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Key parameters describing temperature-dependent sex determination in the southernmost population of loggerhead sea turtles

Lorian Woolgar [a], Sabrina Trocini [b], Nicola Mitchell [a,*]

[a] School of Animal Biology, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia
[b] School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, WA 6150, Australia

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ABSTRACT

All marine turtles have temperature-dependent sex determination (TSD), and there is mounting evidence that climate change has increased sand temperatures at some rookeries, leading to pronounced biases in hatchling sex ratios. Quantification of the variation in the key parameters that describe TSD will be essential to our ability to predict the adaptive capacity of marine turtles, and for implementing conservation programs where necessary. Here we integrate field and laboratory data on the embryonic development of a little-studied population of loggerhead turtles (*Caretta caretta*, Linnaeus 1758) from Western Australia, which is home to a large rookery at the southernmost limit of the species' global range. We determined that the pivotal temperature that produces an equal sex ratio was 29.0 °C, centred within a transitional range of temperatures of 0.67 °C where both sexes are produced. For the first time for a marine turtle, embryonic development rates were modelled with a non-linear function, and were used to define the start and end of the thermosensitive period, where bipotential gonads differentiate into testes or ovaries. The period where gonads were sensitive to a masculinizing trigger occurred between 33 and 64% of development. In general, the TSD parameters for this southernmost population of *C. caretta* were similar to those estimated for other loggerhead populations, reinforcing previous findings that sex determination thresholds and processes are highly conserved.

1. Introduction

Sea turtles lack sex chromosomes and instead exhibit temperature-dependent sex determination (TSD) where incubation temperature triggers the development of either testes or ovaries in embryos (Valenzuela and Lance, 2004). All seven species have Type IA TSD where females are produced above a single temperature threshold. Sexual dimorphism in these reptiles is not externally evident until sexual maturity (Ceriani and Wyneken, 2008). To accurately determine the sex of juvenile sea turtles it is necessary to use laparoscopy, while in hatchlings sex can be determined by identifying histological or morphological characteristics of the gonads of sacrificed hatchlings (Ceriani and Wyneken, 2008) or using non-invasive methods such as hormonal assays of chorioallantoic fluid remaining in eggshells (Gross, 1995; Mrosovsky and Provancha, 1992). Alternatively, data on nest temperatures or the duration of incubation can be used to estimate hatchling sex (Booth and Freeman, 2006; Chu et al., 2008; Godley et al., 2001; Mrosovsky et al., 1999). These latter methodologies incur minimal labour and cost and are therefore a promising avenue for comparing hatchling sex ratios at different times and locations.

The accuracy of hatchling sex estimation from proxies such as nest temperature or incubation period is reliant upon knowledge of TSD parameters, which differ – often subtlety – with respect to latitude, climate and population (Ewert et al., 2004). Key TSD parameters include the pivotal temperature (Tpiv) where hatchlings are produced at a 1:1 sex ratio, and the transitional range of temperatures (TRT) that produce both sexes and beyond which either males or females are produced. Quantification of these two parameters is fundamental to the many approaches for modelling sex ratios of reptiles hatching in the wild, which range from correlative (e.g. Fuentes et al., 2009b) to mechanistic (e.g. Mitchell et al., 2008). Mechanistic methods require additional physiological data that is rarely reported: the rates of embryonic development across the full range of current (and future) environmental temperatures and the precise identification of the developmental stages where bipotential (undifferentiated) gonads are sensitive to temperature cues. This latter parameter – the thermosensitive period (TSP) – is broadly considered to fall in the middle third of incubation (Yntema and Mrosovsky, 1982), but is known to be as short as 4 d in a freshwater turtle (Pieau and Dorizzi, 2004). Hence the precise delineation of the TSP is necessary to identify the particular series of nest temperatures that were most influential in determining offspring sex.

The genetically distinct Western Australian (WA) population of the endangered loggerhead sea turtle (*Caretta caretta*) has been primarily surveyed for its nesting distribution; but its TSD parameters, population size and population status are undefined (Bowen et al., 1994; DEWHA,

* Corresponding author. Tel.: +61 8 6488 4510.
E-mail addresses: lorian.woolgar@gmail.com (L. Woolgar), S.Trocini@murdoch.edu.au (S. Trocini), nicola.mitchell@uwa.edu.au (N. Mitchell).

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Table 1
Sex ratios and incubation times (mean ± s.e.) from two series of experiments on *C. caretta* embryos collected from Gnaraloo Bay.

Experiment type	Back-switch 27 → 31 → 27 °C								Back-switch 31 → 27 → 31 °C								Tpiv 29.2 °C
Length of switch (d)	5	5	5	5	5	10	10	10	5	5	5	5	10	10	10	10	
Day of first switch	17	22	27	32	37	17	22	27	32	17	22	27	32	17	22	27	32	
First series[a] (4 clutches)																		
Number of eggs set	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	12
Number hatched	5	**5**	5	7	7	4	7	6	7	**6**	5	**6**	5	5	5	7	**7**	**8**
Percent female	60	**0**	60	57	43	75	57	50	86	**100**	80	**100**	100	80	80	86	**100**	**67**
Incubation duration (d)	53 ± 1	53 ± 1	53 ± 2	53 ± 2	53 ± 1	51 ± 1	52 ± 1	53 ± 2	53 ± 1	45 ± 1	44 ± 2	43 ± 1	43 ± 1	43 ± 1	45 ± 2	44 ± 1	44 ± 1	45 ± 1	49 ± 2
Mean temperature (°C)	N/A	27.8	27.8	27.8	27.9	28.0	28.2	28.2	N/A	30.2	30.4	30.6	29.8	30.5	30.3	30.1	N/A	30.0	29.09
Second series (5 clutches[b])																			
Box no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
Number of eggs set	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	12
Number hatched	4	5	4	5	4	4	4	3	**5**	3	**8**	**8**	5	1	6	4	2	**6**	**11**
Percent female	50	60	50	40	50	75	25	66	**0**	100	**100**	**100**	100	100	50	75	50	**100**	**64**
Incubation duration (d)	64 ± 1	65 ± 1	64 ± 1	62 ± 3	64 ± 1	62 ± 0	63 ± 1	62 ± 2	64 ± 3	49 ± 1	50 ± 1	49 ± 1	52 ± 1	49 ± 0	52 ± 2	51 ± 1	51 ± 1	51 ± 1	53 ± 2
Mean temperature (°C)	27.1	27.1	N/A	27.0	27.0	27.3	N/A	27.1	27.2	30.1	30.2	30.3	30.1	29.9	29.6	N/A	29.7	29.9	29.02

N/A iButtons in these boxes stopped recording partway through incubation, hence only temperature records for complete incubation periods are included.
Sex ratio and sample size data in bold type indicate the experiments that were used in fitting the TSD model (refer to Section 2.4).
[a] Incubators were housed in a laboratory for the first series of back-switch experiments and temperature control was compromised when nighttime room temperatures rose above 25 °C during a heatwave when central air conditioning was switched off overnight. The incubator programmed at 27 °C rose above the Tpiv when room temperatures exceeded 22 °C, meaning that other periods aside from the back-switch window could potentially have influenced hatching sex. The incubators set to the Tpiv and 31 °C were able to maintain their set points throughout these experiments.
[b] One clutch was infertile.

2010; Prince, 1998). It is important to bridge this knowledge gap, as collectively the WA population represents the third or fourth largest in the world, with 1000–2000 females nesting annually (DEWHA, 2010). As TSD parameters are highly conserved among loggerhead populations (Ewert et al., 1994), we hypothesized that pivotal temperatures of the WA population (the southernmost population of nesting *C. caretta*) would be similar to or lower than those delineated for other populations (Kaska et al., 1998; Marcovaldi et al., 1997; Mrosovsky, 1988; Mrosovsky and Provancha, 1992; Mrosovsky et al., 2002; Yntema and Mrosovsky, 1982). Here we determine the pivotal temperature of the WA population and parameterize a non-linear development rate function using data on incubation periods under laboratory and field conditions. Finally, we use a series of back-switch experiments to improve our understanding of the thermosensitive period in *C. caretta*. Taken together, these new data provide the foundation for assessing the spatial and temporal distribution of hatchling sex ratios in this globally significant population.

2. Material and methods

2.1. Study sites, egg collection and transport

The eggs of *C. caretta* used in our incubation experiments were collected over two years from two major rookeries: Gnaraloo Bay on the WA mainland (−23.82618°S, 113.52629°E) and Turtle Bay (−25.49827°S, 112.98719°E) on Dirk Hartog Island, whilst field temperature data were obtained for two nesting seasons (2006–7 and 2007–8) from Turtle Bay as well as from Bungelup Beach (−22.282331°S, 113.831570°E) in Cape Range National Park. A recent genetic study has confirmed that WA loggerhead turtle populations are one genetic stock (Pacioni et al., 2012) and satellite tracking and tagging data show that sea turtles from different nesting populations share the same foraging grounds (Baldwin et al., 2003; Mau et al., 2012). It is therefore justified to pool data obtained from different WA rookeries.

Eggs of *C. caretta* used in back-switch and pivotal temperature experiments (see below) were collected over two separate trips between 5th December 2010 and January 25th 2011 to the rookery at Gnaraloo Bay. Here, nesting is unpredictable and infrequent, with fewer than 50 nesting females per week during peak nesting in mid-January (Hattingh et al., 2010). We collected 39–40 eggs (about 30% of a clutch) from 4 or 5 just-completed nests on each occasion and the remaining eggs were re-covered and left to hatch.

The eggs were transported from the beach to nearby Gnaraloo Station and held in damp vermiculite within a portable refrigerator (ENGEL 40L; MT45F-S) set to 8 °C to facilitate eggs remaining viable during transport (Harry and Limpus, 1989). Eggs were relocated by road and air to the University of Western Australia in Perth within 44 h of oviposition.

Samples of eggs from four clutches were also collected from Turtle Bay on Dirk Hartog Island in January 2012. Turtle Bay is the major *C. caretta* rookery in WA, with hundreds of females nesting on some nights during the peak of the breeding season (Prince, 1998). These eggs were used in an unrelated experiment conducted by another researcher in our laboratory; 192 of these eggs (48 eggs from each clutch) were incubated at 29 °C, with the exception of a brief 1–3 h heat shock at 34 or 36 °C that occurred at either day 25 or day 50 of incubation (J. Tedeschi, unpublished data). The eggs were then allowed to hatch. Because the heat shock was very brief (0.1 to 0.2% of total development time) and occurred outside of the TSP (see results) we could utilize this experiment to assess the sex ratio arising from incubation at 29 °C.

2.2. Incubation experiments

Over the two field trips eggs were collected from nine nests at Gnaraloo Bay, but one clutch collected on our second trip failed to develop (Table 1). Hence, we incubated approximately 160 eggs (40 eggs