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Assessing the Impacts of Urbanization on Sex Ratios of Painted Turtles (*Chrysemys picta*)

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Abstract: Turtles are particularly susceptible to the negative impacts of urbanization due to low mobility and a life history strategy emphasizing long generation times and high adult survival. In addition to declines directly through habitat loss, urbanization has been hypothesized to limit populations of aquatic turtles through changes in population structure, as adult females are disproportionally killed on and near roads, leading to male-biased populations, which can lead to population declines or local extirpations. The purpose of this study was to better understand how urbanization impacts the sex ratios of painted turtles (*Chrysemys picta*) in an urban ecosystem, as empirical results linking male-biased turtle populations to roads and urbanization are mixed. Using eight years of trapping data from a long-term monitoring program in a suburb of Chicago, IL, USA, we report one of the most male-biased populations ($\bar{x}$ = 75% male) of turtles in the USA, consistent with prevailing road mortality hypotheses. However, we found no evidence that male-biased populations were related to road density or the amount of protected area around a sampling location and found that impervious surface (a metric of urbanization) was weakly related to less male-biased populations. Our results highlight the importance of replicating ecological studies across space and time and the difficulty in assessing population structure in aquatic turtles. We suggest that active conservation measures may be warranted for the continued persistence of urban turtle populations.

Keywords: Chicago; chelonian; conservation; urban ecology; Illinois; impervious surface; preserve; protected area; road density

1. Introduction

Urban ecosystems are notable for their prevalence of anthropogenic features, such as roads, buildings, and other impervious surfaces that influence both biotic and abiotic processes [1]. For example, urban areas are often warmer than the surrounding landscape through changes in reflectance, radiance, and evapotranspiration [2,3]. In addition, the proliferation of roads and other anthropogenic structures causes direct habitat loss, habitat fragmentation, and changes in wildlife communities and behavior [4–7]. Unsurprisingly, urbanization can be a leading cause of species endangerment in some regions. For example, as of 2000, at least 275 species in the USA were listed as threatened or endangered due to urbanization [8].

Turtles are particularly susceptible to the negative impacts of urbanization (primarily via additive mortality) due to low mobility and a life history strategy emphasizing long generation times and high adult survival [9–11]. For example, as little as 10% additional mortality in adult snapping turtles (*Chelydra serpentina*) can lead to a 50% population decline over 15 years [12]. Urbanization can directly reduce or eliminate populations of turtles via habitat loss, fragmentation, and degradation [10,13], road mortality [11,14–16], and increased depredation rates by subsidized predators [17–20]. Understanding how turtles respond to urbanization is imperative in a world with a rapidly increasing urban footprint [4,21] and rapidly shrinking turtle populations [22,23].

Urbanization has been hypothesized to further limit populations of aquatic turtles through changes in population structure, as adult females (relative to males and juveniles) are disproportionally killed on and near roads [24–28], and Gibbs and Steen [27] show that turtle populations in the US have become more male-biased over time, coinciding with the proliferation of roads. This problem is particularly insidious as turtle populations can become functionally extinct well before the last turtle in a population actually dies [22,29]. However, empirical results of urbanization impacting the sex ratios of turtle populations are mixed and limited to two species with a limited geographic scope [25,30–32], and a series of recent papers has failed to find an association between roads and male-biased populations [33–36]. Notably, Bowne et al. [36] found the *opposite* relationship, and hypothesized that that urbanization could be resulting in warmer nesting locations, leading to more females being produced via environmental sex determination [37] (but see Lambert and Steen [38]).

In this study, we attempt to better understand how urbanization impacts the sex ratios of painted turtles (*Chrysemys picta*) in an urban ecosystem (a suburb of Chicago, IL, USA). We test the hypotheses that (1) road density is linked with an increased proportion of males in an urban environment, (2) that impervious surface (a metric of urbanization that might be associated with terrestrial threats, such as increased mesocarnivore abundance) is associated with an increased proportion of males, and (3) that amount of protected area is associated with a *decrease* in the proportion of males (under the assumption that protected areas are protecting turtle habitat and therefore minimizing the negative effects of urbanization on turtle populations). To the best of our knowledge, this is the first study to examine the association of urbanization on the sex ratios of turtles in an intensely urban environment in the United States. This work addresses research needs in turtle biology [39], regional conservation objectives [40], and the call by Belovsky et al. [41] to improve the field of ecology through increased replication of ecological studies across time and space.

2. Materials and Methods

2.1. Study Organism

The painted turtle (Figure 1) is a small (maximum straight-line carapace length (SCL) = 25.4 cm) pond turtle (Emydidae) inhabiting much of the United States and southern Canada. Painted turtles are omnivorous habitat generalists, occupying a wide variety of freshwater environments. Courtship and mating occur in the spring and late summer/early fall, and nesting occurs in late spring/early summer, with females leaving the water to lay eggs in open areas. The incubation period is typically 70 days, and hatchlings in more northern climates are freeze tolerant and may overwinter in the nest chamber. Painted turtles lack sex chromosomes and exhibit temperature sex determination; females are produced at higher incubation temperatures and males at lower incubation temperatures. This species was reviewed by Ernst and Lovich [42].

2.2. Study Location

Our field work took place in Lake County, IL (land area = 1,150 km^2), a suburb of Chicago, IL. Lake County is one of the most densely populated counties in the United States (607 persons/km^2, 97th percentile) [43], and the Chicago Metropolitan Area is the third largest metro area in the USA with nearly 10,000,000 inhabitants (Figure 2). Lake County has a temperate climate; the mean daily temperature in Lake County (1981–2010) ranged from a mean January low of 9.4 °C to a mean July high of 24.4 °C. Annual average precipitation during this time period was 93.0 cm [44]. Prior to European settlement, Lake County was 65% forest/savanna and 30% prairie [45], but today Lake County is dominated by ~75% urban landcover (Figure 1). Within this developed matrix, the Lake County Forest Preserve District manages 120 km^2 of preserves for natural resource conservation and passive recreation. Within these preserves, non-native plant communities (cropland, cool season grasses, and invasive shrubs) were the most common community type (37%), followed by forest (26%), wetland (18%), prairie (7%), and savanna (5%) (J. P. Vanek, unpublished data).

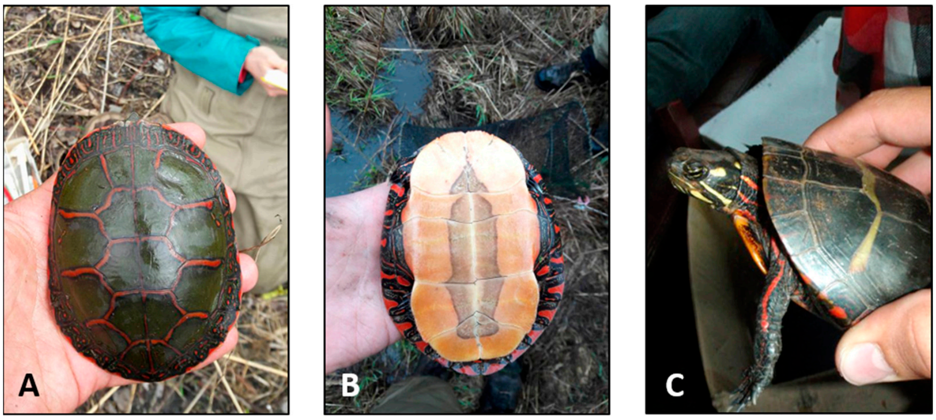

Figure 1. The painted turtle (*Chrysemys picta*). (**A**) Dorsal view displaying the carapace (top shell) of a painted turtle from Lake County, IL, USA. (**B**) Ventral view displaying the plastron (bottom shell) of a painted turtle from Lake County, IL, USA. (**C**) Lateral view of a painted turtle from Dare County, NC, USA. Photos by John P. Vanek.

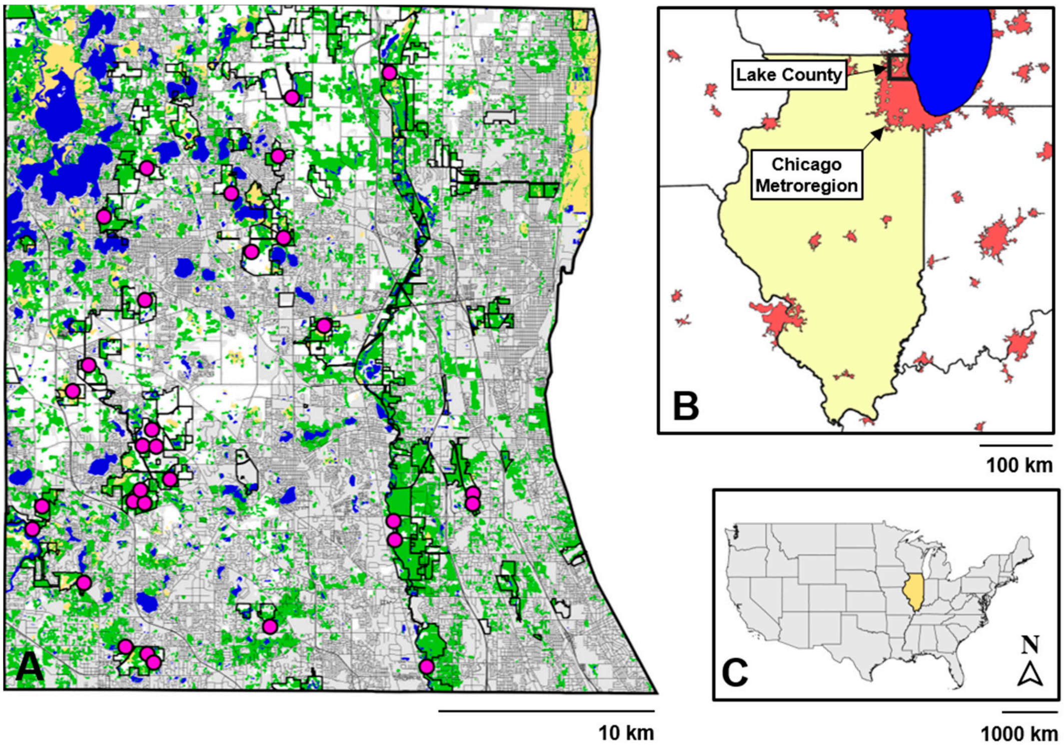

Figure 2. Our study location, Lake County, is a highly urbanized suburb of Chicago, IL, USA. (**A**) Locations of turtle sampling included in the 1000-m scale analyses (magenta circles, n = 31), along with Lake County Forest Preserve District boundaries (black lines), roads (dark grey lines) and major landcover classes pooled from the from the 2011 National Landcover Database (light grey = developed, green = forest, beige = freshwater emergent vegetation, blue = open water, white = other, but mostly row crop agriculture) [46]. Specific capture locations are not listed due to conservation concerns. (**B**) Location of Lake County within Illinois (beige) and in relation to the Chicago metro region (labeled) and other major US Census designated urban areas (maroon). (**C**) Location of Illinois (yellow) within the United States.

2.3. Turtle Sampling

We collected data on turtles from 2009–2018 as part of an ongoing, multi-taxa wildlife monitoring program. In 2009, the Lake County Forest Preserve District (LCFPD) established permanent monitoring plots (n = 235) within 55 preserves. Plots were distributed randomly and spaced at least 400 m apart using a geographic information system (GIS). Additional ad hoc plots (n = 67) were created to sample wetlands missed by random sampling. Preserves were assigned to be sampled either every other year (n = 26) or every four years (n = 29) based on a priori habitat quality and restoration goals (G. A. Glowacki and T. S. Preuss, Lake County Forest Preserve District, unpublished report). All plots within each preserve were sampled on a rotating schedule starting in 2009 as part of the larger monitoring program.

We used hoop nets to sample turtles at all wetlands within 100 m of each monitoring plot (n = 228 plots) and at the ad hoc wetland plots. Hoop nets were baited with canned sardines, which were replaced as needed. Wetlands were sampled for four consecutive days in the spring (April through May) and four consecutive days in the summer (mid-late July through August). We used small hoop nets (throat diameter = 13 cm, stretchable to 20 cm; hoop diameter = 30 cm; mesh size = 1 cm) and large hoop nets (throat diameter >30 cm; hoop diameter >40 cm; mesh size >2.5 cm) to maximize the number of species and size classes captured, as well as to sample wetlands of a variety of depths. Hoop nets typically catch more males than females [47–50], but we assumed this bias was constant across wetlands, so any changes in sex ratio would be apparent by comparing the proportion of males captured [25].

Sampling effort at each plot and sampling period varied but typically consisted of at least one large and one small hoop net for wetlands <5 ha and two large and two small hoop nets for wetlands >5 ha in size. We totaled 11,924 trap nights from 2009–2017 and annual trap nights ranged from 504 in 2013 to 1432 in 2011. The mean number of trap nights per year (excluding 2013) was 1292 (SD = 86.4). Actual trap effort was slightly lower because traps were occasionally stolen, damaged, or lost. We notched the marginal scutes of each captured turtle for individual identification (modified from the protocol described by Cagle [51]), measured the curved carapace length (CCL), and noted the sex as unknown, juvenile, male, or female based on size and secondary sex characteristics [42]. We considered females with CCL >150 mm and males with CCL >95 mm to be adults based on reported sizes at maturity corresponding to PLs of 130 and 81, and respectively [52,53]. We assigned turtles >170 CCL and initially marked as unknown to female based on the maximum size reported for male painted turtles (SCL = 153 mm) [42]. The relationship between PL and CCL (r = 0.99) and SCL and CCL (r = 0.99) was determined by examining *C. picta* specimens (n = 102) maintained by the Chicago Academy of Sciences and Northern Illinois University (Supplementary Materials). We assumed that turtles grew and matured at similar rates across our sampling locations, although Gibbons [52] noted slight changes in growth rates and size at maturity across habitat types.

2.4. Landscape Characteristics

We investigated the impacts of urbanization at two spatial scales: 254 m and 1000 m. The 254-m buffer represented the distance thought to include 95% of nesting forays for painted turtles (154 m; Steen et al. [54]) plus the 100 m sampling buffer built into the monitoring program. The 1000-m buffer represented the broader landscape context of each plot. We assessed both spatial scales to test if the relationship between urbanization and sex ratio was scale dependent and to facilitate comparisons to other studies of urbanization and turtle sex ratios. We used QGIS 3.2 [55] and ArcGIS 10.6 [56] for all geospatial analyses.

For each plot and spatial scale, we calculated road density (km/km^2), impervious surface (ha/km^2), and protected area (%) (Figure 3). We calculated road density around each plot (sum road length within buffer / buffer area) using polyline vector data of paved roads provided by the Illinois Department of Transportation. We calculated the area of impervious surface around each plot (total impervious surface area within buffer / buffer area) using high resolution (1 m) raster data and pooling the road, building, and other paved surfaces categories [57]. We calculated the protected area around each plot (total preserve area within buffer/buffer area) using polygon vector data provided by the LCFPD. In cases where ad hoc wetland plots were located within 300 m of random plots and/or hydrologically connected (n = 5), we calculated 254-m landscape characteristics from the midpoint between plots. In cases when ad hoc and random plots appeared to be hydrologically connected and were within 500 m, we calculated 1000-m landscape characteristics from the midpoint between plots (Table 2).

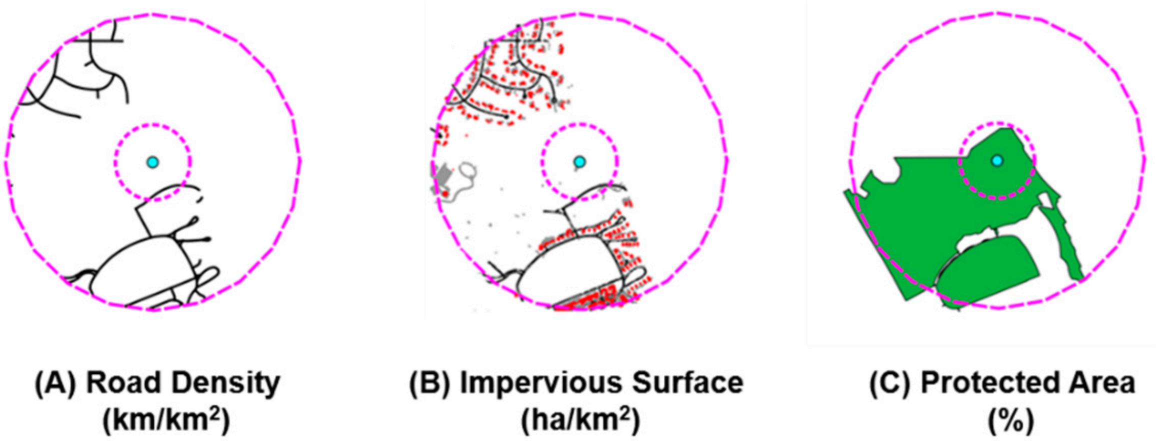

Figure 3. Buffers and landscape variables used in this study. The centroid represents the random monitoring location or ad hoc wetland sampling location, the inner dashed line represents the 254-m buffer, and the outer dashed line represents the 1000-m buffer. (**A**) Example of road polylines used to calculate road density (km/km^2). (**B**) Example of impervious surface raster used to calculate impervious surface (ha/km^2). (**C**) Example of preserve polygons used to calculate protected area (%).

2.5. Data Analysis

All statistics were conducted in the R Statistical Computing Environment version 4.3.4 [58]. We tested for effects of sampling period and hoop net size using a Chi-square test of independence [59]. We tabulated the number of males and females for each plot by pooling the total number of unique adult turtles of each sex from 2010–2018 (Table 2) and used the glm function in R to fit general linear models (logistic regression with binomial errors and a logit-link) [59] to separately test for effects of road density, impervious surface, and protected area on the proportion of males at both spatial scales (254 m and 1000 m). We used the proportion of males (analyzed as binary data with 1 for males and 0 for females) rather than a sex ratio due to the statistically problematic nature of ratios [27,60] and thus did not use an arcsine transformation [59]. We excluded data from 2009 due to >40% of captures without recorded sex data and excluded plots with <20 adult turtles of known sex based on an a priori decision (previous studies have used various sample size requirements of zero [33,34], five [24,30,36], or 10 [25] sexable turtles). Therefore, we wanted to increase our standard of evidence [48], as confidence intervals associated with binary data are sensitive to small sample sizes, and we have captured as many as 13 painted turtles in a single small hoop net (J. P. Vanek personal observation). We also excluded data from plots within one disjunct preserve subject to intensive management specifically designed to benefit turtles [61] to avoid biasing results.

3. Results

3.1. Turtle Sampling

We captured 2685 painted turtles (1537 males, 485 females, 574 juveniles, and 89 unknown) from 2010–2018, excluding recaptures (n = 186) and turtles captured in the preserve extensively managed for turtle conservation (n = 489) (see Section 2.5 in Methods). Males averaged 134 mm CCL (median = 134 mm) ± 17 SD, females averaged 174 mm CCL (median = 174 mm) ± 13 SD, and juvenile CCL was bimodal with a mean of 109 mm CCL (median = 110 CCL) ± 27 SD (Figure 4). The sex ratio of captured turtles was more male-biased in the spring (3.6:1) than in the summer (2.8:1) (χ^2 = 5.62, df = 1, p = 0.02), and we captured relatively more males than females in small hoop nets (3.5:1) relative to large hoop nets (2.6:1) (χ^2 = 6.33, df = 1, p = 0.01).

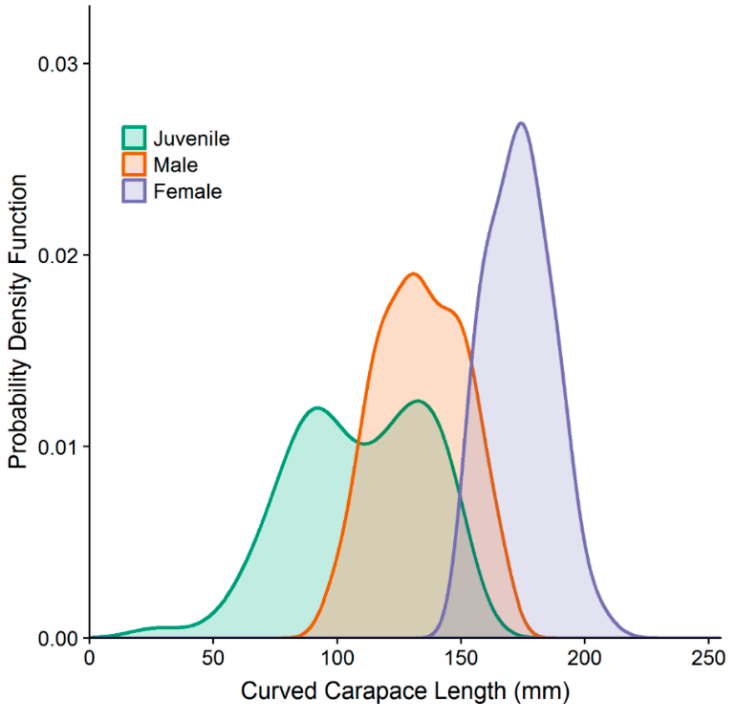

Figure 4. Size distribution of 2596 painted turtles (*Chrysemys picta*) (1537 males, 485 females, 574 juveniles) captured in suburban preserves from 2010–2018 in Lake County, IL. Probability density function calculated using the default smoothing bandwidth and a multiplicative bandwidth adjustment of 1.5 in the "ggplot2" version 3.1.0 R package.

3.2. Landscape Characteristics

We found relatively little correlation ($\bar{x}$ = |0.41| ± 0.14 SD) between landscape variables, which ranged from 0.05 between road density at the 254-m scale and impervious surface at the 1000-m scale to 0.64 between protected area at the 254-m and 1000-m scales and −0.64 between protected area and impervious surface at the 254-m scale (Table 1).

Table 1. Correlation coefficients for each of the six metrics of urbanization used in this study. RD = Road Density (km^2/km), IS = Impervious Surface (ha/km^2), PA = Protected Area (%).

	RD (254 m)	RD (1000 m)	IS (254 m)	IS (1000 m)	PA (254 m)	PA (1000 m)
RD (254 m)		0.52	0.44	0.05	−0.36	−0.37
RD (1000 m)	0.52		0.32	0.37	−0.27	−0.50
IS (254 m)	0.44	0.32		0.57	−0.64	−0.40
IS (1000 m)	0.05	0.37	0.57		−0.33	−0.45
PA (254 m)	−0.36	−0.27	−0.64	−0.33		0.56
PA (1000 m)	−0.37	−0.50	−0.40	−0.45	0.56	

Road density within the 254-m buffer ranged from 0.00 km/km^2 to 4.75 km/km^2 (mean = 0.90, median = 0.27, SD = 1.19) and within the 1000-m buffer ranged from 1.01 km/km^2 to 6.79 km/km^2 (mean = 3.58, median = 3.01, SD = 1.97) (Table 2). Impervious surface within the 254-m buffer ranged from 0.00 ha/km^2 to 13.39 ha/km^2 (mean = 2.05, median = 0.90, SD = 2.71) and within the 1000-m buffer ranged from 1.43 ha/km^2 to 24.35 ha/km^2 (mean = 7.82, median = 5.46, SD = 5.67) (Table 2).

Protected area within the 254-m buffer ranged from 3.34% to 100.0% (mean = 81.5%, median = 84.7%, SD = 33.2%) and within the 1000-m buffer ranged from 21.3% to 86.9% (mean = 45.8%, median = 44.6, SD = 21.3) (Table 2).

Table 2. Demographics of captured painted turtles (*Chrysemys picta*) and landscape characteristics for each capture location. Coordinates for specific capture locations are not listed due to conservation concerns. PM = Proportion Male, RD = Road Density (km^2/km), IS = Impervious Surface (ha/km^2), PA = Protected Area (%).

Plot ID	Scale (m)	# Male	# Female	# Adult	PM	RD (254 m)	RD (1000 m)	IS (254 m)	IS (1000 m)	PA (254 m)	PA (1000 m)
CUB-F-106	254	58	24	82	0.71	0.00	NA	0.00	NA	100.00	NA
CUB-G-201	254	17	11	28	0.61	2.41	NA	2.79	NA	94.20	NA
LAK-P-202	254	18	8	26	0.69	0.00	NA	0.69	NA	100.00	NA
LAK-U-207	254	15	7	22	0.68	1.25	NA	2.59	NA	3.34	NA
CUB-GF-61	1000	75	35	110	0.68	NA	4.91	NA	6.19	NA	63.38
GRT-IF-37	1000	23	9	32	0.72	NA	4.75	NA	10.50	NA	45.39
LAK-P-726	1000	44	11	55	0.80	NA	2.36	NA	5.15	NA	57.41
LAK-UW-57	1000	31	9	40	0.78	NA	3.66	NA	3.00	NA	86.89
ROL-CD-123	1000	47	13	60	0.78	NA	2.28	NA	5.46	NA	49.55
ALM-B-201	Both	57	5	62	0.92	0.00	2.66	1.02	5.26	100.00	45.73
CAH-A-201	Both	24	12	36	0.67	1.16	6.79	4.94	11.07	57.55	23.86
CUB-B-101	Both	14	8	22	0.64	3.41	3.80	3.30	5.17	66.31	37.52
CUB-H-107	Both	33	17	50	0.66	2.00	4.69	5.15	5.98	67.88	51.94
DUC-C-201	Both	41	8	49	0.84	4.75	6.62	5.51	8.04	67.79	35.36
ETH-E-104	Both	13	8	21	0.62	0.27	1.01	0.25	1.43	76.36	45.30
FOX-A-101	Both	18	3	21	0.86	0.00	2.34	0.03	3.73	100.00	41.11
FOX-E-102	Both	95	20	115	0.83	0.00	3.10	0.04	5.23	42.22	23.97
GRS-D-102	Both	27	6	33	0.82	1.68	3.43	2.05	5.08	81.98	21.28
GRT-IF-203	Both	23	9	32	0.72	0.50	4.75	1.37	10.50	90.00	44.90
HER-E-102	Both	15	9	24	0.63	1.73	2.70	3.64	4.39	71.77	23.11
LAK-A-103	Both	35	9	44	0.80	0.00	1.85	0.00	2.37	100.00	65.83
LAK-F-105	Both	22	5	27	0.81	0.00	1.44	0.02	1.89	98.06	69.60
LAK-F-106	Both	43	7	50	0.86	0.00	3.04	0.63	4.51	79.31	74.27
LAK-L-112	Both	15	5	20	0.75	2.26	6.35	3.48	15.86	81.93	28.56
LAK-T-121	Both	192	52	244	0.79	0.00	2.51	0.00	4.90	100.00	68.28
LAK-U-203	Both	20	3	23	0.87	0.00	2.67	0.38	4.72	100.00	61.76
MCD-DE-1_2	Both	24	6	30	0.80	0.28	5.29	0.90	12.94	95.37	38.16
MID-B-103	Both	51	21	72	0.71	1.48	6.73	3.36	13.23	78.40	23.15
MID-C-104	Both	20	4	24	0.83	0.32	6.72	0.37	10.09	87.24	32.93
NIP-B-101	Both	21	10	31	0.68	0.00	3.01	0.00	9.20	97.96	38.46
ROL-FG-67	Both	63	14	77	0.82	0.00	2.47	0.00	6.23	100.00	70.57
SIN-B-103	Both	32	10	42	0.76	2.56	4.47	5.39	12.18	83.96	37.68
SIN-I-102	Both	44	10	54	0.81	0.00	2.47	0.00	5.05	91.95	43.56
WAD-B-102	Both	17	3	20	0.85	1.86	2.17	2.03	7.55	84.69	50.08
WRI-B-101	Both	23	13	36	0.64	0.00	2.10	0.15	21.77	81.14	35.67
WRI-B-102	Both	27	16	43	0.63	0.00	2.54	13.39	24.35	47.21	31.04

3.3. Sex Ratios and Urbanization

After excluding plots with <20 unique captures of sexable adults, we were left with 27 plots analyzed at both buffer distances, four analyzed at the 254-m buffer only, and five at the landscape scale only (Table 2). The mean proportion male across all sites was 0.75 ± 0.09 SD and ranged from 0.61 to 0.92 (Table 2). We found no relationship between the proportion of male turtles and road density (254 m buffer, $p = 0.13$; 1000 m buffer, $p = 0.07$) (Figure 5). The proportion of males decreased slightly with increasing impervious surface at both buffer distances (254 m buffer: $\beta = -0.0648$, $p = 0.002$; 1000 m buffer: $\beta = -0.0387$, $p = 0.001$). We found no relationship between the proportion male and protected area at either scale (254 m buffer, $p = 0.09$; 1000 m buffer, $p = 0.15$) (Figure 4).

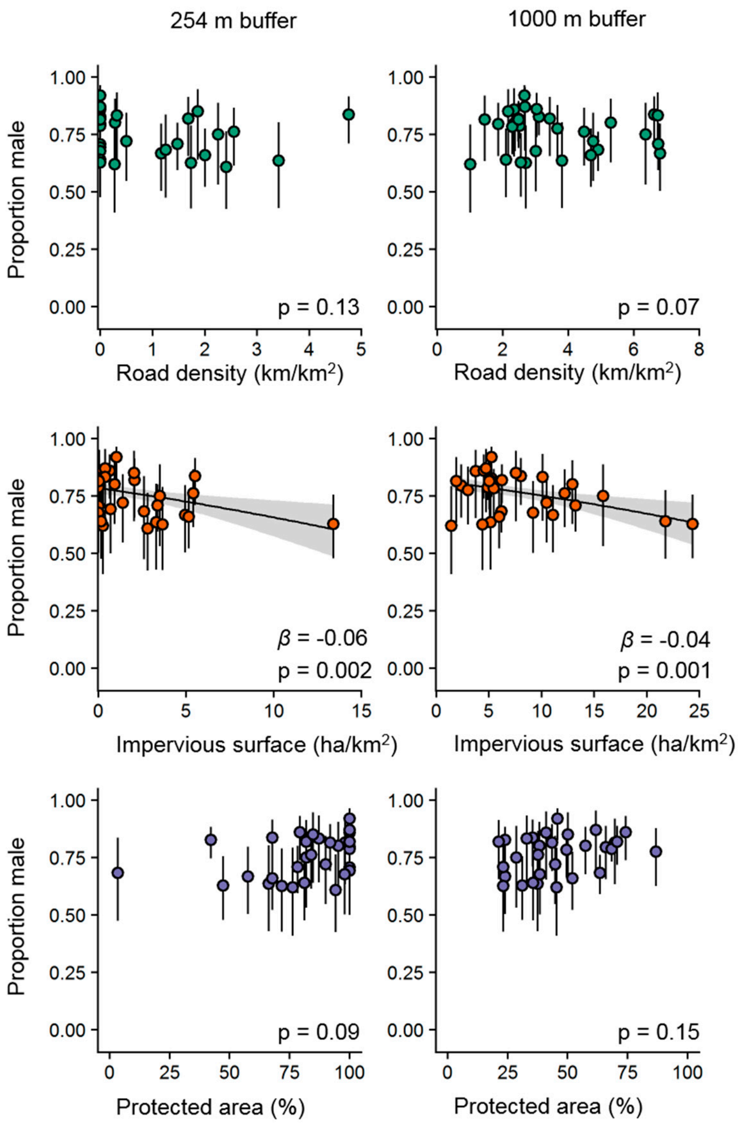

Figure 5. Relationship between the proportion of male painted turtles (*Chrysemys picta*) within a sample and landscape covariates at two spatial scales. Each point represents a sampling location with ≥20 sexable adult turtles and associated binomial confidence intervals. Grey ribbons represent 95% confidence intervals for statistically significant relationships (alpha = 0.05).

4. Discussion

Our results do not support the prevailing hypothesis linking male-biased sex ratios to roads, corroborating more recent studies [33–36]. However, although we were unable to directly link roads to male-biased populations, our average sex ratio of 75% male (of captured adults) is one of the highest reported for turtles in the United States and is higher than all but five of 165 sex ratios collected from 1920 and 2010 as reported by Gibbs and Steen [27]. In addition, our results are similar to the high road density sex-ratio (74% male) reported by Steen and Gibbs [25], which they defined as >1.5 km/km^2 within a 1000 m buffer. By this definition, all but two of our sample sites would be considered high road density (Table 2), despite our random sampling approach. Therefore, while our male-biased populations are consistent with prevailing road mortality hypotheses and might be a result of high road densities, the lack of low road density sites may have prevented us from detecting a trend (but see Reid and Peery [34]). Alternatively, high rates of female depredation by mesocarnivores may be driving male-biased sex ratios in Lake County, as female turtles are at greater risk to not only roads, but terrestrial predators as they leave the relative safety of the water to nest [24,28]). Mesocarnivores (such as raccoons (*Procyon lotor*) reach extremely high densities in urban areas [62], including LCFPD preserves [61], and raccoons were detected at nearly every sampling location used in this study (J. P. Vanek, A. U. Rutter, T. S. Preuss, G. A. Glowacki, unpublished data). Future work should attempt to identify the critical road density threshold associated with detrimental effects to painted turtles, as well as the direct depredation risk of predators on nesting females.

In contrast to our road density results, we did find a relationship between impervious surface and the proportion of males at both spatial scales (Figure 4), although in the opposite direction as hypothesized. This is somewhat consistent with the findings of Bowne et al. [36] who found a negative relationship between a multidimensional index of urbanization and the proportion of male painted turtles. However, though the relationship we identified is statistically significant, we question the biological significance. For example, at the 254-m scale, the proportion of males at sites with no impervious surface (0.79, 95% CI = 0.76–0.81) is very similar to the proportion of males at 5.4 ha/km^2 impervious surface (0.72, 95% CI = 0.68–0.76). Still, at the most extreme values of impervious surface (13.3 ha/km^2 at the 254-m scale and 24.35 ha/km^2 at the 1000-m scale), the proportion of males is noticeably lower (but with wide confidence intervals), at 0.61 (95% CI = 0.48–0.72) and 0.63 (95% CI = 0.54–0.73), respectfully. Urbanization might only begin to impact the proportion of males at very high levels, which would be consistent with the heat island hypothesis, as Bowne et al. [36] suggested to explain their results. However, when Lambert and Steen [38] re-analyzed data in Bowne et al. [35] using more biologically relevant urban covariates, the relationship between urbanization and sex ratio was no longer apparent. Clearly, more empirical work is needed to determine if turtle nests associated with urbanization are indeed warmer (but see recent work by Francis et al. [63]).

We found no relationship between the amount of protected area and the proportion of males at either spatial scale (Figure 4). This is consistent with the results of Reid and Peery [34] who found no evidence linking the proportion of male painted turtles and area of public land within 1000 m of sampled wetlands. If sex ratios in Lake County are indeed male-biased and not simply a result of sampling bias, existing protected areas may not be large enough prevent demographic collapse and more active conservation measures (such as fencing or wildlife underpasses, e.g., Aresco [15], or mesocarnivore removal, e.g., Urbanek et al. [61]) may be warranted [64]. However, we should note that successful reproduction is still occurring in LCFPD preserves, as indicated by juvenile captures (Figure 4) and observations of hatchlings (J. P. Vanek and G. A. Glowacki, unpublished data), although it is unclear if this is enough to maintain viable populations. Still, if painted turtles are not adequately protected, species that nest further from wetland edges, such as snapping turtles and Blanding's turtles (*Emydoidea blandingi*) [54], may be at even greater risk, as predicted by Gibbs and Shriver [11]. Painted turtles, which are easier to sample, may therefore serve as indicator species in urban areas.

The proportion of male painted turtles we captured varied by both trapping season and trap size. We captured more males in the spring than later summer, and more males in smaller hoop nets. This was not unexpected as differential capture rates by trap type and season are well documented [47,49,50,65–68]. We acknowledge that seasonal and trap differences in sex-specific capture rates could be obscuring any impacts of urbanization on sex-ratio, but we do not think these differences were large enough to do so, given our extreme male skew. It is hypothesized that the reason hoop net captures are male-biased is because in addition to being attracted to the bait, males are also attracted to any females in the trap [47,49]. However, we did not expect to catch a larger proportion of males to females in smaller-sized hoop nets. We do not think our smaller hoop nets were excluding females, as the throat diameter was sufficiently wide to accommodate even the largest females. Rather, male painted turtles may have been more likely to encounter the small hoop nets, as our smaller traps could be placed in shallower water, and male painted turtles (which are smaller than females; Figure 4) may use shallower microhabitats more often than females (as smaller red-eared sliders (*Trachemys scripta elegans*) use shallower wetlands relative to larger red-eared sliders; [69]).

Although painted turtles are one of the most studied species of turtles [42], shockingly little is known about their habitat selection and movement patterns (but see Rowe and Dalgarn [70] and Jaeger and Cobb [71] for examples). We recommend more studies investigating the spatial ecology of painted turtles, particularly those incorporating both sexes. Further, future studies need to critically examine the relationship between sampled sex ratios using traps and actual sex ratios [68]. This could be done using experimental ponds stocked with turtles of known sex ratios, or by sampling ponds with natural populations of turtles followed by more exhaustive sampling or censusing (seines, removal sampling, etc.). We also urge authors to report more details about their trapping methodology, including thorough descriptions of trap designs, (e.g., specifying throat diameters), as well as the specific microhabitats associated with trap placement.

In conclusion, we report extremely male-biased sex ratios in painted turtles living in an urban ecosystem, consistent with prevailing hypotheses. However, we found no evidence linking these biases to road density, as has been reported elsewhere, and found no evidence that sex ratios varied by the amount of protected area around a sampling location. In addition, we found a weak link between impervious surface (a measure of urbanization) and a less male-biased sex ratio, supporting the heat island hypothesis, although we question the biological significance of this relationship. We found little correlation between road density, impervious surfaces, and protected areas, and caution urban ecologists to carefully consider which metrics of urbanization are used in analyses. As stated by Gibbons [48], "aberrant sex ratios in turtles should not be accepted lightly." To this extent, our study incorporates over 8 years of field work, a large sample size of over 2000 turtles from 36 trap locations, random sampling, multiple trap types (as recommended by Tesche and Hodges [68]), and trapping over multiple seasons in a year. Therefore, if our sampled populations are indeed extremely male-biased, even in protected areas and despite possible heat island effects, active conservation measures (e.g., fencing, wildlife underpasses, and mesocarnivore removal) may be warranted for the continued persistence of urban turtle populations.

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