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Spatial and temporal variation in nest temperatures forecasts sex ratio skews in a crocodilian with environmental sex determination

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Species displaying temperature-dependent sex determination (TSD) are especially vulnerable to the effects of a rapidly changing global climate due to their profound sensitivity to thermal cues during development. Predicting the consequences of climate change for these species, including skewed offspring sex ratios, depends on understanding how climatic factors interface with features of maternal nesting behaviour to shape the developmental environment. Here, we measure thermal profiles in 86 nests at two geographically distinct sites in the northern and southern regions of the American alligator's (*Alligator mississippiensis*) geographical range, and examine the influence of both climatic factors and maternally driven nest characteristics on nest temperature variation. Changes in daily maximum air temperatures drive annual trends in nest temperatures, while variation in individual nest temperatures is also related to local habitat factors and microclimate characteristics. Without any compensatory nesting behaviours, nest temperatures are projected to increase by 1.6–3.7°C by the year 2100, and these changes are predicted to have dramatic consequences for offspring sex ratios. Exact sex ratio outcomes vary widely depending on site and emission scenario as a function of the unique temperature-by-sex reaction norm exhibited by all crocodilians. By revealing the ecological drivers of nest temperature variation in the American alligator, this study provides important insights into the potential consequences of climate change for crocodilian species, many of which are already threatened by extinction.

1. Introduction

A changing global climate is eliciting widespread impacts on organisms across diverse ecosystems [1–4]. Due to unique features of their life history, species with environmental sex determination and specifically temperature-dependent sex determination (TSD) are especially vulnerable to rapid environmental change [5]. In these species, the temperature embryos experience during incubation determines whether they develop as male or female [6–8]. As a result, thermal regimes associated with climate change have the potential to profoundly impact population sex ratios as well as inter- and intrasexual variation [9,10]. Such skews in population sex ratios can threaten population persistence, and even lead to local extinctions via reduced effective population size and mate limitation [11,12].

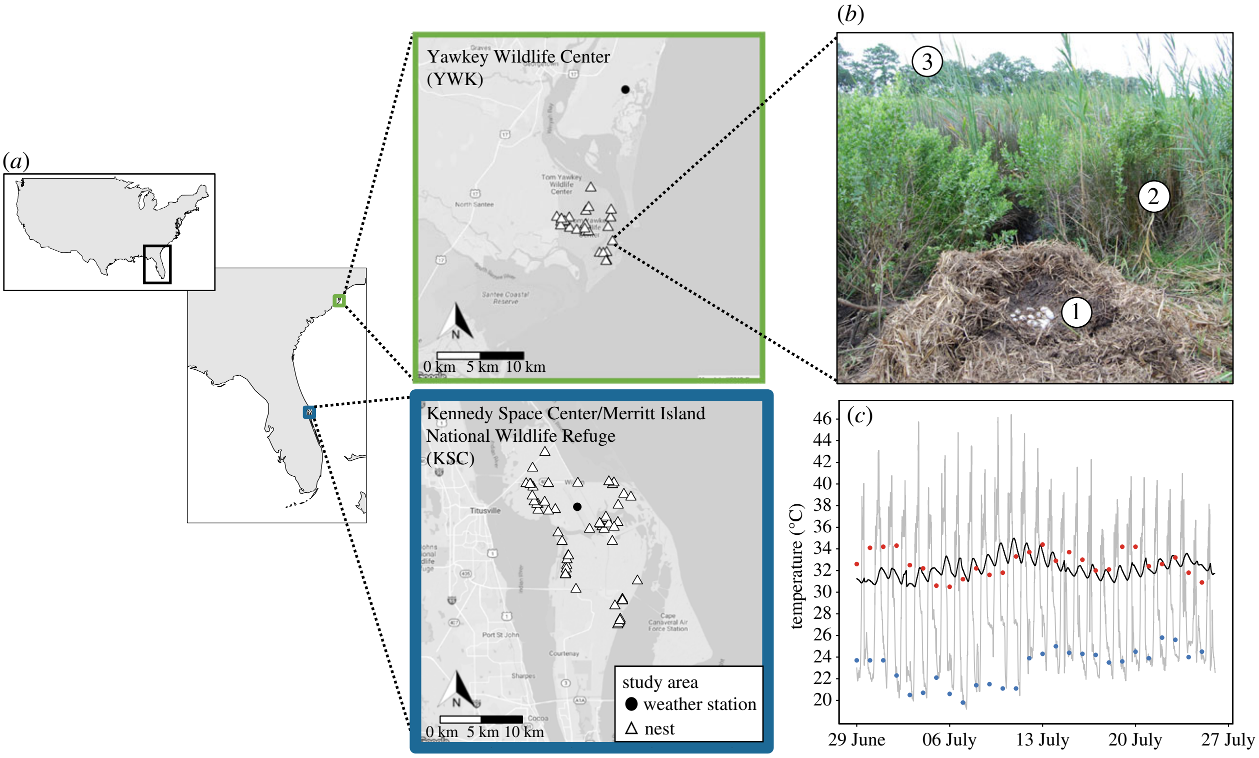

Figure 1. (*a*) Map of nests monitored at KSC (blue) and YWK (green). (*b*) A representative nest with nest cavity exposed. At each nest, at least one thermal logger (1) was placed inside the nest cavity, one thermal logger (2) was placed in close vicinity to the nest and weather station data (3) were accessed for the dates corresponding to the TSP. (*c*) Nest temperature profile during the TSP for one representative nest. Black line is temperature profile inside nest cavity, grey line is microclimate profile in vicinity of the nest, red points are weather station maximum air temperatures and blue points are weather station minimum air temperatures. (Online version in colour.)

Predicting the consequences of future environmental change for incubation temperatures, sex ratios, and population viability, however, remains challenging [5,13,14], in part, because such predictions require an understanding of the state of current nesting environments and the ecological drivers that shape them. Shifts in environmental conditions may affect the nest environment both directly via changes to the nest microclimate and indirectly via influences on components of maternal nesting behaviour including nesting phenology, nest-site choice and nest architecture [15–17]. Patterns in nest temperatures across temporal and spatial ranges reflect these complex interactions and can provide insights into how species respond to environmental variation. However, long-term datasets from actual nest cavities are scarce and taxonomically limited.

Species with TSD are likely to vary widely in their responses to environmental change as a function of variation in their patterns of thermal sensitivity, nesting ecology, geographical distributions and life histories. All crocodilian species studied to date exhibit a unique pattern of TSD in which females are produced at low and high temperatures and males at intermediate temperatures (type F–M–F) [18]. As a result, climate change could bias population sex ratios towards males or females depending on the magnitude of temperature shifts. This uncertainty coupled with crocodilians' long generation times and exclusive reliance on temperature to determine offspring sex contributes to this order's unique vulnerability to rapid environmental change [19,20]. Approximately half of all crocodilian species already face the threat of extinction due to anthropogenic threats including habitat destruction, environmental contaminants and poaching [21–24]. Thus, effectively predicting the consequences of future environmental change for crocodilians represents a timely conservation challenge.

Here, we address the potential impact of future climatic scenarios on the nesting ecology and nest thermal dynamics of the American alligator. With a broad geographical range, the American alligator provides a unique opportunity to examine variation in nest thermal dynamics across multiple spatial and temporal scales. In addition, alligator embryos are exquisitely sensitive to small changes in temperature as incubation below 31.5°C and above 34.5°C produces approximately 100% female offspring, while temperatures between 32.5°C and 33.5°C produce approximately 100% male offspring [7]. The objectives of our study were to (i) characterize temporal and spatial variation in the nest thermal dynamics of the American alligator, (ii) examine the climatic and maternal drivers of variation in nest thermal dynamics, and (iii) apply our understanding of these drivers to predict changes in alligator nest thermal dynamics and resulting offspring sex ratios under future climatic scenarios.

2. Material and methods

(a) Study sites and field methods

Nests were monitored at two sites in the northern and southern regions of the alligator's geographical range (figure 1*a*). Both sites are characterized by extensive upland and wetland coastal marsh habitats but differ in latitude and local ambient conditions. From 2010 to 2017, 56 alligator nests were monitored at Kennedy Space Center/Merritt Island National Wildlife

Refuge (KSC; southern site), Merritt Island, FL (figure 1*a*). From 2015 to 2018, 30 alligator nests were monitored at Yawkey Wildlife Center (YWK; northern site), Georgetown, SC (figure 1*a*).

Nests were located during June via helicopter aerial surveys. At the southern site, physical properties of each nest including nest length, width, height, and shading were assessed prior to deploying the temperature loggers. Nest shading was assessed on a scale of 1–4 based on the estimated percentage of shade a nest would experience throughout a day—1 (0–24%), 2 (25–49%), 3 (50–74%) and 4 (75–100%). While this method of assessing shade is limited in comparison with more quantitative methods [15,25], it was deemed sufficient to represent the scale of variation observed in the field and similar ranking methods have been successfully implemented in other studies of reptile nesting ecology [26,27].

Each nest cavity was carefully exposed, and depth to the topmost, middle and bottommost egg in the nest cavity was recorded, and an Onset (UTBI-001) HOBO temperature logger pre-programmed to record temperature at 5 min intervals was deployed in the middle of the nest cavity. An additional temperature logger was attached to vegetation in close proximity to the nest, out of direct sunlight, to record the air temperature of the nest microclimate. Nests were left undisturbed until after hatchlings had emerged and temperature loggers were retrieved.

(b) Data processing

Nest temperature analyses were limited to the dates encompassing the thermosensitive period (TSP; Ferguson stage 15–stage 24 [7,28,29]), which were estimated for each nest by back-calculating from hatch date using the mean nest temperature during the incubation period [30]. Daily maximum air temperature, daily minimum air temperature and daily precipitation were collected for the dates associated with the estimated TSP for each nest. For the southern site, data were obtained from the National Oceanic and Atmospheric Administration (NOAA) National Centers for Environmental Information for the Titusville 7 weather station. For the northern site, data from the NOAA National Estuarine Research Reserve System (NERRS) system-wide monitoring programme were accessed for the North Inlet–Winyah Bay Oyster Landing weather station.

To obtain elevations of alligator nests, we used a digital terrain model (DTM) generated from LiDAR data acquired during 2007, obtained from the NOAA Digital Coast [31] (https://coast.noaa.gov/digitalcoast/). The DTM was classified as terrain by converting the points directly to raster without interpolation at a cell size of 3 m. To obtain geographical feature data, we used a land cover map of the study area generated from colour infrared aerial imagery acquired on 9 November 2010 and supplemented with imagery from February 2007 and 2009 [32]. To obtain local rainfall data, we used NEXRAD data collected by the National Weather Service. Using a geographic information system (GIS), for each nest location we obtained the elevation from the DTM, extracted the land cover type, measured the distance to the nearest large body of water and summed the rainfall amounts occurring within the 1 km grid containing each nest over the nesting period.

Statistical analyses were conducted using R statistical software v. 3.5.1 [33]. Comparisons of mean nest temperatures, mean microclimate temperatures and weather station parameters at the two sites for 2015–2017 were conducted using two-factor ANOVAs. If necessary, data were transformed to achieve normality and homoscedasticity. Transformations were not sufficient to alleviate non-normality for daily maximum air temperature or daily minimum air temperature for 2015–2017. In addition, transformations were not sufficient to alleviate heteroscedasticity for mean microclimate temperature or daily maximum air temperature for 2015–2017.

The relationships between mean nest temperature and the weather station parameters (mean daily maximum temperature, mean daily minimum temperature and mean daily precipitation) across years were assessed using weighted linear regression, using the number of nests in a year as weights.

(c) Climatic and maternal drivers of nest temperature variation

Penalized linear regression with elastic net regularization using the glmnet package in R was used to model the relationship of ecological parameters to mean nest temperatures [34]. Nonparametric bootstrapping was used to assess the precision of estimates [35]. Data from 42 nests were used in the elastic net regression. Data from nests monitored between 2010 and 2016 at the southern site composed the training set used to fit the elastic net regression model, and data from nests monitored in 2017 at the southern site composed the test set used to test the performance of the model. Factors selected to be included in the model and under the control of maternal nesting behaviour were further assessed for patterns across years. For each year, temperatures of the nests in the top and bottom 50th percentile for each maternally driven nest characteristic were compared.

(d) Climate projections

To model future climatic scenarios, we used the localized constructed analogs (LOCA) statistically downscaled climate projections for 30 climate models in the Coupled Model Intercomparison Project Phase 5 (CMIP5) collection [36] under two emission scenarios, Representative Concentration Pathways (RCPs) 4.5 and 8.5 [37]. Downscaled climate projection data for the years 2006–2100 were accessed via the USGS Geo Data Portal (https://cida.usgs.gov/gdp) [38] for each of the sites using spatial bounding boxes defined by the minimum and maximum latitude and longitude of nest GPS locations at the northern site (minimum latitude = 33.17015°, maximum latitude = 33.24670°, minimum longitude = −79.27430°, maximum longitude = −79.2049333°) and southern site (minimum latitude = 28.48°, maximum latitude = 28.68°, minimum longitude = −80.76°, maximum longitude = −80.61°).

(e) Nest temperature and sex ratio projections

Nest temperatures were projected by applying a weighted regression of mean daily maximum air temperature during the TSP from the weather station and mean nest temperature for each of the sites. A weighting scheme was developed to estimate mean daily maximum air temperatures during the TSP based on the relative representation of individual days in the TSP. The frequency with which a given day was represented in the TSP was determined for both sites, and this frequency was multiplied by the corresponding day's maximum air temperature from the weather station. The sum of the products yielded a weighted mean daily maximum air temperature for a given year based on the days likely to compose the TSP. The mean nest temperature was then regressed against this weighted mean daily maximum air temperature during the TSP from the weather station using weighted linear regression. The equations that described the relationship between the mean nest temperature and the mean daily maximum air temperature ($T_{\max}$) are: mean nest temperature = 0.6755 ($T_{\max}$) + 10.5317 ($F_{1,6} = 16.48$, $R^2 = 0.7331$, p-value = 0.00665) and mean nest temperature = 0.8323 ($T_{\max}$) + 6.1515 ($F_{1,2} = 4.688$, $R^2 = 0.701$, p-value = 0.163), for the southern and northern sites, respectively.

Prior to applying these equations, mean daily maximum air temperatures during the TSP for the years 2006–2100 were calculated from the daily projections for each climate model by multiplying the projected daily maximum air temperatures for each day by the frequency of that day's representation in the

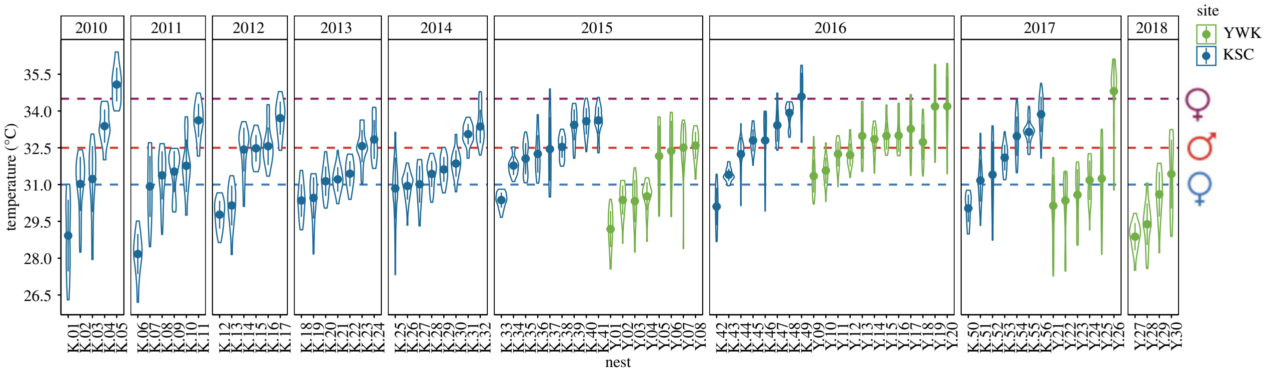

Figure 2. Violin plots of the kernel density of daily temperatures of single alligator nests during the thermosensitive period (KSC nests in blue; YWK nests in green). Central points of violins represent mean nest temperature during the TSP, and vertical lines represent ±1 s.d. Horizontal lines represent threshold temperatures that promote the production of either 100% female or 100% male offspring. The blue line and maroon line represent temperatures that promote female development, and the red line represents a temperature that promotes male development. (Online version in colour.)

TSP and then taking a sum of the products. Individual model projections were then bias-corrected by correcting for the mean difference between the weather station mean daily maximum air temperature and the climate model mean daily maximum air temperature for a reference period (2010–2017 for KSC and 2015–2018 for YWK [39]). This correction factor was calculated for each individual model and applied to the projected mean daily maximum air temperature for the years 2006–2100. The site-specific weighted regression equation relating mean nest temperature to weather station daily maximum air temperature was then applied to these corrected climate projections to predict mean nest temperatures for the years 2006–2100.

To predict offspring sex ratios from mean nest temperature projections, two separate two-parameter log-logistic functions were fit to data from [7] using the drc package in R [40]. The two-parameter log-logistic model is fit using a unified structure with two coefficients—*b*, denoting the steepness of the dose–response curve, and *e*, describing the effective dose yielding 50% males—and has lower and upper asymptotes at 0 and 1, respectively. Thus, the general model function is

$$y = \frac{1}{1 + \exp(b(\log(x) - \log(e)))}. \qquad (2.1)$$

We fit two equations to describe each side of the alligator temperature-by-sex ratio reaction norm. The coefficients for the function relating incubation temperature to sex ratios for incubation temperatures less than 33°C were: $b = -281.81$ (s.e. = 18.50), $e = 31.93$ (s.e. = 0.0064). The coefficients for the function relating incubation temperature to sex ratios for incubation temperatures greater than 33°C were: $b = 152.58$ (s.e. = 6.99), $e = 33.87$ (s.e. = 0.012). These functions were subsequently applied to mean nest temperature projections to predict offspring sex ratios, as it has been shown that sex ratios of alligator hatchlings from nests monitored in the field are highly correlated with predicted sex ratios based on the mean nest temperature during the TSP [41].

3. Results

(a) Broad-scale spatial and temporal trends in nest temperatures

Nest temperature profiles were obtained for 86 nests across 8 years at two sites (figure 1). Mean nest temperatures for individual nests ranged from 28.16 to 35.08°C (figure 2). Across years, the highest yearly mean nest temperature, occurring in 2016 at both sites, was 32.66°C at the southern site and 32.80°C at the northern site, temperatures predicted to yield approximately 99% male offspring. The minimum yearly mean nest temperature was 31.24°C at the southern site occurring in 2011 and 30.07°C at the northern site occurring in 2018, temperatures predicted to produce 100% female offspring. We detected a strong linear relationship between the mean nest temperature and the mean daily maximum air temperature, but not for mean daily minimum air temperature or mean daily precipitation levels, from nearby weather stations across years (figure 3; KSC: $F_{1,6} = 23.94$, $R^2 = 0.7996$, *p*-value = 0.0027; YWK: $F_{1,2} = 6.255$, $R^2 = 0.7577$, *p*-value = 0.130).

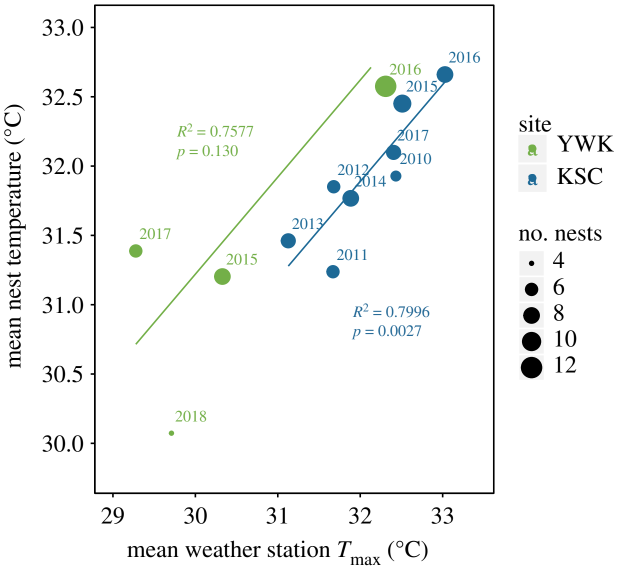

Figure 3. Relationship between yearly mean nest temperatures and mean daily maximum air temperature from the weather stations. The size of points is proportional to the number of nests monitored each year, and lines represent the result of a weighted linear regression. (Online version in colour.)

Alligator nest thermal characteristics also varied considerably within a year. Nests predicted to produce fully male clutches and nests predicted to produce fully female clutches were observed within the same year in 7 of 8 years at the southern site and 2 of 4 years at the northern site. At the northern site, all nests were predicted to produce fully female

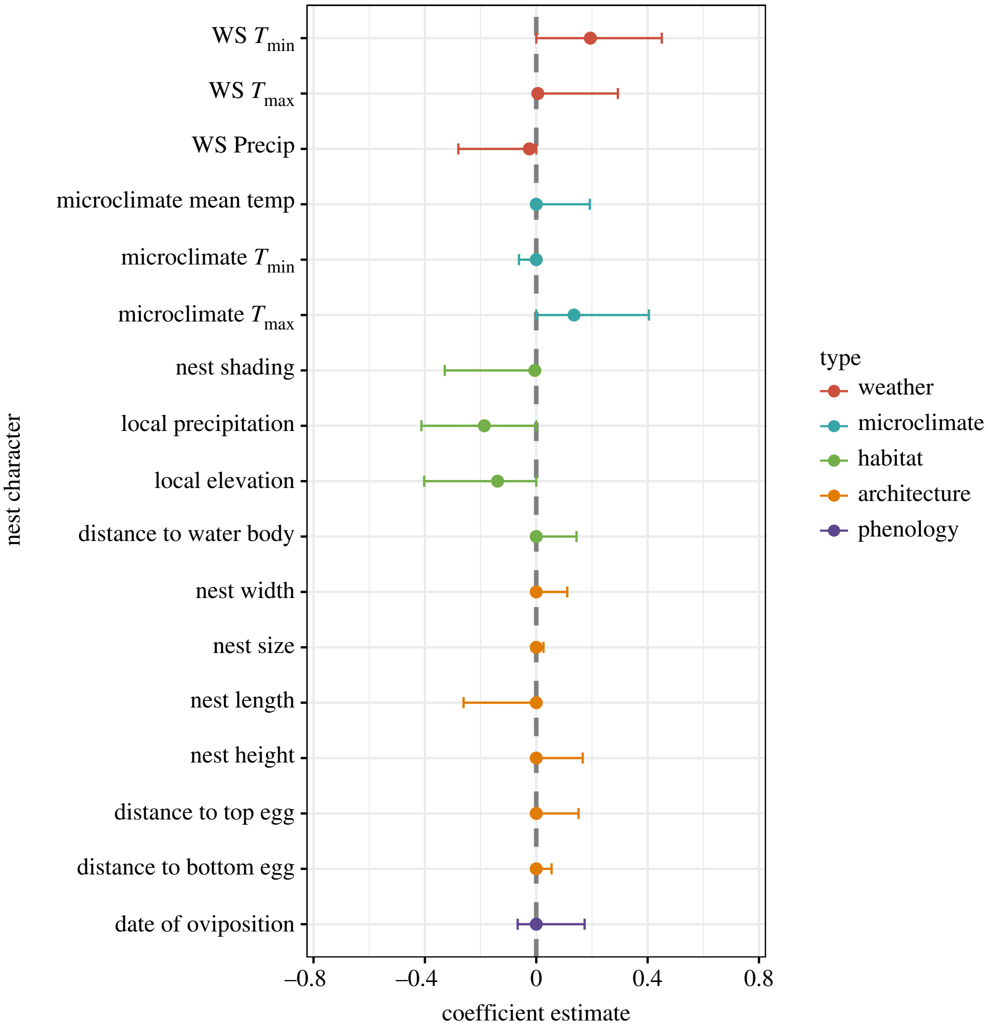

Figure 4. Ecological drivers of alligator nest temperature variation. Standardized coefficient estimates from the elastic net regularized regression model are depicted for all predictors. Error bars represent the middle 95th percentile of coefficient estimates generated from nonparametric bootstrapping with 1000 iterations. (Online version in colour.)

clutches in the years 2017 and 2018. Interestingly, the majority of nests monitored in this study (77% of nests at the southern site; 70% of nests at the northern site) exhibited both male- and female-promoting temperatures over the course of the TSP.

For years during which nests were monitored at both sites (2015–2017), nest temperatures, microclimate temperatures and weather station parameters for each of the sites were compared. There was a significant effect of both site ($F_{1,46} = 154.75$, $p\text{-value} < 0.001$) and year ($F_{2,46} = 47.80$, $p\text{-value} < 0.001$) on mean daily maximum air temperatures, with the southern site on average 1.7°C warmer than the northern site (electronic supplementary material, figure S1). In addition, we detected a significant effect of both site ($F_{1,46} = 9.995$, $p\text{-value} = 0.0028$) and year ($F_{2,46} = 14.869$, $p\text{-value} < 0.001$) on mean microclimate temperature, with the southern site exhibiting higher microclimate temperatures than the northern site (electronic supplementary material, figure S1). We detected a significant effect of year ($F_{2,46} = 3.596$, $p\text{-value} = 0.035$) on mean nest temperatures, but no significant effect of site ($F_{1,46} = 2.311$, $p\text{-value} = 0.135$; electronic supplementary material, figure S1). Collectively, these findings suggest that nest temperatures vary to a lesser degree than ambient environmental conditions across the American alligator's geographical range.

(b) Climatic and maternal drivers of nest temperature variation

Of the 17 predictor variables, seven yielded non-zero coefficients in the elastic net regularized regression and thus were selected to be included in the model describing variation in the mean nest temperature. Consistent with the relationships between the mean nest temperature and weather station parameters observed across years, mean daily maximum air temperature, mean daily minimum air temperature and total precipitation were all included in the model (figure 4). In addition, variables associated with the nest microclimate including microclimate mean daily maximum air temperature and local precipitation were also included in the model (figure 4). Interestingly, factors associated with the maternal nest-site choice (local elevation and nest shading) were included in the model, while factors associated with nesting phenology (estimated date of oviposition) and nest architecture (nest width, length, height and size) were excluded. Overall, variation in the mean temperature of individual nests was more difficult to predict than nest temperature variation across years. The elastic net regularized regression model explained 39.3% of the variation in the mean nest temperature for the training set and 10.4% for the test set.

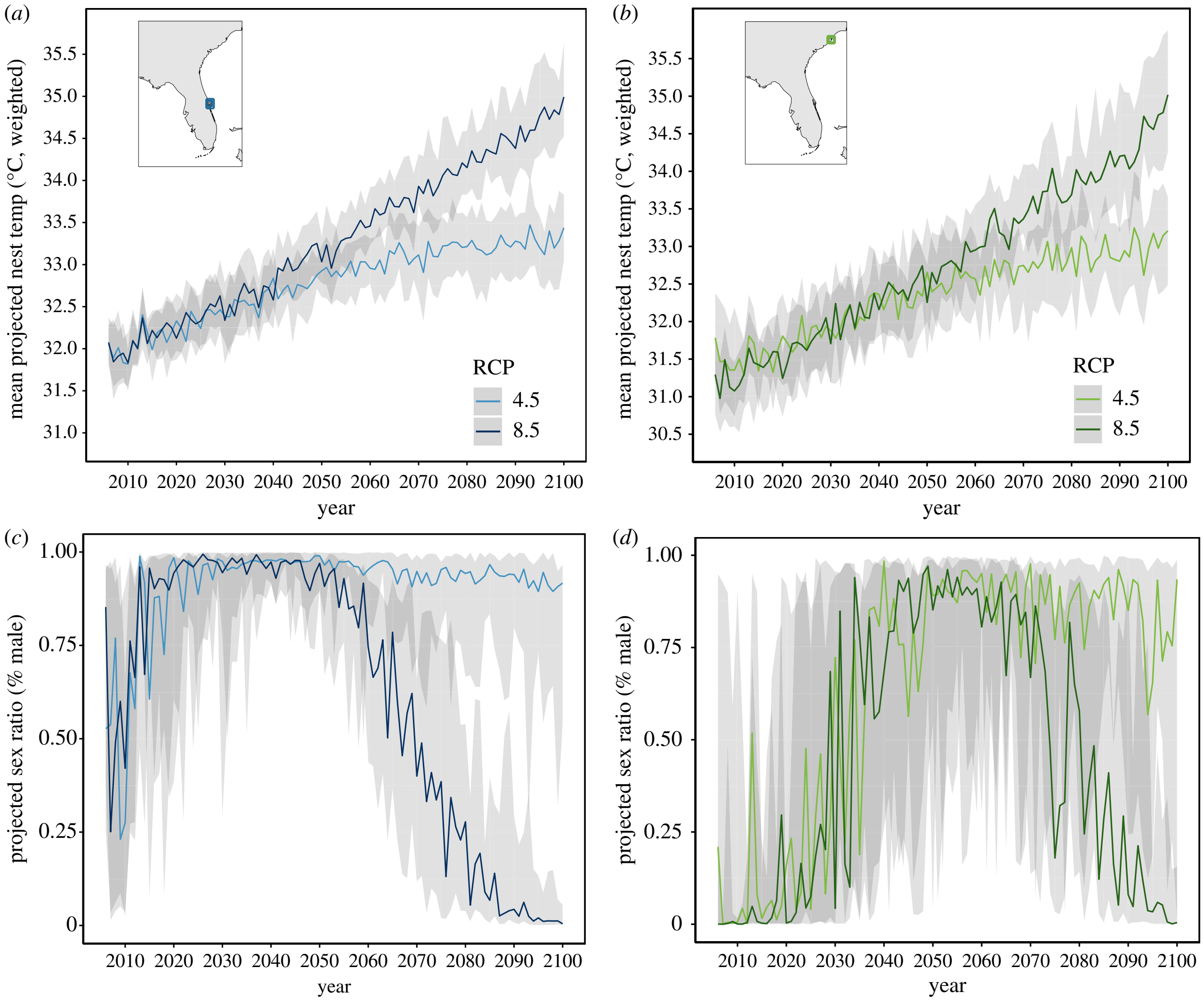

Figure 5. Projected changes in mean nest temperature and offspring sex ratios from 2006 to 2100 for (*a*,*c*) KSC and (*b*,*d*) YWK. For nest temperatures, central lines depict the 30-model mean. For sex ratios, central lines depict 30-model median. Grey shaded area depicts the middle 50th percentile (upper limit is 75th percentile and lower limit is 25th percentile) of model predictions. Different emission scenarios—RCP 4.5 and RCP 8.5—are depicted by light and dark lines, respectively. (Online version in colour.)

The influence of maternal nest-site choice (local elevation and nest shading) on nest temperatures across years was further assessed by comparing nests in the top and bottom 50th percentile of each of these factors. Surprisingly, nests in the top 50th percentile of nest shading were not consistently cooler than those in the bottom 50th percentile across years. This inconsistency was also observed when nests in the top and bottom 50th percentile of local elevation were compared (electronic supplementary material, figure S2).

(c) Climate projections

Based on statistically downscaled climate projections for 30 models in the CMIP5 collection [36], daily maximum air temperatures at both the southern and northern sites are expected to rise by 1.5–2.1°C (under RCP 4.5 and RCP 8.5) by the year 2050. However, by 2100, air temperatures at both the southern and northern sites are expected to increase by as much as 4.6°C under RCP 8.5. Temperatures are still expected be cooler at the northern site, with temperatures predicted to reach 37.58°C by 2100 under RCP 8.5.

(d) Nest temperature and sex ratio projections

Nest temperatures at the southern site are projected to increase by 1.1–1.4°C by the year 2050 and by 1.6°C–3.2°C by the year 2100 (figure 5*a*), and nest temperatures at the northern site are projected to increase to a slightly greater extent with increases of 1.3–1.4°C observed by the year 2050 and by 1.9–3.7°C by the year 2100 (figure 5*b*). Due to the exquisite sensitivity of alligator sex determination to small changes in temperature [7], projected sex ratios fluctuate considerably year to year in parallel with fluctuations in projected nest temperatures. Year-to-year variation in combination with variability in the 30 CMIP5 climate models results in a wide range of projected sex ratios within the 25th and 75th percentiles.

At the southern site, under RCP 4.5, offspring sex ratios are projected to increase to 97.8% male for 2040–2050 and 91.9% male for 2090–2100 (based on the 30-model median; figure 5*c*). Under RCP 8.5 scenarios, offspring sex ratios are projected to increase to 95.6% male for the years 2040–2050 and decrease to 2.2% male for the years 2090–2100 as nest temperatures exceed the second pivotal temperature (figure 5*c*). Changes in sex ratio are expected to be similarly extreme at the northern site. Under RCP 4.5, offspring sex ratios are projected to increase to 80.0% male for 2040–2050 and 80.7% male for the years 2090–2100 (based on the 30-model median; figure 5*d*), and under RCP 8.5, offspring sex ratio is projected to increase to 85.8% male for the years 2040–2050 and then decrease to 6.0% male for the years 2090–2100 (based on the 30-model median; figure 5*d*). Taken together, sex ratios at both sites are expected to initially become highly male-skewed as nest temperatures warm. However, depending on the site and the emission scenario, nest temperatures may exceed the second pivotal temperature resulting in a sharp tilt towards highly female-skewed sex ratios by 2100.

4. Discussion

This study provides insight into the potential consequences of future climate change for the American alligator and other crocodilian species by revealing the ecological factors that contribute to nest temperature variation. Daily maximum air temperatures drive interannual trends in alligator nest temperatures despite contributions of local habitat factors and microclimate characteristics to within-year nest temperature variation. Based on this observed relationship, nest temperatures are predicted to increase by 1.6–3.7°C by the year 2100 concomitant with increasing daily maximum air temperatures. These changes are likely to yield substantial shifts in offspring sex ratio, though the specific outcomes vary widely depending on the site and the emission scenario. While all models generally predict highly male-skewed alligator offspring sex ratios by the year 2050, sex ratio projections for the year 2100 range from nearly 100% female to nearly 100% male. This is, in part, due to the unique temperature-by-sex reaction norm exhibited by all crocodilians [7,18], wherein females are produced at both low and high incubation temperatures. The majority of studies investigating the potential effects of environmental change on nest thermal dynamics in species with TSD have focused on a few focal taxa; and crocodilians have received comparatively little attention, despite exhibiting unique nesting ecology and providing ecological services vital to the structure and function of many wetland ecosystems [42,43]. Our findings highlight the need to consider a range of potential climatic scenarios when predicting the impact of future environmental change on crocodilians and suggest that efforts to actively monitor hatchling sex ratios in the field are warranted.

Predicted shifts in nest temperatures and offspring sex ratio presented here raise questions regarding whether alligator populations possess the capacity to mitigate the effects of future environmental change. Adaptive evolution and plasticity in maternal nesting behaviour provide potential mechanisms by which species with TSD could buffer against adverse climate-induced sex ratio shifts [44–49]. Behavioural plasticity, in particular, probably represents the most viable strategy for long-lived species to respond to rapid climate change [25,50–52]. While a small number of studies suggest that nesting phenology and nest architecture partially compensate for changes in ambient environmental conditions [15,17,53], mounting evidence points to nest-site choice as the dominant mechanism of maternal influence on incubation temperatures [25,26,54,55]. This is consistent with results presented here in which local elevation and nest shading explained individual variation in nest temperatures while the date of oviposition and metrics of nest architecture did not. Canopy cover and shading is an important component of nest-site selection across diverse reptile lineages [54,56–58], and several studies have demonstrated an ability of nesting females to compensate for regional and geographical differences in environmental conditions by selecting nests sites conferring different levels of canopy cover [15,26,59]. While support for behavioural compensation via nest-site selection in other species is intriguing, several lines of evidence suggest that comparable behavioural compensation in this system is unlikely. Though maternal nest-site selection based on shading and elevation may contribute to variation in nest temperature within any particular year, a relatively small amount of variation is explained. Further, a strong relationship between daily maximum air temperature and annual trends in nest temperature persists. This suggests that, collectively, nest-site selection is not currently buffering against ambient environmental variation, though individual females nest in a range of local habitats.

The observed lack of maternal compensation for changes in environmental conditions could result from other ecological constraints and conflicting selective pressures on nesting behaviour. Nest sites promoting embryonic viability, reducing predation pressure and residing in proximity to suitable juvenile habitat may take precedence over sites conferring favourable sex ratios [26,60,61]. In a study of maternal nesting behaviour across a latitudinal gradient in *Chrysemys picta*, variation in nest-site choice suggested that selection for embryonic survival outweighs sex ratio selection [59]. Similarly, *Alligator mississippiensis* embryos face multiple threats to survival including nest predators [62,63] and extreme environmental conditions (inundation and desiccation) [64]; and maternal nesting behaviours aimed at counteracting these threats are probably under strong positive selection. Further work to uncover the ecological drivers and constraints on alligator nesting behaviour would inform predictions regarding the compensatory capacity of this species to respond to a rapid environmental change.

Without compensatory shifts in nesting behaviour, increases in temperatures associated with future climatic scenarios are predicted to result in dramatic changes in alligator nest temperatures. These changes have the potential to not only influence sex ratios but also rates of embryonic mortality. In sea turtles, researchers have suggested that increases in embryonic mortality as a consequence of climate change pose a more immediate threat to population viability than shifts in sex ratios [65,66]. Under constant laboratory conditions, embryonic mortality in *A. mississippiensis* tends to sharply increase as temperatures exceed 35–36°C [7]. Alligator nest temperatures are predicted to rise above 35°C by the year 2100 under RCP 8.5 in 52.3% of models at the southern site and 48.3% of models at the northern site. This raises the possibility that nest temperatures could exceed those promoting viability under the more extreme emission scenario; however, shifts towards highly male-biased sex ratios are predicted to precede these effects. Furthermore, it is unclear how embryonic mortality relates to temperature in the context of wild nest environments. Future investigations into links between clutch viability and nest temperature in ecologically relevant contexts are needed to inform these predictions.

Several open questions contribute additional uncertainty to predictions regarding the future population viability of crocodilians and other TSD species in the face of a rapid environmental change. In particular, how will landscape changes interact with changing environmental conditions to influence future nest environments? Our results suggest that habitat characteristics play a role in shaping the developmental environment of the American alligator, raising the possibility that future anthropogenic landscape alterations including urbanization and sea-level rise could interact with shifting environmental factors to alter incubation conditions in unforeseen ways [67–69]. Furthermore, how will shifts in offspring sex ratio translate to changes in operational sex ratio and, ultimately, population persistence? In turtles exhibiting a M–F pattern of TSD, increased production of females with increasing temperatures may promote population growth via increased egg production in the absence of male limitation [11,66]. Male-biased sex ratios, on the other hand, present a greater threat to population persistence [12]. Across the geographical range of *A. mississippiensis*, juvenile and adult populations already appear to exhibit a slight male bias [70], and results presented here predict highly male-biased offspring sex ratios by the year 2050. Collectively, this underscores the importance of active monitoring of hatchling sex ratios and basic population parameters in order to inform potential management interventions (e.g. artificial incubation programmes). Further work addressing the drivers and constraints on maternal nesting behaviour, the potential of other anthropogenic influences (e.g. habitat alterations) to interact with climate change in shaping the developmental environment and the consequences of shifts in offspring sex ratios for operational sex ratios will greatly inform future wildlife conservation efforts, particularly those focused on crocodilians and other TSD species.

Data accessibility. Data are available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.6t8vb41 [71].

Authors' contributions. R.H.L., L.G., B.B.P. and S.L.B. conceived of the study. R.H.L., T.R.R., P.M.W., S.W., B.B. and B.B.P. collected field data. S.L.B., B.B.P., R.H.L., E.S. and J.M.D. contributed to data analyses. S.L.B. and B.B.P. drafted the manuscript, which was then evaluated and revised for content by all authors.

Competing interests. We declare we have no competing interests.

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