



# Warming conditions boost reproductive output for a northern gopher tortoise population

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**ABSTRACT:** The effects of climate change on at-risk species will depend on how life history processes respond to climate and whether the seasonal timing of local climate changes overlaps with species-specific windows of climate sensitivity. For long-lived, iteroparous species like gopher tortoises *Gopherus polyphemus*, climate likely has a greater influence on reproduction than on adult survival. Our objective was to estimate the timing, magnitude, and direction of climate-driven effects on gopher tortoise reproductive output using a 25 yr dataset collected in southeastern Georgia, USA, near the northern edge of the species' range. We assessed the timing of climate effects on reproductive output (both probability of reproduction and clutch size) by fitting models with climate covariates (maximum temperature, precipitation, and temperature range) summarized at all possible time intervals (in 1 mo increments) within the 24 mo period prior to the summer census date. We then fit a final model of reproductive output as a function of the identified climate variables and time windows using a Bayesian mixture model. Probability of reproduction was positively correlated with the prior year's April–May maximum temperature, and clutch size was positively correlated with the prior year's June maximum temperature. April–May and June maximum temperatures have increased over the past 3 decades at the study site, which likely led to an increase in clutch size of approximately 1 egg (15% increase over a mean of 6.5 eggs). However, the net effect of climate change on gopher tortoise population dynamics will depend on whether there are opposing or reinforcing climate responses for other demographic rates.

**KEY WORDS:** Climate change · Clutch size · Fecundity · *Gopherus polyphemus* · Turtles

## 1. INTRODUCTION

The effects of climate change on the demography and population persistence of at-risk species may depend on whether the seasonal timing of local cli-

mate changes coincides with climate-sensitive physiological processes or life history stages. Some seasons are experiencing climate change more rapidly than others, depending on the location (e.g. warming is accelerating faster in the winter than the summer in the Arctic; Bintanja & van der Linden 2013). If climate-sensitive processes or life stages (e.g. juvenile life stages) occur during seasons when climate is changing most rapidly, popu-

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lation declines may be more likely (Pandori & Sorte 2019, Cordes et al. 2020). Therefore, predicting how a population will respond to climate change will depend on understanding the times of year when fluctuations in temperature and precipitation are likely to impact sensitive processes (Kruuk et al. 2015, van de Pol et al. 2016). In this study, we investigated the seasonal time period in which climate conditions may influence reproductive processes in an at-risk reptile species, the gopher tortoise *Gopherus polyphemus*.

Reptiles have multiple processes and life stages that are influenced by temperature and precipitation, and therefore reptile populations may be particularly vulnerable to climate change (Böhm et al. 2016). Many reptiles have temperature-dependent sex determination, and warming conditions during the critical period for sex determination can have demographic consequences through skewed sex ratios for those species (Janzen 1994, Jensen et al. 2018). For other processes, it is less clear how the timing of temperature and precipitation fluctuations could influence the outcome. Pre-reproductive processes such as gamete production are likely influenced by temperature: as ectotherms, warmer conditions may spur reptiles to acquire more resources, resulting in greater gamete production (Adolph & Porter 1993). However, whether warming results in greater reproduction depends on whether seasonal warming and the timing of pre-reproductive processes coincide.

For many long-lived species, like gopher tortoises, adult survival is likely robust to environmental variation (Sæther & Bakke 2000, Gaillard & Yoccoz 2003), and thus perhaps less affected by a changing climate. However, populations may still be vulnerable if climate change influences reproduction. Many reproductive processes could be affected by climate, including: mating, female condition, follicular development, total female investment (e.g. clutch size, egg size), and hatching success (Spotila et al. 1994, Demuth 2001, Monasterio et al. 2013, Tuberville et al. 2015). However, the effects of climate on these processes and on net reproductive output remains unclear for many species, including gopher tortoises, and long-term datasets on these reproductive processes are needed to understand how climate conditions affect reproductive success for long-lived species and how changing climate conditions may affect future reproductive output (Campos et al. 2017).

Gopher tortoises are endemic to upland habitats of the southeastern US coastal plain. Gopher tortoise populations have declined due to overconsumption and habitat loss and modification, leading

to federal Endangered Species Act listing in 1987 for threatened populations in the western portion of the species' range, and current consideration for federal listing in the rest of the range (Berry & Aresco 2014, Folt et al. 2021). The primary resilience mechanism of gopher tortoises for climate fluctuations may be their burrowing habit, which is at least partially a thermoregulatory behavior to provide refuge from cold winter conditions and hot and dry summers (Douglass & Layne 1978). Although gopher tortoises leave their burrows to thermoregulate (Radzio & O'Connor 2017), as well as to forage and gain access to mates (Castellón et al. 2018), the species spends most of its life in burrows (Douglass & Layne 1978, DeGregorio et al. 2012). Weather conditions may influence tortoise aboveground activity rates, thermoregulation opportunities, and reproductive timing and output directly (Douglass & Layne 1978, Mitchell et al. 2021), but there may also be effects of temperature and precipitation on food resources that could indirectly affect tortoise body condition, survival, and fecundity (Wallis et al. 1999). Whether the pathway of the climate effect is direct or indirect will determine the time window over which changes in climate could influence demographic processes.

Two key aspects of gopher tortoise reproduction, i.e. the probability of reproduction (whether or not a female will produce eggs in a year) and clutch size, have both been studied across the species' range, but most studies only provide a 'snapshot' of these demographic processes, which does not allow for examination of climate effects. Most females (80–90%) reproduce each year in populations in both southern Florida (Rothermel & Castellón 2014) and Mississippi (Smith et al. 1997), but it is not clear what role climate conditions play in year-to-year variability. Among-site variation in clutch size across the range has been attributed to climate both directly and indirectly through female body size (Ashton et al. 2007), but again, the effect of climate conditions on within-site variation in clutch size has not been examined due to a lack of long-term datasets. We studied the magnitude, direction, and timing of effects of climatic conditions (maximum temperature, temperature range, and precipitation) on the probability of reproduction and clutch size at Fort Stewart Army Reserve (FSAR) in the northern portion of the gopher tortoise range in Georgia, USA, with a dataset that spans 3 decades (1994–2019). Understanding how climate conditions influence reproduction will be critical for conservation planning for this at-risk species as the climate continues to change.

## 2. MATERIALS AND METHODS

### 2.1. Study site and species

FSAR is a ~110 000 ha military base in southeastern Georgia, USA, of which approximately 20 000 ha have sandhill habitat suitable for gopher tortoises, consisting of a mix of restored longleaf pine *Pinus palustris*, slash pine *P. elliottii*, loblolly pine *P. taeda*, and turkey oak *Quercus laevis* in the overstory and a wiregrass (*Aristida* spp.)-dominated understory. As of the early 2000s, FSAR contained one of the largest remaining gopher tortoise populations in Georgia (Smith et al. 2006). The gopher tortoise population at FSAR is in the northern part of the species' range (Fig. 1), and tortoises hibernate in the cool months of the year (roughly November through mid-March, Rostal 2014). After females emerge from hibernation, estradiol production slowly increases through the nesting season in May to mid-June, with a large increase following the nesting season in July–September (Ott et al. 2000, Rostal 2014; Fig. 2). The spike in late summer estradiol corresponds to the initiation of vitellogenesis in which follicles begin to grow and mature (Rostal et al. 1994, Rostal 2014). Mating

also typically occurs in the late summer and early fall before hibernation (Rostal 2014).

### 2.2. Field data collection

We surveyed the gopher tortoise population in the western section of FSAR from 1994–2008, 2010–2011, 2014–2016, and 2018–2019. We captured tortoises by hand and occasionally via bucket trapping (see Rostal & Jones 2002, Hunter & Rostal 2021 for details). We triple-marked all captured tortoises with a passive integrated transponder (PIT) tag (AVID Identification Systems), a plastic fish tag epoxied to the carapace (Floy Model FTF-69 Pennant, Floy Tag & Mfg.), and a notch code in the marginal scutes (Rostal & Jones 2002). We measured straight carapace length (SCL) of captured tortoises as an indication of overall size (McRae et al. 1981).

When an adult female tortoise was captured, we determined reproductive status through 2 methods: ultrasonography and radiography. Ultrasound allowed for the detection of shelled eggs as well as follicular development that indicated a female would produce eggs (and therefore reproduce) later in the nesting season (Rostal et al. 1994). When we detected

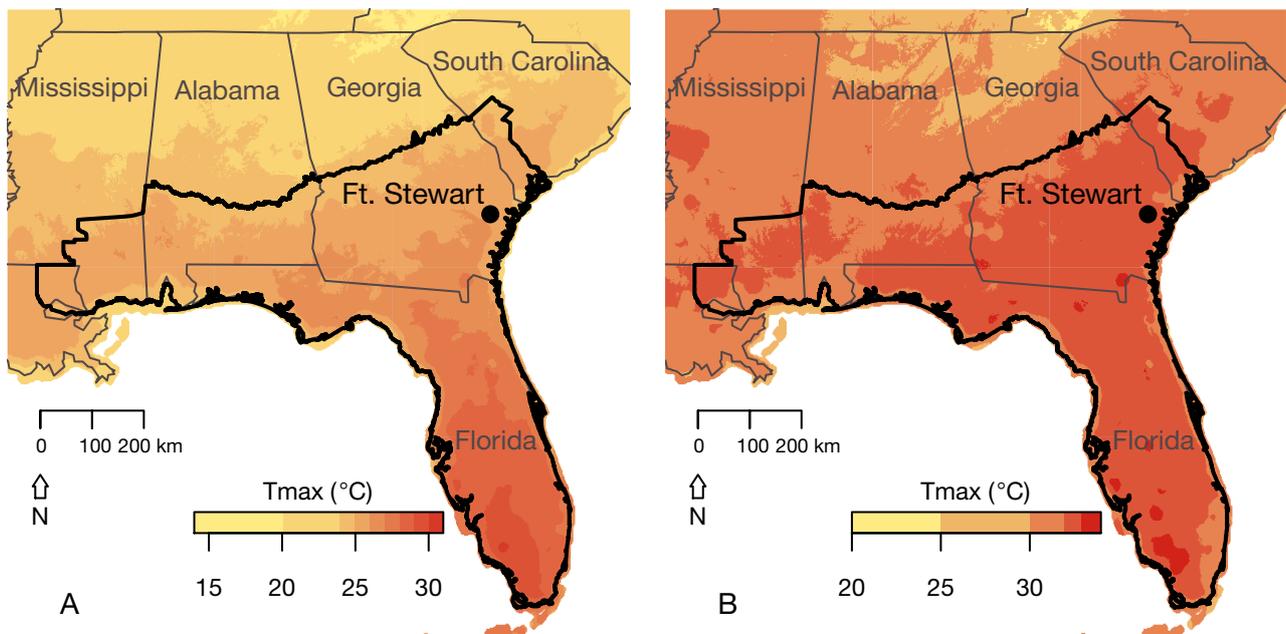


Fig. 1. Climate in the gopher tortoise *Gopherus polyphemus* range (thick black line) in the southeastern USA, with the study site of Fort Stewart Army Reserve indicated in southeastern Georgia. The gradient shows the 30 yr average of the (A) April and (B) June maximum temperature (PRISM 30 yr normals 1980–2010; <https://prism.oregonstate.edu/normals/>), indicating a high degree of correspondence between temperature gradients and the species' range boundary. Temperatures vary across the species' range in the spring, with much cooler conditions in the northern part of the range in April (A), but by June, conditions are consistently warm across the range (B)

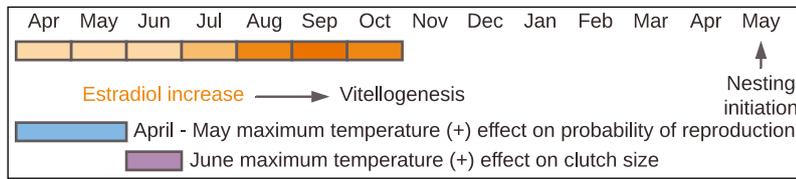


Fig. 2. Reproductive timeline for female gopher tortoises *Gopherus polyphemus* at Fort Stewart Army Reserve, Georgia, USA. Estradiol production begins to increase in July, with a peak in September (orange; following data from Ott et al. 2000, Rostal 2014), which leads to the process of follicle development (vitellogenesis) for eggs that will be laid in the following May and June. Our results (data spanning 1994–2019) indicate that the probability of reproduction for a female is positively influenced by the maximum temperature in April–May prior to vitellogenesis (blue), and clutch size is positively influenced by the maximum temperature in June prior to vitellogenesis (purple)

shelled eggs using ultrasonography, we determined clutch size from radiographs. Fieldwork methods were approved by the Institutional Animal Care and Use Committee of Georgia Southern University (#I19007) and the Georgia Department of Natural Resources (#1000545889 and #1000838720).

### 2.3. Climate data

We obtained daily climate data from the Daymet climate database (Thornton et al. 2018; 1 km<sup>2</sup> resolution): daily maximum temperature (Tmax), daily total precipitation, and daily temperature range (DTR; daily maximum minus daily minimum temperature). We did not include daily minimum temperature in the group of climate variables as it was highly correlated with maximum temperature ( $r = 0.92$ ), and the 3 remaining variables were not correlated ( $|r| < 0.4$ ). We averaged these daily climate variables to a monthly scale for use in analyses.

### 2.4. Identifying appropriate climate ‘windows’

We used sliding window analyses of several climate variables (taking the mean of each variable within each climate window) to determine the optimal time windows for estimating effects on gopher tortoise reproduction (‘climate windows’; implemented in the ‘climwin’ package for R; Bailey & van de Pol 2016, van de Pol et al. 2016). For each climate variable (Tmax, DTR, and precipitation) and for each reproductive process (probability of reproduction, clutch size; see next paragraph), we assessed all possible climate windows (ranging in length from 1 to 24 mo) in the 24 mo period leading up to the 1 May initiation of the nesting season in 1 mo increments (900 models:

3 climate variables × 300 climate windows). We tested for linear effects of all climate variables and windows on probability of reproduction (logistic regressions; see next paragraph) and clutch size (Poisson regressions; see below). We chose a 24 mo maximum time lag because current-year fecundity may be affected by current as well as previous-year plant growth and forage availability, which could be driven by climate conditions from the previous year. In addition, females may not necessarily reproduce every year, and so their reproductive investment may

reflect conditions over a 2 yr time span.

We used logistic regressions to model probability of reproduction where females that were captured with either follicles (only detected with ultrasound) or eggs (detected with both ultrasound and X-ray) were designated as reproducing within a year. We used Poisson regressions to model clutch size (only for females that had shelled eggs and could be X-rayed at the time of capture). The ‘baseline’ models for both the probability of reproduction and clutch size processes (to which climate variables were subsequently added) included female body size (SCL). For the probability of reproduction, date (days since 1 January) was also included to account for the sampling design: tortoises were caught throughout the nesting season, so females that were caught late in the season could have already laid their eggs. Including the date variable accounted for this potential bias, and we predicted that date would have a negative effect on probability of reproduction. We did not have any *a priori* reason to believe that date would influence clutch size, and exploratory analyses confirmed that there was no effect of date on clutch size so it was not included in any additional analyses for that response variable.

We compared all models to the baseline (SCL and date only) model using Akaike’s information criterion corrected for small sample size (AICc). To avoid inflated type I error due to multiple comparisons, we used the built-in permutation test (100 randomizations) in ‘climwin’ that iteratively repeated the climate window analysis with randomized dates (Bailey & van de Pol 2016, van de Pol et al. 2016). The top model (‘optimal’ climate window) was then compared to the null distribution of the  $\Delta$ AICc score to calculate the likelihood that its  $\Delta$ AICc score occurred by chance ( $P\Delta$ AICc). We only interpreted climate covariates with  $P\Delta$ AICc < 0.05.

## 2.5. Full model

After identifying meaningful climate variables, we combined both reproductive processes together in a single Bayesian hierarchical model to account for the linkage between probability of reproduction and clutch size (i.e. only reproductive females can produce a clutch). Modeling the 2 reproductive processes jointly in a Bayesian framework had the benefit of accounting for missing data (e.g. when reproduction was only detected via ultrasound visualization of unshelled follicles so clutch size data was missing for that individual), and so provided more robust estimates of the effects of climate on reproduction than the separate ‘climwin’ analyses. Thus, the Bayesian hierarchical model tested whether the effects identified in the ‘climwin’ variable selection process would be verified when the structure of the data was more properly modeled. Initially, we used a zero-inflated Poisson model (Zuur et al. 2009) in which probability of reproduction was modeled as a Bernoulli process and clutch size was modeled as a Poisson process. However, initial model validations performed poorly and showed that the clutch size data were underdispersed and required a more flexible distribution than the Poisson for modeling clutch size. We chose to use a normal distribution to model clutch size, which allows for separate mean and variance parameter estimation (and thus accounts for underdispersion).

We modeled the expected probability of reproduction for each individual as a logit-linear function of female size (SCL), date, and climate variables identified as meaningful in the climate window analysis. We modeled the expected clutch size for each individual as a linear function of SCL and meaningful climate variables. Prior to analysis, we standardized all continuous covariates (SCL, date, and climate covariates) using z-score scaling to improve model convergence. To account for missing SCL data (6.5% of observations), we sampled from a normal prior probability distribution for each Markov chain Monte Carlo (MCMC) draw. We assigned uninformative priors to all free parameters (Table 1). We fit the model in R version 4.0.4 using an MCMC algorithm implemented in the JAGS software (Plummer 2003, R Core Team 2020), which was called from R using the ‘runjags’ package (Denwood 2016). We ran 3 independent Markov chains, discarding the first 2000 samples as a burn-in, and storing the remaining 5000 iterations for analysis. We tested for Markov chain convergence to a stationary posterior distribution with the Gelman-Rubin diagnostic, and we considered that convergence had occurred when the

Gelman-Rubin statistic was  $<1.1$  for monitored parameters and derived quantities (Bolker 2008). We summarized posterior distributions for all parameters and derived quantities with the median of all MCMC samples as a point estimate and the 2.5 and 97.5 percentiles of the MCMC samples as a 95% credible interval (CI; Bolker 2008).

## 2.6. Model validation and interpretation

To assess the goodness-of-fit of our model, we measured the discrepancy between the actual dataset and data simulated under the estimated parameters to calculate a Bayesian posterior predictive p-value (values close to 0.5, and far from 0 or 1, indicate a well-fitting model; Kéry 2010). We computed the root mean squared error (RMSE) for the simulated datasets and contrasted these values with RMSEs computed for the observed data. We calculated the Bayesian p-value as the fraction of instances in which the RMSE values from simulated datasets exceeded the RMSE values computed using the observed dataset (Kéry 2010).

To assess the predictive performance of the model, we ran a cross-validation procedure in which, for

Table 1. Parameter posterior estimates (95% credible intervals, CI) for a model of gopher tortoise *Gopherus polyphemus* reproduction at Fort Stewart Army Reserve, Georgia, USA, from 1994 to 2019. Prior probabilities for each parameter were normally distributed with mean of 0 and standard deviation of 100. Effect estimates for the probability of reproduction (PR) process are on the logit scale. Date of ultrasound was included as a methodological variable to account for clutches that may not have been detected due to eggs being laid before the capture date. SCL: straight carapace length (used as a measure of female size); Tmax: daily mean maximum temperature for the indicated period. Note that the Baseline PR indicates the probability of reproduction detected at the mean observation date, not at the earliest possible capture date of 10 April (when PR = 97.4%; see Section 3.2)

Parameter	Posterior median (95% CI)
<b>Parameters for PR</b>	
Baseline PR (intercept)	0.77 (0.57–0.99)
Effect of date on PR	−0.93 (−1.17 to −0.70)
Effect of SCL on PR	0.30 (0.09–0.52)
Effect of previous April–May Tmax on PR	0.64 (0.40–0.90)
<b>Parameters for clutch size (CS)</b>	
Baseline CS (intercept)	6.52 (6.38–6.65)
Effect of SCL on CS	0.92 (0.77–1.07)
Effect of previous June Tmax on CS	0.47 (0.33–0.61)

each of 10 iterations, one-tenth of individual tortoises were withheld from the model fitting process. Each resulting model was used to predict both the probability of reproduction and the clutch size produced by the withheld tortoises, and these predictions were compared to the observed reproduction and clutch size for those individuals. We used the area under the curve (AUC; for probability of reproduction process) and  $R^2$  (for clutch size process) metrics to summarize model predictive performance across all withheld samples. Predictive performance for the clutch size process was only evaluated for observations with  $\geq 1$  egg produced.

### 2.7. Change over time

For each climate variable detected as meaningful in the climate window analysis, we estimated its change over the study period (1992–2019, including 2 yr before first tortoise captures) using linear regression. We also estimated the change over time of the climate variables for all other months of the year. We used linear regressions to examine the change in clutch size and body size of reproducing females over the study period (this could not be done for probability of reproduction due to the confounding effect of date of ultrasound on the raw data).

## 3. RESULTS

During nesting seasons from 1994 to 2019, we obtained reproductive data on 221 females from a total of 523 captures. Approximately half of all females ( $n = 104$ ) were captured only once during the study, and the average was 2.4 captures female<sup>-1</sup>. The average body size of all captured females was  $30.5 \pm 1.6$  cm (SD). The average body size of reproducing females was  $30.6 \pm 1.5$  cm, and the smallest reproducing female had an SCL of 25.8 cm.

### 3.1. Climate variable windows

The maximum temperature of April–May prior to the initiation of a nesting season (13–12 mo prior to 1 May) had a positive effect on probability of reproduction ( $P\Delta AICc = 0.05$ ). Maximum temperature of the previous June (11 mo prior to 1 May) had a positive effect on clutch size ( $P\Delta AICc < 0.001$ ). Precipitation and DTR did not influence either reproduction response variable.

### 3.2. Full model

In the Bayesian hierarchical model, the baseline probability of reproduction for an average-sized female caught on the earliest possible date, 10 April (i.e. with no chance of missing a clutch already laid), in a year with average climate conditions was 97.4% (95% CI: 94.7–98.8%, intercept of Fig. 3A). The expected clutch size for an average-sized reproducing female in an average temperature year was 6.5 eggs. As predicted, probability of reproduction decreased substantially with date of capture, likely because some females, when caught later in a nesting season, had already laid their eggs. Female body size had positive effects on both probability of reproduction and clutch size, although the effect on clutch size was much greater (Fig. 3, Table 1).

The Bayesian hierarchical model supported the inclusion of the prior April–May maximum temperature effect on the probability of reproduction and the prior June maximum temperature effect on clutch size, as these effects had 95% CIs that did not overlap zero (Table 1). April–May mean daily maximum temperatures ranged between 20.9 and 25.7°C for the study period and had a positive effect on probability of reproduction, which ranged from 86.9% (95% CI: 72.5–94.5%) at the lowest temperatures to 99.2% (95% CI: 98.1–99.7%) at the highest temperatures for average-sized females (with date set to the earliest possible capture date, 10 April). June mean daily maximum temperatures had a positive effect on clutch size, although body size had a greater effect on clutch size than temperature within the observed range of variability (Fig. 3). Average June maximum temperatures ranged between 26.6 and 31.4°C, and clutch sizes ranged from 5.4 eggs (95% CI: 5.1–5.8 eggs) at the lowest temperatures to 7.3 eggs (95% CI: 7.0–7.6 eggs) at the highest temperatures for average-sized females. Both climate effects occurred in the summer prior to a focal nesting season, which matches with the initiation of increases of estradiol in late summer (Fig. 2).

The Bayesian p-value of 0.52 indicated that the model could plausibly have generated the observed data, and there was no observed lack of fit of the data to the model. From 10-fold cross-validation, the model had a fair performance in predicting the probability of reproduction for an individual (AUC = 0.74; 95% CI: 0.73–0.75). For reproductive individuals, our model explained a high proportion of observed variance in clutch size, with  $R^2 = 0.76$  (95% CI: 0.75–0.77).

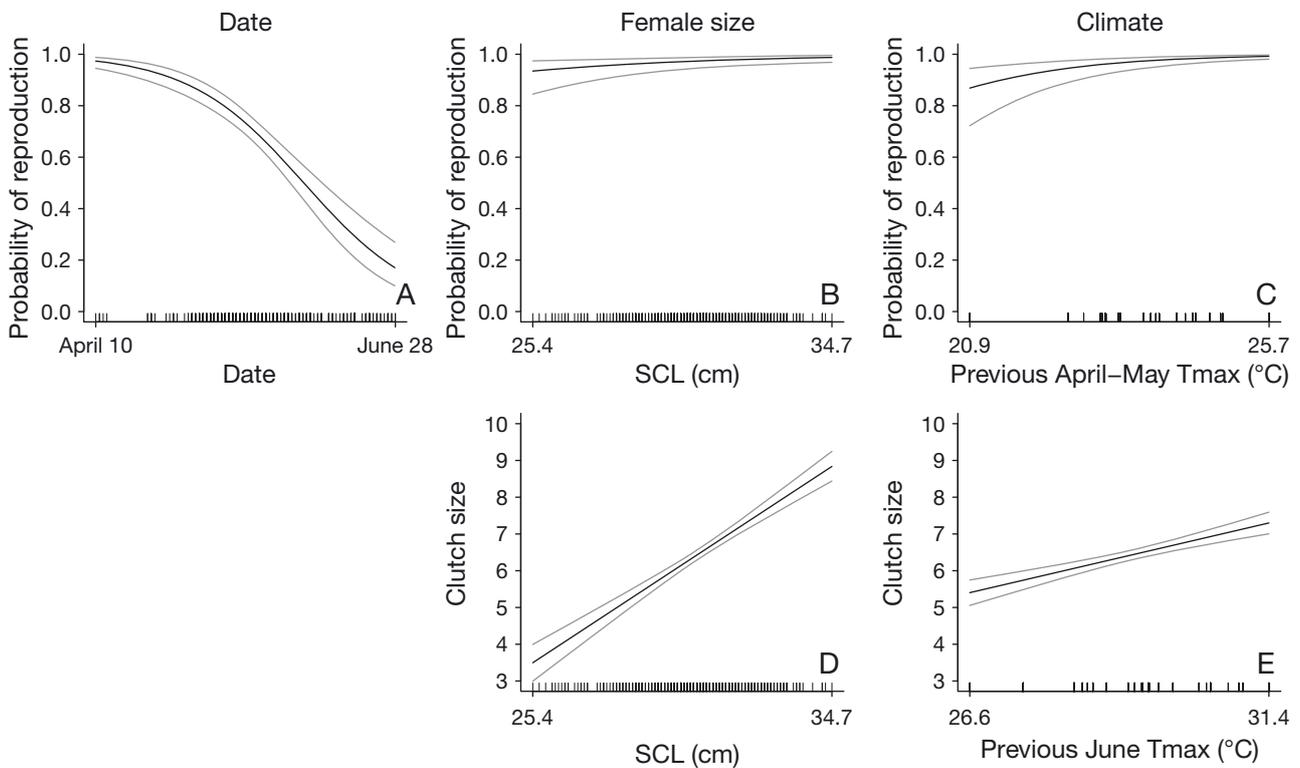


Fig. 3. Effects on female gopher tortoise *Gopherus polyphemus* (A–C) probability of reproduction and (D,E) clutch size at Fort Stewart Army Reserve, Georgia, USA, studied from 1994 to 2019, estimated using a Bayesian hierarchical mixture model. See Section 2.4 for details on variable selection for each reproductive process. Black lines are medians and gray lines are 95% credible intervals for predictions from the model over the observed values for each variable while holding other variables at their mean value (straight carapace length [SCL] = 30.5 cm, April–May maximum temperature [Tmax] = 23.7°C, June Tmax = 29.4°C), except for date, which was held at its earliest value (10 April) to remove the confounding effect of date on detection of reproduction

### 3.3. Change over time

Both climate variables significantly increased in magnitude over the 27 yr of the study period (Fig. 4). April–May maximum temperatures increased by 1.6°C ( $p = 0.01$ ), and June maximum temperatures increased by 2.5°C ( $p < 0.01$ ). This rate of change was greater than for temperatures in other months of the year (Fig. 4F). Observed clutch size significantly changed over the study period, increasing by 1 egg from 1994 to 2019 ( $p = 0.03$ , Fig. 4B), but body size (SCL) of reproducing females did not change ( $p = 0.45$ , Fig. 4C).

## 4. DISCUSSION

We found strong effects of climatic variability on the probability of reproduction and clutch size of gopher tortoises: the prior April–May maximum temperature positively affected probability of reproduction and the prior June maximum temperature posi-

tively affected clutch size. The timing of these temperature effects immediately precedes production of estradiol that begins to elevate in July and peaks in September, leading to vitellogenesis (Fig. 2; Ott et al. 2000, Rostal 2014). This timeline (a temperature effect on the probability of reproduction followed by a temperature effect on clutch size, then initiation of estradiol production) suggests that temperature plays a role in the process of whether to initiate reproduction and how much to invest in it. Detection of this pattern was only possible with a long-term dataset that sampled a wide range of climatic variability at FSAR coupled with a climate window analysis (van de Pol et al. 2016) to discover the timing of effects without *a priori* knowledge of the exact window of the temperature effect.

It is intuitive that temperature effects on probability of reproduction and clutch size would be ‘upstream’ of the hormonal initiation of follicle production, but whether or not females reproduce (and how much, if they do) can be determined at multiple time points, not just the months preceding estradiol pro-

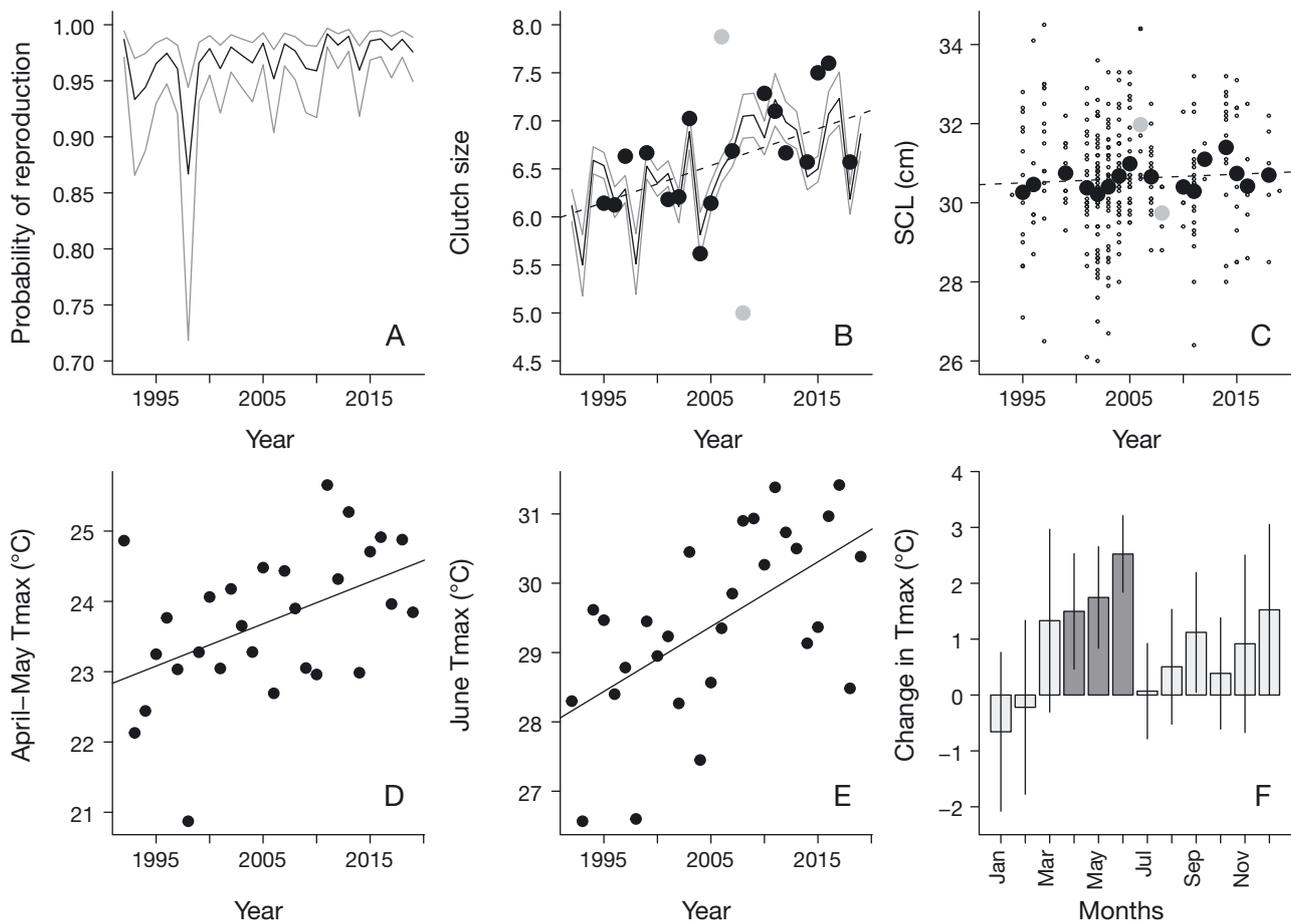


Fig. 4. Change in gopher tortoise *Gopherus polyphemus* (A–C) reproduction and body size and (D–F) climate variables over time at Fort Stewart Army Reserve, Georgia, USA. In (A) and (B), solid black lines indicate the median prediction of a Bayesian hierarchical mixture model of reproduction, and gray solid lines are 95% credible intervals for predictions from the model over the observed values of the climate variable for each year (A: April–May maximum temperature [Tmax]; B: June Tmax) while holding straight carapace length (SCL) at its mean value (30.5 cm), and date at its earliest value (10 April) to remove the confounding effect of date on detection of reproduction. In (B) and (C), large points are means of clutch size (B) and SCL of reproducing females (C) for each year (gray points are outliers due to small sample sizes and biased female SCL in those years; small open circles in C are raw data points of reproducing female SCLs), with dashed lines indicating the slope of linear models of clutch size or reproducing female SCL over time (an analysis of change in probability of reproduction over time could not be conducted because of the confounding effect of date of ultrasound). April–May (D) and June (E) Tmax have been significantly increasing over the study period (solid lines). Bars in (F) are estimates of the change in Tmax for each calendar month over the time period 1992–2019 (intervals are standard errors); dark gray highlighted bars are months in which temperature has an effect on gopher tortoise reproduction (April–June)

duction. Many vertebrates, including gopher tortoises, have the ability to reabsorb follicles after they have been formed (via the process of atresia). Through atresia, females can allocate fewer resources to reproduction up until ovulation (Gouder et al. 1979, Rostal 2014). However, we did not see any temperature effects during fall or winter when females are producing or maintaining follicles, indicating that the determination of whether and how much to reproduce occurs prior to vitellogenesis (i.e. atresia is likely not affected by climatic conditions experienced by this population).

There are multiple potential pathways through which warmer temperatures in April–June could increase the probability of reproduction and clutch sizes. Warmer temperatures during the spring (April–May) may be important for stimulating an endocrine cascade that results in estrogen-driven vitellogenesis by the liver (Jones 2011). As ectotherms, gopher tortoises respond to warmer temperatures through greater activity (Douglass & Layne 1978, DeGregorio et al. 2012), which is necessary for foraging, so females that are more active in the spring and early summer may have gained more mass and be in better

physical condition for greater reproductive investment (Henen 1997, Rostal & Jones 2002). In the southern part of the species' range, body condition is relatively invariant over the course of the year (McCoy et al. 2011), but gopher tortoises in south Florida do not hibernate in burrows as much or as long as tortoises in the northern part of the range due to warmer winter conditions and earlier spring warm-up (Douglass & Layne 1978, DeGregorio et al. 2012; Fig. 1). Given these differences in behavior across the range, it is possible that different climatic conditions would influence the probability of reproduction and clutch size in different parts of the species' range.

We saw no effects of precipitation on the probability of reproduction and clutch size. This result is in stark contrast to a similar study on Mojave desert tortoises *Gopherus agassizii*, in which the total precipitation for the preceding 18 mo had strong positive effects on both probability of reproduction and clutch size (Mitchell et al. 2021). Desert tortoises obviously inhabit much more water-limited environments than gopher tortoises, but the northern part of the gopher tortoise range does experience severe droughts, several of which were captured within the study period (National Integrated Drought Information System 2021). Our results indicate that the studied reproductive processes are not precipitation limited within the ranges recorded in our study years, either directly or through vegetation growth and availability. However, the probability of reproduction sub-model had a relatively weak predictive performance (AUC = 0.74), indicating that other variables (such as more detailed precipitation information) may explain additional variance. We estimated the baseline probability of reproduction in an average year for an average-sized female caught on the earliest possible date, 10 April, to be very high (97.4%); however, this rate may be artificially high, as sampling was not consistent during the early spring. Only 14 data points occurred before 1 May, and when those data points were removed from the analysis, the baseline probability of reproduction dropped to 92% (analysis not shown). It is clear that most (>90%) females are reproducing every year on average, but there may be additional climate-sensitive variation in the probability of reproduction that is not fully captured by our dataset.

April–May and June maximum temperatures have been steadily increasing over the study period (Fig. 4). Given that higher temperatures in these periods led to greater reproductive output, the warming trend in temperatures likely led to an increase in gopher tortoise reproductive output at

FSAR. The change in temperature is a more likely driver of the increased reproductive output than an improvement in habitat, as FSAR has been consistently managed with prescribed burning for the last several decades (Hunter & Rostal 2021). Over the 25 yr study period, mean clutch sizes of reproducing females have increased by approximately 1 egg, a 15% increase based on the mean clutch size of 6.5 eggs. Body size of the population of reproducing females has not changed over the study period, indicating that the change in clutch size is likely due to the changing climate influencing reproduction directly, rather than via an effect on adult size (Fig. 4). Without the climate window analysis, we would not have been able to make this connection, as yearly average temperatures are not warming as rapidly as spring and summer temperatures (due to mostly stable fall and winter temperatures, Fig. 4F). Warming leading to a reproductive boost has been documented in other populations at the 'leading edge' (or northern edge for northern hemisphere species) of species' ranges (Gaston et al. 2005, Dangremond & Feller 2016). Gopher tortoise clutch sizes increase linearly with decreasing latitude across populations (Ashton et al. 2007), and thus spring and early summer warming in northern populations may result in tortoises becoming more 'southern' in their reproductive patterns. It is likely that the northern range limit for gopher tortoises is determined, in part, by temperature – further north, colder conditions may not allow for sufficient incubation temperatures for the duration of nest development (Rostal & Wibbels 2014; Fig. 1). Thus, warming conditions that increase reproduction could not only bolster northern edge populations but also allow for range movement northward.

Whether climate change will be an overall boon for northern populations will depend on many potentially interacting demographic responses, and multiple pathways through which climate could affect the ultimate population growth rate (Stopher et al. 2014, McLean et al. 2016). Warming conditions could have an impact on the growth rate and maximum size for female tortoises – tortoises from warmer climates (lower latitudes) achieve smaller adult sizes (Ashton et al. 2007), although the mechanism underlying this pattern is unclear. We found that body size plays a large role in both probability of reproduction and clutch size, some of which may be due to younger tortoises having smaller body sizes and not yet being reproductively mature, or producing smaller clutches due to inexperience; other studies have also demonstrated a strong body size–clutch size relationship

(Ashton et al. 2007, Rothermel & Castellón 2014, Mitchell et al. 2021). If maximum body sizes become smaller with climate change (following the pattern described by Ashton et al. 2007), this could counteract the boost that warmer temperatures give to egg production.

In addition, overall reproductive success is determined not only by egg production, but also by hatching success and hatchling survival. In lab conditions, hatching success is greatest in middle temperatures (~30°C) and declines at higher and lower temperatures (Spotila et al. 1994, Demuth 2001, Rostal & Wibbels 2014). Natural nests in the field can produce successful hatchlings when eggs experience hot conditions for short periods (Rostal & Wibbels 2014), but longer-term exposure to heat may result in a decline in overall hatching success, potentially cancelling out the boost to reproduction from warmer temperatures that we report here. Finally, adult survival could be influenced by a warming climate in complex ways. Although adult tortoises are unlikely to be directly influenced by changes in climate, their habitats may be. Gopher tortoise habitats are managed with prescribed burning, and burning at FSAR has complex effects on adult population dynamics (Hunter & Rostal 2021). If burning becomes less frequent as temperatures warm, as is predicted (Kupfer et al. 2020), there may be consequences for adult activity rates, foraging opportunities, and overall condition (Howell et al. 2020), which could impact investment in reproduction. Thus, changes to habitat that reduce the quality of forage and cover for adult tortoises may negate any positive impacts on population productivity caused by warming. In addition, a boost in reproductive output may not have biologically meaningful consequences for population growth rates because tortoise (and other turtle) populations are more sensitive to adult female and juvenile survival rates than to other demographic rates (Congdon et al. 1994, Folt et al. 2021). Accounting for these interacting and potentially confounding effects of climate change on all aspects of demography will be important for setting conservation expectations for this at-risk species.

Our results highlight the potential conservation value of leading-edge populations in the face of climate change. Warming conditions may actually improve the population outlook for gopher tortoises at FSAR (barring other mitigating negative responses). Replicated studies in other parts of the species' range are needed to determine whether our observed positive response of reproductive output to warming is limited to the northern part of the range; however, it

is likely that other populations would respond differently to warming, as spatially heterogeneous responses to climate are common (Gaston et al. 2005, Grøtan et al. 2008, Elmendorf et al. 2012). Southern populations of gopher tortoises may already be experiencing temperature conditions that maximize their reproductive output (Ashton et al. 2007), and additional warming could prove to be a detriment. If northern populations are truly more likely to benefit from warming than southern populations, there are implications for conservation practice and policy. Currently, the overwhelming majority of gopher tortoises are in peninsular Florida (Smith et al. 2006), where populations are at continuing risk from development, with translocation being a common mitigation to this threat (Cozad 2018). Translocations may be more successful (in terms of reproductive output of translocated females) if tortoises are moved northward to track their climatic niche as the climate continues to warm. Northward translocations are already occurring (Cozad 2018, McKee et al. 2021), although not as an intentional response to a changing climate, but as a function of where land is available. To understand how movement of tortoises among climates within the species' range will impact the long-term persistence of translocated populations, it will be important to determine whether the effects that we have reported on here (warming conditions leading to increases in reproductive output) translate to other populations from warmer climates. As the climate continues to change, the northern part of the species' range may become an increasingly important stronghold for this at-risk species.

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