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Pivotal temperature of Loggerhead sea turtles, *Caretta caretta*, from Dirk Hartog Island, Western Australia

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ABSTRACT

Investigating responses to different thermal conditions is important for understanding species with temperature-dependent sex determination, including loggerhead sea turtles. We determined the pivotal temperature for loggerhead turtles from Dirk Hartog Island, Western Australia, using constant temperatures in laboratory incubators. Eggs were transported from Dirk Hartog Island, Western Australia to Mon Repos, Queensland, at low temperatures to delay development and minimise movement induced mortality. The pivotal temperature for the Dirk Hartog Island loggerhead sea turtle rookery, is calculated to be 28.7 ± 0.4 °C (SD). The temperature is similar to the eastern Australian population of loggerheads, at the cooler end of a conservative global range. Incubators from 26.4 to 30.1 °C produced both male and female hatchlings, a range also consistent with eastern Australian loggerheads. Incubation periods ranged from 48 days at 31.6 °C to 77 days at 26.4 °C. The pivotal incubation duration to hatching is 63.3 ± 1.2 days (SD). This research indicates Dirk Hartog Island may be a predominantly male producing rookery with lower quantities of females expected.

❒ *Loggerhead sea turtle, Caretta caretta, Western Australia, pivotal temperature, temperature-dependent sex determination*.

Loggerhead sea turtles (*Caretta caretta* Linnaeus, 1758), like all species of sea turtles, have primary sex ratios determined by the incubation temperature during the middle third of development (Miller & Limpus 1981; Yntema & Mrosovsky 1980, 1982). The pivotal temperature is the calculated constant incubation temperature giving a 1:1 sex ratio of offspring (Dobbs *et al.* 2010; Mrosovsky 1988; Yntema & Mrosovsky 1982). Pivotal temperature is species-specific and even population-specific for sea turtles, ranging between breeding units of loggerhead turtles from 28.3 to 30 °C (Limpus *et al.* 1985; Chu *et al.* 2008; Yntema & Mrosovsky 1982; Mrosovsky 1988; LeBlanc *et al.* 2012; Marcovaldi *et al.* 1997; Mrosovsky *et al.* 2002; Fuller *et al.* 2013).

Pivotal temperature is an important biological parameter to quantify because when used in conjunction with nest temperatures, hatchling sex ratios under varying temperature regimes can be predicted (Chu *et al.* 2008), and when included in population models for examining population performance it adds to capacity for considering management options (Chaloupka 2003). Pivotal temperature is part of the critical baseline data required for determining a turtle population's vulnerability to climate and other environmental change (Hamann *et al.* 2008; Hawkes *et al.* 2009; Poloczanska *et al.* 2009). Dirk Hartog Island in Western Australia currently supports the highest density of loggerhead sea turtle nesting in the world (Reinhold & Whiting 2014). The Western Australian loggerhead population is a genetically distinct stock (FitzSimmons & Limpus 2014), with a nesting distribution spanning 5° of latitude from Shark Bay to the Muiron Islands (Baldwin *et al.* 2003), with major densities of the population concentrated on the southern end of their range at Dirk Hartog Island in Shark Bay (Reinhold & Whiting 2014). Woolgar *et al.* (2013) estimated pivotal temperature for the stock using samples from Dirk Hartog Island and Gnaraloo Bay, but their study was limited by only finding mixed sexes at one temperature and low sample sizes at temperatures other than 29 °C, which would inflate the standard error of the estimate.

The work presented herein further refines the pivotal temperature of the Western Australian population, using a robust design with samples spread over a 5 °C incubation range.

METHODS

Egg collection and transport. Eggs were taken from turtles nesting on Turtle Bay on Dirk Hartog Island in Shark Bay (25°29′55″S, 112°59′16″E), Western Australia. Fifty eggs were collected from each of four clutches laid on the night of 11 December 2009. Eggs were preferentially taken from clutches laid closer to the sea and thus potentially vulnerable to tidal inundation. Immediately following laying, each sample of 50 eggs was packed into a Styrofoam esky with the minimum of rotation (Limpus *et al.* 1979) and transferred within one hour of oviposition to two portable refrigerators (two clutches per refrigerator) set at 9 °C on board a boat. The eggs were then transported by boat to Denham (three hours away). In the latter part of the sea voyage from Dirk Hartog Island to the mainland, it was found that one of the refrigerators had lost power supply from the plug being knocked out of the socket during the rough crossing and the eggs had increased in temperature, but the extent of this is not known exactly. This failure to maintain the low temperature in one of the refrigerators may have led to low successes in the two clutches in this refrigerator. The eggs were kept in the refrigerators until packed into a chilled esky (each clutch in individual soft eskies with a damp towel, surrounded by bubble wrap and freezer gel packs) for commercial flights from Denham to Perth, Perth to Brisbane, Brisbane to Bundaberg and then transported by road to the incubation laboratory at Mon Repos Conservation Park in Queensland. Temperatures inside the eskies were recorded using a data logger and uncalibrated digital ProSciTech maximum-minimum thermometers with external sensors, and showed variation between different parts of the esky during air transit ranging from 5 °C to 15 °C. At the end of the journey, the freezer gel packs were an icy slurry. The total time from oviposition until being placed in incubators in Queensland was 34 hours.

Incubation. Upon arrival at the incubation laboratory, each sample of 50 eggs from the same clutch was subdivided to batches of ten eggs and each batch was placed into one of five incubators at 27, 28, 29, 30 and 31 °C. Each group of 10 eggs were set in a single layer (to negate thermal effects of the incubating eggs) within a 2 L plastic container, with 1300 g of sterilised sand and 100 mL of distilled water (after Limpus *et al.* 1985). The damp sand just covered the top of the eggs (so that the tops of the eggs could easily be accessed for visual inspection), and plastic film food wrap covered the top of the container to allow gas exchange, but prevented moisture escaping and hence evaporative cooling. The respective containers of eggs from each of the four clutches were set together on the middle shelf of each of the incubators.

The 28, 29 and 30 °C incubators were Labec Laboratory Equipment Pty Ltd., and the 27 and 31 °C incubators were Qualtex Solidstat (Watson Victor Ltd). Temperatures had been allowed to stabilise before arrival of the eggs. All incubators were housed in a purpose-built air conditioned room with an "airlock" entrance. The temperature during incubation was measured using three methods: temperature data loggers, Nata-certified (National Association of Testing Authorities, Australia) thermometers and maximum-minimum thermometers. Data loggers allowed collection of the greatest amount of data but their accuracy was not as high as the Nata-certified thermometers, so these were used to create a calibration for the data loggers. Maximum-minimum thermometers were used to check for excessive fluctuations in temperatures.

Temperature data loggers (iButton DS1922L, Maxim Integrated Products) were placed both in the air and within the sand of each incubator. These are manufactured to have an accuracy of ± 0.5 °C. They were initialised to log temperature hourly with a precision of 0.0625 °C. The air temperature data logger for each incubator sat on top of the plastic film of one of the containers of eggs in the front half of the incubator. Each sand temperature data logger was located at egg depth in the same container. Although some studies have used a correction factor to take the temperature from inside the egg itself (Mrosovsky 1988; Marcovaldi *et al.* 1997; Mrosovsky *et al.* 2002), we used the ambient sand temperature, as this is the environmental parameter that acts on sex ratios in the field.

Nata-certified thermometers were positioned with a plastic brace in the middle front of each incubator (to enable reading with the inner glass door shut), with the bulb suspended in the air just below the middle shelf. The Nata curves supplied with each thermometer certified accuracy of -0.04 – -0.07 °C. It was assumed that the temperature of the sand inside the egg containers did not differ from the incubator air temperature. Nata-certified readings were taken twice daily.

Maximum-minimum thermometers were placed on the upper shelf of the incubators.

Sexual differentiation occurs during the middle third of embryonic development, but for practicality we used the mean temperature during the middle third time period of incubation. The shortest incubation duration was different for each incubator, so the middle third was calculated separately for each (27 °C: days 23-47; 28 °C: days 22-45; 29 °C: days 20-40; 30 °C: days 18-37; 31 °C: days 16-32). For the middle third of incubation, the hourly temperature readings of the air and sand data loggers were compared with the twice daily Nata-certified thermometer readings. A correction factor added to the sand temperature data loggers was the mean difference between all of the Nata-certified readings and the same-time data logger output over the middle third of shortest incubation duration for each incubator. The incubation temperatures presented throughout are the Nata-certified temperatures at which the incubators stabilised during the middle third of incubation duration. Using Nata-calibrated data loggers gave hourly temperature recordings with an estimated accuracy of ± 0.5 °C.

Sexing hatchlings. When the embryos had straightened out, internalised their yolk sacs and left the eggshell, they were weighed and measured. This is stage 31 ("incubation period complete"), of embryonic development, and the stage at which the term "hatchling" is used to refer to the hatched turtles (Miller, 1985). As there is no current reliable method to sex live hatchling turtles, hatchlings were euthanased with 0.3 mL of Valabarb given via an 18 gauge needle directly into the brain through the foramen magnum, resulting in a chemical and physical pithing effect on the brain. Gonads were cut out of each hatchling or unhatched embryo and sent to a commercial laboratory for sectioning, mounting and staining on microscope slides. Each sectioned gonad was examined for tissue of ovary or testis and photographed. Sex was determined for each gonad by independent examination of the micrographs by three of the authors (CJL, DJL, LR). A subsample of ten hatchlings was lodged with each of the Western Australian Museum (registration numbers: 173019, 173020, 173021, 173022, 173023, 173024, 173025, 173026, 173027, 173028) and the Queensland Museum (registration numbers: QMJ92182, QMJ92183, QMJ92184, QMJ92185, QMJ92186, QMJ90950, QMJ92189, QMJ92190, QMJ92188, QMJ92187).

Calculating pivotal temperature and incubation duration. Pivotal temperature was calculated using the simple logistic equation, Y(Sex ratio)=100/(1+exp((-k*(X(Incubation Temperature)-x0)))), where k and x0 are fitted variables, with 95% confidence intervals estimated using Monte Carlo simulation using the predict *nls* function in R (R development core team 2016). Incubation duration was calculated as the time from being set in the incubators until turtles hatched and left their eggshell.

Ethics and permiting. This project was conducted under Regulation 17 Licence to take fauna for scientific purposes (number SF007142) and Regulation 18 Licence to export fauna interstate (number EA006170) issued by the (former) Western Australian Department of Environment and Conservation. Ethics approval was given by Queensland Animal Research (number DERM/2009/1009).

RESULTS

Temperature validation. The five incubators stabilised at 26.4, 27.4, 29.0, 30.1 and 31.6 °C (Nata-calibrated) during their respective middle thirds of incubation duration. The data loggers consistently read higher than the Nata-certified readings. In three of the incubators, the sand data loggers read higher than the air data loggers, and in two of the incubators the air data loggers read the highest. The average difference between the data loggers and the Nata-certified readings was + 0.9 °C (range + 0.3 – + 1.8 °C). All temperatures referred to throughout are Nata-calibrated.

Pivotal Temperature. Both sexes were produced within the lower ranges of temperatures investigated (26.4 °C to 30.1 °C), with the upper temperature of 31.6 °C producing exclusively female hatchlings (Fig. 1). The pivotal temperature was 28.7 ± 0.4 °C (SD). The transitional range of temperatures could not be calculated because we did not incubate at a temperature low enough to produce 100% males for all clutches.

There appeared to be clutch-specific differences in sex ratios at different temperatures (Fig. 2). Specifically, clutch WA77807 gave a higher ratio of females at the lower two incubation periods (26.4 and 27.4 °C) than the other clutches with all other clutches having no females at these temperatures (Fig. 2).

Neither hatched embryo SCL nor mass were significantly correlated with incubation temperature ($t_{(1,60)}$=1.44, P=0.15, see Table 1 for mean values) or sex (($t_{(1,60)}$= 1.36, P= 0.18)); female mean SCL= 3.95 mm (SD= 0.20, N= 36); male mean SCL= 4.02 mm (SD= 0.18, N= 26); female mean mass= 16.9 g (SD= 1.1, N= 36); male mean mass= 17.3 g (SD= 1.2, N=26).

Incubation duration. The pivotal incubation duration was 63.3 days (SD= 1.2), with shorter incubation durations producing more females and longer incubation durations producing more males (Fig. 3). At incubation durations of 55 days and less, only females were produced. Mixed sexes were produced from 56 days to 70 days.

Incubation duration was highly correlated with temperature (r= 0.96, P<0.001, Fig. 4). There were clutch-specific differences in incubation period, with the two clutches that incubated at 26.4 °C differing by seven days. It was the clutch with the shorter incubation period that produced the females at this temperature. At 27.4 °C incubation duration between clutches differed by only one day, at 29.0 °C all clutches hatched on the same day, at 30.1 °C hatching spanned two days between clutches, and hatching of the four clutches at 31.6 °C spanned four days (Table 1). All hatched turtles in each container of 10 from the same clutch reached stage 31 (Miller 1985) on the same day as each other, meaning there was no variation in incubation duration within clutches at the same temperature.

TABLE 1. Incubation success and sex ratio results for each incubation temperature, pooled for all four clutches.

Incubator temperature	26.4 °C	27.4 °C	29.0 °C	30.1 °C	31.6 °C
Incubation period - days (d)	70-77	67-68	60	55-57	48-52
Number of eggs incubated	40	40	40	40	40
hatched	13	16	12	13	17
unhatched	8	8	15	21	5
undeveloped	19	16	13	6	18
Hatch success	32.5%	40.0%	30.0%	32.5%	42.5%
Hatchling SCL (mean ± SD) mm	4.06 ± 0.20	4.03 ± 0.12	4.03 ± 0.18	3.91 ± 0.19	3.92 ± 0.21
Hatchling mass (mean ± SD) g	17.6 ± 1.3	17.2 ± 0.9	16.8 ± 0.8	16.6 ± 1.4	16.7 ± 1.0
Hatchling size sample size	13	16	12	13	17
Gonads sampled	14	24	18	28	20
not sexed	4	5	3	7	0
male	8	15	8	2	0
female	2	4	7	19	20
Sex ratio: % female	20.00	21.05	46.67	90.48	100.00

Hatching success. Hatching success was reasonable (mean = 56%) only for two of the clutches (64% hatching success for clutch WA77807 and 48% hatching success for clutch WA77813). The other two clutches had a poor hatch success (mean of 15%, comprising 22% from clutch WA41640 and 8% from clutch WA77815). This was associated with a severe fungal infestation that spread from dead eggs to adjacent incubating eggs resulting in death of a number of eggs mid-incubation. As a consequence of an overall low hatch success of 35.5%, the sex ratio results from all four clutches were pooled in Table 1. Individual-based differences were apparent and shown in Fig. 2 however, this may be biased by low sample size.

Identification of sexes. Gonads were able to be sexed from 85 of the eggs (62 out of the 71 hatchlings, and 23 out of the 57 unhatched embryos). Gonads were able to be differentiated from unhatched embryos as small as 28 mm straight carapace length (SCL).

DISCUSSION

Local and global context. The Dirk Hartog Island loggerhead population's pivotal temperature of 28.7 °C is similar to the 28.6/28.3 °C of eastern Australian loggerheads (Limpus *et al.* 1985; Chu *et al.* 2008). This pivotal temperature is also similar to Woolgar *et al.* (2013)'s 29.0 °C for Dirk Hartog Island and Gnaraloo Bay in Western Australia. With samples concentrated at 29 °C in the Woolgar *et al.* study, it was the only temperature at which mixed sexes were detected. The Woolgar *et al.* study also differed by using data loggers with a manufactured accuracy of ± 1 °C rather than Nata-calibrating temperature readings. The data loggers used for our study had a manufactured accuracy of ± 0.5 °C, and differed from the Nata-calibrated thermometers by an average of + 0.9 °C. These Australian studies combined show an overriding similarity in pivotal temperature for a species which has a discrete anti-tropical nesting range either side of the continent.

The 28.7 °C calculated here is towards the lower end of a globally conservative pivotal temperature band for loggerhead turtles,

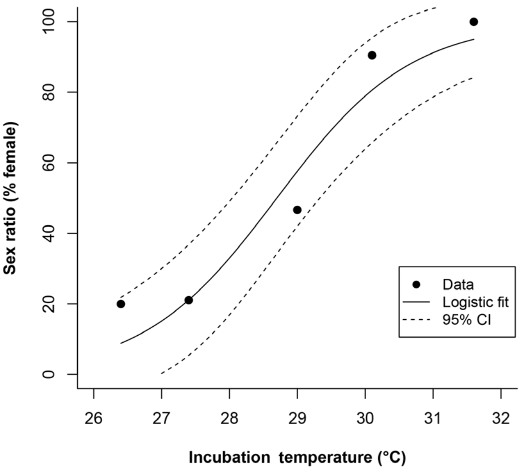

FIG. 1. Sex ratio of *Caretta caretta* hatchlings relative to constant incubation temperature, with logistic regression fit using the *nls* function in R with the logistic equation Y(Sex ratio) = 100/(1+exp((-k*(X(Incubation Temperature)-x0)))), where k and x0 are fitted variables. Results of eggs from four nesting females pooled. 95% confidence limits were calculated using Monte Carlo simulation using the predict *nls* function in R (R development core team 2016).

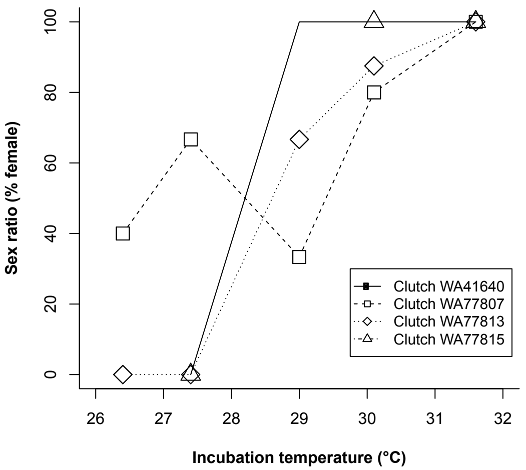

FIG. 2. Clutch-specific sex ratios of *Caretta caretta* hatchlings. Sample sizes for the clutches at 26.4, 27.4, 29.0, 30.1 and 31.6°C incubation temperatures respectively were NA, 3, 1, 5, 5 (Clutch WA41640); 5, 6, 8, 5, 4 (Clutch WA77807); 5, 8, 6, 8, 9 (Clutch WA77813); and NA, 2, NA, 3, 2 (Clutch WA77815).

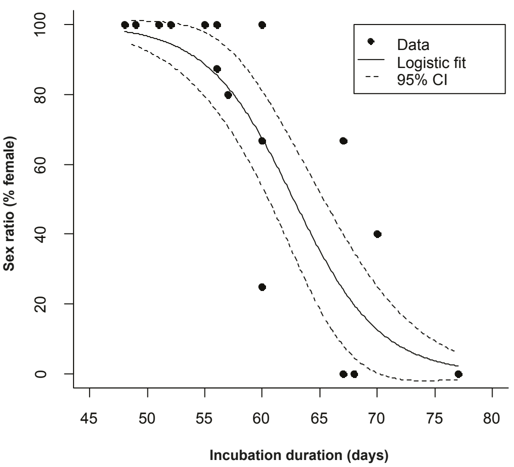

FIG. 3. Sex ratio of *Caretta caretta* hatchlings relative to incubation duration, with logistic regression fit using the *glm* package in R (R development core team 2016).

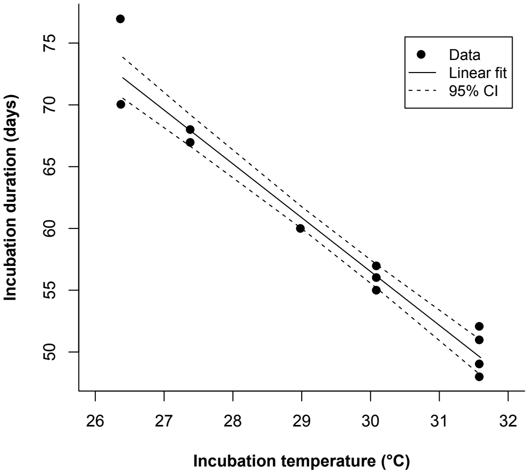

FIG. 4. Incubation duration in days from setting in incubators until hatched embryos left the eggshell, relative to incubation temperature. Solid lines show linear fit (incubation duration = -4.35* incubation temperature + 187.2); dashed lines show 95% confidence limits for linear fit.

differing by no more than a degree either side of 29 °C (Yntema & Mrosovsky 1982; Mrosovsky 1988; LeBlanc *et al.* 2012; Marcovaldi *et al.* 1997; Mrosovsky *et al.* 2002; Fuller *et al.* 2013).

Incubation duration. Pivotal incubation durations described within may be a useful field parameter to estimate the sex ratio production of nesting beaches. Marcovaldi *et al.* (1997) described a method for using pivotal incubation duration to estimate hatchling sex ratios. This method would enable clutch-specific identification of sex production in studies where incubation duration is recorded. To convert our measured incubation durations into incubation duration to emergence, which is used for in situ studies, the number of days it takes hatchlings to climb to the surface of the sand and emerge must be added. This has yet to be defined for Western Australian beaches, but can be estimated comparing the incubation duration and temperature correlations presented herein to incubation durations and temperatures measured for nests in situ. As Jribi *et al.* (2013) pointed out, though, this method may not be as accurate in the field as using pivotal temperature because the temperatures at the first and last thirds of incubation may confound the incubation duration. Field conditions in general mean rates of development may not occur consistently throughout incubation.

For loggerheads at the Dirk Hartog Island rookery, clutches which take from a week either side of 63 days to hatch are likely to have a mixed sex ratio. If clutches are consistently taking less than 56 days to hatch, they are likely to be producing 100% females.

The two-week span of incubation durations producing mixed sexes here is similar to that found on the east coast of the USA (Mrosovsky 1988), and approximately twice as broad as that estimated for loggerhead turtles in Brazil (Marcovaldi *et al.* 1997) and the Mediterranean (Mrosovsky *et al.* 2002; Fuller *et al.* 2013). It is, however, significantly less than the five-week span recorded by Limpus *et al.* (1985), but we did not incubate at a temperature that produced 100% males, so with a greater sample size, the mixed-sex incubation duration for Dirk Hartog Island may also span greater than two weeks. Individual clutch differences can contribute greatly to the spread of sex ratio vs incubation duration curves, and this should be kept in mind when using steep curves to infer sex ratios, as Godley *et al.* (2001) identified.

Transitional range of temperature (TRT). For the Dirk Hartog Island loggerheads, both males and females were produced over a range of 3.7 °C (this is likely to be greater if more clutches were incubated, and at lower temperatures). This is consistent with results from eastern Australian loggerhead turtles (Limpus *et al.* 1985), as well as loggerheads from the east coast of the USA (Girondot 1999). Even our coolest incubator, at 26.4 °C, produced some females. Males were still being produced at 30.1 °C, but very few males are expected to be produced if incubation temperatures exceed 31 °C. Woolgar *et al.* (2013) calculated a much narrower transitional range (0.7 °C) for loggerhead turtles at Dirk Hartog and Gnaraloo. The lower two temperatures for the Woolgar *et al.* study were similar to ours, but the samples of five eggs at each of these lower temperatures produced only male hatchlings.

Clutch-specific differences. Observed clutch-specific differences highlight the importance of using several different clutches when investigating temperature-dependent sex determination. The clutch from adult female WA77807 produced all of the females at the two lowest incubation temperatures, and without this we could have reported an artificially narrow transitional range of temperature and an artificially high pivotal temperature. The contribution of the clutch from this one nesting female increased the range of female-producing temperatures by 2.6 °C. Similar clutch-specific differences were also observed by Limpus *et al.* (1985), Mrosovsky (1988), Maxwell *et al.* (1988) and Wyneken & Lolavar (2015). Limpus *et al.*'s (1985) study on the Australian east coast loggerhead pivotal temperature had three out of four clutch samples incubated at 26 °C producing 100% males, yet one clutch sample produced three females. The same clutch also had a higher proportion of female hatchlings in the 27.5 °C incubator. Mrosovsky (1988) found one clutch from Florida produced significantly more males at middle and feminising temperatures than the other clutch from the same location. Wyneken & Lolavar (2015) found eight out of nine Florida clutch samples incubated at 31.4 °C produced 100% females, yet one clutch sample produced a male. At feminising temperatures, it may be these individuals which increase their relative contribution to the gene pool by producing males when others cannot.

Hulin *et al.* (2009) highlighted the importance of variability between individuals, and of the proportion of mixed nests being correlated to sample size. Whether Australian and eastern USA loggerheads have greater genetic variability between individuals with a broader overall TRT than loggerheads from other parts of the world, or whether this is an artefact of sampling, is yet to be determined. If the difference is real, it would give such populations a better chance of evolving to a changing climate.

Hatching success. The relatively low hatching success of two of the clutches was most likely caused by movement-induced mortality and the spread of a fungal infection. We can only speculate at which point the movement-induced mortality occurred: the clutches in one of the refrigerators warmed up during the rough sea voyage from Dirk Hartog Island to the mainland; the sea voyage itself may have caused far more violent and prolonged movement than turtle eggs can withstand; eggs towards the top of the esky may have warmed up more than those near the thermometer probes – the outside temperature on the day of transit was 41 °C at Shark Bay Airport and 37 °C at Perth Airport, where the outside of the esky was hot to the touch when it was unloaded from the plane; the clutch of WA41640 dipped to 5° C within the esky, but other parts of this same clutch may have been at a lethal cooler temperature, especially as the maximum-minimum thermometers were uncalibrated; parts of the clutch that reached 15 °C in the esky may have been at an even higher temperature, but this was the clutch with the best hatch success, and the clutches with reasonable and poor hatch success alternated within the esky; eggs could have been starting to develop as they warmed up awaiting placement in the incubators. Although this reduced sample sizes, it is unlikely to compromise the experiment as there is no known sex-biased mortality rate for turtle embryos.

Eggs were not randomised within their containers within the incubator, but all clutches were incubated over all temperatures. This means that whether the observed clutch-specific differences were due to the container they were placed in, or inherent to that clutch is unknown. Poor hatching success was attributed to both movement induced mortality and also spread of fungal infection from the non-viable eggs. If the eggs from the two clutches with poor hatching success were placed in containers with viable eggs from the other two clutches, hatching success for all clutches may have been reduced through spread of fungal infection.

In situ nest conditions. Our study was designed to determine how sex ratios change with laboratory-controlled temperatures, but constant temperature may not be the only contributing factor. The environmental conditions that influence the sex ratios of in situ nests are still being described.

Wyneken & Lolavar (2015) found high nest moisture to produce a greater percentage of male loggerhead hatchlings in Florida, even at temperatures above the pivotal. This research means that even if the sand at Turtle Bay was to rise to strongly female-biased dry-sand temperatures, cyclone rainfall at critical times of incubation could not only cool the sand, but enable the production of males because of increased moisture content. Our results are from sand set with a water content of 7.7%, to standardise with Limpus *et al.* (1985), but sand moisture was not tested throughout incubation.

Conservation implications. Recorded temperatures at Dirk Hartog Island from Trocini (2013) indicate that Dirk Hartog Island may be a predominantly male-producing rookery, but produces a mix of males and females across all northern beaches. Trocini (2013) measured sand temperatures at 50 cm depth with data loggers (temperature accuracy ± 0.5 °C) over five beaches at the rookery on Dirk Hartog Island during the 2007/08 season. The mean sand temperature for the middle third of incubation for Beach 1 was 28.3 °C ± 0.5 °C SD (n=4), for Beach 2 was 28.6 °C ± 0.4 °C (n=4), and for Beaches 3 and 4 were 29.1 °C ± 0.4 °C (n=4). Sand temperatures on Beach 5 were higher (n=5), but nests there were not monitored to correlate to the middle third period. These sand temperatures mean that at least for the 2007/08 season, all main nesting beaches along the northern end of Dirk Hartog Island were producing both male and female hatchlings. We borrowed some of these same iButton data loggers from S. Trocini for our laboratory study after her in situ research. When we Nata-calibrated these data loggers, temperatures had to be corrected down by an average of 0.9 °C. This means actual sand temperatures on Dirk Hartog Island may have been cooler than recorded by these data loggers, but still within a range that produces a mix of female and male hatchlings. Trocini (2013) gives in situ data for mean middle-third within-nest temperatures and incubation to emergence period by beach for 77 nests over four beaches over two seasons. Within-nest middle third incubation temperatures of about 28.5 °C had an incubation to emergence period of 63 days, and temperatures of 29.7 °C had an incubation to emergence period of about 59 days. The middle third of development can be kept at a constant temperature in an incubator, but in the field is subject to fluctuations, and in 2007/08 Cyclone Nicholas caused a cooling spike towards the end of this period. Trocini (2013) also found that the temperature inside nests was slightly higher and fluctuated less than sand temperatures at the same beach.

The increase in sand temperatures that Trocini (2013) found from west to east at this northern-facing rookery is likely a result of afternoon shading of the western-most beaches which are backed by 50 m-high limestone cliffs, flattening out towards Beach 5 in the east. The western end of Beach 1 supports a cooler pocket of sand backed by high cliffs to the west. Trocini (2013) also found that nests closer to the cliffs had lower temperatures than nests further out on the beach.

The sand at 50 cm depth on these beaches would have to rise by about 3 °C above 2007/08 temperatures to prevent males from being produced at that time of the season. Trocini's (2013) in situ nest data loggers were deployed mid-January, which means the middle third of incubation duration fell during February, which is the hottest month of the year. The 2007/08 season had a February mean maximum air temperature in Denham (72 km away) 0.4 °C below the mean (mean=32.0 °C), and a mean minimum temperature of 1.0 °C above the mean (mean=23.0 °C), for the 29 years from 1989 to 2017 (http://www.bom.gov.au/climate/data/). Clutches laid when the middle third fell earlier or later in the season would be even more likely to produce males. The temporal span of nesting is broad, with occasional turtle tracks being observed on Dirk Hartog Island throughout the year (R.I.T. Prince, pers. comm.).

Whereas the male-producing beaches are generally thought of as being at the cooler minor extremities of a population's nesting range (Marcovaldi *et al.* 1997; Mrosovsky 1988; Jribi & Bradai 2014), in the case of the Western Australian loggerheads, the cooler part of the range is also the major nesting site for this population.

In the absence of trees at Western Australian loggerhead rookeries, shading from the distinctive cliffs and the chance of cyclone rainfall could become important factors for the continuation of male hatchling production at Dirk Hartog Island in the advent of global warming.

Conclusions. This study provides an estimate of pivotal temperature with high accuracy for loggerhead turtles within the Western Australian stock. Pivotal temperature calculated herein and in situ temperature data collected by Trocini (2013) indicate that the rookery produces predominantly male turtles, with lesser numbers of female turtles also produced. The impact of potential thermal increases in sand temperature from global warming on sex ratios can now be quantified across a 5 °C temperature span.

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