Does urbanization impact the prevalence of defensive behaviours in redbelly *(Storeria occipitomaculata)* and common garter snakes *(Thamnophis sirtalis)*?

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Abstract

Urbanization is the expansion of human presence through the development of infrastructure such as roads and buildings and is a major threat to wildlife. Urbanization can disturb natural habitats and displace species, causing them to either adapt or move elsewhere. Reptiles in Canada are faced with challenges due to their ectothermy and are increasingly affected by the expansion of urban regions. As such, there are major impacts on snake biodiversity (Kjoss *et al,* 2000); however, the effects of urbanization on snake behaviour is less studied. I tested the hypothesis that increasing urbanization will impact the defensive behaviours of redbelly snakes *(Storeria occipitomaculata)* and common garter snakes *(Thamnophis sirtalis)* in the Ottawa-Gatineau area. I captured snakes between May and August 2021 under cover board arrays. During capture, all snakes were filmed continuously, and I used the recordings to tally defensive behaviours. There was no significant relationship between the degree of urbanization and the degree of defense displayed by captured snakes; however, there may be other factors at play that were not accounted for in this study such as foot traffic or noise disturbance.

Key Words

Self-defense, Snake Behaviour, Anthropogenic disturbance, Urban Effects, Hostility

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Introduction

The impacts of urbanization are of great interest. Urbanization describes the phenomenon of expanding human presence through the development of infrastructure such as roads and buildings. In 2021, approximately 73.7% of Canada's population was found to live in urban areas (Statistics Canada, 2022). This world-wide phenomenon begs the question as to what the impacts are of human development on animal populations. Urbanization and human disturbance affect biodiversity as defined by the United Nations Convention on Biological Diversity (2006). A number of threats to biodiversity have been identified such as habitat loss and degradation, over exploitation (direct or indirect), pollution, invasive or problematic species as well as climate change. Different regions have varied prevalence of these threats. For North America, one of the major impacts on biodiversity is habitat loss and degradation (Titeux *et al.*, 2016; Trathan *et al.*, 2015). As such, it is of utmost importance to understand the impacts that anthropogenic changes have on biodiversity. Anthropogenic disturbances are specific consequences resulting from human activities due to deliberate acts or as a result of human presence. Urbanization can have profound impacts on species population sizes as well as their natural ranges. Reptiles in Canada already face a number of challenges due to their ectothermy in a northern country and urbanization is an additional stressor. Urbanization can impact the biodiversity of snakes (Kjoss *et al,* 2000), but its effects on behaviour are less clear (De Meester *et al,* 2018).

Urbanization can shape animal personalities (Lapiedra *et al,* 2016). Notably, it affects behaviour and boldness in birds (Charmantier *et al,* 2017) and in some lizards (Chejanovski *et al,* 2017). These changes in behaviour can be caused by decreasing habitat, crowded populations, changes in predator presence. (Gallo *et al*, 2019). As such, it is of value to document the impact that urbanization on animals as it may improve conservation efforts. Animals living in more urban settings favour behaviours that give them a fitness advantage. As such, the benefits of more defensive behaviours may outweigh the costs to urban species as they compete for more sparse resources (Hurtado *et al,* 2017). I hypothesized that urbanization impacts defensive behaviours of redbelly *(Storeria occipitomaculata)* and common garter snakes *(Thamnophis sirtalis)* in the Ottawa-Gatineau region. More specifically, I predicted that snakes in more urbanized landscapes will be less defensive as they have become acclimated to human disturbance (Łopucki *et al,* 2020) and habituation modified their behaviour significantly (Edwards *et al,* 2012).

Materials & Methods

Study Area - This study was conducted between 18 May and 26 August 2021. I chose 10 sites in Québec and 15 in Ontario, Canada (Fig. 1) all consisting of old field sites, a habitat preferred by redbelly snakes and garter snakes (Halliday *et al,* 2018; Carpenter, 1952). The sites varied in the amount of surrounding urbanization. I visited each study site once per week for a total of 13-15 visits per site. Temperature and weather were controlled to a certain degree as both were optimized for snake collection. I sampled snakes in temperatures between 25-32°C (Halliday *et al*, 2016) and without rain. I collected snakes from under plywood boards (Diaz *et al,* 2017; Cairns *et al,* 2018) measuring 60 x 60 cm and 1.9 or 1.3 cm thick. I placed 20 boards per site with the exception of 2 sites that had 10. The two sites containing only 10 boards were due to a lack of space in the fields. The boards were spaced 20 m apart along a transect that followed the edge of the old fields (Appendix A).

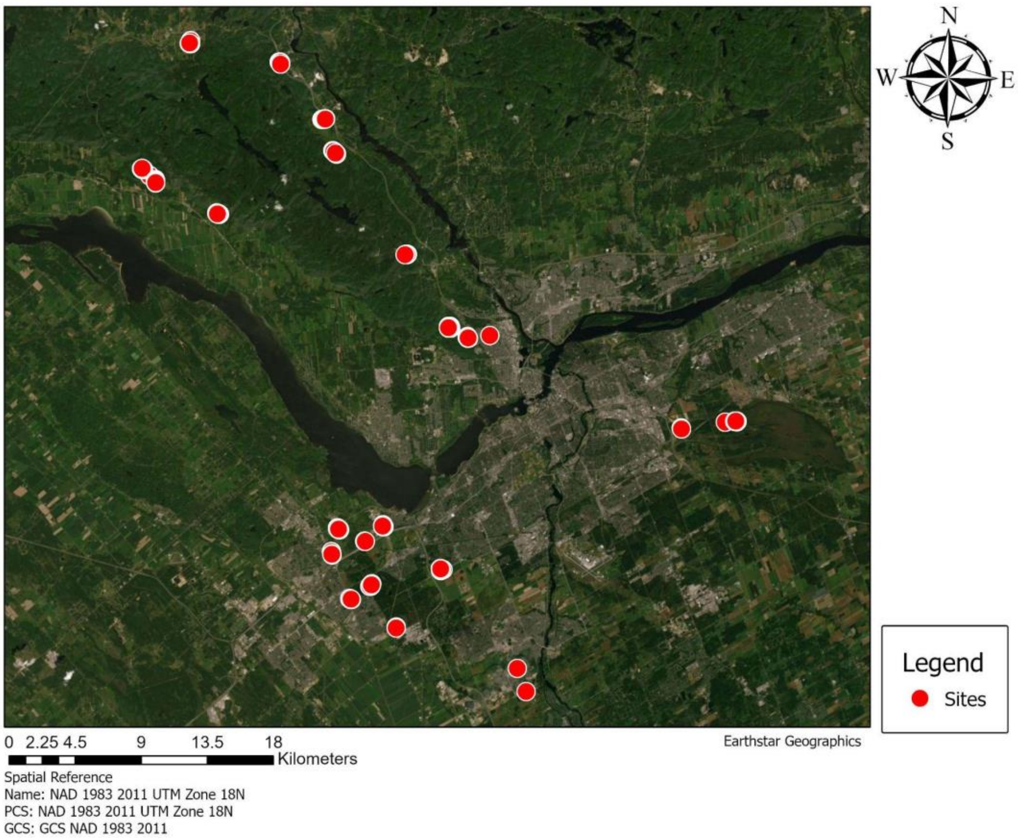

Figure 1. Map of study sites with locations represented by red points. Old fields' locations chosen to collect redbelly (*Storeria occipitomaculata)* and common garter snakes *(Thamnophis sirtalis)* snakes from 10 sites in Gatineau-Wakefield, Québec, and 15 in Ottawa, Ontario (Canada) from May to August, 2021. Map made in ArcGIS Pro ver. 2.9.2 (ESRI, 2022).

Data Collection - Snakes are one of the most precocial animals, demonstrating similar behaviours as a juvenile and adult which meaning behaviours can be easily compared between life stages (Herzog *et al.,* 1986). I used garter and redbelly snakes as they are the most abundant in the Ottawa-Gatineau region (IUCN, 2021). Garter snakes have distinct yellow/green lateral stripes as well as brown/black spots on the back (Ontario Nature, 2021a; Fig. 2). Redbelly snakes are brown with a red belly and have lateral stripes and a pale triangle on the head (Ontario Nature, 2021b; Fig. 2).

Figure 2. Unique colouring of garter snake *(Thamnophis sirtalis;* A) and redbelly snake (*Storeria occipitomaculata;* B) for identification purposes after capture under cover-boards in the Ottawa-Gatineau region (Canada) from May to August, 2021. Photo credits Ella Eberhardt (2021).

Snakes caught from underneath the plywood boards as well as in the old fields were given unique marks by heat-branding specific ventral scales with a cautery unit following Winne *et al.* (2006). Data for the site, capture time, weather, species, length (cm), sex, gravid state, presence of scars, and whether the snake was found under a board were taken for each snake. These factors were included as they were thought to be possible confounds that impact snake behaviour and as such, variation was going to be controlled for using the collected data. The weather was based on The Weather Network's reporting and recorded as a single value for the entire day. Length was calculated from snout to vent (SVL) using a metal ruler and by gently laying the snake along it. Finally, the snake was determined to be gravid or not if a significant bulge was found in the abdomen anterior to the vent indicating the presence of developing embryos. I filmed each capture continuously with a GoPro (GoPro HERO4) to capture the snakes' behaviour. I watched the videos to tally the defensive behaviours. A defensive behaviour was classified as an action taken by the snake with the goal of deterring a predator. I chose nine behaviours to represent defense based on previous research and can be found along with their description in Table 1 (Maillet, *et al.*, 2015; do Amaral, 1999; Delaney, 2019; Greene, 1988). Each of the described behaviour was given a score of 1, with the exception of "bite" and "bite attempt" where each strike was counted as a score of 1. Using this method, I calculated a total "defensive factor" per individual. In the case of re-capture, the first "defensive factor" was used in the analysis to use the same initial reaction for all captures. As such, I was able to obtain a measure for the amount of defense performed for all of the captured snakes with a consistent stimulus as all snakes underwent the same measures and treatment.

Table 1. Ethogram of defensive behaviours of interest performed by redbelly (*Storeria occipitomaculata)* and common garter snakes *(Thamnophis sirtalis)* during capture in the Ottawa-Gatineau region (Canada) from May to August, 2021.

Behaviour	Description
Musk	The release of musk (a strong-smelling glandular secretion) as a defense mechanism.
Bite	A successful bite of the perceived risk.
Bite Attempt	An attempted bite towards the perceived risk.
Lip-Curling	Flicking of the tongue and curling of the lips upwards to show the maxillary teeth.
Head Distortion	The rapid movement of solely the head in a back-and-forth whipping motion.
Feign Death	The body of the snake going limp as a defensive mechanism.
Jerking/Writhing	The excessive jerking and thrashing of the snake's entire body in an escape attempt.
Constrict	The coiling of the snake's body in an attempt to asphyxiate.
Head lock	The hooking of the snake's head around an object (observer's body ie. finger, or environment ie. branch).

Degree of Urbanization – I used the minimum convex polygon method (MCP) to create the smallest convex polygon around all the sampled snakes at each site (Powell, 2000; Row, 2006). I created 20 buffers in ArcGIS vers. 2.9.2 (ESRI, 2022) ranging from 50 m from the MCP to 1000 m with 50 m increments around each site. Degree of urbanization was calculated over all buffers to determine the scale of maximum effect (Fyson *et al.*, 2021). Degree of urbanization was calculated by proxy using the 2015 Land Cover of Canada data. This dataset includes the land usage classified in a range of arbitrary numbers based on the Canada Centre for Remote Sensing's (CCRS) efforts. It was produced through the use of imaging from Operational Land Imager (OLI) Landsat sensor with an accuracy of 80% (Latifovic, 2019).

Statistical Analyses - Nine distinct categories of land use were found within my study area and were given increasing values based on the amount of human disturbance. Regions of urban build-up were assigned a value of 9, while coniferous forest were assigned a value of 1 (Fig. 3). Areas such as grasslands, barren land and cropland fell in the middle of the spectrum. As such, I calculated the total degree of urbanization based on the average land usage proportional to the area of the buffer using summary statistics (ESRI, 2022) in ArcGIS Pro vers. 2.9.2. I ran correlation analyses in R (R3.3.0) to determine at which buffer distance did landscape composition have the strongest effect on defensive behaviours performed by snakes (Fyson *et al.*, 2021; Bewick, 2003). I then ran multivariate analyses using the buffer with the highest effect as well as several control variables. Controls included the snake's length (cm), capture time, date, weather, gravid state, presence of scars, and whether the snake was found under a board. This was done to confirm that the effect of urbanization was being measured rather than a different variable.

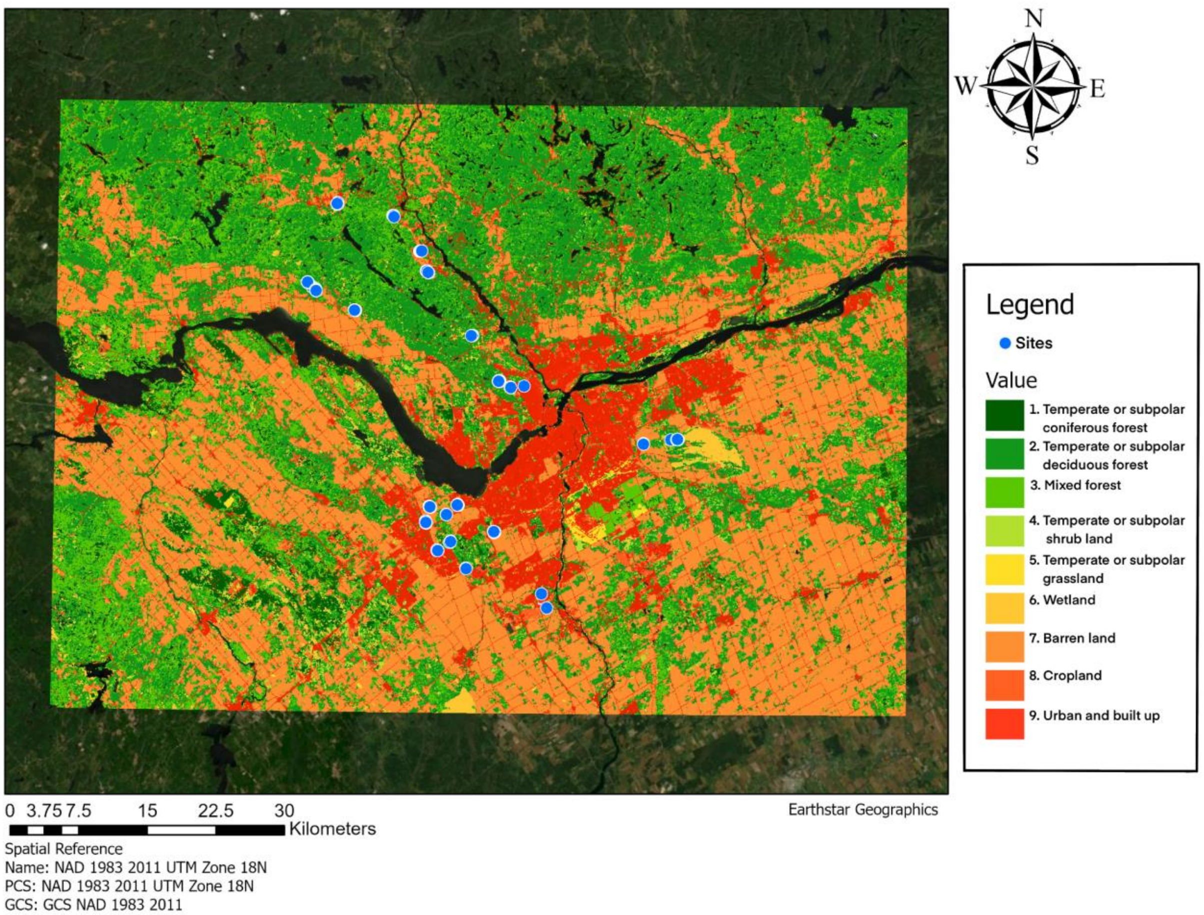

Figure 3. Degree of urbanization surrounding the collection sites in the Ottawa-Gatineau region (Canada) from May to August, 2021. Degree of urbanization represented by increasing values in the legend and obtained from the Land Cover of Canada (2015) dataset (Latifovic, 2019).

Results

By the end of the summer, a total of 549 captures were made, with 235 individual garter snakes and 314 individual redbelly snakes. I found jerking and writhing to be the most prominent defensive behaviour between both redbelly and garter snakes. It occurred a total of 367 times with the second most common behaviour following at 305 occurrences of musking. Musking is the release of a strongly scented musk to deter predators. These top two defensive behaviours were also found separately for both species with 210 redbellies' and 157 garters' jerking around wildly. Musking was done by 53% of redbellies' and 60% of garters' demonstrating the high prevalence of this behaviour and its perceived success as a defense. I found that biting (n = 23) and biting attempts (n = 30) were the least common defensive behaviour for both species, with biting attempts occurring at a slightly higher frequency. While only 1 redbelly used biting as a deterrent behaviour, 22 garters' used this strategy. For garter snakes specifically, lip curling was the least common behaviour as it was only performed by 2 out of the 235 individuals. This stands out in contrast to the 23% of redbellies who performed lip-curling as a manner of defense.

In terms of the effects of urbanization, the 650 m buffer was where the correlation between defensive behaviour and urbanization was the highest (Fig. 4) with a correlation of -0.015.

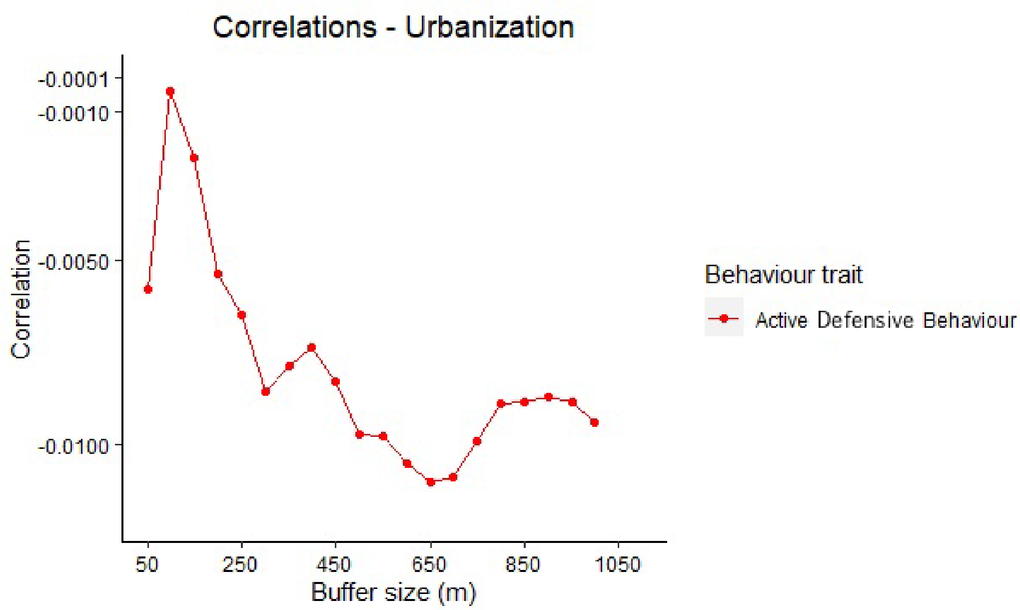

Figure 4. Correlation between active aggressive behaviour and urbanization for redbelly (*Storeria occipitomaculata)* and common garter snakes *(Thamnophis sirtalis).* Analyzed for 20 buffers ranging from 50 meters to 1000 meters surrounding the MCP of each collection site in the Ottawa-Gatineau region (Canada) from May to August, 2021. Graph created in RStudio 2021.09.2 (RStudio, 2020).

Snakes found in areas of higher urbanization were not found to be more or less defensive than those collected in more rural regions (Table 2). I found these results separately for both redbelly snakes ($p = 0.3$) as well as garter snakes ($p = 0.8$). Snake length, as with the other individual characteristics, was initially included to control for any effect of behaviour; however, it was found to be significantly correlated with my variable of interest ($p < 0.05$; Fisher, 1950). I found that longer snakes were more defensive than the shorter ones (Table 2). Redbelly snakes that were longer were more defensive than the shorter one found at all sites ($p = 0.008$; Fig. 5). Longer garter snakes were also seen to perform a larger number of defensive behaviours ($p = 0.006$; Fig. 6).

Table 2. Correlations between redbelly (*Storeria occipitomaculata)* and garter snake *(Thamnophis sirtalis)* defensive factor. Standard error (std. error), t-value and p-value obtained through multivariate regression analysis for scaled length (cm) and degree of urbanization at buffer 16 (650 m) surrounding the MCP of each collection site in the Ottawa-Gatineau region (Canada) from May to August, 2021.

	Redbelly Snakes			**Garter Snakes**		
	Std. error	**t-value**	**p-value**	**Std. error**	**t-value**	**p-values**
Length.scaled (cm)	0.06616	2.689	0.00757	0.09326	2.761	0.00637
Buffer 16 (650 m)	6.77×10^{-2}	1.076	0.28277	9.64×10^{-2}	-0.192	0.848013

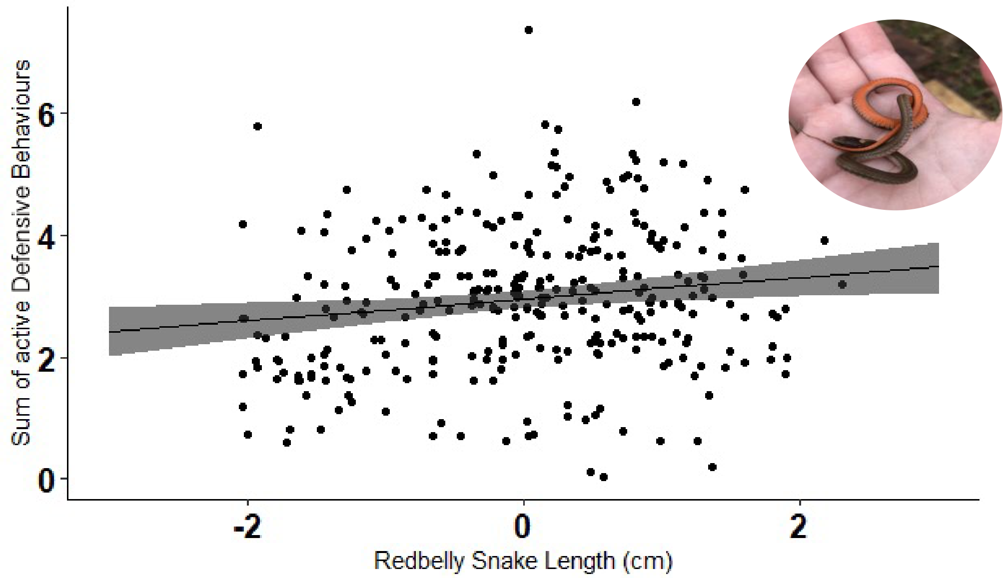

Figure 5. Correlation between redbelly snake (*Storeria occipitomaculata)* length (cm) and sum of active defense. Captures denoted by black dots (n = 314) with length scaled and 0 representing the average. 95% confidence interval demonstrated by grey area and calculated based on predictions in R (Appendix C). Data represents collections from the Ottawa-Gatineau region (Canada) from May to August, 2021.

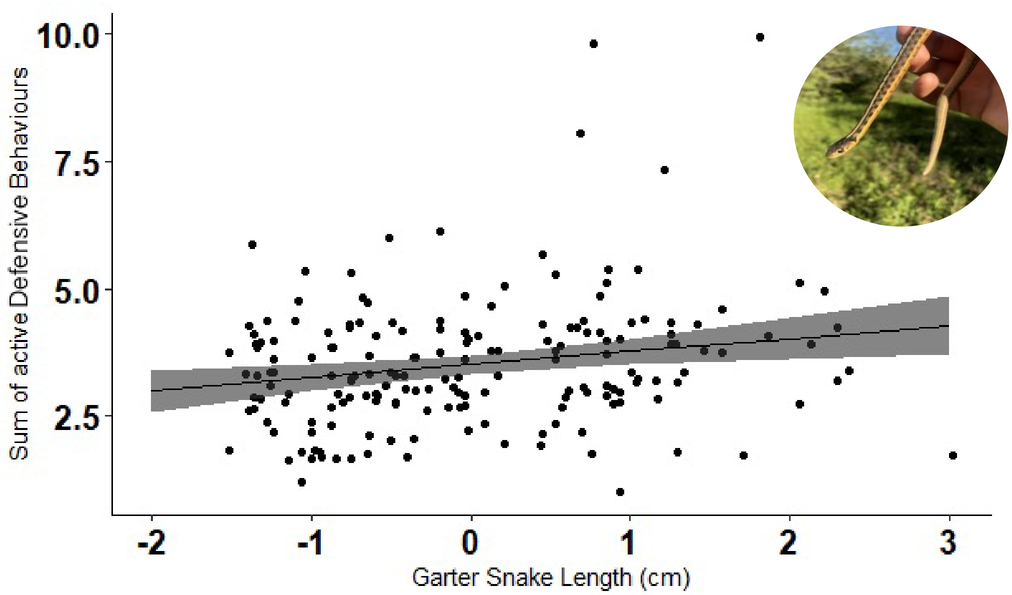

Figure 6. Correlation between garter snake *(Thamnophis sirtalis)* length (cm) and sum of active defense. Captures denoted by black dots (n = 235) with length scaled and 0 representing the average. 95% confidence interval demonstrated by grey area and calculated based on predictions in R (Appendix D). Data represents collections from the Ottawa-Gatineau region (Canada) from May to August, 2021.

Discussion

Urbanization is a well-known threat to wildlife there are no standardized methods to calculate it. Different proxies are used based on the needs and resources of the study which results in a range of quality for this variable. The proxy that I used in this study was an “infrastructure-based” method that focuses on the surrounding environment and urban build-up. There are “population-based” methods that focus more on the density of humans. I do not know if the proxy I used in my study reflected disturbance accurately. Future research would benefit from exploring additional factors such as foot-traffic, noise disturbance, and road density. Combining infrastructure and population density could also be informative.

Sex has a significant impact on snake behaviour (Lee *et al.,* 2019) which may help explain the size effect found in my study because males of both species are generally smaller than females. I was not able to add sex as a variable in my analysis because most of the captured snakes were too small for sex determination by probing. Finally, the life experiences of an individual may be more important in determining their behaviour than the current environment. Past interactions may more strongly dictate reactions rather than a variation in habitat (Sasaki *et al.,* 2009).

Studies have also found links between phenotypic traits and behaviour for eastern garter snakes notwithstanding of their sex. Maillet *et al.* (2014) found that larger female garter snakes were less exploratory than their smaller counterparts. The opposite was also found as larger males were more likely to explore their environment than the shorter ones. Larger snakes may also be more likely to succeed in a display of defense as their size could add to the deterrent effect and a predator may gauge them to be more of a risk than is worth. This could also for longer snakes to be more aggressive in their defense when compared to smaller individuals regardless of their sex. Delaney (2019) also found such a relationship between neotropical snake size and antipredator behaviour; however, it is mentioned that more comparative studies between snakes and their predators would be required to fully understand and explore the variation in behaviour.

To conclude, I found larger snakes to be more defensive, but no effect of landscape composition on behaviour. There are several future works which could explore the impacts of human disturbance on animal behaviour. As individuals modify their behaviour to best suit their environment, they are experiencing new environmental pressures which may impact their lifestyle or range. As such, I anticipate that these data could be used for conservation efforts to mitigate disturbance. By understanding the reaction of wild animals to anthropogenic disturbance, we can modify management to improve the conservation of biodiversity.

Acknowledgement

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Appendix A - Site Locations in Ottawa-Gatineau (Canada)

Table 3. Study Sites along with corresponding GPS coordinates, number of cover boards as well as the number of visits per site. Sites correspond to regions visited for redbelly (*Storeria occipitomaculata)* and garter snake *(Thamnophis sirtalis)* collection from the Ottawa-Gatineau region (Canada) from May to August, 2021.

Site	Name	Latitude (°N)	Longitude (°W)	Number of Cover Boards	Number of Visits
Site 1	Lac Philippe (PH)	45.63876296	-76.0170099	20	15
Site 2	P17	45.62670702	-75.938579	20	15
Site 3	P15	45.59214299	-75.8992579	20	15
Site 4	P16	45.57071096	-75.8883389	20	16
Site 5	P8	45.50964503	-75.8267319	20	15
Site 6	Gatineau Parkway (GP)	45.46581999	-75.7874240	20	14
Site 7	Gabriel-Roy (GB)	45.40947198	-75.5384100	20	12
Site 8	Luskville (LV)	45.532981	-75.9899579	20	14
Site 9	Pilon (PLA)	45.56054598	-76.0578590	10	14
Site 10	Pilon (PLB)	45.55303504	-76.0460469	20	13
Site 11	Stonebridge (SB)	45.24298199	-75.7188670	20	13
Site 12	Kilmarnok Golf Road (KM)	45.24460	-75.72670	20	13
Site 13	Dundonald Golf Road (DD)	45.246920	-75.729735	20	13
Site 14	Golflinks 1 (GL1)	45.257367	-75.724897	10	13
Site 15	Golflinks 2 (GL2)	45.257	-75.726547	20	13
Site 16	Conifer Creek (CC)	45.28162298	-75.8321659	20	14
Site 17	Old Quarry Trail (P5)	45.29844001	-75.8713930	20	14

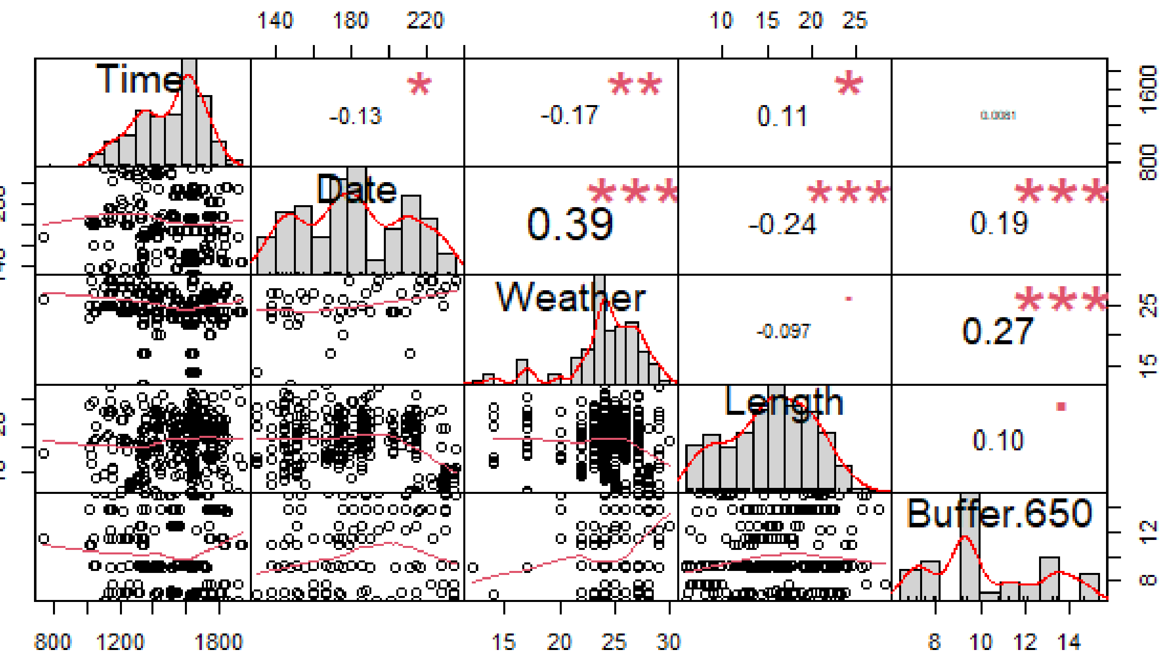
Site 18	Robertson Road (RR)	45.30771702	-75.8537999	20	15
Site 19	Corkstown Road (CT)	45.34286996	-75.8437049	20	15
Site 20	P3	45.333788	-75.8601569	20	15
Site 21	Watts Creek (WC)	45.32537601	-75.8884009	20	15
Site 22	Watts Path (WP)	45.32733	-75.8889879	20	15
Site 23	Sisken Western Path (SK)	45.31728999	-75.7936719	20	14
Site 24	N51	45.40479002	-75.5857589	20	15
Site 25	Mer Bleu P23 (MB)	45.40922698	-75.5382729	20	14

Appendix B - Statistical Analysis for Redbelly snakes (*Storeria occipitomaculata)*

A

```
> anova(mod.ad.null, mod.ad.dummy.site)
Data: Honours.Project.Redbelly
Models:
mod.ad.null: Aggresion ~ Weather.scaled + Length.scaled + Time + Date + Gravid. + Buffe
r.650.scaled + (1 | Dummy)
mod.ad.dummy.site: Aggresion ~ Weather.scaled + Length.scaled + Time + Date + Gravid. +
 Buffer.650.scaled + (1 | Dummy) + (1 | Site)
                  npar    AIC    BIC  logLik deviance Chisq Df Pr(>Chisq)
mod.ad.null          9 980.06 1013.7 -481.03   962.06
mod.ad.dummy.site   10 982.06 1019.4 -481.03   962.06     0  1          1
> |
```

B



C

```
# Predicted values of Aggresion

Length.scaled | Predicted |       95% CI
----------------------------------------
           -3 |      2.41 | [2.00, 2.83]
           -2 |      2.59 | [2.30, 2.89]
           -1 |      2.77 | [2.58, 2.96]
            0 |      2.95 | [2.81, 3.09]
            1 |      3.13 | [2.93, 3.32]
            2 |      3.30 | [3.01, 3.60]
            3 |      3.48 | [3.07, 3.90]

Adjusted for:
* Site = 0 (population-level)
```

D

```
Fixed effects:
                     Estimate Std. Error        df t value Pr(>|t|)    
(Intercept)         2.950e+00  6.752e-01  3.100e+02   4.369  1.7e-05 ***
Length.scaled       2.067e-01  6.788e-02  3.100e+02   3.046  0.00252 ** 
Time               -4.148e-04  3.048e-04  3.100e+02  -1.361  0.17455    
Date                3.394e-03  2.351e-03  3.100e+02   1.443  0.14993    
Buffer.650.scaled  7.288e-02  6.774e-02  3.100e+02   1.076  0.28277    
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

E

```
Random effects:
 Groups   Name        Variance Std.Dev.
 Site     (Intercept) 0.007944 0.08913 
 Residual             1.336195 1.15594 
Number of obs: 310, groups:  Site, 18

Fixed effects:
              Estimate Std. Error        df t value Pr(>|t|)    
(Intercept)    2.94765    0.07174  12.34928  41.090 1.38e-14 ***
Length.scaled  0.17792    0.06616 294.32029   2.689  0.00757 ** 
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

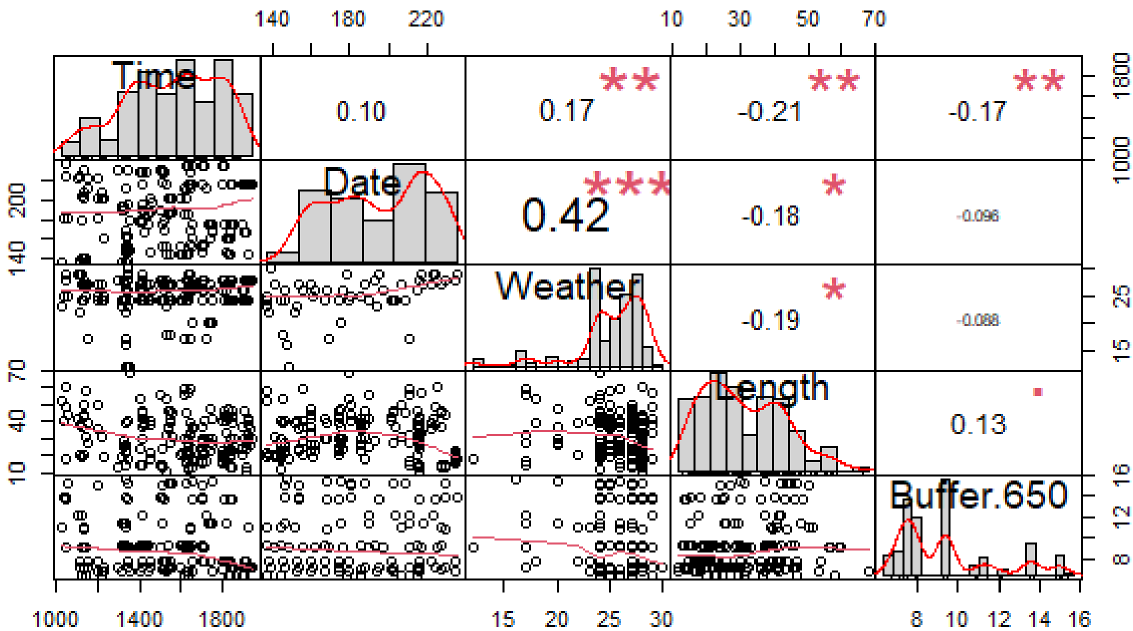
Figure 7. Multivariate analysis in R for Redbelly snake (*Storeria occipitomaculata)* correlation with defensive behaviour and degree of urbanization. (A) ANOVA ran to demonstrate lack of effect by Site number in comparison to a dummy variable. (B) Variance inflation vectors demonstrating elements are not closely correlated and can be included as separate variables. (C) Predicted variables for graphing along with 95% confidence interval. (D) Buffer.650.scaled demonstrates correlation (p-value) of urbanization with the defensive factor. (E) Finally, significant correlation (p-value) between the length.scaled and the defensive factor.

Appendix C - Statistical Analysis for Garter snakes *(Thamnophis sirtalis)*

A

```
> anova(mod.ad.null, mod.ad.dummy.site)
Data: Honours.Project.Garter
Models:
mod.ad.null: Aggresion ~ Weather.scaled + Length.scaled + Time + Date + Buffer.650.scal
ed + (1 | Dummy)
mod.ad.dummy.site: Aggresion ~ Weather.scaled + Length.scaled + Time + Date + Buffer.65
0.scaled + (1 | Dummy) + (1 | Site)
                  npar    AIC    BIC  logLik deviance Chisq Df Pr(>Chisq)
mod.ad.null          8 598.37 623.91 -291.19   582.37
mod.ad.dummy.site    9 600.37 629.11 -291.19   582.37     0  1          1
> 
```

B



C

```
# Predicted values of Aggresion

Length.scaled | Predicted |       95% CI
----------------------------------------
           -2 |      3.00 | [2.59, 3.40]
           -1 |      3.25 | [3.00, 3.51]
            0 |      3.51 | [3.33, 3.69]
            1 |      3.77 | [3.51, 4.03]
            2 |      4.03 | [3.62, 4.43]
            3 |      4.28 | [3.71, 4.86]

Adjusted for:
* Site = 0 (population-level)
>
```

D

```
Fixed effects:
                   Estimate Std. Error         df t value Pr(>|t|)    
(Intercept)       3.001e+00  8.670e-01  1.800e+02   3.461 0.000671 ***
Weather.scaled   -1.425e-01  1.160e-01  1.800e+02  -1.229 0.220854    
Length.scaled     2.485e-01  9.536e-02  1.800e+02   2.606 0.009926 ** 
Time             -4.799e-04  3.766e-04  1.800e+02  -1.274 0.204224    
Date              6.724e-03  3.245e-03  1.800e+02   2.072 0.039690 *  
Buffer.650.scaled -1.850e-02  9.637e-02  1.800e+02  -0.192 0.848013    
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

E

```
Random effects:
 Groups   Name        Variance  Std.Dev.
 Site     (Intercept) 3.534e-20 1.880e-10
 Residual             1.557e+00 1.248e+00
Number of obs: 180, groups:  Site, 18

Fixed effects:
               Estimate Std. Error        df t value Pr(>|t|)    
(Intercept)     3.51111    0.09300 178.00000  37.754  < 2e-16 ***
Length.scaled   0.25745    0.09326 178.00000   2.761  0.00637 ** 
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Figure 8. Multivariate analysis in R for Garter snake *(Thamnophis sirtalis)* correlation with defensive behaviour and degree of urbanization. (A) ANOVA ran to demonstrate lack of effect by Site number in comparison to a dummy variable. (B) Variance inflation vectors demonstrating elements are not closely correlated and can be included as separate variables. (C) Predicted variables for graphing along with 95% confidence interval. (D) Buffer.650.scaled demonstrates correlation (p-value) of urbanization with the defensive factor. (E)Finally, significant correlation (p-value) between the length.scaled and the defensive factor.