

RESEARCH ARTICLE

Is the future female for turtles? Climate change and wetland configuration predict sex ratios of a freshwater species

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Abstract

Climate change and land-use change are leading drivers of biodiversity decline, affecting demographic parameters that are important for population persistence. For example, scientists have speculated for decades that climate change may skew adult sex

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ratios in taxa that express temperature-dependent sex determination (TSD), but limited evidence exists that this phenomenon is occurring in natural settings. For species that are vulnerable to anthropogenic land-use practices, differential mortality among sexes may also skew sex ratios. We sampled the spotted turtle (*Clemmys guttata*), a freshwater species with TSD, across a large portion of its geographic range (Florida to Maine), to assess the environmental factors influencing adult sex ratios. We present evidence that suggests recent climate change has potentially skewed the adult sex ratio of spotted turtles, with samples following a pattern of increased proportions of females concomitant with warming trends, but only within the warmer areas sampled. At intermediate temperatures, there was no relationship with climate, while in the cooler areas we found the opposite pattern, with samples becoming more male biased with increasing temperatures. These patterns might be explained in part by variation in relative adaptive capacity via phenotypic plasticity in nest site selection. Our findings also suggest that spotted turtles have a context-dependent and multi-scale relationship with land use. We observed a negative relationship between male proportion and the amount of crop cover (within 300m) when wetlands were less spatially aggregated. However, when wetlands were aggregated, sex ratios remained consistent. This pattern may reflect sex-specific patterns in movement that render males more vulnerable to mortality from agricultural machinery and other threats. Our findings highlight the complexity of species' responses to both climate change and land use, and emphasize the role that landscape structure can play in shaping wildlife population demographics.

KEYWORDS

adaptive capacity, agriculture, climate change, land use, landscape structure, temperature, turtle, wetland configuration

1 | INTRODUCTION

Climate change and land-use change are leading drivers of biodiversity decline, affecting demographic parameters that are important for population persistence (Selwood et al., 2015). For example, species displaying temperature-dependent sex determination (TSD; Bull et al., 1982) are considered particularly vulnerable to climate and land-use change because variation or directional change in temperatures experienced by developing embryos may skew sex ratios, potentially triggering population decline or extirpation (Janzen, 1994a; Schwanz et al., 2010; Valenzuela et al., 2019). In some species, land use may also induce differential mortality among sexes (Steen & Gibbs, 2004), which has the potential to compound—or conceal—the effects of climate change and land use on primary sex ratio (Reid & Peery, 2014). However, the effects of land use on sex-specific mortality may not be straightforward, with factors such as habitat configuration (i.e., spatial arrangement) potentially influencing movement patterns among sexes (e.g., if one sex is more likely to make inter-wetland movements), which could modulate this relationship. Understanding the factors influencing population sex ratios represents an important research priority that will facilitate

more effective conservation strategies as global change progresses (Janzen, 1994a; Reid & Peery, 2014).

Turtles (Order Testudines) are one of the most threatened vertebrate taxa in the world (Gibbons & Lovich, 2019). Most species display delayed sexual maturity (Ernst & Lovich, 2009), low reproductive output, and a type III survivorship curve (i.e., high juvenile mortality, low adult mortality), and therefore lack the life-history traits necessary to rapidly respond to critical threats (Stanford et al., 2020). Further compounding the vulnerability of global turtle diversity, most species exhibit TSD, and warming climates are expected to dramatically increase the proportion of females in turtle populations (Carter & Janzen, 2021; Janzen, 1994a). However, while there is an abundance of research regarding the effect of climate change on primary sex ratios (e.g., Hawkes et al., 2007; Valenzuela et al., 2019), empirical information linking adult sex ratios in the wild to climate change has proven elusive (although see Jensen et al., 2018), and thus the magnitude of this threat remains unclear.

In some cases, species may possess the adaptive capacity (Beever et al., 2016) to counter the effects of climate change (Mitchell et al., 2013; Patricio et al., 2019; Refsnider & Janzen, 2016; Telemeco et al., 2009; Weishampel et al., 2008). For example, behavioral

plasticity may allow females to place nests in cooler areas by excavating deeper nest chambers (Refsnider et al., 2013) or nesting in shadier environments (Janzen, 1994b; Refsnider & Janzen, 2012). Indeed, some species will nest differentially along an open-closed canopy continuum, apparently in response to the local climate (Ewert et al., 2005). Whether such plasticity is sufficient for turtles to respond to climate change remains uncertain. Complicating the challenge of linking adult sex ratios to climate change is the fact that adaptive capacity may vary intraspecifically among populations. For example, in turtles, some populations may lack the variation in nesting microhabitat necessary to adequately compensate for climatic extremes through plasticity in nest site selection (Refsnider & Janzen, 2012). Therefore, attempts to understand the relationship between climate change and adult sex ratio should not only consider adaptive capacity, but also its potential to vary intraspecifically.

Anthropogenic land-use change can introduce novel conditions, on both local and landscape levels, that may affect adult sex ratios through multiple pathways. For example, the urban heat island effect has the potential to generate female-biased populations in urbanized settings via TSD (for species that produce females at higher temperatures; discussed by Bowne et al., 2018). Conversely, row crops can act as ecological traps by presenting suitable incubation conditions during the nesting period, but producing considerably cooler temperatures once crop growth progresses, ultimately leading to male-skewed clutches (Freedberg et al., 2011; Thompson, Coe, Andrews, Cristol, et al., 2018; Thompson, Coe, Andrews, Stauffer, et al., 2018). Freshwater turtles are also widely considered susceptible to differential mortality among sexes driven by land use, whereby male-biased sex ratios are produced due to increased vulnerability

of females during nesting forays (Aresco, 2005; Steen et al., 2006; Steen & Gibbs, 2004). Notably, however, numerous studies have not found such patterns (e.g., Bowne et al., 2018; Carstairs et al., 2018), suggesting that other factors may influence relationships between sex ratio and land use. One possibility is that variation in landscape structure—specifically wetland configuration—may modulate sex-specific mortality. For example, when wetlands are less aggregated (e.g., a constellation of smaller wetlands), sexes may experience similar mortality if they are both likely to make upland movements to access different wetlands, thus leading to approximately equal sex ratios (Figure 1). However, when wetlands are aggregated (e.g., a single large wetland), males have less need to venture into uplands (because there are fewer or no wetlands to access) while females still need to make upland nesting forays, thus leading to male-biased populations (Figure 1). Therefore, both land use and wetland configuration may represent critical determinants of adult sex ratio.

The aim of this study was to examine the combined influence of climate change and land use on adult sex ratios of a semi-aquatic freshwater turtle, the spotted turtle (*Clemmys guttata*), throughout a large portion of its geographic range. We predicted that adult sex ratios would be female biased in areas that had experienced the most warming compared to mean historical temperature because warmer incubation temperatures produce females (i.e., Type Ia TSD; pivotal temperature = approx. 29°C; Ewert et al., 2004). Additionally, in areas with high levels of anthropogenic land use that may increase mortality (e.g., roads, development, agriculture), we predicted that samples associated with aggregated wetlands would be male biased due to increased vulnerability of females while nest-searching, but unbiased when wetland habitat was less aggregated due to similar

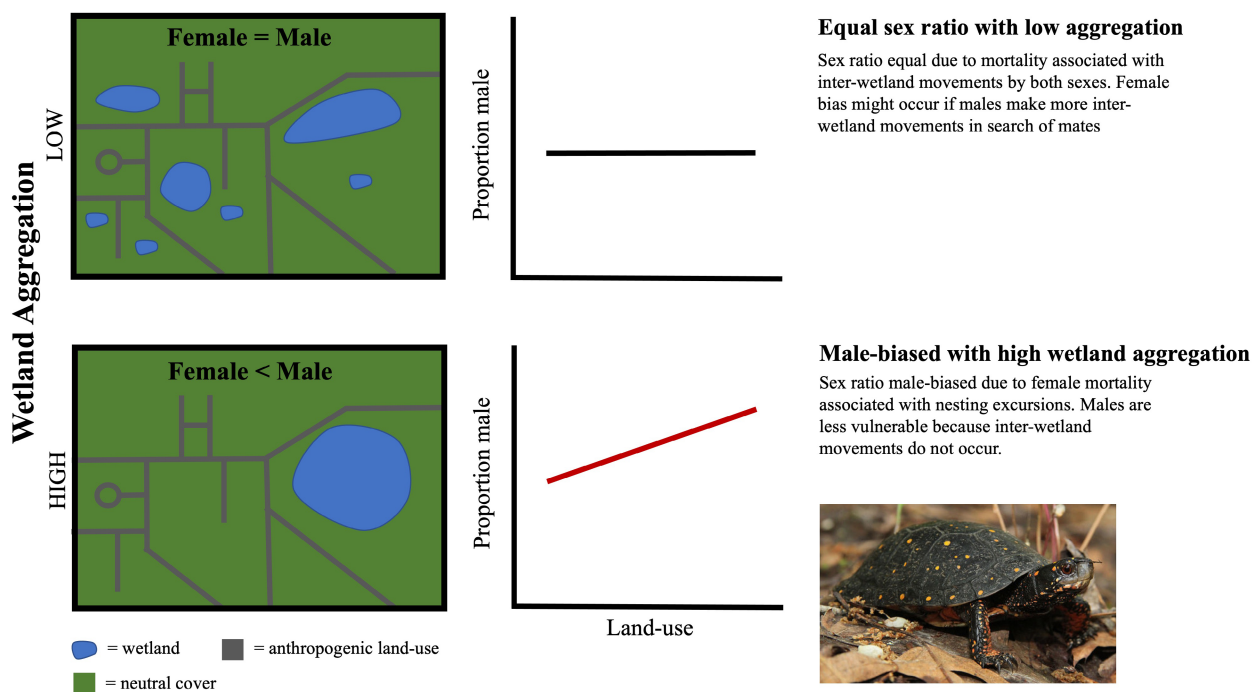


FIGURE 1 Diagram depicting our predictions regarding how landscape structure and land use might influence population sex ratio of the spotted turtle (*Clemmys guttata*). Photo credit: Michael T. Jones. [Colour figure can be viewed at wileyonlinelibrary.com]

rates of mortality between sexes resulting from non-nesting inter-wetland movements (Figure 1).

2 | STUDY AREA

This study was conducted within the spotted turtle's range in the eastern United States from Maine to Florida. We primarily sampled spotted turtle habitats within the Atlantic Coastal Plain and Piedmont, with a small proportion of sampling conducted near the Great Lakes in New York and West Virginia. We do not include any spatially explicit information due to the vulnerability of turtles to poaching for the pet trade (Sung & Fong, 2018).

3 | METHODS

3.1 | Collaborative sampling network

We conducted spotted turtle surveys in association with the Eastern Spotted Turtle Working Group, which is a network of state, non-profit, federal, academic, and volunteer biologists, with the common goal of conserving spotted turtles and their habitat. Conference calls with participating partners were held monthly during active projects to coordinate efforts, develop and refine protocols (northeastturtles.org), and assess progress in achieving objectives. Over 100 state, federal, academic, nonprofit biologists, and volunteers participated in surveys from Maine to Florida.

3.2 | Turtle sampling

3.2.1 | Site selection

We sampled spotted turtles using a standardized protocol developed by the Spotted Turtle Working Group. For each spotted turtle sampling site, we first viewed aerial imagery using Google Earth or ArcGIS to identify four non-overlapping 200-m radius sampling plots (separated by up to 400m) located within target wetlands suitable for spotted turtles (hereafter referred to as "plots"). Plots of this size (200m radius) were approximately 12.6 ha, which is approximately three times the size of the average minimum convex polygon (MCP) measured by Milam and Melvin (2001) in Massachusetts, and similar or larger than the size of male and female MCPs reported by Litzgus and Mousseau (2004) in South Carolina. This plot size was also supported by the state agency biologists throughout the study area (personal observations). Therefore, while plots do not necessarily represent entire populations of interbreeding individuals, their size is intended to represent a scale that could include the movements of typical individual spotted turtles, and potentially encompass multiple non-overlapping home ranges. Thus, we determined

that this size is sufficient to reflect relationships between local sex ratio and the environment. We sampled areas with suitable habitat (generally shallow emergent, shrub, and forested wetlands [Ernst & Lovich, 2009]; see Section 3.2.2 for more detail), many of which were known to be occupied by spotted turtles. Due to competing objectives of a concurrent project, we were unable to randomly sample along a priori environmental gradients of interest. We increased geographic representativeness throughout the study area to the extent possible by encouraging participants to select widely dispersed locations throughout each state (although a specific separation distance was not provided).

3.2.2 | Turtle surveys

We placed five traps in wetlands within each plot. We primarily used collapsible mesh minnow traps (61 × 30.5 cm: ProMar TR-502 or TR-503, Promar). Where mesopredators (e.g., striped skunks [*Mephitis mephitis*], raccoons [*Procyon lotor*]) were common, and predation risk was deemed high, we lined traps with wire mesh. We used wire-based Jones Traps (Chandler et al., 2017) where predators were extremely abundant (Georgia and Florida; four plots (7%) included in analyses). We have no reason to believe Jones Traps would influence the sex ratio of these samples. The specific locations of traps in wetlands within plots were determined by individual surveyors in the field. Surveyors generally placed traps in shallow (≤ 0.2 m) flow channels, at the edge of thick vegetation (e.g., sedges, grasses, shrubs) or structure (e.g., logs, debris), near potential basking sites, and areas with high solar exposure, because these are microhabitats known to be attractive to spotted turtles (Ernst & Lovich, 2009). We separated traps by at least 30 m, which represents the average daily spring movement distance of spotted turtles (Litzgus & Mousseau, 2004). We tethered all traps to stakes and/or adjacent vegetation to prevent movement. We placed flotation devices (typically plastic bottles or foam floats) within traps to ensure breathing space for trapped turtles. We deployed traps for one to three sampling periods of four consecutive nights. In some instances, sites were sampled in more than 1 year (for purposes related to a concurrent project). We baited traps with canned sardines in oil and rebaited and checked for turtles every 24 h. Upon capture, we provided unique identifying codes to all spotted turtles by filing notches into marginal carapace scutes according to local notching systems (e.g., Ernst et al., 1974). We determined the sex of adult turtles based upon the presence (male) or absence (female) of a plastron concavity and the coloration of the chin and throat (tan in males, orange in females; Ernst & Lovich, 2009). We classified turtles as adults using growth ring counts (adults typically have > 8 growth rings) and the extent of new growth visible on the plastron (Ernst & Lovich, 2009). While the number of growth rings does not always correspond to years since birth, they are nevertheless helpful in determining general age class.

3.3 | Environmental covariates

3.3.1 | Climate

We used 4-km resolution PRISM Climate data (PRISM Climate Group) to estimate measures of mean annual deviation from “historical normal” for temperature (daily mean and maximum temperature) and precipitation (daily mean precipitation) in the months of June and July separately. We chose June and July because nests incubate during these months across the range, and previous research has linked hatchling sex ratio to mean July temperature (Janzen, 1994a; Schwanz et al., 2010). We chose to examine precipitation because it has been shown to influence incubation temperatures (Reneker & Kamel, 2016). We defined “historical normal” as the 30-year mean centered around 1959 (1944–1973), and estimated deviation from normal by first (1) calculating the difference from normal for each trapping location in each year from 1960 to 2009, then (2) calculating the mean deviation from normal across all years and traps for each plot. We chose this 50-year temporal window from 1960 to 2009 because, while spotted turtles may live longer (Litzgus, 2006), longevity in the wild is unknown, and we felt this period likely captured when most adult turtles included in this study hatched. We extracted climate values for each trap location using the raster package (Hijmans, 2019) in R statistical software version 4.0.2 (R Development Core Team, 2020).

3.3.2 | Land cover and landscape structure

We calculated land cover variables at multiple spatial scales. “Local” scales consisted of circular buffers ranging from 30 to 300m at 30-m increments and were intended to encompass the typical home range of most individual spotted turtles. We used 30-m increments at the local level because we expected that small differences in scales could potentially yield very different model results. We also wanted to examine scales with greater precision at this level because this range of scales is highly relevant to current management practices (e.g., land protection buffers). “Landscape” scales, which consisted of circular buffers of 480-, 960-, 1920-, 3840-, and 7680-m radii, were intended to reflect the broader landscape beyond a typical spotted turtle home range while encompassing values of extreme long-distance movements (e.g., Milam & Melvin, 2001), the scale of predatory threats (e.g., movements by raccoons [Prange et al., 2004]), and broader landscape-level processes such as disturbance regimes, ecosystem function, and/or dispersal (Roberts et al., 2021). We chose 480-m radius as the lowest “landscape” scale because this diameter (960m) roughly represents an extreme distance that spotted turtles are capable of traveling (Milam & Melvin, 2001). We sequentially doubled scale radii until reaching 7680m, which we arbitrarily chose as the maximum scale. Buffers were applied to trap locations.

We derived land cover variables from the 2016 National Land Cover Database (NLCD), Urban Imperviousness, and Tree Canopy raster data layers developed by the Multi-Resolution Land

Characteristics Consortium (Coulston et al., 2016; Jin et al., 2019; Yang et al., 2018). Land cover variables included road density, percent canopy cover, percent imperviousness, proportion developed, proportion hay/pasture fields, and proportion cultivated crops. We expected that each of these could potentially influence mortality or incubation temperatures (Ewert et al., 2005; Freedberg et al., 2011; Roberts et al., 2021; Willey et al., 2022). We excluded commercial cranberry bogs from the cultivated crops variable because this represents an uncommon crop type throughout the range and, unlike other cultivated crops, may provide suitable habitat for spotted turtles (Massachusetts Natural Heritage and Endangered Species Program, unpubl. data). Developed land included NLCD cover types classified as “Developed, Open Space,” “Developed, Low Intensity,” “Developed, Medium Intensity,” and “Developed, High Intensity.” We calculated each variable at each spatial scale (buffer) for all 30-m raster cells within the study area using the Focal Statistics tool in ArcMap 12.5 (Environmental Systems Research Institute, Inc.). We estimated percent canopy cover and percent imperviousness by calculating the mean of all cells within each buffer. We estimated the remaining variables by taking the proportion of cells within each buffer. We extracted covariate values for each scale for each trap location using the raster package (Hijmans, 2019) in R. We calculated the mean across all five traps within each plot.

We calculated the degree of wetland aggregation using the aggregation index (AI) metric in FRAGSTATS software version 4.2 (McGarigal et al., 2012). This metric characterizes the relative aggregation of a given cover type and is defined as the number of alike raster cell adjacencies divided by the total possible cell adjacencies. We measured AI from the centroid of plot trap locations and only used wetlands that were classified as emergent, shrubland, and forested in the National Wetland Inventory (NWI) database. These represent the primary wetland types that spotted turtles occupy throughout their range (Chandler et al., 2019; Ernst & Lovich, 2009; Milam & Melvin, 2001). We converted NWI wetland shapefiles to a 30-m raster using ArcGIS. We only estimated this class for spatial scales ≥ 300 m. In some contexts, AI can be correlated with the amount of suitable habitat on the landscape (Neel et al., 2004); therefore, we checked Pearson correlations for each scale, which ranged from 0.43 at 300m to 0.7 at 7680m.

3.4 | Statistical analyses

We related adult sex ratio (the proportion of individual male turtles) at plots to environmental covariates using generalized linear mixed models using the “glmmTMB” package in R (Brooks et al., 2017). Because plots were inherently spatially clustered, and a small number of sites (groups of four plots) were placed near each other, we included “macrosite,” which we defined as all plots separated by ≤ 2 km, as a random effect to account for a lack of independence among plots in close proximity. We chose the 2-km separation distance to define macrosites because, upon visual inspection of plot locations, this distance reflected the obvious

spatial clustering pattern (i.e., to include more plots within clusters would have required a much larger separation distance). Plots per macrosite ranged 1–4 in analyses. We removed one plot from analyses because wetlands were not mapped at this location in the NWI dataset. We used a binomial error distribution with proportion of individuals that were male as the response variable. Following Steen and Gibbs (2004), we only modeled sex ratios for plots that captured ≥ 10 unique adult turtles.

We used a multi-stage process to conduct model selection, employing Akaike's information criterion corrected for small sample size (AICc; Burnham & Anderson, 2002) to compare the performance of models. First, we determined the best performing local (30–300m) and landscape-level (480–7680m) scales (hereafter referred to as “conceptual” levels) for each land cover variable (see Section 3.3.2). For each spatial scale we considered models with both linear and quadratic relationships. We also considered interactions with wetland aggregation for scales at which wetland aggregation was estimated (300–7680m). For each respective variable and conceptual level, we retained the scale that had the lowest AICc value, performed better than the null model, and had a coefficient with 95% confidence intervals (CI) that did not overlap zero. Next, to select land cover variables for consideration in final model selection, we conducted an all-subsets model comparison, and retained the variables that appeared in models with $\Delta\text{AICc} < 2$ and 95% confidence intervals that excluded zero (sensu Smetzer et al., 2014). For correlated variables ($r > .7$), we compared univariate models and excluded the variable with the larger AICc value from consideration.

We selected climate variables (see Section 3.3.1) for consideration in final model selection by comparing the performance of models with each climate differential variable alone, as well as including an interaction with the historical normal temperature. We considered these interactions because we suspected that the relationship between male proportion and mean deviation from historical normal might vary depending upon typical local temperature. For example, individuals in areas that are historically warmer (e.g., southeastern United States) might already nest in the coolest locations (e.g., forest

or near water), and therefore not have cooler nesting locations available to maintain an ideal sex ratio. We selected climate variables for consideration in final model selection if they appeared in models with $\Delta\text{AICc} < 2$ and had 95% CI that did not overlap zero.

Last, we conducted final model selection by comparing all variable subsets using the “MuMIN” package (Barton, 2016) in R. We examined variance inflation factor scores of top models to ensure excessive multicollinearity ($\text{VIF} > 10$) was not present. We only considered models with six or fewer fixed effect covariates to limit the potential of over-fitting models. We considered variables to be supported if they appeared in models with $\Delta\text{AICc} < 2$, and strongly supported if the 95% CI excluded zero (Chandler et al., 2009).

4 | RESULTS

We sampled 531 plots between 2018 and 2020 and captured ≥ 10 individual adult turtles at 58 plots (1169 individual turtles). The number of individual turtles per plot ranged 10–76 ($\mu = 20.2$). These plots were distributed across 12 states, including Florida (1), Georgia (3), Virginia (8), West Virginia (6), Maryland (7), Delaware (8), New York (3), Rhode Island (1), Massachusetts (11), Vermont (1), New Hampshire (3), and Maine (6). The proportion of individuals that were males captured at these plots ranged from 0.15 to 0.90 ($\mu = 0.57$) across the study area. To provide a sense of the land cover gradients at these sites, we report the range for each variable (proportions unless otherwise noted) within 300m: cultivated crops = 0–0.62, hay/pasture = 0–0.52, imperviousness = 0%–14.2%, canopy = 10.9%–91.4%, developed = 0–0.26, and road density = 0–0.14.

An interaction between mean maximum temperature differential and historical normal maximum July temperature was strongly supported and appeared in the second-best performing model (Table 1). Where maximum temperatures in July were historically higher, the proportion of male individuals displayed a negative relationship with increasing temperatures, but in historically cooler areas this relationship reversed (Figure 2). An interaction between cultivated crops

TABLE 1 Coefficients (standard error) of best performing models relating the proportion of individual male turtles captured to environmental land cover and climate covariates.

Wetland aggregation ^a (300m)	Crops ^b (300m)	Crops-aggregation interaction ^c	Crops ^b (7680m)	July max. temp. deviation ^d	July max. temp. normal ^e	July max. temp. interaction ^f	AICc ^g	ΔAICc	w ^h
–0.2 (0.09)*	–0.06 (0.05)	0.14 (0.04)*	0.16 (0.05)*				247.1	0	0.58
–0.12 (0.09)	–0.06 (0.05)	0.12 (0.04)*		0.07 (0.1)	0.1 (0.08)	–0.17 (0.07)*	247.7	0.7	0.42

Asterisks (*) indicate coefficients with 95% confidence intervals that do not overlap zero.

^aProportion cultivated crop cover within 300 and 7680m.

^bInteraction between proportion crop cover and wetland aggregation within 300m.

^cMean annual deviation of maximum July temperature from 1959 30-year normal for 1960–2009.

^d1959 30-year normal; mean maximum temperature from 1944 to 1973.

^eInteraction between mean July max. temp. deviation from normal and the 1959 30-year normal.

^fAkaike's information criterion corrected for small sample size.

^gAICc model weights.

^hIndex characterizing the degree of wetland aggregation within 300m.

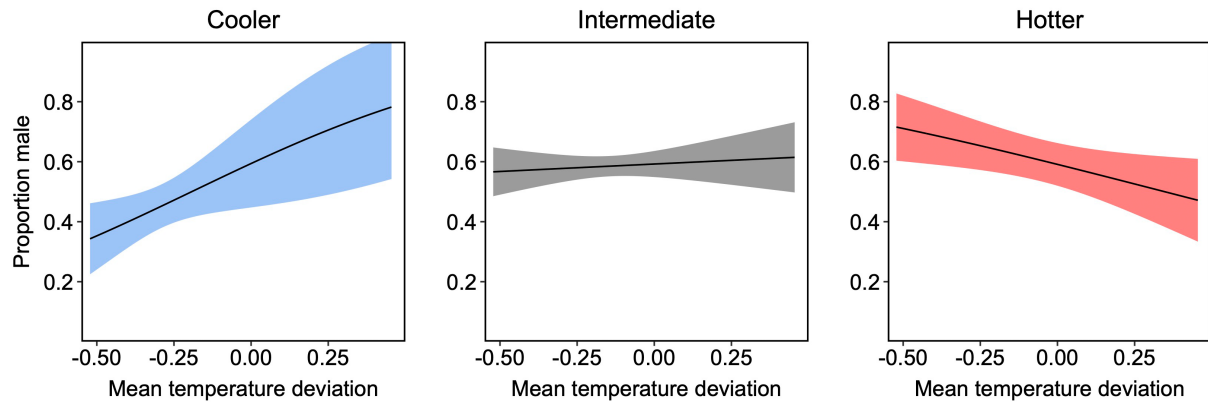


FIGURE 2 Observed relationship between male spotted turtle (*Clemmys guttata*) proportion of captures and the mean annual deviation of maximum July temperature (1960–2009) from the historical normal at low (10th percentile), intermediate (mean), and high (90th percentile) historical normal temperatures. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/gcb.16625)] [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/gcb.16625)]

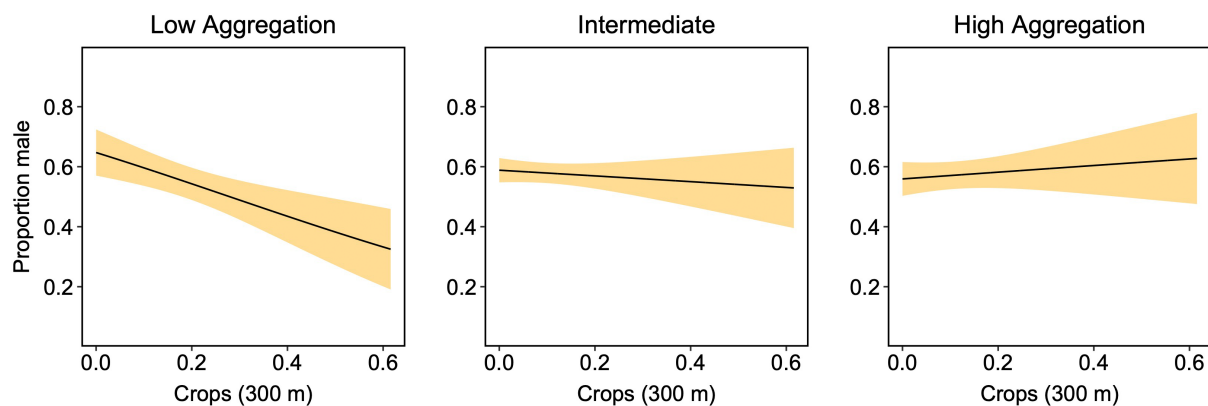


FIGURE 3 Observed relationship between male proportion of captures and the proportion of cultivated crop cover within 300 m at low, intermediate, and high aggregation of wetlands suitable (defined at emergent, shrub, and forested wetlands combined) for spotted turtles (*Clemmys guttata*). [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/gcb.16625)] [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/gcb.16625)]

and wetland aggregation within 300m was strongly supported and appeared in all top models (Table 1). The proportion of male individuals captured was negatively related to cultivated crops at low levels of wetland aggregation, but showed only a slight positive relationship with cultivated crops at high aggregation (Figure 3). Proportion of cultivated crops within 7680m showed a strong positive relationship with male proportion for the best performing model (Table 1, Figure 4).

5 | DISCUSSION

5.1 | Climate change

Despite speculation that climate change will skew adult sex ratios for species with temperature-dependent sex determination (Boyle et al., 2014; Janzen, 1994a), little empirical evidence has emerged suggesting climate change has influenced the adult sex ratio of freshwater turtles with TSD (although see Jensen et al., 2018; Schwanz et al., 2010). We present evidence that suggests climate change (since 1959) might be causing imbalances in the adult sex

ratio of a freshwater turtle across a large portion of its geographic distribution. We found that the plots we studied followed the predicted pattern of greater proportions of females with warming trends (Janzen, 1994a), albeit only in portions of the species range that were historically warmer. Surprisingly, in portions of the range that were historically cooler, the relationship appeared to reverse, with samples becoming more male-biased with increasing temperatures. Because these results are correlative, we cannot definitively conclude that climate change has caused these patterns in sex ratio. However, it is difficult to identify an unmeasured factor during this time period that might confound this relationship.

Adaptive capacity via phenotypic plasticity may explain at least a portion of the observed temperature-dependent variation in the relationship between climate change and adult sex ratio. Many turtles maintain ideal incubation temperatures by locally adjusting nesting behavior (Refsnider et al., 2014) rather than exhibiting genetically determined variation in pivotal temperature (i.e., the temperature at which a 50:50 sex ratio is produced). Some species place nests deeper and closer to sources of water in hot, arid environments (Morjan, 2003), while others appear to utilize shade from vegetation (Janzen, 1994b) to cool nests. For example, the geographically

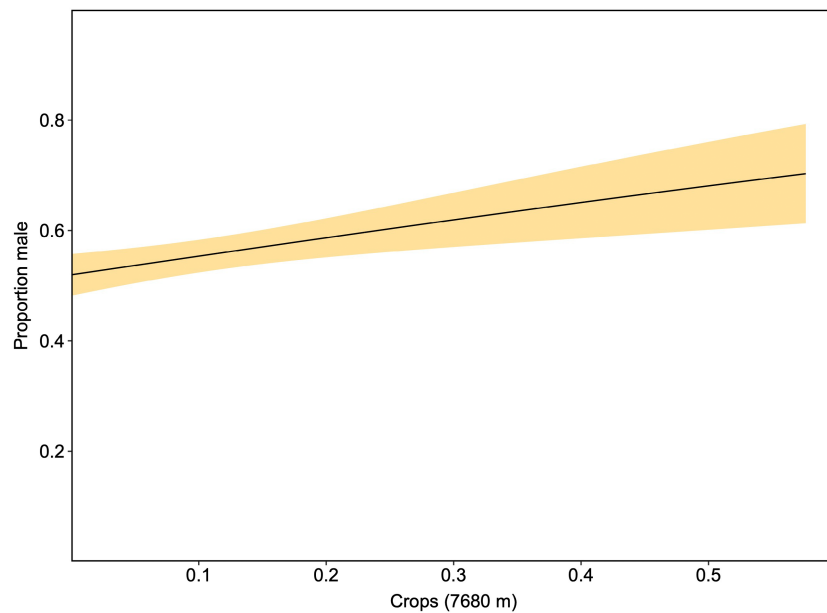


FIGURE 4 Observed relationship between male spotted turtle (*Clemmys guttata*) proportion of captures and proportion cultivated crop cover (7680m) in best performing model. To generate this prediction, all other explanatory variables were held at their mean. [Colour figure can be viewed at wileyonlinelibrary.com]

widespread snapping turtle (*Chelydra serpentina*) has been shown to nest along an open-closed canopy gradient, apparently matching relative shade to local climate (Ewert et al., 2005). Spotted turtles appear to follow a similar pattern, nearly always nesting in shaded (e.g., forest) conditions (Litzgus & Mousseau, 2006) and/or near water (O'Bryan et al., 2016) in warmer portions of their range (Chandler et al., 2022), in varying levels of shade and moisture at intermediate climates (Ernst, 1970; Wilson, 1997), and almost always in dry and exposed (i.e., unshaded) locations in the coolest portions of the range (Beaudry et al., 2010; Joyal et al., 2001; Litzgus & Brooks, 1998; Litzgus & Mousseau, 2006; Milam & Melvin, 2001; Rasmussen & Litzgus, 2010). Such variation in nesting suggests spotted turtles behaviorally adjust to local climate throughout their range and may therefore possess the adaptive capacity to buffer ongoing changes in climate (Escobedo-Galvan et al., 2011). However, the ability to counter the effects of climate change may depend upon the relative position of a population within the broader species range. Populations that occupy the warmest areas may already nest in the coolest potential nesting locations and therefore have no other option but to place nests in suboptimal thermal conditions, thus leading to more female-biased populations in areas experiencing greater warming. In contrast, populations at intermediate climates may still experience enough variation in nesting microclimate to behaviorally adapt.

It is more challenging to explain why more males might be present in areas experiencing warming in cooler portions of the range. This pattern could occur if warming causes females to nest earlier despite cool soils that lag behind air temperatures (Doody et al., 2004). Alternatively, an extended nesting season could cause females to produce additional clutches that incubate under cooler conditions despite overall warming trends (Patricio et al., 2019; Tucker et al., 2008). It is also possible that nesting females could over-adjust to local changes in climate, but it is unclear why this would only occur in cooler portions of the range. These findings

highlight the potentially complex effects of climate change on biota and emphasize the importance of considering intraspecific variation in responses to climate change (Janzen et al., 2018).

5.2 | Land use and wetland configuration

Our study suggests that agriculture—specifically cultivated crop cover—has a complex effect on spotted turtle demographics. Cultivated crops appear to influence sex ratio at multiple spatial scales (300 and 7680m), but the nature of this relationship is scale dependent. In contrast to theory that sources of adult mortality in the surrounding landscape should have a disproportionate effect on females due to elevated vulnerability during nesting excursions (Aresco, 2005; Gibbs & Steen, 2005; Steen & Gibbs, 2004), we found that the proportion of males decreased with greater crop cover within 300m. Notably, this relationship was only apparent when wetland habitat was less aggregated. While all female turtles must find suitable nesting habitat, which can involve extensive and often perilous excursions away from wetlands, males often move at similar or greater frequencies among wetlands throughout the year (Ernst & Lovich, 2009), perhaps associated with mate-searching (Litzgus & Mousseau, 2004). Indeed, male spotted turtles have been shown to move greater distances throughout the year (O'Bryan, 2014), and thus may be more likely to make inter-wetland movements. Therefore, this sex-specific behavior may render males more vulnerable to threats associated with agriculture, such as machinery and mesopredators, when wetland habitat is more spatially disaggregated.

We predicted an increase in the proportion of males at high levels of wetland aggregation and land-use intensity due to female mortality associated with nesting and fewer upland movements by males, yet we observed only a negligible increase at the highest levels of wetland aggregation. While female spotted turtles occasionally

travel substantial distances (hundreds of meters) to nest, most appear to nest very close to wetlands (<40m; Steen et al., 2012), which likely reduces the relative risk of females to threats associated with crops. This finding adds to the growing number of studies that fail to detect male-biased populations near land uses associated with turtle mortality (Carstairs et al., 2018; Hamer et al., 2016; Reid & Peery, 2014; Roe et al., 2011), highlighting that the effects of anthropogenic land use on turtle populations are likely modulated by species life-history, sex-specific traits, and landscape context (Gibbs & Shriver, 2002).

At the landscape level (7680m), the relationship between sex ratio and crop cover appears to follow the opposite trend, with proportion of males captured increasing with greater crop cover. This pattern may reflect the potential cooling effect that mature crops can have on nests (Mui et al., 2016; Thompson, Coe, Andrews, Cristol, et al., 2018). Given that females typically nest near wetlands (Beaudry et al., 2010) and rarely, if ever, travel more than a few hundred meters to nest (Steen et al., 2012), this relationship may reflect a component of spotted turtle ecology that operates at a larger spatial scale, such as male dispersal distance. Large landscapes with high crop cover may produce more males across (sub)populations and thus the number of males immigrating might closely match those emigrating at any given location, producing a skewed adult sex ratio similar to the hatchling/juvenile sex ratios produced. However, if the scale of this relationship was much smaller than the scale of dispersal (e.g., <300m), the surrounding landscape may not have similarly high crop cover and thus the number of males entering a population might be lower than were produced within it, resulting in a less skewed adult sex ratio despite producing male-biased juvenile ratios. It should be noted that there are few, if any, records of spotted turtles nesting within the uniform, exposed soils characteristic of most crops prior to growth, and therefore it is possible that this pattern is driven by another unmeasured factor. However, it is also possible that spotted turtles nest at the edge of crop fields and still become shaded by subsequent crop growth.

Notably, and in contrast to many previous studies of other species (Aresco, 2005; Gibbs & Steen, 2005; Marchand & Litvaitis, 2004; Steen & Gibbs, 2004), road density was not a strong predictor of spotted turtle sex ratio. It seems likely that we simply did not sample a broad enough range of road densities to detect a strong trend. An additional limitation is that we were unable to account for variation in time since land-use change (Gibbs & Steen, 2005), which could bias our results related to roads and other cover types. Finally, if roads cause high mortality, it is also possible there were simply not enough turtles present (≥ 10 adults) at sites with higher road density to be included within analyses.

We demonstrate that the effects of anthropogenic land use on a freshwater turtle's sex ratio were strongly dependent upon wetland configuration, which, to our knowledge, has not previously been reported. Our observation that the relationship between sex ratio and crop cover was dependent upon the relative aggregation of wetland habitat, indicates that existing theory related to turtle sex ratios and anthropogenic land cover is likely too simplistic to be applied

indiscriminately across species and landscape contexts. In particular, the prediction that populations will be male biased in contexts where upland mortality risk is high (e.g., high road density or agriculture), due to disproportionate vulnerability of females during nesting excursions, cannot explain our results alone. This is supported by the growing body of research that reports conflicting results even within the same species. For example, while several studies have linked male-biased painted turtle (*Chrysemys picta*) sex ratios to elevated road densities (Aresco, 2005; Gibbs & Steen, 2005; Marchand & Litvaitis, 2004; Patrick & Gibbs, 2010; Reid & Peery, 2014; Steen & Gibbs, 2004), others have found no relationship (Carstairs et al., 2018; Dorland et al., 2014; Reid & Peery, 2014), or even the opposite trend (Bowne et al., 2018; Buchanan, 2017). Landscape structure may explain these discrepancies. For example, for species where males make frequent overland movements, equal or female-biased populations could be expected in landscapes with high wetland dispersion and high road density, while (although not observed in this study) male-biased populations might occur under high wetland aggregation. On the other hand, for aquatic species that rarely make upland movements, we might expect male-biased populations regardless of the degree of wetland aggregation because only females venture into uplands. Given the influential role that landscape structure may play in determining sex-specific patterns in mortality, future studies should either attempt to control for influential factors (e.g., see Dorland et al., 2014) or incorporate landscape structure directly into analyses. Our results suggest that, when sex ratio is a concern, resource managers and conservationists should prioritize agricultural mitigation for spotted turtles where there is greater configurational heterogeneity of wetlands.

6 | CONCLUSIONS

Our study provides evidence that suggests climate change (among other factors) is potentially driving sex ratio imbalances of a freshwater turtle in a manner that varies depending upon local climate. While warming trends were associated with greater proportions of both females and males at warmer and cooler portions of the range respectively, our results suggest that at intermediate temperatures, spotted turtles may be able to buffer the effects of a changing climate. However, the rate of climate change may overcome the ability to compensate—as might be the case in the warmest portions of the range—and an increasing number of populations may trend toward female bias. While female bias may initially benefit populations through increased growth rates (Tomillo et al., 2015), if ratios become severely skewed it will eventually negatively affect viability (Hays et al., 2017), although it is unclear at what point this will occur. While we examined only one species, we suspect that similar intraspecific relationships with climate change could exist for other turtles and reptiles with TSD, particularly those that occupy broad climatic and environmental gradients.

While climate change represents a major long-term threat to population persistence on multiple fronts (Ihlow et al., 2012), our

results, which include land cover in all top models, support the notion that anthropogenic land use, not climate, is possibly the predominant factor influencing adult sex ratio of spotted turtles and other freshwater turtles (Reid & Peery, 2014). While the effect of climate change on sex ratio may present a future threat to freshwater turtle demographics, the influence of anthropogenic land use represents a more immediate and influential driver of sex ratio imbalances, likely through its effect on sex-specific mortality rates and microclimate.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data not available due to privacy/ethical restrictions.

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