The effect of urbanization on the physiology and behaviour of common garter snakes (*Thamnophis sirtalis*)

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Abstract

The world is in a major biodiversity crisis, mainly driven by anthropogenic land use, including urbanization. To predict future population changes, conservationists often require high-resolution, long-term data on changes in abundance. The predictive timeline framework of population collapse suggests that individual metrics of condition, such as behaviour and physiology, change in a predictable pattern before abundance declines, which would provide conservationists with more time to enact remediation measures. My objective was to determine how urbanization affects the behaviour and physiology of common garter snakes (*Thamnophis sirtalis*), and whether these changes are in line with the predictive timeline framework. I captured 220 snakes from 35 old fields in Ottawa/Gatineau, Canada, from April to October 2025; I chose the sites to vary in the amount of urbanization in the surrounding landscape. I recorded the snakes' antipredator behaviour and took a blood sample. There was no difference in snake abundance across the urbanization gradient. Snakes in areas surrounded by more urbanization used fewer antipredator behaviours, were less likely to be infected with hemoparasites, and those who were infected had lower parasite loads. The likelihood to flee before capture, body condition, heterophil to lymphocyte ratio, and plasma free glycerol did not differ across the urbanization gradient. My results provide some support for a predictable sequence of changes in individuals, in which behaviour is altered before physiology, and both are affected before declines in abundance are apparent. This framework must be tested in the field further to determine its applicability across different taxa and environmental stressors.

Résumé

Le monde est confronté à une crise majeure de la biodiversité, principalement due à l'utilisation anthropique des terres, dont l'urbanisation. Pour prédire les changements futurs des populations, les écologistes ont souvent besoin de données à haute résolution et à long terme sur les variations d'abondance. Le modèle prédictif d'effondrement des populations suggère que certains indicateurs de condition, comme le comportement et la physiologie, changent selon un schéma prévisible avant le déclin de l'abondance, ce qui permettrait aux écologistes de disposer de plus de temps pour mettre en œuvre des mesures correctives. Mon objectif était de déterminer comment l'urbanisation affecte le comportement et la physiologie de la couleuvre rayée (*Thamnophis sirtalis*) et si ces changements sont conformes au modèle prédictif d’effondrement. J'ai capturé 220 couleuvres dans 35 champs en friche de la région d'Ottawa/Gatineau, Canada, d'avril à octobre 2025 ; j'ai choisi ces sites de manière à ce que le degré d'urbanisation des paysages environnants varie. J'ai noté le comportement antiprédateur des couleuvres et prélevé un échantillon de sang. Aucune différence d'abondance n'a été observée le long du gradient d'urbanisation. Les couleuvres vivant dans des zones entourées de plus urbanisation présentaient moins de comportements antiprédateurs, étaient moins susceptibles d'être infectées par des hémoparasites et, lorsqu'elles étaient infectées, la charge parasitaire était moindre. La probabilité de fuir avant la capture, la condition physique, le rapport hétérophiles/lymphocytes et le glycérol libre plasmatique ne différaient pas le long du gradient d'urbanisation. Mes résultats fournissent un certain soutien à une séquence prévisible de changements chez les individus, où le comportement est altéré avant la physiologie, et les deux sont affectés avant que les déclins de l'abondance ne soient apparents. Ce cadre doit être validé plus souvent sur le terrain afin de déterminer son applicabilité à différents taxons et facteurs de stress environnementaux.

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Introduction

The world is currently in a conservation crisis: over a third of vascular plants are threatened with extinction (Nic Lughadha et al. 2020) and conservative estimates indicate that vertebrates are going extinct at over 50 times the background rate (Ceballos et al. 2015). In animals, the main driver of this widespread decline is habitat loss and modification in the form of agricultural development, deforestation, and urban expansion (Cox et al. 2022).

Anthropogenic habitat modifications have negative effects on populations; they diminish the amount of habitat available to animals, reduce the quality of the patches that remain, and limit movement between them (Tilman et al. 2017). These effects reduce survival (Zitske et al. 2011; Breininger et al. 2012) and gene flow between populations (Delaney et al. 2010), which may diminish population persistence (Mitrovich et al. 2018).

Population status is often assessed by measuring temporal trends in population abundance (Clements and Ozgul 2018). These abundance trends are used in analyses of biodiversity declines (Collen et al. 2009) and provide the basis of several International Union for Conservation of Nature (IUCN) Red List classification criteria (Rodrigues et al. 2006). Abundance has been used to assess population status at regional, national, and global scales (Gärdenfors et al. 2001; Gärdenfors 2001; Miller et al. 2007).

Temporal abundance trends only provide warning once declines are strong enough to be detected. When declines are small, population status assessments are often unreliable (Wilson et al. 2011), and struggle to predict precisely future population status (Wauchope et al. 2019). Predictions are more accurate when current data can be compared with pre-decline population baselines (Porszt et al. 2012), but these data are often not available. Assessments also require long-term, high-quality data to be reliable (Coulson et al. 2001; White 2019), which can be expensive and time-consuming to obtain when abundance alone is often not enough to predict accurately population persistence (Keith et al. 2015). Finally, when population declines are strong enough to be detected, the species can already be in a dire situation. These shortcomings limit the usefulness of abundance-based population status assessments when applied within conservation contexts.

Ecologists have begun to incorporate condition-related individual metrics into population status assessments to mitigate the drawbacks associated with traditional assessment methods. I use condition in this context to encompass individual traits that show plasticity to environmental stressors, such as behaviour and physiology (Clements and Ozgul 2016; Cerini et al. 2023). Individual metrics of condition reduce the length of abundance time series data needed to detect changes in abundance trends (Arkilanian et al. 2020). Evidence suggests there may be a predictable sequence in which behaviour, then morphology and physiology, and finally abundance respond to deteriorating environmental conditions (Cerini et al. 2025). Integrating condition-based data can improve the confidence in and reduce the uncertainty of population assessments (Speakman et al. 2025), as well as enhance their ability to anticipate the effects of changing environments (Johnston et al. 2019).

Given their role in improving population status assessments, individual metrics of condition may be used to identify potential factors that push populations towards decline at the landscape scale, such as urban development. Behavioural diversity among individuals changes along an urban-rural gradient in great tit (*Parus major*) populations (Gervais et al. 2025). Birds are more aggressive towards human (Walters et al. 2023) and conspecific (Davies et al. 2018; Lane and Sewall 2022) intruders in urban areas. In human-disturbed habitats, green frogs (*Rana* *clamitans*) have higher parasitic flatworm burdens despite similar abundance to natural habitats (VanAcker et al. 2019). Spotted salamanders (*Ambystoma maculatum)* exhibit markers of an acute stress response when crossing pavement compared to those crossing forest floor (Newcomb Homan et al. 2003). In general, animals are in poorer condition in environments modified by humans (Murray et al. 2019; Macdonald et al. 2023), suggesting that the effects of habitat disturbance can be felt before population metrics, such as abundance, are affected. In other words, the predictable sequence of individual changes that are thought to precede population declines (Clements and Ozgul 2016; Cerini et al. 2023) may also be used to identify landscape effects that contribute to these temporal changes.

While the literature on the effects of human disturbance on individuals is growing, the metrics of interest are limited. The most common physiological measure is stress hormones. Despite their prevalence in the literature, there is no consistency in how stress hormones change in response to urbanization (Iglesias-Carrasco et al. 2020). For example, common side-blotched lizards (*Uta stansburiana*) have higher baseline stress hormones in urban areas (Lucas and French 2012) while tree lizards (*Urosaurus ornatus*) show the opposite relationship (French et al. 2008). The other most common physiological metric is body condition, which shows a more consistent negative relationship with disturbance (Jiménez-Peñuela et al. 2019; Macdonald et al. 2023), although many species also have better body condition in urban areas (Balčiauskas and Balčiauskienė 2024; Chyb et al. 2025).

Other metrics of physiological condition, like hematology and plasma lipids, may reflect changes in habitat quality that are not captured by traditional measures of condition. Ecologists often measure stress using heterophil (neutrophil in anamniotes) to lymphocyte (H:L) ratios, as the changes in peripheral white blood cell composition in response to long-term stressors is well-documented (Davis et al. 2008; Davis and Maney 2018). H:L ratios may be a more accurate metric of chronic stress than hormones, as they do not show the same attenuation of response strength over time that is characteristic of stress hormones (Goessling et al. 2015). Food acquisition can be investigated by measuring plasma lipid metabolites, which is popular among ornithologists assessing stopover success in migrating birds (Jackson et al. 2023; Bouton et al. 2024; Beltran et al. 2024).

Research investigating the link between physiology and behaviour has grown in response to recent advances indicating changes in these metrics may reflect different stages of population collapse (Clements and Ozgul 2016; Cerini et al. 2023; Cerini et al. 2025). Roe deer (*Capreolus capreolus*) in lower quality environments have higher neutrophil to lymphocyte ratios and tend to struggle more during capture and release (Carbillet et al. 2019). Boldness, an aspect of behaviour, is related to parasite infection intensity in pumpkinseed fish (Gradito et al. 2024) and sleepy lizards (Payne et al. 2024). In response to flyover noise, the lizard *Aspidoscelis neotesselatus* increases feeding behaviour, but also accumulates ketones, a marker of fatty acid metabolism (Kepas et al. 2023). In general, animals in poorer nutritional condition tend to exhibit riskier behaviour, especially in the context of predation (Moran et al. 2021). Thus, it is important to include a variety of condition-related individual metrics to better understand the progression from individual effects to population effects.

Conclusions on how anthropogenic changes in habitat quality affect individuals are hindered by a narrow phylogenetic scope, particularly regarding squamate reptiles. Within meta-analyses investigating habitat disturbance and its effects on wild populations, reptiles are either excluded due to insufficient data (Messina et al. 2018) or comprise a miniscule proportion of included species (Keinath et al. 2017; Iglesias-Carrasco et al. 2020). Indeed, a global extinction-risk assessment was only conducted for reptiles over a decade after those for other tetrapod taxa had been published, despite their worldwide decline and high diversity (Cox et al. 2022). Since conservation efforts tend to focus on habitat loss (Hoffmann et al. 2010), these assessments were also likely conducted without information on how the surviving reptile populations fare in the human-modified environments that remain after habitat loss.

A broader variety of physiological metrics of condition have recently been explored in reptiles. H:L ratios have been measured in reptiles in relation to stress (Sparkman et al. 2014; Klukowski et al. 2025) and immunity (Neuman-Lee et al. 2019). H:L ratios are positively correlated with blood parasites in eastern foxsnakes (*Pantherophis gloydi*), but unrelated to body condition (Xuereb et al. 2012). The only comparison of H:L ratios between habitats are in turtles, where *Graptemys flavimaculata* have higher H:L ratios in disturbed areas (Selman et al. 2013) while *Chrysemys picta* show no difference (Turcotte 2023). Parasite load tends to be higher in more disturbed areas (Amo et al. 2006; Amo et al. 2007; Garrido and Pérez-Mellado 2015) though this relationship may be site specific (Putman et al. 2025), potentially because urban areas vary in the use of invertebrate management strategies, such as pesticides. Plasma lipid metabolites have been studied in the lab in relation to experimental food restriction, where garter snakes show decreased true triglycerides and increased glycerol when fasted (Neuman-Lee et al. 2015), which indicate a reduction of fat deposition and an increase in fat metabolism, respectively (Price 2017). Water snakes also show decreased total triglycerides after moderate food deprivation (Webb et al. 2017). There are few studies investigating plasma lipid metabolites in wild populations. The iguana *Cyclura cychlura*, however, has higher triglycerides where there is supplemental feeding by tourists (French et al. 2022), suggesting plasma metabolites may indicate foraging success. Interestingly, red-sided garter snakes show decreased total triglycerides after resumption of feeding post-hibernation (Whittier and Mason 1996), though this may be attributed to the difference between total triglycerides and true triglycerides, as the components of total triglycerides (free glycerol and glycerol associated with triglycerides) show opposite relationships with food deprivation (Neuman-Lee et al. 2015; Price 2017).

Antipredator behaviour is rarely studied at a landscape scale in reptiles, despite its direct relationship with survival (Sih et al. 2011). Flight initiation distance in lizards can increase (Amo et al. 2007) or remain unchanged (Amo et al. 2006) in response to human disturbance. Painted turtles are more likely to exhibit active defensive behaviours in more disturbed habitats (Turcotte et al. 2023; Blanchett et al. 2024). Rattlesnakes living close to humans rattle much later when a human approaches, and are less likely to rattle at all, compared to conspecifics in more natural areas (Atkins et al. 2021); they also are concealed more often (Beale et al. 2016). The snake *Natrix maura* is more likely to exhibit static antipredator behaviours when in worse nutritional condition (Hailey and Davies 1986), which may be due to the metabolic cost associated with active behaviours (Van Zele et al. 2024), and is relevant since most urban reptile populations are in poorer condition than rural or natural populations (Macdonald et al. 2023). Thus, antipredator behaviour may reflect how animals balance physiological costs and risk assessment.

Temperate reptiles, such as those in Canada, may be especially poised to experience physiological and behavioural changes in response to disturbance due to limitations in their dispersal and high fidelity to winter hibernation sites. Being ectotherms, their movement is dependent on available thermal energy, which is lower in higher latitudes. Thus, thermoconformity may come at a higher cost to northern reptiles (Blouin-Demers and Weatherhead 2001; Bulté and Blouin-Demers 2010), leading them to potentially prioritize thermal quality over other habitat characteristics. Due to these thermal constraints, temperate reptiles may be less able to search for better habitat patches compared to other vertebrates. Additionally, northern reptiles show high fidelity to their winter hibernation sites (Brown and Parker 1976; Smith et al. 2009; Blouin-Demers and Weatherhead 2021), possibly exposing them to unsuitable conditions as anthropogenic land modification encroaches. Thus, Canadian reptiles provide an excellent model to investigate the effects of habitat disturbance on individual physiology and behaviour.

Canadian reptiles are a microcosm of the global conservation crisis, as 77% of native reptiles are at risk of extinction nationally (Lesbarrères et al. 2014). This is a striking contrast to the 19% of reptilian species threatened worldwide (Cox et al. 2022). Canada's reptiles face many of the same threats as their global counterparts: habitat destruction and modification via anthropogenic change. These consequences culminate in global reptile abundance being one-third lower in human modified landscapes compared to natural environments (Doherty et al. 2020). Documenting how habitat disturbance may change the physiology and behaviour of Canadian reptiles is thus a promising avenue to uncover the proximate drivers of these population declines, and to identify warning signals that are detectable before the population declines have occurred.

My objective was to determine how urbanization changes the physiology and antipredator behaviour of common garter snakes (*Thamnophis sirtalis*). Garter snakes are a common reptile in North America, several physiological studies have been conducted on them (Sparkman et al. 2014; Halliday et al. 2015; Neuman-Lee et al. 2019), and their behaviour is well-characterized (Shine et al. 2000; Maillet et al. 2015; Gregory 2016). I selected 35 fields around the Ottawa/Gatineau, Canada region that vary along a gradient of urbanization. At each site, I placed coverboards to sample snakes. Each snake underwent a behavioural assay, and a blood sample was obtained. The first question I aimed to answer was: How does urbanization change the physiology of garter snakes? I hypothesized that urbanization reduces habitat quality for snakes, negatively affecting their physiological condition. I predicted that as urbanization increases, H:L ratios would increase, body condition would decrease, and free glycerol (glycerol not associated with triglycerides) would increase. While plasma triglycerides have been more often used to assess health and habitat quality, the small body of evidence in garter snakes indicates that free glycerol may be a more indicative metric of nutritional state (Neuman-Lee et al. 2015). I also documented how hemoparasite load and general hematology parameters (differential leukocyte count, hematocrit) vary with habitat disturbance, though this analysis was exploratory because there is not enough information on these metrics to propose a hypothesis. For example, disruption in the host-parasite relationship may drive any differences in parasite load along the urbanization gradient (Werner and Nunn 2020), independent of any effect of host condition. The second question I aimed to answer was: how does urbanization change the expression of antipredator behaviour in snakes? The evidence of behavioural shifts due to human disturbance is mixed, and multiple hypotheses may apply. Animals may become less defensive (Atkins et al. 2021) or more defensive (Turcotte et al. 2023) in response to disturbance, and it is difficult to suggest a mechanism behind these changes with the small body of research exploring this relationship currently available. Thus, this question was exploratory, and while I expected some sort of behavioural change in response to urbanization, I could not predict the direction of change, nor hypothesize a mechanism behind it. I also estimated abundance to determine whether any changes in physiology and behaviour could be attributed to differences in density across the urbanization gradient. To the best of my knowledge, no study to date has measured metrics of stress, immunology, nutrition, and behaviour at a landscape scale in a reptile.

Methods

Site Selection and Habitat Variables

I selected 35 sites (Figure 1) along an urbanization gradient by combining Ontario (Ontario Ministry of Natural Resources and Forestry 2023) and Québec (Institut de la statistique du Québec 2024) land use datasets in ArcGIS Pro 3.5.3 following Gigeroff and Blouin-Demers (2023) and LaFlamme and Blouin-Demers (2026). Sites consisted of old agricultural or hay fields, which garter snakes prefer (Halliday and Blouin-Demers 2016). This resulted in four land classes: urban, water, forest, and field. I also quantified road density using the 2024 Road Network File from Statistics Canada (https://www12.statcan.gc.ca/census-recensement/2011/geo/RNF-FRR/index-s-eng.cfm?year=24). In addition to impermeable cover (e.g., buildings, concrete), the urban land class included heavily modified greenspace, such as sports fields and lawns. There are no recent data on common garter snake movement within a season, but congenerics have been reported moving on average between 100-200 m in a season, with maxima between 300-400 m (Imlay et al. 2016; Shonfield et al. 2019). Thus, to include the potential area of effect for landscape variables, I constructed 10 buffers from 100 m to 1000 m, in increments of 100 m, around each site using the Buffer function in ArcGIS. I calculated the land composition within each buffer, in m^2, using the Tabulate Area function. I then determined the percent composition of each land class within the buffers. Percent urban within each buffer size varied along a gradient from a minimum of 0% to maxima of 62-90%, depending on buffer size. All buffers were centered around a field or forest edge (Figure 2), as garter snakes tend to prefer these habitats (Halliday et al. 2015; Halliday and Blouin-Demers 2015; Diaz and Blouin-Demers 2018).

Field Sampling

I installed 15 plywood coverboards at each site to sample snakes (Halliday and Blouin-Demers 2015). I visited sites approximately once per week from 23 April to 10 October 2025. I conducted sampling between 08:00 and 17:00 on clear days with air temperatures between 9˚C and 30˚C. Upon detection of a snake (when the coverboard was flipped), I recorded its predator avoidance strategy (crypsis or flee), after which it was captured. I then recorded its predator deterrence strategy (musk, gape, strike) while being held. Each snake was assigned a predator avoidance and predator deterrence score based on their behaviour. Predator avoidance was scored as either a 0 (crypsis, interpreted as a willingness to risk detection to remain in a chosen microhabitat) or a 1 (flee, interpreted as risk-aversion). Predator deterrence was scored as the number of behaviours exhibited by the snake. There were three specific behaviours that I was looking for: musk, gape, and strike (Shine et al. 2000; Maillet et al. 2015; Turcotte et al. 2023; Blanchett et al. 2024). Musking was defined as the expulsion of feces and/or liquid musk out of the cloaca. Gaping was defined as the opening of the mouth towards the person holding the snake, but with no extension of the body (i.e., no attempt to bite). Striking was defined as the extension of the snake's body, with mouth open, towards the person holding the snake (i.e., an attempt to bite). A higher score was interpreted as a greater willingness to invest resources in current survival at the risk of future energy resources for survival/reproduction, as antipredator displays are energetically costly (Van Zele et al. 2024).

Immediately after determining the snake's predator deterrence strategy, I collected a blood sample (200 µl) from the caudal vein using a 0.5 mL syringe fitted with a 28-gauge needle (Halliday et al. 2015). The blood was then transferred slowly back through the needle into a microcentrifuge tube containing 10 µL of 0.8% saline mixed with sodium heparin (catalog

#375095100KU, MilliporeSigma, Oakville, ON, Canada) at a concentration of 2500 U/mL, and stored on ice while in the field. Since it is difficult to take blood samples reliably from small snakes, I only collected blood from snakes 20 g or heavier.

After blood collection, I uniquely marked each snake using a medical cautery unit on the ventral scales for future identification (Winne et al. 2006). I probed snakes to determine sex, measured them from snout to vent, and weighed them using a 600 g Pesola spring scale. I used snout-to-vent length (SVL) and mass to calculate the scaled mass index (SMI), a metric of body condition. SMI accounts for changes in body shape over time that bias other body condition indices, and aids comparison between populations (Peig and Green 2009). I recorded body temperature using an infrared thermometer gun (Halliday and Blouin-Demers 2017).

All protocols were approved by the University of Ottawa Animal Care Committee (protocol BLf-4498) and all animals were handled under an Ontario Wildlife Scientific Collector’s Authorization (permit number 1107132) and Permis Scientifique du Québec (permit number 25-07-SF-002-GR-0).

Plasma Lipid Analysis

I took blood samples to the lab at the end of each day. I placed approximately 70 µL of each sample in a microcapillary tube and centrifuged it at 12 000 rpm for 3 minutes (Clay Adams Autocrit Ultra 3, Becton, Dickinson, and Company, Franklin Lakes, NJ, USA) at room temperature to determine the hematocrit. I centrifuged (Eppendorf Centrifuge 5415D, Eppendorf Canada, Mississauga, ON, Canada) the remainder of each sample at 210 *g* for 10 minutes at 4˚C, after which I collected the plasma, placed it in a new microcentrifuge tube, and stored it at -80˚C until analysis.

I determined plasma free glycerol concentration using a colorimetric assay (catalog #MAK117, Sigma-Aldrich, St. Louis, MO, USA). I analyzed a subset of 63 samples that were selected to maximize the range of urbanization included and ensure only high quality plasma samples were used (e.g., sufficient quantity, minimal hemoglobin contamination). Since this assay has not been used in snakes, I validated the assay by performing a serial dilution to determine parallelism with the standard curve. I diluted samples 9:1 with water and performed the assay with a SpectraMax 340PC384 microplate reader (Molecular Devices, San Jose, CA, USA) according to the manufacturer's instructions. The mean intra-assay and inter-assay coefficients of variation were 7.2% and 8.7%, respectively.

Hematology

To determine H:L ratios, I created 3 blood smears per individual in the field by placing 1-2 drops of blood on a microscope slide and smearing them with the edge of another slide. I air-dried the slides and stored them in the dark until I returned to the lab at the end of the day. Once in the lab, I fixed the smears with 100% methanol and air dried them again before staining them with Wright-Giemsa stain (Fisherbrand™ SureStain™, catalog #CS434D, Fisher Scientific, Pittsburgh, PA, USA) following manufacturer's instructions (Neuman-Lee et al. 2019; Turcotte 2023). I observed smears with an Olympus CX41 compound microscope at 1000x magnification under oil immersion. For the leukocyte differential, I identified the first 100 leukocytes (lymphocytes, heterophils, monocytes, eosinophils and basophils, Appendix A) along a line perpendicular to the blood smear. This was repeated, and I averaged the number of each leukocyte counted over the 200 total observed cells to obtain a proportion (Turcotte 2023). I determined approximate total leukocyte counts by counting the number of leukocytes within 10 fields of view at 400x in the monolayer, and then multiplying the total by 200, to obtain a concentration per microlitre of whole blood (Samour 2016; Winter et al. 2019). I determined hemogregarine infection intensity by counting the number of cells infected along one full line (from edge to edge) within the monolayer at 400x. Hemogregarines are the most common hemoparasite found in reptiles (Campbell 2015).

Statistical Analyses

I conducted statistical analyses using R version 4.4.1 (R Core Team 2024). Full models included potential control variables such as body temperature, snout to vent length (SVL), sex, time of day, and date, as these variables affect behaviour (Shine et al. 2000) and physiology (Whittier and Mason 1996; Price 2017). Full models also included all landscape variables (percent field, forest, water, and urban, as well as total length of roads within the buffer). For each landscape variable, I included the buffer size that had the largest absolute correlation with each respective response variable (Jackson and Fahrig 2015; Gigeroff and Blouin-Demers 2023; LaFlamme and Blouin-Demers 2026). The models often did not converge when including both site and individual identification as random effects, so I removed individual ID as an effect and filtered my data to keep only the first capture of each snake. Thus, random effects included site only to control for the likelihood that snakes at the same sites may be more similar to each other. I centred and scaled all continuous variables to facilitate interpretation.

I constructed all full models using the glmmTMB package (McGillycuddy et al. 2025). Model assumptions were assessed visually using the performance package (Lüdecke et al. 2021) and with DHARMa plots (Hartig 2024). I removed any predictors with a variance inflation factor greater than 2. I also assessed correlation between predictors and removed any that had an absolute correlation of 0.7 or greater. Due to high correlation between road and urban within each buffer (maximum r = 0.88), I decided to include urban only in my models. To reduce overfitting, I dredged each model using the MuMIn package (Bartoń 2025) and the models with a difference in AICc of less than two compared to the best performing model for each response variable were averaged to compute final fixed effect coefficients.

I created a linear model to determine the relationship between abundance and my landscape variables to ensure that any changes in individual metrics could not be attributed to changes in snake density across the landscape. I dredged and averaged the final model following the methods described above. I did not recapture enough snakes to estimate population size based on mark-recapture, so for my abundance metric I used the mean number of unique snakes caught per visit at each site. I included all 35 sites in my abundance analysis, and fixed effects included my four habitat variables.

Results

I caught snakes at 32 of the 35 selected sites. Across these 32 sites, I captured 220 individual snakes. I captured more females (n = 129) than males (n = 90) and one snake that was unsexed due to injury was excluded from the analyses. Overall, 42% of captures occurred under coverboards. Snakes ranged in SVL from 12 cm to 83 cm, with a median SVL of 41.5 cm (Table 1). Excluding the three sites at which no snakes were caught, the mean number of snakes caught per site was 6.9 with a range of 1 to 23. The scale of maximum effect of landscape variables on abundance ranged from 100 m to 1000 m (Figure C1). Abundance did not vary significantly across the urbanization gradient (Figure 3), though I did catch significantly more snakes at sites with more water in the surrounding landscape (Table 2).

Behavioural Responses

The scale of maximum effect of landscape variables on the likelihood to flee ranged from 100 m to 900 m (Figure C2). The final averaged model for the likelihood to flee included sex, body condition, body temperature, date, and the proportion of field, forest, and urban in the surrounding landscape (Table 3). Date and the proportion of field in the surrounding landscape were the only significant predictors, with the likelihood to flee increasing over the field season and decreasing as the proportion of field in the surrounding landscape increased. The likelihood to flee decreased by a factor of 0.71 with every 10% increase in urbanization, but this effect was not statistically significant (95% CI [0.48, 1.04], p = 0.08; Figure 4).

The scale of maximum effect of landscape variables on the number of expressed antipredator behaviours varied from 100 m to 900 m (Figure C3). The final averaged model for behavioural score included sex, SVL, body temperature, date, proportion of forest, and proportion of urban in the surrounding landscape (Table 3). Sex and urbanization were significant predictors of the number of antipredator behaviours exhibited, with males expressing fewer antipredator behaviours than females, and a 10% increase in urbanization predicted to decrease the average behavioural response by a factor of 0.90 (95%CI [0.85, 0.97], p = 0.003; Figure 5).

Physiological Responses

The scale of maximum effect of landscape variables on body condition ranged from 300 m to 1000 m (Figure C4). The final averaged model for body condition included SVL, date, and the proportion of field, water, and urban in the surrounding landscape (Table 4). There were no significant predictors in this model. A 10% increase in urbanization predicted to increase the average SMI by only 0.06 units (95%CI [-0.33, 0.45], p = 0.77; Figure 6).

The scale of maximum effect of landscape variables on H:L ratio ranged from 100 m to 800 m (Figure C5). The final averaged model for H:L ratio included sex, body temperature, SVL, and the proportion of field and urban in the surrounding landscape (Table 4). SVL was the only significant predictor, with longer snakes having higher H:L ratios. With every 10% increase in urbanization, the average H:L ratio (on the log scale) increased by 0.01, but this effect was not statistically significant (95% CI [-0.04, 0.07], p = 0.707; Figure 7).

The scale of maximum effect of landscape variables on parasite load ranged from 100 m to 1000 m (Figure C6). The final averaged model for parasite load included body condition, SVL, date, and the proportion of field and urban in the surrounding landscape (Table 4). SVL and body condition were both significant, with longer snakes having higher parasite loads and snakes with higher body condition having lower parasite loads. Proportion of urban area was also significant, with the average parasite load decreasing by a factor of 0.64 for every 10% increase in urbanization (95% CI [0.49, 0.83], $p < 0.001$; Figure 8).

The scale of maximum effect of landscape variables on infection status ranged from 100 m to 1000 m (Figure C7). The final averaged model for parasite load included body condition, SVL, and the proportion of urban in the surrounding landscape (Table 4). SVL was a significant predictor, with longer snakes being more likely to have parasites. Proportion of urban area was also significant, with the odds of being infected decreasing by a factor of 0.63 for every 10% increase in urbanization (95% CI [0.47, 0.85], $p = 0.002$; Figure 9).

The scale of maximum effect of landscape variables on plasma free glycerol ranged from 100 m to 700 m (Figure C8). The final averaged model for plasma glycerol included date body condition, sex, and the proportion of forest and urban in the surrounding landscape (Table 4). Sex, body condition, and the proportion of forest were significant predictors, with males and snakes in better condition having higher glycerol levels, and snakes in areas with greater forest cover having lower glycerol levels. A 10% increase in urbanization was predicted to increase the average plasma glycerol level by 0.14 mM, but this effect was not statistically significant (95%CI [-0.17, 0.44], $p = 0.38$; Figure 10).

Discussion

In examining the effect of urbanization on individual metrics of condition in garter snakes, I found that both behaviour and physiology change with the degree of urbanization in the surrounding landscape. Furthermore, these changes are independent of local garter snake abundance, as the number of snakes caught at each site, after accounting for sampling effort, did not vary across the urbanization gradient. This is consistent with a previous investigation of garter snake abundance in my study area that found no effect of urbanization on abundance (Gigeroff and Blouin-Demers 2023).

Garter snakes exhibited a dampened antipredator response at sites surrounded by more urban areas. Their likelihood to flee, while marginally non-significant, did decrease as urbanization in the surrounding landscape increased. Concurrently, snakes in areas surrounded by more urbanization used fewer antipredator behaviours after capture. Given that the effect of body condition was not significant in either model, this depressed response in areas surrounded by more urbanization is unlikely to be due to lower energy reserves available to fuel active behaviours. Similar to my results, western rattlesnakes (*Crotalus oreganus*) show a delayed antipredator response (rattling) when encountering a threat (humans) in areas surrounded by urbanization compared to rural areas (Atkins et al. 2021). In the lab, Glaudas (2004) demonstrated that cottonmouths (*Agkistrodon piscivorus*) rapidly habituate to handling by humans. Contrary to my results, several studies in reptiles have found an increased antipredator response in urban-surrounded areas. Painted turtles (*Chrysemys picta*) are bolder and more aggressive in urbanized areas (Blanchett et al. 2024) and use more antipredator behaviours in areas with higher boat traffic (Turcotte et al. 2023). This suggests that responses to urbanization may be species- or population-specific, or may depend on the actual frequency of interactions of the study animals with humans.

A likely mechanism behind the reduced antipredator response in urban-surrounded areas that I observed in garter snakes is habituation to non-lethal stressors. Whether this reflects habituation to predatory threats in general, or just habituation to humans, is unclear. There is evidence that garter snakes may not see humans as predators (Gregory 2016) and, consequently, may behave differently when encountering a human versus a natural predator. In the laboratory, there is no evidence of habituation in antipredator behaviour over repeated trials in common garter snakes when the predatory threat is a simulated bird claw (Maillet et al. 2015). On the other hand, cottonmouths do seem to habituate to humans (Glaudas 2004). Therefore, garter snakes may behave differently in encounters with humans than with their natural predators. Work with captive rattlesnakes (Scudder and Chiszar 1977) and garter snakes (Herzog et al. 1989), however, indicated that antipredator responses were similar regardless of whether snakes were exposed to humans or to predator models. How these laboratory results reflect behaviour in the field has yet to be investigated. There may also be genetic differences between garter snake populations along the urbanization gradient that can explain the differences in behaviour. For instance, individuals with a genetic predisposition for a marked anti-predator response could suffer higher mortality when they encounter humans. Indeed, a combination of learning through habituation and genetics may be contributing to the pattern of behaviour I observed across the urbanization gradient. The specific mechanistic explanations for this variation in behaviour, however, is beyond the scope of the present study.

Body condition did not change across the urbanization gradient; garter snakes in urban-surrounded areas are not in worse physiological condition than snakes in natural areas. This suggests that the quality of habitat patches that are surrounded by urbanization may remain high despite their smaller size, at least for garter snakes. While snakes in urban-surrounded areas tend to have worse body condition (Lomas et al. 2015; Mészáros et al. 2023), the responses to urbanization of reptiles in general are more variable (French et al. 2018; Macdonald et al. 2023). Because some generalist species show the same body condition response to urbanization as some specialists (Macdonald et al. 2023), the variability in reptile responses to urbanization may be due to differing ability to mediate environmental stressors through behaviour (Sih et al. 2011). For example, Iberian wall lizards (*Podarcis hispanica*) that habituate more readily to frequent simulated predatory attacks show increased body condition compared to those that do not habituate (Rodríguez-Prieto et al. 2010). I do not think this is the case in my study, however, as body condition was not a significant predictor of antipredator behaviour.

There was no effect of urbanization on H:L ratio, again suggesting snakes in urban-surrounded areas are in no worse physiological condition than those in natural areas. There are very few studies investigating H:L ratios in response to urbanization in snakes. Bell (2013) did find that northwestern garter snakes (*Thamnophis ordinoides)* have higher H:L ratios in urban-surrounded areas. In turtles, *Graptemys flavimaculata* exhibit higher H:L ratios in areas with more urban cover (Selman et al. 2013), while *Chelodina oblonga* have similar H:L ratios in urban and in natural areas (Refsnider et al. 2026). In contrast, urban lizards often have lower H:L ratios than lizards in natural areas (French et al. 2008; Amdekar et al. 2018). Similar to body condition, the variation in H:L ratio in response to urbanization could be mediated by behaviour (Sih et al. 2011). Further investigation into how urbanization affects the relationship between physiology and behaviour is clearly an avenue for future research.

Garter snakes in urban-surrounded areas had lower hemoparasite loads and were also less likely to be infected compared to snakes in natural areas. This relationship has been documented in lizards (González-Morales et al. 2025; Putman et al. 2025), but is not universal (French et al. 2008; Kaakai et al. 2025). Indeed, most animals have higher parasite loads in urban areas (Murray et al. 2019). A meta-analysis in mammals, however, suggests that parasites requiring multiple hosts to complete their life cycle may be less abundant in urban areas due to a disruption in the parasite-host relationship (Werner and Nunn 2020). It is possible that this disruption plays a role in the lower hemogregarine abundance in garter snakes from urban-surrounded areas; the definitive hosts for hemogregarines are often biting arthropods such as ticks, mites, and mosquitos (Campbell 2015), which are less abundant in urban areas in my study region (Rakotoarinia et al. 2022; Logan et al. 2024).

Longer snakes, and snakes with lower body condition, had higher parasite loads. Given that snake length can be used as a proxy for age, it is likely that infection intensity is driven by the increased likelihood of exposure over time (Brown et al. 2006). Longer snakes may also provide more surface area for parasites to target (Watkins and Blouin-Demers 2019). Snakes with higher infection rates having lower body condition suggests that higher hemogregarine loads incur a fitness effect, or that snakes in poorer condition are less able to combat parasites. Greater hemogregarine infection is associated with lower body condition and slower growth rates in water pythons (*Liasis fuscus*); those with high parasite abundance also exhibited reduced survival (Madsen et al. 2005). Hemogregarine infection, however, has no discernible effect on body condition in several other snake species (Brown et al. 2006; Sperry et al. 2009; Xuereb et al. 2012), suggesting that there may be other environmental or biological factors that mediate the effect of hemogregarines on snake condition, such as exposure to and absorption of toxic substances (Murray et al. 2019).

This is the first study that I am aware of that looked at lipid metabolism in a snake across a full field season. Despite garter snakes showing no difference in plasma glycerol levels across the urbanization gradient, I did find differences between the sexes and a relationship with body condition. In contrast to my finding that garter snakes in better condition have higher glycerol levels, terrestrial garter snakes (*Thamnophis elegans*) have higher plasma glycerol levels when fasted (Neuman-Lee et al. 2015). Given the likelihood that snakes with better body condition eat more often, this suggests that common garter snakes are more similar to green turtles (*Chelonia mydas*), where glycerol levels decrease during fasting (Price et al. 2013). The glycerol levels I documented in common garter snakes were also higher than those found in other reptiles (Price et al. 2013; Neuman-Lee et al. 2015; Butler et al. 2016); this may reflect that the populations in my study prioritize different energetic pathways than those studied previously. For example, garter snakes in Ottawa/Gatineau may rely more heavily on long-term energy storage in triglycerides over short-term storage in glycogen, resulting in higher overall glycerol levels (Price 2017). Together with the lack of effect of urbanization on body condition and H:L ratios, these results do not support my hypothesis that snakes in areas surrounded by more urbanization experience enough stress to show indicators of physiological decline.

Conclusion and Future Directions

My results provide some support for the framework that individual metrics of condition will be affected before population metrics, such as abundance, respond to landscape changes. Cerini et al. (2023) proposed the predictable sequence of individual responses to environmental stress, in which changes in behaviour, then changes in morphology and physiology, and finally changes in population metrics will be detected. My results indicate that urbanization may provide sufficient environmental stress such that garter snakes change their behaviour to mediate its effects. My study also provides some support towards the notion that the physiology of snakes in urban-surrounded areas is altered, though only one of my physiological metrics showed significant differences along the urbanization gradient. This is partly in line with the predictive framework of population collapse (Cerini et al. 2023; Cerini et al. 2025), and indicates that while the populations of garter snakes surrounded by more urbanization in my study area are currently stable, they may face declines in abundance in the future. Alternatively, changes in behaviour may be sufficient for garter snakes to mediate the effect of urbanization, and no further changes in individual metrics may occur. It would be worth revisiting these populations in the future to determine how they change over time.

My study highlights the importance of using multiple metrics of individual condition across several populations. This allows researchers to connect individual responses to population impacts with better confidence (French et al. 2018). Investigating only one or two individual metrics is often not enough to get a detailed picture of how landscape affects condition, especially when changes in these metrics may be driven by different underlying factors (Reid et al. 2024). Additionally, many previous studies on urbanization only focus on a small number of populations (Amo et al. 2007; Atkins et al. 2021; Putman et al. 2025) which amplifies site-specific effects and minimizes the conclusions that can be made about urbanization itself. Indeed, treating urbanization as a binary versus a continuous variable can change the conclusions regarding its effect on individuals (Reid et al. 2024).

There are several avenues of potential research that can further clarify the effect of urbanization on individuals. I was unable to include both roads and urban land cover in my models due to high correlations between them. Roads may exert an effect that is independent of other types of urbanization, however, likely driven by road mortality. For example, Gigeroff and Blouin-Demers (2023) found that garter snakes are less abundant in areas surrounded by more roads, but not in areas with more urbanization. A carefully designed study could select sites to minimize the correlation between roads and urbanization, such that their effects could be separated, which would assist in understanding how different types of urbanization may exert unique pressures on populations.

Additionally, sampling populations for a longer period of time would provide information on how consistent individual metrics are over time, and how they relate to each other. I did not recapture enough snakes to be able to estimate the correlation and consistency between and within metrics of condition. There is some evidence that different physiological metrics do correlate within individuals (Gangloff et al. 2017; Neuman-Lee et al. 2019), but that they are not necessarily consistent over time (Sparkman et al. 2014), suggesting that these metrics may fluctuate along with environmental stressors. This further supports the importance of including multiple metrics when measuring condition in individuals.

In conclusion, I have provided evidence that urbanization is associated with behavioural and physiological changes in garter snakes, consistent with the population collapse timeline framework presented by Cerini et al. (2023). I highlight the need for more studies testing this framework in the field, and the importance of incorporating individual metrics of condition, such as behaviour and physiology, into long-term population monitoring to predict better how changes in individuals will affect future population dynamics.

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Tables

Table 1 Summary values of biological characteristics and response variables of common garter snakes (*Thamnophis sirtalis*) at 35 old fields in the Ottawa/Gatineau, Canada, area in summer 2025.

	Female			Male		
Parameter[a]	N	Mean	SD	N	Mean	SD
SVL (cm)	129	43.8	15.5	90	34.7	11.2
Mass (g)	129	56.6	51.7	90	25.4	19.7
Likelihood to flee	45	0.16	0.37	45	0.24	0.43
Behavioural score	127	1.3	0.77	89	0.88	0.64
SMI	129	33.7	6.8	90	33.4	8.3
H:L ratio	85	0.13	0.11	46	0.11	0.23
Parasite load	85	2.1	7.8	46	3.8	16.1
Infection status	85	0.20	0.40	46	0.15	0.36
Plasma glycerol (mM)	37	5.0	1.7	26	5.7	1.8

[a] SVL = snout-vent length; SMI = scaled mass index; H:L ratio = heterophil to lymphocyte ratio.

Table 2 Summary statistics of the averaged linear model for predicting the abundance of garter snakes at 35 old fields in the Ottawa/Gatineau, Canada, area in summer 2025. Abundance was determined as the mean number of snakes caught per visit at each site. Parameters significant at $\alpha = 0.05$ are in bold.

Parameter	Estimate	Standard error	z-statistic	p-value
Intercept	**0.34**	**0.049**	**6.61**	**<0.001**
%Urban (700 m)	0.06	0.061	1.00	0.316
%Water (100 m)	**0.16**	**0.051**	**3.02**	**0.003**

Table 3 Summary statistics of averaged mixed models for behavioural response variables. The likelihood of common garter snakes (*Thamnophis sirtalis*) to flee upon detection was assessed at 35 old fields in the Ottawa/Gatineau, Canada, area in the summer of 2025, after which they were captured. Once caught, behavioural score was measured as the number of the following behaviours they expressed: musk, gape, strike. Given that these are averaged models, the reported marginal R^2 values are derived only from the model with the lowest AICc. Parameters significant at $\alpha = 0.05$ are in bold.

Parameter[a]	Estimate	Standard error	z-statistic	p-value
Likelihood to flee (n = 90) $R^2 = 0.44$				
Intercept	**-2.10**	**0.54**	**3.85**	**<0.001**
Body temperature	0.72	0.37	1.91	0.056
Date	**0.76**	**0.34**	**2.17**	**0.030**
Sex (male)	0.26	0.55	0.47	0.636
Body condition	0.24	0.30	0.78	0.434
SVL	0.03	0.13	0.20	0.839
%Field (100 m)	**-0.97**	**0.48**	**1.99**	**0.046**
%Forest (700 m)	-0.52	0.48	1.08	0.282
%Urban (800 m)	-0.74	0.42	1.74	0.081
Behavioural score (n = 216) $R^2 = 0.07$				
Intercept	0.19	0.07	2.90	0.004
Body temperature	-0.001	0.01	0.12	0.906
Date	0.001	0.01	0.10	0.924
Sex (male)	**-0.32**	**0.10**	**3.28**	**0.001**
SVL	0.05	0.05	0.93	0.350
%Forest (900 m)	-0.04	0.05	0.88	0.380
%Water (200 m)	-0.02	0.05	0.46	0.645
%Urban (100 m)	**-0.22**	**0.07**	**2.96**	**0.003**

[a] SVL = snout-vent length.

Table 4 Summary statistics of averaged mixed models for physiological response variables of common garter snakes (*Thamnophis sirtalis*) captured at 35 old fields in the Ottawa/Gatineau, Canada, area in the summer of 2025. Body condition was measured using the scaled mass index. Heterophil to lymphocyte (H:L) ratio, parasite load, and infection status were determined by analyzing a blood smear made with whole blood. The parasites of interest were hemogregarines. Plasma free glycerol was determined using a spectrometric endpoint assay. Given that these are averaged models, the reported marginal R^2 values are derived only from the model with the lowest AICc. Parameters significant at $\alpha = 0.05$ are in bold.

Parameter[a]	Estimate	Standard error	z-statistic	p-value
Body condition (n = 219) $R^2 = 0.02$				
Intercept	**33.73**	**0.83**	**40.51**	**<0.001**
Date	-0.90	0.59	1.51	0.130
Sex (male)	0.04	0.34	0.13	0.899
SVL	0.07	0.26	0.26	0.792
%Field (400 m)	0.05	0.28	0.16	0.871
%Water (300 m)	-0.03	0.29	0.10	0.920
%Urban (1000 m)	0.12	0.43	0.29	0.772
Log H:L Ratio (n = 131) $R^2 = 0.12$				
Intercept	**-2.54**	**0.08**	**29.94**	**<0.001**
Body temperature	0.03	0.06	0.47	0.637
Sex (male)	-0.03	0.09	0.28	0.781
SVL	**0.32**	**0.08**	**4.02**	**<0.001**
%Field (200 m)	-0.004	0.03	0.13	0.898
%Urban (400 m)	0.02	0.05	0.38	0.707
Parasite load (n = 131) $R^2 = 0.68$				
Intercept	0.04	0.65	0.06	0.949
Date	-0.06	0.16	0.38	0.702
Body condition	**-0.72**	**0.27**	**2.67**	**0.008**
SVL	**0.66**	**0.22**	**3.04**	**0.002**
%Field (100 m)	-0.07	0.17	0.41	0.684
%Urban (700 m)	**-1.00**	**0.30**	**3.33**	**<0.001**
Infection status (n = 131) $R^2 = 0.31$				
Intercept	**-2.01**	**0.40**	**5.05**	**<0.001**
SVL	**0.64**	**0.29**	**2.19**	**0.029**
Body condition	-0.38	0.36	1.05	0.296
%Urban (700 m)	**-1.03**	**0.33**	**3.06**	**0.002**
Glycerol (n = 63) $R^2 = 0.31$				
Intercept	**4.86**	**0.27**	**17.80**	**<0.001**
Date	0.20	0.24	0.83	0.408

Sex (male)	**1.01**	**0.39**	**2.51**	**0.012**
Body condition	**0.51**	**0.22**	**2.27**	**0.023**
%Forest (200 m)	**-0.66**	**0.22**	**2.91**	**0.004**
%Urban (300 m)	0.22	0.25	0.88	0.380

[a] SVL = snout-vent length.

Figures

Figure 1 Map of 35 old fields from the Ottawa/Gatineau region in Canada where common garter snakes (*Thamnophis sirtalis*) were captured. Sites were visited approximately once a week between April and October of 2025.

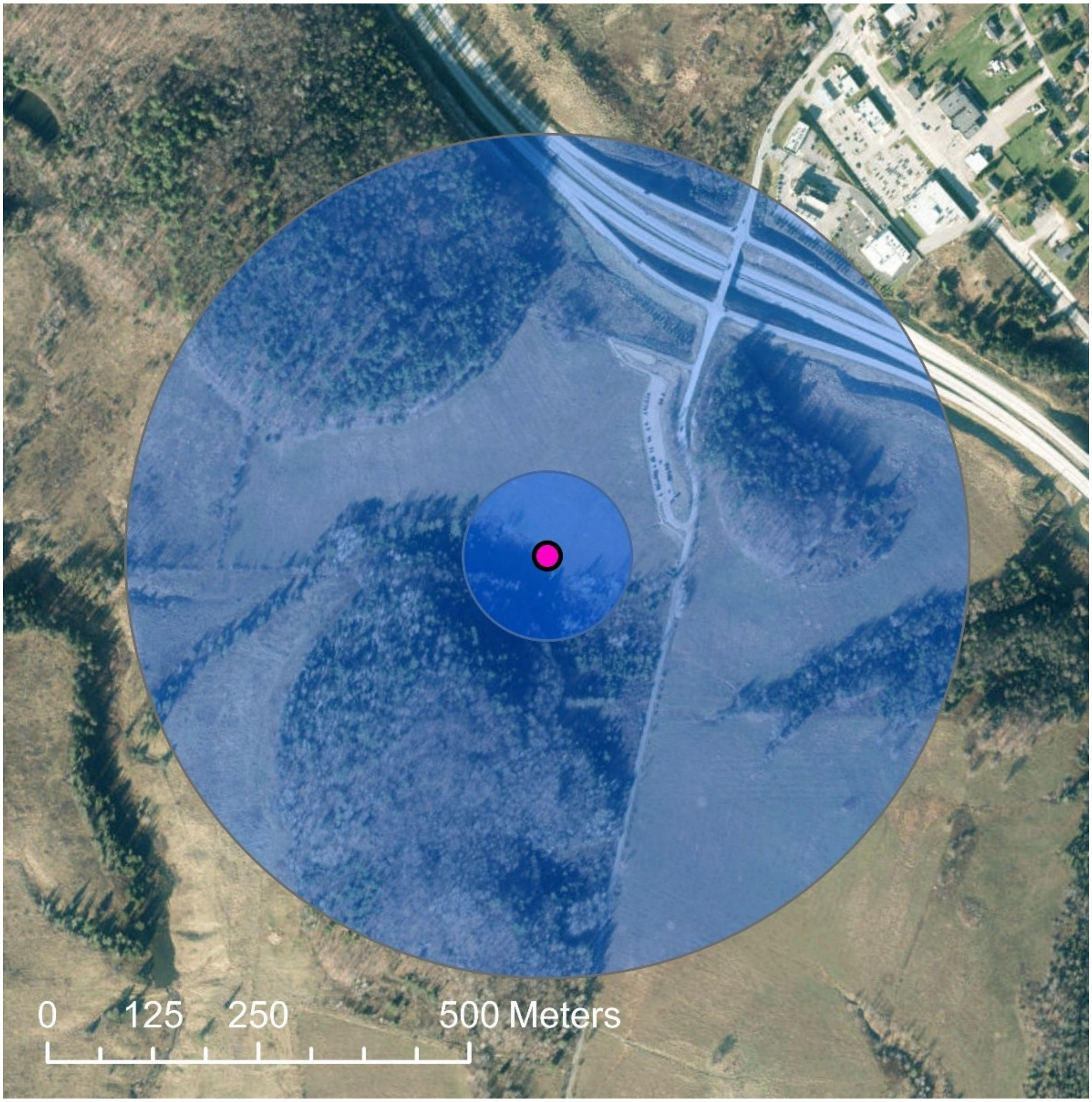

Figure 2 Example of site location and buffer creation in ArcGIS Pro. The pink dot in the center represents the centre of the coverboard array on the habitat edge between forest and field, where common garter snakes (*Thamnophis sirtalis*) were captured in the summer of 2025 in Ottawa/Gatineau, Canada. The two surrounding blue circles represent the 100 m and 500 m buffers, respectively.

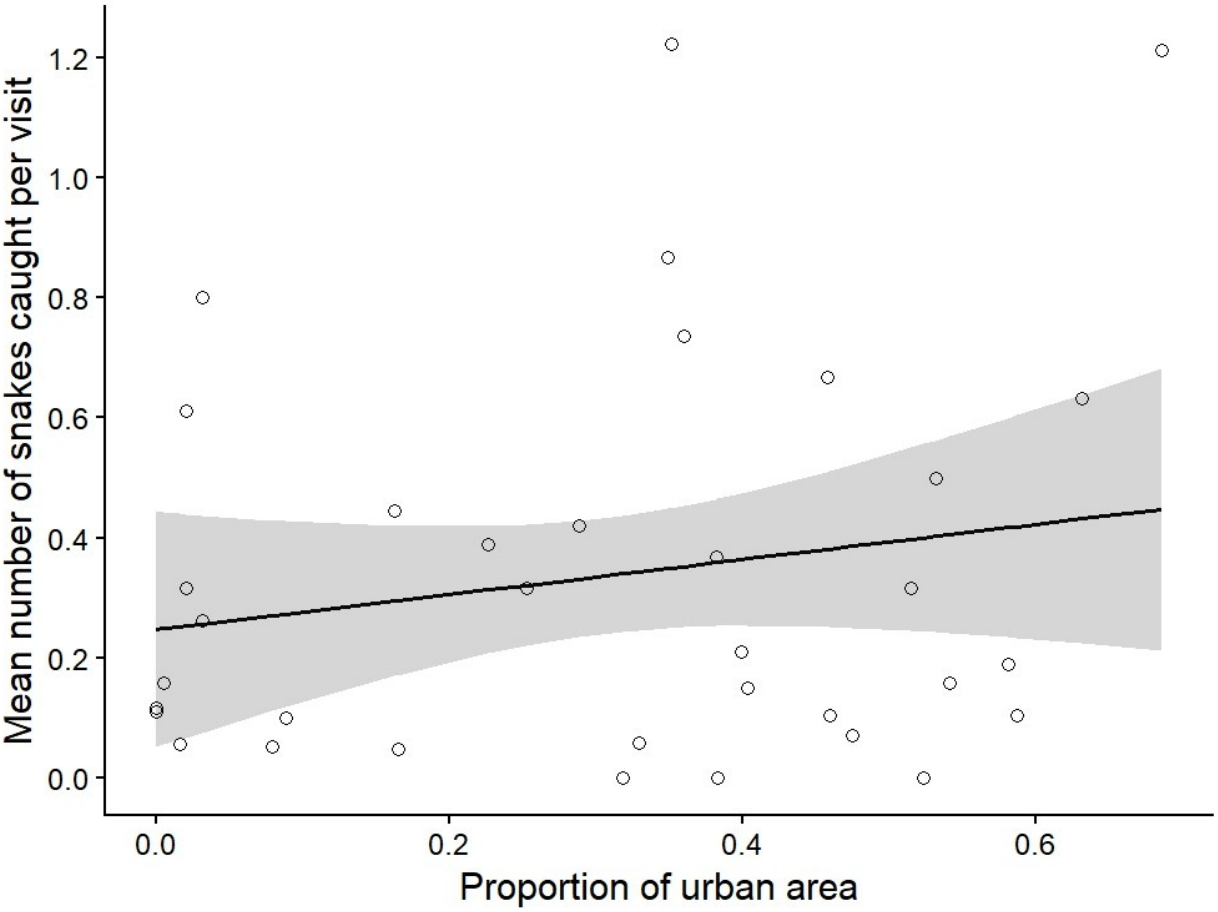

Figure 3 Model-predicted common garter snake (*Thamnophis sirtalis*) abundance at 35 old fields in Ottawa/Gatineau, Canada, along an urbanization gradient. Abundance was measured as the mean number of snakes caught per visit during the summer of 2025. Urban was measured as the proportion of urban land cover in the surrounding landscape. The effect of urbanization on abundance was not significant (p = 0.316).

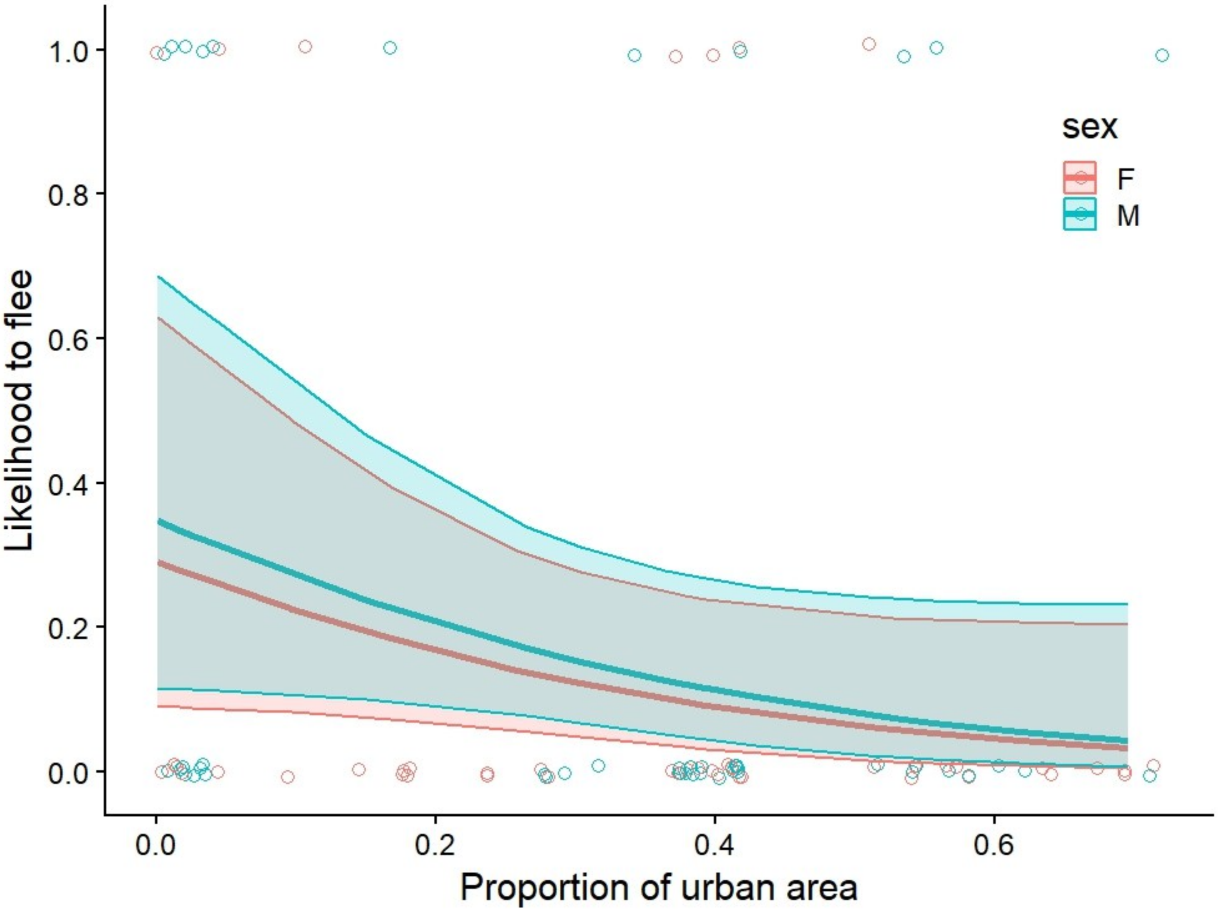

Figure 4 Model-predicted likelihood to flee of common garter snakes (*Thamnophis sirtalis*) against proportion of urbanization in the landscape (1 = flee, 0 = crypsis). Across 21 old fields in Ottawa/Gatineau, Canada, a total of 90 garter snakes were caught in the summer of 2025 and their avoidance tactic upon detection (flee or crypsis) was recorded. The effect of urbanization on likelihood to flee was not significant (p = 0.081).

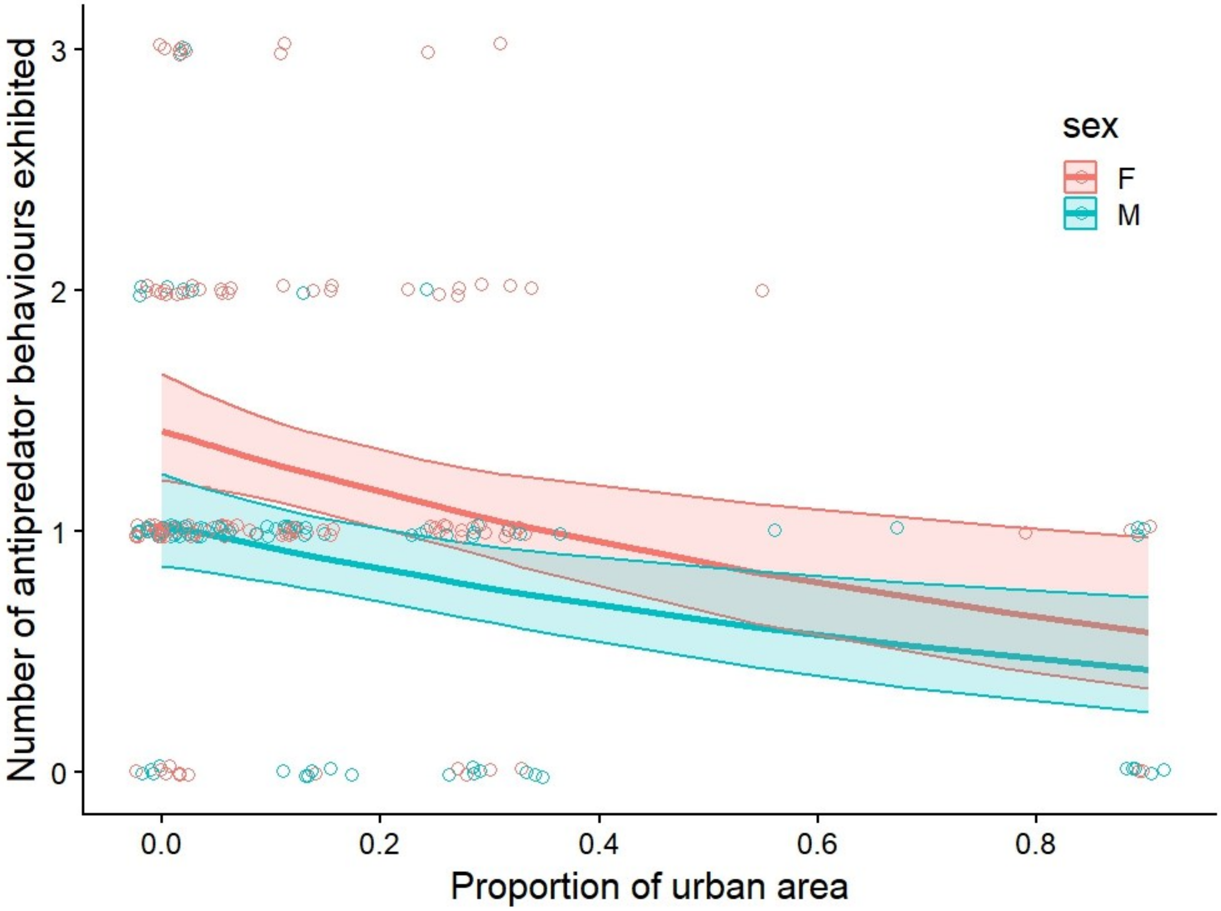

Figure 5 Model-predicted behavioural score (3 = all behaviours exhibited, 0 = no behaviours exhibited) of common garter snakes (*Thamnophis sirtalis*) against proportion of urbanization in the landscape. Across 32 old fields in Ottawa/Gatineau, Canada, a total of 216 garter snakes were caught in the summer of 2025 and the number of antipredator behaviours exhibited after capture (musk, gape, bite) was recorded. The effect of urbanization on behavioural score was significant (p = 0.003).

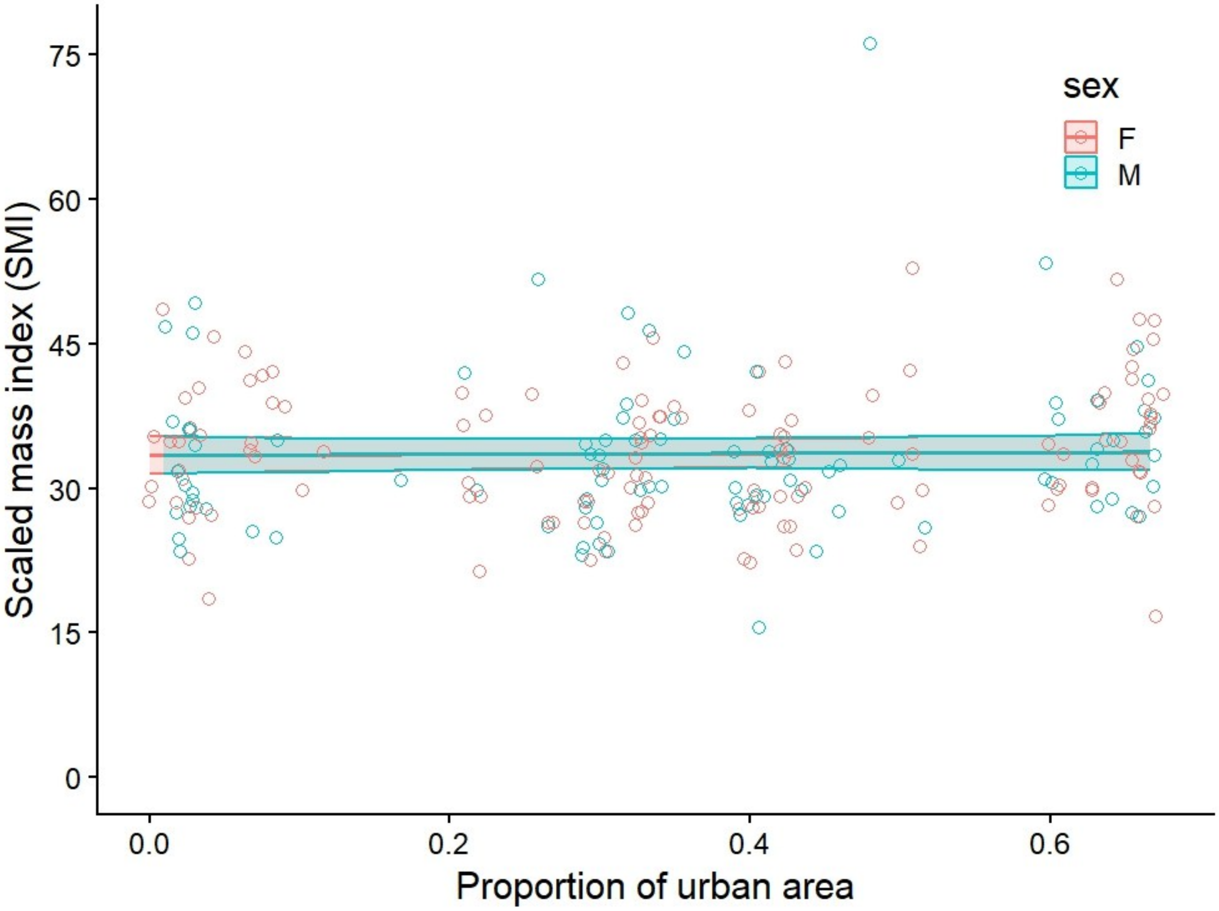

Figure 6 Model-predicted scaled mass index (SMI) of common garter snakes (*Thamnophis sirtalis*) against proportion of urbanization in the landscape. Across 32 old fields in Ottawa/Gatineau, Canada, a total of 219 garter snakes were caught in the summer of 2025 and their snout-vent length and mass were recorded to calculate their SMI. The effect of urbanization on SMI was not significant (p = 0.772).

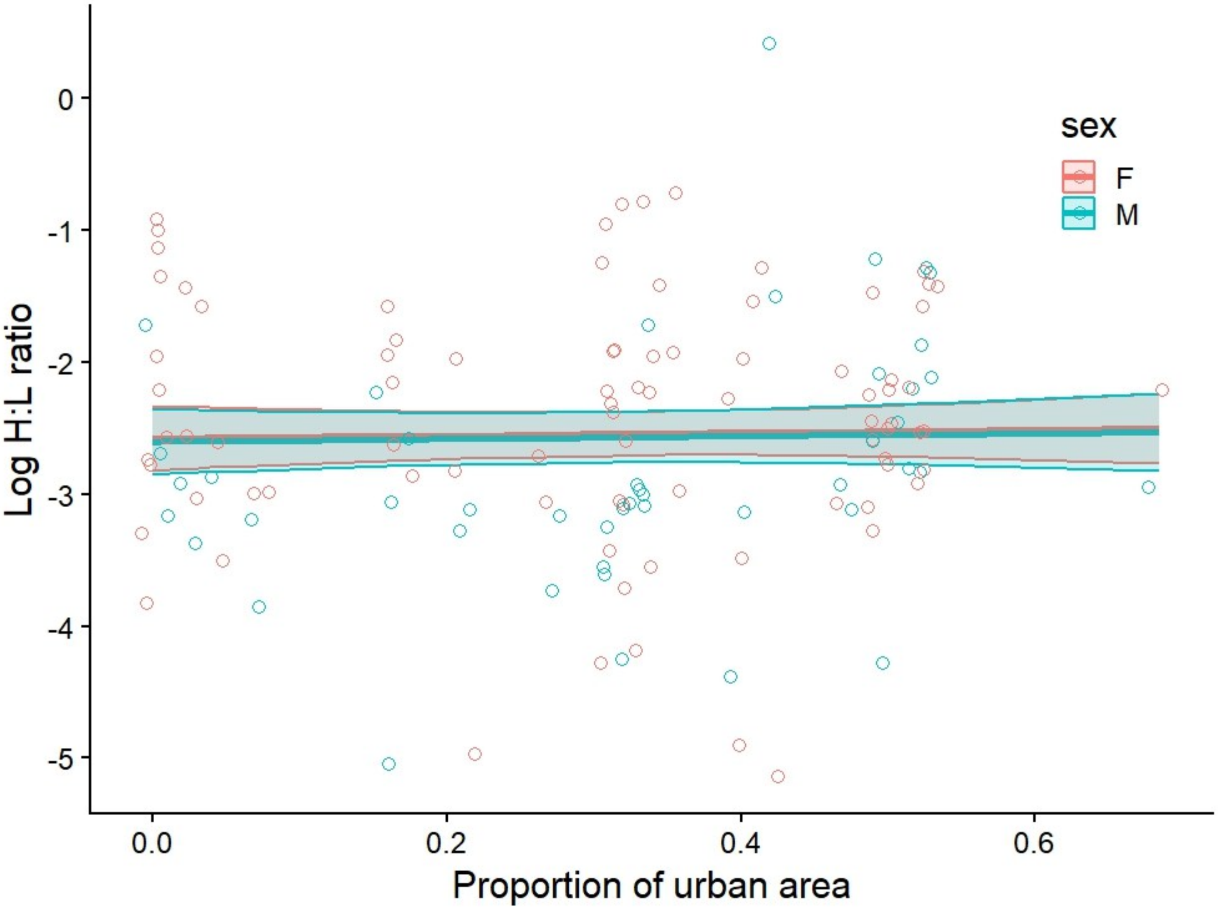

Figure 7 Model-predicted heterophil to lymphocyte (H:L) ratio of common garter snakes (*Thamnophis sirtalis*) against proportion of urbanization in the landscape. Across 31 old fields in Ottawa/Gatineau, Canada, a total of 131 garter snakes were caught in the summer of 2025 and a blood sample was taken to perform a leukocyte differential. The effect of urbanization on H:L ratio was not significant (p = 0.707).

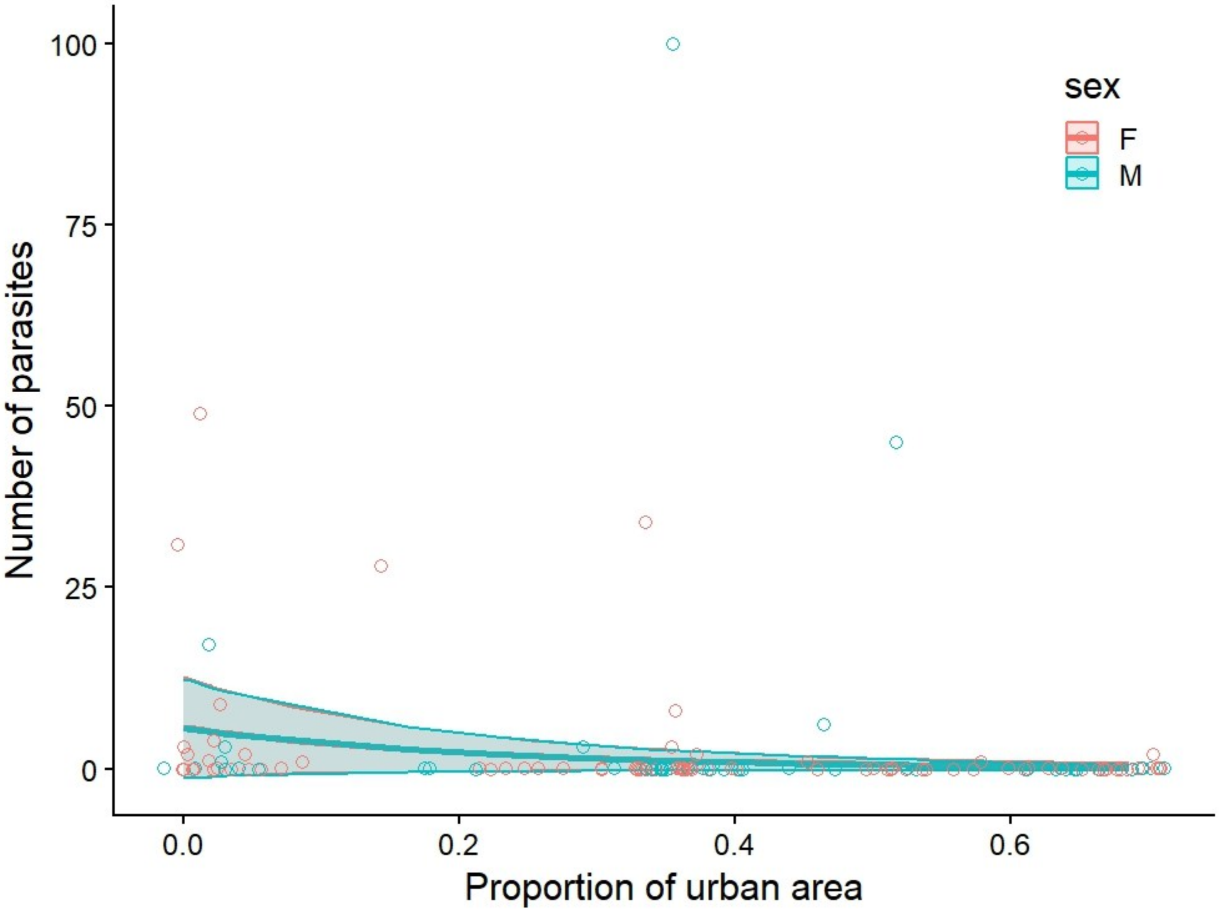

Figure 8 Model-predicted parasite load of common garter snakes (*Thamnophis sirtalis*) against proportion of urbanization in the landscape. Across 31 old fields in Ottawa/Gatineau, Canada, a total of 131 garter snakes were caught in the summer of 2025 and a blood sample was taken to determine the number of hemogregarines along one line in the monolayer of a blood smear. The effect of urbanization on parasite load was significant ($p < 0.001$).

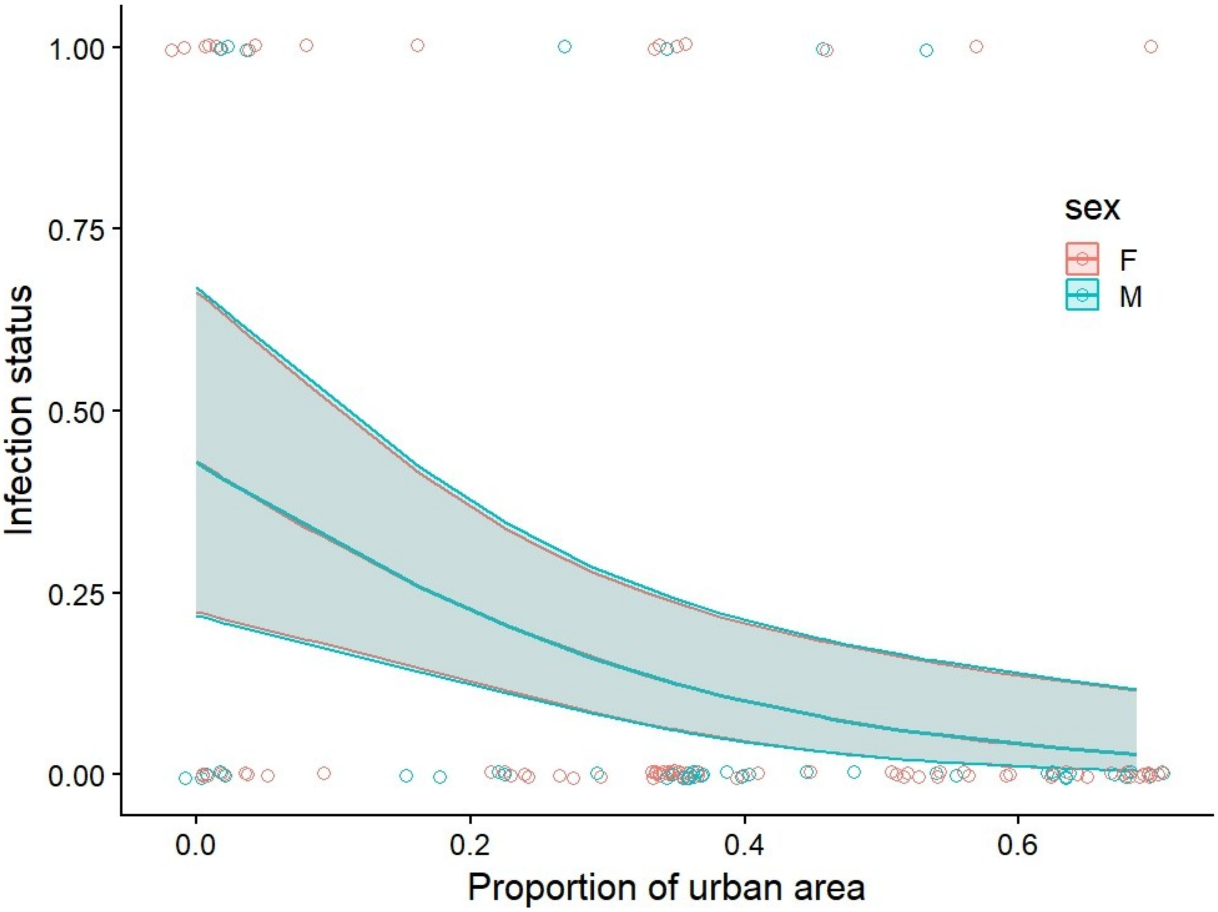

Figure 9 Model-predicted parasite infection status of common garter snakes (*Thamnophis sirtalis*) against proportion of urbanization in the landscape (1 = infected, 0 = not infected). Across 31 old fields in Ottawa/Gatineau, Canada, a total of 131 garter snakes were caught in the summer of 2025 and a blood sample was taken to determine the presence of hemogregarines along one line in the monolayer of a blood smear. The effect of urbanization on infection status was significant (p = 0.002).

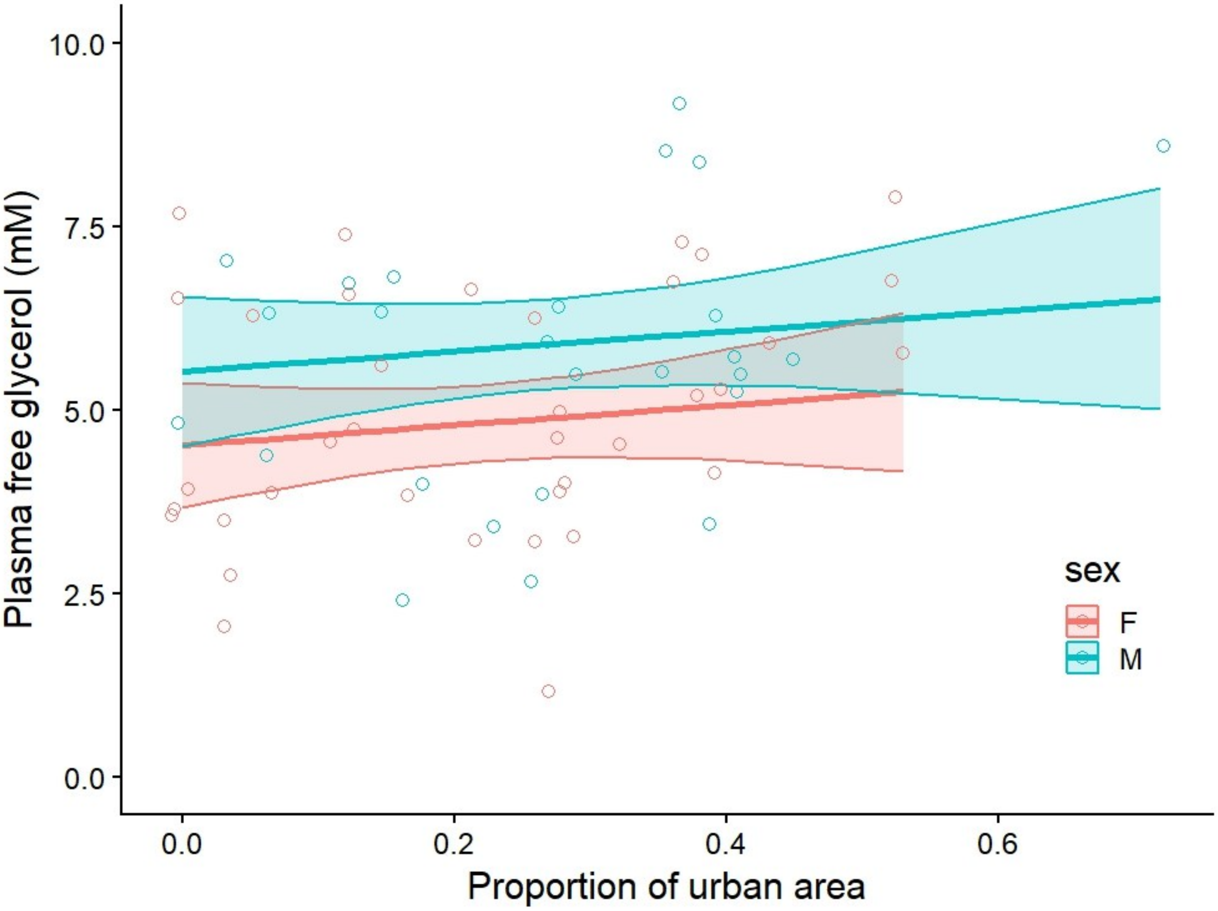

Figure 10 Model-predicted plasma free glycerol in common garter snakes (*Thamnophis sirtalis*) against proportion of urbanization in the landscape. Across 25 old fields in Ottawa/Gatineau, Canada, a total of 63 garter snake blood samples collected in the summer of 2025 were assessed for plasma glycerol. The effect of urbanization on plasma glycerol was not significant (p = 0.380).

Appendix A: Leukocyte Differential and Morphology

Note: A modified version of this appendix is currently accepted pending revision in *Journal of Wildlife Diseases*

Hematology is useful to assess the condition of wild animals, and it has applications in disease ecology and in conservation. Health assessments are conducted with reference intervals, which are often derived from single populations (Neuman-Lee et al. 2019) or from captive animals (Dervas et al. 2023). This can be problematic, as animals from different populations and experiencing different conditions are likely to exhibit different hematological profiles (Scheelings 2024).

Our objective was to quantify the hematological profiles of common garter snakes (*Thamnophis sirtalis*), and to determine how biological and environmental factors may affect them. Garter snakes are found across North America and are often the focus of physiological studies (Halliday et al. 2015; Neuman-Lee et al. 2019). Biological factors such as sex, size, body condition, and parasite load affect the health of individuals (Neuman-Lee et al. 2019), but their effects on leukocyte counts have rarely been explored. Similarly, environmental factors, such as landscape composition and time of year, can have strong influences on animal health (Turcotte 2023), but are often not considered when presenting hematological reference intervals for wild animals.

We chose 31 sites along an urbanization gradient by combining Ontario (Ontario Ministry of Natural Resources and Forestry 2023) and Québec (Institut de la statistique du Québec 2024) land use datasets in ArcGIS Pro 3.5.3 following Gigeroff and Blouin-Demers (2023). We used four land classes: urban, water, forest, and field. Data on common garter snake movement are scant, but congenerics move on average 100-200 m in a season, with maxima of 300-400 m (Shonfield et al. 2019). Thus, to include the potential area of effect for landscape variables, we constructed buffers from 100 m to 1000 m, in increments of 100 m, around each site in ArcGIS. We calculated the land composition within each buffer, then determined the percent composition of each land class within the buffers. All buffers were centered around a field or forest edge, as garter snakes prefer these habitats (Halliday and Blouin-Demers 2015; Halliday et al. 2015).

We installed 15 60 cm x 60 cm plywood boards at each site to sample snakes (Halliday and Blouin-Demers 2015). We visited sites approximately once a week from 23 April until 10 October 2025. We conducted sampling between 08:00 and 17:00 on clear days with air temperatures between 9˚C and 30˚C. We captured snakes found under coverboards as well as those found opportunistically.

After a snake was captured, we collected a blood sample (200 µl) from the caudal vein using a 0.5 mL syringe fitted with a 28-gauge needle (Halliday et al. 2015), which was transferred to a microcentrifuge tube containing 10 µL of 0.8% saline mixed with sodium heparin (catalog #375095100KU, MilliporeSigma, Oakville, Ontario, Canada) at a concentration of 2500 U/mL, and stored on ice while in the field. We only collected blood from snakes that weighed at least 20 g.

We uniquely marked each snake using a medical cautery unit on the ventral scales for future identification (Winne et al. 2006). We probed snakes to determine sex, measured them from snout to vent length (SVL), and weighed them using a spring scale. We used SVL and mass to calculate the scaled mass index (SMI), a metric of body condition (Peig and Green 2009). We recorded body temperature using an infrared thermometer (Halliday and Blouin-Demers 2017). Protocols were approved by the University of Ottawa Animal Care Committee (protocol BLf-4498), and all animals were handled under an Ontario Wildlife Scientific Collector's Authorization (permit number 1107132) and Permis Scientifique du Québec (permit number 25-07-SF-002-GR-0).

We created whole blood smears in the field immediately after blood collection, air dried the slides and then stored them in the dark until we returned to the lab at the end of the day. Once in the lab, we fixed the smears with 100% methanol and stained them with Wright-Giemsa stain (Fisherbrand™ SureStain™, catalog #CS434D, Fisher Scientific, Pittsburgh, Pennsylvania, USA) following manufacturer's instructions, and stored them in the dark until analysis. We observed the smears with an Olympus CX41 compound microscope at 1000x magnification under oil immersion. We estimated total white blood cell (WBC) counts by counting the number of leukocytes within 10 fields of view in the monolayer, and then multiplied the total by 2×10^9 to obtain a concentration per litre of whole blood (Winter et al. 2019). For the WBC differential, we identified the first 100 leukocytes (lymphocytes, azurophils, heterophils, monocytes, eosinophils, and basophils) along a line perpendicular to the blood smear. We did this twice and used the number of each leukocyte counted over the 200 total observed cells to obtain a proportion (Turcotte 2023). We quantified hemogregarine infection intensity by counting the number of cells infected along one full line (from edge to edge) within the monolayer at 400x, and then standardizing this count per 100 red blood cells (RBCs). We placed a portion of each blood sample in a microcapillary tube and centrifuged it at 12 000 rpm for 3 minutes (Clay Adams Autocrit Ultra 3, Becton, Dickinson, and Company, Franklin Lakes, New Jersey, USA) at room temperature to determine the packed cell volume (PCV).

WBC morphology was similar to that found in other reptiles (Figure A1). Lymphocytes were small (7.9 ± 1.2 μm) with little light-blue cytoplasm that tended to be concentrated at one pole of the cell (Figure A1A). Lymphocytes were usually round, but sometimes exhibited small cytoplasmic protrusions. They were similar in appearance to thrombocytes, however the latter had more and clearer cytoplasm, and the nucleus tended to stain darker (Figure A1B).

Azurophils were large (12.8 ± 2.0 μm), with small azurophilic granules in the cytoplasm. The cytoplasmic granules varied from light pink around the edges of the cell to a deep magenta throughout the cell, obscuring the light blue cytoplasm. Azurophils were often round, and the nucleus could be central or pushed to the poles of the cell (Fig. A1C).

Heterophils were the largest (17.0 ± 2.5 μm) and most distinct leukocyte. They were round, with a small, eccentric nucleus that stained lightly basophilic in the center with a white rim. The cytoplasm was obscured by abundant heterophilic granules that were variably shaped (Fig. A1D).

Basophils were medium-sized (11.3 ± 1.8 μm) and round or oblong. They had large, round, basophilic granules throughout the entire cell. When the nucleus was visible, it was pale blue (Fig. A1E). Degranulated basophils were also present in some smears, but faint basophilic outlines were still visible throughout the cytoplasm.

Monocytes were large (13.5 ± 1.5 μm) and usually oblong with an eccentric nucleus, similar in appearance to lymphocytes, but larger and with more cytoplasm (Fig. A1F). The cytoplasm was light blue and often contained several small vacuoles.

Eosinophils were similar in size (11.9 ± 1.9 μm) and shape to basophils. They had small, round, lightly eosinophilic granules throughout the cytoplasm. The nucleus could be central or eccentric and was usually covered by granules but still visible with a violet colouring (Fig. A1G).

We conducted statistical analyses using R version 4.4.1 (R Core Team 2024). We first determined whether the time between capture and acquisition of the blood sample affected any hematological values. Time to sample ranged from 2.5 minutes to 19 minutes, with a mean of 7.7 minutes. We calculated the Spearman's correlation between each response variable and the time to sample, and there were no significant correlations, except for the number of eosinophils (rho = -0.20, p = 0.0173). Thus, we included time to sample as a predictor in the eosinophil model. To ensure our analyses represented a suitable reference population, we excluded outliers determined using interquartile ranges, adjusted for skewed distributions where needed (Hubert and Vandervieren 2008). Reference intervals were determined following the American Society of Veterinary Clinical Pathology guidelines (Friedrichs et al. 2012).

We fitted generalized linear mixed effects models for all variables. We used a beta distribution for PCV, which is appropriate for proportional data (Douma and Weedon 2019), a lognormal distribution for the number of total leukocytes, lymphocytes, heterophils, and azurophils, and a Tweedie distribution for the number of total monocytes, basophils, and eosinophils to account for the presence of zeros in these datasets.

Full models included body temperature, body condition, parasite load, SVL, sex, and date as predictors because these variables can affect physiology (Neuman-Lee et al. 2019; Turcotte 2023), and all landscape variables (percent field, forest, water, and urban). For each landscape variable, we used the buffer size that had the largest absolute correlation with each respective response variable (Jackson and Fahrig 2015; Gigeroff and Blouin-Demers 2023). Full models also included total WBC count as a predictor to control for variation in total leukocyte counts between individuals. We included site as a random effect. We centred and scaled all continuous variables.

We constructed models using the glmmTMB package (McGillycuddy et al. 2025). We assessed model assumptions visually with DHARMa plots (Hartig 2024). We removed any predictors with a variance inflation factor greater than two. We also assessed correlation between predictors, and removed any that had an absolute correlation of > 0.7. To reduce overfitting, we dredged the models using the MuMin package (Bartoń 2025) and averaged the models within two AICc of the best performing model to compute final coefficients.

We captured 131 snakes (85 females, 46 males) at the 31 sites. Hemogregarines (Fig. A1H) were present in 18% of snakes. Lymphocytes were the most common leukocyte, followed by azurophils, heterophils, basophils, monocytes, and eosinophils (Table A1). Lymphocytes, heterophils, and azurophils were present in all snakes, basophils were present in most snakes, and monocytes and eosinophils were present in few snakes. Leukocyte estimates between repeated smear analyses were close for most parameters (Table B1).

Warmer snakes, snakes caught later in the season, and snakes in sites with more water in the surrounding landscape had significantly higher PCV (Table A2). Snakes at sites surrounded by more field had lower total leukocyte counts (Table A2). Snakes caught later in the year had fewer lymphocytes, and males had fewer heterophils (Table A2). Longer snakes had more azurophils, but had fewer eosinophils (Table A2). No predictors of interest were significant in the monocyte model.

We have presented robust hematological reference intervals based on several populations of common garter snakes, and identified several biological and environmental factors that affect them. Our WBC differentials are similar to those found in other garter snake species (Wack et al. 2012; Bell and Gregory 2014), although our total WBC estimates were higher, and our PCV estimates lower. In contrast to previous investigations of garter snake hematology, we did find eosinophils, albeit in small numbers.

Longer snakes showed hematological differences consistent across several WBC types. Snakes continue to grow past maturity, suggesting that WBC differentials may change with age. While *Boa constrictor* exhibit differences between age classes in some hematological parameters (Dervas et al. 2023), few other studies have investigated potential ontogenetic changes in hematological profiles in reptiles; the relationship between hematology and age remains unclear.

There were some differences between sexes, including males having fewer heterophils and basophils. There are sex differences in WBC differentials in *Naja naja* (Dissanayake et al. 2017) as well as sex differences in the blood chemistry of *Vipera ammodytes* (Lisičić et al. 2013), but little consistency regarding which parameters vary, suggesting the specific differences between sexes may be species-specific.

Blood samples collected later in the field season had higher PCVs and lower total lymphocytes. Many hematological studies do not investigate the effect of time of year (Wack et al. 2012; Dissanayake et al. 2017), making comparisons difficult. However, blood chemistry in carpet pythons (*Morelia spilota*) changes across seasons (Bryant et al. 2012) and WBC differentials in *Boa constrictor* are different between the summer and winter (Machado et al. 2006). Given the extreme temperature variation garter snakes in Canada experience over the year, including the time of year in hematological investigations of temperate North American reptiles may be especially important.

The proportion of water and field in the surrounding area was related to differences in PCV and total WBC, respectively. Previous studies (Wack et al. 2012; Scheelings 2024) have found differences in hematology between sites, but given the small site replication of these investigations (both studies used fewer than five sites), it is difficult to separate site-specific effects from landscape effects. Given that we included over 30 sites, our results suggest that hematology may be affected by landscape characteristics that are independent of specific sampling location.

We have demonstrated that the hematology of garter snakes is affected by biological characteristics, such as age and sex, as well as environmental characteristics, such as the time of year and landscape composition. Our results indicate that future investigations of hematology in wild animals should take these factors into account when developing reference intervals or assessing population health.

Table A1 Hematology values for common garter snakes (*Thamnophis sirtalis*) at 31 old fields around Ottawa/Gatineau, Canada. Sample size for each parameter was 131 except PCV, for which the sample size was 117.

Parameter[a]	N	Mean	SD	Median	Min	Max	LRL of RI	URL of RI	CI 90% of LRL	CI 90% of URL
PCV (%)	116	27.5	5.99	27.64	13.57	41.94	15.50	39.35	13.91 - 17.00	37.83 - 40.98
WBC (x10^9/L)	129	16.18	6.17	14.8	6.0	39.4	6.55	30.75	6.0 - 7.6	28.0 – 39.4
Parasites/100 RBCs	131	0.011	0.046	0	0	0.40	0	0.17	0 - 0	0.068 – 0.40
Lymphocyte (%)	127	61.59	11.38	61.0	34.5	86.0	39.6	83.3	34.5 – 44.0	81.0 – 86.0
Lymphocyte (x10^9/L)	126	9.68	3.97	8.90	3.44	22.32	3.58	19.48	3.44 - 3.95	16.8 - 22.32
Heterophil (%)	120	6.62	4.61	5.0	1.5	24.5	1.5	18.98	1.5 - 2.0	17.0 – 24.5
Heterophil (x10^9/L)	108	1.08	0.80	0.80	0.27	3.45	0.28	3.35	0.21 - 0.29	3.26 – 3.65
Azurophil (%)	129	27.39	10.20	26.0	7.5	60.0	9.13	52.88	7.5 – 14.0	44.5 – 60.0
Azurophil (x10^9/L)	124	4.40	2.22	3.84	1.37	11.06	1.58	9.93	1.37 - 1.80	8.64 - 11.06
Basophil (%)	131	3.80	3.40	3.0	0	16.0	0	13.20	0 - 0	10.5 - 16.0
Basophil (x10^9/L)	131	0.57	0.51	0.44	0	2.27	0	2.06	0 - 0	1.60 – 2.27
Monocyte (%)	131	1.0	1.19	0.5	0	6.0	0	4.85	0 - 0	4.0 – 6.0
Monocyte (x10^9/L)	131	0.16	0.20	0.089	0	0.95	0	0.86	0 - 0	0.59 - 0.95
Eosinophil (%)	131	0.80	1.72	0	0	11.5	0	6.70	0 - 0	4.0 - 11.5
Eosinophil (x10^9/L)	131	0.12	0.25	0	0	1.25	0	1.10	0 - 0	0.72 - 1.25

[a] SD = standard deviation; PCV = packed cell volume; RI = reference interval; LRL = lower reference limit; URL = upper reference limit; CI = confidence interval; WBC = white blood cell; RBC = red blood cell.

Table A2 Summary statistics of averaged mixed models exploring potential predictors of hematology parameters in common garter snakes (*Thamnophis sirtalis*) at 31 old fields around Ottawa/Gatineau, Canada. Site of capture was included as a random effect. All continuous variables were centred and scaled. Predictors that are significant at α = 0.05 are in bold.

Parameter[a]	Estimate	Standard error	z-statistic	p-value
Model: PCV (n = 116)				
Intercept	**-1.00**	**0.034**	**29.40**	**<0.001**
Body temperature	**0.071**	**0.026**	**2.71**	**0.007**
Sex (male)	0.077	0.065	1.18	0.239
Date	**0.10**	**0.027**	**3.66**	**<0.001**
Body condition	0.009	0.021	0.42	0.674
SVL	0.003	0.013	0.26	0.797
Parasites/100 RBCs	0.004	0.013	0.30	0.765
%Field (200 m)	0.047	0.032	1.43	0.154
%Water (900 m)	**0.060**	**0.026**	**2.26**	**0.024**
%Forest (700 m)	-0.033	0.033	1.01	0.312
%Urban (100 m)	0.001	0.007	0.13	0.893
Model: Total WBC (n = 129)				
Intercept	**2.78**	**0.034**	**81.12**	**<0.001**
Date	-0.008	0.020	0.42	0.676
Body condition	0.001	0.006	0.096	0.924
SVL	0.009	0.021	0.45	0.651
Parasites	0.015	0.022	0.71	0.478
%Field (1000 m)	**-0.069**	**0.029**	**2.38**	**0.018**
%Water (1000 m)	0.011	0.022	0.50	0.615
%Urban (600 m)	-0.002	0.011	0.21	0.834
Model: Total lymphocytes (n = 126)				
Intercept	**2.24**	**0.027**	**81.21**	**<0.001**
Total WBC	**0.24**	**0.022**	**10.85**	**<0.001**
Body temperature	0.032	0.025	1.24	0.216
Date	**-0.10**	**0.028**	**3.65**	**<0.001**
Body condition	0.020	0.027	0.74	0.462

SVL	-0.015	0.027	0.55	0.586
Parasites/100 RBCs	-0.001	0.009	0.09	0.929
%Field (300 m)	0.011	0.019	0.55	0.580
%Water (100 m)	-0.003	0.010	0.25	0.805
Model: Total heterophils (n = 108)				
Intercept	0.13	0.065	1.90	0.058
Total WBCs	**0.18**	**0.045**	**3.95**	**<0.001**
Body temperature	0.052	0.047	1.09	0.275
Sex (male)	**-0.18**	**0.084**	**2.07**	**0.039**
%Water (500 m)	-0.016	0.031	0.53	0.598
%Urban (1000 m)	0.040	0.045	0.89	0.374
Model: Total azurophils (n = 124)				
Intercept	**1.40**	**0.064**	**21.65**	**<0.001**
Total WBCs	**0.29**	**0.034**	**8.54**	**<0.001**
Sex (male)	-0.019	0.046	0.41	0.686
Date	-0.007	0.021	0.35	0.723
Body condition	0.016	0.027	0.58	0.560
SVL	**0.075**	**0.028**	**2.62**	**0.009**
Parasites/100 RBCs	-0.009	0.030	0.30	0.768
%Forest (200 m)	0.001	0.012	0.12	0.905
%Water (200 m)	0.003	0.018	0.17	0.869
%Urban (100 m)	0.002	0.015	0.16	0.876
Model: Total basophils (n = 131)				
Intercept	**-0.50**	**0.095**	**5.27**	**<0.001**
Total WBCs	**0.19**	**0.072**	**2.62**	**0.009**
Sex (male)	**-0.39**	**0.16**	**2.39**	**0.017**
Date	0.017	0.051	0.33	0.738
SVL	**-0.29**	**0.080**	**3.58**	**<0.001**
Body condition	0.054	0.083	0.65	0.517
%Forest (700 m)	-0.16	0.10	1.59	0.112
%Urban (200 m)	0.027	0.061	0.43	0.665

Model: Total monocytes (n = 131)				
Intercept	**-1.91**	**0.10**	**19.01**	**<0.001**
Total WBCs	**0.40**	**0.089**	**4.47**	**<0.001**
Body temperature	-0.040	0.080	0.50	0.8617
Date	-0.002	0.023	0.10	0.918
SVL	0.004	0.029	0.14	0.889
Body condition	-0.027	0.071	0.38	0.705
%Field (100 m)	0.014	0.050	0.27	0.784
%Water (800 m)	-0.015	0.052	0.29	0.771
%Urban (700 m)	0.057	0.094	0.60	0.547
Model: Total eosinophils (n = 131)				
Intercept	**-2.52**	**0.26**	**9.72**	**<0.001**
Total WBCs	0.23	0.20	1.14	0.255
Body temperature	-0.21	0.20	1.06	0.288
SVL	0.011	0.060	0.18	0.857
Body condition	**-0.59**	**0.21**	**2.72**	**0.007**
Parasites/100 RBCs	-1.22	0.65	1.86	0.064
%Water (100 m)	0.028	0.094	0.30	0.765
%Urban (700 m)	0.015	0.072	0.21	0.838

[a] PCV = packed cell volume; WBC = white blood cell; SVL = snout-vent length; RBC = red blood cell.

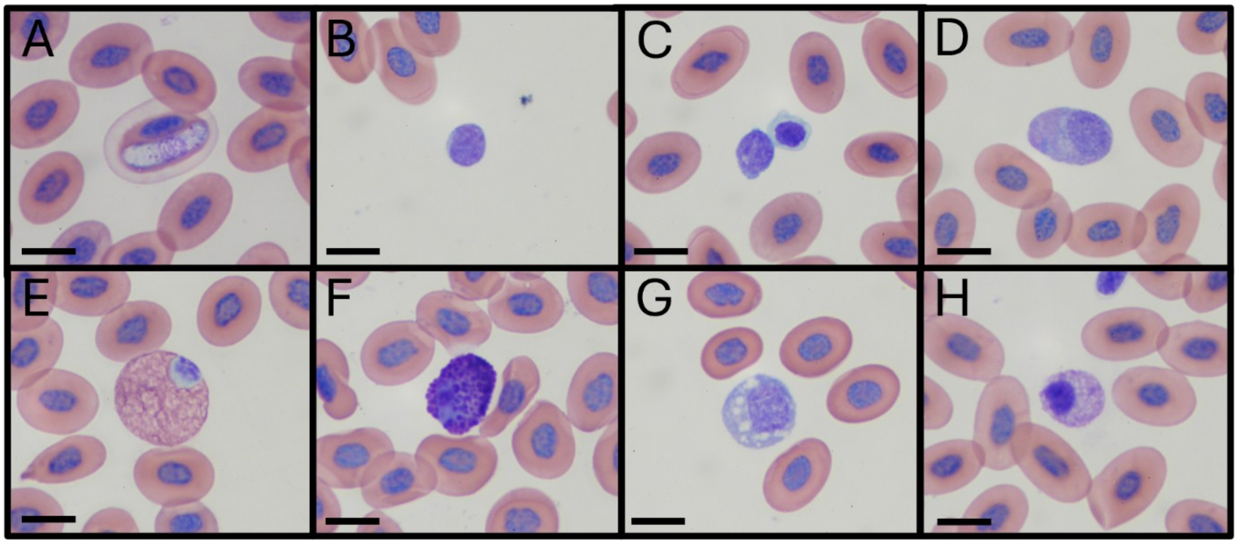

Figure A1 Blood cells observed in common garter snakes (*Thamnophis sirtalis*) at 31 old fields around Ottawa/Gatineau, Canada. A. A hemogregarine inside an erythrocyte, displacing the nucleus. B. A small lymphocyte. C. A small lymphocyte (left) beside a thrombocyte (right). D. An azurophil. E. A heterophil. F. A basophil. G. A monocyte with cytoplasmic vacuoles. G. An eosinophil. All photos were taken at 1000x magnification under oil immersion. Scale bars = 10 µm.

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Appendix B: Leukocyte Differential Repeatability Analysis

To ensure that my blood smear assessments were reliable, I conducted a repeatability analysis to determine how consistent I was in my leukocyte identification. To do this, I reanalyzed the first 20 smears immediately after I completed them, and then approximately five weeks after I completed all assessments, I reanalyzed 10 randomly selected smears. I combined these datasets for analysis. Two smears were analyzed during both the first and second reassessment, resulting in 60 observations of 28 different smears.

I conducted all analyses in R version 4.4.1 (R Core Team 2024). First, I created a linear mixed model using the lme4 package for each leukocyte parameter (H:L ratio, total WBC, and proportion of lymphocytes, heterophils, azurophils, basophils, monocytes, and eosinophils) to assess model assumptions. A transformation was applied to some response variables to better meet model assumptions. Each model included individual ID as a random effect and no fixed effects. Once model assumptions were met, I assessed repeatability using the rptR package (Stoffel et al. 2017). I estimated confidence intervals around each repeatability estimate by parametric bootstrapping with 5000 iterations.

Repeatability estimates were high for H:L ratio, and the proportion of lymphocytes, heterophils, and azurophils (Table B-1). Total WBC count and the proportion of basophils were moderately repeatable, while the proportion of monocytes and eosinophils were low and imprecise. This is likely due to the low numbers of these cells seen in the smears, making their observation much less consistent compared to the more common leukocytes.

Table B1 Repeatability estimates for hematology parameters. A total of 28 blood smears were analyzed 60 times (26 analyzed twice, 2 analyzed 4 times) to determine estimates. Repeatability was calculated using the mixed model method in the rptR package in R. Bootstrapping with 5000 iterations was used to determine 95% confidence intervals (CI).

Parameter[a]	Model Formula	R	95% CI
H:L ratio	sqrt(y) ~ 1|ID	0.92	0.84 – 0.96
Total WBC	y ~ 1|ID	0.69	0.45 – 0.84
Lymphocytes	y ~ 1|ID	0.94	0.87 – 0.97
Heterophils	y ~ 1|ID	0.92	0.84 – 0.96
Azurophils	y ~ 1|ID	0.92	0.84 – 0.96
Basophils	y ~ 1|ID	0.63	0.35 – 0.80
Monocytes	sqrt(y) ~ 1|ID	0	0 – 0.33
Eosinophils	sqrt(y) ~ 1|ID	0.56	0.26 – 0.76

[a] H:L ratio = heterophil:lymphocyte ratio; WBC = white blood cell.

Appendix B References

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Appendix C: Scale of Maximum Effect Correlations

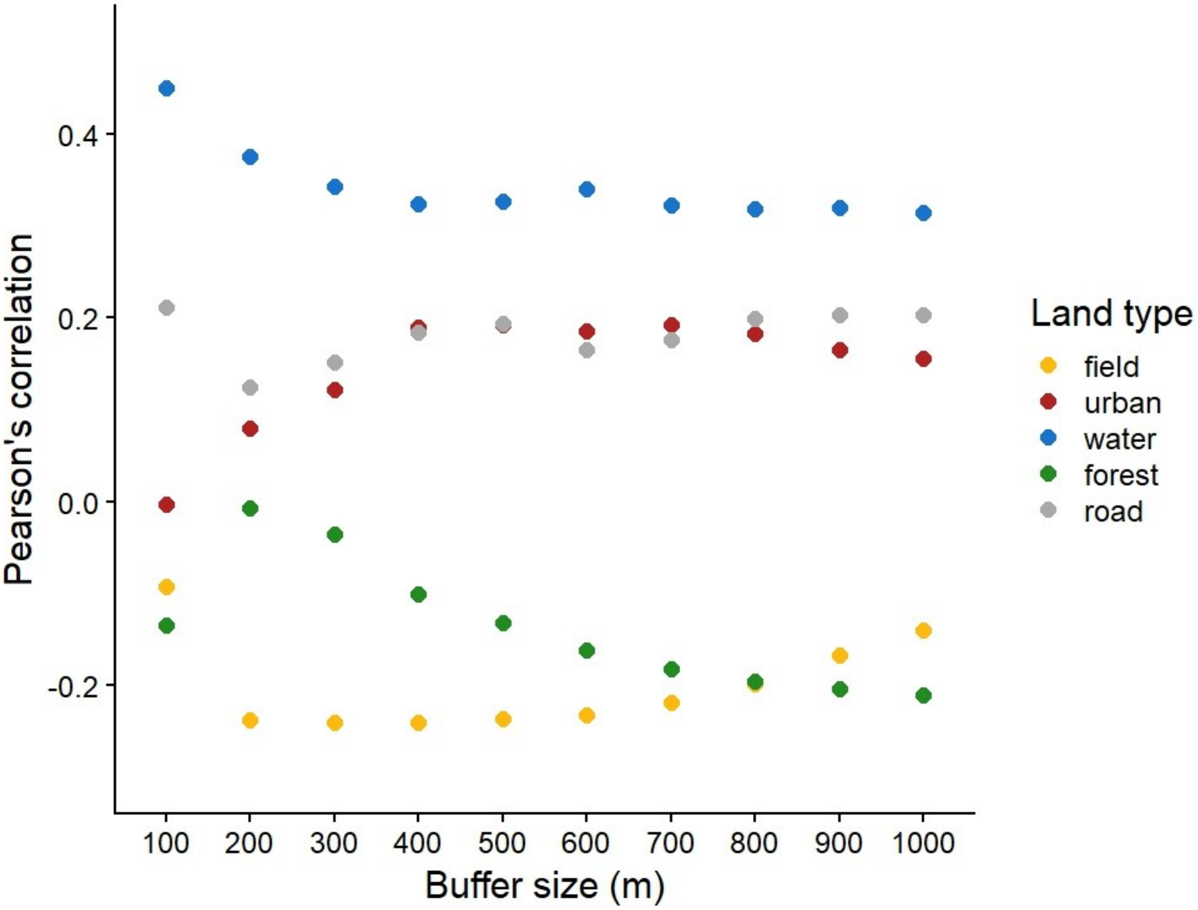

Figure C1 Pearson's correlation of habitat variables (field, forest, water, urban, roads) with number of unique snakes caught per visit at each site.

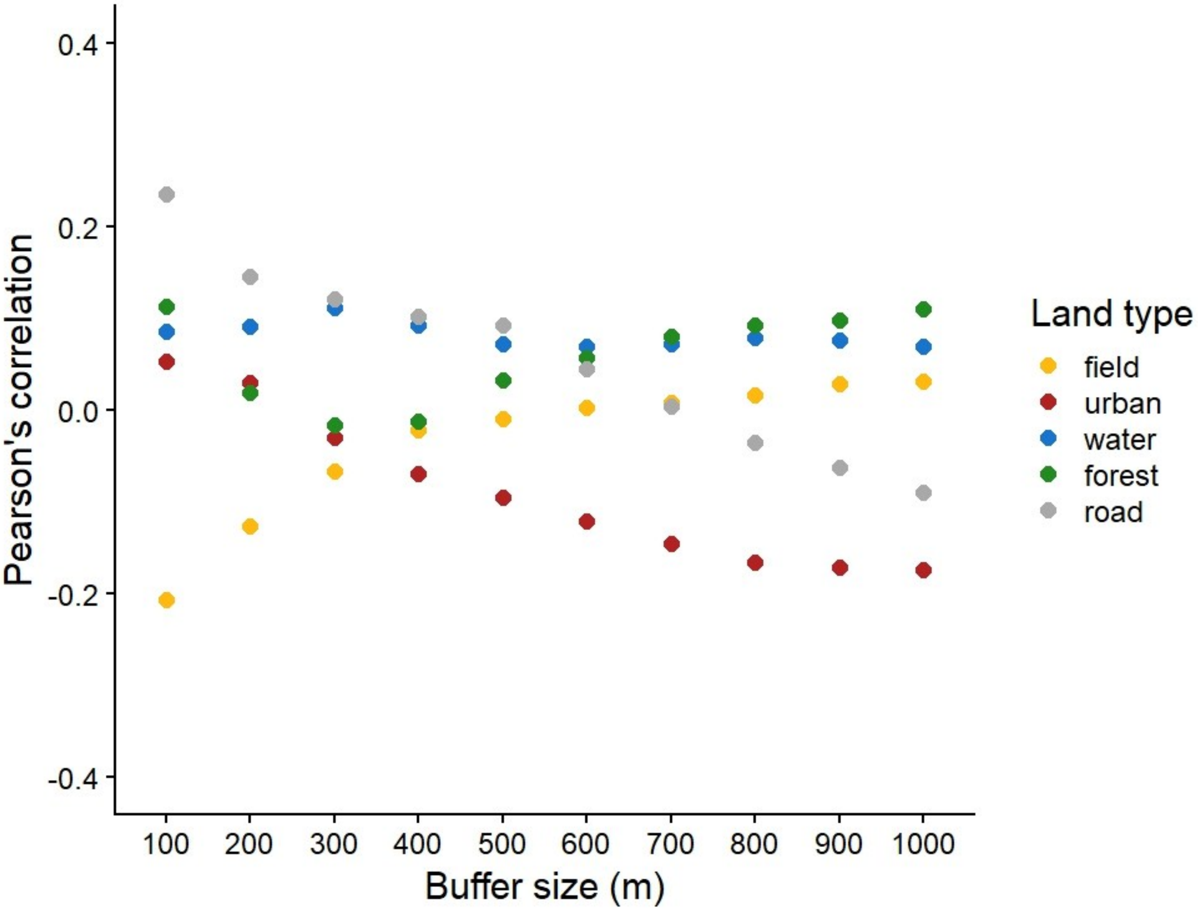

Figure C2 Pearson's correlation of habitat variables (field, forest, water, urban, roads) with likelihood to flee.

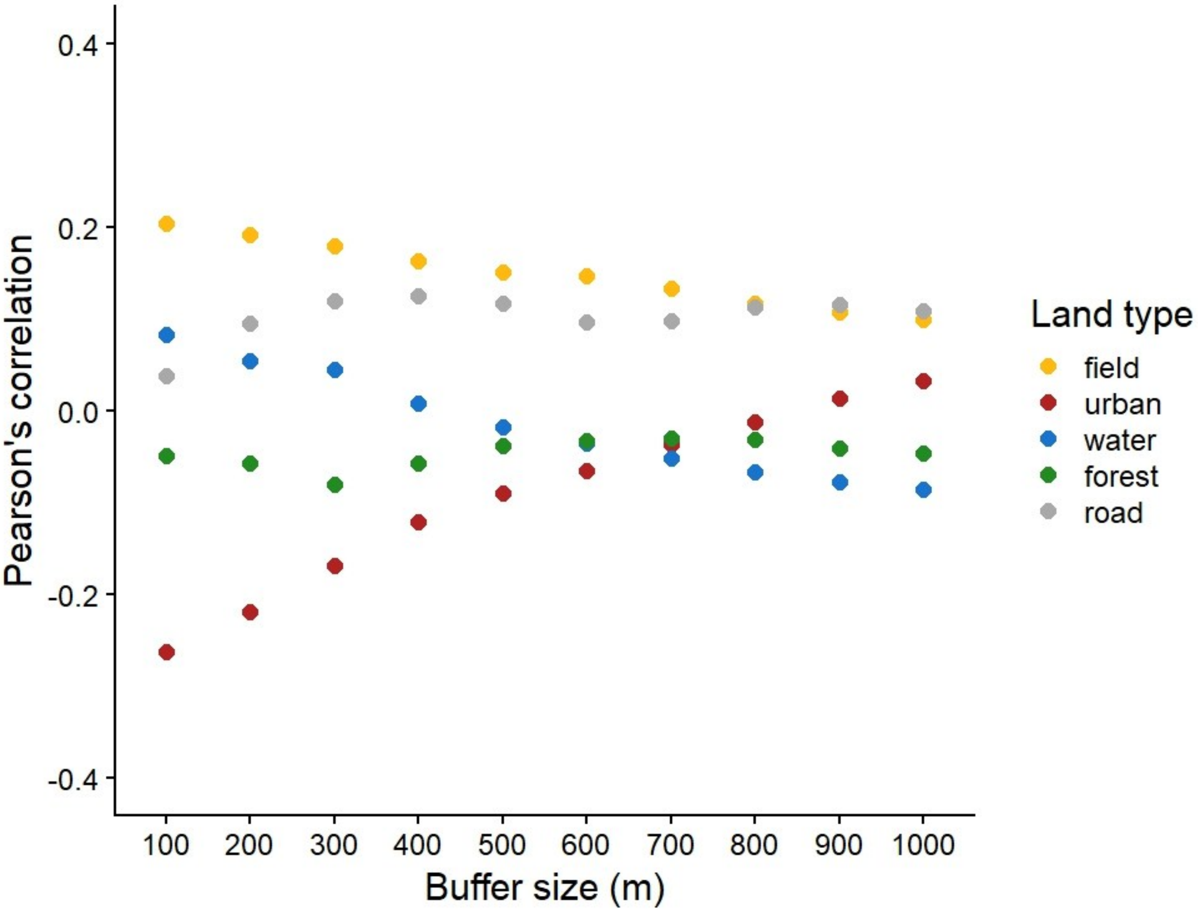

Figure C3 Pearson's correlation of habitat variables (field, forest, water, urban, roads) with behavioural score.

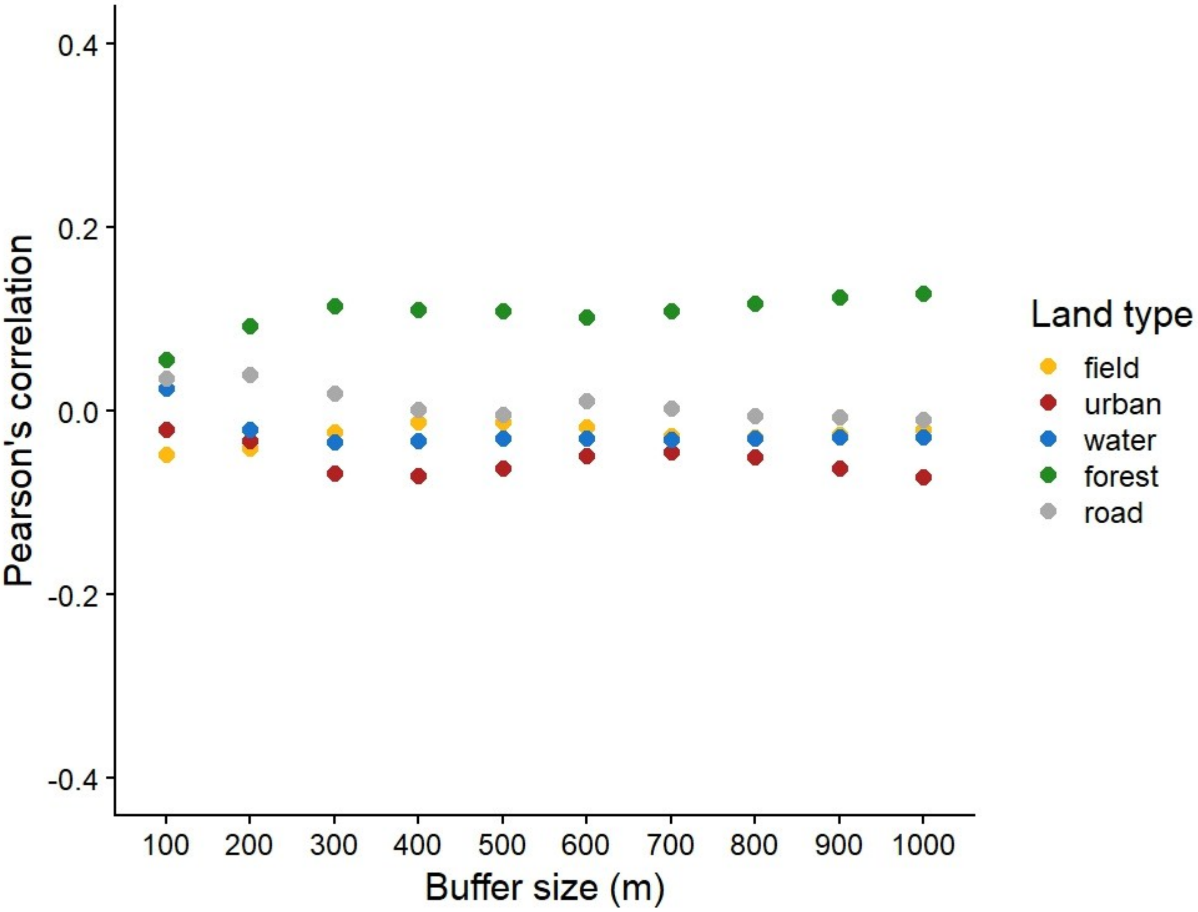

Figure C4 Pearson's correlation of habitat variables (field, forest, water, urban, roads) with scaled mass index.

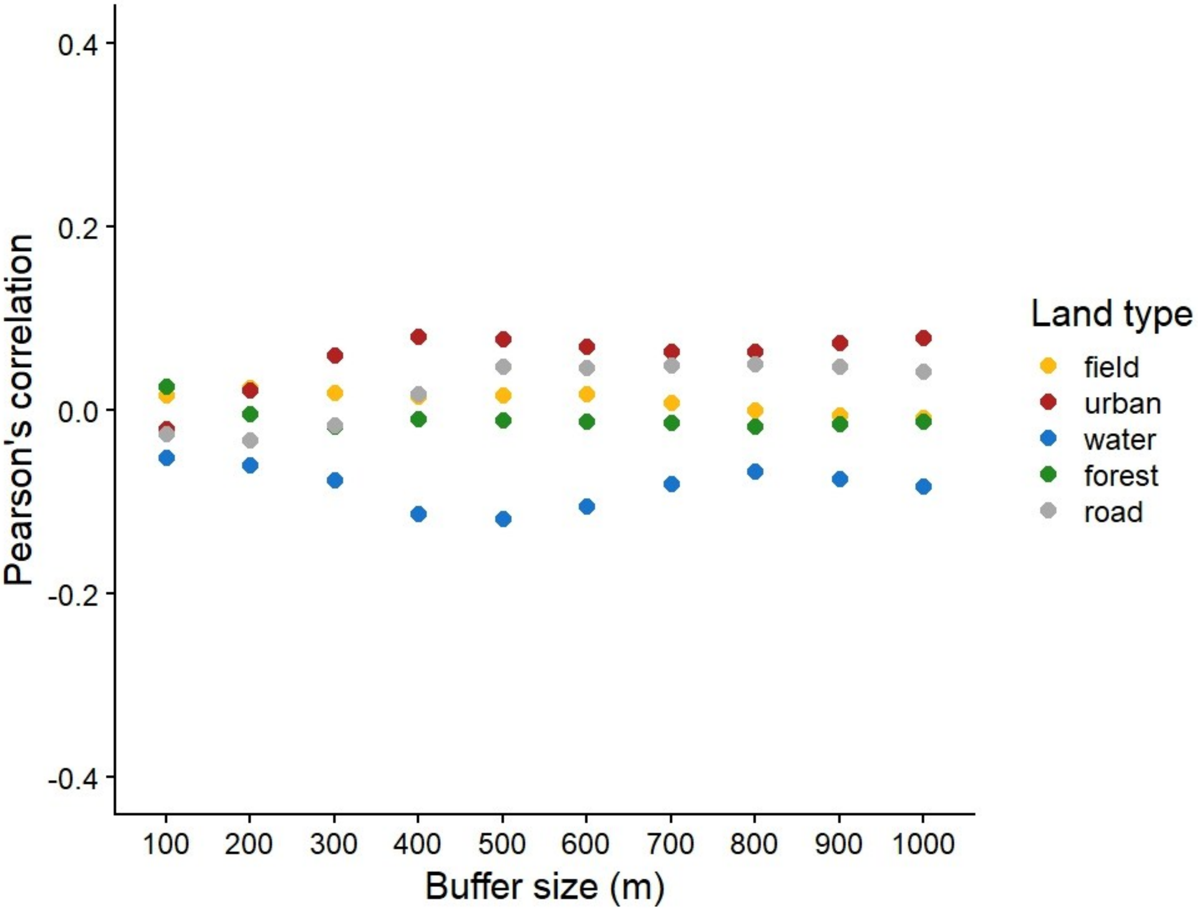

Figure C5 Pearson's correlation of habitat variables (field, forest, water, urban, roads) with H:L ratio.

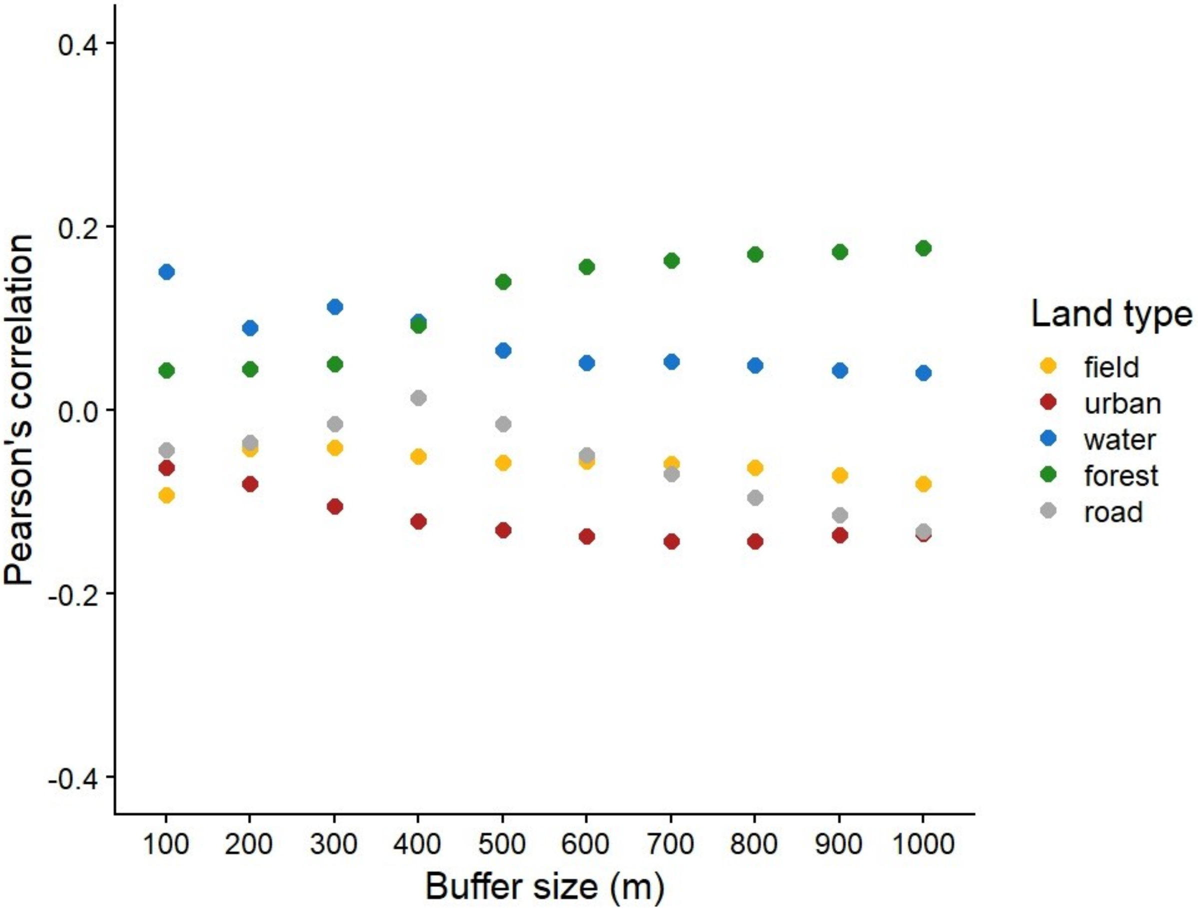

Figure C6 Pearson's correlation of habitat variables (field, forest, water, urban, roads) with parasite load.

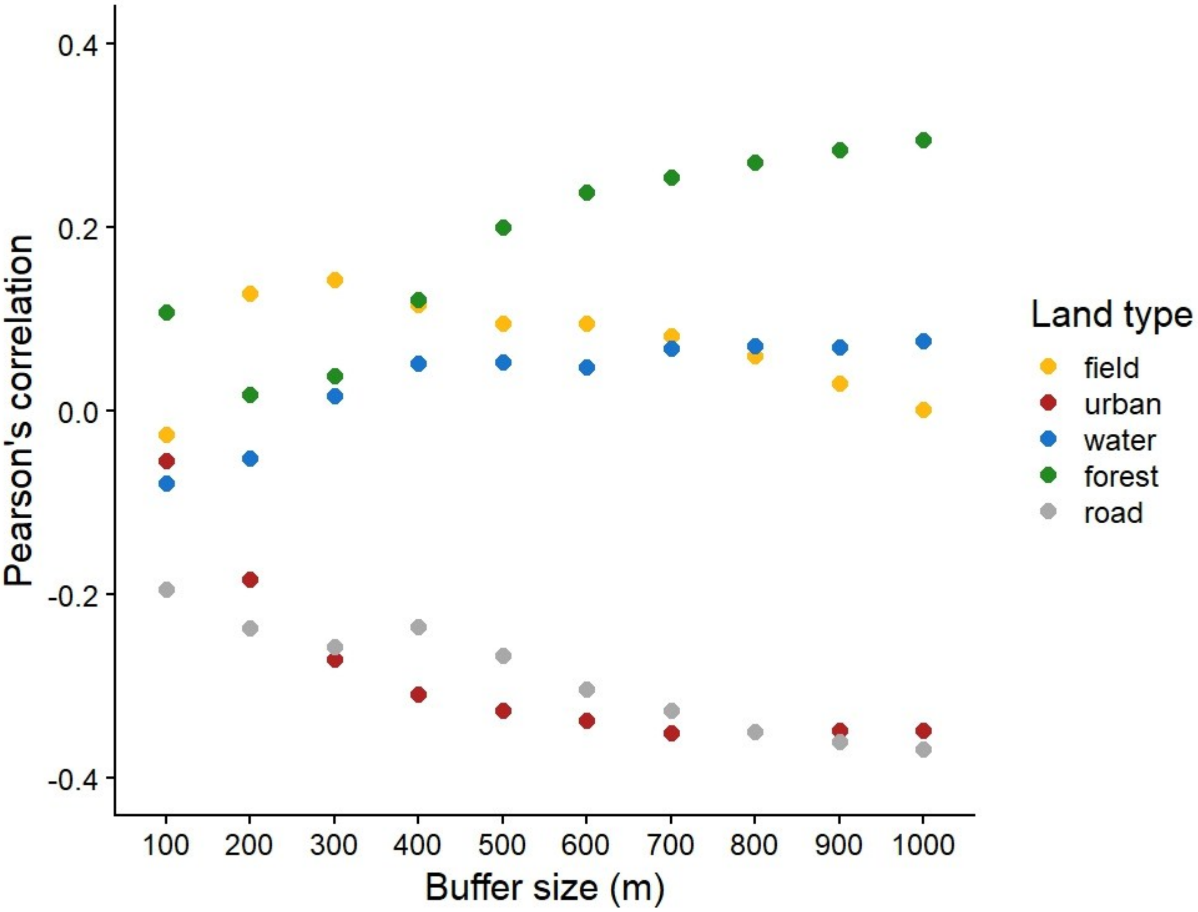

Figure C7 Pearson's correlation of habitat variables (field, forest, water, urban, roads) with parasite infection status.

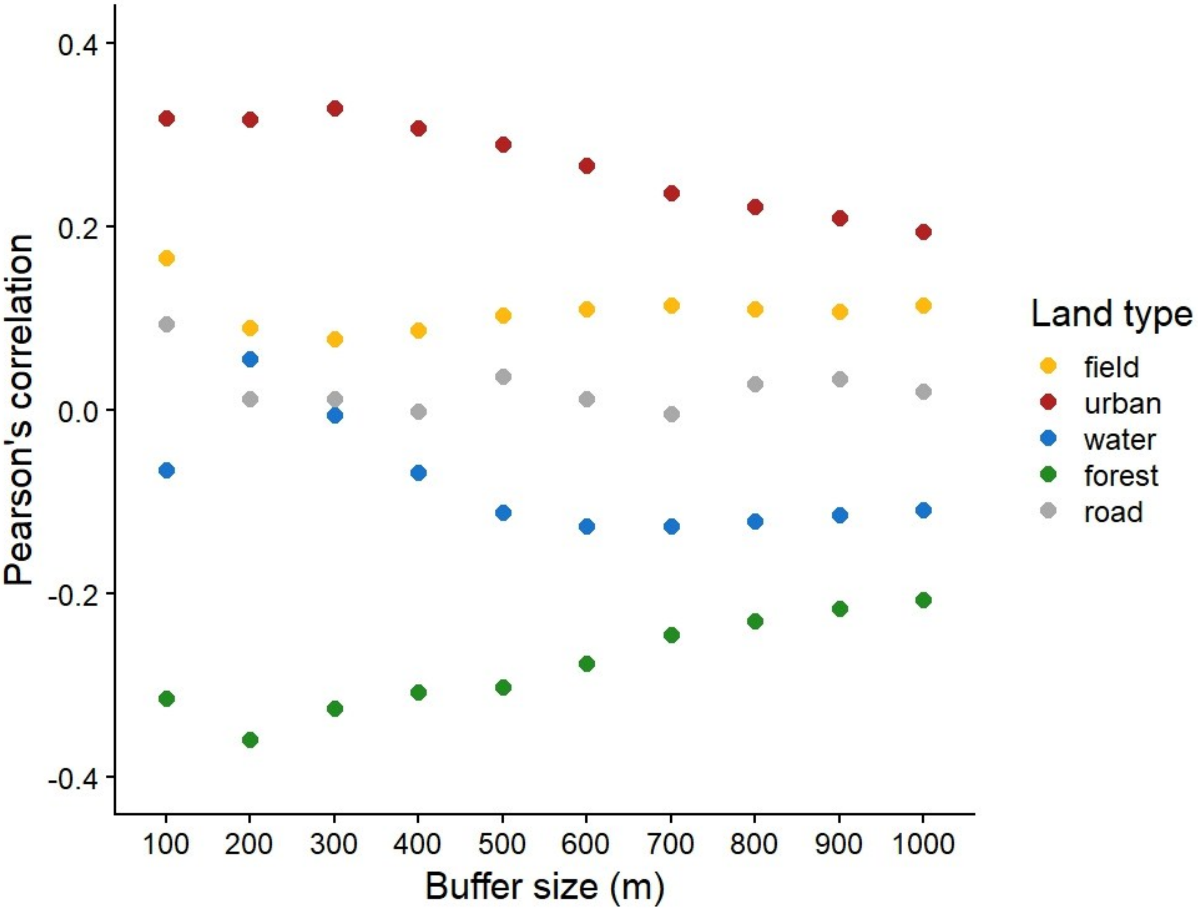

Figure C8 Pearson's correlation of habitat variables (field, forest, water, urban, roads) with plasma glycerol.

Appendix D: R Scripts

Script 1: Behavioural Models

```
library("readxl")
library("lme4")
library("lmerTest")
library("DHARMa")
library("car")
library("performance")
library("tidyverse")
library("glmmTMB")
library("parameters")
library("smatr")

data <- read_xlsx("Data/snakedat.xlsx")
#removing the single observation that was not sexed
data <- data[-(55),]

#Calculating SMI
plot(data$svl, data$weight)
data$logsvl <- log(data$svl)
data$logweight <- log(data$weight)

#Step 2: calculate log SMA regression
smamodel <- sma(logweight ~ logsvl, data = data)
plot(smamodel)

#Step 3: extract slope from regression, calculate mean
bsma <- coef(smamodel)[2]
L0 <- mean(data$svl)

#Step 4: calculate SMI
data$smi <- data$weight * (L0 / data$svl)^bsma

#Behavioural Score Models ----

#Want to model:
#DV = behaviour score - count data
#Fixed effects: svl, smi, body temp, date, tod, sex, water, field, urban, road, forest
#Random effects: site, id
```

```
#making a dataframe with the variables relevant to behavioural score data
scoredat <- data %>%
  select(id, date, site, total, svl, btemp, tod, sex, smi)

#Changing data to proper classes
scoredat$id <- as.factor(scoredat$id)
scoredat$site <- as.factor(scoredat$site)
scoredat$total <- as.numeric(scoredat$total)
scoredat$date <- as.Date(scoredat$date)
scoredat$svl <- as.numeric(scoredat$svl)
scoredat$btemp <- as.numeric(scoredat$btemp)
scoredat$sex <- as.factor(scoredat$sex)
scoredat$tod <- hms::as_hms(scoredat$tod)

#Read in scale of effect data
scalescore <- read_csv("maxscalesum.csv")

#Append scale of effect buffers to behavioural data
scoredat <- merge(scoredat, scalescore, by = "site")

scoredatna <- scoredat %>%
  filter(!is.na(btemp))

scoredatun <- scoredatna %>%
  distinct(id, .keep_all = TRUE)

#make dataframe of scaled variables to be used in model
scoredatun <- scoredatun %>%
  mutate(
  totalsc = scale(total),
  datesc = scale(date),
  svlsc = scale(svl),
  btempsc = scale(btemp),
  todsc = scale(tod),
  fieldsc = scale(field100),
  urbansc = scale(urban100),
  watersc = scale(water200),
```

```
  forestsc = scale(forest900),
  roadsc = scale(road400),
  smisc = scale(smi)
)

#Removing non-numeric columns to calculate correlations between independent variables
scorenum <- scoredatun %>% select(-c(id, site, date, sex, tod))
cor(scorenum, method = "pearson")

#Making a dataframe to visually look at the correlations
scoremat <- scoredatun %>% select(-c(totalsc, datesc, svlsc, btempsc, todsc, fieldsc, urbansc,
watersc, forestsc, roadsc, smisc))
scatterplotMatrix(scoremat,
                  regLine = TRUE, smooth = TRUE, diagonal = TRUE, data = scoremat)

#Making full model
fullmodsc <- glmmTMB(total ~ datesc + svlsc + btempsc + todsc + sex + smisc +
                     fieldsc + urbansc + watersc + forestsc +
                      (1|site), family = genpois(), data = scoredatun)

check_model(fullmodsc)
testDispersion(fullmodsc)
check_convergence(fullmodsc)
check_collinearity(fullmodsc)
plot(simulateResiduals(fullmodsc))
summary(fullmodsc)
diagnose(fullmod)

#VIF: field
redmodsc <- glmmTMB(total ~ svlsc + datesc + btempsc + todsc + sex + smisc +
                     forestsc + urbansc + watersc +
                     (1|site),
                    family = genpois(), data = scoredatun, na.action = na.fail)

summary(redmodsc)
check_model(redmodsc)
check_convergence(redmodsc)
testDispersion(redmodsc)
plot(simulateResiduals(redmodsc))
check_collinearity(redmodsc)
```

```
check_distribution(redmodsc)
plot(residuals(redmodsc))

#Model average to get more certain results
redsc <- dredge(redmodsc)
top_models_redsc <- get.models(redsc, subset = 1:10)
avgmodelredsc <- model.avg(top_models_redsc) # compute average parameters
summary(avgmodelredsc) # display averaged model
confint(avgmodelredsc, full = TRUE)

# Flee models ----------

fleedat <- data
fleedat$flee <- as.numeric(fleedat$flee)
fleedat <- fleedat %>% filter(!is.na(flee))
fleedat$flee <- as.factor(fleedat$flee)

#Read in scale of effect data
scaleflee <- read_csv("maxscaleflee.csv")

#Append scale of effect buffers to behavioural data
fleedat <- merge(fleedat, scaleflee, by = "site")

#Changing data to proper classes
fleedat$id <- as.factor(fleedat$id)
fleedat$site <- as.factor(fleedat$site)
fleedat$total <- as.numeric(fleedat$total)
fleedat$date <- as.Date(fleedat$date)
fleedat$svl <- as.numeric(fleedat$svl)
fleedat$btemp <- as.numeric(fleedat$btemp)
fleedat$sex <- as.factor(fleedat$sex)
fleedat$tod <- hms::as_hms(fleedat$tod)

fleedat <- fleedat %>%
  mutate(
    totalsc = scale(total),
    datesc = scale(date),
    svlsc = scale(svl),
    btempsc = scale(btemp),
    todsc = scale(tod),
```

```
    fieldsc = scale(field100),
    urbansc = scale(urban800),
    watersc = scale(water900),
    forestsc = scale(forest700),
    roadsc = scale(road100),
    smisc = scale(smi)
  )

fleedatcomp <- fleedat %>%
  filter(!is.na(btemp))

fleedatun <- fleedatcomp %>%
  distinct(id, .keep_all = TRUE)

fullmodfl <- glmmTMB(flee ~ datesc + svlsc + sex + btempsc + smisc +
                   fieldsc + urbansc + watersc + forestsc +
                   (1|site), data = fleedatun, family = binomial())

summary(fullmodfl)
check_model(fullmodfl)

#Reduced model
#VIF variables: water

redmodflee <- glmmTMB(flee ~ datesc + svlsc + sex + btempsc + smisc +
                    fieldsc + urbansc + forestsc +
                    (1|site), data = fleedatun, family = binomial(), na.action = na.fail)

summary(redmodflee)
check_model(redmodflee)
check_convergence(redmodflee)
testZeroInflation(redmodflee)
testDispersion(redmodflee)
plot(simulateResiduals(redmodflee))

#Model average for better confidence
ddredfl <- dredge(redmodflee)
top_models_redfl <- get.models(ddredfl, subset = 1:8)
avgmodelredfl <- model.avg(top_models_redfl) # compute average parameters
summary(avgmodelredfl)# display averaged model
```

```r
confint(avgmodelredfl, full = TRUE)

#Abundance Models ----

abundancedat <- read_xlsx("Data/abundance.xlsx")

abddat <- abundancedat %>%
  select(site, abund)

#Changing data to proper classes
abddat$site <- as.factor(abddat$site)

abddat <- abddat[-(36),]
colnames(abddat)[2] <-  "abd"

#Read in scale of effect data
scaleabd <- read_csv("maxscaleabd.csv")

#Append scale of effect buffers to behavioural data
abddat <- merge(abddat, scaleabd, by = "site")

#make dataframe of scaled variables to be used in model
abddat <- abddat %>%
  mutate(
    fieldsc = scale(field300),
    urbansc = scale(urban700),
    watersc = scale(water100),
    forestsc = scale(forest1000),
    roadsc = scale(road100)
  )

#Removing non-numeric columns to calculate correlations between independent variables
abdnum <- abddat %>% select(-c(site))
cor(abdnum, method = "pearson")

#Making a dataframe to visually look at the correlations
scatterplotMatrix(abdnum,
                  regLine = TRUE, smooth = TRUE, diagonal = TRUE, data = abdnum)

fullmodabd <- lm(abd ~ urbansc + forestsc + fieldsc + watersc,
```

```
                data = abddat)

check_model(fullmodabd)
abddat$forestsc <- as.numeric(abddat$forestsc)

vifmodabd <- lm(abd ~ urbansc + fieldsc + watersc,
            data = abddat, na.action = na.fail)

check_model(vifmodabd)
check_distribution(vifmodabd)
summary(vifmodabd)
testDispersion(vifmodabd)
plot(simulateResiduals(vifmodabd))
shapiro.test(residuals(vifmodabd))
library(lmtest)
bptest(vifmodabd)
dwtest(vifmodabd)
resettest(vifmodabd, type = "regressor", data = abddat)

ddredabd <- dredge(vifmodabd)
top_models_redabd <- get.models(ddredabd, subset = 1:2)
avgmodelredabd <- model.avg(top_models_redabd) # compute average parameters
summary(avgmodelredabd)# display averaged model
confint(avgmodelredabd, full = TRUE)

# Current Final Results -------

scoreresults <- summary(avgmodelredsc, full = TRUE)
scoreresultsconf <- confint(avgmodelredsc, full = TRUE)


fleeresults <- summary(avgmodelredfl, full = TRUE)# display averaged model
fleeresultsconf <- confint(avgmodelredfl, full = TRUE)


abdresults <- summary(avgmodelredabd) # display averaged model
abdresultsconf <- confint(avgmodelredabd, full = TRUE)

#Interpreting results
```

```
#Score
score10 <- ((exp(coef(avgmodelredsc, full = TRUE)[5])))^(1/(sd(scoredatun$urban100)/0.1))
score10
#For every 10% increase in urbanization, median behavioural response decreases by a factor of 0.90
#(conditional that other predictors are held at their mean)
score10up <- ((exp(scoreresultsconf[5,2])))^(1/(sd(scoredatun$urban100)/0.1))
score10low <- ((exp(scoreresultsconf[5])))^(1/(sd(scoredatun$urban100)/0.1))

#Flee
flee10 <- (exp(coef(avgmodelredfl, full = TRUE)[7]))^(1/(sd(fleedatun$urban800)/0.1))
flee10

#For every 10% increase in urbanization, odds of fleeing decrease by by a factor of 0.71
#(conditional that other predictors are held at their mean)
flee10up <- (exp(fleeresultsconf[7,2]))^(1/(sd(fleedatun$urban800)/0.1))
flee10low <- (exp(fleeresultsconf[7]))^(1/(sd(fleedatun$urban800)/0.1))

#Abundance
abd10 <- ((coef(avgmodelredabd)[2]))/(sd(abddat$urban700)/0.1)
abd10
#For every 10% increase in urban, the median snakes per visit increases by about 0.04

#Making Graphs -----

#SCORE

library(ggplot2)
install.packages("scales")
library("scales")

#Plot of Response
urbanscsc <- scoredatun$urbansc
urbanscsc <- as.numeric(urbanscsc)
forestscsc <- mean(scoredatun$forestsc)
forestscsc <- as.numeric(forestscsc)
waterscsc <- mean(scoredatun$watersc)
waterscsc <- as.numeric(waterscsc)
btempscsc <- mean(scoredatun$btempsc)
btempscsc <- as.numeric(btempscsc)
```

```
svlscsc <- mean(scoredatun$svlsc)
svlscsc <- as.numeric(svlscsc)
datescsc <- mean(scoredatun$datesc)
datescsc <- as.numeric(datescsc)
sexsc <- scoredatun$sex
sexsc <- as.factor(sexsc)

new_datasc <- cbind(urbanscsc, forestscsc, waterscsc, btempscsc, svlscsc, datescsc, sexsc)
new_datasc <- as.data.frame(new_datasc)

new_datasc$sexsc <- gsub("1", "F", new_datasc$sexsc)
new_datasc$sexsc <- gsub("2", "M", new_datasc$sexsc)
new_datasc$sexsc <- as.factor(new_datasc$sexsc)

colnames(new_datasc) <- c("urbansc", "forestsc", "watersc", "btempsc", "svlsc", "datesc", "sex")
new_datasc$total <- scoredatun$total
new_datasc$urban <- scoredatun$urban100

predictscl <- predict(avgmodelredsc, newdata = new_datasc, type = "link", se.fit = TRUE, re.form
= NA)
new_datasc$predictscl <- predictscl$fit
new_datasc$lower_cil <- predictscl$fit - 1.96 * predictscl$se.fit
new_datasc$upper_cil <- predictscl$fit + 1.96 * predictscl$se.fit

new_datasc$predictscr <- exp(new_datasc$predictscl)
new_datasc$lower_cir <- exp(new_datasc$lower_cil)
new_datasc$upper_cir <- exp(new_datasc$upper_cil)

plotrespsc <- ggplot(new_datasc, aes(x = urban, y = predictscr, group = sex)) +
  geom_line(aes(colour = sex), lwd = 1.5) +
  geom_ribbon(aes(ymin = lower_cir, ymax = upper_cir, colour = sex, fill = sex), alpha = 0.2) +
  scale_x_continuous(breaks = seq(0, 1, by = 0.2), labels = label_comma()) +
  geom_jitter(aes(x = urban, y = total, colour = sex), width = 0.025, height = 0.025, shape = 1) +
  labs(
      x = "Urban",
      y = "Behavioural score") +
  theme_classic(base_size = 18)
plotrespsc
```

Script 2: Physiological Models

```
library("readxl")
library("lme4")
library("lmerTest")
library("DHARMa")
library("car")
library("performance")
library("tidyverse")
library(ggplot2)
library("MuMIn")
library("scales")
library("smatr")
library("glmmTMB")

data <- read_xlsx("Data/snakedat.xlsx")
#removing the single observation that was not sexed
data <- data[-(55),]

#making a dataframe with the variables relevant to physiological data
physdat <- data %>%
  select(-c(flee, musk, gape, strike, total, notes))

#Changing data to proper classes
physdat$id <- as.factor(physdat$id)
physdat$site <- as.factor(physdat$site)
physdat$date <- as.Date(physdat$date)
physdat$svl <- as.numeric(physdat$svl)
physdat$btemp <- as.numeric(physdat$btemp)
physdat$sex <- as.factor(physdat$sex)
physdat$tod <- hms::as_hms(physdat$tod)

#Scaled Mass Index ------

#Read in scale of effect data
scalesmi <- read_csv("maxscalesmi.csv")

#Append scale data to phys data
smidat <- merge(physdat, scalesmi, by = "site")
```

```
smidat <- smidat %>%
  mutate(datesc = scale(date),
         svlsc = scale(svl),
        btempsc = scale(btemp),
        todsc = scale(tod),
        fieldsc = scale(field400),
         urbansc = scale(urban1000),
         watersc = scale(water300),
         forestsc = scale(forest900),
         roadsc = scale(road400)
         )

#Step 1: make plot of length vs mass

plot(physdat$svl, physdat$weight)
physdat$logsvl <- log(physdat$svl)
physdat$logweight <- log(physdat$weight)

#Step 2: calculate log SMA regression
smamodel <- sma(logweight ~ logsvl, data = physdat)
plot(smamodel)

#Step 3: extract slope from regression, calculate mean
bsma <- coef(smamodel)[2]
L0 <- mean(physdat$svl)

#Step 4: calculate SMI
physdat$smi <- physdat$weight * (L0 / physdat$svl)^bsma
smidat$smi <- physdat$smi

smidat <- smidat %>%
  mutate(smisc = scale(smi)
  )

#Univariate models

#Removing non-numeric columns to calculate correlations between independent variables
sminum <- smidat %>% select(c(datesc, svlsc, fieldsc, urbansc, watersc, forestsc, roadsc))
cor(sminum, method = "pearson")
```

```
#Making a dataframe to visually look at the correlations
smimat <- smidat %>% select(c(smisc, datesc, svlsc, btempsc, todsc, fieldsc, urbansc, watersc,
forestsc, roadsc,
                             ))
scatterplotMatrix(smimat,
                  regLine = TRUE, smooth = TRUE, diagonal = TRUE, data = smimat)

#SMI Models ------

#Want to model:
#DV: smi
#Fixed effects: sex, svl, urban, field, forest, water, road, date
#Random effects: site

smidatun <- smidat %>%
  distinct(id, .keep_all = TRUE)

fullmodsmi <- glmmTMB(smi ~ datesc + svlsc + sex +
                     fieldsc + urbansc + watersc + forestsc +
                     (1|site), family = gaussian(), data = smidatun)

summary(fullmodsmi)
check_model(fullmodsmi, verbose = TRUE)
vif(fullmodsmi)

#Removing forest due to high VIF
vifmodsmi <- glmmTMB(smi ~ datesc + svlsc + sex +
                     fieldsc + urbansc + watersc +
                     (1|site), family = gaussian(), data = smidatun, na.action = "na.fail")

summary(vifmodsmi)
vif(vifmodsmi)
check_heteroscedasticity(vifmodsmi)
testDispersion(vifmodsmi)
plot(simulateResiduals(vifmodsmi))
check_model(vifmodsmi)

#Model selection and averaging to reduce overfitting
na.action = na.fail
```

```
ddsmi <- dredge(vifmodsmi)
topmodsmi <- get.models(ddsmi, subset = 1:7)
avgmodelredsmi <- model.avg(topmodsmi) # compute average parameters
summary(avgmodelredsmi) # display averaged model
confint(avgmodelredsmi, full = TRUE)

#H:L Ratio Models -----
#Want to model:
#DV: hlr
#Fixed effects: sex, svl, urban, field, forest, water, road, date, btemp, tod
#Random effects: site

scalehlr <- read_csv("maxscalehlr.csv")

#Append scale data to phys data
hlrdat <- merge(physdat, scalehlr, by = "site")

#Making dataframe no NA in HL
hlrdatcomp <- hlrdat %>%
  filter(!is.na(hlr))

hlrdatcomp$hlrsc <- scale(hlrdatcomp$hlr)

hlrdatcomp <- hlrdatcomp %>%
  mutate(
              datesc = scale(date),
               svlsc = scale(svl),
               btempsc = scale(btemp),
               todsc = scale(tod),
        fieldsc = scale(field200),
        urbansc = scale(urban400),
        watersc = scale(water500),
        forestsc = scale(forest100),
        roadsc = scale(road800)
  )

hlrdatun <- hlrdatcomp %>%
  distinct(id, .keep_all = TRUE)

fullmodhlr <- glmmTMB(hlr ~ datesc + svlsc + sex + btempsc + todsc +
```

```
                  fieldsc + urbansc + watersc + forestsc +
                  (1|site), family = gaussian(), data = hlrdatun)

summary(fullmodhlr)
check_model(fullmodhlr, verbose = TRUE)
vif(fullmodhlr)
plot(fullmodhlr)

#VIF: forest
vifmodhlr <- glmmTMB(log(hlr) ~ datesc + svlsc + sex + btempsc + todsc +
                fieldsc + urbansc + watersc +
                (1|site), family = gaussian(), data = hlrdatun, na.action = "na.fail")

summary(vifmodhlr)
check_model(vifmodhlr)
plot(simulateResiduals(vifmodhlr))
testDispersion(vifmodhlr)
check_heteroscedasticity(vifmodhlr)
testDispersion(vifmodhlr)

#Model average to get more certain results
na.action = na.fail
redhlr <- dredge(vifmodhlr)
top_models_redhlr <- get.models(redhlr, subset = 1:10)
avgmodelredhlr <- model.avg(top_models_redhlr) # compute average parameters
summary(avgmodelredhlr)# display averaged model
confint(avgmodelredhlr, full = TRUE)

#Parasite Models ----

#Parasite Prevalence
#Want to model:
#DV: par
#Fixed effects: sex, svl, urban, field, forest, water, road, date,
#Random effects: site

scalepar <- read_csv("maxscalepar.csv")

#Append scale data to phys data
pardat <- merge(physdat, scalepar, by = "site")
```

```
#Making dataframe no NA in HL
pardatcomp <- pardat %>%
  filter(!is.na(par))

pardatcomp <- pardatcomp %>%
  mutate(
    datesc = scale(date),
    svlsc = scale(svl),
    fieldsc = scale(field100),
    urbansc = scale(urban700),
    watersc = scale(water100),
    forestsc = scale(forest1000),
    roadsc = scale(road1000),
    smisc = scale(smi)
  )

pardatun <- pardatcomp %>%
  distinct(id, .keep_all = TRUE)

fullmodpar <- glmmTMB(par ~ svlsc + sex + smisc + datesc +
                     fieldsc + urbansc + watersc + forestsc +
                     (1|site), family = genpois(), data = pardatun)

summary(fullmodpar)
check_model(fullmodpar)
testDispersion(fullmodpar)
check_overdispersion(fullmodpar)
testZeroInflation(fullmodpar)
check_collinearity(fullmodpar)
plot(residuals(fullmodpar))

#Variables removed due to VIF: forest

vifmodpar <- glmmTMB(par ~ svlsc + sex + smisc + datesc +
                      fieldsc + urbansc + watersc +
                      (1|site), family = genpois(), data = pardatun, na.action = na.fail)

check_model(vifmodpar)
summary(vifmodpar)
```

```
check_convergence(vifmodpar)
check_collinearity(vifmodpar)
testDispersion(vifmodpar)
check_overdispersion(vifmodpar)
plot(simulateResiduals(vifmodpar))
testZeroInflation(vifmodpar)
check_zeroinflation(vifmodpar)
plot(residuals(vifmodpar))
check_autocorrelation(vifmodpar)
check_distribution(vifmodpar)

#Model average to get more certain results
na.action = na.fail
redpar <- dredge(vifmodpar)
top_models_redpar <- get.models(redpar, subset = 1:3)
avgmodelredpar <- model.avg(top_models_redpar) # compute average parameters
summary(avgmodelredpar)# display averaged model
confint(avgmodelredpar, full = TRUE)

#For graphing
top_models_redparsex <- get.models(redpar, subset = 1:4)
avgmodelredparsex <- model.avg(top_models_redparsex) # compute average parameters
summary(avgmodelredparsex)

#Infection Status Models ----
#Want to model:
#DV: ifs
#Fixed effects: sex, svl, urban, field, forest, water, road, date,
#Random effects: site

scaleifs <- read_csv("maxscaleifs.csv")

#Append scale data to phys data
ifsdat <- merge(physdat, scaleifs, by = "site")

#Making dataframe no NA in HL
ifsdatcomp <- ifsdat %>%
  filter(!is.na(par))

ifsdatcomp <- ifsdatcomp %>%
```

```
  mutate(
    ifs = case_when(par > 0 ~ 1, TRUE ~ 0)
  )

ifsdatcomp <- ifsdatcomp %>%
  mutate(
    datesc = scale(date),
    svlsc = scale(svl),
    fieldsc = scale(field300),
    urbansc = scale(urban700),
    watersc = scale(water100),
    forestsc = scale(forest1000),
    roadsc = scale(road1000),
    smisc = scale(smi)
  )

ifsdatun <- ifsdatcomp %>%
  distinct(id, .keep_all = TRUE)

fullmodifs <- glmmTMB(ifs ~ datesc + svlsc + sex + smisc +
                      fieldsc + urbansc + watersc + forestsc +
                      (1|site), family = binomial(), data = ifsdatun, na.action = na.fail)

summary(fullmodifs)
check_model(fullmodifs)
testDispersion(fullmodifs)
testZeroInflation(fullmodifs)

#VIF: forest
vifmodifs <- glmmTMB(ifs ~ datesc + sex + smisc + svlsc +
                     fieldsc + urbansc + watersc +
                     (1|site), family = binomial(), data = ifsdatun, na.action = na.fail)

summary(vifmodifs)
check_model(vifmodifs)
testDispersion(vifmodifs)
testZeroInflation(vifmodifs)
vif(fullmodifs)
plot(simulateResiduals(vifmodifs))
```

```
#Model average to get more certain results
na.action = na.fail
redifs <- dredge(vifmodifs)
top_models_redifs <- get.models(redifs, subset = 1:2)
avgmodelredifs <- model.avg(top_models_redifs) # compute average parameters
summary(avgmodelredifs)# display averaged model
confint(avgmodelredifs, full = TRUE)

#For graphing
top_models_redifssex <- get.models(redifs, subset = 1:6)
avgmodelredifssex <- model.avg(top_models_redifssex) # compute average parameters
summary(avgmodelredifssex)# display averaged model

#Glycerol Models ----

#Want to model:
#DV: gly
#Fixed effects: sex, svl, urban, field, forest, water, date, smi
#Random effects: site

scalegly <- read_csv("maxscalegly.csv")

#Append scale data to phys data
glydat <- merge(physdat, scalegly, by = "site")

#Making dataframe no NA in HL
glydatcomp <- glydat %>%
  filter(!is.na(gly))

glydatcomp <- glydatcomp %>%
  mutate(
    datesc = scale(date),
    svlsc = scale(svl),
    fieldsc = scale(field100),
    urbansc = scale(urban300),
    watersc = scale(water700),
    forestsc = scale(forest200),
    roadsc = scale(road100),
    smisc = scale(smi)
  )
```

```
glydatun <- glydatcomp %>%
  distinct(id, .keep_all = TRUE)

#Making dataframe to manage selection of data subset to be measured for glycerol (not used for
models)
physdat$bvol <- as.numeric(physdat$bvol)

#Making a csv to select the samples I want to analyze
glydatunsel <- physdat %>%
  distinct(id, .keep_all = TRUE)

glydatunsel <- glydatun %>%
  filter(bvol > 10)

summary(glydatunsel)

write.csv(glydatunsel, "glycerol_selection.csv", row.names = FALSE)

#Models
fullmodgly <- glmmTMB(gly ~ datesc + svlsc + sex + smisc +
                      fieldsc + urbansc + watersc + forestsc +
                      (1|site), family = gaussian(), data = glydatun, na.action = na.fail)

summary(fullmodgly)
check_model(fullmodgly)
testDispersion(fullmodgly)
testZeroInflation(fullmodgly)

#VIF: field
vifmodgly <- glmmTMB(gly ~ datesc + sex + smisc + svlsc +
                     forestsc + urbansc + watersc +
                     (1|site), family = gaussian(), data = glydatun, na.action = na.fail)

summary(vifmodgly)
check_model(vifmodgly)
testDispersion(vifmodgly)
testZeroInflation(vifmodgly)
plot(simulateResiduals(vifmodgly))
```

```
#Model average to get more certain results
na.action = na.fail
redgly <- dredge(vifmodgly)
top_models_redgly <- get.models(redgly, subset = 1:4)
avgmodelredgly <- model.avg(top_models_redgly) # compute average parameters
summary(avgmodelredgly)# display averaged model
confint(avgmodelredgly, full = TRUE)

#Current Final Results ------

smiresults <- summary(avgmodelredsmi, full = TRUE)
smiresultsconf <- confint(avgmodelredsmi, full = TRUE)

hlrresults <- summary(avgmodelredhlr, full = TRUE)
hlrresultsconf <- confint(avgmodelredhlr, full = TRUE)

parresults <- summary(avgmodelredpar, full = TRUE)
parresultsconf <- confint(avgmodelredpar, full = TRUE)

ifsresults <- summary(avgmodelredifs, full = TRUE)
ifsresultsconf <- confint(avgmodelredifs, full = TRUE)

glyresults <- summary(avgmodelredgly, full = TRUE)
glyresultsconf <- confint(avgmodelredgly, full = TRUE)

#Interpreting Results

#SMI
smi10 <- ((coef(avgmodelredsmi, full = TRUE)[3]))/(sd(smidatun$urban1000)/0.1)
smi10
#For every 10% increase in urban, the median SMI increases by about 0.06 units
smi10up <- (smiresultsconf)[3,2]/(sd(smidatun$urban1000)/0.1)
smi10low <- (smiresultsconf)[3]/(sd(smidatun$urban1000)/0.1)

#HLR
hlr10 <- ((coef(avgmodelredhlr, full = TRUE)[4]))/(sd(hlrdatun$urban400)/0.1)
hlr10
#For every 10% increase in urban, the median log HLR increases by about 0.02
hlr10up <- (hlrresultsconf)[4,2]/(sd(hlrdatun$urban400)/0.1)
hlr10low <- (hlrresultsconf)[4]/(sd(hlrdatun$urban400)/0.1)
```

```
#Parasites
par10 <- ((exp(coef(avgmodelredpar, full = TRUE)[4])))^(1/(sd(pardatun$urban700)/0.1))
par10
#For every 10% increase in urbanization, median parasite load decreases by a factor of 0.64
#(conditional that other predictors are held at their mean)
par10up <- ((exp(parresultsconf[4,2])))^(1/(sd(pardatun$urban700)/0.1))
par10low <- ((exp(parresultsconf[4])))^(1/(sd(pardatun$urban700)/0.1))

#Infection Status
ifs10 <- ((exp(coef(avgmodelredifs, full = TRUE)[4])))^(1/(sd(ifsdatun$urban700)/0.1))
ifs10
#For every 10% increase in urbanization, odds of being infected decreases by a factor of 0.63
#(conditional that other predictors are held at their mean)
ifs10up <- ((exp(ifsresultsconf[4,2])))^(1/(sd(ifsdatun$urban700)/0.1))
ifs10low <- ((exp(ifsresultsconf[4])))^(1/(sd(ifsdatun$urban700)/0.1))

#Glycerol
gly10 <- ((coef(avgmodelredgly, full = TRUE)[6]))/(sd(glydatun$urban300)/0.1)
gly10
#For every 10% increase in urban, the average gly by about 0.14 mM
gly10up <- (glyresultsconf)[6,2]/(sd(glydatun$urban300)/0.1)
gly10low <- (glyresultsconf)[6]/(sd(glydatun$urban300)/0.1)
```

Script 3: Leukocyte Models (Appendix A)

```
library("readxl")
library("lme4")
library("lmerTest")
library("DHARMa")
library("car")
library("performance")
library("MuMIn")
library("tidyverse")
library(ggplot2)
library("scales")
library("parameters")
library(glmmTMB)

data <- read_xlsx("Data/snakedat.xlsx")
#removing the single observation that was not sexed
data <- data[-(55),]

plot(data$svl, data$weight)
data$logsvl <- log(data$svl)
data$logweight <- log(data$weight)

smamodel <- sma(logweight ~ logsvl, data = data)
plot(smamodel)

#Step 3: extract slope from regression, calculate mean
bsma <- coef(smamodel)[2]
L0 <- mean(data$svl)

#Step 4: calculate SMI
data$smi <- data$weight * (L0 / data$svl)^bsma

#making a dataframe with the variables relevant to physiological data
leukdat <- data %>%
  select(c(site, id, date, svl, btemp, sex, hct, wbc, lym, het, mon, az, bas, eos, par, tbleed, smi))

#Changing data to proper classes
leukdat$id <- as.factor(leukdat$id)
leukdat$site <- as.factor(leukdat$site)
```

```
leukdat$date <- as.Date(leukdat$date)
leukdat$id <- as.factor(leukdat$id)
leukdat$svl <- as.numeric(leukdat$svl)
leukdat$btemp <- as.numeric(leukdat$btemp)
leukdat$sex <- as.factor(leukdat$sex)
leukdat$tbleed <- as.numeric(leukdat$tbleed)

leukdat <- leukdat %>%
  filter(!is.na(wbc))

#Checking Effect of Time to Bleed on Response Variables ----

plot(leukdat$tbleed, leukdat$hct)
cor.test(leukdat$tbleed, leukdat$hct, method = "spearman")

plot(leukdat$tbleed, leukdat$wbc)
cor.test(leukdat$tbleed, leukdat$wbc, method = "spearman")

plot(leukdat$tbleed, leukdat$lym)
cor.test(leukdat$tbleed, leukdat$lym, method = "spearman")

plot(leukdat$tbleed, leukdat$het)
cor.test(leukdat$tbleed, leukdat$het, method = "spearman")

plot(leukdat$tbleed, leukdat$az)
cor.test(leukdat$tbleed, leukdat$az, method = "spearman")

plot(leukdat$tbleed, leukdat$bas)
cor.test(leukdat$tbleed, leukdat$bas, method = "spearman")

plot(leukdat$tbleed, leukdat$mon)
cor.test(leukdat$tbleed, leukdat$mon, method = "spearman")

plot(leukdat$tbleed, leukdat$eos)
cor.test(leukdat$tbleed, leukdat$eos, method = "spearman")

plot(leukdat$tbleed, (leukdat$het/leukdat$lym))
cor.test(leukdat$tbleed, (leukdat$het/leukdat$lym), method = "spearman")
```

```
#Hematocrit Models ----

#DV: hct
#IV: date, svl, smi, btemp, sex, par
#Random effects: site

scalehct <- read_csv("maxscalehct.csv")

#Append scale data to phys data
hctdat <- merge(leukdat, scalehct, by = "site")

#Making dataframe no NA in HL
hctdatcomp <- hctdat %>%
  filter(!is.na(hct))

hctdatcomp$hctsc <- scale(hctdatcomp$hct)
hctdatcomp$btemp <- as.numeric(hctdatcomp$btemp)

hctdatcomp <- hctdatcomp %>%
  mutate(
    datesc = scale(date),
    svlsc = scale(svl),
    smisc = scale(smi),
    btempsc = scale(btemp),
    parsc = scale(par),
    fieldsc = scale(field200),
    urbansc = scale(urban100),
    watersc = scale(water900),
    forestsc = scale(forest700),
    roadsc = scale(road600)
  )

hctdatcomp$hctprob <- hctdatcomp$hct/100
hctdatun <- hctdatcomp %>%
  distinct(id, .keep_all = TRUE)

#Checking correlations between predictors
hctdatcor <- hctdatcomp %>% select(c(datesc, svlsc, btempsc, smisc, parsc,
                                     fieldsc, urbansc, watersc, forestsc, roadsc))
cor(hctdatcor)
```

```
fullmodhct <- glmmTMB(hctprob ~ datesc + svlsc + smisc + btempsc + parsc + sex +
                fieldsc + urbansc + watersc + forestsc +
                (1|site), family = beta_family(), data = hctdatun)

summary(fullmodhct)
check_model(fullmodhct, verbose = TRUE)
plot(fullmodhct)

#VIF variables: none
vifmodhct <- glmmTMB(hctprob ~ datesc + svlsc + smisc + btempsc + parsc + sex +
                fieldsc + urbansc + watersc + forestsc +
                (1|site), family = beta_family(), data = hctdatun, na.action = na.fail)

summary(vifmodhct)
check_model(vifmodhct, verbose = TRUE)
check_convergence(vifmodhct)
check_collinearity(vifmodhct)
testDispersion(vifmodhct)
check_overdispersion(vifmodhct)
plot(simulateResiduals(vifmodhct))
testZeroInflation(vifmodhct)
check_zeroinflation(vifmodhct)
plot(residuals(vifmodhct))
check_autocorrelation(vifmodhct)
check_distribution(vifmodhct)

#Model averaging to reduce overfitting
na.action = na.fail
redhct <- dredge(vifmodhct)
top_models_vifmodhct <- get.models(redhct, subset = 1:10)
avgmodelvifmodhct <- model.avg(top_models_vifmodhct) # compute average parameters
summary(avgmodelvifmodhct) # display averaged model
confint(avgmodelvifmodhct, full = TRUE)

#Total WBC Models ----

#DV: wbc
#IV: date, svl, smi, btemp, sex, par
```

```
#Random effects: site

scalewbc <- read_csv("maxscalewbc.csv")

#Append scale data to leuk data
wbcdat <- merge(leukdat, scalewbc, by = "site")

#Making dataframe no NA in HL
wbcdatcomp <- wbcdat %>%
  filter(!is.na(wbc))

wbcdatcomp$wbcsc <- scale(wbcdatcomp$wbc)
wbcdatcomp$btemp <- as.numeric(wbcdatcomp$btemp)

wbcdatcomp <- wbcdatcomp %>%
  mutate(
    datesc = scale(date),
    svlsc = scale(svl),
    smisc = scale(smi),
    btempsc = scale(btemp),
    parsc = scale(par),
    fieldsc = scale(field1000),
    urbansc = scale(urban600),
    watersc = scale(water1000),
    forestsc = scale(forest1000),
    roadsc = scale(road900)
  )

wbcdatun <- wbcdatcomp %>%
  distinct(id, .keep_all = TRUE)

#Checking correlations between predictors
wbcdatcor <- wbcdatcomp %>% select(c(datesc, svlsc, btempsc, smisc, parsc,
                                     fieldsc, urbansc, watersc, forestsc, roadsc))
cor(wbcdatcor)

fullmodwbc <- glmmTMB(wbc ~ datesc + svlsc + smisc + btempsc + parsc + sex +
                      fieldsc + urbansc + watersc + forestsc +
                      (1|site), family = gaussian(), data = wbcdatun)
```

```
summary(fullmodwbc)
check_model(fullmodwbc, verbose = TRUE)
plot(fullmodwbc)

#VIF variables: forest
vifmodwbc <- glmmTMB(wbc ~ datesc + svlsc + smisc + btempsc + parsc + sex +
                fieldsc + urbansc + watersc +
                (1|site), family = gaussian(), data = wbcdatun, na.action = na.fail)

summary(vifmodwbc)
check_model(vifmodwbc, verbose = TRUE)
check_convergence(vifmodwbc)
check_collinearity(vifmodwbc)
testDispersion(vifmodwbc)
check_overdispersion(vifmodwbc)
plot(simulateResiduals(vifmodwbc))
testZeroInflation(vifmodwbc)
check_zeroinflation(vifmodwbc)
plot(residuals(vifmodwbc))
check_autocorrelation(vifmodwbc)
check_distribution(vifmodwbc)

#Model averaging to reduce overfitting
na.action = na.fail
redwbc <- dredge(vifmodwbc)
top_models_vifmodwbc <- get.models(redwbc, subset = 1:13)
avgmodelvifmodwbc <- model.avg(top_models_vifmodwbc) # compute average parameters
summary(avgmodelvifmodwbc) # display averaged model
confint(avgmodelvifmodwbc, full = TRUE)

#Lymphocyte Models ----

#DV: lym
#IV: date, svl, smi, btemp, sex, par
#Random effects: site

scalelym <- read_csv("maxscalelym.csv")

#Append scale data to leuk data
lymdat <- merge(leukdat, scalelym, by = "site")
```

```
#Making dataframe no NA in HL
lymdatcomp <- lymdat %>%
  filter(!is.na(lym))

lymdatcomp$lymsc <- scale(lymdatcomp$lym)
lymdatcomp$btemp <- as.numeric(lymdatcomp$btemp)

lymdatcomp <- lymdatcomp %>%
  mutate(
    datesc = scale(date),
    svlsc = scale(svl),
    smisc = scale(smi),
    btempsc = scale(btemp),
    parsc = scale(par),
    fieldsc = scale(field300),
    urbansc = scale(urban400),
    watersc = scale(water100),
    forestsc = scale(forest900),
    roadsc = scale(road200)
  )

lymdatcomp$lymprob <- lymdatcomp$lym/100
lymdatun <- lymdatcomp %>%
  distinct(id, .keep_all = TRUE)

#Checking correlations between predictors
lymdatcor <- lymdatcomp %>% select(c(datesc, svlsc, btempsc, smisc, parsc,
                                     fieldsc, urbansc, watersc, forestsc, roadsc))
cor(lymdatcor)

fullmodlym <- glmmTMB(lymprob ~ datesc + svlsc + smisc + btempsc + parsc + sex +
                      fieldsc + urbansc + watersc + forestsc +
                      (1|site), family = beta_family(), data = lymdatun)

summary(fullmodlym)
check_model(fullmodlym, verbose = TRUE)
plot(fullmodlym)

#VIF variables: forest,
```

```
vifmodlym <- glmmTMB(lymprob ~ datesc + svlsc + smisc + btempsc + parsc + sex +
                fieldsc + urbansc + watersc +
                (1|site), family = beta_family(), data = lymdatun, na.action = na.fail)

summary(vifmodlym)
check_model(vifmodlym, verbose = TRUE)
check_outliers(vifmodlym)
check_convergence(vifmodlym)
check_collinearity(vifmodlym)
testDispersion(vifmodlym)
check_overdispersion(vifmodlym)
plot(simulateResiduals(vifmodlym))
testZeroInflation(vifmodlym)
check_zeroinflation(vifmodlym)
plot(residuals(vifmodlym))
check_autocorrelation(vifmodlym)
check_distribution(vifmodlym)

#Model averaging to reduce overfitting
na.action = na.fail
redlym <- dredge(vifmodlym)
top_models_vifmodlym <- get.models(redlym, subset = 1:18)
avgmodelvifmodlym <- model.avg(top_models_vifmodlym) # compute average parameters
summary(avgmodelvifmodlym) # display averaged model
confint(avgmodelvifmodlym, full = TRUE)

#Heterophil Models ----

#DV: het
#IV: date, svl, smi, btemp, sex, par
#Random effects: site

scalehet <- read_csv("maxscalehet.csv")

#Append scale data to leuk data
hetdat <- merge(leukdat, scalehet, by = "site")

#Making dataframe no NA in HL
hetdatcomp <- hetdat %>%
  filter(!is.na(het))
```

```
hetdatcomp$hetsc <- scale(hetdatcomp$het)
hetdatcomp$btemp <- as.numeric(hetdatcomp$btemp)

hetdatcomp <- hetdatcomp %>%
  mutate(
    datesc = scale(date),
    svlsc = scale(svl),
    smisc = scale(smi),
    btempsc = scale(btemp),
    parsc = scale(par),
    fieldsc = scale(field100),
    urbansc = scale(urban1000),
    watersc = scale(water500),
    forestsc = scale(forest400),
    roadsc = scale(road200)
  )

hetdatcomp$hetprob <- hetdatcomp$het/100
hetdatun <- hetdatcomp %>%
  distinct(id, .keep_all = TRUE)

#Checking correlations between predictors
hetdatcor <- hetdatcomp %>% select(c(datesc, svlsc, btempsc, smisc, parsc,
                                     fieldsc, urbansc, watersc, forestsc, roadsc))
cor(hetdatcor)

fullmodhet <- glmmTMB(hetprob ~ datesc + svlsc + smisc + btempsc + parsc + sex +
                      fieldsc + urbansc + watersc + forestsc +
                      (1|site), family = beta_family(), data = hetdatun)

summary(fullmodhet)
check_model(fullmodhet, verbose = TRUE)
plot(fullmodhet)

#VIF variables: forest
vifmodhet <- glmmTMB(hetprob ~ datesc + svlsc + smisc + btempsc + parsc + sex +
                     urbansc + watersc + fieldsc +
                     (1|site), family = beta_family(), data = hetdatun, na.action = na.fail)
```

```
summary(vifmodhet)
check_model(vifmodhet, verbose = TRUE)
check_convergence(vifmodhet)
check_collinearity(vifmodhet)
testDispersion(vifmodhet)
check_overdispersion(vifmodhet)
plot(simulateResiduals(vifmodhet))
testZeroInflation(vifmodhet)
check_zeroinflation(vifmodhet)
plot(residuals(vifmodhet))
check_autocorrelation(vifmodhet)
check_distribution(vifmodhet)

#Model averaging to reduce overfitting
na.action = na.fail
redhet <- dredge(vifmodhet)
top_models_vifmodhet <- get.models(redhet, subset = 1:13)
avgmodelvifmodhet <- model.avg(top_models_vifmodhet) # compute average parameters
summary(avgmodelvifmodhet) # display averaged model
confint(avgmodelvifmodhet, full = TRUE)

#Azurophil Models ----

#DV: az
#IV: date, svl, smi, btemp, sex, par
#Random effects: site

scaleaz <- read_csv("maxscaleaz.csv")

#Append scale data to leuk data
azdat <- merge(leukdat, scaleaz, by = "site")

#Making dataframe no NA in HL
azdatcomp <- azdat %>%
  filter(!is.na(az))

azdatcomp$azsc <- scale(azdatcomp$az)
azdatcomp$btemp <- as.numeric(azdatcomp$btemp)
```

```
azdatcomp <- azdatcomp %>%
  mutate(
    datesc = scale(date),
    svlsc = scale(svl),
    smisc = scale(smi),
    btempsc = scale(btemp),
    parsc = scale(par),
    fieldsc = scale(field200),
    urbansc = scale(urban100),
    watersc = scale(water200),
    forestsc = scale(forest200),
    roadsc = scale(road200)
  )

azdatcomp$azprob <- azdatcomp$az/100
azdatun <- azdatcomp %>%
  distinct(id, .keep_all = TRUE)

#Checking correlations between predictors
azdatcor <- azdatcomp %>% select(c(datesc, svlsc, btempsc, smisc, parsc,
                                   fieldsc, urbansc, watersc, forestsc, roadsc))
cor(azdatcor)

fullmodaz <- glmmTMB(azprob ~ datesc + svlsc + smisc + btempsc + parsc + sex +
                     fieldsc + urbansc + watersc + forestsc +
                     (1|site), family = beta_family(), data = azdatun)

summary(fullmodaz)
check_model(fullmodaz, verbose = TRUE)
plot(fullmodaz)

#VIF variables: field
vifmodaz <- glmmTMB(azprob ~ datesc + svlsc + smisc + btempsc + parsc + sex +
                    forestsc + urbansc + watersc +
                    (1|site), family = beta_family(), data = azdatun, na.action = na.fail)

summary(vifmodaz)
check_model(vifmodaz, verbose = TRUE)
check_convergence(vifmodaz)
check_collinearity(vifmodaz)
```

```
testDispersion(vifmodaz)
check_overdispersion(vifmodaz)
plot(simulateResiduals(vifmodaz))
testZeroInflation(vifmodaz)
check_zeroinflation(vifmodaz)
plot(residuals(vifmodaz))
check_autocorrelation(vifmodaz)
check_distribution(vifmodaz)

#Model averaging to reduce overfitting
na.action = na.fail
redaz <- dredge(vifmodaz)
top_models_vifmodaz <- get.models(redaz, subset = 1:9)
avgmodelvifmodaz <- model.avg(top_models_vifmodaz) # compute average parameters
summary(avgmodelvifmodaz) # display averaged model
confint(avgmodelvifmodaz, full = TRUE)

#Basophil Models ----

#DV: bas
#IV: date, svl, smi, btemp, sex, par
#Random effects: site

scalebas <- read_csv("maxscalebas.csv")

#Append scale data to leuk data
basdat <- merge(leukdat, scalebas, by = "site")

#Making dataframe no NA in HL
basdatcomp <- basdat %>%
  filter(!is.na(bas))

basdatcomp$bassc <- scale(basdatcomp$bas)
basdatcomp$btemp <- as.numeric(basdatcomp$btemp)

basdatcomp <- basdatcomp %>%
  mutate(
    datesc = scale(date),
    svlsc = scale(svl),
    smisc = scale(smi),
```

```
    btempsc = scale(btemp),
    parsc = scale(par),
    fieldsc = scale(field500),
    urbansc = scale(urban200),
    watersc = scale(water200),
    forestsc = scale(forest700),
    roadsc = scale(road700)
  )

basdatcomp$basprob <- basdatcomp$bas/100
basdatun <- basdatcomp %>%
  distinct(id, .keep_all = TRUE)

#Checking correlations between predictors
basdatcor <- basdatcomp %>% select(c(datesc, svlsc, btempsc, smisc, parsc,
                                     fieldsc, urbansc, watersc, forestsc, roadsc))
cor(basdatcor)

fullmodbas <- glmmTMB(basprob ~ datesc + svlsc + smisc + btempsc + parsc + sex +
                      fieldsc + urbansc + watersc + forestsc +
                      (1|site), family = ordbeta(), data = basdatun)

summary(fullmodbas)
check_model(fullmodbas, verbose = TRUE)
plot(fullmodbas)
check_collinearity(fullmodbas)

#VIF variables: field

vifmodbas <- glmmTMB(basprob ~ datesc + svlsc + smisc + btempsc + parsc + sex +
                      forestsc + urbansc + watersc +
                      (1|site), family = ordbeta(), data = basdatun, na.action = na.fail)

summary(vifmodbas)
check_model(vifmodbas, verbose = TRUE)
check_convergence(vifmodbas)
check_collinearity(vifmodbas)
testDispersion(vifmodbas)
check_overdispersion(vifmodbas)
plot(simulateResiduals(vifmodbas))
```

```
testZeroInflation(vifmodbas)
check_zeroinflation(vifmodbas)
plot(residuals(vifmodbas))
check_autocorrelation(vifmodbas)
check_distribution(vifmodbas)

#Model averaging to reduce overfitting
na.action = na.fail
redbas <- dredge(vifmodbas)
top_models_vifmodbas <- get.models(redbas, subset = 1:4)
avgmodelvifmodbas <- model.avg(top_models_vifmodbas) # compute average parameters
summary(avgmodelvifmodbas) # display averaged model
confint(avgmodelvifmodbas, full = TRUE)

#Monocyte Models ----

#DV: mon
#IV: date, svl, smi, btemp, sex, par
#Random effects: site

scalemon <- read_csv("maxscalemon.csv")

#Append scale data to leuk data
mondat <- merge(leukdat, scalemon, by = "site")

#Making dataframe no NA in HL
mondatcomp <- mondat %>%
  filter(!is.na(mon))

mondatcomp$monsc <- scale(mondatcomp$mon)
mondatcomp$btemp <- as.numeric(mondatcomp$btemp)

mondatcomp <- mondatcomp %>%
  mutate(
    datesc = scale(date),
    svlsc = scale(svl),
    smisc = scale(smi),
    btempsc = scale(btemp),
    parsc = scale(par),
    fieldsc = scale(field100),
```

```
    urbansc = scale(urban700),
    watersc = scale(water800),
    forestsc = scale(forest100),
    roadsc = scale(road700)
  )

mondatcomp$monprob <- mondatcomp$mon/100
mondatun <- mondatcomp %>%
  distinct(id, .keep_all = TRUE)

#Checking correlations between predictors
mondatcor <- mondatcomp %>% select(c(datesc, svlsc, btempsc, smisc, parsc,
                                     fieldsc, urbansc, watersc, forestsc, roadsc))
cor(mondatcor)

fullmodmon <- glmmTMB(monprob ~ datesc + svlsc + smisc + btempsc + parsc + sex +
                        fieldsc + urbansc + watersc + forestsc +
                        (1|site), family = ordbeta(), data = mondatun)

summary(fullmodmon)
check_model(fullmodmon, verbose = TRUE)
plot(fullmodmon)

#VIF variables: forest

vifmodmon <- glmmTMB(monprob ~ datesc + svlsc + smisc + btempsc + parsc + sex +
                       fieldsc + watersc + urbansc +
                       (1|site), family = ordbeta(), data = mondatun, na.action = na.fail)

summary(vifmodmon)
check_model(vifmodmon, verbose = TRUE)
check_convergence(vifmodmon)
check_collinearity(vifmodmon)
testDispersion(vifmodmon)
check_overdispersion(vifmodmon)
plot(simulateResiduals(vifmodmon))
testZeroInflation(vifmodmon)
check_zeroinflation(vifmodmon)
plot(residuals(vifmodmon))
check_autocorrelation(vifmodmon)
```

```
check_distribution(vifmodmon)

#Model averaging to reduce overfitting
na.action = na.fail
redmon <- dredge(vifmodmon)
top_models_vifmodmon <- get.models(redmon, subset = 1:4)
avgmodelvifmodmon <- model.avg(top_models_vifmodmon) # compute average parameters
summary(avgmodelvifmodmon) # display averaged model
confint(avgmodelvifmodmon, full = TRUE)

#Eosinophil Models ----

#DV: eos
#IV: date, svl, smi, btemp, sex, par
#Random effects: site

scaleeos <- read_csv("maxscaleeos.csv")

#Append scale data to leuk data
eosdat <- merge(leukdat, scaleeos, by = "site")

#Making dataframe no NA in HL
eosdatcomp <- eosdat %>%
  filter(!is.na(eos))

eosdatcomp$eossc <- scale(eosdatcomp$eos)
eosdatcomp$btemp <- as.numeric(eosdatcomp$btemp)

eosdatcomp <- eosdatcomp %>%
  mutate(
    datesc = scale(date),
    svlsc = scale(svl),
    smisc = scale(smi),
    btempsc = scale(btemp),
    parsc = scale(par),
    fieldsc = scale(field100),
    urbansc = scale(urban700),
    watersc = scale(water100),
    forestsc = scale(forest1000),
    roadsc = scale(road900),
```

```
  tbleedsc = scale(tbleed)
 )

eosdatcomp$eosprob <- eosdatcomp$eos/100
eosdatun <- eosdatcomp %>%
 distinct(id, .keep_all = TRUE)

#Checking correlations between predictors
eosdatcor <- eosdatcomp %>% select(c(datesc, svlsc, btempsc, smisc, parsc,
                                     fieldsc, urbansc, watersc, forestsc, roadsc))
cor(eosdatcor)

fullmodeos <- glmmTMB(eosprob ~ datesc + svlsc + smisc + btempsc + parsc + sex + tbleedsc +
                      fieldsc + urbansc + watersc + forestsc +
                      (1|site), family = ordbeta(), data = eosdatun)

summary(fullmodeos)
check_model(fullmodeos, verbose = TRUE)
plot(fullmodeos)

#VIF variables: water

vifmodeos <- glmmTMB(eosprob ~ datesc + svlsc + smisc + btempsc + parsc + sex + tbleedsc +
                     forestsc + urbansc + fieldsc +
                     (1|site), family = ordbeta(), data = eosdatun, na.action = na.fail)

summary(vifmodeos)
check_model(vifmodeos, verbose = TRUE)
check_convergence(vifmodeos)
check_collinearity(vifmodeos)
testDispersion(vifmodeos)
check_overdispersion(vifmodeos)
plot(simulateResiduals(vifmodeos))
testZeroInflation(vifmodeos)
check_zeroinflation(vifmodeos)
plot(residuals(vifmodeos))
check_autocorrelation(vifmodeos)
check_distribution(vifmodeos)

#Model averaging to reduce overfitting
```

```
na.action = na.fail
redeos <- dredge(vifmodeos)
top_models_vifmodeos <- get.models(redeos, subset = 1:15)
avgmodelvifmodeos <- model.avg(top_models_vifmodeos) # compute average parameters
summary(avgmodelvifmodeos) # display averaged model
confint(avgmodelvifmodeos, full = TRUE)

#Summary Statistics ----

#Calculating leukocyte type / uL
leukdat$lymwbc <- leukdat$wbc*(leukdat$lym/100)
leukdat$hetwbc <- leukdat$wbc*(leukdat$het/100)
leukdat$azwbc <- leukdat$wbc*(leukdat$az/100)
leukdat$monwbc <- leukdat$wbc*(leukdat$mon/100)
leukdat$baswbc <- leukdat$wbc*(leukdat$bas/100)
leukdat$eoswbc <- leukdat$wbc*(leukdat$eos/100)

#Summary Stats
summary(leukdat)
leukdatcomp <- leukdat %>%
  filter(!is.na(wbc))
sd(leukdatcomp$lymwbc)
sd(leukdatcomp$hetwbc)
sd(leukdatcomp$azwbc)
sd(leukdatcomp$monwbc)
sd(leukdatcomp$baswbc)
sd(leukdatcomp$eoswbc)

#Current Final Results ----

hctresults <- summary(avgmodelvifmodhct, full = TRUE)
hctresultsconf <- confint(avgmodelvifmodhct, full = TRUE)

wbcresults <- summary(avgmodelvifmodwbc, full = TRUE)
wbcresultsconf <- confint(avgmodelvifmodwbc, full = TRUE)

lymresults <- summary(avgmodelvifmodlym, full = TRUE)
lymresultsconf <- confint(avgmodelvifmodlym, full = TRUE)

hetresults <- summary(avgmodelvifmodhet)
```

```
hetresultsconf <- confint(avgmodelvifmodhet)

azresults <- summary(avgmodelvifmodaz)
azresultsconf <- confint(avgmodelvifmodaz, full = TRUE)

basresults <- summary(avgmodelvifmodbas)
basresultsconf <- confint(avgmodelvifmodbas, full = TRUE)

monresults <- summary(avgmodelvifmodmon) # display averaged model
monresultsconf <- confint(avgmodelvifmodmon, full = TRUE)

eosresults <- summary(avgmodelvifmodeos) # display averaged model
eosresultsconf <- confint(avgmodelvifmodeos, full = TRUE)
```