the sand and (2) interior metabolic heating from the developing embryos which is insulated by the sand (Booth and Freeman 2006). To mitigate against excessive solar heating due to climate change, management strategies have already been developed and proven at sea turtle rookeries to reduce nest temperatures. Hatcheries covered with artificial shade cloth and tall-growing trees planted on the hind dunes of nesting beaches, reduced nest temperatures by up to 2 °C (Maulanly et al. 2012; Patino-Martinez et al. 2012; Hill et al. 2015; Wood et al. 2015). Ideally, sand-cooling strategies need to be more practical for high-density nesting beaches to cover a large area. In the less advanced places of east Asia and the Indo-Pacific, they need inexpensive materials that can reduce sand temperatures similar to what has been demonstrated with artificial shade cloth and trees.

In this study, we investigated the sand-cooling effectiveness of two under-studied strategies; (1) seawater-irrigation and (2) palm-frond-shade. Seawater-irrigation would be ideal for Raine Island, as managers could cool sand over a large nesting area. Palm-fronds could be used in developing countries, where artificial shade cloth materials are expensive and less accessible. We compared these two sand-cooling treatments to two well-used strategies; (3) artificial-shade cloth and (4) tree-shade. A (5) sun-exposed treatment was added as a control. Seawater-irrigation can reduce nest temperature by two mechanisms; (1) with light irrigation, the evaporation of water cools the surface sand ultimately cooling the nest temperature; and (2) with heavy irrigation, the bulk influx of cool water flows down through the sand to nest depth, resulting in heat loss from nests. In this experiment, our seawater-irrigation protocol used the first mechanism.

We relocated clutches of eggs from two sea turtle species with nesting populations in the nGBR; (1) the vulnerable nGBR green turtle (C. mydas, Qld Department of Environment, 2019) and (2) the critically endangered hawksbill turtle (Eretmochelys imbricata, IUCN 2008) population from north Queensland (FitzSimmons & Limpus 2014). The primary aim was to discover if seawater-irrigation and palm-frond-shade lowered nest temperatures enough during the middle third of development (thermal-sensitive period, TSP) to produce more male hatchlings than the other three treatments. Secondly, we aimed to confirm the predicted sex-ratios derived from TSP nest temperature using logistic models for each species and to compare it to the observed sex-ratios of clutches in the field. Lastly, we aimed to determine if these under-studied strategies resulted in increased incubation success of eggs and improved the overall ‘quality’ and survivorship of hatchlings, as it is expected that larger, faster sea turtle hatchlings have greater survivorship in the first 48 hours after emerging (Booth et al. 2004).
METHODS

Milman Island Study Site

Milman Island is a coral sand cay located 23 km off the east coast of the Cape York Peninsula, Queensland Australia (11.1697° S, 143.0157° E, 17 m above sea level) and within the Great Barrier Reef Marine Park (Dobbs et al. 1999). The island is 2.3 km around (Limpus 1980) and contains a variety of sea turtle nesting environments such as grassy raised dunes and bare sand areas partially shaded by pemphis sp. and Guettarda speciose trees.

Initially site A on the northern-end of the island (Fig. 1) was selected as a proxy for the northern-end of Raine Island, due to the similar incubation environment.

Three weeks of persistent strong north-westerly monsoon winds eroded this section of beach in late January and early February 2019. This combined with the largest high tides of the year in mid-February, resulted in wave wash-over of all nests in the study. Stronger winds and similar tides were forecasted for the following day, so all 51 clutches were relocated to site B (Fig. 1) within 28 hours to avoid further embryonic mortality.

Panasesa Island Study Site

Part of this study was also replicated on the eastern side of Panasesa Island (10.739583° S, 151.724111° E, 18 m above sea level), a small vegetated coral island that forms part of the Conflict Island group, Papua New Guinea (Fig. 1). The Conflict Island group resides ~ 500 km East of the capital, Port Moresby. The majority of nesting on Panasesa Island are green turtles, with several hawksbills nesting each season.

The experiment was placed within an open-top hatchery (Fig. 3.a) built by the coordinators of the Conflict Islands Conservation Initiative (CICI) and was exposed to sunlight from ~ 09:00 to 16:00 each day. This site did not experience any wave wash-over during the experiment.

![Map of Queensland, Australia and Papua New Guinea (PNG) with the location of the two study sites. (1) Milman Island in the nGBR, identified by the orange circle. Area A was the location of the initial experiment and area B was the relocation site after the wave wash over event. (2) Panasesa Island, within the Conflict Island group of PNG, identified by the pink circle. Also shown is an image of Raine Island, the largest green turtle rookery in the world, and identified on the map by the green circle.](image-url)
Experimental Design

On Milman Island within site A, treatments were set up amongst five plots. Treatment plots included; (1) sun-exposed control, (2) sun-exposed with seawater-irrigation, (3) natural tree-shade, (4) artificial-shade and (5) palm-frond-shade. Both sun-exposed and seawater plots received full-sunlight from 07:00 to sunset each day and all three shade treatments received shade from 9:00 onwards.

In the seawater-irrigation treatment for the first 36 days of incubation (Fig 2.a), a plastic watering can with 8 litres of freshly collected seawater was used to sprinkle water directly above each nest for 1 minute at dusk each day (~21 mm of daily rainfall). Irrigation at night reduces sand temperature more effectively than if watering was done during the day (Jourdan and Fuentes 2015).

The shade structure (hatchery) was 1.4 m high, 3.3 m wide and 8.4 m long and was constructed from driftwood found on the island. The western-half of the hatchery was shaded with artificial-shade cloth (90% of sunlight was blocked; Fig 2.b) and the eastern-half was shaded with palm-fronds (Fig 2.c). Dried palm-fronds (Arecaceae sp.) were collected in Cairns, Australia, and sprayed with Virkon™ virucidal bactericidal fungicidal disinfectant prior to arriving on the island (in compliance with Milman Island’s pink zone status under the Queensland Government Parks and Wildlife Department regulations). Shade coverage for palm-frond and tree-shade treatments (Fig. 2d) was ~ 52% and ~ 68% respectively and calculated in python from images taken at midday.

Of the 51 clutches relocated, 36 were from hawksbill turtles, and 15 from green turtles. Six hawksbill turtle clutches were relocated into the sun, seawater-irrigation, palm-frond and artificial-shade treatments, whilst 12 clutches were relocated into the tree-shade. Only 15 green turtle clutches were collected in this study due to the low number of nesting green turtles across the nGBR region. Six green turtle clutches were relocated into the sun-exposed treatment, six clutches were relocated into the seawater-irrigation treatment and three clutches were relocated into the palm-frond hatchery.

Fig 2: (a) Seawater-irrigation treatment being applied to nest plots in the afternoon (17:30 – 19:00). Both seawater (visible) and sun (control) sites (not visible) had no shade coverage until sunset. (b) Nests shaded by an artificial hatchery with 90% shade cloth (made from polypropylene). (c) Nests shaded by a natural-material hatchery with palm-fronds placed above. (d) Nests shaded by a single G. speciose tree in the tree-shade treatment.
On Panasesa Island (PNG), only two of the five treatments were replicated, whereby 12 green turtle clutches were relocated into either a seawater-irrigated \( (N = 6) \) or sun-exposed control site \( (N = 6) \). Nest positions were alternated within a 4 x 6 m area (Fig 3.a). The seawater-irrigation protocol was identical to that of Milman Island with the exception of a 35-day watering period (not 36 days).

**Clutch Relocation**

Relocations on Milman Island occurred between the 6 – 19 of January 2019 and occurred between 13 – 31 January 2019 on Panasesa Island. Each night, the beach was patrolled for nesting sea turtles. Once a sea turtle was found laying, her flipper tag numbers (in QLD, issued by QLD Department of Environment and Science) and curved carapace length (cm) were recorded. When the sea turtle moved off the nest, the eggs were carefully excavated by hand and placed into a plastic bucket. The total clutch count (TCC) was recorded and every tenth egg counted was placed into a separate bucket until 15 eggs were set aside for measuring egg mass.

Within two hours of oviposition, clutches were relocated with minimal rotations to avoid the risk of movement-induced embryonic mortality \( (\text{Limpus and Miller 1979}) \). Artificial egg chambers were dug by hand to a standard depth of 65 cm for green turtle clutches and 50 cm for hawksbill turtle clutches. A temperature data logger \( \text{(iButton® model DS1922L, Maxim Integrated, San Jose, CA, USA)} \) was enclosed with in a watertight balloon, placed into the centre of each clutch and programmed to log temperature for every hour of incubation.

**Clutch Rescue**

On the 21 February 2019, a 3.6 m high tide combined with 13 knot north-westerly winds caused wave wash over of all five treatments on Milman Island. Subsequently, seawater pooled over the nests and all 51 clutches were relocated (rescued) within 28 hours. Clutches were carefully excavated by hand and reburied in artificially dug nests at site B (Fig. 3.b - c). Clutches were 33 – 45 days old when the rescue occurred, which posed no risk of movement-induced embryonic mortality \( (\text{Limpus and Miller 1979}) \) and inundation occurred after the mid-trimester when sex is determined.

A new artificial-shade structure was built over all shaded nests (Fig 3.b), whilst seawater and sun-exposed nests were left in un-shaded bare sand area (Fig 3.c). The new shade structure was comprised of five metal stakes in a square formation and was draped with artificial shade cloth. Nests were not shaded by trees at site B due to the risk of damaging pre-existing natural nests.

**Fig 3:** (a) ‘Open’ hatchery structure on Panasesa Island in Papua New Guinea where the sun (control) and seawater – irrigation treated nests were relocated to. Structure was built to protect the nests from ground predators. (b) Shaded area in site B where clutches from the artificial shade, palm-frond shade and tree-shaded treatments were relocated to after the wave wash over event. Hatchery was built using five metal stakes with 90% shade cloth draped over top. (c) Sun-exposed area in site B where sun and seawater-irrigation treated clutches were relocated to after the wave wash over event. Photograph was taken at 17:00, after enclosures were installed to capture hatchlings that were expected to emerge at night.
Nest Temperature

Prior to the experiment, temperature data loggers had their calibration checked in a water bath at temperatures of 20, 25, 30, 35 and 40°C and found to be accurate to ± 0.5°C. All nest temperature data from data loggers was recovered on Milman Island, except for one failed data logger from a green turtle clutch in the seawater treatment. No nest temperature data was recovered from Panasesa Island due to a programming failure of data loggers. Daily rainfall (mm) was recorded using a DigiTech mobile weather station located on Milman Island within site A.

Average nest temperature was calculated for four different periods of incubation: (1) whole incubation period (oviposition to hatching), which included the inundation period, (2) the mid-trimester of embryonic development (thermal-sensitive period, TSP) when sex is determined, (3) the dry period of incubation from 18 - 21 January 2019 and (4) the rainy period of incubation from 24 January – 12 February 2019. The time of hatching was calculated from the nest temperature trace using a temperature-development algorithm (Booth and Freeman 2006).

Predicted Sex-ratios

The temperature of a sea turtle nests determines the rate of embryonic development and length of the incubation period. The relationship between constant incubation temperature and incubation period was used to develop an algorithm that calculates the increment in development per hour at any given temperature (Booth and Freeman 2006). Following the methods of Booth and Freeman (2006), the mid-trimester of development was calculated for each nest using development rates of sea turtle embryos. A logistic equation was then used to relate the proportion of females to mean nest temperature. The mean nest temperature ($T_x$) during the mid-trimester (TSP) of field nests from the present study were then inserted into the following equation using constants (b and c) derived from observed sex-ratios from hawksbill eggs in a lab setting (Dobbs et al. 2010) and from sex-ratios of green turtle eggs in the field from this study:

$$Y = b(T_x) - c$$

The proportion of females that would have been produced at $T_x$ was then described as:

$$\text{Proportion of females} = \frac{\exp(Y)}{1+ \exp(Y)}.$$

A logistic model was also developed for each of the stocks (hawksbill and green turtles) to demonstrate the relationship between incubation temperature and proportion of females, this was derived from observed sex-ratios (from gonad histology) of field nests of the present study. From this we were also able to estimate the pivotal temperature of each stock.

Observed Sex-ratios

Sea turtle embryos and hatchlings cannot be sexed by their external morphology. To collect empirical data on sex-ratios of clutches from field nests, a small sample ($n = 4 - 8$) of dead undecomposed late-stage embryos and hatchlings were obtained during nest excavations and dissected for gonad extraction (i.e. no hatchlings were euthanized for histology). Gonads were removed and placed into specimen jars with 70% ethanol in water solution. Histology was conducted at the School of Medicine’s histological facility located at the University of Queensland. The tissues were processed following the standard protocol for reptilian gonad specimens (Whiteley et al. 2018) and gonadal sex of each sample was identified ‘blindly’ following the descriptions by Miller and Limpus (2003) (Fig. 4).

Fig 4 (left): Examples of the histological images of premature gonads from hawksbill and green turtle hatchlings/embryos that died in field nests on Milman Island, Queensland. (a) Premature testis from a male embryo, with a thin cortex and medulla filled with bundles of seminiferous tubules. Scale bar = 200µm. (b) Premature ovary from a female embryo, ‘hook-shaped’ with a thick cortex layer and dense medulla. Scale bar = 300µm.
Hatchling Performance and Morphology

From each nest, 15 emerged hatchlings were haphazardly selected for morphological and locomotive performance measurements which included; carapace size measured as an index (CSI, cm²; Fig 5.a), live body mass (g; Fig. 5.b), crawling speed (cm sec⁻¹), swimming speed (Fig. 5.c) and self-righting propensity (Fig. 5.d) measured as an index (SRPI). Hatchling selection and subsequent measurements followed the protocols described by Ischer et al. (2009), Booth et al. (2013) and Read et al. (2013).

As mentioned previously, on Milman Island the final two to four weeks of incubation for all clutches were completed under an entirely different treatment regime to the original experiment site. Hence, the analysis of treatment effects on hatchling quality were compromised by the change of treatment site. Hatchling quality became a minor focus of the Milman Island study and results should be regarded as ‘shaded’ or ‘un-shaded’ treatments (although original treatment groups were still used in analyses). However, the results from Panasesa Island, are representative of their initial treatments, as these nests were not affected by inundation.

Nest Excavations

One to two days post the emergence of a clutch, the nest was excavated by hand to assess incubation success. On Milman Island, a compromise was made on the analysis of the incubation success with consideration that survivorship of all clutches was impacted by the inundation event. Hatched eggs were counted, and unhatched eggs were opened by hand to determine the embryonic stage of development at which mortality occurred. Six developmental phases were identified following the field guide of Dunstan and Robertson (2017), which was summarised from more extensive staging criteria (Miller et al. 2017). The mortality of embryos prior to inundation was attributed to mortality induced by the treatment, mortality of late stage embryos and hatchlings due to inundation were considered as ‘survived’. The ‘survived’ count was added to the hatched count and was a measure of estimated embryonic survival (%).

On Panasesa Island, incubation success was measured using the following:

\[
\text{Hatching success (\%) = } \frac{(\text{TCC} - (\text{Unhatched eggs + Undeveloped eggs})}{\text{TCC}}
\]
Emergence success (%) = (TCC – (Unhatched eggs + Live hatchlings +Dead hatchlings))/TCC

RESULTS

Nest Temperature

Whole incubation nest temperature differed between treatments for green turtle clutches (Tables 1 & 5). Nests in the sun-exposed treatment were ~0.5 °C warmer than nests in the seawater-irrigation treatment, while nest temperatures in the palm-frond-shade did not differ from either sun-exposed or seawater-irrigation treatments (P < 0.05; Tables 1 & 5). Whole incubation nest temperatures also differed between treatments for hawksbill turtle clutches (Tables 3 & 5). Nest temperatures in the seawater-irrigation treatment were similar to all treatments, while nests in the shaded treatments (artificial, palm-frond and tree) were up to 0.9 °C cooler than the sun-exposed treatment (Tables 3 & 5). Small daily temperature fluctuations were conspicuous in hawksbill turtle nests, but not obvious in the green turtle nests (Fig. 6). A monsoonal trough in late January caused a large spike in rainfall and dropped nest temperatures, which remained low until early February (Fig. 6). When the persistent rain cleared, nest temperatures rapidly increased up until 21 February, when wave wash-over flooded all nests and caused a second drop in nest temperature (Fig. 6). Following this, nest temperatures remained almost constant until hatching.

Sex-ratios

In green turtle nests, the average nest temperature during TSP varied between treatments (Tables 1 & 5). Green turtle nests in the seawater-irrigation treatment were on average ~0.6 °C cooler than both sun-exposed and palm-frond-shade treatments (Tables 1 & 5). As a result, there was a difference in green turtle clutches between treatments in the proportion of female hatchlings predicted by TSP nest temperature (Tables 1 & 5). The proportion of females predicted for clutches in the seawater-irrigation treatment was ~30% lower than the sun-exposed and palm-frond shaded treatments (P < 0.05; Tables 1 & 5; Fig. 7.a).

For hawksbill turtle clutches, TSP nest temperature also varied between treatments (Tables 3 & 5; Fig. 6a). The TSP temperature in the seawater treatment was similar to artificial-shade, but 0.8 – 0.3 °C cooler than that of clutches in the sun, tree and palm-frond-shade treatments (P < 0.05; Tables 3 & 5). As a result, the proportion of female hatchlings predicted from TSP temperature in hawksbill turtle nests was different across treatments (Table 5). The lowest proportion of females was predicted in the seawater treatment. Palm-frond and tree-shade had higher female hatching production than the sun-exposed and artificial-shade treatment (Tables 3 & 5; Fig. 7.b).

Analysis of the observed sex-ratios (determined from gonad histology) of embryos indicted no differences between treatments in the proportion of females produced for either species (Tables 1,3 & 5; Fig. 7).

Hypothetical Sex-ratios

As clutches of eggs were laid sequentially, the heavy rainfall from the 22 January confounded the results for predicted sex-ratios. Two standardised periods (dry and wet) were used to understand the effects of the treatments. During the dry period (18 - 21 January), nest temperatures were very warm and ranged from 29.1 – 31.5 °C for both species, differences amongst the treatments were also detected (Tables 2, 4 & 5). Nests in the shaded treatments were cooler than the sun and seawater-irrigation treated nests (Tables 2, 4 & 5). Despite this, for both species, nests were still strongly female-biased (> 90% female) and only tree and artificial-shade were able to produce a few hawksbill nests that were 20 – 53 % male (Tables 2 & 4; Fig. 7).

During the wet period (24 January – 12 February), nest temperatures of both species were much cooler than during the dry period and ranged between 27.7 – 29.2 °C (Tables 2 & 4). There were significant differences in nest temperature between treatments during this period for both species (Table 5). Green turtle nests in the palm-frond shade were cooler than the seawater-irrigation and sun-exposed treatments. As such, the palm-frond shade is predicted to have produced almost completely male-biased clutches, between 87 – 92 % male, compared to the control, 81% male (Fig. 7.a). The cooling effect was more obvious for hawksbill turtle nests as the temperature difference between treatments was greater (Table 4). It was predicted that artificial-shade would have produced the most males (98 % male), followed closely by palm-frond shade (96.9 % male) and tree-shade (85.7 % male) (Fig. 7.b). Sun and seawater-irrigated nests are predicted to have had produced fewer males (75 – 79% male) than the palm-frond and artificial-shade treatments, but were still male-biased (Fig. 7.b).
Fig 6: (a) Line traces for average hourly nest temperature from hawksbill turtle clutches placed into five incubation environment treatments on Milman Island, QLD: sun-exposed (N = 6, yellow), seawater-irrigated (N = 6, blue), tree-shaded (N = 12, green), artificially-shaded (N = 6, red) and palm-frond-shaded (N = 6, pink); (b) Line traces for average nest temperature from green turtle clutches placed into three incubation environment treatments on Milman Island, QLD: sun-exposed (N = 6, yellow), seawater-irrigated (N = 6, blue), palm-frond-shaded (N = 3, pink). The pivotal temperature (P) used for modelling sex ratios of each species is denoted by the dashed lines. Daily rainfall is denoted by the dark blue bars. The shaded area denotes the period of seawater inundation from wave wash over.
Fig 7: Box and whisker plot depicting the alternative predictions of the sex-ratios (a) in green turtle nests in three different treatments and (b) in hawksbill turtle nests under five different treatments on Milman Island, QLD. The real (true) thermal-sensitive period (TSP) occurred at vastly different time points, as each nest was laid at different times, the light blue bars depict the hypothetical sex-ratios of each treatment using the average temperature during the real TSP. The bright blue bars depict the hypothetical sex-ratios when the TSP was standardized for all nests (regardless of age) to the driest period (18/01/19 – 21/01/19). The deeper blue bars depict the predicted sex-ratios when TSP was standardized for all nests to the wet period (24/01/19 - 12/02/19). The dark blue bars represent the observed sex-ratios (verified through histology) of nests (samples from the artificial treatment were not collected). Boxes contain the inner quartiles of sampled values and an X represents the mean value for each treatment group. The sample range is represented by error bars and extreme outliers are denoted by circles.
Pivotal Temperature

A logistic curve was fitted to data from the present study of observed proportions of females in field nests from the north Queensland stock of hawksbill turtles (Fig. 8.a) and nGBR stock of green turtle (Fig 8.b) that nested on Milman Island. For hawksbill turtle clutches on Milman Island, the observed sex-ratios from the field nests had a relationship where P = 28.6 °C (Fig. 8.a). For green turtle clutches on Milman Island, the observed sex-ratios from the field nests had a relationship where P = 29.2 °C (Fig. 8.b).

![Fig 8: Scatter plots of observed sex-ratios and fitted logistic regression lines that depict the relationship between incubation temperature during the thermal-sensitive period (TSP) and the proportion of female hatchlings from clutches collected from (a) the north Queensland stock of hawksbill turtles and (b) the northern Great Barrier Reef green turtles that nest on Milman Island, Queensland. Dark circles represent observed sex-ratios in field nests and the respective nest temperature during the TSP. The dark line is the respective logistic curve (model) fitted to the field nest data. The grey dashed line represents the pivotal temperature (P) where a 1:1 sex-ratio was predicted from the field nest data.](image)
Incubation Success

For green turtle clutches on Milman Island, there was no effect of treatment on embryonic survival up until the inundation event (Table 5; Fig. 9), although three of the six seawater-irrigation nests had 58 – 72 embryos die during the first stage of development. There was a significant difference between treatments in embryonic survival for hawksbill turtle clutches up until the inundation event (Table 5; Fig. 9). Hawksbill turtle clutches in the palm-frond shade had the highest survivorship of the five treatments and was significantly greater than embryonic survival in the seawater-irrigation treatment ($P < 0.05$; Table 3).

On Panasesa Island, there was a significant difference between the two treatments in hatching and emergence success ($P < 0.05$, Table 9; Fig. 10). Nests in the sun treatment had hatching and emergence success ~10% higher on average than nests in the seawater-irrigation treatment (Table 9; Fig. 10). Embryonic survival was higher for nests in the seawater treatment on Panasesa Island than for seawater-irrigation nests on Milman Island (79% and 46.4% respectively; Tables 1 & 9).

Fig 9 (above): Box and whisker plot representing incubation success measured as estimated embryonic survival (%) from green turtle (dark bars) and hawksbill turtle (light bars) clutches placed in five incubation environment treatments on Milman Island, Queensland. Boxes contain the inner quartiles of sampled values and an X represents the mean value for each treatment group. The sample range is represented by capped vertical lines and outliers are denoted by circles.

Fig 10 (left): Box and whisker plot representing hatching (light bars) and emergence (dark bars) success of green turtle clutches placed into sun (control) or seawater irrigation treatments on Panasese Island, Papua New Guinea. Boxes contain the inner quartiles of sampled values and an X represents the mean value for each treatment group. The sample range is represented by capped vertical lines.
Hatchling Morphology

On Milman Island, initial egg mass of hawksbill and green turtle clutches did not vary between treatments (Tables 6, 7 & 8), but was included as a covariate in the analyses for hatchling mass and CSI. Hatchling mass and CSI of hawksbill and green turtle hatchlings did not vary between treatments (Tables 6, 7 & 8). Although there was a lack of treatment effects, hawksbill hatchlings from the seawater-irrigation and sun treatments had slightly smaller carapace dimensions than hatchlings from shaded treatments (Table 7).

On Panasesa Island, initial egg mass of green turtle clutches did not vary between treatments (Table 8) but was included as a covariate for hatchling mass and CSI. Both hatchling mass and CSI did not vary between treatments (Table 8).

Hatchling Locomotion Performance

On Milman Island, only crawling speed of green turtle hatchlings was significantly different between treatments (Table 8). The seawater-irrigation treatment had the poorest crawlers of the three treatments, however sample sizes were small ($P < 0.05$; Table 6). Swimming speed and SRPI were similar across all treatments (Table 8), although the hatchlings from the seawater-irrigation treatment were the slowest swimmers and the poorest at self-righting (Table 6).

Both crawling and swimming speed of hawksbill turtle hatchlings on Milman Island differed between treatments (Table 8; Fig. 11). The hatchlings from sun-exposed and seawater-irrigation treatments were equally slower at crawling than hatchlings from the shaded treatments ($P < 0.05$; Table 7; Fig. 11). Swimming speeds of hawksbill hatchlings from the tree-shade, sun and seawater-irrigation treatments were equally slower at swimming than hatchlings from the artificial and palm-frond-shade ($P < 0.05$; Table 7; Fig. 11). SRPI did not differ between treatments for hawksbill turtle hatchlings (Table 8). However, hawksbill hatchlings from the sun and seawater-irrigation treatments had similar SRPI but lower than that of hatchlings from shaded treatments (Table 7). Meaning it took slightly longer and more attempts for hatchlings from un-shaded treatments to self-right successfully three times (if at all).

On Panasesa Island, there were no significant differences between crawling speed and SRPI score of green turtle hatchlings from the seawater – irrigation and sun treatments (Table 9). Swimming speed was not reported for green turtle hatchlings on Panasesa Island.

Fig 11: Box and whisker plot representing the crawling (light bars) and swimming speed (dark bars) of hawksbill turtle hatchlings from five incubation environment treatments on Milman Island, Queensland. Seawater and sun-exposed nests experienced the same ‘un-shaded’ treatment in the last three weeks of incubation, whilst the nests in palm-frond, tree and artificial-shade treatments all experienced the same ‘shaded’ (90% artificial shade cloth) treatment in the last three to four weeks of incubation. Boxes contain the inner quartiles of sampled values and an X represents the mean value for each treatment group. The sample range is represented by capped vertical lines and extreme outliers are denoted by circles.
Table 1. Mean (± SE), minimum and maximum values (range) for variables measured from nGBR green turtle clutches on Milman Island, for each experimental treatment group. Sample size (N) of clutches used for analysis of each variable are also reported.

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<th>Sun</th>
<th>Seawater</th>
<th>Palm-Frond</th>
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<td></td>
<td>$\bar{x}$</td>
<td>range</td>
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<td>Whole incubation</td>
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<td>28.6 – 34.3</td>
<td>30.4 ± 0.0</td>
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<td></td>
</tr>
<tr>
<td>Incubation period (days)</td>
<td>52.3 ± 0.2</td>
<td>53 – 52</td>
<td>53 ± 0.0</td>
</tr>
<tr>
<td>Estimated embryonic</td>
<td>76.6 ± 6.2</td>
<td>55.8 – 95.3</td>
<td>46.4 ± 14.4</td>
</tr>
<tr>
<td>survival (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSP nest temperature</td>
<td>29.8 ± 0.1</td>
<td>29.6 – 30.1</td>
<td>29.2 ± 0.2</td>
</tr>
<tr>
<td>(°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predicted percentage</td>
<td>82.2 ± 2.5</td>
<td>74.0 – 91.0</td>
<td>52.0 ± 10.9</td>
</tr>
<tr>
<td>of females (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed percentage</td>
<td>100.0 ± 0.0</td>
<td>100.0</td>
<td>44.3 ± 22.1</td>
</tr>
<tr>
<td>of females (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Mean (± SE), minimum and maximum values (range) for hypothetical variables measured for green turtle clutches on Milman Island, from experimental treatment groups. Sample size (N) of clutches used for analysis of each variable are also reported. Dry period occurred 18/01/19 – 21/01/19, wet period occurred 24/01/19 - 12/02/19 (excluded inundation event).

<table>
<thead>
<tr>
<th></th>
<th>Sun</th>
<th>Seawater</th>
<th>Palm-Frond</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$</td>
<td>range</td>
<td>$\bar{x}$</td>
</tr>
<tr>
<td>Nest temperature</td>
<td>31.3 ± 0.1</td>
<td>31.1 – 31.4</td>
<td>31.4 ± 0.0</td>
</tr>
<tr>
<td>during dry period (°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nest temperature</td>
<td>28.6 ± 0.1</td>
<td>28.3 – 28.8</td>
<td>28.5 ± 0.1</td>
</tr>
<tr>
<td>during wet period (°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypothetical percentage</td>
<td>99.4 ± 0.0</td>
<td>99.0 – 99.6</td>
<td>99.5 ± 0.0</td>
</tr>
<tr>
<td>of females during dry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>period (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypothetical percentage</td>
<td>18.8 ± 0.0</td>
<td>10.6 – 28.6</td>
<td>17.2 ± 0.0</td>
</tr>
<tr>
<td>of females during wet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>period (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Mean (± SE), minimum and maximum values (range) for variables measured from hawksbill turtle clutches on Milman Island, from experimental treatment groups
Sample size (N) of clutches used for analysis of each variable are also reported.

<table>
<thead>
<tr>
<th></th>
<th>Sun</th>
<th>Estimation</th>
<th>Palm-Frond</th>
<th>Artificial</th>
<th>Tree</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x</td>
<td>range</td>
<td>N</td>
<td>x</td>
<td>range</td>
</tr>
<tr>
<td>Whole incubation nest temperature (°C)</td>
<td>30.9 ± 0.1</td>
<td>27.6–35.2</td>
<td>6</td>
<td>30.4 ± 0.1</td>
<td>27.6–33.2</td>
</tr>
<tr>
<td>Incubation Period (days)</td>
<td>51.0 ± 0.0</td>
<td>51 ± 31</td>
<td>6</td>
<td>51.0 ± 0.0</td>
<td>51 ± 31</td>
</tr>
<tr>
<td>Estimated embryonic survival (%)</td>
<td>86.9 ± 4.2</td>
<td>67.2–96.4</td>
<td>6</td>
<td>60.4 ± 12.2</td>
<td>7.8–90.7</td>
</tr>
<tr>
<td>TSP nest temperature (°C)</td>
<td>29.2 ± 0.1</td>
<td>29.1–29.4</td>
<td>6</td>
<td>28.9 ± 0.2</td>
<td>28.5–29.1</td>
</tr>
<tr>
<td>Predicted percentage of females (%)</td>
<td>55.2 ± 3.3</td>
<td>47.6–70.1</td>
<td>6</td>
<td>31.1 ± 4.3</td>
<td>11.9–40.7</td>
</tr>
<tr>
<td>Observed percentage of females (%)</td>
<td>91.7 ± 11.8</td>
<td>83.3–100</td>
<td>2</td>
<td>88.3 ± 12.6</td>
<td>75.0–100.0</td>
</tr>
</tbody>
</table>

Table 4. Mean (± SE), minimum and maximum values (range) for hypothetical variables measured for hawksbill turtle clutches on Milman Island, from experimental treatment groups Sample size (N) of clutches used for analysis of each variable are also reported. Dry period occurred 18/01/19 – 21/01/19, wet period occurred 24/01/19 - 12/02/19 (excluded inundation event).

<table>
<thead>
<tr>
<th></th>
<th>Sun</th>
<th>Estimation</th>
<th>Palm-Frond</th>
<th>Artifical</th>
<th>Tree</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x</td>
<td>range</td>
<td>N</td>
<td>x</td>
<td>range</td>
</tr>
<tr>
<td>Nest temperature during dry period (°C)</td>
<td>31.3 ± 0.1</td>
<td>31.1–31.5</td>
<td>6</td>
<td>31.1 ± 0.0</td>
<td>31.0–31.2</td>
</tr>
<tr>
<td>Nest temperature during wet period (°C)</td>
<td>28.7 ± 0.1</td>
<td>28.6–28.9</td>
<td>6</td>
<td>28.8 ± 0.1</td>
<td>28.4–29.0</td>
</tr>
<tr>
<td>Hypothetical percentage of females during dry period</td>
<td>99.9 ± 0.0</td>
<td>99.8–99.9</td>
<td>6</td>
<td>99.8 ± 0.0</td>
<td>99.8–99.8</td>
</tr>
<tr>
<td>Hypothetical percentage of females during wet period</td>
<td>20.9 ± 0.0</td>
<td>14.7–28.2</td>
<td>6</td>
<td>24.7 ± 0.0</td>
<td>8.3–34.8</td>
</tr>
</tbody>
</table>
Table 5. Nested generalised linear model (GLM) probability statistics reported for whole incubation nest temperature, TSP nest temperature, predicted and observed percentage of females, incubation period estimated survival for green and hawksbill clutches. Clutch (random) was nested within treatment (fixed).

<table>
<thead>
<tr>
<th>Probability statistic between treatments</th>
<th>Green turtles</th>
<th>Hawksbill turtles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole incubation nest temperature (°C)</td>
<td>$F_{1,2} = 4.8, P = 0.03^*$</td>
<td>$F_{1,4} = 6.5, P &lt; 0.001^*$</td>
</tr>
<tr>
<td>Incubation period (days)</td>
<td>$F_{1,2} = 3.28, P = 0.098$</td>
<td>$F_{1,4} = 22.7, P &lt; 0.001^*$</td>
</tr>
<tr>
<td>Estimated embryonic survival (%)</td>
<td>$F_{1,2} = 3.08, P = 0.083$</td>
<td>$F_{1,4} = 3.01, P = 0.032^*$</td>
</tr>
<tr>
<td>TSP nest temperature (°C)</td>
<td>$F_{1,2} = 7.1, P = 0.01^*$</td>
<td>$F_{1,4} = 7.9, P &lt; 0.001^*$</td>
</tr>
<tr>
<td>Predicted percentage of females (%)</td>
<td>$F_{1,2} = 7.25, P = 0.009^*$</td>
<td>$F_{1,4} = 9.53, P &lt; 0.001^*$</td>
</tr>
<tr>
<td>Observed percentage of females (%)</td>
<td>$F_{1,2} = 0.44, P = 0.460$</td>
<td>$F_{1,3} = 0.49, P = 0.701$</td>
</tr>
<tr>
<td>Nest temperature during dry period (°C)</td>
<td>$F_{1,2} = 32.3, P &lt; 0.001^*$</td>
<td>$F_{1,4} = 38.2, P &lt; 0.001^*$</td>
</tr>
<tr>
<td>Nest temperature during wet period (°C)</td>
<td>$F_{1,2} = 6.2, P = 0.016^*$</td>
<td>$F_{1,4} = 14.9, P &lt; 0.001^*$</td>
</tr>
<tr>
<td>Hypothetical percentage of females during dry period</td>
<td>$F_{1,2} = 31.6, P &lt; 0.001^*$</td>
<td>$F_{1,4} = 9.86, P &lt; 0.001^*$</td>
</tr>
<tr>
<td>Hypothetical percentage of females during wet period</td>
<td>$F_{1,2} = 4.4, P = 0.039^*$</td>
<td>$F_{1,4} = 4.78, P = 0.004^*$</td>
</tr>
</tbody>
</table>

*Represents statistical significance (P<0.05) detected for the variation between groups.
### Table 6. Mean (± SE), minimum and maximum values (range) for variables measured from green turtle clutches on Milman Island, from experimental treatment groups

Sample size (N) of hatchlings used for analysis of each variable are also reported.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sun</th>
<th>Seawater</th>
<th>Palm-Frond</th>
<th>Artificial Tree</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$</td>
<td>range</td>
<td>$N$</td>
<td>$\bar{x}$</td>
</tr>
<tr>
<td>Initial egg mass (g)</td>
<td>43.6 ± 0.5</td>
<td>42.6 – 44.5</td>
<td>90</td>
<td>42.7 ± 1.1</td>
</tr>
<tr>
<td>Hatchling mass (g)</td>
<td>23.3 ± 0.2</td>
<td>17.5 – 27.0</td>
<td>79</td>
<td>21.9 ± 0.5</td>
</tr>
<tr>
<td>Carapace Size Index (cm²)</td>
<td>17.8 ± 0.1</td>
<td>14.4 – 20.4</td>
<td>79</td>
<td>16.0 ± 0.3</td>
</tr>
<tr>
<td>Crawling speed (cm s⁻¹)</td>
<td>22.5 ± 0.6</td>
<td>6.7 – 25.1</td>
<td>79</td>
<td>12.0 ± 1.44</td>
</tr>
<tr>
<td>Swimming speed (cm s⁻¹)</td>
<td>17.6 ± 0.6</td>
<td>2.4 – 19.7</td>
<td>79</td>
<td>8.2 ± 1.4</td>
</tr>
<tr>
<td>Self-righting Propensity Index</td>
<td>2.0 ± 0.2</td>
<td>0 – 6.7</td>
<td>79</td>
<td>1.0 ± 0.4</td>
</tr>
</tbody>
</table>

### Table 7. Mean (± SE), minimum and maximum values (range) for variables measured from hawksbill turtle clutches on Milman Island, from experimental treatment groups

Sample size (N) of hatchlings used for analysis of each variable are also reported.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sun</th>
<th>Seawater</th>
<th>Palm-Frond</th>
<th>Artificial Tree</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$</td>
<td>range</td>
<td>$N$</td>
<td>$\bar{x}$</td>
</tr>
<tr>
<td>Initial egg mass (g)</td>
<td>25.7 ± 0.2</td>
<td>25.4 – 26.0</td>
<td>90</td>
<td>25.6 ± 0.4</td>
</tr>
<tr>
<td>Hatchling mass (g)</td>
<td>13.4 ± 0.1</td>
<td>11.7 – 15.3</td>
<td>90</td>
<td>14.0 ± 0.2</td>
</tr>
<tr>
<td>Carapace Size Index (cm²)</td>
<td>11.0 ± 0.1</td>
<td>7.7 – 12.4</td>
<td>90</td>
<td>10.9 ± 0.2</td>
</tr>
<tr>
<td>Crawling speed (cm s⁻¹)</td>
<td>6.0 ± 0.2</td>
<td>2.1 – 10.4</td>
<td>90</td>
<td>5.5 ± 0.5</td>
</tr>
<tr>
<td>Swimming speed (cm s⁻¹)</td>
<td>5.1 ± 0.4</td>
<td>1.3 – 15.3</td>
<td>90</td>
<td>5.3 ± 0.8</td>
</tr>
<tr>
<td>Self-righting Propensity Index</td>
<td>1.3 ± 0.1</td>
<td>0 – 4.7</td>
<td>90</td>
<td>0.7 ± 0.3</td>
</tr>
</tbody>
</table>
Table 8. Nested GLM probability statistics reported for hatchling morphological and locomotion performance measurements of green and hawksbill turtle clutches, where clutch (random) was nested within treatment (fixed).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Probability statistic between treatments</th>
<th>Covariates for analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Green turtle</td>
<td>Hawksbill turtle</td>
</tr>
<tr>
<td>Initial egg mass (g)</td>
<td>$F_{1,2} = 1.31, \ P = 0.327$</td>
<td>$F_{1,4} = 1.31, \ P = 0.3$</td>
</tr>
<tr>
<td>Hatchling mass (g)</td>
<td>$F_{1,2} = 0.25, \ P = 0.782$</td>
<td>$F_{1,4} = 0.46, \ P = 0.766$</td>
</tr>
<tr>
<td>Carapace Size Index (cm$^2$)</td>
<td>$F_{1,2} = 1.2, \ P = 0.354$</td>
<td>$F_{1,4} = 1.58, \ P = 0.21$</td>
</tr>
<tr>
<td>Crawling speed (cm s$^{-1}$)</td>
<td>$F_{1,2} = 5.25, \ P = 0.041^*$</td>
<td>$F_{1,4} = 4.21, \ P = 0.009^*$</td>
</tr>
<tr>
<td>Swimming speed (cm s$^{-1}$)</td>
<td>$F_{1,2} = 4.41, \ P = 0.058$</td>
<td>$F_{1,4} = 3.51, \ P = 0.021^*$</td>
</tr>
<tr>
<td>Self-righting Propensity Index</td>
<td>$F_{1,2} = 0.5, \ P = 0.626$</td>
<td>$F_{1,4} = 2.53, \ P = 0.065$</td>
</tr>
</tbody>
</table>

*Represents statistical significance ($P < 0.05$) detected for the variation between groups.*
**Table 9.** Nested GLM probability statistics for incubation period, hatching and emergence success of Conflict Islands’ green clutches (N = 12). Hatching and emergence success data was arc sine transformed prior to analysis. Nested GLM probability statistics for hatchling morphological and locomotion performance measurements of Conflict Islands’ green clutches. Means (± SE) and sample size (n) represented for each treatment.

<table>
<thead>
<tr>
<th></th>
<th>Sun</th>
<th></th>
<th>Seawater</th>
<th></th>
<th>Probability statistic between treatments</th>
<th>Covariates for analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$</td>
<td>range</td>
<td>N</td>
<td>$\bar{x}$</td>
<td>range</td>
<td>N</td>
</tr>
<tr>
<td>Incubation Period (days)</td>
<td>50.7 ± 0.1</td>
<td>49 – 53</td>
<td>6</td>
<td>49.8 ± 0.1</td>
<td>48 – 52</td>
<td>6</td>
</tr>
<tr>
<td>Hatching Success (%)</td>
<td>88.7 ± 2.3</td>
<td>83.5 – 97.7</td>
<td>6</td>
<td>78.9 ± 3.5</td>
<td>68.0 – 92.5</td>
<td>6</td>
</tr>
<tr>
<td>Emergence Success (%)</td>
<td>85.0 ± 2.1</td>
<td>77.2 – 91.8</td>
<td>6</td>
<td>75.3 ± 2.8</td>
<td>61.9 – 80.0</td>
<td>6</td>
</tr>
<tr>
<td>Initial egg mass (g)</td>
<td>41.8 ± 2.8</td>
<td>30.4 – 51.7</td>
<td>90</td>
<td>43.3 ± 0.6</td>
<td>41.4 – 45.5</td>
<td>90</td>
</tr>
<tr>
<td>Hatchling mass (g)</td>
<td>20.4 ± 0.3</td>
<td>15.0 – 25.0</td>
<td>90</td>
<td>22.3 ± 0.2</td>
<td>17.9 – 29.0</td>
<td>90</td>
</tr>
<tr>
<td>Carapace Size Index (cm²)</td>
<td>17.0 ± 0.3</td>
<td>12.0 – 23.0</td>
<td>90</td>
<td>17.7 ± 0.1</td>
<td>15.3 – 20.0</td>
<td>90</td>
</tr>
<tr>
<td>Crawl Speed (cm s⁻¹)</td>
<td>8.1 ± 0.5</td>
<td>0.8 – 22.1</td>
<td>90</td>
<td>13.2 ± 0.7</td>
<td>4.25 – 27.0</td>
<td>90</td>
</tr>
<tr>
<td>Self-righting Propensity Index</td>
<td>3.9 ± 0.3</td>
<td>0.0 – 11.5</td>
<td>90</td>
<td>3.1 ± 0.3</td>
<td>0.0 – 10.34</td>
<td>90</td>
</tr>
</tbody>
</table>

*Represents statistical significance (P<0.05) detected for the variation between groups.*
DISCUSSION

On Milman Island, all hawksbill turtle nests experienced daily cyclic 0.2 – 0.4 °C fluctuations in nest temperature, but these fluctuations were absent in the green turtle nests. This difference can be attributed to the nest depth of each species, where the deeper below the sand surface the less prominent cyclic daily temperatures become (Booth 2006). As incubation progressed, nest temperatures increased for both species (most obvious after persistent rainfall). This trend occurred due to an increase in metabolic heat produced by the rapidly growing embryos during the second half of incubation (Booth and Astill 2001; Broderick et al. 2001). Heavy rainfall reduced nest temperature in all treatments by as much as 4.5 °C and remained low (28 – 29.5 °C) whilst the rain continued. This phenomenon is commonly observed in sea turtle nests and results from the combination of cloud cover and rainwater penetrating the sand (Godfrey et al. 1996; Lolavar and Wyneken 2017; Staines et al. 2019). However, once sunshine prevailed, nest temperatures increased.

For both species, nest temperature during the TSP in the palm-frond treatment was warmer than nests in the sun, seawater-irrigated, artificial and tree-shade treatments. This was due to the lag time in oviposition and the significant rainfall event in late January. As a result, clutches in palm-frond-shade had a strong female-bias. For hawksbill turtle clutches in the palm-frond-shade, we found 82.3 % female predicted from temperature traces but 100% female determined by histological examination of embryos, and for green turtle clutches, we found 83.1 % female predicted from temperature traces and 83 % female observed by histological examination of embryos. Studies in Malaysia where a hatchery was built with coconut palm leaves (100% opacity) over green turtle nests and were able to reduce nest temperatures below P and produce male-biased clutches (Van De Merwe et al. 2005; Van De Merwe et al. 2006). On Milman Island, all three shade treatments had a strong female-biased result predicted by models and observed for both species. Even artificial-shade cloth (which blocks 90% of sunlight) failed to greatly reduce TSP nest temperatures and produce significant proportions of males in all nests.

A study conducted at the Mon Repos loggerhead turtle (C. caretta) rookery in south-east Queensland used 70% artificial shade cloth (similar to the shade coverage of palm-fronds in this study) and had nest temperature reductions of up to 2 °C and 45% male production predicted in clutches (Wood et al. 2014). Model predictions based on TSP nest temperature, however, fail to detect the potential ‘feminising’ effect that polymers leached from plastic shade cloth may have on incubating clutches. The presence of plastic polymers in the nesting environment has
recently concerned conservationists (reviewed in Nelms et al. 2015). Laboratory research conducted on red-eared slider turtles (Trachemys scripta) found that two different polychlorinated biphenyls (PCBs) from plastic had an estrogenic effect on embryos and resulted in a female-bias of hatchlings produced from eggs incubated at male-producing temperatures when the compounds were in high concentration (Bergeron et al. 1994; Crews et al. 1995; Matsumoto et al. 2014). Future research could investigate if PCBs from artificial shade cloth have potential antagonistic effects on male hatchling production in field nests of sea turtles using histological methods. Although, it seems unlikely that these compounds could accumulate in sufficient concentrations at the nest depth of sea turtles.

The greatest proportion of males was predicted for green and hawksbill turtle clutches in the seawater-irrigation treatment (52 % and 31.1 % female respectively), as these nests experienced the lowest TSP nest temperatures. The low nest temperatures in the seawater-irrigation treatment was likely due to two possible reasons; (1) the heaviest rainfall period overlapped completely with the TSP for clutches in the seawater treatment and confounded the effect of seawater, or (2) the daily application of seawater to the sand during TSP resulted in nest cooling. This would be the first indication that seawater could reduce nest temperatures, as previous studies have exclusively used freshwater to irrigate bare sand and reduced sand temperatures at 45 cm (the nest depth of Lepidochelys kempiii), 60 cm (C. mydas) and 70 cm (Dermochelys coriacea) below the surface (Naro-Maciel et al. 1999; Jourdan and Fuentes 2015; Hill et al. 2015).

There are two potential mechanisms for cooling sand using irrigation; (1) evaporation of water from the sand surface, which cools the surface sand layer and thus reduces the heat conducted to from the surface to nest depth, (2) a bulk influx of cool water that penetrates the sand all the way down to nest depth. The latter explanation is the phenomenon that occurs with heavy rainfall events and was demonstrated in the nest temperature traces in late January through to mid-February on Milman Island. The application of seawater in this study was an example of evaporative cooling from the surface sand and resulted in a moderate ~0.5 °C decrease in nest temperature of both green and hawksbill turtle nests. A greater cooling effect might be obtained by the application of a larger volume of seawater, which would result in direct cooling by a bulk flow of water at nest depth.

We used hypothetical TSP periods that were standardized for all nests to better represent the cooling effects of the treatments in the presence and absence of rainfall (i.e. wet vs dry period). During the dry period, both species were predicted to produce highly female-biased sex-ratios regardless of the treatment applied. The application of shading and seawater-irrigation for the majority of nests were not enough to cool nests below 29 °C and produce males.

During the wet period, the hypothetical sex-ratios were vastly different to the dry period, as all clutches from both species were predicted to be male-biased. For hawksbill turtle clutches, nests in the palm-frond and artificial shade treatments were predicted to be > 95 % male. Both palm-frond and artificial shade treatments were cooler than the tree, sun and seawater-irrigation treatments during the wet period and were predicted to produce more males (85.7 %, 79.1 % and 75.3 % male respectively). Due to the lack of detectable differences in sex-ratios predicted for the seawater and sun (control) treatments during this period, it is assumed that seawater had minimal to no cooling effect on nest temperature. Seawater produced similar proportions of males to the sun treatment under both wet and dry periods. However, with the addition of natural rainfall, palm-frond and artificial-shade proved to be the most effective methods of producing male hatchlings. This implies that a combination of irrigation and shaded hatcheries would be an ideal management strategy to maximise male hatchling production.

The sex-ratios of hatchlings from clutches incubated in field nests have rarely been determined by more than one method (reviewed in Wibbels 2003; Wyneken and Lolavar 2015). Even fewer studies have validated the sex-ratios predicted for field nests from models using the observed sex-ratios determined directly by gonad histology of hatchlings from field nests (Park et al. 2004; Kaska et al. 2006; LeBlanc et al. 2012; King et al. 2013; Sari and Kaska 2015; Tilley et al. 2019). In the current study, only one of the 16 nests examined had a different observed sex-ratio then compared to the sex-ratio predicted from the nest temperature profile.

Logistic curves were calculated using nest temperature data during the TSP and the hatchling sex-ratio determined using gonad histology that describes the relationship between incubation temperature and hatchling sex-ratio. Using this method, the P for the nGBR green turtle stock was estimated to be 29.2 °C. The P for the north Queensland stock of hawksbill turtles was estimated to be 28.6 °C which is lower than the P of 29.2 °C reported for the same population using data gathered from constant temperature incubation experiments (Dobbs et al. 2010), and for other low latitude hawksbill nesting populations, 29.2 °C for Brazil (Mrosovsky et al. 1992) and 29.6 °C for Antigua, (Godfrey et al. 1999). Our lower estimate for P may be because we did not have data for nest temperatures below 28.8 °C, so the statistical fit of the logistic curve function does not include data.
from male dominant temperatures that may result in a poor extrapolation at these lower temperatures.

For the Milman Island study, the six developmental phases of dead embryos were used to estimate embryonic survival up until the point of inundation. For both hawksbill and green turtle clutches, the seawater-irrigation treatment had the highest embryonic mortality (39.6% and 53.6% respectively). Higher embryonic mortality associated with seawater-irrigation treatment could be due to; (1) decreased nest ventilation in the sand above the nest (Miller et al. 2003). The seawater added to the sand layer above the nest may have impeded gas diffusion to the nest depth of green turtles, however, a recent laboratory study conducted on loggerhead and green turtle eggs showed that early-stage embryos can survive PO2 levels as low as 10% (A. Archibald-Binge, UQ, unpublished). (2) Embryo dehydration, as seawater has a higher osmotic concentration (~1000 mOsmol) than embryonic fluids (~300 mOsmol). If there is continuous fluid contact across the eggshell, this will dehydrate the egg and cause embryonic death. Although, the hatching success of green turtle clutches in the seawater-irrigation treatment on Panasesa Island, PNG, was much greater than that reported on Milman Island, meaning if seawater had a significant detrimental effect on embryonic survival (<50 % hatching success), it would have been obvious across both studies. (3) Egg inviability, as research conducted on the viability of nesting leatherback turtles (D. coriacea) in Costa Rica confirmed that inviable clutches occurred frequently due to egg retention (Rafferty et al. 2011). Nesting success was ~ 50% for green turtles nesting on Milman Island (Ian Bell, pers. comm.), which may have resulted in greater egg retention and failure of early-stage embryos.

Palm-frond-shade had the lowest early-stage embryonic mortality for both hawksbills and green turtle clutches (8.5% and 13.4% respectively). As anticipated, incubation success was reduced in natural tree-shade due to the accumulation organic (humus) content in the sand. Decaying leaves and organic matter reduce sand ventilation (Marco et al. 2017) and increases the clutch’s susceptibility to bacterial or fungal infection (Bézy et al. 2015). Hence, for the purpose of maximising incubation success, shade structures should be used in preference to tree-shade in developing countries. This reinforces the need for more research into alternatives like palm-frond-shade hatcheries which can increase the incubation success of sea turtle clutches.

Hatchling mass and carapace size (CSI) did not vary between treatments, however hatchlings that emerged from un-shaded nests had slightly smaller carapaces than hatchlings from shaded treatments. This small variation is likely due to the greater conversion of yolk to tissue in embryos incubated in cooler, shaded nests for a longer incubation period (Booth 2006). Although this effect was not significant because incubation period was only 1 – 3 days longer in shaded nests. Sea turtle hatchlings also experience body composition trade-offs; larger hatchlings will be available to fewer predators than smaller hatchlings, meaning their survivorship should be greater in ‘predator-dense’ environments, but smaller hatchlings have greater energy stores for greater survival in food-limited environments at sea (Gyuris 2000). Moist nests may also stunt embryonic development, as a previous study had artificially watered freshwater turtle (C. picta) nests which produced smaller hatchlings (Bodensteiner et al. 2015). However, the hatchlings from both the Milman Island and Panasesa Island studies showed no difference in carapace size between sun and seawater-irrigated nests. This was predicted, as the majority of embryonic growth occurs during the last trimester of incubation (Booth and Astill 2001), when the seawater was no longer being applied.
On Milman Island, the un-shaded treatments produced slow crawling and slow swimming hatchlings compared to hatchlings from shaded nests. This was not surprising, as previous studies on hatchling performance have demonstrated that high nest temperatures during late-stage development causes hatchlings to become lethargic (Burgess et al. 2006; reviewed in Booth 2017). The index for self-righting ability (SRPI) weakly demonstrated that performance in self-righting ability was reduced in hawksbill turtle hatchlings from un-shaded nests compared to shaded treatments. Smaller carapace size and higher nest temperatures in late-stage development could explain the poorer self-righting ability of hatchlings from the un-shaded treatments, however the effect was not significant overall. Based on these findings, one might expect hatchlings from shaded nests had greater survivorship in the first 48 hours post emergence as they can potentially self-right, crawl and swim faster which will assist with evading predators on the beach and offshore (Davenport 1997). On Panasesa Island, the clutches were not affected by wave wash over, yet seawater did not reduce the locomotive performance of green turtle hatchlings when compared to the sun treatment.

CONCLUSION

For all sea turtle species, their innate nesting behaviour, long generation time and ability to navigate back to the same region where they had hatched 20 – 30 years earlier to lay their eggs is something to both marvel at and become concerned about. The life history traits of the turtle lineage have remained relatively unchanged for over 150 million years, and in that time, major environmental changes have occurred in both the aquatic and terrestrial environments that they depend on (reviewed in Poloczanska et al. 2009). Sea turtles in some areas now need to cope with a rapidly changing climate, fewer nesting areas and increased inundation events due to sea level rise and erosion (Pike et al. 2015; Patrício et al. 2019). These sea turtle populations will need to shift nesting to higher latitudes and/or cooler months of the year or adapt their thermal sensitivity in the incubation environment and maintain hatching success to recruit more males into the population (Fuentes et al. 2010; Poloczanska et al. 2009). If shifting nesting sites is not possible, and thermal mitigation is implemented, sea turtle populations will likely perish within two or three generations.

Our seawater-irrigation protocol resulted in a modest ~0.5 °C decrease in nest temperature and produced the highest proportions of males when the TSP coincided with the heaviest rainfall period. Outside of this period, hypothetical predictions were more female-biased, meaning the cooling effect of seawater-irrigation was not large enough to result in male hatchling production. This result coupled with the possible trade-offs of reduced embryonic survival suggests that seawater-irrigation protocol we used may not be a viable strategy to cool nests. Palm-fronds are an inexpensive material that can be used to shade nests in a hatchery, effectively increasing the incubation success and hatching quality of sea turtle nests. Maximising cooling effects need to be investigated further with alternative structure designs and greater volumes of water applied during irrigation in order to mimic the temperature drops observed during heavy rainfall events.

This study demonstrated that a heavy rainfall event was the greatest contributor to male hatchling production compared with applied treatments. Hence, a seawater-irrigation protocol that mimics heavy rainfall events should reduce nest temperature enough to result in greater male hatchling production. More palm-frond hatcheries could be implemented immediately in sea turtle rookeries in countries with limited resources like Papua New Guinea, Solomon Islands and Malaysia which are at the highest risk to producing female-biased clutches from increased sand temperature associated with climate change.
FUTURE RESEARCH

1. **How many breeding males are needed in a viable population?**

Conducting observational studies of the operational sex-ratio in the breeding grounds will help us to gain better insight into what proportion of the population needs to be male in order for populations to persist into the future.

2. **How many males are produced outside the peak of the nesting season?**

Currently, we know that hawksbills nest year-round in the nGBR and Torres Strait, whereas green turtles nest primarily Nov–Jan. Surveys could be conducted year-round on nesting beaches to monitor sand temperatures and aerial surveys to determine the proportion of females nesting at non-peak cooler times of the year.

3. **What is the critical time in the thermal-sensitive-period (in days) when sex is determined during embryonic development?**

Currently, we have no finite indication (in days) when the gonadal sex of a sea turtle embryo is determined, as previous research has only narrowed it down to the middle-third of development (which could be anywhere from four days to two weeks). Conducting a comprehensive study incubating eggs in a lab setting will assist with narrowing the TSP and improving the models used to predict sex-ratios.

4. **How much water is needed to cool nests down to male-producing temperatures?**

The seawater-irrigation protocol used in this study mimicked light rain, and the resulting evaporative cooling was not great enough to produce males during clear sunny weather. Future studies could investigate this further by comparing the evaporative cooling effects to the ‘bulk water’ cooling that has been consistently associated with natural heavy rainfall. Also, compare fresh and seawater without using live clutches of eggs.

5. **Does watering and shade combined maximize the cooling effect and produce all male clutches?**

By conducting a more comprehensive study using a combination of seawater, freshwater, artificial and natural shade cloth (without live clutches), the best possible strategy for maximising male hatchling production could be achieved.

6. **Where are the warmest nesting beaches and which of these need the most immediate intervention?**

Achievable by implementing data loggers on low-latitude beaches, model the predicted sex-ratios and make suggestions for management strategies of these high-risk rookeries. The investigations could also include other important variables e.g. erosion rates, sea level rise and anthropogenic impacts.
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REFERENCES


PERMITS & ETHICS APPROVAL

Access to a pink zone (Milman Island, Queensland) and experimental manipulations conducted on sea turtle clutches in Australia were covered by scientific permits held by QLD DES Conservation officer, Dr Ian Bell. This study was approved by the University of Queensland Animal Ethics Committee in the August 2018 meeting (AEC Approval Number: SBS/332/18).


Gyuris E. 2000. The relationship between body size and predation rates on hatchlings of the green turtle (Chelonia mydas): is bigger better? Pages 143-147 in: NJ Pilcher, MG Ismail (Eds.), Sea Turtles of...


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