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Contributed Paper

Effects of urbanization on the population structure of freshwater turtles across the United States

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Abstract: *Landscape-scale alterations that accompany urbanization may negatively affect the population structure of wildlife species such as freshwater turtles. Changes to nesting sites and higher mortality rates due to vehicular collisions and increased predator populations may particularly affect immature turtles and mature female turtles. We hypothesized that the proportions of adult female and immature turtles in a*

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*population will negatively correlate with landscape urbanization. As a collaborative effort of the Ecological Research as Education Network (EREN), we sampled freshwater turtle populations in 11 states across the central and eastern United States. Contrary to expectations, we found a significant positive relationship between proportions of mature female painted turtles (*Chrysemys picta*) and urbanization. We did not detect a relationship between urbanization and proportions of immature turtles. Urbanization may alter the thermal environment of nesting sites such that more females are produced as urbanization increases. Our approach of creating a collaborative network of scientists and students at undergraduate institutions proved valuable in terms of testing our hypothesis over a large spatial scale while also allowing students to gain hands-on experience in conservation science.*

Keywords: population structure, roads, sex ratio, turtle, urbanization

Efectos de la Urbanización sobre la Estructura Poblacional de Tortugas de Agua Dulce en los Estados Unidos

Resumen: *Las alteraciones a escala de paisaje que acompañan a la urbanización pueden afectar negativamente a la estructura poblacional de las especies silvestres como las tortugas de agua dulce. Los cambios en los sitios de anidación y las altas tasas de mortalidad causados por colisiones con vehículos y el incremento poblacional de depredadores pueden afectar particularmente a las tortugas inmaduras y a las tortugas hembras maduras. Supusimos que las proporciones de hembras adultas y de tortugas inmaduras en una población se correlacionan negativamente con la urbanización del paisaje. Como un esfuerzo colaborativo de la Investigación Ecológica como Red Educativa (EREN, en inglés) muestreamos las poblaciones de tortugas de agua dulce en once estados del centro y el este de los Estados Unidos. Contrario a lo esperado, encontramos una relación positiva significativa entre las proporciones de hembras maduras de* Chrysemys picta *y la urbanización. No detectamos una relación entre la urbanización y la proporción de tortugas inmaduras. La urbanización puede alterar el ambiente térmico de los sitios de anidación de tal forma que se producen más hembras conforme incrementa la urbanización. Nuestra estrategia de crear una red colaborativa de científicos y estudiantes en instituciones universitarias resultó ser valiosa para probar nuestra hipótesis a lo largo de una gran escala espacial mientras permitió también que los estudiantes ganaran experiencia de primera mano en la ciencia de conservación.*

Palabras Clave: carreteras, estructura poblacional, proporción de sexos

Introduction

The landscape in which many wildlife species occur is dramatically different from that experienced over the majority of their evolutionary histories (Ellis 2015; Waters et al. 2016) because human activities have transformed ecosystems into novel types with altered form and function (Radeloff et al. 2015). Understanding how organisms respond to novel ecosystems is important to basic ecology and conservation efforts (Hobbs et al. 2009). Examining patterns of landscape change and the response of biota to those transformations will help improve wildlife conservation and maintain ecosystem function.

Freshwater turtles are increasingly used as a model taxon to explore the effects of landscape transformations because of their discrete primary habitat (i.e., ponds and lakes), use of upland habitat for nesting and dispersal, and vulnerability to novel, anthropogenic threats such as collisions with vehicles (Bowne et al. 2006; Dorland et al. 2014). Urbanization, most notably the road network, is often predicted to negatively affect freshwater turtle populations (Marchand & Litvaitis 2004*a*; Gibbs & Steen 2005). High road density is linked to male-skewed sex ratios in some studies, putatively due to increased road-induced mortality of females during nesting movements (Steen & Gibbs 2004; Aresco 2005; Gibbs & Steen 2005). Based on the diversity of genetic markers, Laporte et al. (2012) concluded that fewer painted turtle (*Chrysemys picta*) females reproduce when populations are near roads. Roads, however, do not affect turtle population structure uniformly (e.g., Dorland et al. 2014).

Human activities also change the quality and quantity of nesting sites and influence predation risk (Marchand & Litvaitis 2004*b*). The ability of freshwater turtles to select appropriate nesting sites depends on the availability and proximity of terrestrial habitat. Fields and tilled soils are used as nesting sites, but turtles using such sites risk exposure to human activity (Joyal et al. 2001). Sex determination in most freshwater turtles is type 1a temperature dependent: females are produced at higher incubation temperatures than males (Refsnider & Janzen 2016). Consequently, nesting sites with warmer microclimates arising from human modifications may produce more females than males (Freedberg & Bowne 2006). Landscapes dominated by anthropogenic activities may also affect the spatial distribution of nests, which can increase the risk of predation (Marchand & Litvaitis 2004*b*). Raccoons (*Procyon lotor*) and other generalist predators thrive in suburban areas and can increase depredation of turtle nests and juvenile mortality (Seigel 1980; Congdon et al. 1993). Browne and Hecnar (2007) report a decline in juveniles of several turtle species over 30 years due primarily to predation by a large raccoon population.

We investigated the population structure of freshwater turtles in central and eastern United States along a gradient of anthropogenic landscape modification surrounding their core lentic habitat. We hypothesized that the proportions of adult female and immature turtles in a population negatively correlate with landscape urbanization. This hypothesis has been tested previously (Marchand & Litvaitis 2004*b*; Dorland et al. 2014; Hamer et al. 2016) in relatively small geographic areas. Here, we investigated simultaneously freshwater turtle population structure across 11 states in central and eastern United States through the Ecological Research as Education Network (EREN), a collaboration of faculty and students at primarily undergraduate institutions. Our approach of concurrently collecting data on multiple turtle populations across the United States while engaging undergraduates in authentic research has not been reported in the literature. The large spatial scale of our study allowed us to investigate the extent to which the response of turtle population structure to urbanization was consistent across sites with varying species assemblages and degrees of urbanization. We did not a priori select specific turtle species, but the painted turtle became the focal species due to its wide distribution and abundance across sites.

Methods

Building Collaboration

We conducted this research as project TurtlePop within the Ecological Research as Education Network (EREN), the mission of which is to improve ecological research and teaching at primarily undergraduate institutions by creating collaborations through ecological projects in which the same data are collected using common methods across a range of sites (Bowne et al. 2011; Lindquist et al. 2011; Simmons et al. 2016). All collaborators used standard protocols (Bowne 2012) and received professional training via hands-on workshops, videos, and manuals. We incorporated TurtlePop into undergraduate courses and independent student research (Supporting Information), and shared pedagogical approaches to the project among group members and with the broader community (available from www.erenweb.org).

Pond Selection

Each collaborator chose at least one lentic environment, hereafter referred to as ponds, based on logistical factors such as proximity to campus and access. We did not a priori select ponds to establish a specific gradient of urbanization across all sites or target individual species; rather, we investigated the gradients represented by available ponds. Although a stratified random design would have been desirable, it was not feasible given the large geographic spread of our sites and accessibility to participating faculty and students. Participants provided pond-specific information (Supporting Information) such as geographic coordinates, approximate age of pond, dominant substrate type (inorganic or organic), number of potential basking spots, water retention (ephemeral or permanent), and presence of common snapping turtle (*Chelydra serpentina*). All the ponds retained water over the duration of the study and nearly all had organic substrate.

We further characterized the location of each pond by its elevation, mean annual rainfall, mean annual air temperature (www.usclimatedata.com), and amount of light pollution. We determined light pollution with 2 satellite-based measurements, night-sky brightness and surface radiance (www.lightpollutionmap.info). Night-sky brightness is a consequence of anthropogenic light sources (Falchi et al. 2016*a*). We used brightness data provided by the World Atlas 2015 (Falchi et al. 2016*b*). Surface radiance is a measure of nighttime visible and near infrared light from both anthropogenic and nonanthropogenic sources (Miller et al. 2013). We used data on surface radiance collected from the Visible Infrared Imaging Radiometer Suite (VIIRS) collected by the Suomi National Polar-orbiting Partnership (NPP) satellite in 2013 (Miller et. al 2013).

Turtle Trapping

We trapped turtles using standardized protocols that followed guidelines for use of reptiles in field research (ASIH 2004) and approved by the Animal Use and Care Committees of respective institutions. A minimum of four hoop traps (0.9 m diameter with 2.5 cm mesh) baited with sardines were deployed in each pond for a minimum of 2 consecutive nights from 20 August to 6 October in 2012 and 2013. Captures of snapping turtles were noted, but individuals were not measured out of concern for the safety of participating students. For all other turtle species, the length of the plastron, carapace, middle-right foreclaw, and precloacal tail were measured with dial calipers to the nearest 0.1 mm. If visible, the number of plastron annuli were counted. These metrics were used to determine sex and age class (mature, immature) as appropriate for each species (Frazer et al. 1990). Each turtle was marked by filing notches into three marginal scutes in a locally unique pattern. Photographs of the plastron and carapace of turtles were taken for verification of species and sex. All students were supervised by participating faculty members to ensure data integrity. Each faculty member then sent the trapping data to the lead scientist, who checked all measurements for consistency and verified age class and sex for each turtle based on morphological measurements.

Landscape Analysis

We characterized the landscape surrounding each pond with ArcGIS version 10.2 (ESRI, Redlands, California). After selecting each pond from the National Wetland Inventory (Cowardin et al. 1979), we then extracted land-use data from the 2006 National Land Cover/Land Use Dataset (Fry et al. 2011) of buffered areas within 250 and 1000 m around each pond. Because turtles are long-lived species, their exposure to land-use characteristics from 2006 would still be relevant to our 2012 and 2013 trapping season. Over 95% of nesting by painted turtles (Steen et al. 2012), our most common species, was within 250 m of a water body. Painted turtles can move more than 1000 m overland, but that distance likely captures the majority of interpond movements (Bowne et al. 2006). We collapsed land-use types to eight broad categories: forest, agriculture, developed, shrubland, herbaceous, barren, open water, and wetland. We obtained data on road length in each of the buffered distances from road data sets from each state's transportation department.

Extent of Urbanization

Because no one universally applicable definition of *urbanization* exists, we used a variety of metrics to characterize the urbanization context of each pond. We used categorical metrics such as the Bortle dark-sky scale, which assigns a value along the rural-urbanization gradient based on light pollution (Bortle 2001), and the U.S. Census Bureau urbanization designation (Table 1) to describe qualitatively the landscape context of each pond, but we did not incorporate them in statistical analyses. According to the Bortle scale (www.lightpollutionmap.info), our ponds were distributed among rural-suburban transition, suburban, bright suburban, and suburban-urban transition (Table 1). From the perspective of the U.S. Census Bureau, our ponds were categorized almost equally into rural and urbanized areas; a few were in urban clusters (Table 1; Fig. 1). We used the continuous metrics of the proportion of developed land and total length of roads within 250 m of each pond to describe the landscape context of each pond (Table 1) and in statistical analyses.

Data Analyses

We set a minimum requirement of $n = 10$ unique individuals per species per pond for inclusion in the analysis (Marchand & Litvaitis 2004*a*) and a minimum of 1000 m distance between ponds. This requirement yielded 26 ponds across 11 states (Fig. 1) containing either painted turtles or pond sliders (*Trachemys scripta*). The minimum number of pond sliders was found in only four ponds for adults and six ponds for immatures. The painted turtle was the only species common enough, with the minimum number of adults found in 19 ponds and adult plus immature individuals in 22 ponds, to analyze on urbanization effects (Table 1).

We then calculated the proportion of adult females and immature turtles per species per pond, pooling data across both years (2012 and 2013). We restricted statistical analyses to data collected during the late summer and early fall to avoid possible seasonal differences in capture probability. This removes the possibility of capture bias associated with nesting, which occurs in mid-May to mid-July for painted turtles (Moll 1973). Our trapping period coincides with peak spermatogenesis regardless of latitude (Moll 1973), so any influence on capture probability by autumnal courtship by males should be similar across sites. Emergence of painted turtle hatchlings from the nest occurs in the fall or the following spring (Gibbons & Nelson 1978), but this is irrelevant to our study because hatchlings are too small to be captured with hoop traps. Although turtle activity within our late summer and early fall trapping period could potentially differ between sites due to latitudinal effects, we had no reason to expect it would affect measured sex ratio. Although our capture data were based on only this late summer and early fall season, analysis of a subset of sites ($n = 7$ [Supporting Information]) with long-term data (3-7 years) showed sex ratio was more variable among sites than across seasons at the same site (intraclass correlation coefficient = 0.57; 95% CI = $0.23 - 0.89$). We thus concluded surveys from a single season provide a reasonable measure of sex ratio at a site. Any bias introduced by sex-dependent differences in capture probability should be consistent across all sites because we used the same methods.

We performed a principal component analysis (PCA) with varimax rotation to identify correlated sets of explanatory variables at the pond and landscape scale. Pond-level variables included in the PCA were pond age, number of basking spots, and pond area. Context variables included mean annual temperature, mean annual rainfall, elevation, brightness, and radiance. Landscape variables, measured within 250 m and 1000 m of ponds, included road length and proportion of open water, developed, forest, agriculture, and wetland. We excluded other land-cover categories (shrubland, herbaceous, and barren) because they accounted for minimal area. We excluded presence and absence of snapping turtles because only two sites did not have them. Separate PCAs were conducted for all variables measured at 250 m and 1000 m. We used the psych package (Revelle 2015) in R (R Development Core Team 2016) to conduct the PCAs. We then used general linear models (GLM) to examine how variation in proportions of adult female and proportions of immature turtles responded to the principal components. For each response variable, we ran a full model and then used likelihood ratio tests to test the significance of each covariate. We checked model assumptions (normal residuals and heteroscedasticity) with

Table 1. Location and landscape context of each sampled pond by turtle species.

State[a]	*Pond*	*Proportion of adult female*[b]	*Proportion of immature turtles*	*Lat. (°)*	*Long. (°)*	*Bortle*[c]	*Dev*[d]	*Roads (m)*[e]	*Census designation*[f]
Chrysemys picta									
NY	Ann Lee	0.04	0.17	42.7368	−73.8135	6	0.43	2450	3
MA	Clear	0.14	0.27	41.8317	−70.7401	4	0.14	1911	3
MA	FA	0.15	0.17	41.8727	−70.6610	4	0.06	1142	1
MI	Stu Visser	0.16	0.23	42.7996	−86.1431	5	0.29	349	3
MA	B	0.17	0.06	41.8632	−70.6727	4	0.09	1004	1
MA	C	0.20	0.13	41.8634	−70.6643	4	0.05	887	1
MA	Ice	0.20	0.00	41.9601	−70.9548	5	0.06	14	1
MA	Shady Acres	0.20	0.21	41.8576	−70.7275	4	0.12	1806	1
NC	Golf Course	0.21	0.26	36.0079	−79.9496	6	0.45	4	3
MN	Lochness	0.28	0.28	45.1724	−93.1541	6	0.19	1201	1
NY	Odell's	0.30	0.21	42.8565	−76.9906	5	0.67	1996	2
WI	Detention	0.39	0.69	42.5197	−89.0687	5	0.84	2886	3
MD	Turtle	0.47	0.29	39.6831	−77.3448	5	0.34	2086	1
PA	Mallard	0.49	0.28	40.7875	−75.6483	4	0.04	666	1
NJ	Centennial	0.50	0.23	40.2800	−74.7400	6	0.76	149	3
OH	Dempsey	0.52	0.21	40.3100	−83.0848	5	0.47	349	2
PA	Kreiderheim	0.52	0.12	40.3361	−76.5207	5	0.54	1628	3
NJ	Abbott's	0.55	0.00	39.7089	−75.1146	7	0.91	1704	3
PA	Weird	0.56	0.14	40.1492	−76.5852	5	0.56	1219	2
MN	Sunfish	NA	0.46	45.2362	−93.4278	6	0.57	3209	3
NC	Meredith	NA	0.59	35.7970	−78.6859	7	0.99	2216	3
NY	Barracks	NA	0.67	44.6501	−73.4496	4	0.77	657	1
Trachemys scripta									
OK	Health Sciences	0.18	0.33	36.3170	−95.6361	6	0.60	1060	2
OK	Oxley	0.33	0.28	36.2241	−95.9027	6	0.07	412	1
OK	Ranger Station	0.33	0.48	36.4302	−95.6839	4	0.13	778	1
NC	Meredith	0.75	0.41	35.7970	−78.6859	7	0.99	2216	3
PA	Green	NA	0.66	40.6796	−75.3096	5	0.23	1215	3
NC	Golf Course	NA	0.47	36.0079	−79.9496	6	0.45	4	3

[a] *Abbreviations: MA, Massachusetts; MD, Maryland; MI, Michigan; MN, Minnesota; NJ, New Jersey; NY, New York; NC, North Carolina; OH, Ohio; OK, Oklahoma; PA, Pennsylvania; WI, Wisconsin.*
[b] *Cells with NA had too few turtles to calculate.*
[c] *Bortle dark sky scale: 4, rural to suburban transition; 5, suburban; 6, bright suburban; 7, suburban to urban transition.*
[d] *Proportion of developed land within 250 m of pond.*
[e] *Total length of roads within 250 m of pond.*
[f] *Urban designation according to the U.S. Census Bureau: 1, other; 2, urban cluster; 3, urbanized area.*

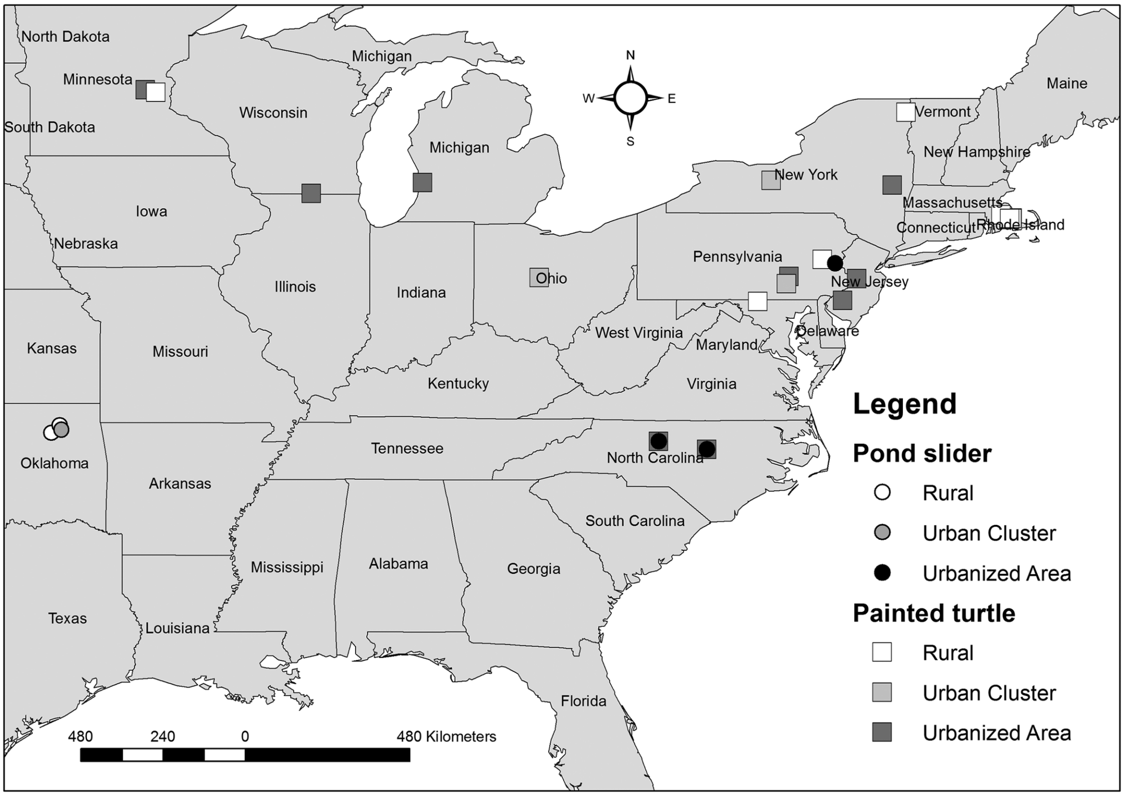

Figure 1. Distribution of ponds with turtle species included in the analysis of sex ratio. Urbanization designation is from the U.S. Census Bureau (2000).

the global models and applied a logit transformation to proportion of immatures to minimize heteroscedasticity. Proportions of adult females met assumptions without a transformation. All GLMs were analyzed in R, and we used the AICcmodavg package in R (Mazerolle 2016) to assess model selection results.

Results

Variables within 250 m of each pond

The PCA yielded five components with eigenvalues >1 accounting for 82% of the variation at the 250-m scale (Table 2). The PCA indices were as follows: PC1, urbanization, high values indicated high development, low forest cover, high radiance, and high brightness; PC2, pond age and size (large and old ponds tended to be surrounded by more roads); PC3, basking sites, wetland cover, and air temperature, high values reflected fewer basking sites, lower wetland cover, and higher air temperatures; PC4, annual rainfall and elevation, high values indicated relatively higher elevation and lower rainfall; and PC5, agricultural cover, high values reflected low agricultural cover and moderate brightness.

Proportion of adult female painted turtles was significantly related to PC1 ($p = 0.054$) (Table 3) and PC3 ($p = 0.040$) (Table 3). The proportion of adult females was greatest in urban areas where annual temperature was relatively higher and there were few basking spots and low wetland cover (Fig. 2). Proportion of immature painted turtles was significantly related to PC4 ($p = 0.008$) (Table 3). Proportionally more immature painted turtles were found at relatively higher elevations and in drier locations (Fig. 3).

Variables within 1000 m of each pond

The PCA yielded 5 components with eigenvalues >1 accounting for 85% of the variation at the 1000-m scale (Table 2). High values for PC1 indicated urbanization with high levels of developed cover, high brightness, and high radiance, whereas low values indicated high levels of forest and open water. The PC2 was positively related to mean air temperature and negatively related to number of basking spots. The PC3 is positively related to age and size of ponds. The PC4 was positively related to elevation and negatively related to annual rainfall. The PC5 was an index of agriculture with high values related to low agricultural cover.

Proportion of adult female painted turtles at 1000 m was significantly related to PC1 ($p < 0.001$) (Table 3) and PC2 (basking sites, wetland cover, mean temperature, $p = 0.023$) (Table 3). Proportion female was greatest in

Table 2. Results of principal component analysis (PCA) of pond and landscape variables at 250 and 1000 m distance from sampled ponds.

		Factor Loadings				
Spatial scale	*Variable*	*PC1*	*PC2*	*PC3*	*PC4*	*PC5*
250 m	open water	−0.70	−0.06	0.23	0.01	0.27
	developed	0.87	−0.17	0.11	0.26	0.21
	forest	−0.87	−0.12	0.20	−0.21	0.11
	agriculture	0.00	0.21	0.15	−0.15	−0.86
	wetland	−0.01	0.23	−0.77	−0.15	0.13
	road length	0.42	0.58	0.14	0.16	−0.32
	pond age	0.04	0.90	−0.32	0.08	−0.01
	basking spots	0.11	−0.06	−0.87	0.13	0.05
	pond area	0.01	0.91	−0.05	0.21	−0.09
	temperature	0.22	−0.21	0.62	−0.28	0.47
	rainfall	−0.29	−0.19	0.38	−0.84	−0.02
	brightness	0.71	0.25	0.12	0.05	0.60
	radiance	0.82	0.01	0.18	0.00	0.22
	elevation	0.10	0.19	0.14	0.87	0.10
	eigenvalue	3.51	2.29	2.20	1.80	1.63
	proportion of variance explained	0.31	0.20	0.19	0.16	0.14
1000 m	open water	−0.66	0.40	−0.10	0.06	0.52
	developed	0.87	−0.10	−0.14	0.34	0.22
	forest	−0.83	0.22	−0.09	−0.36	0.22
	agriculture	−0.09	0.08	0.07	0.02	−0.93
	wetland	0.03	−0.64	0.55	−0.19	−0.07
	road length	0.66	0.19	−0.15	0.33	−0.06
	pond age	0.00	−0.23	0.92	0.09	−0.02
	basking spots	0.03	−0.88	0.02	0.04	0.22
	pond area	−0.01	−0.03	0.89	0.23	−0.07
	temperature	0.27	0.74	−0.23	−0.16	0.31
	rainfall	−0.32	0.47	−0.20	−0.77	0.00
	brightness	0.82	0.20	0.27	0.05	0.37
	radiance	0.86	0.11	−0.02	−0.09	0.03
	elevation	0.17	0.12	0.17	0.92	−0.01
	eigenvalue	3.94	2.33	2.19	1.93	1.52
	proportion of variance explained	0.33	0.20	0.18	0.16	0.13

Table 3. Deviance and p-values from likelihood ratio tests for each principal component versus proportions of adult females and proportions of immature turtles at spatial scales of 250 m and 1000 m from each pond.

Scale (m)	*Principal component*	*Adult females (p)*	*Immatures (p)*
250	PC1 (urbanization)	0.06 (0.054)*	0.07 (0.372)
	PC2 (pond size, age)	0.03 (0.210)	0.04 (0.828)
	PC3 (air temperature, basking spots, wetlands)	0.07 (0.041)*	0.004 (0.945)
	PC4 (elevation, rain)	0.03 (0.170)	5.92 (0.008)*
	PC5 (agriculture)	0.002 (0.720)	0.02 (0.891)
1000	PC1 (urbanization)	0.14 (<0.001)*	0.14 (0.698)
	PC2 (air temperature, basking spots, wetlands)	0.06 (0.023)*	0.46 (0.480)
	PC3 (pond size, age)	0.03(0.087)	0.00 (0.998)
	PC4 (elevation, rain)	0.01 (0.310)	4.85 (0.022)*
	PC5 (agriculture)	0.04 (0.066)	0.1 (0.737)

*Significant at $\alpha = 0.05$.

urban areas with higher mean temperature, few basking sites, and low wetland cover. Proportion of immature painted turtles was significantly related to PC4 ($p = 0.022$) (Table 3); proportionally more juveniles occurred at sites with higher elevations but lower annual rainfall (Fig. 3).

Discussion

We successfully sampled ponds embedded in landscapes of varying degrees of urbanization by collaborating through EREN. Nesting painted turtles in our sampled ponds would encounter developed land within 250 m in

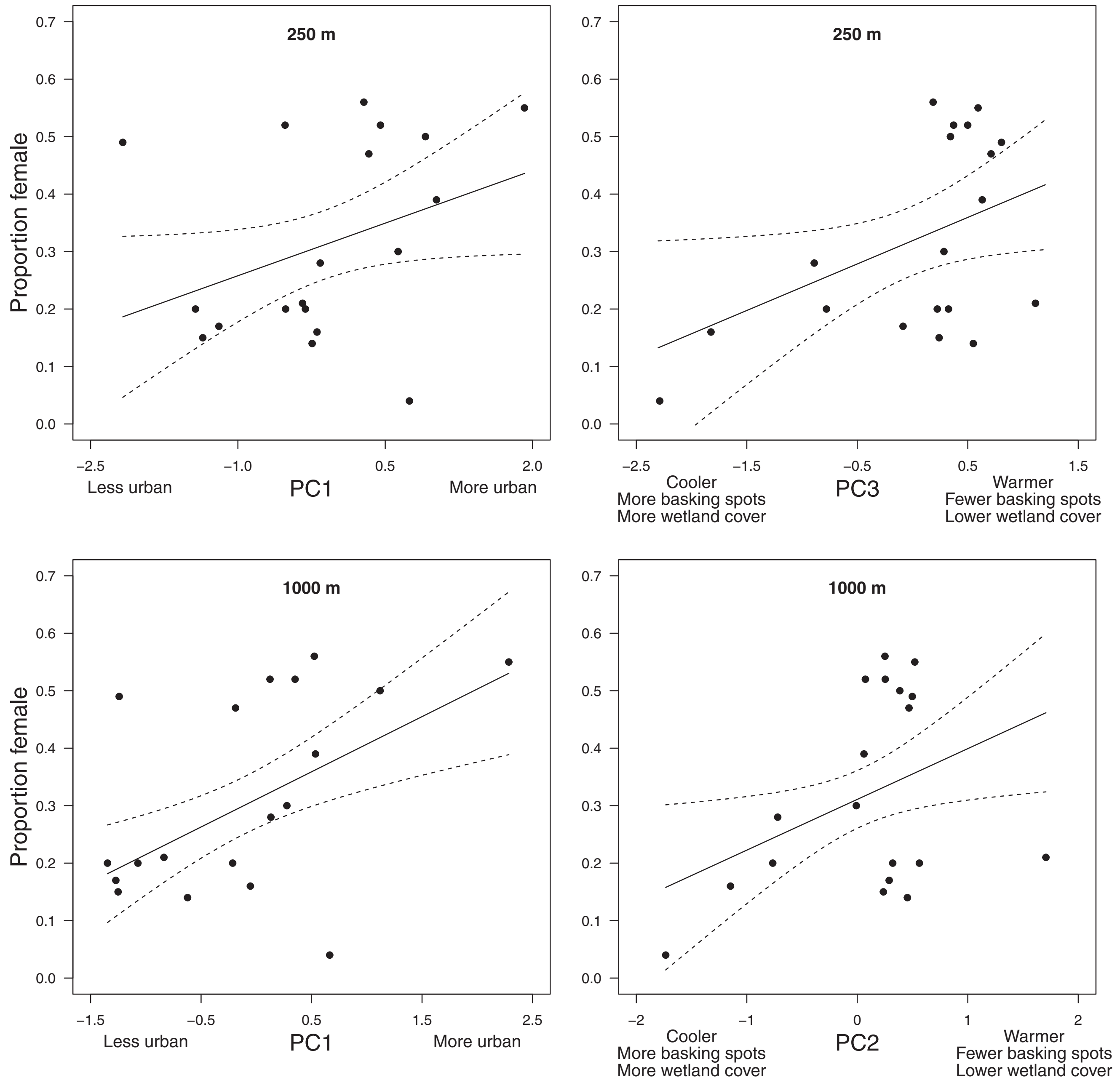

Figure 2. Relationship of proportion adult female painted turtle to principal components representing urbanization and mean annual air temperature, basking sites, and wetland cover at 250 and 1000 m (Table 2). Best-fit lines (solid) and 95% confidence intervals (dotted) were generated from a full model including effects of all principal components. Principal components not of interest were held at their means.

4–99% of the landscape. They would encounter from 4 to 3208 m of total road length. Our sampled portion of the rural–urban gradient was most associated with the rural–suburban through suburban–urban transitions. Urbanization characteristics such as proportion of developed land, night-sky brightness, and surface radiance had the largest impact on adult sex ratio of painted turtles but in an opposite manner to our hypothesis. The proportion of adult females increased with urbanization metrics measured at 250 and 1000 m. Our results contradict others who found either a negative relationship between urbanization and proportion females in painted turtles (Marchand & Litvaitis 2004*b*; Steen & Gibbs 2004; Patrick & Gibbs 2010; Winchell & Gibbs 2016) or no impact (Dorland et al. 2014; Reid & Peery 2014). Although our data are insufficient to analyze urbanization effects on pond sliders, others have found conflicting results for this species; roads either correlate with male-bias (Aresco 2005) or

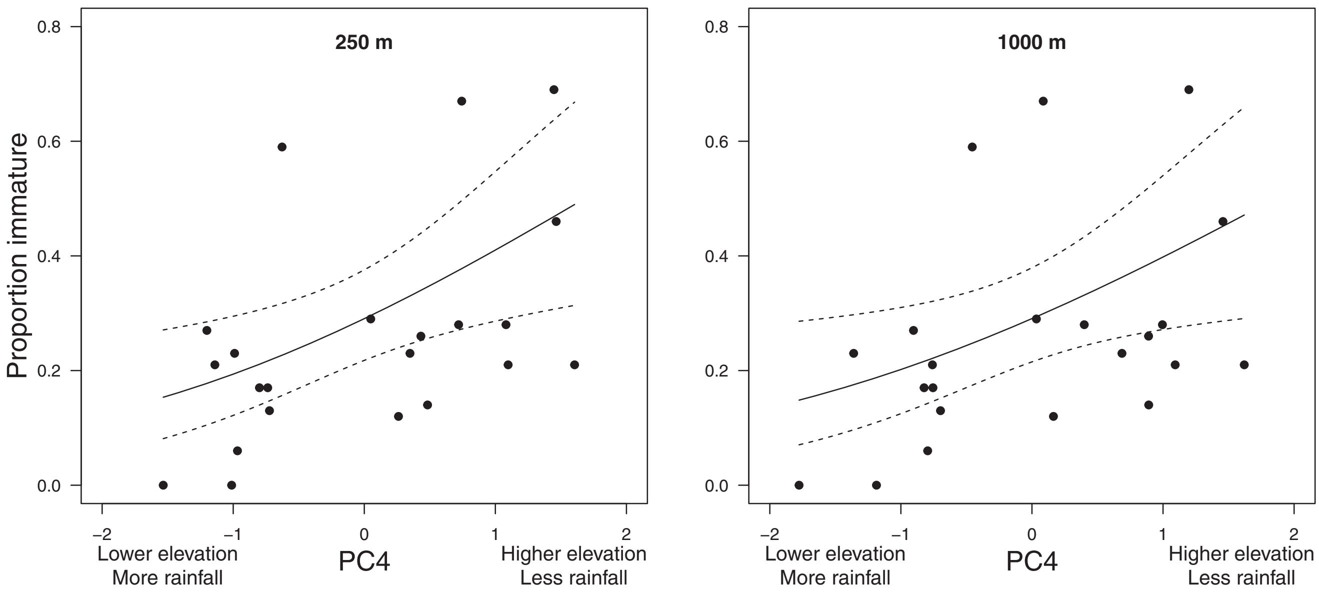

Figure 3. Relationship of proportion immature painted turtle to principal components representing elevation and mean annual rainfall at 250 and 1000 m from sampled ponds (Table 2). Best-fit lines (solid) and 95% confidence intervals (dotted) were generated from a full model including effects of all principal components. Principal components not of interest were held at their means.

have no effect (Mali et al. 2013). Reports on other turtle species have similarly detected no impact of urbanization (Roe et al. 2011; Hamer et al. 2016). Ours is the first to detect a positive relationship between urbanization and proportion of adult female turtles.

Increased mortality of adult females due to vehicular collisions is the primary proposed mechanism for male-biased sex ratios (Steen & Gibbs 2004; Aresco 2005; Gibbs & Steen 2005), yet we found female-biased populations in urban areas. Although roads load positively onto the principal component representing urbanization, we think the detected urbanization effect is unlikely linked to the road network. Other urbanization characteristics, such as higher night-sky brightness and higher surface radiance, are positively related to proportion of adult females, but a causative link between light pollution and population structure is difficult to imagine for painted turtles. Light pollution affects sea turtles by reducing nest number (Brei et al. 2016), increasing predation risk, and disrupting land-to-sea movement behavior (Silva et al. 2017), but to our knowledge, no studies have detected similar relationships between light pollution and freshwater turtles.

The positive relationship between urbanization and the proportion of adult female painted turtles could be linked to temperature-dependent sex determination, by which higher incubation temperatures produce female hatchlings (Ewert et al. 1994). Urbanization reduces forested area and consequently increases more open land uses, such as lawns and agricultural fields. The increase in thermally absorptive impervious surfaces in urbanized areas causes elevated temperatures (Heisler & Brazel 2010). For example, Savva et al. (2010) found higher soil temperatures in urban sites than in rural ones near Baltimore, Maryland (U.S.A.). By increasing urban soil temperatures, these factors could increase the availability of nesting sites that favor the production of females (Freedberg & Bowne 2006). Although turtles may choose nesting sites at the microsite level that offset the overall greater availability of open habitats (Refsnider & Janzen 2012), the thermal soil environment in more urbanized areas may be elevated to a point where lower temperature sites are not available to nesting females. Our finding that higher mean annual air temperature at weather stations close to our sites, as incorporated into principal components, is positively associated with proportion of adult females tentatively supports this argument. Of course, general air temperature and specific incubation temperature are two different things. Additional research on the thermal characteristics of nesting sites along an urbanization gradient is needed to test our hypothesis that more females are produced in more urbanized areas. This hypothesis has the advantage of providing a mechanistic cause (elevated incubation temperatures) for the observed pattern based on known turtle biology.

Another possible explanation for our unexpected outcome regarding urbanization is the wide geographic scope of our project (Fig. 1). We sampled landscapes that varied in urbanization characteristics and were spatially independent of each other. All previous studies on the effects of urbanization on turtle sex ratios examined ponds in one local area. The three previous studies that detected a negative relationship between urbanization and proportion of adult female painted turtles were in either upstate New York (Steen & Gibbs 2004; Patrick & Gibbs 2010) or southern New Hampshire (Marchand & Litvaitis 2004*a*). Recognizing the importance of scale in determining ecological pattern (Levin 1992), their results may be influenced by factors specific to their local area. In our study, six of our sampled ponds were in eastern Massachusetts and all had proportions of adult females from 0.14 to 0.20. We found more equal sex ratios in the Mid-Atlantic states of New Jersey, Pennsylvania, Maryland, and Ohio. Even with these regional differences, our data revealed an overall urbanization effect.

We also found the proportion of adult females was positively related to PC2. Sites with high mean annual air temperature, low wetland cover, and few basking sites had proportionally more adult females. Low wetland cover could increase the proportion of adult females if males need to search large areas to find mates (Morreale et al. 1984; Gibbons et al. 1990) and have low survival while traveling. Higher air temperature should lead to higher surface-water temperature (Piccolroaz 2016). Because basking is performed mostly to match body temperature to environmental temperature (Edwards & Blouin-Demers 2007), higher water temperatures may reduce the need for painted turtles to bask. Carriere et al. (2008) found basking is sex-biased; adult females bask longer than adult males. Adult female painted turtles would thus benefit from not needing to bask. If warmer ponds also had fewer basking spots than cooler ponds, the costs of finding a basking location would increase while the benefit of basking would decrease. Not basking may also have the added benefit of reducing predation risk to terrestrial predators (Ibáñez et al 2015). Having fewer basking sites coupled with reduced need to bask could thus increase the proportion of adult females by reducing predation associated with basking.

The proportions of immature painted turtles were consistently influenced by elevation and annual rainfall at both the 250-m and 1000-m scales. Higher locations with less rainfall had proportionally more immatures. We cannot think of a biologically meaningful mechanism to explain how these variables could affect the age structure of turtle populations. It is more likely these variables are correlated with variables we did not measure. More importantly, the proportions of immatures were not influenced by any variables associated with urbanization. Our finding is in agreement with Hamer et al. (2016), who found no effects of road density on frequency of immature long-necked turtles (*Chelodina longicollis*) throughout Melbourne, Australia. We had expected the proportion of immature turtles to be lower in more highly urbanized areas due to purported higher nest predation (Congdon et al. 1993; Marchand & Litvaitis 2004*b*). It is possible that any increase in nest predation in urbanized areas by generalist predators is not sufficiently large to affect population characteristics relative to less urbanized sites. The proportion of immature turtles in populations of painted turtles is highly variable (e.g., 0.09–0.70 [Ernst 1971]) even in less-urbanized areas. Our results fall within the reported range.

Our findings may be influenced by the limited intensity of our sampling efforts. We sampled each pond with a minimum of 4 hoop traps per night for 2 nights during the late fall and early fall season (8 trap nights), for a total of 16 trap nights per pond. Most ponds were sampled in 2012 and 2013. This effort is similar to those reported in other published work. For example, Steen and Gibbs (2004) sampled each of their ponds for 12 trap nights from May to August 2002. Dorland et al. (2014) employed 6 trap nights per site from June to August 2011 and captured some turtles by hand. We, along with researchers in nearly all published research on urbanization effects on turtle populations, do not have the data to formally estimate population size or sex- and age-specific capture probabilities. This limitation is inherent in the trade-off of infrequent sampling of many ponds across an urbanization gradient versus intense sampling of a few ponds within a similar landscape.

Because common methods for capturing freshwater turtles (e.g., hoop traps, basking traps, dip netting) are all biased in some fashion (Frazer et al. 1990; Gamble 2006; Tesche & Hodges 2015), sampling by multiple methods has been recommended to minimize overall bias in a study (Ream & Ream 1966; Koper & Brooks 1998; Tesche & Hodges 2015). We used only one sampling method (hoop traps) given the large geographic scale and collaborative nature of this project. Our single sampling method focused effort and consistency among all collaborators despite the inherent bias associated with hoop traps. This approach underestimates the abundance of all sex and age classes (Tesche & Hodges 2015) and may be biased against females (Frazer et al. 1990) and juveniles (Tesche & Hodges 2015). Nevertheless, assuming the bias is consistent in space and time, we remain confident in our conclusions regarding the relationship between urbanization and sex ratios. Future studies can include other sampling methods that are amenable to the scale and collaborative nature of this study and provide additional skills development for undergraduate researchers.

The detection of spatial differences in the effects of urbanization on turtle populations illustrates a key advantage of the EREN approach to this research question and provides an example that could be easily reproduced using different research questions and locations. All EREN projects test ecological questions that have the potential to be influenced by the local context in which they occur. The EREN's scientific goals include revealing ecological patterns that cannot be detected by conducting the study at a single site and doing so in a cost-effective way by engaging local scientists and undergraduate students as researchers across a range of sites (Bowne et al. 2011; Simmons et al. 2016). Over 1000 students across 24 institutions in 13 states experienced authentic scientific discovery over the course of this project (Supporting Information). The larger spatial extent of this study provides evidence that the effects of urbanization on painted turtles vary across the country and that we cannot make the broad generalization that urbanization has a consistent negative effect on female turtles. More work at multiple sites needs to be conducted to understand the mechanisms underlying this pattern and whether regional effects exist. Networks such as EREN can serve a central role in this effort.

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Supporting Information

Information on faculty and student participation in TurtlePop (Appendix S1), characteristics of individual ponds (Appendix S2), and longer-term trapping records (Appendix S3) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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