No evidence of fine scale thermal adaptation in green turtles

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ABSTRACT

Adaptation to increasing temperatures may enable species to mitigate the long-term impacts of climate change. Sea turtles have temperature dependent sex determination (TSD) and variation in the thermal reaction norm, which influences offspring sex ratio, has been suggested as a potential adaptive mechanism to rising global temperatures. Here, we investigate the sex ratio of green turtle *Chelonia mydas* offspring from nests on beaches with notable differences in their thermal properties, to look for evidence of localised adaptation. We compared pivotal temperatures and hatch success in both the laboratory and *in situ* using eggs laid on two nesting beaches (dark *vs.* pale sand) at Ascension Island that represent the extremes of the range of incubation temperatures experienced by this population. We found no effect of beach of origin on pivotal temperatures, hatch success, or hatchling size in the laboratory or the wild. This suggests that turtles from the same rookery are not locally adapted to different thermal conditions experienced during incubation. Under predicted climate change scenarios, this will result in reduced hatch success and an increased proportion of female offspring unless temporal or spatial range shifts occur.

1. Introduction

Clear patterns of spatiotemporal shifts in biotic and abiotic trends have unequivocally been associated with a response to climate change (Laloë et al., 2014; Parmesan and Yohe, 2003; Rosenzweig et al., 2008), as species are forced to adapt, disperse or disappear (Parmesan, 2006). Broad scale responses include changes in phenologies (Scheffers et al., 2016), distributions (Poloczanska et al., 2013) and trophic mismatches (Edwards and Richardson, 2004). Species that exhibit temperature dependent sex determination (TSD) are sensitive to climatic variation (Refsnider and Janzen, 2016) and there are concerns that increasingly imbalanced sex ratios may affect the long term viability of some populations (Laloë et al., 2016; Nelson et al., 2004). Plasticity in the thermal reaction norm, which influences offspring development and sex, has been suggested as one mechanism that may allow adaptation to changing climatic conditions (Refsnider and Janzen, 2016). For instance, painted turtles (*Chrysemys picta*) inhabit a wide geographic area and are thought to exhibit some degree of heritability in pivotal temperature (Refsnider and Janzen, 2016). Marine turtles have inhabited the oceans for the last 100 million years (Naro-Maciel et al., 2008) and, as such, have had to contend with climate change over evolutionary

timescales. So far, sea turtles have been shown to respond to climate change by changing the phenology of nesting (Weishampel et al., 2010), which may ensure clutches incubate under conditions within their thermal norm. Although not yet documented as a response to climate change, plasticity in nest site selection has also been observed with some lizards actively selecting nest sites with specific thermal conditions (Doody et al., 2006; Warner and Shine, 2008). In the short term, this behavioural plasticity may mitigate changes in thermal conditions, which, combined with restricted maternal gene flow through natal philopatry, could create the conditions that lead to localised adaptation. For instance loggerhead turtles (*Caretta caretta*) at the northern extreme of their range produce near balanced offspring sex ratios (Hawkes et al., 2007), but green turtles (*Chelonia mydas*) show a marked contrast in offspring sex ratio from the northern to southern end of the Australian Great Barrier Reef, likely as a result of differing incubation temperatures (Jensen et al., 2018).

1.1. Pivotal temperature and transitional range of temperature

For marine turtles, thermal tolerance limits (beyond which embryonic development and hatching is unlikely to occur) are thought to

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range between 25 °C and 35 °C (Howard et al., 2014), with a greater proportion of females produced at temperatures above the pivotal temperature, at which an equal proportion of males and females are produced. Although many nesting populations are yet to be assessed, pivotal temperatures for green turtles appear to be relatively consistent across the species range, and typically estimated to be between 28.5 - 30 °C (laboratory data: 28.75 °C–29.5 °C Godfrey and Mrosovsky, 2006; Mrosovsky et al., 1984; Stubbs and Mitchell, 2018; Xia et al., 2011; field data: 27.6 °C–30.3 °C Broderick et al., 2000; Candan and Kolankaya, 2016; Godley et al., 2002; Kaska et al., 1998; King et al., 2013; Limpus, 2008; Patrício et al., 2017; Spotila et al., 1987). The transitional range of temperature (TRT), during which a mixed proportion of offspring is produced (Mrosovsky and Pieau, 1991), has been found to span the pivotal by ≈1 °C to 5 °C (Godfrey and Mrosovsky, 2006; Hulin et al., 2009). Furthermore, the TRT can be used to infer resilience in a population, as with greater ranges in temperature during which a mixed sex brood can be produced, there is more potential to respond to the changing thermal conditions and increase the chances of producing the rarer sex (Hulin et al., 2009). It is important to note that the concepts of pivotal temperature and TRT were originally defined for constant incubation temperature conditions (Mrosovsky and Pieau, 1991; Yntema and Mrosovsky, 1982), but are sometimes used to infer sex ratios for field conditions (Hulin et al., 2009). Therefore, comparison between field and laboratory data need to be treated with caution as they are not necessarily determined using the same methods and fluctuations in thermal regimes in the field may influence results (Bull, 1985; Stubbs et al., 2014).

1.2. Population growth, philopatry and thermal adaptation

The lack of parental care in many reptiles means that maternal investment of resources in eggs and abiotic properties of the nest environment are the dominant external influences on embryonic development (Deeming and Ferguson, 1988; Lolavar and Wyneken, 2017; Mitchell, 2015; Nelson et al., 2010). Various theories have been proposed to explain the occurrence of TSD in reptiles (Shine, 1999); one theory is that TSD may confer maternal fitness advantages by enabling the sexual differentiation of embryos best suited to the thermal conditions; combined with philopatry, adaptive fitness may be further enhanced (Shine, 1999). It has been suggested that fine scale philopatry can confer an adaptive advantage both for males and females, as it maintains genetic diversity and facilitates the retention of locally adapted genetic polymorphism (Stiebens et al., 2013). For example, at Ascension Island, Weber et al. (2012) found that success of green turtle eggs, incubated under constant laboratory conditions, differed with beach of origin and hypothesised that philopatry combined with contrasting thermal regimes among nesting beaches may have facilitated local adaptation to specific beach conditions (Weber et al., 2012).

We hypothesise that turtles are adapted to the local conditions of their beach of origin, and therefore maternal output would be optimised to the incubation conditions leading to variation in the thermal reaction norm that influences offspring sex ratios. To investigate this, we build on Weber et al. (2012), and use laboratory conditions to assess how eggs from differing thermal backgrounds, due to female philopatry to beaches of contrasting physical characteristics, perform under controlled thermal conditions. We then compare the output from *in-situ* clutches in the field to investigate how the pivotal temperature of green turtles at Ascension Island may vary between beaches and among females which can provide an insight into mechanisms for adaptation to climate change.

2. Materials and methods

2.1. Study site and species

Ascension Island (14°20′ W, 7°55′ S) is a volcanic island situated midway between the African and South American continents and home

to one of the largest green turtle rookeries in the world (Broderick et al., 2006; Weber et al., 2014). The study beaches, Long Beach (LB) and North East Bay (NEB), collectively support over 55% of nesting activity on the island and exhibit widely differing sand characteristics (LB: 46% of nesting; pale, biogenic sand; NEB: 10% of nesting; dark grey volcanic sand (Stancyk and Ross, 1978; Weber et al., 2014)). The different albedos of these beaches means that sand temperature on NEB is consistently ~2 °C warmer than LB (Hays et al., 1995; Weber et al., 2012) with conditions approaching the limit of known thermal tolerance. A more detailed description of the study site is available from previous publications (Broderick et al., 2001; Godley et al., 2001, 2002, Hays et al., 1995, 1999; Mortimer and Carr, 1987; Weber et al., 2014). Nesting at Ascension Island occurs from late December till June, with a peak in nesting around mid-March.

2.2. Beach incubation

During the 2015 and 2016 nesting seasons, a total of 88 clutches laid above the high tide line were selected at random across each study beach (2015: LB n = 23, NEB n = 21; 2016: LB n = 23, NEB n = 21). A Tinytag Plus 2 data logger (models: TGP4017 and TGP4500; Gemini Data Loggers Ltd., Chichester, UK) was placed in the centre of each clutch during laying (after approximately 50 eggs were deposited) and female curved carapace length (CCL notch to tip - Bolten, 1999) was recorded. Once the turtle had finished covering the clutch, wooden stakes were positioned around the nest to prevent it being destroyed by other nesting turtles and GPS location recorded. After 40 (NEB) or 50 (LB) days of incubation, to encompass minimum previously recorded incubation duration for each beach (Godley et al., 2002), a wooden corral (ESM 1 - Fig. S1) was placed on the surface of the sand above the clutch and checked daily at first light to monitor for hatching. A random sample of hatchlings (n = 10 per clutch) was collected from each hatched nest for measurement and histological sexing and the remainder released the following night. After hatching, nests were excavated and all contents were removed and classified as hatched or unhatched eggs. Unhatched eggs were opened to determine development stage as either early term embryo (embryo smaller than residual yolk) or late term embryo (embryo larger than residual yolk). Hatch success was defined as the number of hatched eggs divided by the clutch size (Miller, 1999).

2.3. Laboratory incubation

2.3.1. Incubation set up

For the duration of this study four sets of incubation experiments were carried out using the following design. In 2015, two sets of incubation experiments using eight incubation temperatures ranging from 26 °C to 33 °C at 1 °C increments was carried out (except 26 °C where n = 1 replicate; time constraints precluded a second round of incubation at that temperature). In 2016, two sets of incubation experiments using a restricted range of three temperatures from 29 °C to 31 °C was carried out, with three replicates of each temperature.

All incubations were carried out in custom-made forced air incubators (ESM1 - Fig. S2), set at different constant temperatures. Each incubator contained two boxes filled with humidified vermiculite (water:vermiculite ratio 1.7:1,--50 kPa, Booth, 2004). Temperature was recorded using a Tinytag Plus 2 data logger at 30 min intervals. Prior to and after each season, each temperature datalogger was checked against a calibrated datalogger in a constant temperature room to verify accuracy and precision of readings. Any data logger varying by > 0.3 °C was excluded from the study.

A total of 528 eggs were sampled from 40 clutches over two nesting seasons, as follows. During the 2015 nesting season, 16 clutches were sampled (8 from LB and 8 from NEB) and eight eggs taken from each clutch at the point of laying. One egg from each study clutch was placed into each of the eight incubators (n = 1 egg/clutch/beach/temperature

treatment); note for replicate 2, only seven eggs per clutch were collected as only seven temperature treatments were used; total of 240 eggs collected and incubated in 2015. During the 2016 nesting season, 24 clutches were sampled (12 from LB and 12 from NEB) and 12 eggs taken from each clutch at the point of laying. Four eggs from each study clutch were placed into each of the three temperature treatments (n = 4 eggs/clutch/beach/temperature treatment); total of 288 eggs collected and incubated in 2016.

2.3.2. Sample collection and management

In the field, eggs from each clutch were placed in labelled sample bags within an insulated box and transported back to the laboratory. They were brushed free of sand and organic material, patted dry and weighed to the nearest 0.01 g (PGW 4502e Adam Scales, d = 0.01 g). Each egg was randomly allocated to a box within an incubator. Eggs from each beach were distributed around the edge of each box and labelled with a pencil. The central position in each box was reserved for the data logger and thermostat or thermometer probe (Fig. S3A&3B). Eggs were buried in the vermiculite to two thirds of their height, to avoid desiccation whilst enabling them to be monitored for fungal or bacterial growth. Placement in the incubator occurred within six hours of oviposition. Incubators were checked daily to ensure the temperature was adequate, opened to allow for ventilation, and to monitor the condition of the incubating eggs. From 40 days (or first sign of pipping) onwards, separators were placed between the eggs to isolate any hatchlings that emerged and inspected at up to four-hour intervals to monitor signs of hatchling emergence.

2.4. Sexing

All laboratory incubated hatchlings and ten hatchlings from each *in situ* study clutch were weighed to the nearest 0.01 g (PGW 4502e Adam Scales, d = 0.01 g) and measured (SCL: straight carapace length in mm) to the nearest 0.1 mm with electronic callipers (Digitronics Caliper, Polycal Series). Hatchlings were then euthanised using a modified version of Work and Balazs (2013) and dissected to excise the adrenal-kidney-gonad (AKG) complex and the yolk residue, with the latter weighed to the nearest 0.01 g. The AKG was fixed in 10% formalin for a minimum of 48 h, dehydrated in a series of alcohol baths and cleared in xylene, before embedding in paraffin wax, sectioning (at 3 to 10 μm; Shandon Finesse 325 microtome; blade: MX35 ultra, 34˚, 80 mm) and staining. Sex was then determined using histological criteria by examining the sections under a light microscope. Male gonads were distinguished by a thin smooth cortex and the presence of immature seminiferous tubules in the medulla whereas female gonads exhibited a thickened and infolded cortex with a fairly homogenous medulla (Godfrey et al., 1999; Godfrey and Mrosovsky, 2006; Miller and Limpus, 2002; Yntema and Mrosovsky, 1980). If no sex could be determined, further sections and staining were carried out until a clear readable slide was obtained. Slides were read independently by two researchers and if they did not agree the slides were read again or the gonad reprocessed until a consistent result was obtained.

2.5. Statistical analysis

For *in situ* clutches, clutch sex ratio was evaluated at a clutch level (proportion female) using a binomial (logit link) generalised linear mixed model (GLMM) with incubation temperature during the thermosensitive period (TSP), beach of origin as a fixed effect with a random effect of female identity, starting with the temperature*beach interaction.

For the laboratory analysis, a generalised linear mixed model (GLMM) with a binomial error structure (logit link) was carried out to assess the importance of temperature (mean middle third of incubation), beach of origin (LB or NEB), adult female size (CCL), replicate (*e.g.* season 1 replicate 1), with female identity as random effect, as

predictors of offspring sex, starting with a temperature*beach interaction effect.

The pivotal temperature and transitional range of temperatures for each beach were determined separately, and for the combined dataset, using function *tsd* in R package '*embryogrowth*' (Girondot, 2016). The sex-temperature curves and associated field pivotal and field transitional range of temperatures for *in situ* conditions were then produced using this same approach.

Pivotal temperature is defined for constant incubation conditions, but the term is also erroneously used for field conditions to describe the mean temperature during the middle third of incubation that produces a 1:1 sex ratio. Given that this term is widely used in the literature, we clarify it by determining the field pivotal temperature as the time weighted temperature during the thermosensitive period for each clutch, and use these data as constant temperature equivalent combined with histology data to produce a temperature-sex curve and infer field pivotal temperature and the range of temperatures producing mixed sex clutches. This analysis is carried out using package '*embryogrowth*' (Girondot et al., 2018), following methodology described by Girondot et al., 2018; Girondot and Kaska, 2014; Monsinjon et al., 2017.

For the laboratory study, hatch success was analysed at the egg level (hatched/unhatched) using a generalised linear mixed model (GLMM) with a binomial error structure (logit link) assessing the impact of temperature and beach of origin, starting with the interaction between these, with female identity as a random effect. For *in situ* clutches, hatch success was analysed at a clutch level (number of hatched eggs, number of eggs not hatched) using a binomial generalised linear mixed model (GLMM) with mean incubation temperature, beach of origin, and nest depth as a fixed effect with a random effect of female identity. *Post hoc* analysis of hatch success and mean incubation temperatures between beaches was evaluated with a Wilcoxon test for non-normal distribution.

For both the laboratory analysis and *in situ* clutches, we carried out a linear mixed model (LMER) to assess the importance of incubation temperature, beach of origin and sex on hatchling size (Straight Carapace Length), starting with the interaction between temperature and beach of origin.

Models were evaluated using information theoretic model selection, implemented with package '*MuMIn*' (Bartoń, 2018), ranked by AIC value, and graphically checked for the relative importance of terms. All analyses were carried out in R version 3.4.1 (R Core Team, 2017).

3. Results

3.1. Beach incubation

For clutches incubated *in situ* from which we sampled hatchlings for sexing (n = 26 clutches; LB = 12, NEB = 14), we obtained a best fit model suggesting a field pivotal temperature of 28.9 °C with a mixed proportion of sexes occurring between 27.1 °C and 30.6 °C for both LB and NEB combined (Fig. 1 - A). Note, we could not fit the model for individual beaches as no NEB nests sampled produced male hatchlings. The result of the GLMM indicates a significant effect of temperature ($\chi^2_{(1)}$: 24.6, $p < 0.001$), but no temperature*beach interaction ($\chi^2_{(1)}$: 0, $p = 0.99$) or beach effect ($\chi^2_{(1)}$: 0.8, $p = 0.4$).
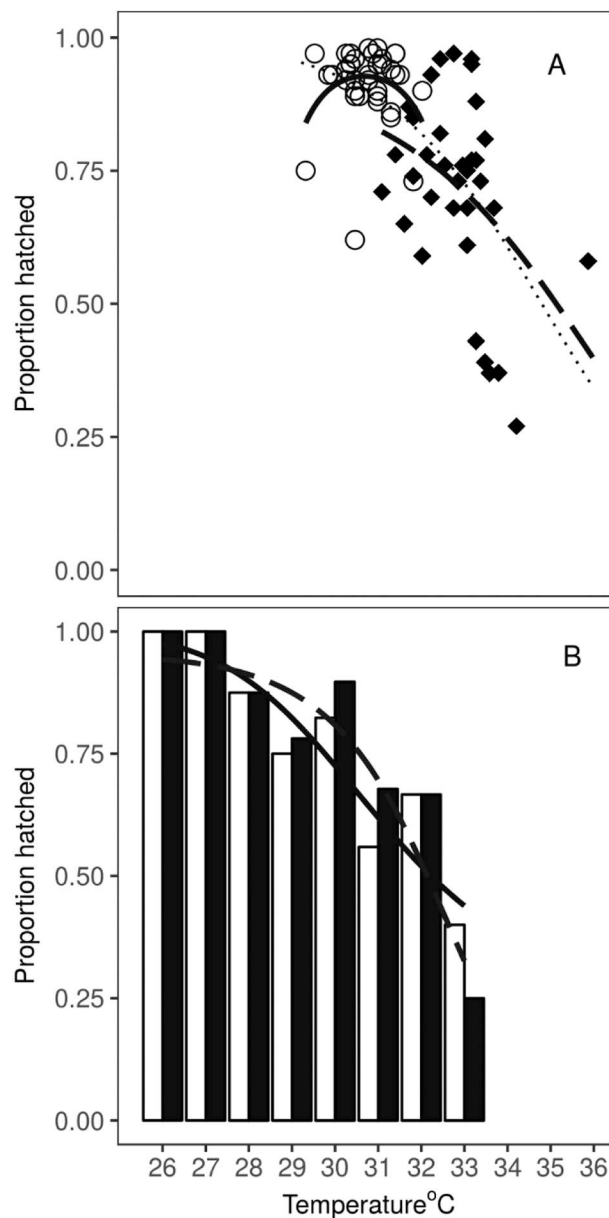
In situ clutches had a mean hatch success of 81% (SD 17%, n = 72 clutches), with clutches on LB having significantly higher success than nests on NEB (W = 1199, $p < 0.001$; LB: 91%, SD = 7%, n = 37, NEB: 71%, SD = 18%, n = 35) (Fig. 2 - A), but also experiencing significantly cooler incubation temperatures (mean temperature: W = 77.5, $p < 0.001$; LB: 31 °C, SD = 0.6 °C, n = 37, NEB: 33 °C, SD = 0.9 °C, n = 35). The effect of temperature ($\chi^2_{(1)}$: 4.92, $p = 0.03$) and beach ($\chi^2_{(1)}$: 5.88, $p = 0.02$) had a significant negative impact on hatch success (Fig. 2-A). There was however no effect of mean incubation temperature*beach interaction ($\chi^2_{(1)}$: 1.74, $p = 0.18$).
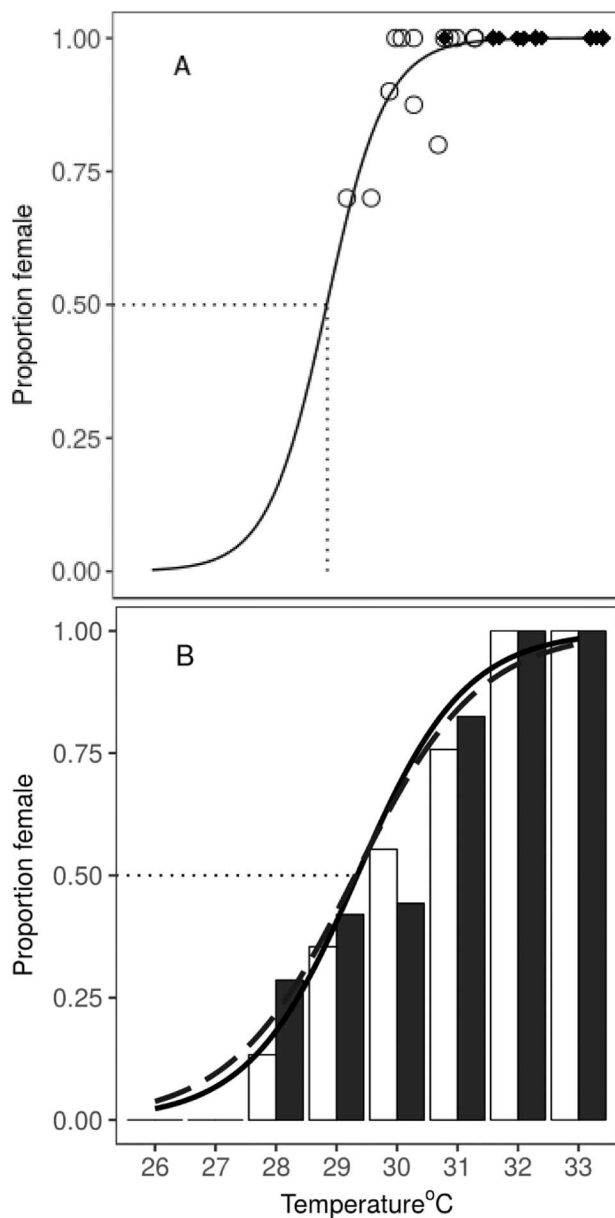
Fig. 1. Proportion of females obtained from eggs in relation to incubation temperature in field (A) and laboratory (B) conditions. A - Beach: Proportion of females obtained in clutches on LB (open circles) and NEB (full diamonds) in relation to mean middle third of incubation temperature. The trend line is a binomial generalised linear model regression, where data were not split between beaches for lack of males in sampled nests on NEB. Dotted lines indicate temperature at which equal sex ratio is obtained (field pivotal temperature). B -Laboratory: Proportion of females in relation to mean incubation temperature binned by 1° increments for LB eggs (white fill, solid trend line) and NEB eggs (black fill, dashed trend line). The trend lines are based on a binomial generalised linear model. Dotted line indicates pivotal temperature, at which balanced sex ratio is obtained.
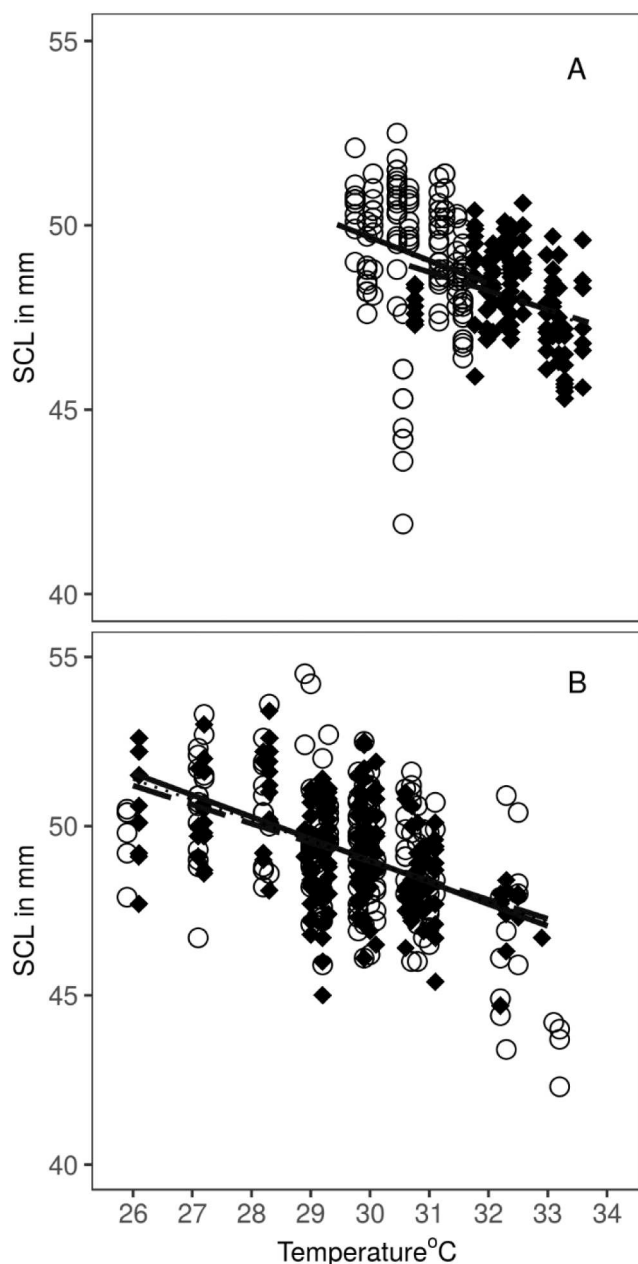

Fig. 2. Hatching success for eggs in relation to incubation temperature in field (A) and laboratory (B) conditions. A - Beach - Proportion of LB (open circles, full black line) and NEB (full diamonds, dashed line) eggs hatched in relation to mean incubation temperature. Dotted line is the combined hatching success, fit with a binomial general linear model. Trend lines start at coldest and end at the hottest recorded temperature, rounded to the closest full °C, on each beach (LB: 29.5 °C–32 °C, NEB: 31 °C–36 °C). B - Laboratory - Proportion of LB (white bar, solid trend line) and NEB (black bar, dashed trend line) eggs hatched in relation to mean incubation temperature. Data are binned in 1° increments, fit with a binomial general linear model.

3.2. Laboratory incubation

In our laboratory incubated eggs, multi model inference determined that there was no statistical significance for the interaction between beach and temperature ($\chi^2_{(1)}$: 0.29, $p = 0.56$), group ($\chi^2_{(3)}$: 3.88, $p = 0.27$), beach of origin ($\chi^2_{(1)}$: 0.03, $p = 0.85$), or female size ($\chi^2_{(1)}$: 4.37, $p = 0.04$) on offspring sex (ESM2 - Table S1, S2) (Johnson, 2013), however there was a significant effect of incubation temperature ($\chi^2_{(1)}$: 132.0, $p < 0.001$).

From our laboratory study we estimate the pivotal temperature to be 29.75 °C [TRT 27.45 °C – 32.07 °C] and 29.74 °C [TRT 26.97 °C –

Further analysis of nest content revealed that late stage arrest is more common in clutches incubated on NEB (W = 223.5, $p < 0.001$; LB: 3.6 embryos, SD = 6.1, n = 37 clutches, NEB: 20.9 embryos, SD = 24.1, n = 34 clutches).

Temperature had a negative effect on hatchling size ($\chi^2_{(1)}$: 8.62, $p < 0.005$), with no effect of beach of origin ($\chi^2_{(1)}$: 0.1, $p = 0.75$), sex ($\chi^2_{(1)}$: 0.25, $p = 0.61$), or mean incubation temperature*beach interaction ($\chi^2_{(1)}$: 0.01, $p = 0.9$) (Fig. 3 - A).

Fig. 3. Straight carapace length of offspring in relation to different incubation temperatures in field (A) and laboratory (B) conditions. A - Beach - Straight Carapace Length (SCL) in mm of hatchlings from LB (n = 120 offspring, 12 clutches; open circles, full black line) and NEB (n = 128 offspring, 13 clutches; full diamonds, dashed line) in relation to mean incubation temperature. Trend lines start at coldest and end at the hottest recorded temperature, rounded to the closest full °C, on each beach (LB: 29.5 °C – 32 °C, NEB: 31 °C - 36 °C). B - Laboratory - Straight Carapace Length (SCL) in mm of hatchlings from LB (n = 188; open circles, full black line) and NEB (n = 201; full diamonds, dashed line) in relation to mean incubation temperature.

Previous laboratory based studies suggest that, as a result of evaporative cooling, the core egg temperature is between 0.25 °C and 0.5 °C cooler than the air, thus a correction factor approximating to the mean difference of 0.4 °C was applied (Mrosovsky et al., 2009), and we obtained a corrected pivotal temperature of 29.35 °C [TRT 26.8 °C – 31.89 °C] (Fig. 1 - B).

Mean incubation temperature had a significant negative effect on hatching success (GLM, $\chi^2_{(1521)}$: 59.6, p < 0.001). Neither beach of origin (GLM, $\chi^2_{(1520)}$: 1.4, $p = 0.24$) nor the interaction between beach * temperature (GLM, $\chi^2_{(1521)}$: 0.48, $p = 0.49$) had any effect (Fig. 2 - B).

Temperature was found to have a negative effect on hatchling size ($\chi^2_{(1)}$: 70.9, $p < 0.001$), but with no effect of beach of origin ($\chi^2_{(1)}$: 2.76, $p = 0.1$), sex ($\chi^2_{(1)}$: 0.57, $p = 0.45$), or mean incubation temperature*-beach interaction ($\chi^2_{(1)}$: 2.8, $p = 0.1$) (Fig. 3 - B).

4. Discussion

4.1. Key findings

In this study we tested whether variation in pivotal temperatures in green turtles may provide a mechanism for adaptation to predicted rising temperatures. Comparing the pivotal temperatures obtained in laboratory condition for eggs from two beaches with different thermal conditions, we find them to be consistent between beaches, with some variation in the transitional range of temperature. All differences in offspring sex, size and hatching success of clutches recorded between the two beaches were a result of incubation temperature.

4.2. Context

Over the last 150 years, or 3 to 5 sea turtle generations (Seminoff, 2004), temperatures have progressively risen (Hays et al., 2003). The lack of difference in pivotal temperature that we recorded between the nesting beaches suggests adaptation to specific nesting beaches does not occur or that gene flow through paternal influence or maternal exploratory behaviour between the distinct nesting aggregations masks the specific long term adaptation. If there is a lack of natural plasticity in pivotal temperatures between greatly differing thermal environments, it may be more difficult for long lived species with extended generation times to deal with the rapid pace of contemporary climate change, as overall fewer males will be produced. The short geographic separation (≈ 7 km straight line distance) between the beaches in our study may not lead to selective pressure on adaptation of pivotal temperatures as there is still a production of males from nearby beaches. Thus, we may expect isolated rookeries to be more labile as presumably increasing the production of the rarer sex would provide fitness benefits to the population. Recent studies suggest that differing thermal conditions in nesting grounds at the extremes of the Great Barrier Reef (Jensen et al., 2018) and in Malaysia through the use of shading in hatcheries (Jensen et al., 2016) are responsible for the different observed sex ratios on foraging grounds. Similarly, divergence in loggerhead populations using thermally distinct conditions in the Mediterranean has not led to any specific adaptation to local conditions (Monsinjon et al., 2017). However it must be noted that male mediated gene flow has been shown to occur at ocean basin level (Roberts et al., 2004), and therefore selection of pivotal temperature may not be required, as males may prove to be less philopatric.

In the absence of any pre-existing genetic adaptation, turtles may need to respond to rapid climate change through range shifts, altered phenology, or nest site selection, although the former will be problematic for populations using isolated rookeries such as Ascension Island. It is the plasticity of each individual which in the long run may confer the adaptive potential of the population (capturing this among female variation would require sampling full clutches of multiple females which would be ethically questionable and logistically challenging). Sea turtles inhabit all major temperate oceans, with different nesting

32.51 °C] for LB and NEB respectively (ESM2 - Table S3 for data; model output). Contrary to our hypothesis, there was no significant difference in pivotal temperatures between beaches, but NEB data suggest a wider transitional range of temperature. Thus, we fitted the data in a single model without differentiating between beach of origin to obtain a pivotal temperature of 29.75 °C [TRT 27.20 °C – 32.29 °C] for this population (AICc accounting for beach = 414.40, AICc excluding beach = 410.93).

aggregations found along vast geographic areas, and varied biotic and abiotic conditions (*e.g.* different thermal conditions, rainfall and vegetation levels between Florida, Ascension, Poilão, and the Eastern Mediterranean) thus it would be surprising if each rookery responded in the same manner and exhibited the same thermal tolerances.

Ectotherms are highly sensitive to thermal conditions and respond to changes in different ways; for instance brown anoles (*Anolis sagrei*) (Logan et al., 2018) and snow skinks (*Niveoscincus* species) (Caldwell et al., 2017) show strong phenotypic plasticity as an immediate response to changing thermal conditions. However the long term response in anoles is slow, meaning that they may not be able to keep up with the rate at which climate change is occurring (Logan et al., 2018), but the skinks may be showing signs of adaptive evolution (Caldwell et al., 2017). In contrast, *Pleurodema thaul*, a south American frog, exhibits a behavioural change to contend with thermal variation (Barria and Bacigalupe, 2017), and the Andean toad (*Rhinella spinulosa)* showed little response, and generally coped with a wide variation in temperatures (Riquelme et al., 2016). Conversely Asian sea bass (*Lates calcarifer*) exhibit underlying molecular adaptation to cooler temperatures (Newton et al., 2013). Sessile species, such as larch (*Larix gmelinii*) may face more pressures as spatial displacement is not possible for established individuals, leading to interesting situations where the trees are adapted to their local provenance, but acclimatise to current conditions (Xiankui and Chuankuan, 2018).

Thus it is clear that there is not a single response to increasing temperatures, and understanding the mechanisms driving the selection is complex; whether sea turtles truly adapt to climate change is yet to be conclusively determined, and whether they can on such a rapid scale is debatable.

5. Conclusion

Our findings for the laboratory data are consistent with pivotal temperatures obtained from other green turtle rookeries using similar methodology (*e.g.* Suriname 29.2 °C (Godfrey and Mrosovsky, 2006), China 29 °C (Xia et al., 2011)). Field pivotal temperature for *in-situ* clutches was also consistent with what was previously found for Ascension (28.8 °C - Godley et al., 2002) nearly two decades earlier and may show slight variation from other sites (*e.g.* Poilão, Guinea Bissau 29.4 °C (Patrício et al., 2017), Heron Island, Australia < 28.7 °C (Limpus et al., 1983)).

Contrary to our hypothesis, and the findings of Weber et al. (2012) that suggested there may be a genetic basis to heat tolerance of turtles nesting on NEB, we found no evidence of fine scale adaptation to thermal conditions. We do however find reduced hatch success at higher temperatures which suggests that increasing temperatures will have a detrimental impact on overall hatchling production in addition to skewing the already very highly female bias, as has been previously suggested (Broderick et al., 2001; Hawkes et al., 2007; Hays et al., 2017; Kobayashi et al., 2017). Therefore to contend with increasing temperatures, turtles on Ascension may need to shift spatially or temporally.

Ethics

Research was carried out under Ascension Island Government Conservation Department Research Permits #ERP-2014-11, #ERP-2015-17, and in accordance with the University of Exeter CLES Ethics Committee # 2015/890.

Data

Data are stored in the Ascension Island Government Conservation Department database. Data are available on figshare, under embargo until publication. Beach data: https://figshare.com/s/dc16fbd027a59560ce1d; Lab data: https://figshare.com/s/24788e81a4140547b38b.

Competing interests

The authors declare that the research was carried out without any competing interests.

Authors contributions

ACB, BJG and SW conceived the study and were awarded a NERC icase studentship. DT conducted the field work with assistance and/or guidance from all co-authors. DT led the data analysis and writing of the manuscript with assistance from all co-authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jembe.2019.04.001.

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