Effect of roads on painted turtle (*Chrysemys picta*) sex ratios

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Abstract

Urbanization is a major threat to wildlife and roads play an important part in the demise turtles are facing. During the nesting period, female turtles are often found near roads and are more prone to vehicle strikes than males. I tested the hypothesis that due to higher risk of road mortality of females, there will be more males than females in painted turtle populations closer to roads. I predict that turtle populations surrounded by more roads will be more male biased. I collected data from 12 populations of painted turtles in Ottawa and determined the sex ratio of each population. For each site, I created buffers from 100 m to 4000 m with a 100 m increment. I classified the land cover of each buffer using 6 categories and identified scale of maximum effect for each category. I used a multiple linear regression to conduct the analysis to determine the effect of the environmental variables on turtle sex ratios. Roads and other environmental variables did not affect turtle sex ratios. Future studies, done on a larger scale, could provide more knowledge on the effects of roads as other factors could affect sex ratios.

Keywords: Urbanization, Wetlands, Reptile, Roadkill, Nesting

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Introduction

As the human population keeps increasing, urbanization is expanding and increasingly threatens wildlife. Only 5% of the terrestrial area on Earth remains unmodified by human activities (Kennedy et al., 2019). Since the beginning of the 20th century, the human footprint alters ecosystems directly, especially through land cover changes (Leu, Hanser, & Knick, 2008). Earth biodiversity is now experiencing its 6th mass extinction where 32% of 27,600 vertebrate species experienced decreases in population size and range with human activities (Ceballos et al., 2017). Modification and destruction of natural habitat are the main drivers of biodiversity loss and are associated in part with the expansion of transportation infrastructure (Haddad et al., 2015). In fact, because of roads, more than half of the remaining habitat patches are less than 1 km^2 and only 7% are more than 100 km^2 (Schwartz, Shilling, & Perkins, 2020).

Roads are a major threat to wildlife populations because they fragment the habitat (Beaudry et al., 2008). This modification to land impacts biodiversity and ecological processes at many scales (Mcgarigal & Cushman, 2002). Fragmentation reduces areas of suitable habitat and creates isolated fragments bordered by human settlements, agricultural lands, and anthropogenic infrastructures (Marzluff & Ewing, 2001). Therefore, species abundance and density decrease with the size of the fragment. Because of edge effects, wildlife is also more exposed to threats such as predation, invasion, or human presence, leading them to avoid roads. Fragmentation restrains species in their movements since it creates obstacles between patches (Debinski & Holt, 2000). Roads lead to mortality by involving animals in vehicle strikes. (Beaudry, deMaynadier, & Hunter, 2008). In the United States, over a million vertebrates are killed by vehicle strikes per day (Schwartz, Shilling, & Perkins, 2020). In Canada, 13.8 million birds are killed annually on roads (Schwartz, Shilling, & Perkins, 2020). By 2050, 25 million km of new roads are expected, thus road mortality will increase (Schwartz, Shilling, & Perkins, 2020). Because of noise avoidance, road surface avoidance, or vehicle avoidance, roads isolate populations into their subdivided habitat (Jaeger et al., 2005) and smaller populations face a higher risk of extinction (Kenneth Dodd, 1990). In fact, turtles are more likely to cross unpaved roads than paved ones because of traffic volume (Paterson et al., 2019). The populations more at risk are those in which animals avoid roads because of noise and surface type, traffic volume also influences population behavior (Jaeger et al., 2005). Over time, the consequences of road avoidance and road mortality can lead to changes in population dynamics (Jackson & Fahrig, 2011).

Reptiles are greatly affected by road expansion. At a global scale, populations of reptiles are declining, and this is partly caused by habitat transformation following road expansion, fragmentation, and roadkill (Paterson et al., 2019). According to the IUCN Red List of Threatened Species, 21% of reptile species are threatened by extinction (IUCN, 2021). More specifically, roadkill puts reptiles particularly at risk because they tend to be slow and seem unaware of the danger of a vehicle in movement (Paul & Robinson, 1996). Roadkill is especially a problem for turtles (Beaudry, deMaynadier, & Hunter, 2008). Turtles in general are in peril according to IUCN (IUCN, 2021). Eight species of freshwater turtles are found in Canada (Dorland, Rytwinski, & Fahrig, 2014). Six of them are species at risk according to *COSEWIC* (COSEWIC, 2021). Because turtles have a long lifespan and populations have a slow growth rate, small increases in adult mortality have a large impact on population persistence (Carstairs et al., 2018). Nesting makes female turtles more vulnerable to vehicle strikes. During the nesting season, females move more than males when looking for nesting sites and are more likely to cross roads (Carrière, Bulté, & Blouin-Demers, 2009). Moreover, females sometimes use the substrate along the road as nesting sites (Dorland, Rytwinski, & Fahrig, 2014). In a wetland in Ontario, Canada, the painted turtle population sex ratio was 84% in favor of males which differs significantly from the 0.5 ratio that would be expected (Dupuis-Désormeaux et al., 2017). The authors associated this skewed sex ratio to higher road mortality in females (Dupuis-Désormeaux et al., 2017). Another study, conducted in New York state, found that painted turtle populations had a significant male-biased sex ratios (74% males; Steen & Gibbs, 2004).

In this study, I determined whether roads create a male-biased sex ratio in painted turtles in the Ottawa, Ontario, Canada, area. I hypothesized that due to higher risk of road mortality in female painted turtles, there should be more male than female painted turtles at sites surrounded by more roads. Thus, I predicted that turtle populations surrounded by more roads should be more male-biased.

Methods

Study sites and study species

I sampled 13 populations of painted turtles (*Chrysemys picta*) in wetlands in the City of Ottawa, Ontario, Canada. I selected sites with the most sightings of painted turtles in a previous study (Čapkun-Huot et al 2021). Sites were at least 1.5 km apart to prevent catching the same turtle at multiple sites and to minimize spatial auto-correlation (Capkun-

Huot 2021). Sites were distributed along a gradient of urbanization from natural wetlands in conservation areas to artificial ponds in golf courses. (Figure 1)

Painted turtles are generalist; thus, they can be found in a variety of environments. Compared to other species of turtles, they tend to nest more on the shoulder of roads which makes them more vulnerable to vehicle collisions (Browne & Hecnar, 2007). Over three years, the Canadian Wildlife Federation reported over 1400 roadkill turtles in eastern Ontario and most of them were painted turtles (Sebrun, 2020). The painted turtle is considered least concern by *The IUCN Red List of Threatened Species* and has stable populations (Van Dijk, 2011). However, populations of painted turtles in Ontario are considered of special concern (COSEWIC, 2018).

Sampling

I sampled one site per week from early June to mid-August 2021. I used 3 sets of unbaited fyke nets to capture turtles and checked them daily. Buoys were placed in every net so that turtles could always access air.

Measurements

To establish the sex ratio of each population, each painted turtle was sexed based on external morphology. Male and female painted turtles differ in some key morphological traits. Males have a longer distance between their cloaca and their plastron, and they have longer foreclaws. Males also have a flatter carapace and are smaller (Valenzuela, 2009). Using a file and by making small notches on the carapace scutes, all turtles were uniquely marked following the North American coding system (Nagle, 2017) and then released at their site of capture.

Statistical analyses

Landscape composition - To determine the landscape composition surrounding each site, I used Arcmap 10.8.1 (ESRI, 2020; http://www.esri.com). I used the Southern Ontario Land Resource Information System (SOLRIS) Version 3.0 layer (OMNRF, 2019), the 2015 Land Cover of Canada layer (Canada Centre for Remote Sensing, 2015) and the Road Network File (Minister of Industry, 2017). The 29 land classes were merged into seven (Table 1). I then created buffers from 100 m to 4000 m with 100 m increment for all 15 sites. I tabulated the landscape composition of each incremental buffer at each site.

Sex Ratio - I determined the sex ratio of each population as the proportion of males among the captured turtles. Turtles that were too small to be sexed were excluded and released. To have more accurate estimates of population sex ratios, I only used sites where I captured at least 10 turtles for the analyses (n = 12) (Table 2).

Scale of maximum effect - Using R (The R Foundation, 2018), I calculated correlations between sex ratios and each landscape variable for each buffer. Because of the modest number of sites, I had to select only one buffer distance per variable for the analysis of the effect of landscape composition. Therefore, I determined the scale of maximum effect of every variable on sex ratio as the buffers with the highest correlations in absolute values. (Figure 2; Table 3; Table 5).

Statistical model - To assess the effect of landscape composition on turtle sex ratios, I used a multiple linear regression. Again, I had to reduce my number of predictor variables because my sample size was too modest to integrate all potential variables in my model at once. To avoid multicollinearity, when a pair of variables had a Spearman rank correlation coefficient with an absolute value over 0.6 (Akoglu, 2018), I removed the variable, but I always kept road density if that variable was involved because this is the variable of interest for my study (Figure 4). I removed the landscape categories Wetlands, Others, and Forest because they were highly correlated with other variables (Figure 4). To further reduce my number of predictor variables, I performed simple linear regressions for each remaining variable and the population sex ratio. When a predictor variable had a p-value over 0.25, the variable was not considered for the model, again because of the size of my sample that was too small (Table 6). After removing the anthropogenic cover, agriculture cover and population size variables, I ran a model with road density and open water cover.

Results

The mean sex ratio, calculated as the proportion of males, was 0.545 (0.261 to 0.818; Table 2). The characteristics of the environment did not have any significant effects on the sex ratios. To establish the model describing the sex ratio, I computed the variance inflation factors (VIF) to see if there was an issue of multicollinearity (Table 4) and there was none. I used an ANOVA to compare this model, which had sex ratio as response variable and road density and open water cover as predictor variables with a second model with only road density as a predictor variable. The ANOVA was not significant ($p = 0.17$) and allowed me to remove the Open water variable. With only Road left, I compared this new model to a null model with an ANOVA. The test was not significant ($p = 0.14$)

Discussion

Understanding how roads affect turtle populations is important. This knowledge could allow road and urban development to happen while preserving the populations at risk. My study focused on the effects of roads on painted turtle population sex ratios. The landscape variables considered had no significant effects on population sex ratios.

Contrary to other studies (Dupuis-Désormeaux et al., 2017; Steen & Gibbs, 2004), I did not detect any effects of roads on the population sex ratios. A study conducted on anurans found that traffic density had an important negative effect on species richness and that forest cover had a smaller positive effect on species richness. However, the response to roads and forest cover varies a lot between species (Eigenbrod, Hecnar, & Fahrig, 2008). Maybe female painted turtles are not very sensitive to road density.

Confounding variables

Contrary to my hypothesis, the sex ratios turned out to be balanced and many factors could explain this result.

Precision of predictor - It is possible that the choice of my predictor variable was not the best to evaluate the effect of roads on population sex ratios and it could explain the non-significant results. To measure the effects of roads on turtle sex ratios, I used road density as a predictor. However, traffic density could be a better predictor to use when evaluating road effects since it has a more direct effect on mortality (Eigenbrod, Hecnar, & Fahrig, 2008). Other studies observed that traffic volumes have a more important effect than road size on population survival (Jaeger et al., 2005). Using a better predictor of female road mortality to test the effects of road on turtle sex ratios could provide more representative results.

Predation near roads - Populations might not exhibit a biased sex ratios because of road avoidance behavior. If females do not approach or cross roads, they are less likely to be killed, thus the 1:1 sex ratio of the population will be maintained (Steen et al., 2006). Also, because roads are open area and a boundary between forest and human activities, females might be more susceptible to predation by racoons and domestic dogs (Steen & Gibbs, 2004). Therefore, female could tend to stay away from these open areas. However, contrary to Blanding's turtles, no road avoidance behavior has been documented in painted turtle (Dorland, Rytwinski, & Fahrig, 2014). It is therefore unlikely that this type of behavior explains the similar sex ratios I found between populations.

Lower mortality in females due to selective pressure and site availability - Road mortality could also be reduced because of selective pressures. Because road mortality could be highest in females nesting on the side of the road or crossing roads, females nesting closer to ponds at site with high road density could be favored (Dorland, Rytwinski, & Fahrig, 2014). On the long term, persisting females would be the ones that nest near the pond, and thus away from roads. Therefore, populations in which females nest closer to the pond would suffer from fewer roadkill females. These population sex ratios would then be less or not male-biased even near high road density. Mortality in females could also be lower if they do not have to migrate long distances. Sites with higher proportion of forest or wetland can have more suitable nesting sites. The distance traveled by female painted turtles is negatively correlated with the number of nesting sites near ponds (Baldwin, Marchand, & Litvaitis, 2004). Therefore, female turtles do not have to travel long distances to find a nest and they stay closer to the pond, which lower their exposition to road (Dorland, Rytwinski, & Fahrig, 2014).

Sampling method leads to biased sex ratio - Biased sex ratios could be due to sampling methods. To estimate the population sex ratios, I only sampled in ponds of interest. Because females, in general, leave their ponds to find nesting sites, it would be expected to have more males in ponds as they do not migrate like females. This would result in finding more males in each population. Another possible confounding variable is the fact that when females are caught in the fyke nets, their presence attracts more males, resulting in more male caught than female (Vanek & Glowacki, 2019). However, these two phenomena would only occur during the nesting period which last a few weeks so this would only affect some sites as each were sampled at different moments of the summer.

Temperature-dependant sex determination - Another confounding variable that could modify the expected male biased sex ratio is the temperature at which the eggs develop. In painted turtles, when the eggs incubate at warm temperatures, it produces females while at lower temperatures it produces males (Steen & Gibbs, 2004). Warm conditions could be linked with urbanization. Because urbanization increases the number of open areas, which absorb and concentrate solar radiation, nesting sites in these urban environments might have the thermal conditions that favor the development of females (Bowne et al., 2018). The amount of vegetation surrounding the nest is significantly negatively correlated with temperatures above the threshold temperature at which females are produced (Weisrock & Janzen, 1999). Because of the negative correlation I found between the forest cover and road density, it is possible that the roadside, warmer because of less vegetation cover, produces more female (Steen & Gibbs, 2004). This could result in female biased sex ratios where sites are closer to road or could help maintain even sex ratios.

Lack of power

I might have been unable to detect significant effect because of my modest sample size. I was not able to assess the effect of the 8 predictor variables together on population sex ratios because my sample size was limited. I had one observation per site, no matter how many turtles were captured. Based on a study that found significant male biased sex ratios caused by urbanization (Steen & Gibbs, 2004), I had not enough power with my sample of ponds. Using the effect size of my study (Figure 3), I conducted post-hoc analysis and identified that I should have sampled 43 sites to have power of 0.95. To be able to test my hypothesis, future research should be based on a larger sample size.

Conclusion

Overall, sex ratios in my study did not seem to be related to road density. The sex ratios I found were not male-biased and did not seem influenced by urbanization. Moreover, none of the environmental characteristics had an impact on sex ratios. Although many studies found significant male biased population sex ratios in response to road mortality, long-term consequences of skewed sex ratios in painted turtle populations remain unknown. More investigations are required as many factors could influence the populations sex ratio of turtles and large-scale studies could provide such information (Steen & Gibbs, 2004). This knowledge would greatly help conservation efforts and future protection measures given that urbanization is a continuing threat.

Table 1. Landcover classes of Southern Ontario Land Resource Information System (SOLRIS) Version 3.0 layer) and the 2015 Land Cover of Canada layer merged into 7 categories. Only the values present within the incremental buffers were classified. Buffers were done at each of the 12 sites sampled from June to mid-August 2021 in the city of Ottawa.

	SOLRIS	
Value	**Class name**	**Category**
90	Forest	Forest
91	Coniferous Forest	Forest
92	Mixed Forest	Forest
93	Deciduous Forest	Forest
131	Treed Swamp	Wetland
135	Thicket Swamp	Wetland
140	Fen	Wetland
150	Bog	Wetland
160	Marsh	Wetland
170	Open Water	Open Water
191	Plantations – Tree Cultivated	Forest
192	Hedge Rows	Forest
193	Tilled	Agriculture
201	Transportation	Anthropogenic
202	Built -Up Area – Pervious	Anthropogenic
203	Built -Up Area – Impervious	Anthropogenic
204	Extraction – Aggregate	Anthropogenic
205	Extraction – Peat/Topsoil	Anthropogenic
250	Undifferentiated	Other

2015 Land Cover of Canada

Value	Class name	Category
1	Temperate or sub-polar needleleaf forest	Forest
5	Temperate or sub-polar broadleaf deciduous forest	Forest
6	Mixed forest	Forest
8	Temperate or sub-polar shrubland	Other
10	Temperate or sub-polar grassland	Other
14	Wetland	Wetlands
15	cropland	Agriculture
16	Barren land	Other
17	Urban and built-up	Anthropogenic
18	Water	Water

Table 2. Sex ratios of the 12 populations of painted turtles sampled from June to mid-August in the city of Ottawa, Ontario, Canada. Sex ratio is calculated as the proportion of male. Population sizes range from 14 to 44 individuals.

ID	**Site**	**Male**	**Female**	**Total**	**Sex ratio**
1	Chapman Mills Conservation Area	25	14	39	0,641
2	Richmond Conservation Area	6	17	23	0,261
3	Baxter River	12	7	19	0,632
4	South March Highlands Conservation Area	36	8	44	0,818
5	Carp Barrens (C)	12	5	17	0,706
6	Cumberland forest	27	31	58	0,466
7	Nepean Creek	9	8	17	0,529
8	Beaver Pond	4	10	14	0,286
9	Meadow Breeze	11	4	15	0,733
10	Long Island	29	13	42	0,690
11	Dow's lake	9	16	25	0,360
12	Marais en quartier résidentiel	11	15	26	0,423

Table 3. Buffer with the maximal scale effect on sex ratios for each category of landcover and their p-values. Sex ratios are from the 12 populations of painted turtles sampled from June to mid-August in the city of Ottawa, Ontario, Canada.

	Road	Forrest	Wetlands	Open water	Agriculture	Anthropogenic	Other
Buffer	1600	4000	4000	2700	4000	100	3400
Correlation	-0.456	0.270	0.263	0.497	-0.303	0.353	0.482
p-value	0.136	0.397	0.408	0.100	0.339	0.260	0.113

Table 4. Initial model of the variation of the sex ratio and the VIF test performed. I used a multiple linear regression to assess the effect of 8 predictor variables on the sex ratios of 12 populations of painted turtles I sampled from June to mid-August in the city of Ottawa, Ontario, Canada.

Variable	Estimate	Std. Error	t value	Pr(>|t|)
Road	-0.8684	0.6664	-1.303	0.2249
Open water	2.7483	1.8334	1.499	0.1681

	Road	**Open water**
VIF	1.06269	1.062685

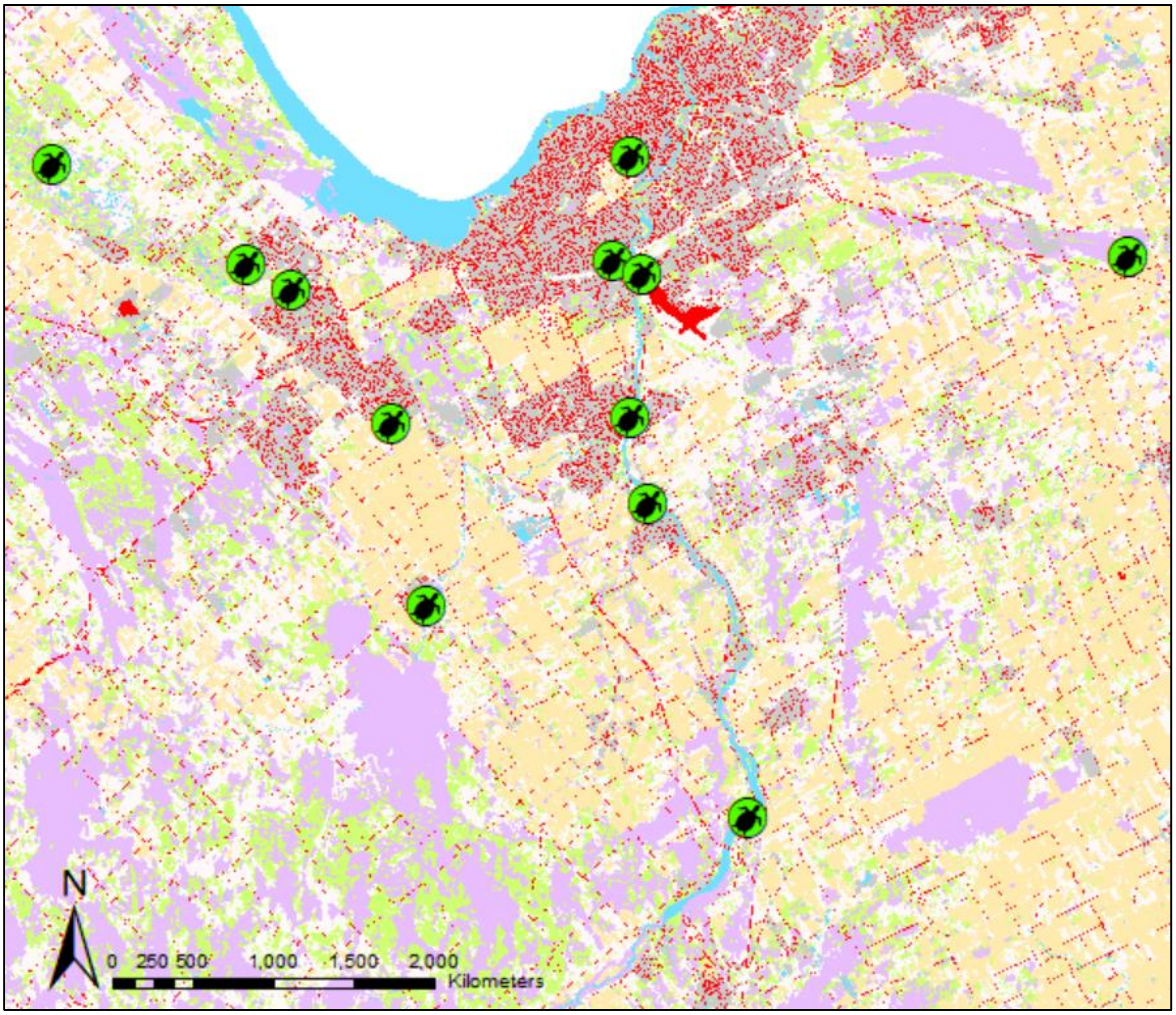


Figure 1. Locations of the sites studied in Ottawa, Ontario, Canada, where 12 populations of painted turtles (*Chrysemys picta) (*) were studied in summer 2021. Each type of landcover is represented by a color; Forest (green), Wetlands (purple), Anthropogenic (grey), Road (red), Open water (blue), Other (white), Agriculture (tan).

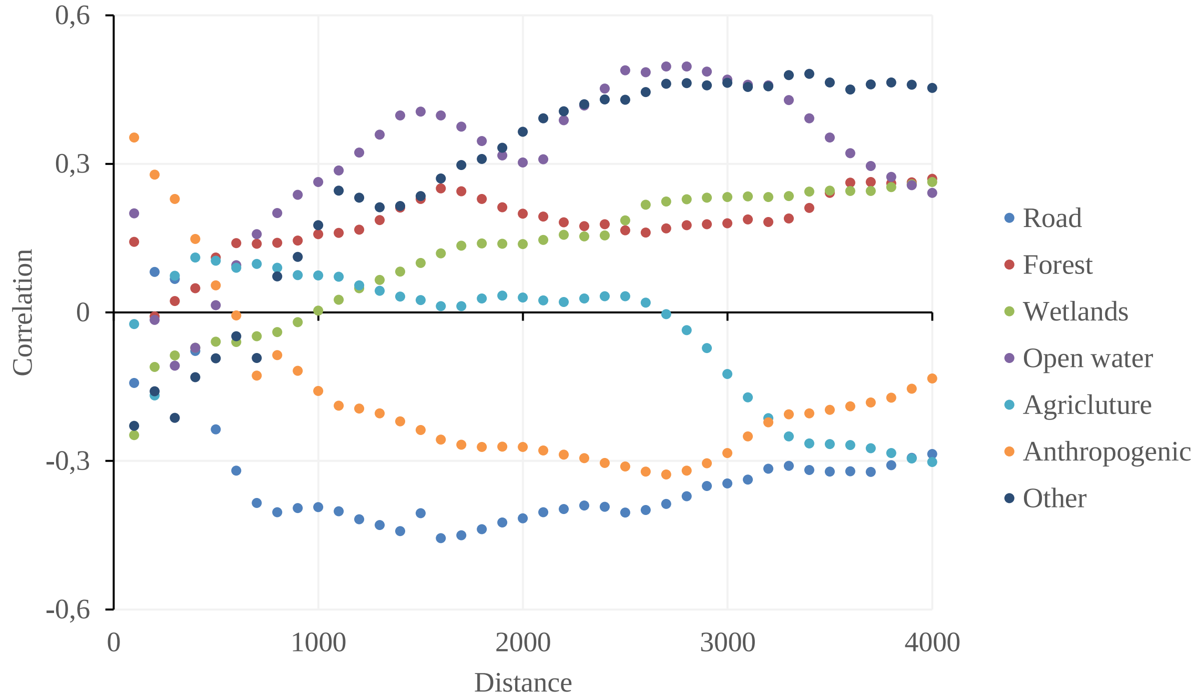


Figure 2. Correlation between the sex ratios of the 12 populations of painted turtles sampled from June to mid-August in the city of Ottawa, Ontario, Canada, and each landscape category for each buffer (100 m to 4000 m with a 100 m increment). The buffers used were the ones with the maximum scale effect on sex ratios (Table 3).

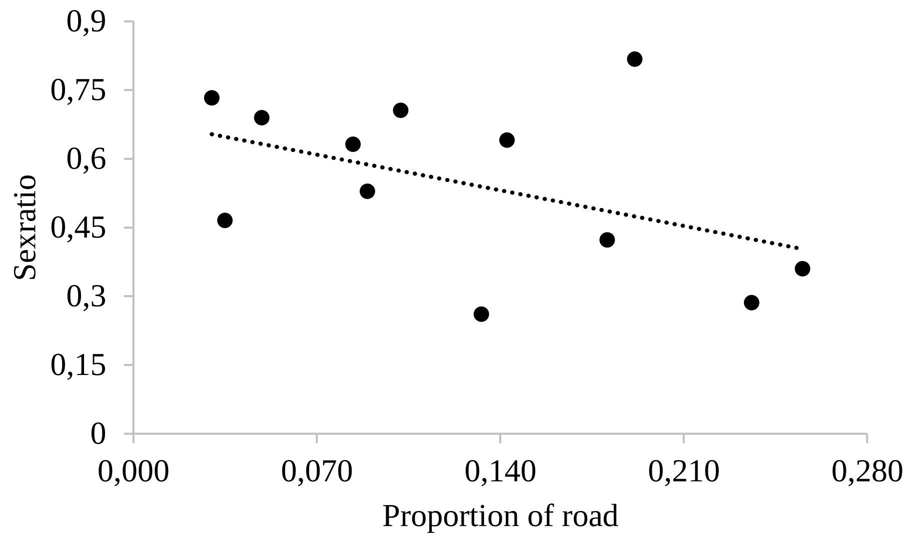
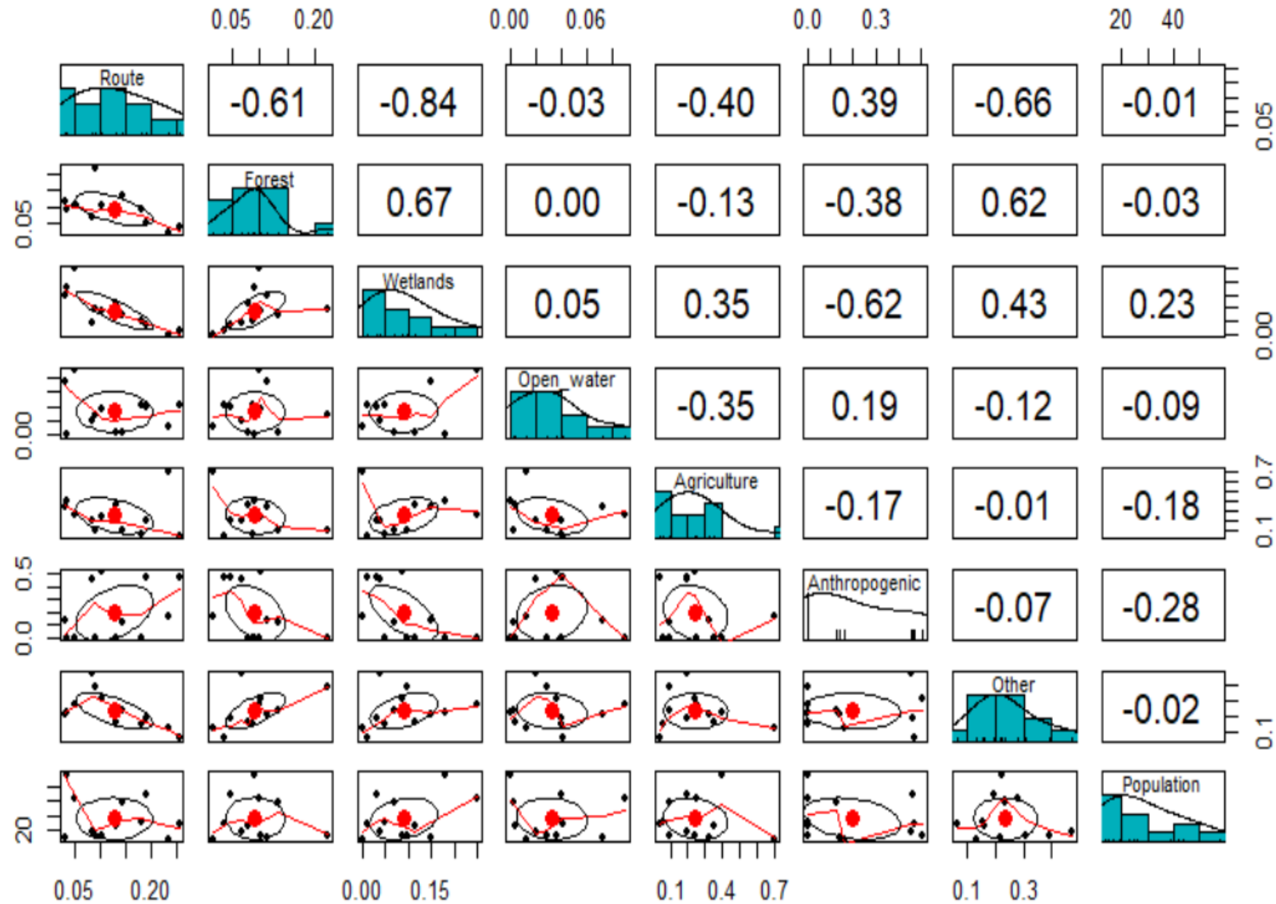


Figure 3. Relationship between the sex ratios of the 12 populations of painted turtles sampled from June to mid-August in the city of Ottawa, Ontario, Canada, and the proportion of road at each site. Proportion of road was calculated with the buffer 1600 and data was taken from the Road Network File of Canada (2016).

Figure 4. Spearman correlation matrix between the predictor variables. Population is the number of painted turtles that were captured at each from June to mid-August in the city of Ottawa, Ontario, Canada.



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Appendix

Table 5. Correlation between the sex ratios of the 12 populations of painted turtles sampled from June to mid-August in the city of Ottawa, Ontario, Canada, and the environmental variables which were taken from the buffer with the maximum scale effect. Number of captured turtles was also considered as a variable named Population, but no correlation had to be made.

Site	Road	Forest	Wetlands	Open water	Agriculture	Anthropogenic	Other	Population
1	0.1426	0.1345	0.0700	0.0028	0.0978	0.1232	0.2364	39
2	0.1328	0.0795	0.1161	0.0036	0.3558	0.0000	0.1617	23
3	0.0838	0.0685	0.0396	0.0201	0.1987	0.4599	0.4690	19
4	0.1913	0.0488	0.0308	0.0400	0.1966	0.4638	0.1937	44
5	0.1020	0.1022	0.0877	0.0360	0.2381	0.5109	0.3105	17
6	0.0349	0.0894	0.1803	0.0006	0.3994	0.0000	0.2251	58
7	0.0893	0.2217	0.0982	0.0283	0.0934	0.0000	0.3907	17
8	0.2359	0.0174	0.0000	0.0124	0.7018	0.1606	0.1351	14
9	0.0299	0.1139	0.1493	0.0726	0.3269	0.1377	0.2182	15
10	0.0490	0.1005	0.2489	0.0897	0.2500	0.0000	0.2777	42
11	0.2553	0.0369	0.0107	0.0412	0.0371	0.4745	0.0665	25
12	0.1807	0.0882	0.0488	0.0403	0.0562	0.0000	0.1585	26

Table 6. Results of the linear regression with the predictor variables left after the spearman rank correlation test. Male is the sex ratio of 12 populations of painted turtles sampled from June to mid-August in the city of Ottawa, Ontario, Canada.

Model	**R (adjusted)**	**df**	**F**	***p***
Male~Road	0.1288	10	2.626	**0.1362**
Male~Open_water	0.1713	10	3.274	**0.1005**
Male~Agriculture	0.0007095	10	1.008	0.3391
Male~Anthropogenic	0.037	10	1.423	0.2605
Male~Population	-0.0505	10	0.4711	0.5081

Supplementary material

Methods

Sampling - Starting with 2 sets of nets on the first day, a third set of nets could be installed if not many turtles were caught on the first day of capture. Nets were installed in a site for 5 days and they were checked and emptied every 24 h for a total of 4 days of capture. All animals were released except the painted turtles that were kept for further measurements.

Analyses

Landscape composition - The Forest category was all vegetation cover that was at least 2 m high. Wetlands were all areas that had a water table near or above their substrate surface seasonally or permanently. Agricultural land were areas where annual crops were cultivated. Anthropogenic cover was determined as any infrastructures that serve human activities such as residential, industrial, recreational and resource extraction areas. The Other category was everything that did not fit into the other categories. Finally, there were the Road and Open water categories.