Chapter 4 – Climate Change

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Executive Summary

Contemporary declines of marine turtle populations are expected to be exacerbated through a number of processes associated with anthropogenic climate change. A rapid increase in ambient temperatures will adversely impact all life history stages of marine turtles, with the embryonic stage being the most vulnerable due to narrow physiological thresholds and their inability to avoid excess heat during incubation. Incubation temperatures now often occur close to the upper thermal limits of the embryo. Further, incubation temperatures are important drivers of population demography, as marine turtles have a temperature-dependent mechanism of sex determination (TSD), where females are produced at warmer temperatures and males are produced at cooler temperatures (type-MF TSD). As a consequence, increasing temperatures have been predicted to lead to rookery feminization and increased embryonic mortality, and this is already being observed at many marine turtle rookeries within and outside of Australia.

The effects of climate change will vary over regional scales, with geographical and temporal variation in nesting behaviours expected to influence the severity of these impacts. Here, by incubating eggs from various rookeries across a range of temperatures, we find that the parameters defining the TSD reaction norm vary between and within two species of marine turtle in the Kimberley region of Australia, and that the differences are correlated with temperatures at typical nesting depths at their respective rookeries. The resulting sex-ratios we predict using a mechanistic niche model also differ between rookeries, with high female production at Eighty Mile Beach (flatback turtles) and the Lacepede Islands (green turtles), while winter nesting populations (flatback turtles) and Cassini Island (green turtles) appear to produce more balanced sex-ratios. We present the details of our mechanistic model and show how it can be used to explore the effects of increasing ambient temperature on the embryonic life stage. Winter nesting rookeries are at the highest risk from climate change, as their nesting phenology can only shift slightly to avoid highly female-skewed sex-ratios. Under the most severe climate change scenario for 2070, the narrow temporal window where embryonic mortality could be avoided disappears, very likely meaning that no current marine turtle rookeries in the Kimberley would be viable.

1. Introduction

Marine turtle populations are declining globally as a consequence of anthropogenic activities, with contemporary declines attributed to processes such as habitat loss and by-catch through fisheries (Mazaris et al. 2017). These declines will be exacerbated by a number of processes associated with anthropogenic climate change. However, there is expected to be considerable variation in the magnitude of climate change effects at regional and local scales (Hawkes et al. 2009). For example, in Australia mean air temperatures have increased by approximately 1°C since 1910, with extreme heat events also increasing in frequency and severity. This trend is expected to continue over the coming century (BOM and CSIRO 2016). The impacts of these temperature changes on marine turtles will vary with their life history stage due to differences in habitat requirements and physiological thresholds between adult and embryonic stages (Howard et al. 2014; Howard et al. 2015; Pike 2014; Telemeco et al. 2013). Additionally, the embryonic stages of oviparous species that lack parental care, such as marine turtles, are particularly vulnerable to the effects of climate change as they remain in the nest for extended periods prior to hatching and are unable to 'behaviourally buffer' themselves against suboptimal nest environments (Fuentes et al. 2011; Hawkes et al. 2009; Tedeschi et al. 2016).

Temperature increases are of profound importance when considering the development of marine turtle embryos, as all extant species possess a temperature-dependent mechanism of sex determination (TSD; Yntema and Mrosovsky 1980). All species of marine turtle have a male-female pattern of TSD, where female phenotypes are produced at higher temperatures, and males are produced at lower temperatures (Wibbels 2003). Two parameters are typically used to characterize and compare TSD reaction norms between and within species (Hulin et al. 2009). The first is the transitional range of temperatures (TRT), which describes the range of temperatures that produce both sexes, with incubation temperatures above or below this range producing either males or females respectively (Godfrey and Mrosovsky 2006; Hulin et al. 2009; Mrosovsky and Pieau 1991). The TRT varies from abrupt transitions between male and female-producing temperatures, to broader ranges of temperatures that produce mixed-sexes (Ewert et al. 2004), with larger TRTs suggesting greater resilience and potential capacity to adapt to climate change (Patrício et al. 2017). Within the TRT is the 'pivotal temperature' (TPIV), defined as the constant incubation temperature that produces a balanced sex-ratio. The TPIV generally lies between 29-30°C in marine turtles (Wibbels 2003; Yntema and Mrosovsky 1982), but can also occur outside of this range (e.g. Howard et al. 2015). Resolving the TRT and TPIV of marine turtle populations allows for accurate prediction of sex-ratios in natural nests, and the information can be used to enhance the reproductive output of a population for conservation purposes (Wibbels 2003).

Due to the narrow temperature ranges associated with thermal traits in marine turtles, even subtle changes in incubation temperature will have a significant influence on the resulting sex-ratios (Hewavisenthi and Parmenter 2002), with climate change anticipated to lead to widespread rookery feminization, potentially threatening population persistence (Fuentes et al. 2010; Hulin et al. 2009; Nelson et al. 2004). Many rookeries already show highly female-skewed primary sex-ratios (e.g. Broderick et al. 2000; Godfrey and Mrosovsky 1999), and identifying the cause of the skew requires that TSD parameters are resolved not only the species level, but also at a population level. Further, temperatures in marine turtle nests are often close to the upper thermal limits for successful embryonic development, and lethal temperatures may be reached more regularly under climate change (Pike 2014). Early studies suggested that temperatures above 33-35°C decreased embryonic survival (Ackerman 1997; Miller 1997), however nest temperatures often exceed these limits, towards the end of development when metabolic heating increases (Broderick et al. 2001; Howard et al. 2014). Hence, changes in air (and/or sea surface) temperatures may result in beaches that are currently suitable for nesting becoming too hot for successful incubation, and similarly, unsuitable beaches may ultimately become new rookeries (e.g. Butt et al. 2016).

Modelling approaches are an important tool for developing management strategies to mitigate the impacts of climate change on reptiles with TSD (e.g. Botkin et al. 2007; Mitchell et al. 2008). Many earlier studies on the impacts of climate change on marine turtle rookeries employed correlative models, where climatic variables such as air temperatures, sea surface temperatures and rainfall are correlated with sand temperatures and the associated sex-ratios and mortality (Fuentes and Porter 2013). However, a mechanistic modelling approach allows predictions to be made outside of the range of typical environmental variables, which is not possible for a correlative approach (Buckley et al. 2010; Kearney et al. 2010; Mitchell et al. 2008). Mechanistic models incorporate environmental data in the form of gridded climate surfaces or point data derived from weather stations (Kearney et al. 2014b), and predict soil temperatures based on laws of thermodynamics. These predictions are then used to drive a model of embryonic development that can be customised with population-specific physiological parameters to allow predictions of hatching sex-ratios and mortality (e.g. Mitchell et al. 2008). The gridded climate surfaces can also be adjusted to simulate future climate change scenarios, and the developmental models can be altered to consider the effects of changed nesting phenology, or nest depth (e.g. Mitchell et al. 2008, Mitchell et al. 2016). These adjustments allow the exploration of impacts of climate change on sex-ratios and mortality at individual rookeries.

Six of the seven extant species of marine turtle forage in water waters along the Kimberley coast in Western Australia, with the Kemp's Ridley marine turtle (*Lepidochelys kempii*) being the only exception. Five of these species also nest on island and mainland beaches throughout the Kimberley, with globally significant rookeries of flatback *(N. depressus*) and green (*Chelonia mydas*) turtles (see Limpus 2009). Despite this high density of marine turtles, relatively few studies have focused on nesting populations, and as a consequence, there are substantial knowledge gaps in the basic biology, ecology and physiology of these populations. This is particularly important as both species are listed as Vulnerable under the Australian Environment Protection and Conservation Act (EPBC 1999), and *C. mydas* is listed as Endangered under the International Union for Conservation of Nature Red List (IUCN 2015) while *N. depressus* has been listed as Data Deficient. An understanding of how nesting sites may change under different climate change scenarios has been identified as an important consideration for threat abatement and recovery of marine turtle populations (Hamann et al. 2007). This is particularly important in the Kimberley, where *N. depressus* rookeries show a distinct nesting peak in the summer months (November to January) south of the Lacepede Islands, while nesting occurs primarily in winter and spring (August to October) north of this location (Chapter 2 of this report; Whiting et al. 2008).

Due to the remoteness of most Kimberley rookeries, reliable predictions of sex-ratios, hatching success, and the impacts of climate change are critical for the development of conservation and management strategies. Here we describe differences in TSD patterns between two populations of flatback turtles (*N. depressus*) and one population of green turtle (*C. mydas*), and integrate this information within a mechanistic model to predict current sex-ratios. We also model the effects of increasing ambient temperature expected under climate change scenarios for 2030 and 2070, and show how hypothetical changes in nesting phenology could mitigate high embryonic mortality and strongly feminised hatching sex ratios.

2 Materials and Methods

Ethics statement

All procedures described in this report were reviewed and approved by the University of Western Australia's Animal Ethics Committee (RA/3/100/1323; RA/3/100/1145) and collection permits were issued by the Western Australian Department of Parks and Wildlife (SF008844, SF010081, SF009952, SF010620 and 01-000005-4).

Study sites

Marine turtle rookeries throughout the Kimberley were visited during nesting seasons between 2013 and 2016. This included both summer- and winter-peaking rookeries of *N. depressus* and summer-peaking rookeries of *C. mydas*. Rookeries were selected based on aerial surveys, previous studies and Indigenous Knowledge, and their locations are shown in Figure 1.

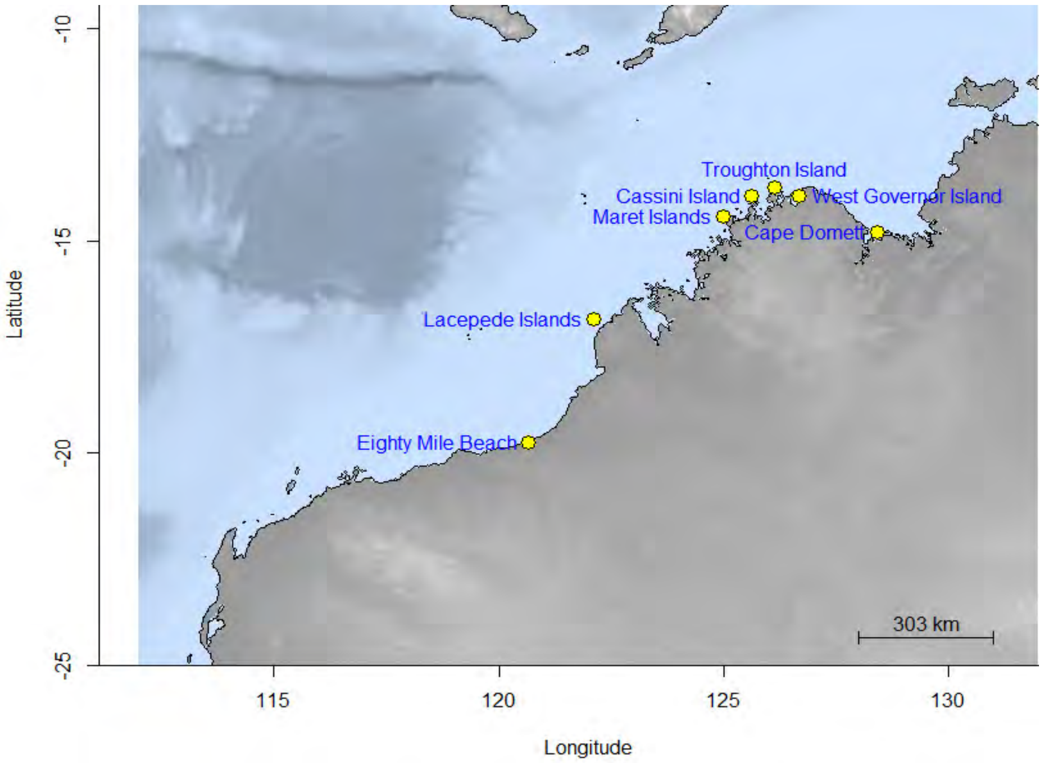

Figure 1. Marine turtle rookeries targeted for egg collection for physiological experiments. Due to low nesting numbers at a number of sites, egg were collected from larger *N. depressus* rookeries at Eighty Mile Beach, Cape Domett, and West Governor Island, while *C. mydas* eggs were collected from West Lacepede Island (Table 1).

Table 1. Collection sites and dates for each rookery in the Kimberley. Number of eggs refers to the total number of eggs collected from that rookery, and number of females describes the number of nesting females the eggs were collected from.

Rookery	**Species**	**Latitude**	**Longitude**	**Collection date(s)**	**No of eggs**	**Number of females**
Cape Domett (CD)	N. depressus	-14.798	128.415	Aug 2012, Aug 2014, Aug 2015	614	24
Eighty Mile Beach (EMB)	N. depressus	-19.753	120.672	Nov 2014, Nov 2015	300	17
West Governor Island (WGI)	N. depressus	-13.949	126.684	Aug 2016	62	1
West Lacepede Island (LI)	C. mydas	-16.853	122.125	Dec 2015	300	10

Egg collection

Eggs were collected during the process of oviposition where possible, with the back of the egg chamber dug away and eggs caught with a bowl, or by hand, as they were laid. We attempted to randomly collect eggs to sample across the entire clutch – with eggs collected at the start of oviposition, in the middle of oviposition and at the end of the process. In some cases we encountered a nesting female that had already initiated oviposition. In these events, we dropped a string attached to a relatively heavy object (e.g. a metal washer) into the egg chamber and tied the other end to a stake or held onto it. Once the female had covered the nest, we carefully excavated the top of the nest within three hours, removed the top 20 or 30 eggs, and re-covered the remaining clutch. In all cases we expected the hatching success of the remaining eggs to reflect those of undisturbed nests (Koch et al. 2007; Stancyk et al. 1980). Collections preferentially targeted nesting females laying at sub-optimal positions on the beach, included nests below the high tide mark and high up in dunes.

Care was taken during collection not to rotate or jostle the eggs to avoid movement induced mortality (see Limpus et al. 1979). The top of each egg was individually labelled using a soft (4B) pencil, which later assisted in maintaining the correct orientation during incubation. All eggs were transferred within three hours of collection to a portable refrigerator (models ENGEL MT45F-S and MT60F-G4P with 40L and 60L capacity respectively) cushioned and covered with damp vermiculite (~1 L H2O kg-1) and cooled to 7-15°C to arrest development during transport (Harry and Limpus 1989). Eggs were transported from the nesting beaches to commercial aircraft via boat, vehicle and or small aircraft. Temperature was continuously monitored using K-Type Thermocouples (MAKE) until eggs were repacked into ice chests for commercial flights, at which point the temperature within the ice chest was also logged using iButtons (DS1921G; accuracy ±1°C; resolution 0.5°C) for the duration of transport. In all cases, eggs were transported back to the Crawley campus of The University of Western Australia within 96 hours of collection.

Incubation experiments

Upon arrival to the laboratory, eggs were weighed (± 0.01g) and their diameter was measured using digital callipers. Eggs were then randomly allocated to plastic containers, with 5-10 eggs being allocated to each container depending on the collection trip. Incubation containers were half filled with washed white sand and covered with a plastic lid to ensure high humidity within the box during incubation. Containers were partially sealed to allow for respiratory gas exchange, and therefore required weekly re-wetting of sand via light spraying of deionized water. Containers were placed at target temperatures (see below) inside incubators (Steridium models i140 and i500) and viability was initially determined by the development of a ‘white spot’ (Yntema 1981) which appears on the top of the eggs within the first few days of development. Eggs were monitored every 2-3 days, then daily after day 40 of development, and then several times per day once hatching within a container commenced. Containers were rotated within the incubators to account for fine-scale heterogeneity at different positions.

Natural incubation temperatures differed slightly between rookeries, so we used subtly different sets of incubation temperatures for each rookery, including one daily fluctuating temperature regime to simulate more natural incubation conditions (Table 2). Temperatures inside incubation containers were measured with Thermochron^{TM} iButtons (DS1921H model). Eggs were incubated for the entirety of development, the end point of which was characterized by the hatchling breaching the egg shell with the egg tooth (i.e. "pipping"; Miller and Limpus 1981). This occurred for all eggs with the exception of a small subset of the 29°C treatment (which were used a heat shock experiment not reported here). Hatchlings were euthanised within 48 hours of pipping by intra-muscular injection of Zoletil® (Provet; 10mg/kg) followed by pithing, and cerebral dislocation. Labelled hatchlings were preserved in 10% neutral buffered formalin until dissection.

Table 2. Target laboratory incubation temperatures for each rookery.

Rookery	**Species**	**Laboratory incubation temperatures (°C)**	**Eggs set per temperature**
Cape Domett*	N. depressus	28.0, 28.4, 28.8, 29.0, 29.2, 32±5	60, 60, 60, 78, 60, 60
Eighty Mile Beach	N. depressus	28.0, 28.6, 29.0, 29.4, 29.8, 32.0, 30±5	15, 48, 48, 78, 48, 15, 48
West Governor Island	N. depressus	28.0, 29.0, 30.0, 31.0, 32.0, 30±5	10, 12, 10, 10, 10, 10
Lacepede Islands	C. mydas	28.0, 29.0, 31.0, 32.0, 30±5	60, 60, 60, 60, 60

* N.B. for Cape Domett, field data sourced from Stubbs *et al*. (2014) were included in subsequent analyses.

Sex identification through histology

Marine turtles show no external sexual dimorphism until sexual maturity (Wibbels 2003), which occurs at 15-50 years of age, depending on the species (Chaloupka and Musick 1997). As such, histological examination of hatchling gonads is required to reliably assign their sex. Gonads are fully differentiated at around 80% of embryonic development, and are small (<500µm) and attached to the kidney. Entire left kidneys with gonads attached were removed from formalin-fixed specimens by making abdominal incisions through the plastron, before being transferred to labelled histology cassettes and stored in 70% ethanol. Kidneys were then prepared as paraffin-embedded sections and stained with haematoxylin and eosin for light microscopy (Stubbs et al. 2014). Individuals were classified as either male, female or unknown based on the criteria by Ceriani and Wyneken (2008) and Ikonomopoulou et al. (2012) where males were characterized by the presence of seminiferous tubules in the medulla and a regressed cortex, while females had a relatively disorganised medulla with a thick, well developed cortex. The identification process was repeated three times for each sample without reference to previous assignment, and a repeatability analysis was performed to determine the reliability of sex assignment. Any specimen where gonads were absent or unable to be distinguished were re-sectioned and re-examined until sex could be determined. If this was still not possible, then entire right kidneys from that individual were removed and the process was repeated.

2.1 Resolution and comparison of TSD parameters

We used the EMBRYOGROWTH package for R (v6.5.8; Girondot and Kaska 2014) to determine the relationship between incubation temperature and sex ratio. This method allows for up to six threshold models to be fitted to the data (Logistic, Hill, Richards, Double-Richards, GSD and Hulin) using maximum likelihood, and requires at least two temperatures producing mixed sexes (Girondot 1999a). AIC criteria are then used to select the model with the strongest fit (Girondot 1999a; Godfrey et al. 2003; Hulin et al. 2009). All models are based on equations with a sigmoidal shape, with differences between models based around the asymmetry of the inflection point (Hulin et al. 2009). For each rookery, the model with the best fit to the data was selected and used to estimate TSD parameters.

The first publication generated from Project 1.2.2 (Stubbs et al. 2014) focused on identifying the thermosensitive period (TSP) for *N. depressus* , which occurred between 43 and 66% of development, consistent with other studies that state that the TSP occurs within the middle third of incubation (Mrosovsky and Pieau 1991). Hence, for each incubation treatment we calculated the average temperatures during the TSP and used this value during model fitting, with the exception of Cape Domett, where constant temperature equivalents (CTEs; Georges 1994) derived from field sex ratio data collected by Stubbs et al. (2014) were also included for TSD model fitting. For simplicity, we also assumed that the TSP of *C. mydas* fell between 43 and 66% of development, although this has not been demonstrated experimentally.

The output parameters from the best fitting maximum-likelihood model were used as priors for Markov chain Monte Carlo (MCMC) iterations using the Metropolis-Hastings algorithm for sex-ratios to compare TPIV and TRT correlations. To determine whether there was variation in TSD parameters between rookeries, we combined all data into a single ‘meta-dataset’, as well as compiled data from *N. depressus* rookeries. The TSD model fittings described above for each population were then applied to these two combination datasets. AICc comparisons were utilized to determine whether the combined datasets or the separate datasets were the best fitting model for the data. Where separate models were identified as the better fit, it was assumed that there were differences between rookeries. The outputs from the MCMC analyses were also used to compare pivotal temperature and transitional range of temperatures between rookeries by calculating a probability differential.

2.2 Fitting nonlinear development rate functions for each population

The effect of temperature on incubation duration was tested using a generalized linear mixed model (GLMM) for each rookery, where maternal ID and incubation box were included as random factors, using the LME4 package for R (Bates et al. 2014). Development rate (the inverse of incubation time), expressed as a function of temperature, was calculated using the program DEVARA (Dallwitz and Higgins 1992). This program allows for non-linear curve fitting and incorporation of incubation times that result from fluctuating or variable temperatures (e.g. Mitchell et al. 2008; Neuwald and Valenzuela 2011). The average incubation time (in days) and hourly temperature data from constant and fluctuating incubations were used as model inputs. DEVARA fits a nonlinear model expressing development rate (r_a) as a percentage per day, as a function of temperature (T):

$$r\_a = b\_1 \ 〖10〗\text{\textasciicircum}(-v2(1-b_5+b_5v2)) \qquad (1)$$

where

$$u = (T-b\_3)/(b\_2-b\_3\ )-c\_1 \qquad (2)$$

$$v = (u+e\text{\textasciicircum}(b\_4\,u))/c\_2 \qquad (3)$$

$$c\_1 = 1/(1+0.28b\_4+0.72\ ln(1+b\_4\ )) \qquad (4)$$

and

$$c\_2 = 1+b\_4/(1+1.5b\_4+0.39\ 〖b\_4)〗\text{\textasciicircum}2 \qquad (5)$$

These parameters fitted by DEVARA define the maximum development rate (b_1) and its corresponding temperature (b3), and the temperature at which development approaches zero (b_2). The asymmetry and steepness of the curve are controlled by parameters b_4 and b_5, which were fixed at 6 and 0.4 respectively, as is recommended when development rates at extreme temperatures are unknown (Dallwitz and Higgins 1992), as was the case here.

Microclimate model

To model temperatures of beach sand we used NicheMapR (Kearney and Porter 2016) - a version of the Niche Mapper^{TM} mechanistic (process-explicit) microclimate model (Porter and Mitchell 2006) adapted to operate within the R environment (R Development Team 2016). The microclimate model component of NicheMapR utilizes climate data and other inputs such as the physical properties of beach sand to calculate sand temperatures at any specified depth, via a one-dimensional partial differential equation that simultaneously solves heat and mass balance equations (Kearney et al. 2014a; Kearney and Porter 2016; Kearney et al. 2014b; McCullough and Porter 1971; Porter et al. 1973; Porter et al. 2002). A summary of the modelling framework employed in this project is shown in Figure 2, and further details of the model assumptions are outlined below.

We modelled sand temperatures at 31 island and mainland beaches throughout the Kimberley region of Western Australia, extending from Pardoo Station, north of Port Headland, to Cape Domett, close to the Northern Territory border (Figure 3). Five sites were selected as focal beaches based on the abundance of nesting females; these were Cape Domett, Cassini Island, Eighty Mile Beach, West Lacepede Island, and West Governor Island.

Topographic parameters

The topographic parameters of the microclimate model, which includes measures of elevation, slope, aspect, and horizon angle in order to calculate the clear sky radiation, were kept constant for all simulations of sand temperatures. Foe ease of comparison we assumed that all beaches were flat (slope = 0) and at sea level (elevation = 0 m), although in reality beach slopes were likely between 1-5 degrees. Clear sky solar radiation was predicted based on the specified point location (latitude, longitude) using algorithms described by McCullough and Porter (1971). Scatter from atmospheric particles was additionally accounted for by computing aerosol attenuation using the Global Aerosol Data Set (GADS) (Koepke et al. 1997). All models were run assuming no beach shading, as a related study suggested that running soil temperature models with a shade component reduced the accuracy of the simulations (Carter et al. 2015), but we acknowledge that some sections of Kimberley beaches can be shaded by vegetation and/or rock ledges. We ran the models with the 'runmoist' parameter equal to 1 and 0 to determine if using a soil moisture model within the calculations had an impact on the sand temperature predictions.

Soil property inputs

With the exception of sand reflectance, we modelled all sites with a set of general soil parameters (Kearney et al. 2014a; Kearney et al. 2014b) rather than 'fine tuning' each site, as customised soil inputs do not tend to improve the accuracy of the microclimate model predictions (Carter et al. 2015). The general soil properties included a thermal conductivity of 2.5 W mC-1, a density of 2560 kg m-3, and a specific heat of 870 J kg-1 –K, and assumed a bulk density of 1400kg m-3. The colour of beach sand at marine turtle rookeries influences nest temperature (e.g. Hays et al. 2001) and NicheMapR's microclimate model allows this parameter to be adjusted on a site-by-site basis to account for its effect on sand temperatures. As such, surface sand samples were collected from many Kimberley marine turtle nesting beaches, and their visual and non-visual reflectance was measured using methods described in Stubbs et al. (2014), or were inferred through interpolation. Briefly, where reflectance was measured directly, solar reflectance was measured in the wavelength range of 300 – 2,100 nm using two spectrometers (Ocean Optics USB2000 for the UV-visible range and NIRQuest for the NIR range) and two light sources (Ocean Optics PX-2 pulsed xenon light for the UV-visible range and HL-2000 tungsten halogen light for the visible-NIR range) all connected with a quadrifurcated fibre optic. To estimate the reflectance of the remaining samples, all sand samples were photographed under a dissection microscope (Olympus SZ-CTV) using Olympus DP20 digital camera with consistent exposure settings and lighting (Figure A3.1). A software program that operates in the Java environment (http://matkl.github.io/average-color/) was then used to estimate the average colour of each image in the RGB channels. A linear regression analysis was applied to determine the correlation of each of the channels of the spectrum with the measured reflectance values. The red colouration explained 84.3% of the reflectance values ($p < 0.01$) and was subsequently used to estimate the reflectance of the remaining samples with the linear equation: $REFL=0.3824 \times RED-7.1687$ (Figure A3.2).

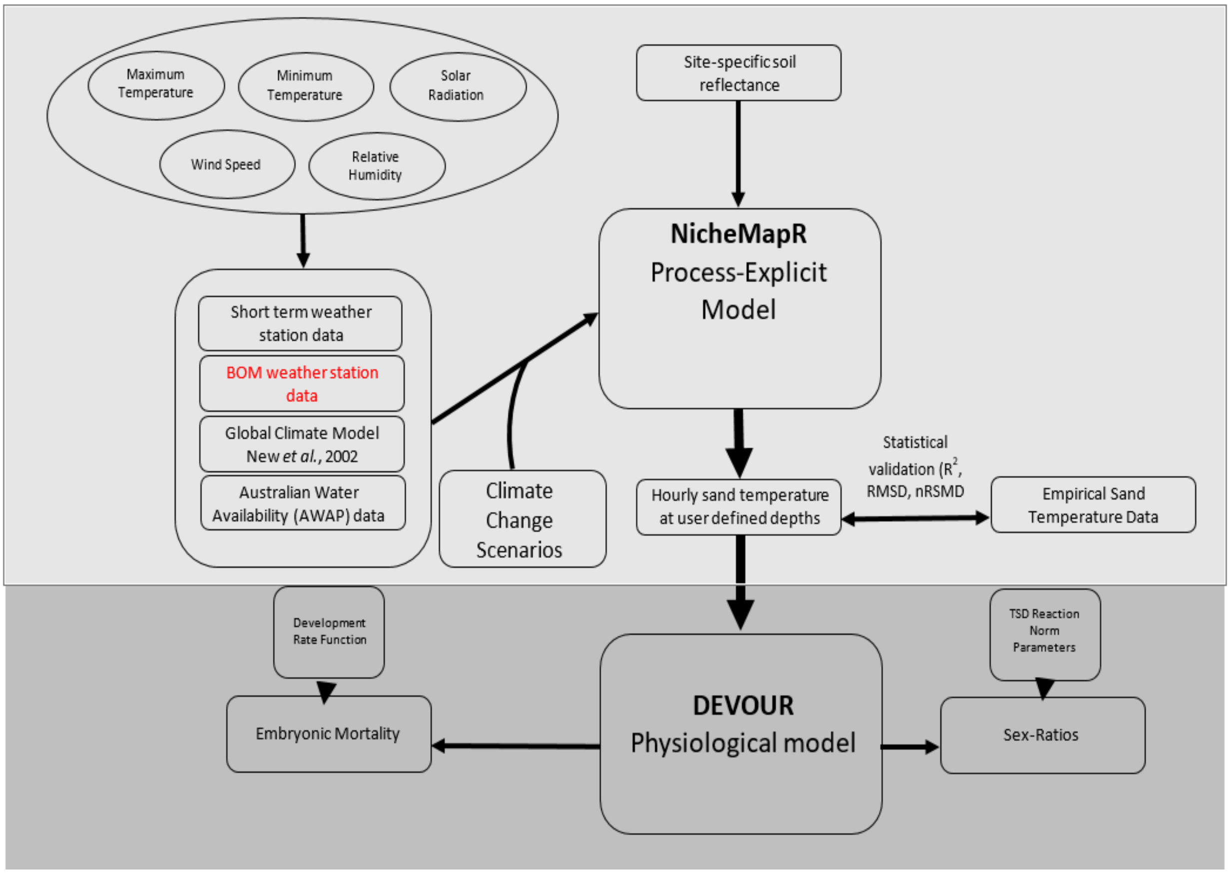

Figure 2. The mechanistic modelling framework employed in this research to predict sand temperatures throughout the Kimberley, and subsequently the sex-ratios and embryonic mortality of *Natator depressus* and *Chelonia mydas*. Figure is adapted from Stubbs et al. (2014) and Mitchell et al. (2016). The DEVOUR script was written by Anna Carter, based on a methodology described in Mitchell et al. 2008.

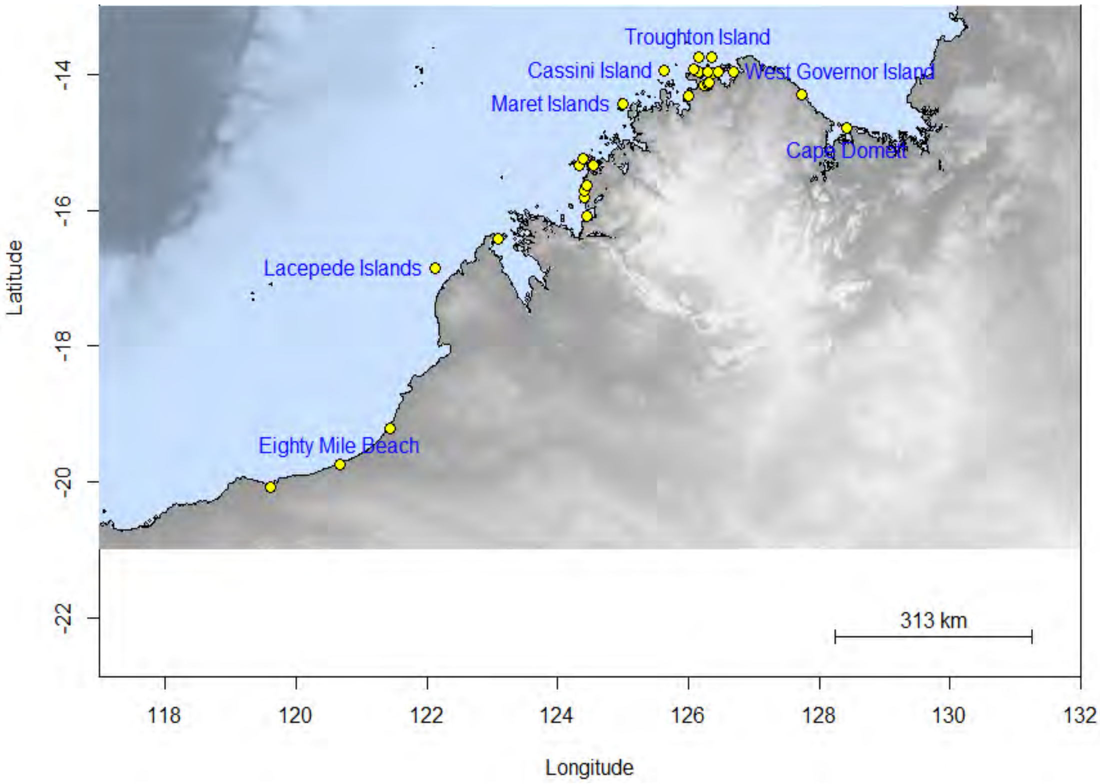

Figure 3. Rookery locations (n=29) where sand temperatures were modelled using both a global climate model (New et al. 2002) and the Australian Water Availability Project climate surface (AWAP; Raupach et al. 2009). Surface sand was collected from each rookery, and its reflectance was measured directly, or was interpolated using a linear regression (see Materials and Methods).

Climate inputs

NicheMapR's microclimate model was run using two alternative climate databases, both of which provided seasonally dynamic climate variables at a given location, and included daily maximum and minimum values of wind speed, air temperature, relative humidity, rainfall and cloud cover. The first of these was a global averaged climate surface with a resolution of 10' (~17km) derived from empirical data collected between 1961 and 1990 (New et al. 2002). The second database was a climate surface for Australia based on daily data collected between 1990 – 2016 from weather stations across the continent (the Australian Water Availability Project, AWAP; Raupach et al. 2009). The AWAP data provides higher resolution (~5km) climate surfaces for daily maximum and minimum temperatures, relative humidity, rainfall, and solar radiation. Daily wind speed data were not available, so data were splined from a gridded monthly database of long-term average 9:00 and 15:00 h 10 m wind speed surfaces for Australia, obtained from Australian National University Climate software package (ANUCLIM; Houlder et al. 1999). These values were scaled from the 10 m reference height to a 1.2 m reference height so that they were the same as all the other climate inputs (see Kearney et al. 2014b for scaling equations and descriptions). Maximum winds speeds were taken as the highest of the two daily data points, with minimum wind speeds assumed to be 10% of the maximum wind speed. Daily cloud cover for the AWAP surfaces were derived as the ratio of daily integrated clear sky solar radiation compared to daily solar radiation grids obtained through the AWAP surface model. To approximate daily cycles, the minima half was assumed to be the average daily cloud cover and the maxima was inferred as double this value. Maximum air temperature and wind speed were assumed to occur one hour after local solar noon, as were relative humidity and cloud cover minima. Likewise, minimum air temperature and wind speed were assumed at dawn, as were maximum values of relative humidity and cloud cover. This dataset has previously demonstrated accurate predictions of soil temperatures across Australia (Kearney et al. 2014b), including at marine turtle rookeries (Stubbs et al. 2014). The AWAP climate surface does not extend to offshore islands, so island locations were modelled based on the AWAP inputs for the nearest grid point on the mainland.

In addition to these two climate surface inputs, we also ran the NicheMapR microclimate model with hourly weather station data retrieved from weather stations deployed for short periods at various Kimberley marine turtle rookeries (Table 3). Any missing data from the weather stations were approximated using the ‘na.approx’ function of the R package ‘ZOO’ (Zeileis and Grothendieck 2005). In all cases, climate data were used to predict sand temperatures at standardized depths (0, 2.5, 5, 10, 15, 20, 30, 50, 100, 200 cm) as well as at customised depths that matched the depths where sand temperatures had been measured empirically (Table 4, and see below).

Sand temperature measurements and model validation

In order to test the accuracy of the modelled sand temperatures, we buried temperature data loggers (Thermochron^{TM} DS1921H iButtons; resolution: 0.125°C, accuracy: 1°C) on beaches throughout the Kimberley (Table 4). Temperature loggers were programmed to record hourly, or every 4.25 hours in order to capture data over a calendar year. Loggers were buried at depths between 30 and 70 cm, with the majority at 50cm, which is a typical nest depth for a marine turtle (Limpus 2009) and is a consistent reference depth for marine turtle rookeries globally.

To test the accuracy of the microclimate model we generated a series of hourly sand temperature predictions at the same depths as our temperature logger deployments, as well as at the depths at which loggers were retrieved (where known, see Table 4). In all cases, the site-specific sand reflectance was used as an input, along with the general soil parameters described above, and we assumed 0% shade. Two or three models were run for each site, depending on whether data from weather stations were available. Summary statistics were generated to compare the predicted sand temperatures to the empirical data (e.g. Carter et al. 2015; Kearney et al. 2014a; Kearney et al. 2014b; Mitchell et al. 2016); these being the coefficient of determination (r^2) and the root-mean-squared deviation (RMSD). We also calculated the normalized-RSMD (nRMSD), which is the RMSD value divided by the range of observed temperatures, and was used to compare the model fit by depth and by the type of climate input used (global, AWAP or weather station). All summary statistics were calculated using the ‘HYDROGOF’ and ‘PLYR’ packages in R (Wickham 2011; Zambrano-Bigiarini 2014). Closer agreement between observed and predicted values were indicated by lower RMSD and nRSMD values, and higher r^2 values.

Table 3. Locations and deployment durations of local weather stations throughout the Kimberley region of Western Australia.

Weather station deployment	GPS coordinates (lat, long)	Weather station model	Start date	End date	Number of recordings (intervals/errors)
Cape Domett 2013 – 2014	S 14.816 /E 128.583	MEA	02/11/2013	09/08/2014	41,909 (every 10 minutes)
Cape Domett 2015 – 2016	S 14.816 / E 128.583	MEA	NA	NA	0 (logger malfunction)
Cassini Island 2015 – 2016	S 14.317 / E 125.583	WeatherHawk	29/08/2015	23/05/2016	6,432 (hourly)
Deception Bay	S 15.633/ E 124.442	MEA	NA	NA	0 (battery malfunction)
Eighty Mile Beach 2015	S 19.753 / E 120.673	WeatherHawk	10/02/2015	25/11/2015	6,936 (hourly)
West Lacepede Island 2015 – 2016	S 17.150 / E 122.417	Em50 data logger	16/12/2015	25/01/2016	984 (hourly)

Table 4. Locations, duration, recording intervals and depths of temperature loggers used for validation of sand temperature models.

Site	Number of loggers	Start date	End date	Logging interval (hrs)	Depths at deployment (cm)	Depths at retrieval (cm)
Cape Domett	8	12/08/2013	09/08/2014	4.25	50	44, 45.5, 46, 53
Cape Domett	5	17/08/2014	09/08/2015	4.25	50	10, 40, 50
Cassini Island	1	21/08/2015	12/11/2015	1	50	unknown
Deception Bay	11	01/10/2014	08/07/2015	4.25 h	50	36, 40, 44, 47, 48, 49
Eighty Mile Beach	10	28/11/2014	21/02/2015	1	30, 40, 50, 60, 68, 70	44, 57, 58, 62, 66, 67, 68, 70, 74, 79
Eighty Mile Beach	6	28/05/2015	27/11/2015	4.25	50	55, unknown
Eighty Mile Beach	6	27/11/2015	07/01/2016	1	35, 43, 46, 52, 63, 68	35, 45, 48, 57, 62

Thevenard Island	6	25/11/2016	04/02/2017	1	40, 50, 60	40, 50, 60
Troughton Island	10	07/08/2016	30/10/2016	1	25, 30, 32, 40, 50, 60, 70	26, 30, 34, 35, 39, 40, 45, 52, 60
West Lacepede Island	3	12/12/2014	09/12/2015	4.25	50	5, unknown
West Lacepede Island	6	15/12/2015	25/01/2016	1	30, 40, 50, 58, 62	5, 29, 31, 33, 42, 55
Vanisttart Bay	2	05/08/2014	20/10/2014	1	50	unknown

2.3 Sex-ratio and mortality predictions under climate change

Sand temperatures estimated from microclimate models, as well as sand temperature data measured empirically were used as inputs for a physiological model of embryonic development (DEVOUR, see Figure 3). In brief, DEVOUR is an R script which calculates a developmental increment for each hour (using the equations presented in section 2.7) and a constant temperature equivalent (CTE) for the thermosensitive period. The script then calculates a sex-ratio based on the CTE, in accordance with the TSD reaction norm fitted for specified marine turtle populations (Figure 4).

We first simulated sex-ratios at five focal beaches across the study period (2013 and 2016), with nesting dates varying between Julian day 1-365. To simulate sex ratios under climate change, we calculated the maximum and minimum temperature from 1990 to 1999 using the AWAP data, to produce average values for each day of the year. This produced a reference climate for ~1995. We then increased the maximum and minimum input temperatures according to future climate projections for Australia for 2030 and 2070 (CSIRO and BOM 2014). For 2030, we added either 0.6 or 1.5°C (upper and lower predictions), while for 2070 we simulated a low emissions scenario by adding 1.0 or 2.5°C (upper and lower predictions) and a high emissions scenario by adding 2.2 or 5.0°C (upper and lower predictions). These adjusted inputs were then run through NicheMapR's microclimate model, and sand temperature outputs were used to drive the DEVOUR model to predict historic (~1995) and future (2030 or 2070) sex-ratios, depending on the date of nesting.

We modelled embryonic mortality in a similar way, by assuming that oviposition could occur on any day of the year, and that embryos would develop at the 50cm sand temperatures predicted on that day, and for each day afterward, until hatching stage was reached (100% development). If, during this period, sand temperatures were 35°C or above for at least one third of the time, then we assumed that a clutch would not survive. As for sex ratios, we modelled the outcome (survival or mortality) for each day of nesting, for the five focal rookeries, and under the ~1995 reference climate and under the same 2030 and 2070 climate projections described above.

3 Results

3.1 Pivotal temperature and transitional range of temperatures

For *N. depressus* rookeries at Cape Domett and Eighty Mile Beach, the asymmetrical Richards model provided the best fit for the sex ratio data generated from laboratory incubation experiments, while a logistic model provided the best fit for the *C. mydas* data (Table 5, Figure 4). ΔAIC values were no greater than 5 between all models in all rookeries, with the exception of the models that assumed genotypic sex determination (GSD; i.e. 50% at all temperatures). These results suggest that the pivotal temperature for *N. depressus* populations nesting in the Kimberley region varies by 1°C (29.5 °C and 30.5°C); while the pivotal temperature for *C. mydas* at the Lacepede Islands was estimated to be 29.4°C (Table 5). The transitional range of temperatures (RT) also varied between nesting populations and species, ranging from 6.3°C in *C. mydas* to 2.1°C for *N. depressus* nesting at Cape Domett rookery.

A comparison of AICc values suggested that separate models for each rookery fitted the data better than models that combined all studied rookeries (AIC: 208.68 vs 241.79), as well as when only the two *N. depressus* rookeries were combined (AIC: 167.57 vs 186.73). This suggests that the TSD reaction norms differ between populations, with the pivotal temperature being highest at the Eighty Mile Beach *N. depressus* rookery, and the TRT broadest for the *C. mydas* rookery (Table 5).

3.2 Development rate

Incubation duration decreased significantly as incubation temperature increased (r^2 = 0.89, p < 0.01) for all rookeries and ranged from 40.5 days at ~33°C to 64 days at ~28°C. There were small differences in the development rate between rookeries at similar temperatures. Eggs from West Governor Island appeared to have a relatively faster development rate, however this observation is based on a single clutch and consequently this rookery was not included when calculating a nonlinear development rate function using the DEVARA software. Figure 5 shows the development rate data and the reaction norm for each rookery, with the fitted parameters b1, b2 and b3 presented in Table 6.

Table 5. Model scores for each rookery according to Akaike information criterion (AIC) and their associated TSD reaction norm parameters with standard errors. TPIV and TRT refer to pivotal temperature and transitional range of temperatures respectively.

		Model Selection (AIC)						TSD Parameters	
Species	Rookery	Logistic	Hill	Richards	GSD	Hulin	Double-Richards	T_{PIV} (°C) (SE)	TRT (°C) (SE)
Natator depressus	Cape Domett	54.0	54.4	53.0	243.4	55.0	55.0	29.54 (0.14)	2.13 (0.10)
	Eighty Mile Beach	68.1	68.5	67.6	141.2	69.6	69.6	30.54 (0.26)	4.00 (0.19)
Chelonia mydas	Lacepede Islands	44.9	45.0	46.1	62.0	48.1	48.1	29.41 (0.3)	6.31 (0.28)

3.3 Thermal environments of Kimberley beaches

All microclimate models driven with the Australian Water Availability Project (AWAP) climate surfaces outperformed those run using the global climate model (GCM) (see Figures A3.3-A3.13, which include summary statistics). Including a soil moisture subroutine in the microclimate model decreased the accuracy of the sand temperature predictions, irrespective of whether the soil moisture input was generated through the AWAP or

GCM climate data. As such, all subsequent modelling was done using AWAP climate data, with the soil moisture subroutine disabled. Plots of sand temperatures predicted at 50 cm depth showed substantial variation in the thermal environments of beaches throughout the Kimberley region of Western Australia, where data were aggregated for a ‘winter’ nesting period, and a ‘summer’ nesting period (Figure 6).

3.4 Sex-ratios and embryonic mortality at five Kimberley marine turtle rookeries

The modelled sex-ratios at five focal rookeries within the Kimberley (Cape Domett, Eighty Mile Beach, West Lacepede Island, Cassini Island and West Governor Island; see Figure 1) for the study period (2013-2016) are presented in Figure 7. *N. depressus* nesting on West Governor Island were assumed to have the same TSD parameters as Cape Domett, while *C. mydas* nesting on Cassini Island were assumed to have the same TSD parameters as the Lacepede Islands. Figures 8 to 12 show the predicted sand temperatures at 50 cm depth, and the associated sex-ratios for ~1995, as well as under climate change scenarios for 2030 and 2070 . Sex-ratios were highly variable between rookeries, and were predicted to shift to 100% female for nesting at all days of the year under a high emission scenario for 2070 at Cape Domett.

Models that focussed on predicting embryonic survival and mortality showed that under the ~1995 reference climate, and the 2030 low warming scenario, all focal rookeries had sand temperatures that promoted embryonic survival (Figure 13). Green turtle rookeries (Lacepede and Cassini Islands) had suitable nesting temperatures year-round in all but the extreme high emissions 2070 climate scenario, where the model predicted mortality, irrespective of the date of nesting. The situation for flatback rookeries was more complex, and varied by rookery and climate change scenario. Eighty Mile Beach was mostly suitable year-round except under high emissions scenarios, while West Governor Island and Cape Domett showed diminishing periods of the year that could support nesting, primarily in autumn and winter months. The simulations for Cape Domett were most alarming, with even a 2030 scenario showing just a small portion of the year in winter that could result in successful nesting (Figure 13).

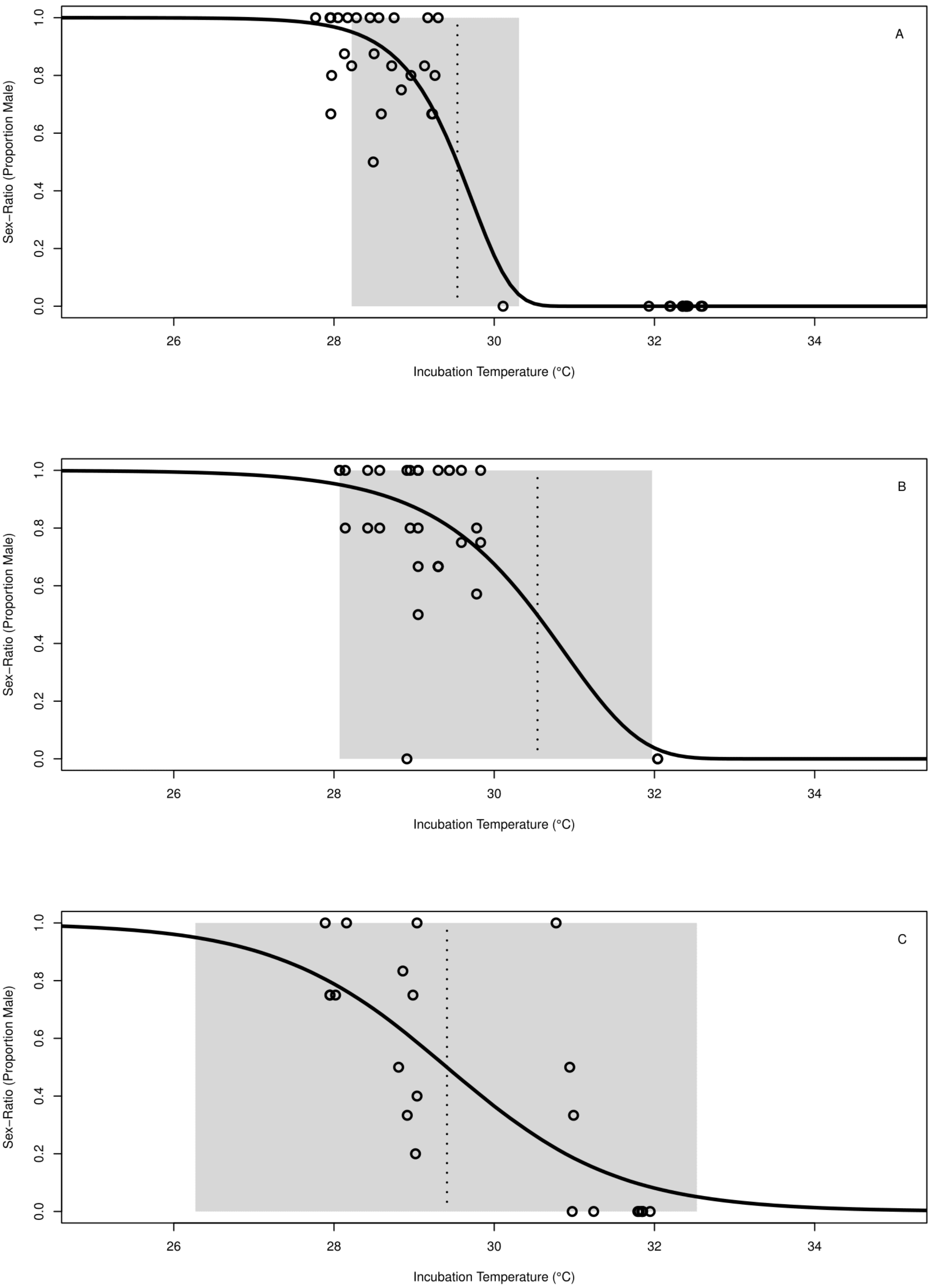

Figure 4. Sex-ratio reaction norms and observed sex-ratios (open circles) from laboratory incubations for the three study rookeries: (a) Cape Domett (*N. depressus*), (b) Eighty Mile Beach (*N. depressus*), and (c) Lacepede Islands (*Chelonia mydas*). Cape Domett observations include field data collected and published by Stubbs et al. (2014), as well as more recent data. The dashed lines indicate the pivotal temperatures, and the shaded region represents the transitional range of temperatures (TRT).

Table 6. Fitted parameters* for the nonlinear development rate function calculated using DEVARA software.

	Fitted parameters		
Rookery (species)	b_1	b_2	b_3
Cape Domett (ND)	2.37	18.56	33.88
Eighty Mile Beach (ND)	2.29	17.97	33.41
Lacepede Islands (CM)	2.38	16.24	33.98

* b_4 and b_5 (which control the slope and asymmetry of the curve) were fixed at 6.0 and 0.4 respectively for all models, as recommended by Dallwitz & Higgins 1992 when data at high temperatures are unavailable. b_3 can be interpreted as an approximation of the temperature at which development rate peaks.

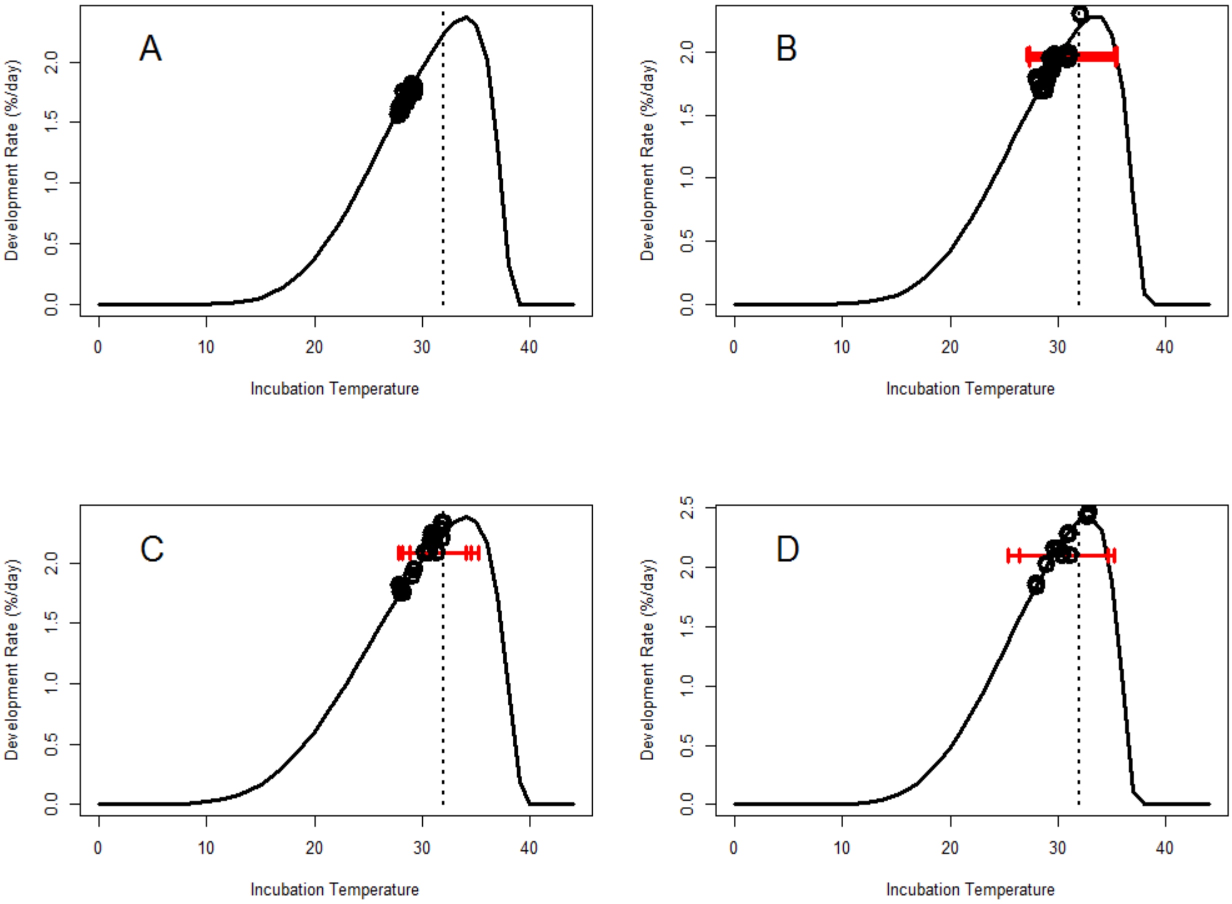

Figure 5. Nonlinear development rate functions fitted by DEVARA for three rookeries of *N. depressus* (a, Cape Domett; b, Eighty Mile Beach; d, West Governor Island) and one rookery of *C. mydas* (c, Lacepede Islands) from the Kimberley region of Western Australia. Black circles represent constant temperature incubations, and red diamonds represent cycling temperature regimes where horizontal lines show the daily temperature fluctuation. Dashed lines at 32°C are included to aid comparison among plots.

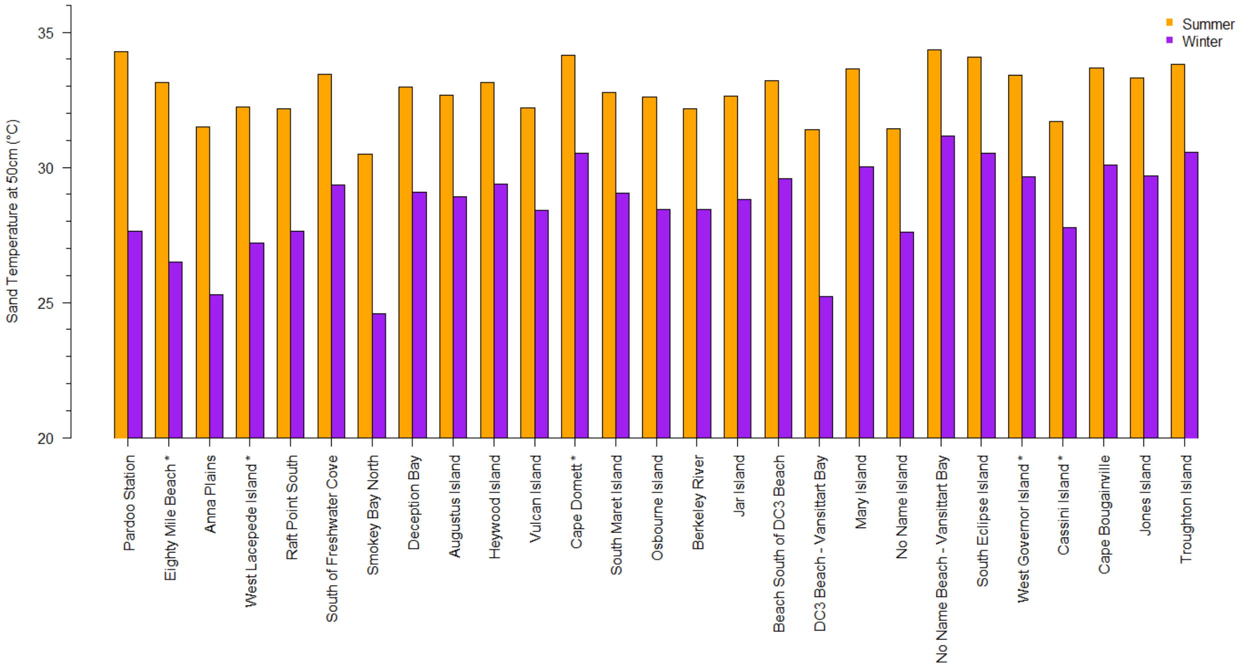

Figure 6. Modelled sand temperatures at 50 cm for 31 Kimberley beaches. ‘Winter’ refers to temperatures between August 1st and October 31st (purple) and ‘summer’ refers to temperatures occurring between November 1st and January 31st (orange).

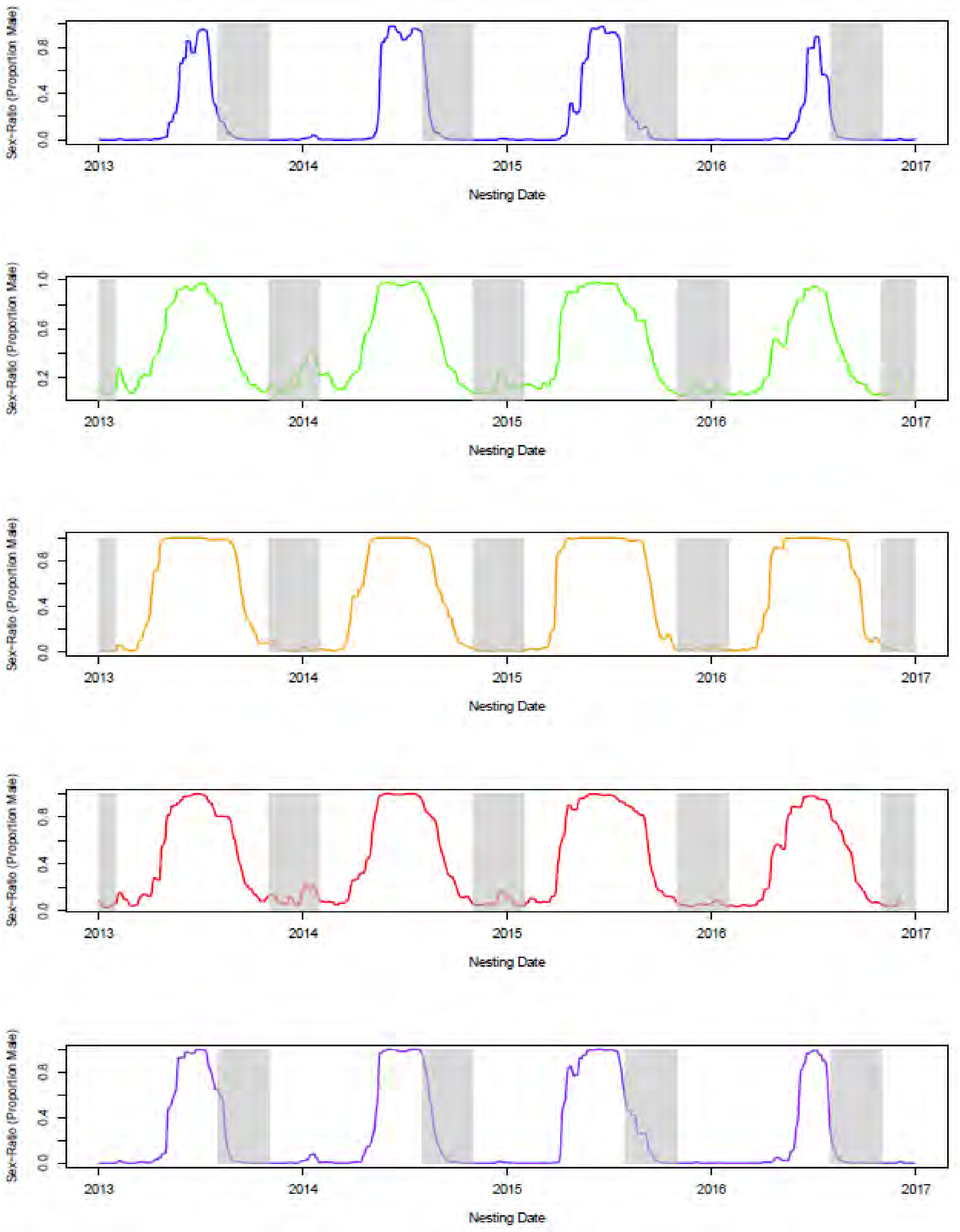

Figure 7. Modelled sex-ratios for five focal rookeries in the Kimberley for the period 2013 and 2016: Cape Domett (blue), Cassini Island (green), Eighty Mile Beach (orange), West Lacepede Island (red) and West Governor Island (purple). Grey shading represents the typical nesting period at each rookery (i.e. winter or summer).

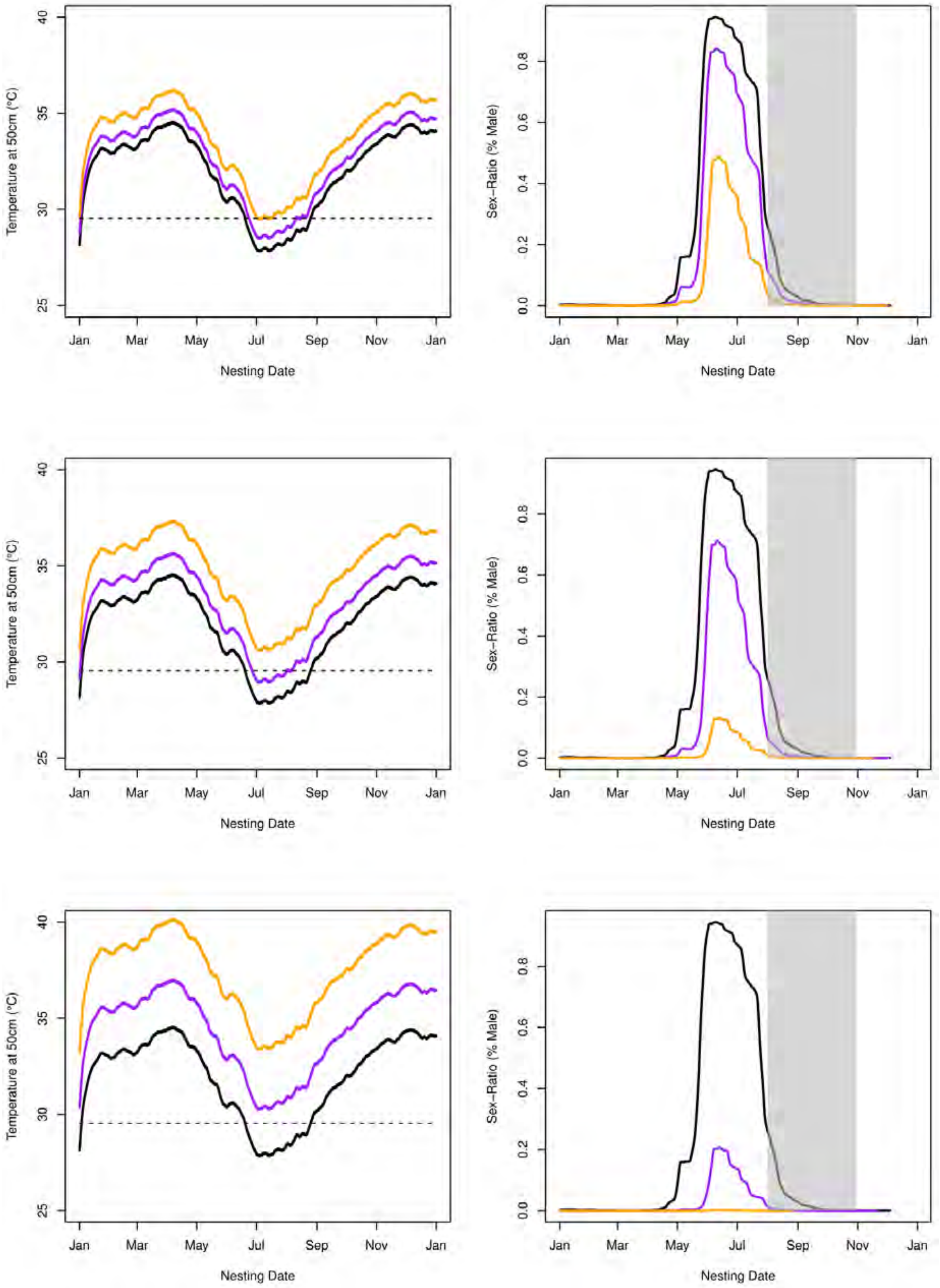

Figure 8. Current and future sand temperature and associated sex-ratio predictions for ***Natator depressus*** nesting at **Cape Domett**: (top) 2030, (middle) 2070 low emission scenario, and (bottom) 2070 high emission scenario. All climate change scenarios are presented relative to ~1995 (black lines); and represent either conservative warming (purple lines) or more extreme warming (orange lines). Grey shading represents the typical winter-spring nesting period.

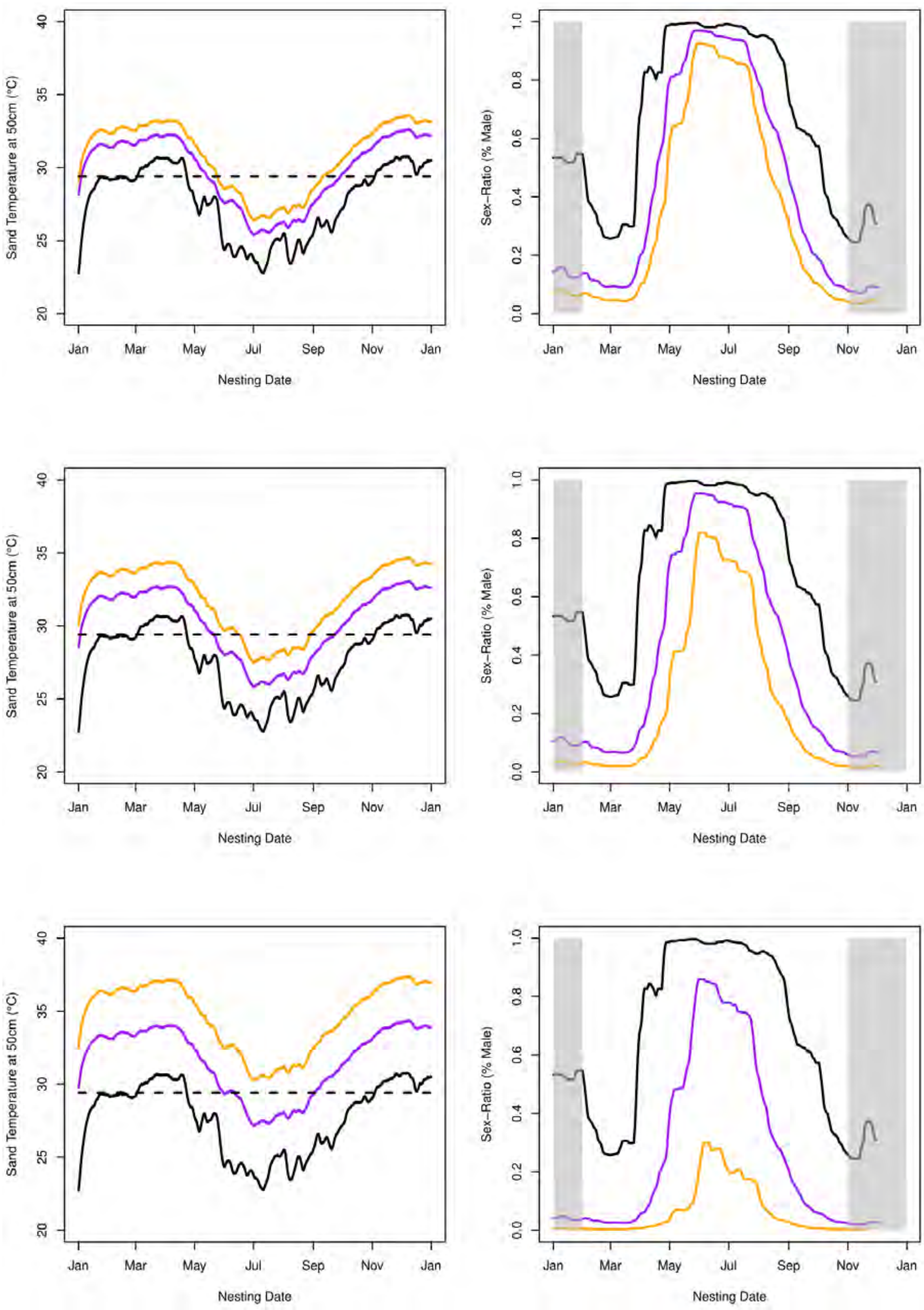

Figure 9. Current and future sand temperature and associated sex-ratio predictions for ***Chelonia mydas*** nesting on **Cassini Island**: (top) 2030, (middle) 2070 low emission scenario, and (bottom) 2070 high emission scenario. All climate change scenarios are presented relative to ~1995 (black lines); and represent either conservative warming (purple lines) or more extreme warming (orange lines). Grey shading represents the typical summer nesting period.

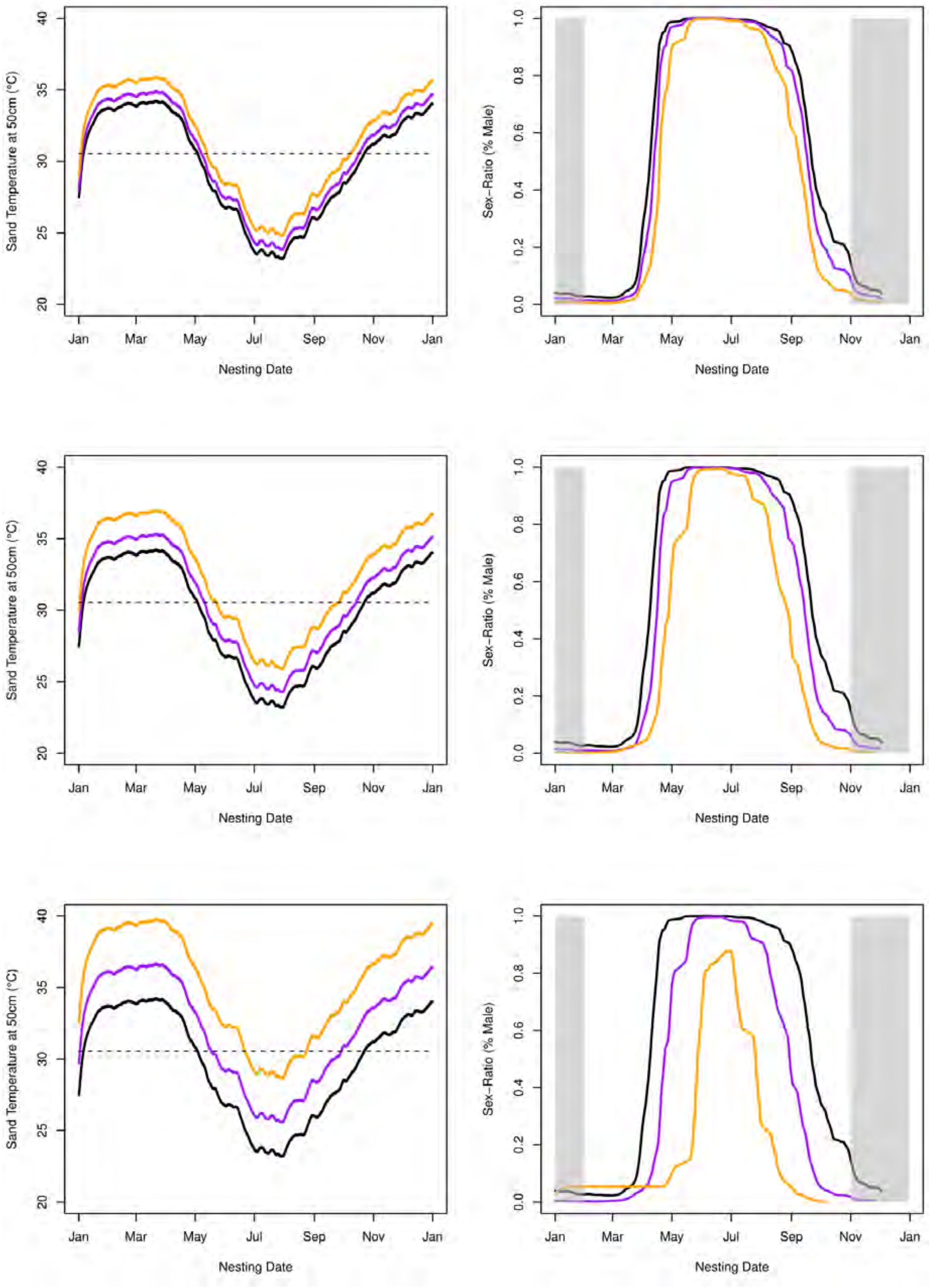

Figure 10. Current and future sand temperature and associated sex-ratio predictions for ***Natator depressus*** nesting at **Eighty Mile Beach**: (top) 2030, (middle) 2070 low emission scenario, and (bottom) 2070 high emission scenario. All climate change scenarios are presented relative to ~1995 (black lines); and represent either conservative warming (purple lines) or more extreme warming (orange lines). Grey shading represents the typical summer nesting period.

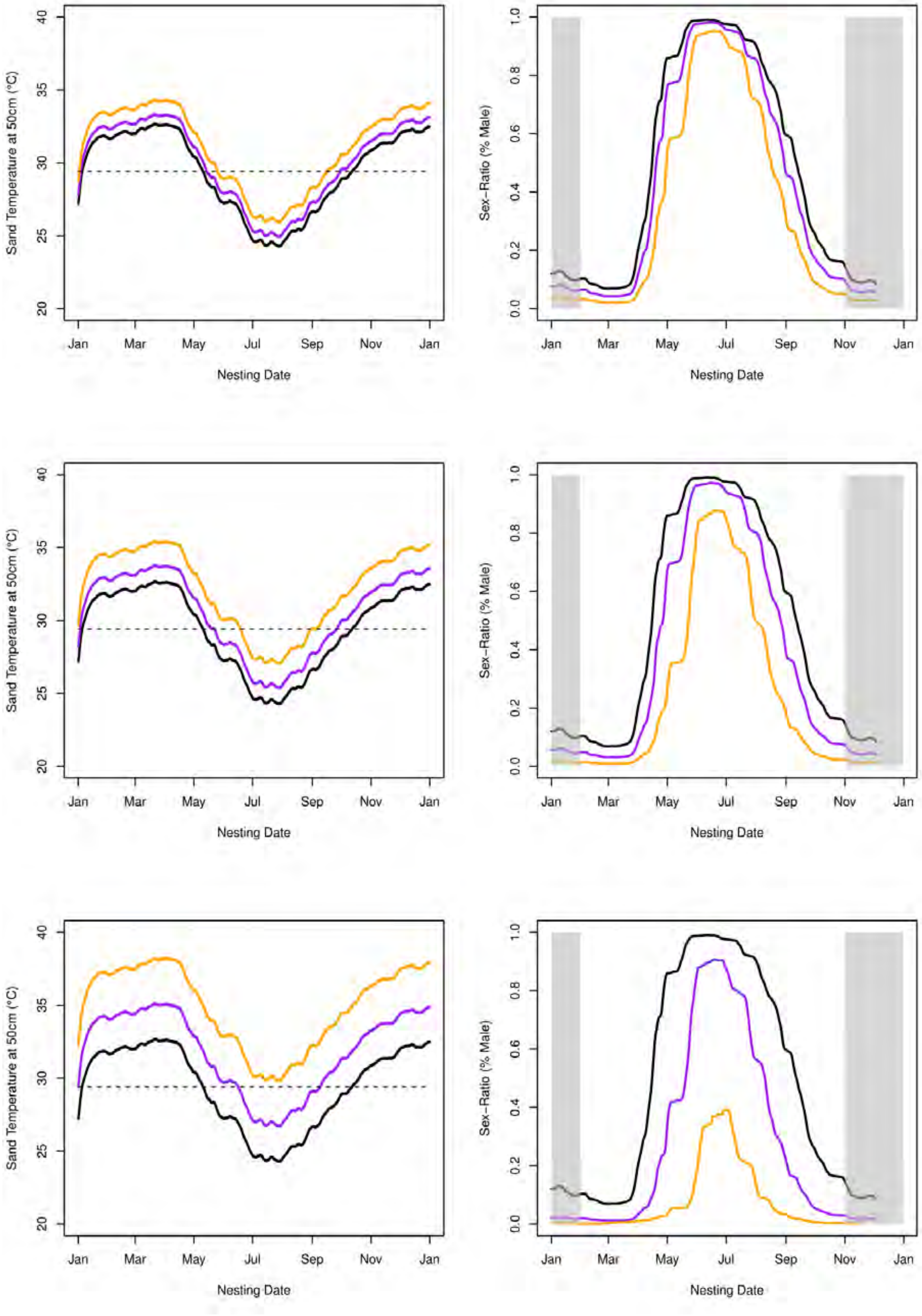

Figure 11. Current and future sand temperature and associated sex-ratio predictions for ***Chelonia mydas*** nesting on the **Lacepede Islands**: (top) 2030, (middle) 2070 low emission scenario, and (bottom) 2070 high emission scenario. All climate change scenarios are presented relative to ~1995 (black lines); and represent either conservative warming (purple lines) or more extreme warming (orange lines). Grey shading represents the typical summer nesting period

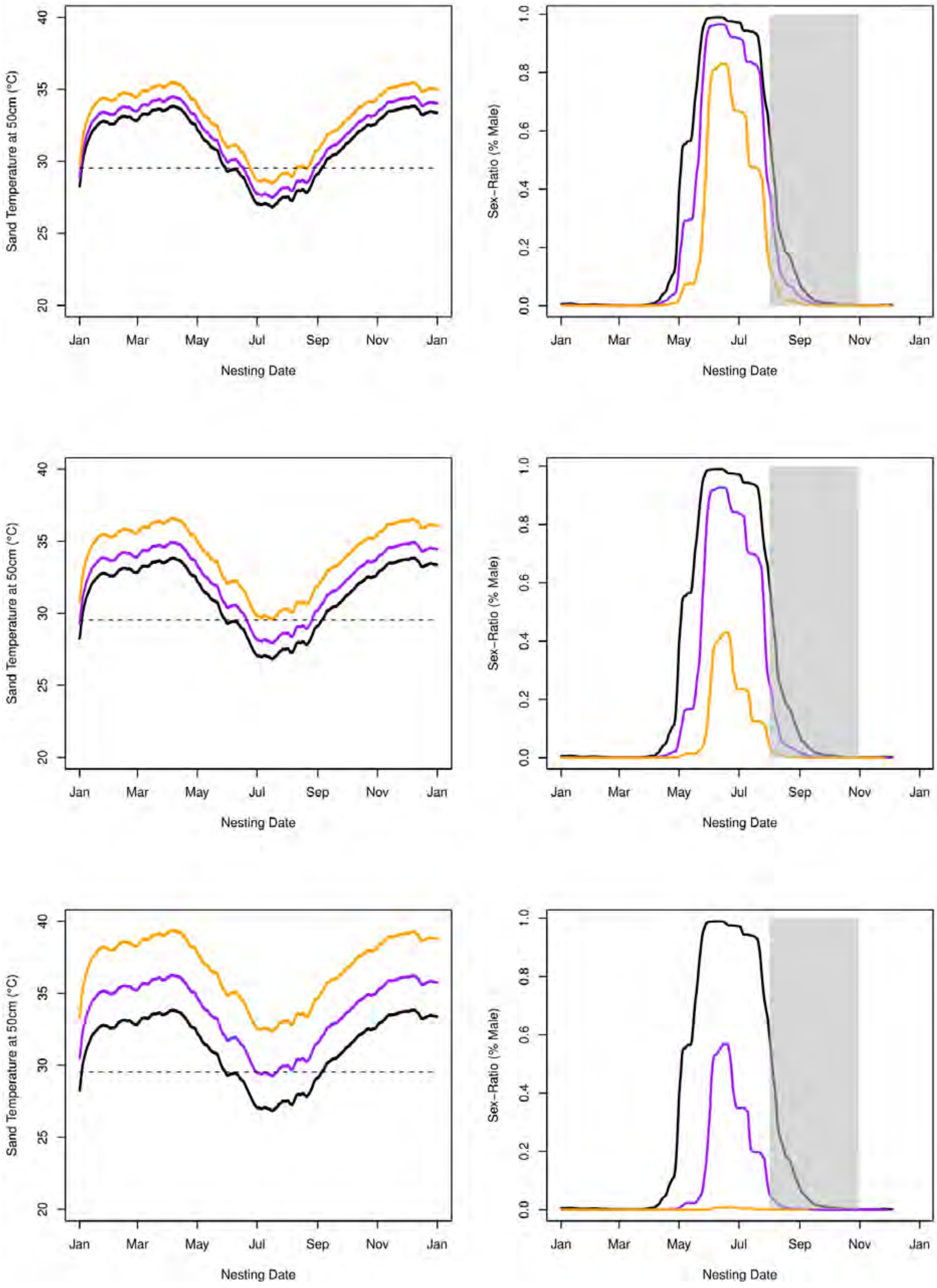

Figure 12. Current and future sand temperature and associated sex-ratio predictions for ***Natator depressus*** nesting at **West Governor Island**: (top) 2030, (middle) 2070 low emission scenario, and (bottom) 2070 high emission scenario. All climate change scenarios are presented relative to ~1995 (black lines); and represent either conservative warming (purple lines) or more extreme warming (orange lines). Grey shading represents the typical winter-spring nesting period.

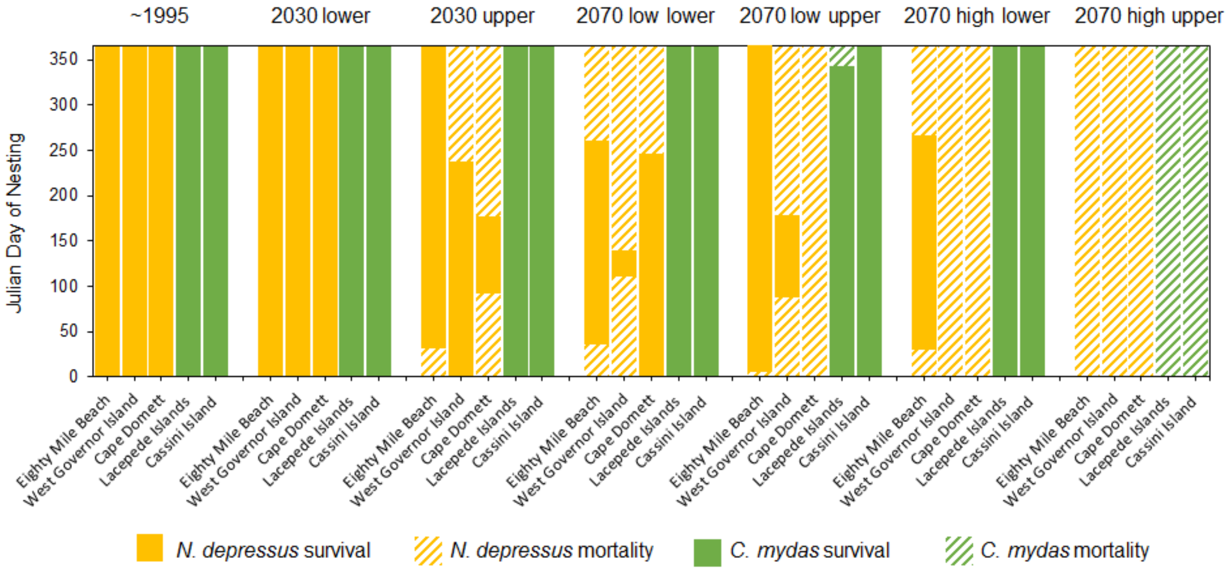

Figure 13. Expected outcomes of nesting on different days of the year (1-365), depending on rookery location, and climate. The current climate is represented by the period 1990-1999 (~1995) while six future climates for either 2030 or 2070 are also modelled. Mortality was assumed to occur if incubation temperatures exceed 35°C for at least one third of the estimated development time (oviposition-hatching), and this assumption was consistent between species.

4 Discussion and Conclusions

Here we have demonstrated a mechanistic modelling approach for predicting sex-ratios for two marine turtle species nesting extensively in the Kimberley region of Western Australia. We parameterized the model for one genetic stock of *C. mydas* (represented by the Lacepede Island population), and for two genetic stocks of *N. depressus* (represented by the Eighty Mile Beach and Cape Domett population). Our models suggest that the risk of adverse impacts from higher beach temperatures due to climate change are variable across the region, ranging from a high impact on the winter nesting population at Cape Domett to lower impacts at summer nesting rookeries. Other winter nesting rookeries may be similarly vulnerable to the effects of rising ambient temperatures, as nesting females cannot avoid high nest temperatures by shifting their nesting to a cooler time of year.

4.1 Variation in physiological parameters

4.1.1 *Sex ratios and development rates*

Pivotal temperatures and, more recently, the transitional range of temperatures have been used as repeatable metrics for comparing intra- (Bull et al. 1982; Burke and Calichio 2014; Ewert et al. 2005) and inter-specific (Sarre et al. 2004) variation in reptiles with TSD. Differences in TSD parameters are hypothesized to reflect adaptations to local climates, with selection favouring balanced sex-ratios according to Fisherian theory (Charnov 1982; Ewert et al. 2005). We show that the pivotal temperature of *N. depressus* varies by 1°C between two rookeries that are separated by 5 degrees of latitude. Cape Domett (14.8°south) had a lower pivotal temperature than at the higher latitude rookery at Eighty Mile Beach (19.8°south; 29.5 vs 30.5°C), which may be related to the timing of nesting. The rookery at Cape Domett has peak nesting in winter, around August/September (Whiting et al. 2008), while Eighty Mile Beach peaks around late November/December (Limpus 2009). We show that modelled sand temperatures during the nesting periods differed between these two rookeries, with an average of 33.2°C at Eighty Mile Beach, and 30.5°C at Cape Domett. These patterns may represent alternative strategies for avoiding skewed primary sex-ratios: with *N. depressus* from Cape Domett nesting earlier when sand temperatures are more favourable (Whiting et al. 2008), while the population nesting at Eighty Mile Beach can tolerate summer sand temperatures at this more southerly location.

High proportions of mixed-sex nests favour the expression of heritable genetic variation between embryos and promotes the presence of both sexes in the breeding population (Hulin et al. 2008). We show marked variation in the TRT for the marine turtle rookeries we studied, with variation of almost 2°C between the *N. depressus* rookeries (2.1 vs 4.0 at Cape Domett and Eighty Mile Beach respectively), while the TRT in the Lacepede Island *C. mydas* population was substantially higher at 6.3. While these estimates should be considered as preliminary given limited data points below the lower TRT limit, they provide important insights into the primary sex-ratios that are likely to be generated at these locations. The higher TRT in the *C. mydas* rookery, and at the southernmost *N. depressus* rookery suggest they have a higher capacity to avoid strongly skewed sex ratios associated with climate change.

Development rates were relatively similar at Eighty Mile Beach (19.8 °south) and Cape Domett (14.8 °south), but were slightly slower in Lacepede Island green turtles. The temperatures at which development rates peaked (fitted parameter b3) were also similar between each rookery (33.4 - 34.0°C), and implied that embryos experiencing temperatures above this threshold would be subject to thermal stress and consequently slower rates of development.

4.1.2 *Comparisons with other populations*

Despite the discovery of TSD in marine turtles by Yntema and Mrosovsky (1979) over 25 years ago, few studies have directly estimated pivotal temperatures or TRTs (see review by Wibbels 2003). Estimates of these traits are particularly sparse for Australian marine turtle populations. For *N. depressus*, estimates of pivotal temperature range from 29.3 to 30.4°C (Box et al. 2010; Hewavisenthi and Parmenter 2000; Howard et al. 2015; Limpus 2009; Reinhold et al in Press, Stubbs et al. 2014), however these studies primarily relied on sex-ratios derived from natural nests or laboratory experiments using small numbers of nesting females. The results presented here from constant incubation provide additional evidence for variation in this trait between populations of *N. depressus*. The TRT for *N. depressus* also varies between populations, with an estimated TRT of 1°C in Queensland stocks (Hewavisenthi and Parmenter 2000) up to 3.6°C for a population the Pilbara region of Western Australia (Box et al. 2010). Our estimate for the Eighty Mile Beach population is slightly higher (4.0°C), while the population at Cape Domett has a TRT of ~2°C.

The TSD parameters for *C. mydas* populations from Australia are less well defined, with estimates of pivotal temperature ranging between 27.6 to 29.3°C on the Great Barrier Reef (Limpus 2009; Miller and Limpus 1981). On a global scale, the pivotal temperature ranges from between 28.0 to 30.3°C (Broderick et al. 2000; Godfrey and Mrosovsky 2006; Kaska et al. 1998; Mrosovsky et al. 1984; Patrício et al. 2017; Spotila et al. 1987; Standora and Spotila 1985). Our Tpiv estimate was similar to other populations (29.4 °C) but our TRT estimate (6.3°C) is much higher than any other previous estimate for this species, with TRTs for populations from Costa Rica and Suriname estimated to be 1.5 and 3°C respectively (Mrosovsky et al. 1984; Standora and Spotila 1985). We suggest that additional incubation experiments at the upper and lower extremes of the incubation regime (i.e. 28 and 32°C) would better resolve this parameter.

4.2 Performance of the microclimate model

As expected, microclimate models that utilized the Australian Water Availability Project climate surface data produced sand temperature predictions that were better correlated with empirical data than models that were forced with a global climate surface. There are two likely reasons for this observation, the first being related to the resolution of the surfaces. The AWAP surfaces have been specifically generated for the Australian continent at a resolution of approximately 5 km (Kearney et al. 2014b), while the global model is gridded at lower resolution (approximately 17 x 17 km pixels). Higher resolution surfaces will generally produce more reliable outputs as they have a higher probability of accounting for subtle variation in local climates. The second explanation relates to the nature of the input, with the AWAP data interpolated from hourly weather station data from across Australia, while the global model simulates an average climate for each day based on interpolation of historic data (1961 - 1990; New et al. 2002). Surprisingly, the weather stations that were deployed simultaneously with the temperature loggers were less effective than the AWAP climate surfaces at predicting sand temperatures.

We show that models forced with the AWAP climate surfaces were variable in the reliability of the sand temperature predictions. For a number of sites, particularly islands off the northern Kimberley coast, correlations between the predicted and observed sand temperatures were high. At other sites, such as mainland beaches, predictions were less reliable. Eighty Mile Beach for example, shows a strong correlation between modelled and observed sand temperatures for 2015 ($r^2 = 0.98$), however predictions are lower than observed in winter, and higher than observed in summer (see Figure A3.8). These discrepancies may be due to a number of stochastic processes, such as movement of beach sand causing a change in the depth of the temperature logger. For example, during the summer of 2014/2015 at Eighty Mile Beach, we observed large shifts in beach sand associated with a storm surge and strong winds, and loggers were recovered at depths approximately 15 - 30 cm deeper than they were deployed at. We were unable to pinpoint the timing of the depth change, and assume that the sand movement was gradual.

4.3 Thermal environments of beaches

Nesting of *N. depressus* in the Kimberley region has two peaks, with a winter (Whiting et al. 2008) and summer peak (Chapter 2 of this report). It appears that the split occurs around the King Sound (see Chapter 2), with summer nesting at latitudes south of this point, and winter nesting occurring at lower latitudes. Our results suggest that summer sand temperatures across this range are similar (ranging between ~31-35°C), which despite being around the upper thermal limits of marine turtles (Ackerman 1997; Howard et al. 2014; Miller 1997), result in rapid embryonic development, which is likely favoured by natural selection, as it reduces the risk of mortality through environmental perturbation or predation. Therefore, winter nesting at latitudes higher than the Lacepede Islands may be selected against as sand temperatures are too cool (less than 28°C), with incubation expected to take 65-70 days based on the development models presented here. At these same sites (i.e. Pardoo Station, Eighty Mile Beach, Anna Plains, and the Lacepede Islands), temperatures during the summer should result in hatching after approximately 42-45 days, which is consistent with field observations in both *N. depressus* and *C. mydas*.

The temperature increases associated with climate change are expected to push beach temperatures considerably higher than those of historic (i.e. ~1995) and contemporary levels. Our models of focal rookeries suggest that beach temperatures will rise to unprecedented levels under all climate change scenarios, particularly beaches in the tropical northern and eastern Kimberley, where temperatures at 50 cm depth will consistently exceed 35°C for a large proportion of the year. However we also show substantial local variation in beach temperatures throughout the Kimberley (Figure 6), and it is possible that some beaches will be increasingly suitable for nesting as the climate warms.

4.4 Impact of climate change on sex ratios and mortality

Our results show that climate change will have variable impacts on the sex-ratios and embryonic mortality of marine turtle rookeries in the Kimberley region. Of particular concern is the impact on winter nesting rookeries of *N. depressus* in the northern and eastern Kimberley, as these populations currently nesting during the coolest time of year, and therefore shifts in nesting will have limited impacts on embryonic mortality and primary sex-ratios (Stubbs et al. 2014). All other focal rookeries modelled showed a shift towards producing more female offspring, but sites such as Eighty Mile Beach and Cassini Island still have a viability window where shifting to earlier nesting should contribute to production of male hatchlings, and thereby maintain balanced primary sex-ratios.

More alarmingly, we show that under high carbon emission scenarios, sand temperatures at depths where marine turtle eggs are commonly deposited (~50cm) will exceed putative upper thermal limits for embryonic survival (Ackerman 1997; Howard et al. 2014; Miller 1997; this study) at a higher frequency, duration and magnitude than occurs currently. Embryonic mortality is therefore expected to increase at most focal rookeries, with a consequent decrease in the window for viable development as climate change becomes more pronounced. For example, by 2030 it is expected that mortality will impact the winter nesting rookeries of *N. depressus* if upper predictions of ambient increases are reached. By 2070, low emission scenarios predict increasing mortality at Cape Domett, West Governor Island and Eighty Mile Beach, while Cassini Island and the Lacepede Islands only show marginal changes. Under 2070 high emission scenarios, the impacts range from minimal for the Lacepede Islands and Cassini Island *C. mydas* rookeries (conservative warming), to the extreme situation for the winter nesting *N. depressus* rookeries at Cape Domett and West Governor Island, where no matter which day of the year nesting occurs, mortality would be expected. These predictions of mortality effectively negate the predictions of entirely female primary sex ratios by 2070 (as shown in Figures 8 and 12), as without micro evolutionary change in thermal tolerance, embryos will die before their sex is determined.

5 References

Ackerman, R.A. 1997. The nest environment and the embryonic development of marine turtles. pp. 83-106 in P. Lutz and J. Musick, eds. The Biology of Marine turtles.

Bates, D., M. Mächler, B. Bolker and S. Walker. 2014. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67: 1-48.

Bentley, B.P., B. Haas, J.N. Tedeschi and O. Berry. 2017. Loggerhead marine turtle embryos (*Caretta caretta*) regulate expression of stress-response and developmental genes when exposed to a biologically realistic heat stress. Molecular Ecology 26: 2978-2992.

Bobyn, M.L., and R.J. Brooks. 1994. Interclutch and interpopulation variation in the effects of incubation conditions on sex, survival and growth of hatchling turtles (*Chelydra serpentina*). Journal of Zoology 233: 233-257.

Box, T. 2010. A mechanistic approach to predicting hatchling sex ratios of the Flatback Turtle *(Natator depressus*).Honours Thesis, School of Animal Biology. The University of Western Australia, Perth, Western Australia.

Broderick, A.C., B.J. Godley and G.C. Hays. 2001. Metabolic heating and the prediction of sex ratios for green turtles (*Chelonia mydas*). Physiological and Biochemical Zoology 74: 161-170.

Broderick, A.C., B.J. Godley, S. Reece and J.R. Downie. 2000. Incubation periods and sex ratios of green turtles: highly female biased hatchling production in the eastern Mediterranean. Marine Ecology Progress Series 202: 273-281.

Bull, J., R.C. Vogt and C. McCoy. 1982. Sex determining temperatures in turtles: a geographic comparison. Evolution 36: 326-332.

Burke, R.L., and A.M. Calichio. 2014. Temperature-dependent sex determination in the diamond-backed terrapin (*Malaclemys terrapin*). Journal of Herpetology 48: 466-470.

Butt, N., S. Whiting and K. Dethmers. 2016. Identifying future marine turtle conservation areas under climate change. Biological Conservation 204: 189-196.

Carter, A., M. Kearney, N. Mitchell, S. Hartley, W. Porter and N. Nelson. 2015. Modelling the soil microclimate: does the spatial or temporal resolution of input parameters matter? Frontiers of Biogeography 7: 138-154.

Ceriani, S.A., and J. Wyneken. 2008. Comparative morphology and sex identification of the reproductive system in formalin-preserved marine turtle specimens. Zoology 111: 179-187.

Charnov, E.L. 1982. The theory of sex allocation. Princeton University Press, Princeton, NJ.

Chaloupka, M. Y. and J. A. Musick. 1997. Age, growth, and population dynamics. pp. 233 – 276 in P. Lutz and J. Musick eds. The Biology of Marine turtles.

Commonwealth Scientific and Industrial Research Organisation (CSIRO) and the Bureau of Meteorology (BOM). 2014. State of the Climate 2014. Commonwealth of Australia, Australia.

Commonwealth Scientific and Industrial Research Organisation (CSIRO) and the Bureau of Meteorology (BOM). 2016. State of the Climate 2016. Commonwealth of Australia, Australia.

Dallwitz, M., and J. Higgins. 1992. DEVAR: a computer program for estimating development rate as a function of temperature.

Deeming, D.C., and M.W.J. Ferguson. 1990. Morphometric analysis of embryonic development in *Alligator mississippiensis*, *Crocodylus johnstoni* and *Crocodylus porosus*. Journal of Zoology 221: 419-439.

Doody, J.S., E. Guarino, A. Georges, B. Corey, G. Murray and M. Ewert. 2006. Nest site choice compensates for climate effects on sex ratios in a lizard with environmental sex determination. Evolutionary Ecology 20: 307-330.

Du, W.-G., D.A. Warner, T. Langkilde, T. Robbins and R. Shine. 2010. The physiological basis of geographic variation in rates of embryonic development within a widespread lizard species. The American Naturalist 176: 522-528.

Environment Protection and Biodiversity Conservation Act (EPBC). 1999. Commonwealth of Australia, Australia.

Ewert, M.A. 1979. The embryo and its egg: development and natural history. Turtles: Perspectives and research: 333-413.

Ewert, M.A. 1985. Embryology of turtles. pp. 75-267 in C. Gans, F. Billett and P.F.A. Maderson, eds. Biology of the Reptilia. Wiley, New York, NY.

Ewert, M.A., C.R. Etchberger and C.E. Nelson. 2004. Turtle sex-determining modes and TSD patterns, and some TSD pattern correlates. pp. 21-32 in N. Valenzuela and V.A. Lance, eds. Temperature-dependent sex determination in vertebrates. Smithsonian Books, Washington, DC.

Ewert, M.A., J.W. Lang and C.E. Nelson. 2005. Geographic variation in the pattern of temperature-dependent sex determination in the American snapping turtle (*Chelydra serpentina*). Journal of Zoology 265: 81-95.

Fuentes, M.M.P.B., C. Limpus and M. Hamann. 2011. Vulnerability of marine turtle nesting grounds to climate change. Global Change Biology 17: 140-153.

Fuentes, M.M.P.B., and W.P. Porter. 2013. Using a microclimate model to evaluate impacts of climate change on marine turtles. Ecological Modelling 251: 150-157.

Georges, A. 1994. The influence of fluctuating temperatures on hatchling sex ratios-a model and proposed test using *Caretta caretta*. pp. 156-162. Proceedings of the Australian Marine Turtle Conservation Workshop; Gold Coast.

Girondot, M. 1999a. A fifth hypothesis for the evolution of TSD in reptiles. Trends in ecology & evolution 14: 359-360.

Girondot, M. 1999b. Statistical description of temperature-dependent sex determination using maximum likelihood. Evolutionary Ecology Research 1: 479-486.

Girondot, M., and Y. Kaska. 2014. A model to predict the thermal reaction norm for the embryo growth rate from field data. Journal of thermal biology 45: 96-102.

Godfrey, M.H., V. Delmas and M. Girondot. 2003. Assessment of patterns of temperature-dependent sex determination using maximum likelihood model selection. Ecoscience 10: 265-272.

Godfrey, M.H., and N. Mrosovsky. 2006. Pivotal temperature for green marine turtles, *Chelonia mydas*, nesting in Suriname. The Herpetological Journal 16: 55-61.

Hamann, M., M. Godfrey, J. Seminoff, K. Arthur, P. Barata, K. Bjorndal, A. Bolten, A. Broderick, L. Campbell and C. Carreras. 2010. Global research priorities for marine turtles: informing management and conservation in the 21st century. Endangered Species Research 11: 245-269.

Hamann, M., C. Limpus and M. Read. 2007. Vulnerability of marine reptiles to climate change in the Great Barrier Reef. in J. Johnson and P. Marshal, eds. Climate change and the Great Barrier Reef: a vulnerability assessment. Great Barrier Reef Marine Park Authority and the Australian Greenhouse Office, Townsville.

Harry, J., and C. Limpus. 1989. Low-temperature protection of marine turtle eggs during long-distance relocation. Wildlife Research 16: 317-320.

Hawkes, L.A., A.C. Broderick, M.H. Godfrey and B.J. Godley. 2009. Climate change and marine turtles. Endangered Species Research 7: 137-154.

Hays, G., J. Ashworth, M. Barnsley, A. Broderick, D. Emery, B. Godley, A. Henwood and E. Jones. 2001. The importance of sand albedo for the thermal conditions on marine turtle nesting beaches. Oikos 93: 87-94.

Hewavisenthi, S., and C.J. Parmenter. 2000. Hydric environment and sex determination in the flatback turtle (*Natator depressus* Garman)(Chelonia: Cheloniidae). Australian Journal of Zoology 48: 653-659.

Hewavisenthi, S., and C.J. Parmenter. 2002. Thermosensitive period for sexual differentiation of the gonads of the flatback turtle (*Natator depressus* Garman). Australian Journal of Zoology 50: 521-527.

Houlder, D., M. Hutchinson, H. Nix and J. McMahon. 1999. ANUCLIM User Guide, Version 5.0. in A.N.U. Centre for Resource and Environmental Studies, ed. Centre for Resource and Environmental Studies, Australian National University, Canberra.

Howard, R., I. Bell and D.A. Pike. 2014. Thermal tolerances of marine turtle embryos: current understanding and future directions. Endangered Species Research 26: 75-86.

Howard, R., I. Bell and D.A. Pike. 2015. Tropical flatback turtle (*Natator depressus*) embryos are resilient to the heat of climate change. Journal of Experimental Biology 218: 3330-3335.

Hulin, V., V. Delmas, M. Girondot, M.H. Godfrey and J.-M. Guillon. 2009. Temperature-dependent sex determination and global change: are some species at greater risk? Oecologia 160: 493-506.

Hulin, V., M. Girondot, M.H. Godfrey and J.M. Guillon. 2008. Mixed and uniform brood sex ratio strategy in turtles: the facts, the theory and their consequences. pp. 279-300 in J. Wyneken, V. Bels and M.H. Godfrey, eds. Turtles: from structures to strategies of life. CRC, Boca Raton.

Ikonomopoulou, M.P., R.C. Aland, K. Ibrahim, E. Gosden and J.M. Whittier. 2012. Quantitative sex identification of hatchling green marine turtles (*Chelonia mydas*). Journal of Herpetology 46: 331-337.

International Union for Conservation of Nature (IUCN). 2015. IUCN Red List of threatened species. Version 2015.2.

Kaska, Y., R. Downie, R. Tippett and R.W. Furness. 1998. Natural temperature regimes for loggerhead and green turtle nests in the eastern Mediterranean. Canadian Journal of Zoology 76: 723-729.

Kearney, M.R., A.P. Isaac and W.P. Porter. 2014a. microclim: Global estimates of hourly microclimate based on long-term monthly climate averages. Scientific Data 1: 140006.

Kearney, M.R., and W.P. Porter. 2016. NicheMapR-an R package for biophysical modelling: the microclimate model. Ecography 40: 664-674.

Kearney, M.R., A. Shamakhy, R. Tingley, D.J. Karoly, A.A. Hoffmann, P.R. Briggs and W.P. Porter. 2014b. Microclimate modelling at macro scales: a test of a general microclimate model integrated with gridded continental-scale soil and weather data. Methods in Ecology and Evolution 5: 273-286.

Koch, A. U., M. L. Guinea and S. D. Whiting. 2007. Effects of sand erosion and current harvest practices on incubation of the flatback marine turtle (*Natator depressus*). Australian Journal of Zoology 55: 97-105.

Koepke, P., M. Hess, I. Schult and E. Shettle. 1997. Global aerosol data set. p. 44. Max-Planck-Institut fur Meteorologie, Hamburg.

Limpus, C.J. 2009. A biological review of Australian marine turtles. Environmental Protection Agency (EPA).

Limpus, C.J., V. Baker and J.D. Miller. 1979. Movement induced mortality of loggerhead eggs. Herpetologica: 335-338.

Matsuzawa, Y., K. Sato, W. Sakamoto and K. Bjorndal. 2002. Seasonal fluctuations in sand temperature: effects on the incubation period and mortality of loggerhead marine turtle (*Caretta caretta*) pre-emergent hatchlings in Minabe, Japan. Marine Biology 140: 639-646.

McCullough, E.C., and W.P. Porter. 1971. Computing clear day solar radiation spectra for the terrestrial ecological environment. Ecology 52: 1008-1015.

Miller, J., and C. Limpus. 1981. Incubation period and sexual differentiation in the green turtle *Chelonia mydas* L. pp. 66-73. Proceedings of the Melbourne Herpetological Symposium. Zoological Board of Victoria, Parkville, Victoria, Australia.

Miller, J.D. 1997. Reproduction in marine turtles. pp. 51-81 in P. Lutz and J. Musick, eds. The biology of marine turtles. CRC Press, Boca Raton.

Mitchell, N.J., M.R. Kearney, N.J. Nelson and W.P. Porter. 2008. Predicting the fate of a living fossil: how will global warming affect sex determination and hatching phenology in tuatara? Proceedings of the Royal Society B: Biological Sciences 275: 2185-2193.

Mitchell, N.J., N. Rodriguez, G. Kuchling, S.G. Arnall and M.R. Kearney. 2016. Reptile embryos and climate change: modelling limits of viability to inform translocation decisions. Biological Conservation 204: 134-147.

Mrosovsky, N., P. Dutton and C. Whitmore. 1984. Sex ratios of two species of marine turtle nesting in Suriname. Canadian Journal of Zoology 62: 2227-2239.

Mrosovsky, N., and C. Pieau. 1991. Transitional range of temperature, pivotal temperatures and thermosensitive stages for sex determination in reptiles. Amphibia-Reptilia 12: 169-179.

Neuwald, J.L., and N. Valenzuela. 2011. The Lesser Known Challenge of Climate Change: Thermal Variance and Sex-Reversal in Vertebrates with Temperature-Dependent Sex Determination. PLoS One 6: e18117.

New, M., D. Lister, M. Hulme and I. Makin. 2002. A high-resolution data set of surface climate over global land areas. Climate research 21: 1-25.

Patrício, A.R., A. Marques, C. Barbosa, A.C. Broderick, B.J. Godley, L.A. Hawkes, R. Rebelo, A. Regalla and P. Catry. 2017. Balanced primary sex ratios and resilience to climate change in a major marine turtle population. Marine Ecology Progress Series 577: 189-203.

Pike, D.A. 2014. Forecasting the viability of marine turtle eggs in a warming world. Global Change Biology 20: 7-15.

Porter, W., J. Mitchell, W. Beckman and C. DeWitt. 1973. Behavioral implications of mechanistic ecology. Oecologia 13: 1-54.

Porter, W.P., and J.W. Mitchell. 2006. Method and system for calculating the spatial-temporal effects of climate and other environmental conditions on animals. p. 155:377 in U.P.a.T. Office, ed, USA.

Porter, W.P., J.L. Sabo, C.R. Tracy, O. Reichman and N. Ramankutty. 2002. Physiology on a landscape scale: plant-animal interactions. Integrative and Comparative Biology 42: 431-453.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Raupach, M.R., P.R. Briggs, V. Haverd, E.A. King, M. Paget and C.M. Trudinger. 2009. Australian water availability project (AWAP): CSIRO marine and atmospheric research component: final report for phase 3; CAWCR Technical Report No. 013. in BOM and. CSIRO, ed. Bureau of Meteorology and CSIRO, Canberra, Australia.

Sarre, S.D., A. Georges and A. Quinn. 2004. The ends of a continuum: genetic and temperature-dependent sex determination in reptiles. Bioessays 26: 639-645.

Spotila, J.R., E.A. Standora, S.J. Morreale and G.J. Ruiz. 1987. Temperature dependent sex determination in the green turtle (*Chelonia mydas*): effects on the sex ratio on a natural nesting beach. Herpetologica: 74-81.

Stancyk, S.E., O.R. Talbert and J.M. Dean. 1980. Nesting activity of the loggerhead turtle *Caretta caretta* in South Carolina, protection of nests from raccoon predation by transplantation. Biological Conservation 18: 289-298.

Standora, E.A., and J.R. Spotila. 1985. Temperature dependent sex determination in marine turtles. Copeia: 711-722.

Stubbs, J.L., M.R. Kearney, S.D. Whiting and N.J. Mitchell. 2014. Models of primary sex ratios at a major flatback turtle rookery show an anomalous masculinising trend. Climate Change Responses 1: 1-18.

Tedeschi, J., W. J. Kennington, J. Tomkins, O. Berry, S. Whiting, M. Meekan and N. Mitchell. 2016. Heritable variation in heat shock gene expression: a potential mechanism for adaptation to thermal stress in embryos of marine turtles. Proceedings of the Royal Society B: Biological Sciences 283: 20152320.

Telemeco, R.S., D.A. Warner, M.K. Reida and F.J. Janzen. 2013. Extreme developmental temperatures result in morphological abnormalities in painted turtles (*Chrysemys picta*): a climate change perspective. Integrative Zoology 8: 197-208.

Whiting, A.U., A. Thomson, M. Chaloupka and C.J. Limpus. 2008. Seasonality, abundance and breeding biology of one of the largest populations of nesting flatback turtles, *Natator depressus*: Cape Domett, Western Australia. Australian Journal of Zoology 56: 297-303.

Wibbels, T. 2003. Critical approaches to sex determination in marine turtles. pp. 103-134. The biology of marine turtles.

Wickham, H. 2011. The split-apply-combine strategy for data analysis. Journal of Statistical Software 40: 1-29.

Yntema, C. 1981. Characteristics of gonads and oviducts in hatchlings and young of *Chelydra serpentina* resulting from three incubation temperatures. Journal of Morphology 167: 297-304.

Yntema, C., and N. Mrosovsky. 1982. Critical periods and pivotal temperatures for sexual differentiation in loggerhead marine turtles. Canadian Journal of Zoology 60: 1012-1016.

Yntema, C.L., and N. Mrosovsky. 1979. Incubation temperature and sex ratio in hatchling loggerhead turtles: a preliminary report. Marine Turtle Newsletter 11: 9-10.

Yntema, C.L., and N. Mrosovsky. 1980. Sexual differentiation in hatchling loggerheads (*Caretta caretta*) incubated at different controlled temperatures. Herpetologica 36: 33-36.

Zambrano-Bigiarini, M. 2014. hydroGOF: Goodness-of-fit functions for comparison of simulated and observed hydrological time series. R package version 0.3-8.

Zeileis, A., and G. Grothendieck. 2005. zoo: S3 infrastructure for regular and irregular time series. Journal of Statistical Software 14: 1-27.

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Marine Turtles in the Kimberley: key biological indices required to understand and manage nesting turtles along the Kimberley coast

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