Stress physiology and anti-predator behaviour in urban Northwestern Gartersnakes
(*Thamnophis ordinoides*)

by

Katherine Bell
B.Sc., University of Guelph, 2010

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Supervisory Committee

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Abstract

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Over 50% of the world's human population resides in urban centres, and this is expected to increase as the global human population grows and people migrate from non-urban to urban centres. Concentrated in these urban areas are anthropogenic disturbances that impose additional challenges on wildlife compared to their non-urban counterparts. These challenges can be stress provoking. Through the release of corticosterone (CORT) reptiles can adapt to these stressors, physiologically and behaviourally, both in the short- and long-term. To investigate the relationships between stress activation and defensive tactics in wild urban Northwestern Gartersnakes (*Thamnophis ordinoides*) I conducted visual encounter surveys, along edge-focused transects, following a semi-constrained random sampling method. I sampled snakes at five sites, each with a different level of anthropogenic disturbance, in the Greater Victoria Area, BC. I sampled blood, observed anti-predator behaviour, and collected data on characteristics of snakes. The most disturbed site (with the most people, pets, and natural predators) also had the most snakes: those snakes also had highest H:L values (a proxy of CORT) in their blood compared to the other populations. Nevertheless, none of the snakes had H:L values that indicated chronic stress. Stress physiology was not correlated with anti-predator behaviour. More important to anti-predator behaviour was the size, sex/reproductive condition, and cloacal temperature of snakes. Although anthropogenic development can reduce habitat quality for some reptiles, Northwestern Gartersnakes coexist with recreationists at many sites in the District of Saanich. A multi-disciplinary approach is of paramount importance to understand the full effect of anthropogenic influences on wildlife.

Table of Contents

List of Tables

List of Figures

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CHAPTER 1 – NORTHWESTERN GARTERSNAKES AND THE URBAN SITES THAT THEY INHABIT

INTRODUCTION

Humans are major drivers of environmental change (Shochat et al. 2006). Effects of our impacts on the environment are most apparent in urban areas (Ditchkoff & Wakeling 2001); natural undisturbed lands are overtaken by introduced flora or are paved to support houses and other urban developments, leaving areas of high- and low-density buildings and fragmented vegetated patches (Ditchkoff & Wakeling 2001; Endriss et al. 2007; Werner 2011). Concentrated in these urban areas are anthropogenic disturbances that impose additional challenges on wildlife compared to their non-urban counterparts (Koenig et al. 2002; Mollov 2005; French et al. 2008; Sol et al. 2013).

Already over 50% of the world's human population resides in urban centres (United Nations 2011). Over the next four decades, urban centres are expected to absorb all population growth (projected 30% global increase) and, at the same time, draw in some of the non-urban populations (United Nations 2011). To accommodate urban population growth, more 'wildlands' will become developed (Dearborn & Kark 2010), and more wildlife will be exposed to anthropogenic disturbances. How wildlife will be affected by and respond to these challenges is a complex problem (Minton 1968).

Recreational activities, such as wildlife viewing, photography, hiking, and biking are usually viewed as 'benign, non-consumptive' anthropogenic disturbances that do not permanently remove wildlife or alter their behaviour or physiology (Parent & Weatherhead 2000; Reed & Merenlender 2008; Knight 2009). For instance, Great Tit (*Parus major*), Blue Tit (*P. caeruleus*) and Chaffinch (*Fingilla coelebs*) individuals in suburban and rural settings react to an approaching human in the same way (Cooke 1980). Cases like these, of unaffected urban wildlife, are few and far between because any animal that lives among or near humans is likely disturbed in one way or another (Knight & Cole 1995; Gibbons et al. 2000; Koenig et al. 2002). Some urban animals are tamer than their counterparts that live in undisturbed 'wildlands' (Adams 2005) and benefit from the increased access to resources that people provide, either accidentally or

intentionally (Ditchkoff et al. 2006). Other wildlife are less fortunate and respond negatively to habitat that is altered by hikers trampling vegetation and/or leaving garbage (Boyle & Samson 1985; Garner et al. 2008). Various wildlife appear to be threatened by humans, as interpreted from their flight response, especially when intentionally sought out: wildlife may respond to an approaching camera lens and/or the viewers' eyes by fleeing to a refuge sooner than animals that are not directly approached (Cooper 1997; Boyle & Samson 1985; Fernández-Juricic et al. 2001; Stankowich & Blumstein 2005; Knight 2009). Specifically, suburban Blackbirds (*Turdus merula*), Starlings (*Sturnus vulgaris*), and House Sparrows (*Passer domesticus*), are less tolerant of an approaching observer (i.e., flee sooner) than their rural counterparts (Cooke 1980). The anticipated spread of urbanization into less urban areas (United Nations 2011) coupled with the growing popularity of outdoor recreation (Knight & Cole 1995; Parent & Weatherhead 2000) will increase the number of recreationists who visit wildlife habitat and thus expose more wildlife to anthropogenic disturbances.

Many bird species and other wildlife respond to disturbances caused by recreationists as a form of predation risk (Garber & Burger 1995; Fernández-Juricic et al. 2001; Gill et al. 2001): when threatened, wildlife weigh the costs (e.g., lost foraging opportunities) and benefits (e.g., not injured or eaten) of fleeing (Frid & Dill 2002). As such, many wildlife species, such as the Eastern Massasauga Rattlesnake (*Sistrurus catenatus catenatus*), respond to people as if they were potential predators (Parent & Weatherhead 2000; Frid & Dill 2002). The increased occurrence of human-wildlife encounters is one of many sources of changed predation pressure in urban centres (Seress et al. 2011); human presence decreases the abundance of native carnivore species in many urban green spaces (Reed & Merenlender 2011) at the same time as it introduces new predators, including Domestic Dogs (*Canis familiaris*) and Cats (*Felis catus*). Cats have direct negative impacts on reptile abundance; cats increase mortality rates in Bluetongue Lizards, *Tiliqua scincoides* (Koenig et al. 2002), and alter anti-predator behaviour of Lava Lizards (*Tropidurus* spp.; Stone et al. 1994). Although dogs are the most common carnivores in urban centres (Vanak & Gompper 2009), they have a generally lesser negative impact on wildlife than cats (Bekoff & Meaney 1997; Miller et al. 2001).

Predators seek out prey for sustenance. When predators kill or injure prey (unsuccessful predation event), the survival rates and/or population densities of prey species are altered (Lima & Dill 1990; Maritz & Scott 2010). Unsuccessful predation events can affect the physiology and/or behaviour of prey (Lima & Dill 1990; Maritz & Scott 2010). For instance, predator-prey interactions can be stressful (Scheuerlein et al. 2001). Our understanding of stress has mostly come from studies of mammals and birds: stress is a physiological response to unfavourable environmental conditions, or stressors, that is measured by changes in glucocorticoid (GC, e.g., corticosterone, CORT, in birds, reptiles, and amphibians, and cortisol in humans and teleost fish) levels and the subsequent alteration of other physiological and behavioural processes (Bailey et al. 2009; Lupien et al. 2009). Reptiles display this 'classical stress response' (Moore & Jessop 2003; Taylor & Denardo 2010).

Research on reptiles stressed by challenges of urban environments is limited, especially when compared to the number of studies on birds and mammals (Ditchkoff & Wakeling 2001; Magle et al. 2012). This is in part due to the solitary and secretive nature of reptiles, which makes them less observable (Burger 2001). Although difficult to study (Burger 2001), reptiles may be especially vulnerable to human disturbance because of their limited dispersal ability and extended periods of basking out in the open to keep warm (Parent & Weatherhead 2000). Many reptiles are constrained to specific areas (e.g., refuges and hibernacula) and activity regimes that may make them more exposed to anthropogenic disturbances (Parent & Weatherhead 2000). Continual exposure to human-induced stressors can have biological costs for reptiles: individuals suffer from reduced wellbeing and fitness that can have consequences for the population (Yao & Denver 2007; Dedovic et al. 2009; Linklater 2010). In less than 20 years, a population of 104 North American Wood Turtles (*Clemmys insculpta*) was reduced to zero after a forested watershed in New Haven County, Connecticut, was opened to the public for hiking and fishing (Garber & Burger 1995a). The intrusion of humans and their pets into wildlife habitat is a major contributor to reptile declines and is recognized as a worldwide crisis (Gibbons et al. 2000).

Anthropogenic disturbances, however, are not always detrimental to reptiles. The tight interplay between hormones and alterations in physiology and behaviour enables wildlife to adapt to these stressors both in the short and long term (Moore & Jessop 2003; Ditchkoff et al. 2006; Romero & Butler 2007; Yao & Denver 2007; French et al. 2008; Lupien et al. 2009; Thaker et al. 2009b; Sol et al. 2013): Tree Lizards (*Urosaurus ornatus*) are highly urban-adapted and reach high densities in cities (Ditchkoff & Wakeling 2001); and, free-ranging Cottonmouths (*Agkistrodon piscivorus*) are not threatened by short-term encounters with humans (Bailey et al. 2009).

Despite the fact that urbanized landscapes can be stressful for some reptile species, affecting their physiology (immunity – French et al. 2008; reproductive hormones – Moore et al. 2000) and behaviour (activity patterns – Ditchkoff et al. 2006; courtship – Greenberg 2002), there is limited information about the consequences of stress activation for reptilian defence. To date, no study has established a relationship between elevated stress caused by exposure to potential predators (people, pets, and natural predators) and anti-predator tactics in wild urban reptiles (Thaker et al. 2009a). To address this gap in research I investigated the relationships between stress activation and defensive tactics in wild urban Northwestern Gartersnakes (*Thamnophis ordinoides*) at sites with different levels of disturbance (i.e., relative exposure to people, pets, and natural predators). My general research (all chapters) questions were:

1) Is repeated human presence stressful for Northwestern Gartersnakes?
2) Are Northwestern Gartersnakes wary of humans?
3) Is there a relationship between stress and wariness?

More specific to this chapter, I wanted to know:

1) Is the disturbance regime at a site (i.e., abundance of people, pets, and/or natural predators) related to the abundance of Northwestern Gartersnakes?
2) Is the proportion of injured snakes related to the disturbance regime at a site?

Predictions

Assuming that snakes respond to recreationists and pets similarly to how they respond to natural predators (Garber & Burger 1995a; Parent & Weatherhead 2000; Fernández-Juricic et al. 2001; Gill et al. 2001; Frid & Dill 2002), I predicted that counts of Northwestern Gartersnakes would be lower in more disturbed sites. If snakes were habituated to disturbance, then I expected the counts of snakes would be statistically unrelated to the number of each of people, pets, and natural predators at the sites.

Humans provide important resources (e.g., food and habitat) for both pets and wildlife (Garber & Burger 1995; Love & Bird 2000, as cited in Hager 2009; Chace & Walsh 2006). I anticipated a positive correlation between the numbers of people and of pets. If predators are neither deterred by nor attracted to anthropogenic disturbances I predicted that the numbers of people and of natural predators would be unrelated.

STUDY SITES

I conducted this study in the Greater Victoria Area, focusing on three parks and one nature sanctuary (geographically divided into two sites) in the District of Saanich, BC (Figure 1). The five sites were Mount Douglas Park (MDP), Mount Tolmie Park (MTP), Layritz Park (LP), and the Swan Lake/Christmas Hill Nature Sanctuary (SLNS and CHNS, respectively; Figure 1).

Figure 1. Map of study sites (indicated by red circles with site name beside). Image amended from: http//www.saanich.ca/parkrec/parks/trails/pdf/FullMapofSaanichParksandTrails2012.pdf

The sizes of the sites vary considerably. Mount Douglas Park is much larger than each of the other sites: 181.57 ha compared to CHNS, SLNS, MTP, and LP, which are 1.84 ha, 3.29 ha, 18.25 ha, and 29.10 ha, respectively (District of Saanich 2012). Mount Douglas Park is also the oldest site, established in 1889, and incorporates a network of paths through a forested mountain with bare rock at the top (District of Saanich 2012). Mount Tolmie Park was designated a Saanich park in 1891, predominantly composed of Gary Oak habitat on a small mountain with a street, Mayfair Drive, running through the middle (District of Saanich 2012). In 1975 the SL/CHNS became a controlled green space for humans and urban wildlife with forest, grassland, bare rock, and paths. Also, SLNS has a lake in the middle of the site. Lastly, in 1997, LP was established as a mixed-green area with baseball diamonds, a soccer field, undeveloped open grasslands, and a forested hill (District of Saanich 2012): an abundance of trails fragment the different habitat types.

STUDY SPECIES

Three species of Gartersnake (*Thamnophis* spp.) inhabit the Greater Victoria Area: the Northwestern Gartersnake (*Thamnophis ordinoides*; Figure 2), the Common Gartersnake (*T. sirtalis*), and the Western Gartersnake (*T. elegans*). My research focuses on *T. ordinoides* because it was the most abundant species at all sites. Snakes that were seen and not caught, and could not be confidently identified to the species level, are here referred to as *Thamnophis* spp. The Northwestern Gartersnake is a diurnal terrestrial snake (Stewart 1968; Gregory 1978). It is the smallest of the three Gartersnake species (Hebard 1950) and is found predominantly in meadows and along forest edges (Stewart 1968; Gregory 1984b; Matsuda et al. 2006).

Figure 2. Northwestern Gartersnake, *Thamnophis ordinoides*. Note the blunt-snouted head, which is indistinct from the neck.

The body is generally dark brown with dorsal and ventral stripes, which vary between individuals from light yellow to dark orangey-red (Figure 3). Some lack stripes altogether. There are also albino and melanistic (completely black) morphs. These snakes prey on slugs (Figure 3) and earthworms (Gregory 1978; Gregory 1984b; Matsuda et al. 2006).

Figure 3. *T. ordinoides* preying on a Black Slug, *Arion ater*.

METHODS

Locating and catching Gartersnakes

With the aid of a colleague, I conducted visual encounter surveys, along edge-focused transects, following a semi-constrained random sampling method. We visited CHNS, MDP, and LP, and MTP 14 times, and SLNS 13 times between 9am and 8pm on sunny, cloudy, and lightly raining days from May to August 2012, when Gartersnakes were most active (Stewart 1968; Gregory 1984a; Lind et al. 2005). To account for the potential variation in human visitation rates between week and weekend days every site was visited at least once for every day of the week (Monday through Sunday).

Repeated searching began from a fixed point at each site throughout the season (except for MDP, which had two starting locations because of its comparatively larger size). First, the ambient temperature (°C) was measured by placing a Traceable Digital Thermometer (VWR Scientific Inc.) in the shade so that the heat of the sun's rays would not alter the reading. At the beginning of each day, an 8-sided die was rolled to determine the number of paces (50 times the number on the die, X) walked along a path into the site. The time of day was recorded and we walked 50X paces, one behind the other, at approximately 0.3 m/s, while searching the ground and surrounding vegetation

for snakes. In an effort to sample as much of the site as possible, every alternative path from the parked car was taken throughout the field season. Once the 50X paces were completed, one of us, without looking, spun the bezel on a compass until the other said 'stop'. This provided a haphazard bearing (between 0° and 360°) for the first transect.

Snakes are often found on edges between habitat types, because edges provide opportunities for thermoregulation, foraging, and predator avoidance (Weatherhead & Charland 1985; Durner & Gates 1993; Blouin-Demers & Weatherhead 2001). To increase the chances of finding and capturing a Gartersnake, edge searching therefore was incorporated into the random transect method. An edge occurred when two of any of the following six habitat classes met: vegetation heights of 0-10cm, 10-30cm, and >30cm each with and without canopy cover. When the transect intersected an edge, the transect was interrupted and the edge was searched; the habitat class that was searched while on the transect was kept on the right hand side in an attempt to return to where the transect was paused. If this location was lost or if the transect was blocked by a structure (e.g., large body of water, dense bush, fence, or site border), a new compass bearing was randomly determined and a new transect was started.

When a snake was seen lying still, approach distance (AD) was measured before it was caught by hand. Upon exposure to predators and other threats, animals weigh the risks and benefits of abandoning current activities, and when the risks exceed the benefits, flee (Frid & Dill 2002). Propensity to flee is measured as AD, also termed flight-initiation distance (Bulova 1994; Stankowich & Blumstein 2005). By definition, AD is the distance between observer and animal when the animal starts to flee and is used to assess fear (i.e. wariness) and/or anti-predator tactics of animals in response to disturbance (Ydenberg & Dill 1986; Blumstein et al. 2003; Stankowich & Blumstein 2005). Snakes that were moving when first seen were caught right away without measuring AD.

Processing snakes

A GPS measurement (including accuracy, m) was taken at the site of capture, and the time and current weather conditions (air temperature in °C, % cloud cover, and degree of precipitation) were recorded to control for time of day and weather.

I collected the following information from each captured snake: sex/reproductive condition (i.e., palpated abdomen of females to determine if pregnant), snout-vent length (SVL, cm), mass (g), cloacal temperature (°C), shedding status (cloudy eyes or venter indicating impending shed – Figure 4, or if skin is currently shedding), and presence of an injury (Gregory & Isaac 2005; Santos et al. 2011). I also took a blood sample from the heart to measure H:L (see CHAPTER 2).

Figure 4. *Thamnophis ordinoides* with cloudy eyes.

I discriminated between 'injuries' (Figure 5) and 'nicks' (minimal damage; Figure 6). 'Nicks' were small, more superficial and found on the scales on the ventral surface of their body. I excluded individuals with 'nicks' from the dataset because it was highly likely that these marks were caused by scraping against hard substrate (Gregory & Isaac 2005), as opposed to being caused by a predator (Greene 1988).

Figure 5. Injuries inflicted on *T. ordinoides*, most likely due to a failed predation attempt.

Figure 6. Nick on the ventral side of a *T. ordinoides*. The snake's head is towards the left of the image.

All captured snakes were individually marked using implanted passive integrated transponders (PIT-tags) to avoid pseudoreplication (through repeat observations of the same individuals) in the statistical analyses (Ford 1995; Bailey et al. 2009). The PIT-tag was injected at 3/4 SVL into the peritoneal cavity using a syringe with a 12-gauge needle (Keck 1994). To do this, the needle was inserted between two ventral scales about 10 mm deep and the tag was released to one side of the ventral midline (Keck 1994; Jemison et al. 1995).

Disturbance regime

To obtain a general index of the disturbance regime at each site the following were counted throughout each sampling day, both while searching for and while sampling the snakes: the number of people and their pets (only Domestic Dogs, as I did not see Domestic Cats), and the number of natural predators. Of the possible natural predators of Gartersnakes (small mammals, medium and large birds, and snake-eating-snakes; Greene 1988; Robert et al. 2009), only five avian species were observed in the field: Red-Tailed Hawks, *Buteo jamaicensis*; Bald Eagles, *Haliaeetus leucocephalus*; America Crows, *Corvus brachyrhynchos*; Common Ravens, *Corvus corax*; and, Turkey Vultures, *Cathartes aura*. Of the natural avian predators encountered, only a Crow was seen actively preying on a Gartersnake (Figure 7; Shine et al. 2000). I included Turkey Vultures as a potential natural predator because, although they are scavengers and do not attack live snakes, I made the assumption that snakes do not differentiate between species of medium-large birds, and therefore equate Turkey Vultures with the other types of avian natural predators. I did not include smaller avian species (e.g., American Robins, *Turdus migratorius*) because their bill and overall body sizes are too small to accommodate Gartersnakes other than young-of-the-year.

Figure 7. American Crow, *Corvus brachyrhynchos* preying on a Gartersnake. Photos were taken by Jenna Cragg, M.Sc. (University of Victoria).

Statistical analyses

All analyses and figures were completed using R version 2.14.1 and a statistical significance level of 0.05. Data are presented in the text as mean±1 standard error (SE). Tests for the equality of variances (F-test) and of normality (i.e., skew, kurtosis, and a normal Q-Q plot) were carried out prior to conducting further statistical tests. The majority of my data were not normally distributed. Instead of transforming the non-normal data, I ran non-parametric tests (e.g., Wilcoxon rank-sum test, Spearman rank correlations coefficients, and Kruskal-Wallis test) on all of the data.

I used notched box-and-whisker plots to portray the variability in the data by site (or other categories). In these plots, the main 'box' is the interquartile range, and comprises 50% of the data; the bottom boundary is the 25th percentile, below which is 25% of the data (bottom 'whisker'); the middle line is the median; and, the upper boundary is the 75th percentile, above which is the last 25% of the data (upper 'whisker'). The dots beyond the 'whiskers' are possible outliers. The notched part of the 'box' portrays the 95% confidence interval around the median. As a rough rule of thumb, and a method for informal hypothesis testing, if the notches of two box-and-whisker plots do not overlap, this provides strong evidence that the medians of the data sets are significantly different ($p<0.05$), even when the requirements for the hypothesis are not strictly met (Chambers et al. 1983).

I used the binom.test() function in R to determine average (including 95% confidence interval) percentages of snakes that were injured at each site. I also used logistic regression (e.g., glm(injury~SVL, family=Binomial)) to determine how SVL influenced injury (N=126).

I also modelled how the presence of each of people, pets, natural predators, and potential predators influenced the number of snakes encountered (number of *T. ordinoides* caught plus the number of *Thamnophis* spp. seen) per hour searching. I ran the model only for all sites combined (N=69) because the sample sizes were too small when I separated sites (N=14 for each of CHNS, LP, MDP, MTP, and N=13 for SLNS). A generalized additive model (gam() function in R) was used to determine the appropriate family type (e.g., Gaussian) to be applied to the model. I ran a series of single predictor models using the generalized linear model (glm() function in R; e.g.,

glm(number of snakes encountered per hour searching ~ number of natural predators seen per hour spent at the site, family=Gaussian)). The fit of each model was assessed by two methods: for the Akaike Information Criterion (AIC), a smaller value indicates that the model fits better and models that are within 2 of the model with the lowest AIC are all equally important to consider when making inferences (Burnham & Anderson 2004); and, with a Chi-squared (χ^2) Test, models that fit the data have p-values > 0.05. The p-value was calculated using the residual deviation and its degrees of freedom.

RESULTS

Over the entire field season, I caught 147 Northwestern Gartersnakes and saw another 103 Gartersnakes (*Thamnophis* spp.). The average number of snakes that were caught and seen per hour at each site varied among the 69 sampling days, but there was no obvious temporal trend (Figure 8). Between May 4th and August 30th, on average (±1SE), 1.392±0.345 (N=69) *T. ordinoides* were captured per hour searching and 0.790±0.177 (N=69) *Thamnophis* spp. were seen per hour searching (Figure 8).
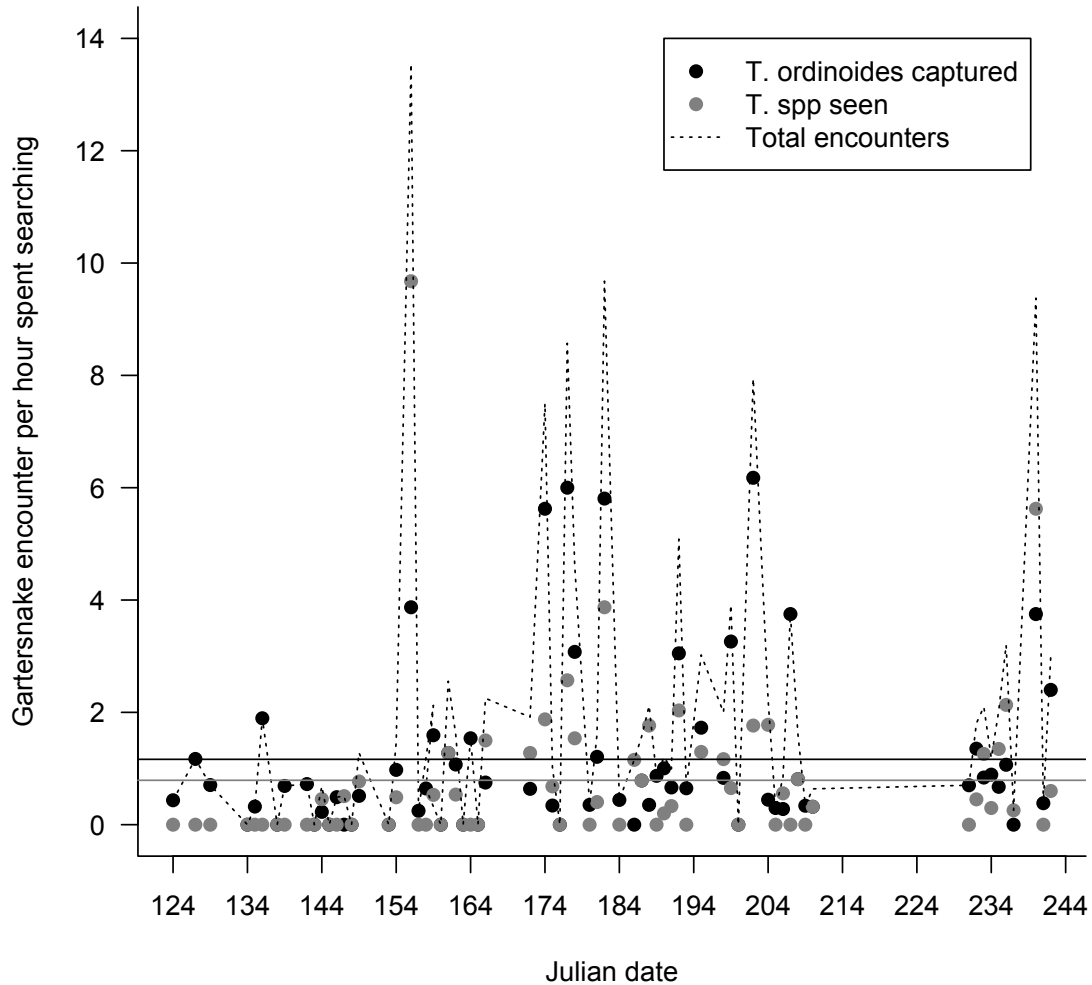
Figure 8. Variability in the number of snakes encountered (*T. ordinoides* captured plus *T.* spp. seen) per hour spent searching by Julian date.

Overall, disregarding site, every type of disturbance (people, pets, natural predators, and their cumulative influence) was positively related to the average number of snakes encountered (*T. ordinoides* captured plus *Thamnophis* spp. seen) per hour spent searching (Table 1). However, none of these relationships was significant ($p > 0.05$).

Table 1. Snake encounters were modelled against four different measures of disturbance for all sites combined (N=69). The 'potential predator' category is the sum of natural predators, people, and pets. The '+' sign indicates that the independent variable has a positive influence on the number of snakes encountered. Models within 2 AIC values of the lowest AIC value are bolded. No model fit the data (χ^2; p<0.05).

Measure of disturbance (independent variable)	Influence of independent variables ('+' OR '-') on snake encounters	P-value	AIC	P-value (χ^2; degrees of freedom = 67)
Pets	+	0.158	376.51	0.000
Potential predators	+	0.311	377.51	0.000
People	+	0.435	377.95	0.000
Natural predators	+	0.620	378.32	0.000

I encountered more snakes, potential predators, and people at LP than at any other site. On average I saw/caught 2.891±0.768 snakes per hour spent searching (median=1.732, N=14), and saw 41.138±7.991 potential predators (median=30.254, N=14) and 36.602±8.073 people (median=23.622, N=14) per hour spent at LP. There were significantly more snakes at LP than at both MDP (1.260±0.565, median=0.643, N=14, Wilcoxon rank-sum test; W=154, p<0.02; Figure 9) and MTP (0.542±0.172, median=0.310, N=14, W=175, p<0.0005; Figure 9). I encountered the fewest snakes at MTP in comparison with every other site. There were significantly fewer snakes at MTP than at SLNS (1.954±0.319, median=1.915, N=13; W=28, p<0.003) and at CHNS (2.205±0.756, median=1.410, N=14; W=155, p<0.01; Figure 9). Similarly, significantly fewer snakes were at MDP than at SLNS (W=44.5, p<0.03; Figure 9).
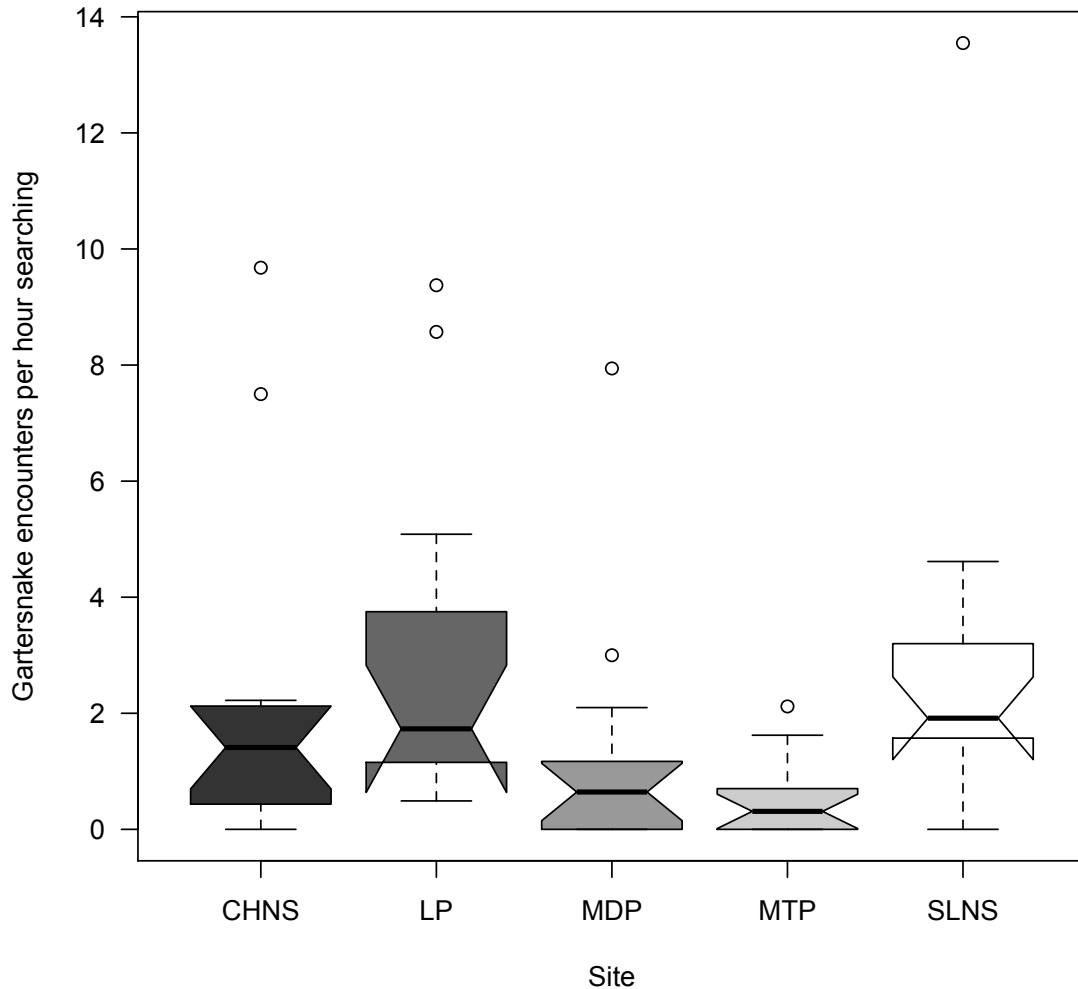
Figure 9. Notched box-and-whisker plots of the number of snakes encountered (*T. ordinoides* captured plus *Thamnophis* spp. seen) per hour searching at each site. See explanation of plots in text. The sites are Christmas Hill Nature Sanctuary (CHNS, N=14), Layritz Park (LP, N=14), Mount Douglas Park (MDP, N=14), Mount Tolmie Park (MTP, N=14) and, Swan Lake Nature Sanctuary (SLNS, N=13).

The number of potential predators seen at LP was significantly greater than at CHNS (3.502 ± 0.597, median=3.510, N=14, W=0, $p<0.0001$), at MDP (12.568 ± 1.727, median=13.029, N=14, W=174, $p<0.0003$), and at SLNS (14.522 ± 1.911, median=14.318, N=13, W=157, $p<0.0009$; Figure 10). Also, there were significantly more potential predators seen for every hour spent at MTP (23.669 ± 2.561, median=23.713, N=14), than at CHNS (W=0, $p<0.0001$), MDP (W=30, $p<0.002$), and at SLNS (W=140, $p<0.02$; Figure 10).
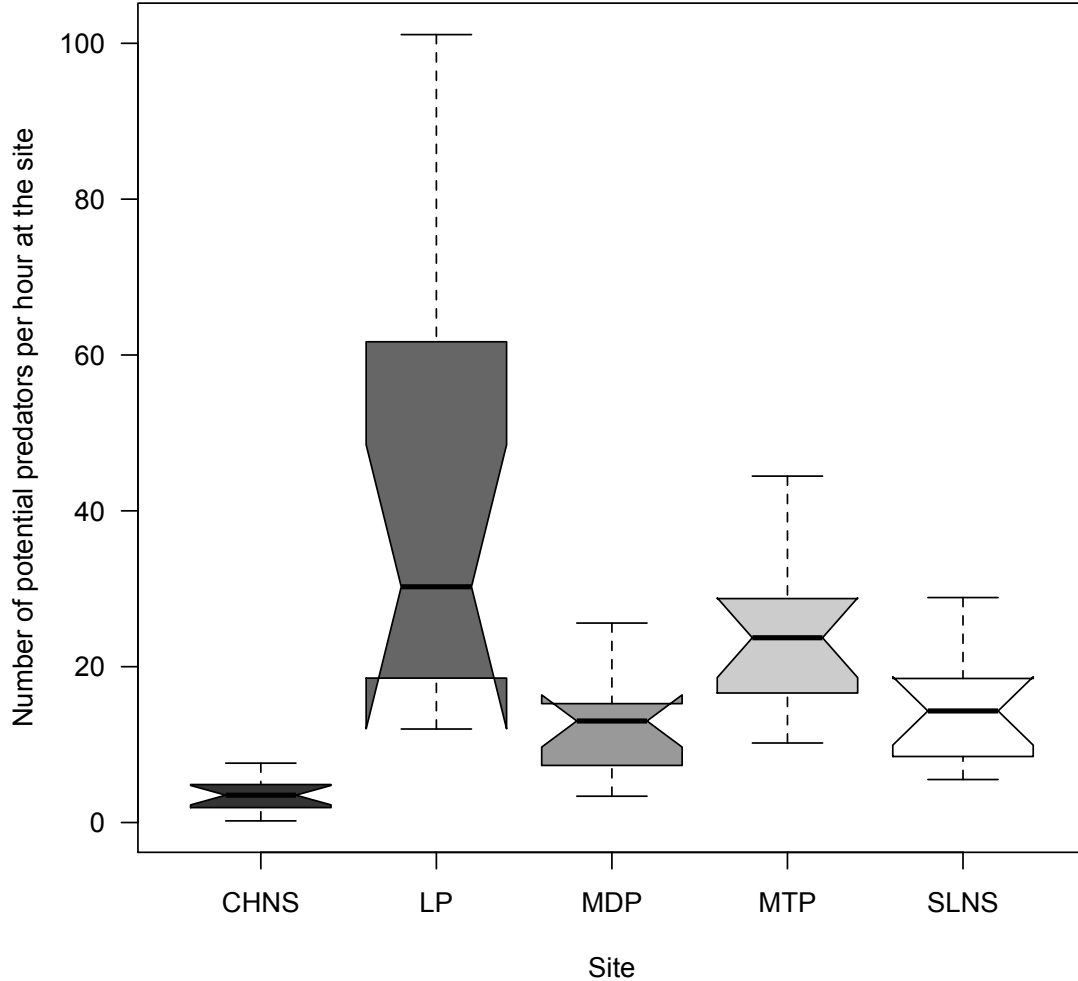
Figure 10. Notched box-and-whisker plots of the number of potential predators (natural predators plus people plus pets) seen per hour at each site. See explanation of plots in text. Site names (with sample size) are abbreviated as in Figure 9.

There also were significantly more people at LP than at every other site (Figure 12): 2.075±0.452 (median=1.815, N=14, W= 0, p<0.0001) people at CHNS; 8.451±1.543 (median=7.520, N=14, W=182, p<0.0001) people at MDP; 10.514±1.213 (median=9.379, N=14, W=167, p<0.002) people at MTP; and 11.307±1.351 (median=11.591, N=13, W=152, p<0.003) people at SLNS (Figure 11).
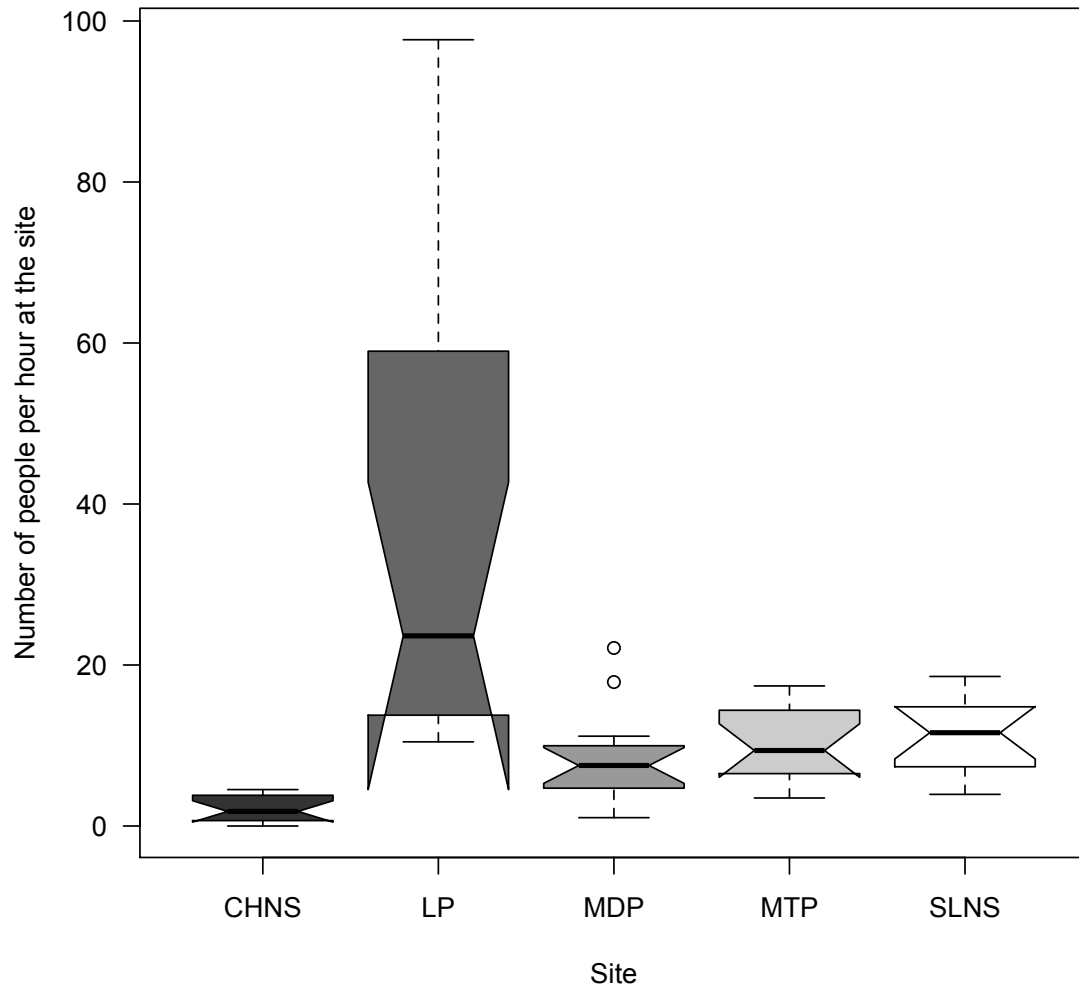
Figure 11. Notched box-and-whisker plots of the number of people seen per hour at each site. See explanation of plots in text. Site names (with sample size) are abbreviated as in Figure 9.

The largest number of natural predators was seen at MTP (11.786±2.060 individuals per hour spent at MTP, median=9.671, N=14; Figure 12). This was significantly more than the rate of natural predator sightings at CHNS (1.333±0.310, median=1.081, N=14, W=9, p<0.0001), at LP (2.410±0.738, median=1.042, N=14, W=23, p<0.003), at MDP (1.910±0.509, median=1.423, N=14, W=14, p<0.0001), and at SLNS (3.117±0.975, median=1.346, N=13, W=155, p<0.002; Figure 12).
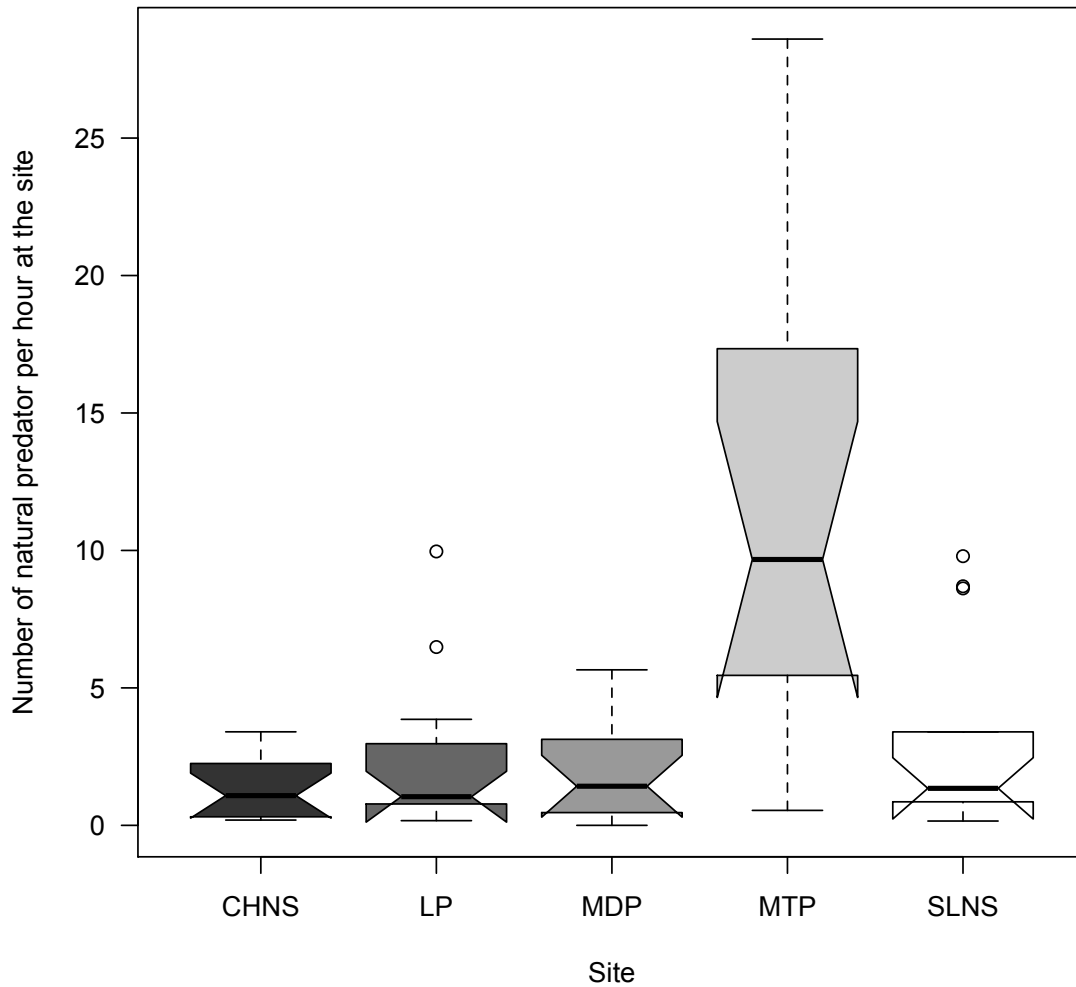
Figure 12. Notched box-and-whisker plots of the number of natural predators (see text for species) seen per hour at each site. See explanation of plots in text. Site names (with sample size) are abbreviated as in Figure 9.

At MTP there were significantly more pets seen than at SLNS (0.0979 ± 0.0468, median=0, N=13; W=178, p<0.0001) and at CHNS (0.0946 ± 0.0361, median=0, N=14; W=5, p<0.0001; Figure 13).
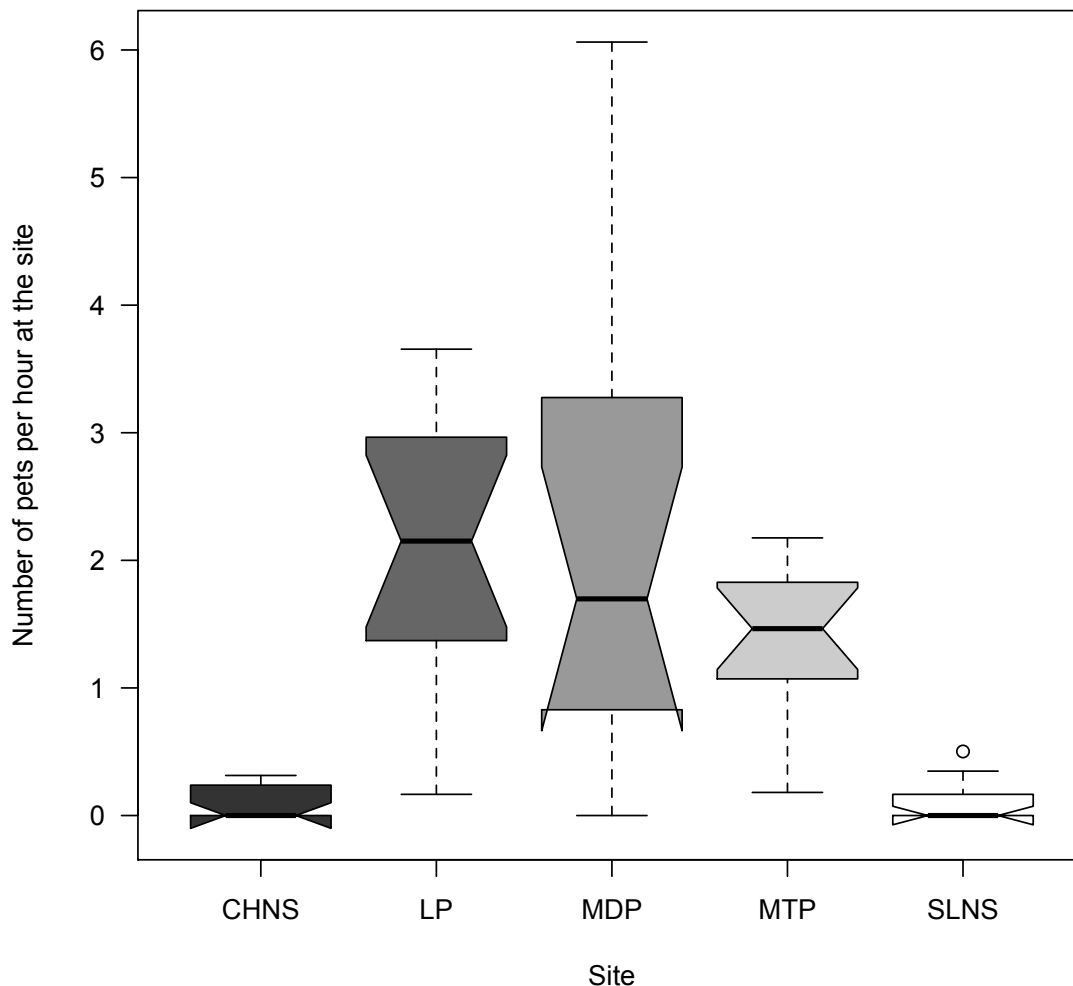
Figure 13. Notched box-and-whisker plots of the number of pets (only Domestic Dogs) seen per hour at each site. Site names (with sample size) are abbreviated as in Figure 9.

The fewest potential predators, natural predators, people, and pets were seen per hour spent at CHNS compared to at every other site. I saw significantly fewer potential predators per hour at CHNS than at MDP (2.207±0.460, median=1.698, N=14; W=14, p<0.0001) and at SLNS (W=6, p<0.0001; Figure 10). The rate that I saw people at CHNS was significantly less than the rate at which I saw people at MDP (W=18, p<0.0003), at MTP (W=4, p<0.00002), and at SLNS (W=3, p<0.00003; Figure 12).

People and pets (average number seen per hour at the site) were positively correlated (significant, p<0.05) at every site and at all sites combined (Table 2). People

and natural predators (average number seen per hour at the site) were positively correlated (non-significant, p>0.05) at CHNS, at MTP, and at SLNS (Table 2). Pets and naturals predators (average number seen per hour at the site) were negatively (non-significant, p>0.05) correlated at CHNS and LP, and people and natural predators (average number seen per hour at the site) were negatively (non-significant, p>0.05) correlated at LP, MDP (Table 2).

Table 2. Spearman rank correlations between types of disturbances (people, pets, and natural predators) for each site and for all sites combined. The '+' and '-' signs represent positive and negative correlations, respectively. Significant correlations (p<0.05) are indicated by a *. Site names are abbreviated as in Figure 9.

Comparison between types of disturbances (# seen/ hr spent at the site)	Site					
	CHNS	LP	MDP	MTP	SLNS	All sites combined
People-Pets	+	+	+	+	+	+*
People-Natural predators	+	-	-	+	+	-
Pets-Natural predators	-	-	+	+	+	+

Injuries inflicted by a predator were most often on the lateral or dorsal sides of the snake's body and both larger and deeper than 'nicks'. Most snakes had no injures (30.1% injured across all sites; Figure 14). Significantly fewer snakes were injured at MDP (15.8%) compared to at SLNS (44.4%, Wilcoxon rank-sum test; W=183, p=0.0449; Figure 14). There were comparable percentages of injured snakes for all other site combinations (p>0.05; CHNS – 20%, LP – 37.0%, and MTP – 15.8%). Of the snakes with injuries, most were larger snakes; the presence of an injury was positively associated with SVL (glm(family=Binomial); p=0.0736, N=126) and the model fit the data (χ^2; p=0.0504, DF = 124). The proportion of injured snakes captured each day was weakly correlated (rho=-0.0436, p=0.769, N=48) with the average number of natural predators seen per hour at all sites combined. I did not consider sites separately because the sample size was too small (N<30).
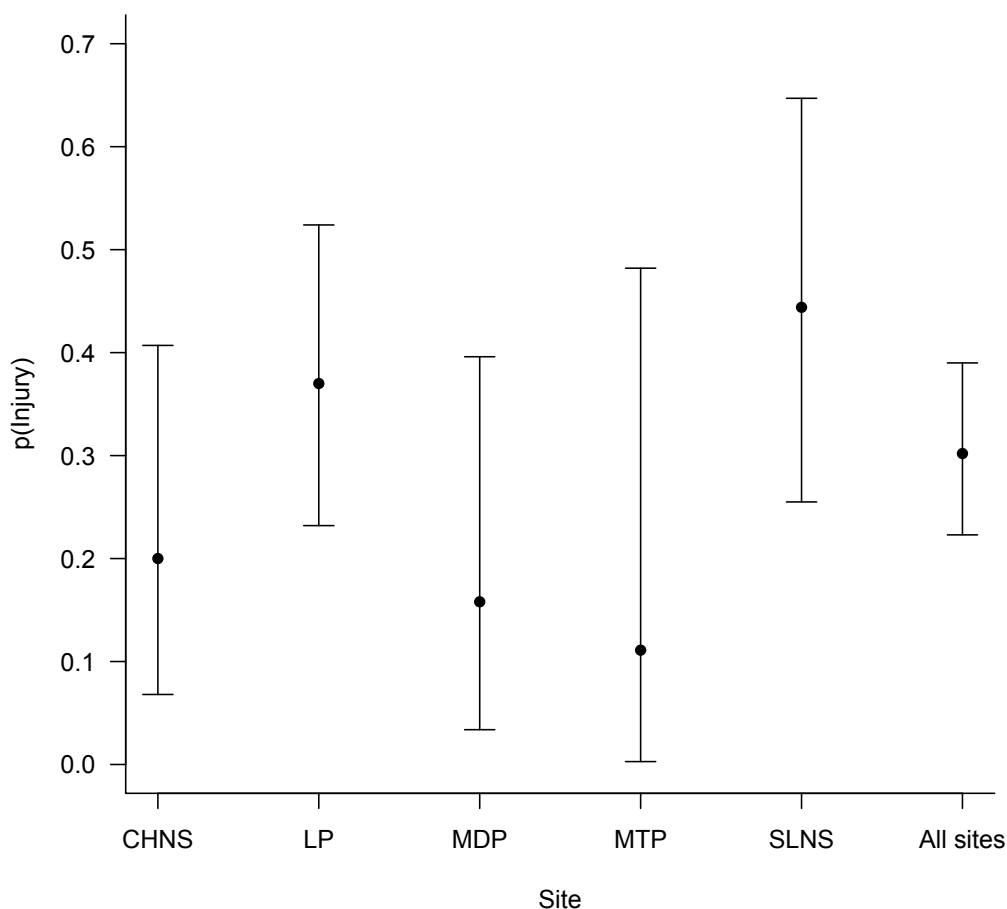
Figure 14. Frequency of injured snakes at each site. Values represent averages with 95% confidence intervals. Sample size are as follows: CHNS (N=25); LP (N=46); MTP (N=19); MDP (N=9); and, SLNS (N=27). Site names are abbreviated as in Figure 9.

DISCUSSION

Northwestern Gartersnakes and people were most abundant at LP; the counts of both snakes and people were significantly higher at LP than the respective counts of each at the other sites. It seems as though these snakes are well adapted to living among people. Dogs are strongly associated with people (Reed & Merenlender 2011) but are often nonthreatening to wild animals (Bekoff & Meaney 1997; Miller et al. 2001). In general, predator counts are a poor predictor of injuries in snakes.

The average number of potential predators seen per hour (N=69) predicted the average number of snakes that were encountered per hour on any given day; sites with more people, pets, and natural predators also had more snakes. This suggests that the combined level of disturbance at these sites was not detrimental to the counts of Northwestern Gartersnakes. When broken down by disturbance type, the average rates at which I saw people, pets, and natural predators were positive predictors, although non-significant, of the average rate of encounters with snakes. Because the AIC values of the four models were each within 2 AIC values of the other, each type of disturbance was equally unimportant when considering the average rate of snake encounters across all sites (Burnham & Anderson 2004).

Reaching conclusions about predation risk and perceived threat from counts alone can be misleading (Shochat et al. 2006). Whether or not Northwestern Gartersnakes are threatened by the presences of each of people, pets, or natural predators may be a question of behaviour more than of counts. The positive relationship between the counts of potential predators and of snakes was probably confounded by how weather can affect activity patterns of wildlife and of recreationists: on warmer, clearer days there are more people outdoors (pers. obs.), more snakes basking in the sun (Bogert 1949; Brattstrom 1965; Gregory 1984a; Waye 1999), more active prey (Shine et al. 2000), and more natural predators seeking prey (Chace & Walsh 2006). When animals are more active, they are more likely to be observed. Therefore, I suspect that the model outputs describe correlative, as opposed to causative, positive relationships.

The degree to which a habitat is used for recreation has variable impacts on reptile individuals and populations (Neill 1950; Mollov 2005). North American Wood Turtles became extinct in parts of Connecticut 10 years after a nature reserve opened for recreational use (e.g., hikers and fishers; Garber & Burger 1995b), whereas the Butler's Gartersnake, *T. butleri*, in the US Midwest does well in both urban and suburban sites (Minton 1968). The Northwestern Gartersnake may also be well adapted to recreationists. This species was most abundant at LP, where there were significantly more people than at each of the other sites. The number of interactions that an animal has with a stimulus influences how quickly it learns how to react (Knight et al. 1987). Perhaps snakes at LP have had more non-threatening experiences with people than snakes

at the other sites, and have thus learned to not fear people's presence. Learning to tolerate interactions with people has been documented in other reptiles: Cottonmouths are increasingly passive towards people following repeated exposures (Glaudas 2004); and lizards (*Liolaemus* spp.) that are exposed to high densities of humans are less threatened by people than lizards that interact less with people (Labra & Leonard 1999).

High recreation use at LP also may indirectly lessen the predator pressure by displacing natural predators (Muhly et al. 2011). Raptors exhibit lower abundance in areas that are more urban and are used more by recreationists (e.g., Buehler et al. 1991) and carnivores are less abundant in more disturbed sites (Reed & Merenlender 2011). Therefore, there were perhaps more snakes at LP because they were less likely to be eaten, given the low counts of natural predators at this site.

Correspondingly, the percentage of injured snakes at MTP was lower than at SLNS, where there were significantly fewer natural predators and significantly more snakes seen. Non-lethal injuries can be a physical indicator of an unsuccessful predation attempt (Gregory & Isaac 2005; Gregory 2013). It is possible that natural predators at MTP were more successful than predators at SLNS. However, the proportion of injured snakes across sites (high at SLNS and comparatively low at MTP) did not parallel the counts of natural predators at the sites (low at SLNS and comparatively high at MTP); the proportion of injured snakes was non-significantly correlated with natural predator counts across sites. The proportion of injured wildlife is not necessarily a good predictor of the severity of predation pressure (Schoener 1979; Gregory & Isaac 2005).

Urban development can increase the abundance and diversity of resources for wildlife (Chace & Walsh 2006). Wildlife that have their food requirements met in an urban setting (e.g., human food hand-outs or waste) can be present in higher densities (Garber & Burger 1995b; Chace & Walsh 2006). There were significantly higher average rates of natural predator sightings, most of which were crows (pers. obs.), at MTP compared to the other sites. Crows are commensal with humans and depend on anthropogenic sources of food (Marzluff & Neatherlin 2006); these birds had a better chance of meeting their food requirements from direct handouts or garbage (pers. obs.; Marzluff & Neatherlin 2006) at MTP than at sites where there were fewer people. This supplementation of food for crows could thus have an indirect effect on Gartersnakes by

increasing predation pressure on them (e.g., Andrén et al. 1985; Kristan & Boarman 2003; Lepczyk et al. 2004).

Apparent low counts of snakes at MTP could also have been due to the road that passes through that site. Roads are detrimental to snakes (e.g., mortality from collisions and barrier to dispersal; Gregory 1984b; Andrews & Gibbons 2005).

Overall, the conclusions that I draw from comparing the counts of snakes with the counts of people, of pets, and of natural predators at each site are speculative. My failure to control for habitat quality/type and weather likely confounds my ability to draw meaningful conclusions about how the presence of potential predators influences these snakes. For instance, changes in habitat features along the urban gradient affect lizard assemblages in Tucson, Arizona (Ditchkoff & Wakeling 2001). Land development in urban areas removes ground cover and underbrush that can make habitat less suitable for small mobile reptiles, such as Northwestern Gartersnakes, which rely on cover to escape predators (Webb & Whiting 2005; Webb et al. 2009).

Although anthropogenic development can reduce habitat quality for some reptiles, the Northwestern Gartersnake apparently does well at many sites in the District of Saanich. The sites in this study contain many general habitat components that are suitable for Northwestern Gartersnakes (e.g., light forestation and edges; Gregory 1984a). In the following chapters, I address the stress physiology and anti-predator displays of Northwestern Gartersnakes at these sites to make more informed conclusions about the impacts of disturbance on them.

CHAPTER 2 – PROFILING WHITE BLOOD CELLS IN GARTERSNAKES TO INFER STRESS

INTRODUCTION

One way to gauge the impact of various disturbances (presence of people, pets, and natural predators) on snakes is to measure their physiological stress responses. Corticosterone (CORT) is the main stress hormone in snakes (Greenberg & Wingfield 1987; Preest et al. 2005). There are three alternative methods for interpreting the relative stress levels of wildlife: analyzing CORT levels in plasma; determining fecal concentrations of glucocorticoid metabolites; or conducting a leukocyte profile of blood smears to indirectly infer CORT levels from the ratio of two types of white blood cells.

The most common method to interpret stress levels is to measure plasma CORT (Davis & Maerz 2008). However, recent literature urges caution in its use when the goal is to determine baseline CORT levels (as my aim was for this study). This is because capture and handling during blood sampling are stressors in themselves and thus enhance activation of the HPA axis (Romero & Reed 2005).

Quantification of fecal concentrations of glucocorticoid metabolites is more suitable for determining baseline stress levels. These metabolites reveal an integrated level of glucocorticoids from several hours prior to capture (Romero & Reed 2005) and thus provide a more reliable assessment of chronic stress (Atkins et al. 2002; Rittenhouse et al. 2005). However, analysis of feces to determine the concentration of glucocorticoid metabolites is complex and expensive.

A cost-effective and manageable alternative to determine stress levels is the application of a haematological approach that involves a leukocyte profile. Conducting a leukocyte profile requires a microscope, stained slides, and a minuscule amount of blood (5–10 μL) that can realistically be obtained from a captured snake while in the field (Davis et al. 2011; Davis et al. 2008). The leucocyte profile method is also highly reliable given the tight relationship between circulating leukocytes and the adrenal stress response; increased CORT levels induce a rise in circulating heterophils and a decrease in circulating lymphocytes (Davis et al. 2008; Davis et al. 2011). Because of this opposing

effect of elevated CORT levels on the numbers of these leukocytes, researchers use the ratio of heterophils to lymphocytes (H:L) to indirectly infer the degree of chronic HPA axis activation in reptiles (Davis et al. 2008; Davis & Maerz 2008). Additionally, because hormone-controlled proliferation of leukocytes in circulation takes hours to days for reptiles, there is minimal potential for elevated CORT caused by capture and handling to influence changes in H:L (Davis et al. 2008; Davis et al. 2011). Leukocyte profiling is a consistent and predictable method (Davis et al. 2008), and is my method of choice to infer stress levels in Northwestern Gartersnakes.

To obtain accurate measures of white blood cell abundance, it is important to collect whole blood that is not diluted by lymph fluid (Thrall et al. 2004). Blood drawn from veins is often diluted given the close association between blood and lymphatic vessels (Thrall et al. 2004). Therefore, I utilized cardiocentesis (puncturing the heart) instead of caudal venipuncture to collect blood from snakes (Thrall et al. 2004).

Snakes can have up to six different types of white blood cells: heterophils, lymphocytes, basophils, eosinophils, monocytes, and azurophils (Davis et al. 2008). Because the morphology and relative abundances of each cell type vary both inter- and intra-specifically (Sykes & Klaphake 2008), published leukocyte parameters in even closely related species provide only limited information about what the cells in Northwestern Gartersnakes look like and in what relative abundance they exist in circulation. I addressed the following questions:

1) What are the key morphological characteristics of each type of white blood cell in Northwestern Gartersnakes?
2) What is the relative abundance of leukocytes in wild Northwestern Gartersnakes?

METHODS

Gartersnakes and the study sites they inhabit

I searched for Northwestern Gartersnakes using visual encounter surveys, along edge-focused transects at five sites in the Greater Victoria Area, BC: Christmas Hill/Swan Lake Nature Sanctuary (CHNS and SLNS, respectively), Mount Douglas Park (MDP), Layritz Park (LP), and Mount Tolmie Park (MTP). Snakes were caught by hand throughout May-August, 2012. Various data on snakes and capture location were recorded. I also took a blood sample from the heart of each captured snake.

Sampling blood

I firmly held the snake on its back, elevated at about 45° to the ground (head up) between the thumb and index finger of my non-dominant hand. I then located the heart, which resides in the anterior 1/3 of the body, just craniad the lungs (Campbell & Ellis 2007; Sykes & Klaphake 2008). I detected the heart either by observing the movement of the ventral scutes (indicating heartbeats) or by palpating the ventral surface, starting at the base of the head and moving caudally (Campbell & Ellis 2007). In the rare event that the heart was not detected by one of these aforementioned methods, I held the snake out in front of me to identify the most cranial area of lung movement – the heart is just above this. When using this method to locate the heart, more puncture attempts were required to collect blood because the exact location of the heart was not known.

I collected blood from snakes by cardiocentesis (puncturing the heart) using Becton, Dickonson and Company (BD) Ultra-Fine insulin syringes (0.3 cc, 12.7 mm length, and 29-gauge needle). I chose this method because it is non-lethal, safe to use on non-anesthetized snakes (Campbell & Ellis 2007), and is manageable for one person, at least for small snakes (snout-vent length, SVL < 1 m, e.g., Gartersnakes; Campbell & Ellis 2007). Also, since the blood that is obtained from the heart is not diluted with lymph fluid, it is ideal for preparing blood smears in the field for leukocyte profiling.

To collect the blood, I held a syringe with the needle pointed cranially, and then inserted it slightly between two ventral scutes at an angle of 30° from the snake's body surface. I increased the angle of the needle to 45° and slowly inserted it until it touched

the snake's spine. The plunger was slowly pulled back as the needle was slowly pulled out of the snake until blood started to enter the syringe. The syringe was held steady until about 3 units (0.03 mL) of blood was collected. This is well below the safe amount of blood to collect from these snakes: reptiles can tolerate removal of up to 10% of the blood volume, which corresponds to 0.5-0.8 mL for a 100 g individual (Sykes & Klaphake 2008). The syringe was set aside briefly (in the shade if outside, keeping it vertical with the needle end down) while I recorded other measurements from the snake.

Preparing blood smears

I prepared two to six blood smears per snake by placing one drop of blood onto a microscope slide and used the bevel-edge slide technique to create a smear (Perpinan et al. 2006). I then air-dried the slides and labelled them.

In the laboratory, I stained the smears on the same day that they were prepared using CAMCO Quik Stain II (buffered differential Wright-Giemsa stain). A Wright's-Giemsa stain is sufficient for identifying most leukocytes with ease (Alleman et al. 1999). I submerged the smears in stain for 10 seconds, and then immediately transferred them to tap water for 20 seconds, after which I left them to air-dry. Once dry, I lightly wiped the smears using a Kim Wipe to remove excess stain from the backs of the slides. I stored the slides in slide boxes for later leukocyte profiling.

Leukocyte profiling

Leukocyte profiles provide information about the abundance of white blood cells. By comparing values between individuals and/or to accepted basal values, one can learn about an individual's physiological status (e.g., stress and immune responses).

I profiled only the one smear with the largest area of monolayer cells for each snake under 1000X oil immersion (Zeiss immersionsoel), using a Leitz Laborlux S compound microscope. I started the leukocyte profile at the most distal edge of the feather end of the smear and proceeded one field of view at a time, across the entire smear in an 'S' fashion. I considered only fields of view with 15^+ erythrocytes in a monolayer (Davis & Maerz 2008). I recorded the number of lymphocytes, heterophils,

monocytes, azurophils, basophils, and eosinophils using a Unico 8-key manual cell counter until I counted 100 white blood cells (WBC). I counted only those leukocytes that I could identify with 100% confidence. I also counted the number of fields viewed while identifying the cells. I determined a total WBC by counting the number of WBCs in 10 fields of view (with erythrocytes dispersed in a monolayer across the entire field).

I used a DD12NLC camera (model 15.2) and SPOT software (version 4.5.9.9) to take photographs of the different leukocyte types. The camera was attached to a Zeiss microscope and was hooked up to a Macintosh computer (OS X version 10.4.11). All leukocyte images were captured using an immersion oil objective lens (100X). The photos were edited using Photoshop CS3 (version 10.0.1). The mean (±1 standard error, SE) was calculated for the number of each type of leukocyte.

RESULTS

I was not able to consistently draw blood from the hearts of very small snakes. Therefore, I report results only from individuals with SVL > 20 cm. Also, no eosinophils were seen in Northwestern Gartersnake blood, nor were there any blood-borne parasites in the red blood cells of these snakes.

Lymphocytes were the most abundant cell type in circulation (55.667±1.409 per individual, median=57; Figure 15). Next, ordered from higher to lower average abundance per snake, were azurophils (22.968±0.999, median=22), basophils (13.135±0.615, median=12), heterophils (6.508±0.448, median=5), and lastly monocytes (1.722±0.227, median=1; Figure 15). The sample size was 126 in all cases.
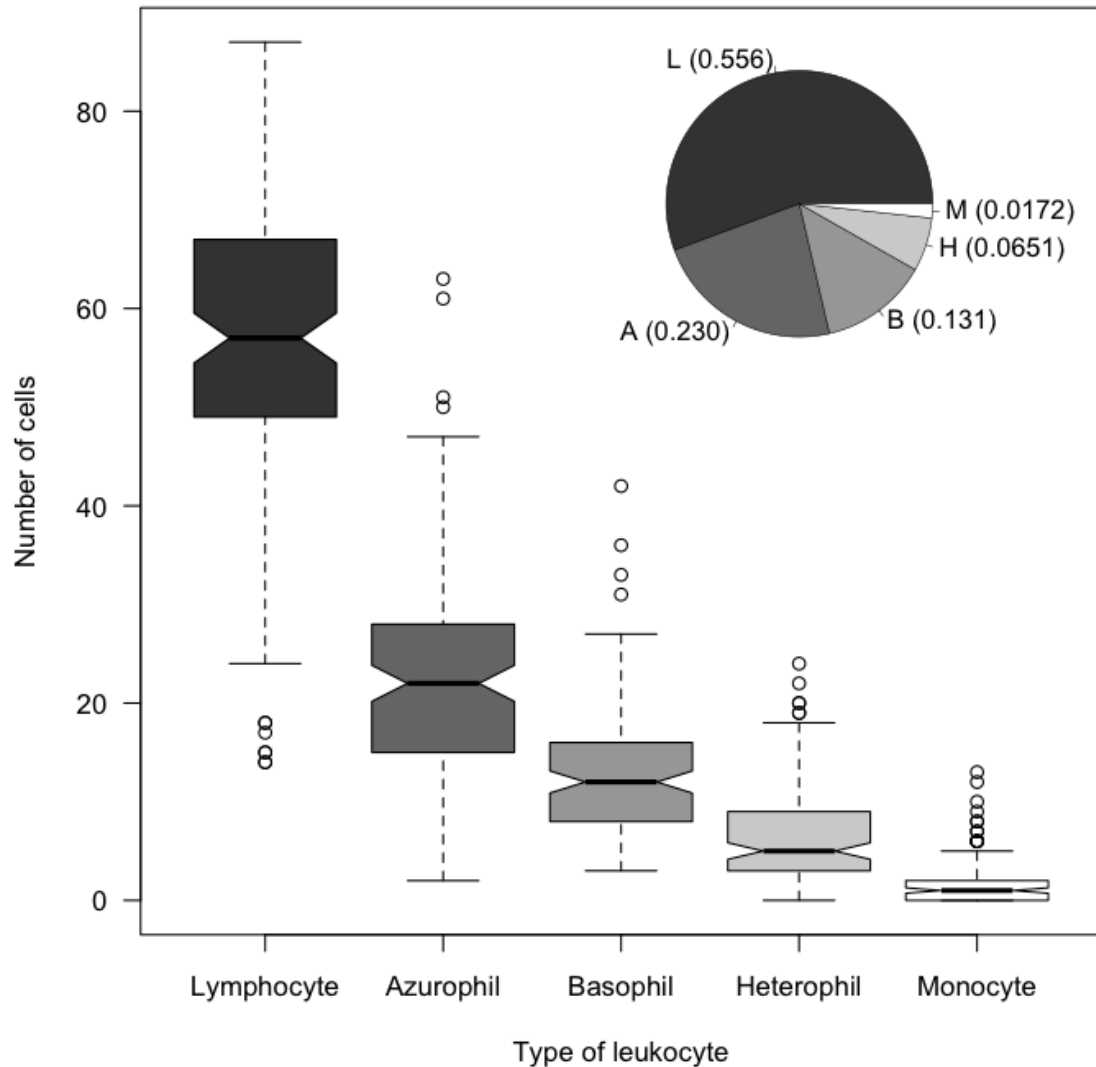
Figure 15. Notched boxplots of the five different types of leukocytes (Lymphocyte: L, Azurophil: A, Basophil: B, Heterophil: H, and Monocyte: M) in blood of Northwestern Gartersnakes. See CHAPTER 1 for a description of a boxplot. The pie chart in the top right displays the relative proportion of each white blood cell of all types in blood.

Lymphocytes varied in size (5-10 μm; Campbell & Ellis 2007) from about half to equal the size of erythrocytes but were most often on the smaller end (Figure 16). These cells have a high nucleus-to-cytoplasm ratio. The cytoplasm (sparse) was blue without granules and the nucleus was purple-pink with dense nuclear chromatin. These comprised the most abundant cell type in circulation.
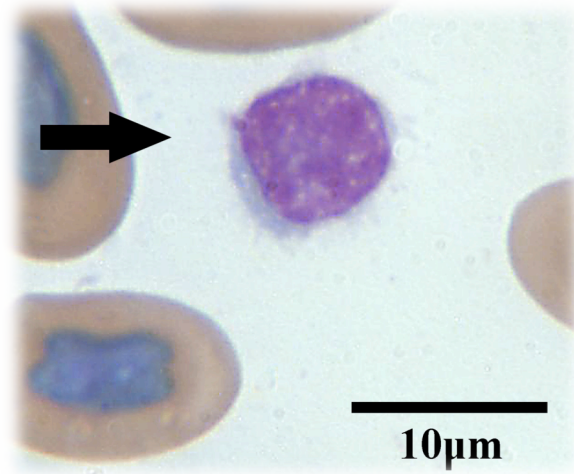
Figure 16. Gartersnake lymphocyte (black arrow) surrounded by red blood cells – CAMCO Quik Stain II (buffered differential Wright-Giemsa stain).

The azurophil is a type of monocyte distinct to reptiles and found in especially high numbers in snakes (Campbell & Ellis 2007). It is moderately sized, of comparable size to erythrocytes (Figure 17). Azurophils have blue cytoplasm and are easily recognized by the azurophilic (pink/purple) indistinct cytoplasmic granules, typically occupying the peripheral areas of the cytoplasm (Figure 17). The nucleus is dark pink with dense chromatin. Non-azurophilic monocytes are of comparable size to azurophils and erythrocytes (Figure 18). The cytoplasm is non-granulated and transparent clear to light purple. The nucleus is purple-pink with dense chromatin (Figure 18).
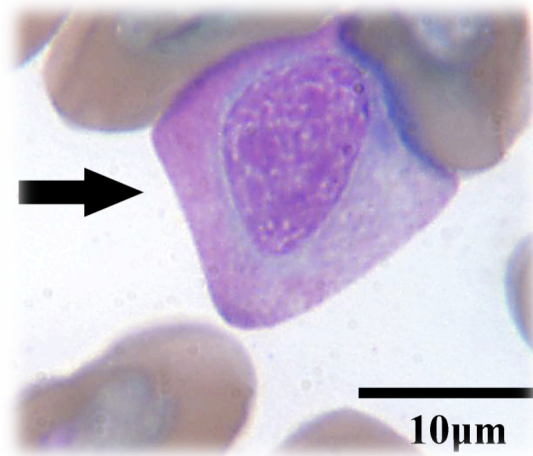
Figure 17. Gartersnake azurophil (black arrow) surrounded by red blood cells – CAMCO Quik Stain II (buffered differential Wright-Giemsa stain).
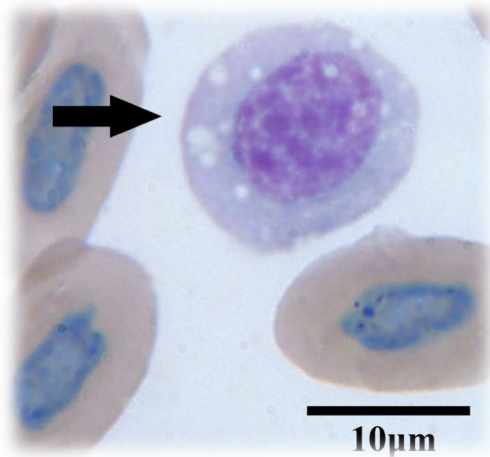
Figure 18. Gartersnake monocyte (black arrow) surrounded by red blood cells – CAMCO Quik Stain II (buffered differential Wright-Giemsa stain).

Basophils were of comparable size to lymphocytes, and perhaps a little larger (8-15 μm; Campbell & Ellis 2007). This cell has basophilic (burgundy) cytoplasmic granules. Sometimes the granule contents are expelled during blood processing and granules appear as clear transparent vacuoles. The nucleus is dark pink, with dense chromatin, and is often visually obscured by the dark granules (Figure 19).
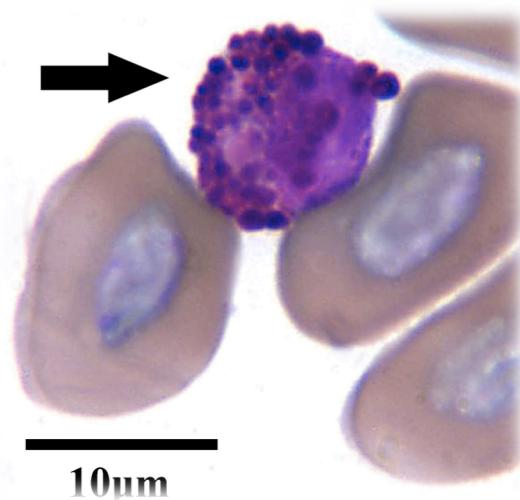
Figure 19. Gartersnake basophil (black arrow) surrounded by red blood cells – CAMCO Quik Stain II (buffered differential Wright-Giemsa stain).

Heterophils were the largest leukocytes (10-23 μm; Campbell & Ellis 2007), about 1.5X the size of erythrocytes and are distinguished by round eosinophilic (orange) granules that fill the cytoplasmic space (Figure 20). These granules often displace the light blue nucleus to one side of the cell, and may completely obscure the nucleus.
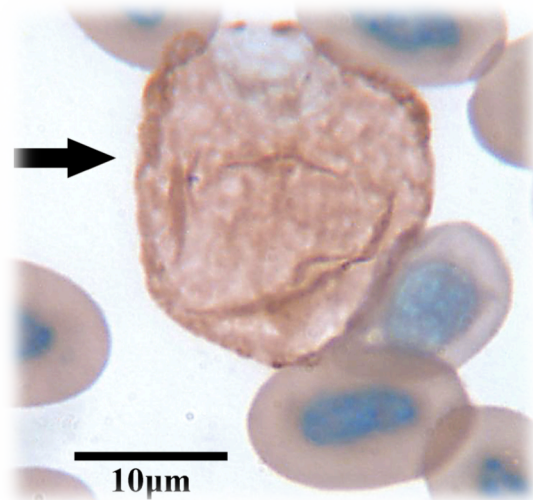
Figure 20. Gartersnake heterophil (black arrow) surrounded by red blood cells – CAMCO Quik Stain II (buffered differential Wright-Giemsa stain).

DISCUSSION

There is considerable interspecific variation in the leukocyte parameters and in the morphological characteristics of white blood cells among reptilian species, even within Squamata (Bounous et al. 1996; Salakij et al. 2002; Campbell & Ellis 2007; Claver & Quaglia 2009). The abundance and morphology of blood cells can be affected by the health, age, sex/reproductive status of the individual, the venipuncture site, the staining (type of stain: e.g., Wright's versus Wright's-Giemsa stains; see Salakij et al. 2002) and evaluation of the slides, the season, in addition to environmental conditions (Sykes & Klaphake 2008). Therefore, I cannot directly compare the abundances and

morphology of white blood cells reported in this study on Northwestern Gartersnakes with those in studies about other species. Additionally, I am the first to examine lymphocytes in Northwestern Gartersnakes; there is no published information about leukocyte parameters in this species. I therefore focus on general comparisons with the relative abundance of leukocytes in other snake species.

Leukocytes are present in the blood of King Cobras (*Ophiophagus hannah*) in the same order of abundance as I report here: lymphocytes, followed by azurophils, then basophils, then heterophils, then monocytes, and finally eosinophils (Salakij et al. 2002). Additionally, since a Wright's-Giemsa stain was used to treat the blood smears of the King Cobras there are some notable similarities in the morphological characteristics of some cell types between King Cobras and Northwestern Gartersnakes: heterophils have dull eosinophilic granules and lymphocytes have a very small amount of cytoplasm surrounding the nucleus (Salakij et al. 2002).

The absence of eosinophils is not surprising because eosinophils are present in only some squamate species (Claver & Quaglia 2009) and are often absent in snakes (Sykes & Klaphake 2008). Eastern Diamondback Rattlesnakes (*Crotalus adamanteus*; Alleman et al. 1999) and Yellow Ratsnakes (*Elaphe obsoleta quadrivitatta*; Bounous et al. 1996) do not have eosinophils. However, there is also the possibility of misidentifying eosinophils by confusing them with basophils. The use of Wright's-Giemsa stain can make it difficult to differentiate between the granules of basophils and eosinophils (Salakij et al. 2002). It is likely that this is a species-specific attribute because eosinophils are known for their round eosinophilic granules, which usually stain orange-brown, as in heterophils (Campbell & Ellis 2007). Another potential reason for the absence of eosinophils in circulation might be season. Generally, eosinophils are lowest during the summer months, which is when I collected blood from the Gartersnakes, and highest during hibernation (Thrall et al. 2004). Finally, eosinophils fight against parasitic infections (Thrall et al. 2004). The absence of parasites on the external body, or in the blood of the Northwestern Gartersnakes may also be why no eosinophils were identified in circulation.

It is well established that elevations in stress hormones increase the number of circulating heterophils (or neutrophils in mammals and amphibians) and decrease the

number of circulating lymphocytes across all vertebrate taxa (Davis et al. 2008; Davis et al. 2011). The abundances of azurophils (a type of monocyte only in snakes; Sykes & Klaphake 2008), monocytes, basophils, and eosinophils are not dictated by changing concentrations of CORT in circulation. These cells function specifically in response to infections and injury: monocytes defend against bacterial infections; basophils aid with a variety of inflammatory responses; and, eosinophils defend against parasitic infections (Davis et al. 2008). Therefore, comparative analysis of the ratio of heterophils to lymphocytes between individuals is a way to indirectly infer stress levels.

CHAPTER 3 – INTERPRETING STRESS LEVELS OF URBAN GARTERSNAKES

INTRODUCTION

Stress is a vital component of all vertebrates' wellbeing. It facilitates the recognition of, and response to, threatening stimuli (Lupien et al. 2009), whether environmental, developmental, or physiological (Greenberg & Wingfield 1987), through the recruitment of endocrine, behavioural, autonomic, immune, and metabolic processes (Yao & Denver 2007). This recruitment is achieved by the hierarchical integration of the hypothalamus, the pituitary gland, and the adrenal gland in tetrapods (Romero & Wikelski 2002; Dedovic et al. 2009). Together, these structures are referred to as the stress axis, or the Hypothalamo-Pituitary-Adrenal (HPA) axis, or as the HP-Interrenal (HPI) axis in teleost fish (Yao & Denver 2007). Activation of this three-tiered system culminates in the increased release of glucocorticoids (corticosterone, CORT in non-human tetrapods, and cortisol in teleost fish and humans) into blood plasma (Greenberg & Wingfield 1987; Preest et al. 2005). Once in circulation, glucocorticoids travel throughout the body enhancing survival-promoting responses (e.g., mobilizing energy stores) and suppressing functions that are not crucial to immediate survival (e.g., growth, reproduction, and immune function) to re-establish homeostasis (Romero & Wikelski 2002; Moore & Jessop 2003; Gesquiere et al. 2008; Thaker et al. 2009a). This in turn limits the costs of unfavourable influences and thereby enables the individual to recover from the stressful situation, given that the stressor was not fatal (Romero & Wikelski 2002; Landys et al. 2006).

Stress can affect organisms in both the short and long term. Acute increased release of CORT can be adaptive for tetrapods (Romero & Reed 2005; Gesquiere et al. 2008). A classical example is a predator attack: activation of the HPA axis activates gluconeogenesis for the immediate mobilization of energy that the organism may utilize in the 'fight or flight' response to avoid predation (Gesquiere et al. 2008). However, prolonged CORT elevations following chronic exposure to stressors can lead to impaired reproduction, suppressed immunity, and neuronal cell death (Romero & Wikelski 2002;

Bailey et al. 2009; Thaker et al. 2009a). These deleterious consequences of chronic stress are avoided through negative feedback loops that limit the respective release of neuropeptides and hormones from the hypothalamus and the pituitary gland, and overall decrease the release of CORT (Yao & Denver 2007; Dedovic et al. 2009). Although this self-regulation of the HPA axis reduces the impact of prolonged CORT elevation (Moore & Jessop 2003), a dampened stress response also means delayed physiological and behavioural responses, which can increase the cost(s) of stressor exposure.

The structure of the HPA axis is conserved in vertebrates, but the way in which it is activated and its actions vary both inter- and intra-specifically (Moore & Jessop 2003). Even in response to the same stressor, individuals exhibit varied rates, durations, and magnitudes of the synthesis and release of glucocorticoids into circulation because of differences in their physiological state (e.g., body condition, sex/reproductive condition, disease status), environmental conditions (e.g., resource availability, habitat quality), and even with the season and time of day (Woodley & Moore 2002; Moore & Jessop 2003; Landys et al. 2006). For instance, most reptiles display elevated concentrations of CORT coinciding with the onset of feeding and activity cycles (Landys et al. 2006). Additionally, the influence of glucocorticoids on physiology and behaviour is determined by the amount of hormone released, the distribution and type of receptor, the levels of corticosteroid binding globulin, and the concentration of site-specific enzymes (Landys et al. 2006). These conditions contribute to natural fluctuations in basal glucocorticoid levels that fall well below those characteristic of an emergency stress response (Magiakou et al. 1997; Woodley & Moore 2002; Preest et al. 2005; Landys et al. 2006). Predictable (e.g., seasonal change) and unpredictable (e.g., predator attack) stressors require different modulation of the HPA axis (Robert et al. 2009); although commonly assumed, not all HPA activation is deleterious (Greenberg & Wingfield 1987). Allostasis addresses the actions of alternative levels of GC release (Landys et al. 2006).

The variability in the rate, duration, and magnitude of the stress response reflects the sensitivity and plasticity of the HPA axis (Moore et al. 2001) and is termed adrenocortical modulation (Moore & Jessop 2003). For instance, males of two subspecies of the Common Gartersnake respond to capture stress differently depending on the time of year (Moore et al. 2001). The male Red-Spotted Gartersnake, *T. sirtalis*

concinnus responds to capture stress with elevated CORT levels during all times of the year supposedly, whereas male *T. s. parietalis*, the Red-Sided Gartersnake, exhibits an emergency stress response when exposed to capture stress only during the non-breeding season (Moore et al. 2001). This results from different effects of climate on the length of the breeding season in different subspecies; *T. s. concinnus* has an extended breeding season compared to *T. s. parietalis* because it inhabits a more southerly region where temperatures are higher for longer (Moore et al. 2001). With more limited reproductive opportunities, male *T. s. parietalis* seem to suppress the hormonal stress response during the brief breeding season (Moore et al. 2001).

The majority of studies have assessed CORT responses to acute invasive stressors (e.g., capture and handling; Moore et al. 2000). For instance, plasma CORT levels of male Red-Sided Gartersnakes subjected to 4 hours of capture stress are significantly greater than levels measured prior to stressor exposure for the same individual (Moore et al. 2000). Short-term confinement also enhances HPA axis activation in many other reptile species: Galapagos Marine Iguanas, *Amblyrhynchus cristatus*; Freshwater Crocodiles, *Crocodylus johnstoni*, Western Fence Lizards, *Sceloporus occidentalis*; and, Tree Lizards, *Urosaurus ornatus* (Moore & Jessop 2003).

Studies of whether chronic exposure to non-invasive human disturbance (e.g., people walking through reptile habitat) is stressful for reptiles are scarce and highly concentrated on only a few reptile species (Bailey et al. 2009): continual human presence from tourist visits does not alter baseline CORT levels in Galapagos Marine Iguanas (Romero & Wikelski 2002), and Cottonmouths are not stressed by occasional encounters with humans walking along footpaths (Bailey et al. 2009). Although these studies suggest that perhaps reptiles are adaptable to low-level disturbances, and are not stressed by the presence of humans, additional research is required in a variety of taxa, notably in a range of reptile and amphibian species.

I carried out leukocyte profiles to indirectly determine the concentration of CORT in circulation. Through analysis of the relative abundance of different white blood cells (specifically the ratio of heterophils to lymphocytes, H:L), stress between individuals can be indexed comparatively (higher H:L coincides with a higher concentration of CORT, where a ratio > 2:1 implies a stressed individual; Davis et al. 2008; Davis 2009; Davis et

al. 2011). Because hormone-controlled proliferation of leukocytes in circulation takes one to a few days to change in reptiles (Davis et al. 2008; Davis et al. 2011; Davis & Maerz 2011), the H:L is not influenced by stress incurred by capture and handling and thus provides information about long-term or baseline stress activation in reptiles (Davis et al. 2008; Davis & Maerz 2008). Leukocyte profiling is being implemented more and more in ecological studies as a reliable method to assess the stress level of wildlife (Davis et al. 2008). I focused on the following questions:

1) Are Northwestern Gartersnakes in urban parks chronically stressed (e.g., H:L > 2:1; Davis 2009)?
2) Is there an effect of potential predator abundance on H:L values?
3) Do other factors influence H:L? Such factors may include sex/reproductive condition, snout-vent length (SVL), mass, body temperature, presence of an injury, and/or shedding status.

Predictions

Predation threat has indirect positive effects on circulating CORT levels (Clinchy et al. 2011). I expected that snakes that were exposed to more predators would have higher H:L values compared to snakes that encountered predators less often (Table 3).

Females and males respond to stressors differently, partly because oestrogen enhances, whereas testosterone inhibits, HPA function (Handa et al. 1994). Because females have more oestrogen, and males have more testosterone in circulation, males generally have lower concentrations of plasma CORT compared to females (Handa et al. 1994; Clinchy et al. 2011). Additionally, pregnant females, in comparison with non-pregnant females, have a suppressed HPA axis to avoid the deleterious effects of high concentrations of plasma CORT on developing offspring (Magiakou et al. 1997; Woodley & Moore 2002; Preest et al. 2005). I expected that both males and gravid females would have lower H:L values than non-gravid females (Table 3).

Experience enables animals to learn from previous encounters and thus better differentiate a threat from a non-threat (e.g., Greenberg 2002; Thaker et al. 2010). I assumed that longer snakes are generally older, and that older snakes have had more stress-inducing encounters than younger snakes. I expected that longer Northwestern Gartersnakes would have a down-regulated stress axis (i.e., lower H:L) to ensure that excess CORT is released only in the face of a true threat (Table 3). Furthermore, given that mass is generally positively correlated with SVL in snakes (e.g., Kaufman & Gibbons 1975; Guyer & Donnelly 1990; Feldman & Meiri 2013), I anticipated that mass would also be negatively associated with H:L (Table 3).

Furthermore, attack by a predator can be stressful for prey (Scheuerlein et al. 2001). It then follows that non-lethal injuries inflicted on prey by predators trigger the HPA axis and increase plasma CORT levels. I predicted that injured Northwestern Gartersnakes would have higher H:L values than uninjured snakes (Table 3).

Skin shedding is not directly affected by CORT or other adrenalcorticoids (Halberkann 1954; Chiu & Lynn 1972), but is indirectly related to CORT release through their common relation to metabolism: the frequency of shedding is associated with the metabolic status of the animal (Chiu et al. 1986); and, CORT is an important hormone for mobilizing energy stores (King & Turmo 1997; Moore et al. 2000). Although indirect, I anticipated a positive association between CORT and skin shedding (Table 3).

Because I did not find any literature about relationships between body temperature and CORT levels I did not make any specific predictions about how it might have been related in Northwestern Gartersnakes (Table 3).

Table 3. Predicted influence (positive: '+', negative: '-', or unknown: '?') of independent variables (site: level of disturbance, sex/reproductive condition, snout-vent length: SVL, mass, cloacal temperature, presence of an injury, and skin shedding) on the baseline stress levels of Northwestern Gartersnakes. The categories of the sex/reproductive condition factor are F (non-gravid female), FG (gravid female), and M (male), where F is the reference level.

Potentially influential variables	Direction of predicted influence on H:L ('+' OR '-')
Site (level of disturbance)	+
Sex/reproductive condition	FG: - M: -
SVL	-
Mass	-
Body temperature	?
Injury	+
Shedding	+

METHODS

Gartersnakes and the study sites they inhabit

I searched for Northwestern Gartersnakes using visual encounter surveys, along edge-focused transects at five sites in the Greater Victoria Area, BC: Christmas Hill/Swan Lake Nature Sanctuary (CHNS and SLNS), Mount Douglas Park (MDP), Layritz Park (LP), and Mount Tolmie Park (MTP). I caught snakes by hand throughout May-August, 2012. For each captured snake I took a blood sample (see CHAPTER 2) and recorded information about other characteristics. I also determined the disturbance regime at each site (see CHAPTER 1) and compared H:L among sites.

Statistical analyses

I used R and a statistical significance level of 0.05 when analyzing my results. I calculated the mean H:L for non-gravid females, gravid females, and males both overall and for each site and compared values using Wilcoxon rank-sum tests. This test was also used to compare the H:L of individuals (same sex/reproductive condition) between sites.

I used a series of generalized linear models to determine how site, sex/reproductive condition, mass, SVL, skin shedding (see CHAPTER 1), presence of an injury (see CHAPTER 1), or cloacal temperature individually influence H:L (e.g., glm(H:L ~ injuries, family=Gaussian). I determined the appropriate family type (Gaussian for all cases) using the gam() function in R. I compared Akaike Information Criterion (AIC) values to determine which variable best predicted H:L. Also, to determine the fit of each model I performed a Chi-squared (χ^2) Test by using the residual deviance and its degrees of freedom to calculate a p-value (e.g., 1-pchisq(residual deviation, degrees of freedom) in R). Following these single predictor models I examined the combined influence of the variables that significantly affected H:L (p<0.05) by using the glmulti() function in R. Again, I used the residual deviance and its degrees of freedom to calculate a p-value, following a χ^2 distribution.

RESULTS

The mean H:L (±1SE) for all individuals and sites combined was 0.163±0.204 (N=126; Figure 21). The mean H:L values for non-gravid females, gravid females, and males for all sites combined were 0.218±0.0510 (N=42, median=0.101), 0.0670±0.00944 (N=30, median=0.0577), and 0.173±0.0235 (N=54, median=0.102), respectively (Figures 21 & 22). Gravid females had a significantly lower H:L than both non-gravid females (Wilcoxon rank-sum test; W=895.5, p=0.00247) and males (W=1149, p=0.00158). Males had lower H:L values than females, but this difference was not significant (W=1135, p=0.997).
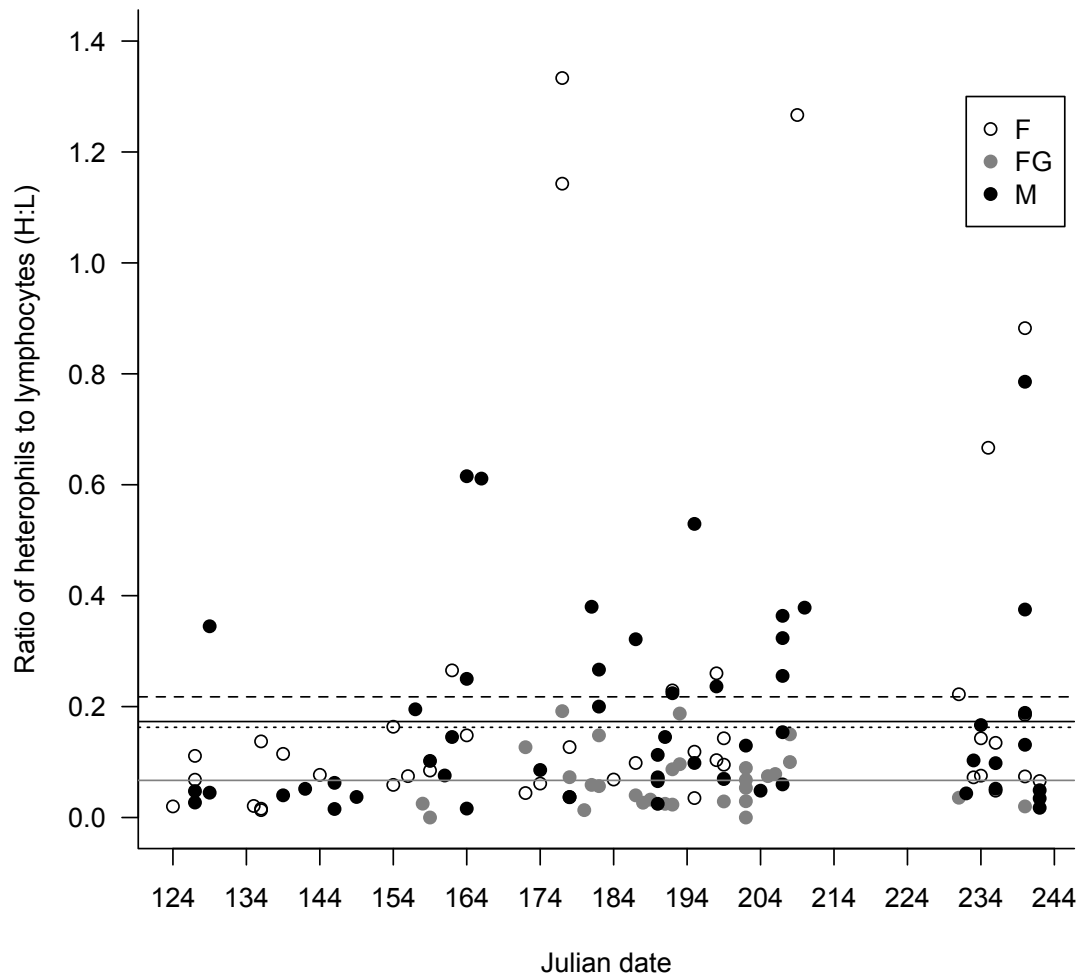
Figure 21. Ratio of heterophils to lymphocytes (H:L) by Julian date. The data was collected on May 4th to August 30th (Julian date = 124-242). The horizontal lines represent mean H:L values.
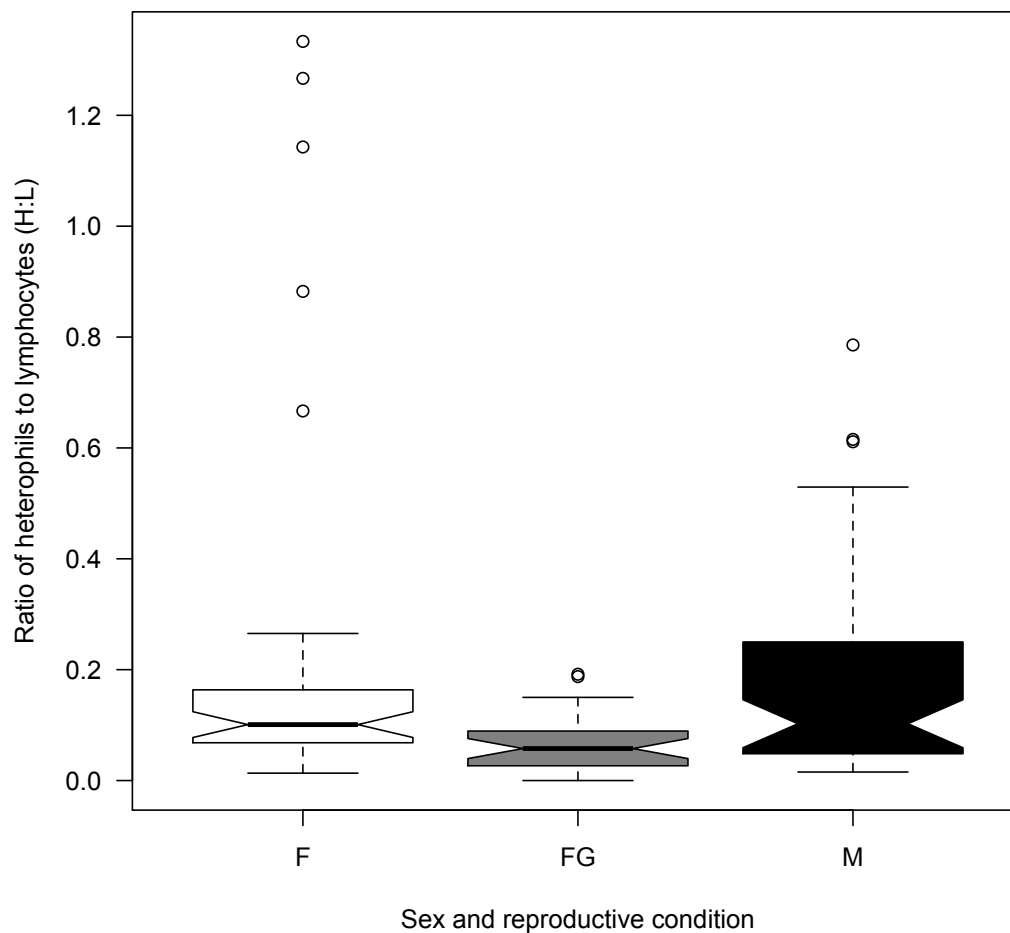
Figure 22. Notched box-and-whisker plots of the ratio of heterophils to lymphocytes (H:L) for non-gravid female (F), gravid female (FG), and male (M) Gartersnakes. All snout-vent lengths > 20 cm.

Shedding best predicted the variability in H:L (Table 4), compared to the other variables that I individually modelled against H:L. The relationship between shedding and H:L was significantly positive. Mass was also significantly related to H:L, but this relationship was negative (Table 4). Similarly, gravid females had a significantly lower H:L value than non-gravid females (Figure 22 & Table 4). Males also had lower H:L values than non-gravid females, but not significantly so (Figures 22). Snout-vent length was also a non-significant negative predictor of H:L (Table 4). Positive, non-significant predictors of the variability in H:L included cloacal temperature and injuries excluding nicks (Table 4). Although not significant, individuals at LP had higher H:L values than

individuals at CHNS, whereas individuals at MDP, MTP, and SLNS had lower H:L values compared to CHNS (Table 4). Results from the χ^2 Test indicated that all of these above-described single predictor models for H:L fit the data (p>0.05).

Table 4. Various independent variables were modelled to determine their influence on the ratio of heterophils to lymphocytes (H:L) using a generalized linear model (family=Gaussian). The '+' or '-'sign indicates that the independent variable has a positive or negative influence on the number of snakes encountered. Model results are ordered from best to worst, by AIC value. Significant variables (p<0.05) are indicated by a *. All models fit the data (χ^2; p>0.05). N=108 for all cases. The site names are abbreviated as in Figure 9. Abbreviations for sex/reproductive conditions are the same as in Table 3.

Independent variable	Influence of independent variables on H:L ('+' OR '-')	P-value	AIC	P-value (χ^2), degrees of freedom
Shedding*	+	0.0294	-10.015	1.000, 106
Sex/reproductive condition*	FG*: - M: -	FG*: 0.0127 M: 0.476	-9.955	1.000, 105
Mass*	-	0.0420	-9.393	1.000, 106
SVL	-	0.240	-6.574	1.000, 106
Cloacal temperature	+	0.497	-5.634	1.000, 106
Injury	+	0.907	-5.175	1.000, 106
Site	LP: + MDP: - MTP: - SLNS: -	LP: 0.474 MDP: 0.240 MTP: 0.363 SLNS: 0.571	-4.920	1.000, 103

The independent variables that significantly predicted H:L were sex/reproductive condition (specifically gravid females), mass, and shedding. I combined these variables to determine how they cumulatively predicted the variability in H:L (Table 5). All shedding individuals had significantly lower H:L values than non-shedding individuals, and shedding males and shedding gravid females each had a significantly lower H:L values than shedding females. Mass was no longer significant when included as a predictor variable with shedding and sex/reproductive condition. However, the model that included mass fit the data marginally better (lower AIC value; Table 5).

Table 5. Variables that were significantly related to the ratio of heterophils to lymphocytes (H:L) from the single predictor modelling for H:L (see Table 4) were combined into a new model, including second order interactions: glmulti (H:L ~ sex/reproductive condition + mass + shedding, level=2, family=Gaussian). Covariates that are significant (p<0.05) are indicated by a *. Both models fit the data (χ^2; p>0.05). N=108 for all cases. Abbreviations for sex/reproductive conditions are the same as in Table 3.

Model	Independent variables	Influence of independent variables on H:L ('+' OR '-')	P-value	AIC	P-value (χ^2), degrees of freedom
H:L ~ Shedding* + [(Sex/ reproductive condition)(Shedding)]*	Shedding*	+	<0.0001	-24.277	1.000, 102
	FG	-	0.238		
	M	+	0.389		
	[(Shedding)(FG)]*	-	0.00265		
	[(Shedding)(M)]*	-	0.00040		
H:L ~ Mass + Shedding + [(Sex/ reproductive condition)(Shedding)]*	Mass	+	0.964	-22.280	1.000, 101
	Shedding*	+	<0.0001		
	FG	-	0.290		
	M	+	0.405		
	[(Shedding)(FG)]*	-	0.00354		
	[(Shedding)(M)]*	-	0.00045		

DISCUSSION

Snakes in this study were not chronically stressed because all H:L values were less than 1.4, lower than the minimum of 2 generally associated with stress (Davis 2009). That said, drawing comparisons between individuals can elucidate information about how sex/reproductive condition and exposure to environmental stressors (disturbance by humans, pets, and/or natural wildlife predators at the sites) may influence the activity of the HPA axis. In turn, this can provide information about the health of wild individuals and of wild populations (Davis et al. 2011).

The stress axis in all vertebrates, not just in reptiles, is a very flexible system; the functioning of the HPA axis differs relative to the physiological status of the individual and the environmental conditions to which it is exposed (Love et al. 2013). One such

physiological influence is sex/reproductive condition (Magiakou et al. 1997). Because male snakes had, on average, lower H:L values than non-gravid females, there was a potential biological difference in the activities of their stress axes.

The Hypothalamo-Pituitary-Gonadal (HPG) and HPA axes are highly interconnected (Handa et al. 1994; Moore & Mason 2001). One way by which the HPG axis affects the HPA axis is exemplified by differences in HPA functioning between males and females that emerge from differences in the concentrations of circulating gonadal steroid hormones: testosterone, a male-dominant sex hormone, inhibits the stress axis, whereas oestrogen, the female-dominant sex hormone, enhances its functionality (Handa et al. 1994; Magiakou et al. 1997). This hormonal difference is expressed as females having a more robustly responsive stress axis than males following exposure to the same stressor (Handa et al. 1994). Thus, female reptiles generally have higher levels of CORT in circulation than male reptiles. This was the case with Northwestern Gartersnakes living in a disturbed environment. Similarly, female New Zealand Common Geckos (*Hoplodactylus maculatus*) respond to capture, handling, and confinement with higher concentrations of CORT in circulation than males (Preest et al. 2005). However, the conventional inhibitory effect of testosterone and excitatory effect of oestrogen on the functioning of the HPA axis may not always apply (Moore et al. 2001).

Differences in the responsiveness of the stress axis were more pronounced when I considered female reproductive state. Gravid females had, on average, significantly lower H:L values than both non-gravid females and males. The relationship between the HPA axis and the female reproductive system varies depending on the reproductive state of the individual (Magiakou et al. 1997; Woodley & Moore 2002); activity of the HPA axis decreases as pregnancy proceeds in lizards, turtles, and caimans (Preest et al. 2005).

Possibly the most biologically relevant reason why gravid female Northwestern Gartersnakes have significantly lower H:L values, and thus lower concentrations of CORT in circulation, compared to non-gravid females and males, is to protect the developing embryo(s) from exposure to high levels of CORT. In viviparous placental vertebrates, including reptiles such as the Gartersnake (Hoffman 1970), CORT can be transferred from mothers through the placenta to their offspring (Woodley & Moore

2002; Robert et al. 2009). Embryos that are developing within stressed mothers are exposed to high levels of CORT that have detrimental impacts on the offspring's development, affecting the programming of their stress axis, and altering morphological and behavioural traits that can lead to reduced fitness later in life (Preest et al. 2005; Robert et al. 2009; Love et al. 2013). For instance, pregnant female Wandering Gartersnakes (*T. elegans*) that were treated with CORT had 15% higher rates of unsuccessful pregnancy (still births and dead offspring within the first month of life) than mothers that were not treated with CORT (Robert et al. 2009). Of the successful births, offspring of treated mothers reversed significantly less than the offspring of non-treated mothers (Robert et al. 2009). Reversing is an anti-predator behaviour employed by these snakes to avoid capture (Robert et al. 2009). With compromised defensive tactics, it is more likely that an encounter with a predator would result in death.

There are a number of mechanisms by which mothers might temporarily resist the influences of environmental stressors to avoid the potential negative side effects that high concentrations of CORT can have on their offspring (Wingfield & Sapolsky 2003): a gravid snake might not perceive a challenge as stressful so as to not activate the stress response; her HPA axis might be down-regulated; her HPG axis might be resistant to the actions of the stress hormones; and/or her HPG axis might be up-regulated to counteract the inhibitory effects of the stress hormones. The basis for the relationship between plasma CORT levels and reproductive processes in live-bearing reptiles remains theoretical, with few studies testing these hypotheses, especially in snakes (Smith et al. 2012).

The obscure nature of the causal link between the female's reproductive condition and the level of circulating stress steroid hormones is exhibited across all vertebrate taxa (Wilson & Wingfield 1992). It is also an excellent example of adrenocortical modulation in vertebrates and exemplifies the high level of integration between physiological systems, specifically between the HPA and HPG axes. There exists a multitude of possible biological reasons for why and mechanisms for how gravid female Northwestern Gartersnakes have significantly less CORT in circulation (i.e., lower H:L values) compared to both non-pregnant females and males. It is clear that this result stems from a hormonal interaction as well as altered behaviour. Gravid females, compared to non-

pregnant females, eat less and spend more time maintaining a high body temperature to support their developing offspring (Gregory et al. 1999).

To examine the overall trends in H:L variability between sites I modelled site against H:L, with sex/reproductive condition combined. Snakes at LP had more CORT in their blood (as inferred from the higher H:L values, on average). Also, LP was significantly more disturbed (number of people, pets, and natural predators seen per hour spent at the site) than all other sites (see CHAPTER 1). Thus, Northwestern Gartersnakes seem to be responding to these disturbances with an up-regulated HPA axis.

Information about the effects of long-term predator exposure on average stress responses of birds and mammals, let alone of reptiles, is in short supply (Clinchy et al. 2011). Mean CORT levels of Song Sparrows (*Melospiza melodia*) were significantly higher in a high predator pressure location compared to a low predator pressure location, independent of sex and habitat structure (Clinchy et al. 2011). An attenuated stress response has also been associated with adaptation to urbanization in a variety of bird species (Schoech et al. 2004; Partecke et al. 2006; Atwell et al. 2012). These results support my findings that higher potential predator exposure at LP, compared to the other sites, has a positive effect on baseline CORT levels in Northwestern Gartersnakes

Mass is a significantly positive (p<0.05) predictor of the variability in H:L for Northwestern Gartersnakes. Male tropical Stonechats (*Saxicola torquata axillaris*) with more body fat (and therefore heavier) had higher plasma CORT levels than males without visible body fat (Scheuerlein et al. 2001). Birds with less fat had a blunted stress response perhaps to avoid depleting energy stores below a threshold level (Scheuerlein et al. 2001). This may also apply to Northwestern Gartersnakes because increased CORT levels trigger gluconeogenesis and lipolysis in all vertebrates to mobilize energy (Landys et al. 2006; Breuner et al. 2013). Few studies report on mass alone (Feldman & Meiri 2013); mass is usually combined with SVL into a metric of body condition and then compared to baseline and stress-induced CORT levels. Snout-vent length was a non-significant predictor of the variability in H:L.

As with mass, shedding is significantly positively related to H:L values in Northwestern Gartersnakes. Corticosterone levels in circulation are heightened when snakes begin to shed their skin (Whittier et al. 1987). However, this relationship appears

to be indirect. First, the administration of adrenalcorticoids, the family into which CORT is classified, has no effect on skin shedding in squamate reptiles (Halberkann 1954; Chiu & Lynn 1972). Additionally, the process of skin shedding is controlled by the pituitary-thyroid axis, and therefore not by concentrations of CORT (Maderson et al. 1970; Chiu & Lynn 1972). The significant relationship between CORT and shedding in Northwestern Gartersnakes appears to be an indirect result of the actions of the adrenocorticotropic hormone (ACTH). This hormone is released from the adrenal pituitary gland and stimulates the adrenal gland to release CORT. Therefore, the actions of ACTH on CORT release must be greater than its actions on shedding directly in Northwestern Gartersnakes. This is very possible given that ACTH has no effect on shedding in a closely related species, the Common Gartersnake (Chiu & Lynn 1972). Additionally, the frequency of shedding relates to the metabolic status of the animal (Chiu et al. 1986); corticosterone is an important hormone for mobilizing energy stores (Moore et al. 2000), the same energy stores that are needed to facilitate the process of shedding (King & Turmo 1997). Again, this points to an indirect relationship between CORT levels and shedding in Northwestern Gartersnakes.

I differentiated between minimal marks, which I called 'nicks', from more severe 'injuries' (see CHAPTER 1), in an attempt to focus on injuries that were more likely to elicit a stress response, and result in increased levels of CORT in circulation. Although there is much debate about whether injuries are suggestive of the intensity of predation pressure, or are better indicative of an unsuccessful predator attack (Gregory & Isaac 2005), predators can cause non-lethal injuries to their prey. Furthermore, attack by a predator is stressful for prey (Scheuerlein et al. 2001). Theoretically, it is possible that Northwestern Gartersnakes with injuries have higher H:L values, but this area is generally unstudied.

Shedding remained a significantly positive predictor of H:L in multi-predictor models. Moreover, both shedding gravid females and shedding males had significantly lower H:L values compared to shedding non-gravid females. Mass, however, when included with shedding and shedding*sex/reproductive condition, had no influence on H:L. Thus, the significant effect of mass on H:L in single predictor factor models may be spurious, presumably due to correlation with other factors.

In addition to the above-described factors that are involved in adrenocortical modulation in snakes and other reptiles, baseline levels of plasma CORT vary with season. For example, both male Red-Sided and Red-Spotted Gartersnakes have higher levels of CORT in their blood during the fall than in the summer (Moore et al. 2000). More specifically, heterophils are present in circulation in higher abundance during the summer than during hibernation in reptiles (Thrall et al. 2004). Because abundance of heterophils is directly influenced by concentrations of CORT in the bloodstream, this finding may be interpreted as reptiles having higher levels of CORT in circulation during the summer than when hibernating. These seasonal differences in the responsiveness of the HPA axis are suggested to be adaptations to reproduction, such that the hormonal, as well as behavioural responses are attenuated during reproductively active periods of the year (Moore et al. 2001; Taylor & Denardo 2011).

Results from previous studies demonstrate temporal variation in the stress response. Therefore, to obtain a full picture of HPA axis activity in Northwestern Gartersnakes, information needs to be gathered for more than one season (Taylor & Denardo 2011). Re-sampling throughout one year and/or for multiple years would yield more information about how an individual animal's susceptibility to stress changes with time (Taylor & Denardo 2011). This is something to consider for future studies that aim for a more in-depth understanding of an animal's stress response.

CHAPTER 4 – ANTI-PREDATOR BEHAVIOUR OF URBAN GARTERSNAKES IN RESPONSE TO AN ENCOUNTER WITH A HUMAN

INTRODUCTION

Behaviours are immediate and plastic traits that animals implement to deal with current environmental challenges (e.g., foraging behaviours to gain access to resources or anti-predator behaviours to avoid potentially harmful situations; Sol et al. 2013). In the urban environment, wildlife are challenged by a suite of disturbances different from those encountered by non-urban wildlife, that interfere with how wildlife 'naturally' spend their time and energy (Miller et al. 2001; Frid & Dill 2002; Knight 2009; Sol et al. 2013). Some urban disturbances include foreign predators (e.g., Domestic Dogs and Cats), increased noise and light pollution, vehicle traffic, and recreationists (Sol et al. 2013). Wildlife detect and respond to these situation-specific threats (Whittaker & Knight 1998) using many different types of behaviours. The flexibility of these behaviours enables short-term adaptation in wildlife (Sol et al. 2013). Any change in behaviour that decreases the chances of capture by another animal by making an attempt at predation less worthwhile and/or successful is recognized as a defensive behaviour (Edmunds 1974; Greene 1988).

Learning capabilities of animals have been documented for a variety of taxa (Herzog et al. 1989; Glaudas 2004; Raderschall et al. 2011; Sol et al. 2013). Many urban wildlife species have become used to the presence of people and are 'habituated' (Knight 2009). This taming effect of urban wildlife takes time (Herzog et al. 1989). Humans can have more immediate control over the behaviour of wildlife by attracting them with incentives (Whittaker & Knight 1998). Whether through habituation or attraction, interactions with humans in urban centres alter the 'wildness' of wildlife.

Although some animals tolerate or may even be attracted to humans, many increase vigilance and default to avoidance and flight behaviours when around people (Carpenter 1952; Mollov 2005; Knight 2009; Sol et al. 2013). Humans are threatening for reptiles: lizards evade human and nonhuman predators using similar tactics (Schall &

Pianka 1980); Massasauga Rattlesnakes employ typical anti-predator behaviours toward humans (Prior & Weatherhead 1994). Humans have therefore been used as surrogate predators to study defensive behaviour in animals (Labra & Leonard 1999).

Studies about defensive behaviour have been limited by the rarity of observations of natural predator-prey interactions and our inability to experimentally control factors that affect the interactions (Frid & Dill 2002). Our understanding of anti-predator behaviours in animals has grown by observing and measuring animals' responses to humans. For animals to avoid injury or death, it is paramount that they effectively detect and efficiently respond to the potential threat (Greene 1988; Placyk & Burghardt 2005). Animals encountering a challenge assess the risks of the potential threat (e.g., injury or death) against the costs of lost fitness-enhancing activities (e.g., mating, or foraging; Frid & Dill 2002). Depending on that assessment, animals may ignore the predator and continue current behaviours, adjust behaviours, or flee (Zug 1993; Frid & Dill 2002; Cooper & Peréz-Mellado 2004; Cooper 2008; Sol et al. 2013).

Reptiles may employ various anti-predator tactics depending on the stage of the predator-prey encounter (Chiszar et al. 1976; Greene 1988; Herzog et al. 1989; Zug 1993). Many animals are cryptic and avoid detection. Others, whether seen or not, may flee to a refuge. The distance between observer and animal when the animal starts to flee is approach distance (AD) and is used to assess fear (i.e. wariness) and/or anti-predator tactics of animals in response to disturbance (Ydenberg & Dill 1986; Blumstein et al. 2003; Stankowich & Blumstein 2005). Defensive displays that range from 'passive' to 'aggressive' are also common anti-predator tactics in reptiles.

Encounters with predators are stressful (Scheuerlein et al. 2001). However, there is limited information about how activation of the Hypothalamo-Pituitary-Adrenal (HPA) axis (or 'stress axis') engages reptilian defence. The only study conducted thus far shows that elevated corticosterone (CORT) levels enhance anti-predator responses (e.g., flight) in male Tree Lizards following attack by an inanimate object (Thaker et al. 2009a). To date, no study has experimentally established a relationship between elevated stress caused by human exposure and anti-predator tactics in reptiles (Thaker et al. 2009a).

In this study I describe defensive behaviours of wild Northwestern Gartersnakes living in urban parks and nature sanctuaries in the Greater Victoria area, BC. I also

determine how these behaviours are affected by a variety of independent variables, including one indicator of stress, H:L (see CHAPTERS 2 & 3). I examined how these snakes responded to an encounter with a human observer (myself) during three phases of an interaction (approach, handling, and post-release). I addressed the following questions:

1) Are Northwestern Gartersnakes wary of humans?
2) Do these snakes respond more passively or aggressively during an encounter with a human?
3) Is there an effect of handling on post-release flight in these snakes?
4) Do other factors influence a snake's anti-predator behaviour during encounters? Such factors include physiological state (e.g., body temperature, sex/reproductive condition), body size, presence of an injury, and distance to paths or to cover.

Predictions

I assumed that with higher concentrations of CORT in circulation, snakes could more readily mobilize energy stores for defensive behaviours (Wingfield et al. 1995) and therefore I predicted that individuals with higher H:L values would flee later when approached than those with lower H:L values (Table 6).

The influence of body temperature on AD of ectotherms has not been widely studied and no consistent trends have been shown (e.g., Stankowich & Blumstein 2005; Gregory 2013; but see Zug 1993). I predicted that these snakes would respond similarly to Common Gartersnakes (Heckrotte 1967; Nelson & Gregory 2000) and flee sooner when warmer (Table 6).

In general, bigger snakes are older than smaller snakes of the same sex (Waye 1999; Roth & Johnson 2004), and they presumably have had more experience with predators on which to base their 'decision' about when to flee. Experience with predators should increase the perception of risk (Stankowich & Blumstein 2005), so larger animals

should flee sooner. Similarly, I predicted that injured snakes would flee sooner than uninjured snakes because of their previous experience with predators (Gregory 2013; Table 6). I also expected that pregnant females would flee sooner than non-pregnant females (Table 6) because the increased mass of the former hinders their mobility (Shine 1980).

Generally, when closer to a refuge, animals flee later when approached (Frid & Dill 2002; Stankowich & Blumstein 2005). I therefore predicted that Northwestern Gartersnakes would also flee later when closer to cover (Table 6). On the other hand, I could not make a clear prediction about how distance to path would affect AD. Depending on how snakes perceived people and other disturbances that were more common on than off trails and paths, the distance to path might have a positive or negative effect on AD of Northwestern Gartersnakes (Table 6).

Table 6. Predicted influence (positive: '+', negative: '-', or unknown: '?') of independent variables (snout-vent length: SVL, cloacal temperature, ratio of heterophils to lymphocytes: H:L, injury, distance to cover, and distance to path) on approach distance (AD).

Potentially influential variables	Predicted influence on AD
SVL	-
Cloacal temperature	+
Injury	-
H:L	+
Pregnancy	-
Distance to cover	+
Distance to path	?

I also tested the effect of the factors in Table 6 on defensive behaviours of captured and released animals, but, based on limited and conflicting literature, made no specific predictions about directional effects.

METHODS

Gartersnakes and the study sites they inhabit

I searched for Northwestern Gartersnakes using visual encounter surveys, along edge-focused transects in the Greater Victoria Area, BC. I visited five sites throughout May-August, 2012: Christmas Hill/Swan Lake Nature Sanctuary (CHNS and SLNS), Mount Douglas Park (MDP), Layritz Park (LP), and Mount Tolmie Park (MTP). I captured snakes by hand and measured various characteristics (see CHAPTER 1). I also recorded the number of each of people, pets, and natural predators that I saw throughout each sampling day to determine the disturbance regime at each site.

Anti-predator behaviour

When a snake was seen lying still, I (observer), with the help of a graduate student colleague (assistant), stopped to avoid further immediate disturbance of the snake. I then placed a marker on the ground to note my starting distance (SD, the distance between the observer and snake when the snake was first seen). The SD was measured to account for possible collinearity with AD (Møller 2012).

To control for the influence of predatory stimulus on AD, I consistently approached each snake slowly, directly (with eyes fixed on the snake; Labra & Leonard 1999), and at a constant speed (0.5-1 m/s; Cooper 1997; Blumstein et al. 2003), while the assistant watched the snake from a stationary position (Bulova 1994; Blumstein et al. 2003; Smith & Lemos-Espinal 2005). Then, once the snake began its escape I stopped and placed a marker at my feet and at the snake's initial position, to later measure AD, while the assistant captured the snake by hand.

I also recorded the following measures: whether the head was visible, if the snake was in the sun or not, and, distance (cm) to paths and cover. Cover included any vegetation that obstructed my view of the snake (Parent & Weatherhead 2000).

I then recorded behaviours displayed by snakes while they were being handled. Each behaviour was either displayed (scored a '1') or not displayed (scored a '0'). I here present and discuss only behaviours that were clearly defensive, based on the literature (Table 7).

Table 7. Behaviours displayed by Northwestern Gartersnakes during handling. Each snake was scored a '1' when the behaviour was displayed and a '0' when it was not. Behaviours were not mutually exclusive (i.e., snakes could display every behaviour during handling).

Behaviour	Definition
Bite	Teeth penetrate handler's skin
Hide head	Head is hidden by unevenly stacked body coils, which sometimes form a ball (Dirksen et al. 1998; Figure 23)
Musk	The animal discharges an odorous scent gland secretion from the vent (may include feces and/or urea)
Strike	Head extends towards the handler quickly and with force, but without making contact (mouth may or may not be gaped)
Tongue flick	Tongue extends out of the mouth, moves up and down, and is quickly pulled back into the mouth (repeated at varying intervals; (Gove 1979; Figure 24)
Twirl	Body spins lengthwise, with the axis of rotation running through the middle of the body from head to tail (snake may be held at any part of the body)

Figure 23. Northwestern Gartersnake displaying head hide defensive behaviour by forming an incomplete 'ball'

Figure 24. Northwestern Gartersnake displaying tongue flick defensive behaviour.

Once processed, each snake was released at its capture location. The time it took the snake to flee out of sight was recorded to the nearest millisecond. I observed individuals fleeing for a maximum of 5 minutes. Snakes that were still visible after 5 minutes were excluded from the analyses because the exact flight time was unknown.

Statistical analyses

I used R and a nominal rejection level of $\alpha = 0.05$ for all statistical analyses. I first tested the collinearity between AD and SD using the Spearman rank-sum correlation test. I also used this test to determine the influence of the following continuous independent variables on AD and on time to flee out of sight: cloacal temperature, ratio of heterophils to lymphocytes (H:L), snout-vent length (SVL), distances to cover and a path. For binomial independent variables (injury and pregnancy) I applied the Kruskal-Wallis test (e.g., kruskal.test() function in R). I ran these correlations for all sites and sexes/reproductive conditions combined because the sample sizes were too small (N<30)

when I separated data by these categories (except when I compared gravid females with non-pregnant snakes, with males and non-gravid females pooled in the latter category).

I determined the correlation between the antipredator behavioural displays (e.g., bite, strike, hide head, tongue flick, musk, and twirl) using a Fisher Test (e.g., fisher.test(bite,strike) in R). For highly positively correlated behaviours (e.g., rho>0.5), I considered only one of the behaviours for the remainder of the analysis, and assumed that the highly correlated behaviour would be similarly affected by the independent variables. Also, I calculated the probability of display for each of the defensive behaviours (except for bite) and plotted these values with 95% confidence limits. I tested whether the probabilities of display were significantly different between defensive tactics using a binomial proportions test.

For binomial dependent variables, I used logistic regression. I ran single predictor generalized linear models (e.g., glm(hide head ~ SVL, family=binomial)) to determine how the following independent variables influenced the display of each defensive behaviour during handling: cloacal temperature, H:L, injury, SVL, sex/reproductive condition, and pregnancy. I assessed the fit of each model using a Chi-squared (χ^2) Test. For this test I calculated a p-value using the residual deviation and its degrees of freedom. When p>0.05, the model fit the data. I again ran these models for all sites combined because the sample sizes were too small (N<30) when I separated data by site.

I also ran a glm with multiple independent variables (cloacal temperature and H:L and injury and SVL and sex/reproductive condition and pregnancy) and without interactions. The output of this full model was not any more informative than the results from the single variable models (e.g., did not provide any different significant results). Therefore, I do not present the results about the full model here.

In favour of using the maximum sample size for each model, I did not compare AIC values between models. To compare AIC values between models the sample sizes need to be the same. However, I had more information about some independent variables (e.g., SVL and distance to cover) than others (e.g., limited data on cloacal temperature and H:L for small snakes).

RESULTS

The correlation between approach distance (AD) and starting distance (SD) was positively significant (rho=0.323, p=0.0150, N=56; Figure 25). Approach distance and SD were related in three distinct ways (Figure 25): AD equalled SD (i.e., individuals that fled immediately); AD equalled zero, and did not equal SD (i.e., snakes that did not flee); and, AD equalled neither SD nor zero (i.e., intermediate group). I ran another correlation test only for the intermediate group of snakes. Approach distance was still significantly related to SD for these snakes (rho=0.821, p<0.0001, N=19; Figure 25).
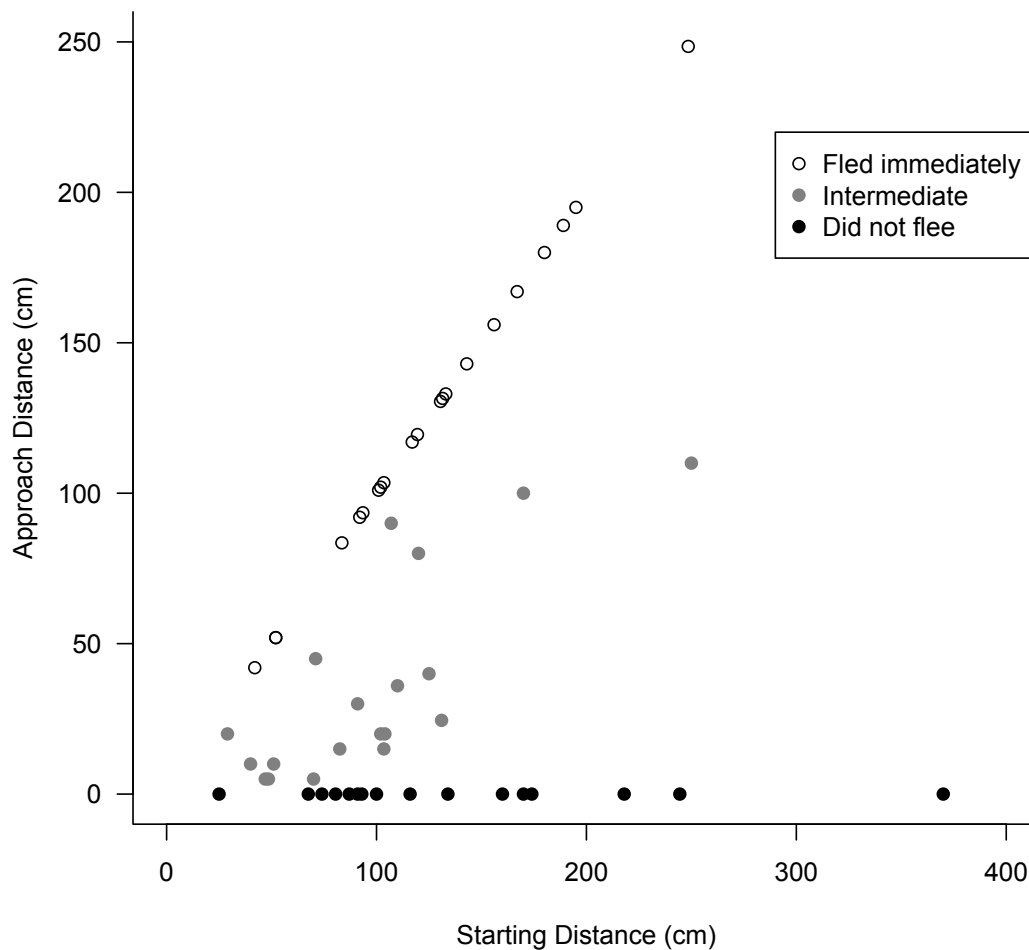
Figure 25. The relationship between approach distance (AD) and starting distance (SD) for Northwestern Gartersnakes of all sexes and at all sites combined.

Every other variable was non-significantly (p>0.05) related to AD (Table 8).

Table 8. Various independent variables were modelled to determine their influence on approach distance (AD) using the Spearman's rank correlation test (S; includes a correlation coefficient, rho) or the Kruskal-Wallis test (K-W). Relationships are ordered by p-value, from lowest to highest.

Independent variable	N	Type of test used	Rho	Direction of influence on AD ('+' OR '-')	P-value
Pregnancy	59	K-W	NA	-	0.178
SVL	60	S	0.164	-	0.212
Distance to path	51	S	0.126	+	0.379
Injury	53	K-W	NA	-	0.790
H:L	52	S	0.098	+	0.488
Distance to cover	60	S	0.029	+	0.824
Cloacal temperature	53	S	0.018	-	0.897

Of the various behaviours that the snakes displayed during handling, three pairs of behaviours were significantly positively correlated: bite and strike (Fisher test; p<0.0001, rho=0.720); twirl and hide head (p=0.0022, rho=0.260); and, twirl and musk (p=0.0304, rho=0.194). Of the individuals that struck at the handler (N=6), five bit. Given these highly positive correlations between strike and bite, I consider only strike hereafter.

The proportion of snakes that displayed a defensive behaviour during handling, for all sites combined, differed by the type of behaviour (N=147; Figure 26). Almost all snakes flicked their tongues. This behaviour was displayed by significantly more snakes compared to any other display (Binomial Proportions Test: Musk – χ^2=18.414, p<0.0001, DF=1; Hide head – χ^2=49.758, p<0.0001, DF=1; Twirl – χ^2=54.147, p<0.0001, DF=1; Strike – χ^2=237.105, p<0.001, DF=1; Figure 26). Musking was another common behaviour, and significantly more common than head hiding (χ^2=8.958, p=0.003, DF=1), twirling (χ^2=11.158, p=0.0008, DF=1), and striking (χ^2=156.074, p<0.0001, DF=1; Figure 26). Just over half of the snakes hid their head. A comparable proportion of snakes twirled (χ^2=0.0559, p=0.8131, DF=1; Figure 26). Striking was the least common behaviour displayed by the snakes during handling, and significantly so compared to all other behaviours (Musk – χ^2=156.074, p<0.0001, DF=1; Hide head – χ^2=100.658,

p<0.0001, DF=1; Twirl – χ^2=94.941, p<0.0001, DF=1; Figure 26). Additionally, of the snakes that struck, half also hid their head (Figure 26). Overall, aggressive behaviours (strike and bite) were far less common than more passive behaviours.
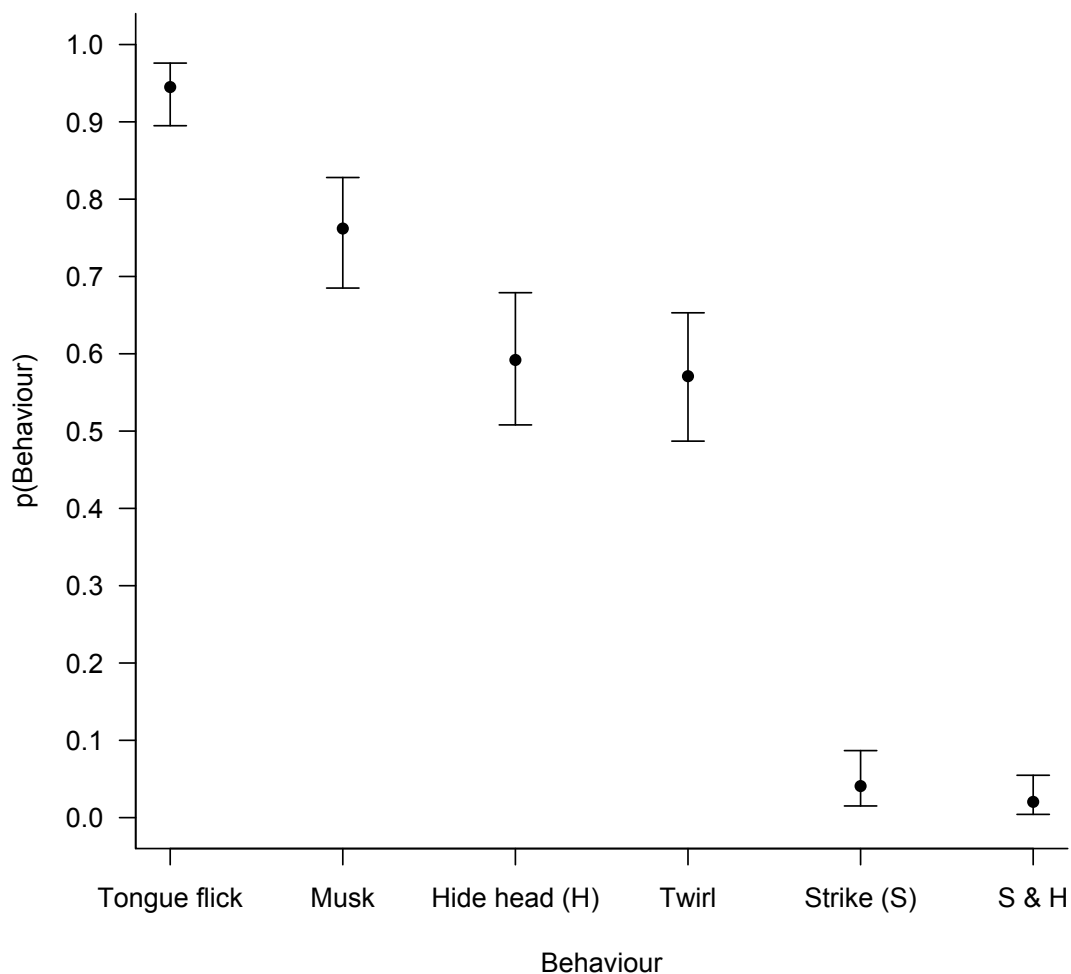
Figure 26. Probability of anti-predator behaviours displayed by snakes when handled. Values represent averages for all snakes combined (N=147) with 95% confidence intervals.

The glm models for tongue flick, musk, and strike fit the data (χ^2; p>0.05), whereas the models for head hide and twirl did not (χ^2; p<0.05; Table 9). Whether the independent variable had a positive or negative, or significant or non-significant influence on the display of a behaviour varied by behaviour type.

Three relationships were significant (p<0.05). Males hid their head significantly more than non-gravid females (p=0.035; Table 9). Longer snakes musked significantly more than shorter snakes (p<0.0001; Table 9). Cloacal temperature was a significantly negative predictor of tongue flicking (p=0.0209; Table 9).

Most relationships were non-significant (p>0.05; Table 9), but there were some generalities that I briefly summarize here. Snout-vent length was a positive predictor of every behavioural display (Table 9). Additionally, every behaviour was negatively influenced by cloacal temperature; individuals that were cooler were more likely to display than individuals that were warmer. Snakes with an injury were generally less likely to display a defensive behaviour, except that injured individuals twirled more than uninjured individuals (Table 9). Snakes that had larger H:L values were more likely to display a behaviour, except that snakes with lower H:L values hid their head more than snakes with higher H:L values (Table 9). Males were less likely to musk than non-gravid females and gravid females were less likely to tongue flick than non-gravid females (Table 9). Additionally, gravid females displayed all behaviours more than non-pregnant individuals, except for tongue flicking (Table 9): gravid females tongue flicked less than non-gravid individuals.

Table 9. Various independent variables were modelled to determine their influence on the display of the behaviours using a generalized linear model (family=binomial). Model outputs are ordered by p-value, from lowest to highest for each behavioural display. Relationships that are significant (p<0.05) are indicated by a *. Models that fit the data (χ^2; p>0.05) are in bold. Abbreviations for sex/reproductive condition are the same as Table 3.

Behaviour	Independent variable	N	Direction of influence on behaviour ('+' OR '-')	P-value	P-value (χ^2), degrees of freedom
Hide head	Sex/reproductive condition*	145	FG: + M*: +	FG: 0.148 M*: 0.035	0.004, 142
	SVL	147	+	0.257	0.002, 145
	Injury	126	-	0.522	0.004, 124
	Pregnancy	145	+	0.563	0.003, 143
	H:L	126	-	0.986	0.006, 124
	Cloacal temperature	125	-	0.988	0.006, 123
Musk	**SVL***	**147**	**+**	**<0.0001**	**0.799, 145**
	Cloacal temperature	**125**	**-**	**0.101**	**0.935, 123**
	H:L	**126**	**+**	**0.172**	**0.862, 124**
	Injury	**126**	**-**	**0.181**	**0.115, 124**
	Pregnancy	**145**	**+**	**0.325**	**0.241, 143**
	Sex/reproductive condition	**145**	**FG: + M: -**	**FG: 0.450 M: 0.736**	**0.225, 142**
Strike	**SVL**	**147**	**+**	**0.186**	**1.000, 145**
	Sex/reproductive condition	**145**	**FG: + M: +**	**FG: 0.313 M: 0.416**	**1.000, 142**
	Pregnancy	**145**	**+**	**0.472**	**1.000, 143**
	Cloacal temperature	**125**	**-**	**0.508**	**1.000, 123**
	H:L	**126**	**+**	**0.527**	**1.000, 124**
	Injury	**126**	**-**	**0.820**	**1.000, 124**
Tongue flick	**Cloacal temperature***	**125**	**-**	**0.0209**	**1.000, 123**
	Pregnancy	**145**	**-**	**0.173**	**1.000, 143**
	SVL	**147**	**+**	**0.203**	**1.000, 145**
	Sex/reproductive condition	**145**	**FG: - M: +**	**FG: 0.234 M: 0.878**	**1.000, 142**
	H:L	**126**	**+**	**0.292**	**1.000, 124**
	Injury	**126**	**-**	**0.862**	**1.000, 124**
Twirl	Sex/reproductive condition	145	FG: + M: +	FG: 0.319 M: 0.167	0.002, 142
	Injury	126	+	0.371	0.003, 124
	SVL	147	+	0.478	0.001, 145
	H:L	126	+	0.540	0.005, 124
	Pregnancy	145	+	0.669	0.002, 143
	Cloacal temperature	125	-	0.902	0.004, 123

One third of variables tested had a significant (p<0.05) effect on the time it took snakes to flee out of sight (Table 10). Of the independent variables with significant effects (SVL, distance to cover, and pregnancy), each positively affected time to flee out of sight; snakes that were longer, further from cover, pregnant, or male fled out of sight significantly more slowly than snakes that were shorter, closer to cover, or not pregnant females (Table 10).

Of the variables that had non-significant (p>0.05) effects on the time to flee, distance to path, H:L, and cloacal temperature negatively impacted the time to flee, whereas injury positively affected the average time to flee (Table 10).

Table 10. Various independent variables were modelled to determine their influence on the time it took snakes to flee out of sight when released using a Spearman's rank correlation test (S, includes a correlation coefficient, rho) or a Kruskal-Wallice test (K-W). Results are ordered by p-value, from lowest to highest. Significant relationships (p<0.05) are indicated by a *. 'Interesting' relationships (0.05<p<0.1) are indicated by parentheses.

Independent variable	N	Type of test used	Rho	Direction of influence on time to flee out of sight ('+' OR '-')	P-value
SVL*	129*	S	0.341	+*	<0.0001*
Distance to cover*	129*	S	-0.165	+*	0.00878*
Pregnancy*	127*	K-W	NA	+*	0.0124*
(Distance to path)	(118)	S	0.230	(-)	(0.0740)
Injury	109	K-W	NA	+	0.247
H:L	108	S	-0.101	-	0.297
Cloacal temperature	107	S	0.0538	-	0.582

DISCUSSION

In response to handling by a human, Northwestern Gartersnakes display sensory (e.g., tongue flick), passive (e.g., head hide), disruptive (e.g., musk and twirl), and/or aggressive (e.g., strike/bite) defensive behaviours. This is not the full suite of behaviours that these snakes display, but represents those that were most straightforward to classify as defensive.

Tongue flicking is a conspicuous behaviour performed by snakes (and lizards) to gain information about the environment (Meredith & Burghardt 1978; Scudder & Burghardt 1983) by collecting and delivering chemical odours to the vomeronasal organ (Chiszar et al. 1976; Gove 1979). Plains Gartersnakes (*T. radix*), Hognose Snakes (*Heterodon platyrhinos*) and Rattlesnakes (*Crotalus* spp.) flick their tongues in environments that do not contain food or sexual odours, which suggests that this behaviour is involved in investigation of surroundings (Chiszar et al. 1976). In the context of my study, therefore, tongue flicking is an indirect defensive behaviour associated with assessment of the situation. Not surprisingly, given its sensory role, tongue flicking is also set apart from other defensive behaviours by the much higher frequency at which it was displayed (Meredith & Burghardt 1978).

Behaviours that Northwestern Gartersnakes displayed that make them less likely to be further attacked by a potential predator include head hiding (Bustard 1969; Carpenter 1977; Arnold & Bennett 1984), musking (Greene 1988; Sullivan & Sullivan 2012) and twirling (Carpenter 1977). Predators tend to target the head of prey (Bustard 1969; Dirksen et al. 1998), so head-hiding snakes are protecting the most vulnerable part of the body (Carpenter 1977; Arnold & Bennett 1984; Dirksen et al. 1998). Head-hiding is a passive behavioural display that does not directly affect the predator, other than the lost feeding opportunity when prey successfully defend against the predator.

Musking behaviour contributes to decreased palatability of snakes to predators; some dogs (Sullivan & Sullivan 2012), as well as other snake predators (Greene 1988), reject snakes that have released a strong smelling fluid from their cloaca. Twirling, by contrast, indicates an active attempt to escape from the predator's grasp (Carpenter 1977). Both of these behaviours are disruptive; musking and twirling are displayed to release the snake from the predator.

Finally, striking is a display of aggression that, similar to other defensive displays, confuses and/or distracts the predator by startling or intimidating it (Carpenter 1977; Arnold & Bennett 1984; Dirksen et al. 1998). Very few of the Northwestern Gartersnakes that I sampled struck; striking occurred significantly less often than any other defensive behaviour. Thus, these snakes rely more on other defensive behaviours (e.g., flight, passive, and/or disruptive displays) than on aggressive displays to avoid or

deter a predator (Fitch 1940). Alternatively, my handling may not have been perceived as threatening enough for these snakes to elicit aggressive behaviours.

Sensory, passive, disruptive, and aggressive defensive behaviours are not mutually exclusive (e.g., half of the snakes that struck also hid their head). How physiological and other factors influenced a display differed by behaviour type. Notably, cloacal temperature, sex/reproductive condition, and body size had significant respective influences on tongue flicking, head hiding, and musking.

Body temperature plays an important role in anti-predator behaviour for ectotherms as it affects their ability to perceive, deter, or escape from a predator (Weldon & Burghardt 1979; Shine et al. 2000). Tongue flicking is a unique trait of snakes (and lizards) for sensing chemicals in the immediate environment (Gove 1979; Weldon & Burghardt 1979). These two important aspects of physiology were significantly negatively correlated in Northwestern Gartersnakes. Locomotory ability of snakes is compromised at low temperatures (Layne & Ford 1984; Hailey & Davies 1986), so increased vigilance via tongue flicking perhaps helps compensate for that shortcoming. More information about the potential threat may be useful in determining what further course of action to take; the more snakes tongue flick, the more information they collect about the stimulus (Gove 1979). There was much variation in the rate at which snakes flicked their tongues in my study, but I did not measure tongue flick rates, which, in retrospect, would have been more informative. Perhaps measurements of tongue flick rates would reconcile my results with those for Western Gartersnakes (Stevenson et al. 1985); in that species, warmer individuals tongue flicked more often than cooler snakes.

Head hiding also varied between individuals. Specifically, males hid their heads significantly more than both non-gravid and gravid females. Perhaps male Northwestern Gartersnakes were more passive than non-gravid females, as is the case with the Common Gartersnake (King 2002). However, what it is about being male that could account for the increased display of head hiding is unclear, especially given that male Watersnakes are more aggressive than female conspecifics (Scudder & Burghardt 1983).

Musking behaviour in relation to body size also varies interspecifically. I found that longer Northwestern Gartersnakes musked significantly more than shorter individuals, but larger Cottonmouths musk significantly less than smaller individuals

(Roth & Johnson 2004). Perhaps Cottonmouths rely more on aggressive behaviours than Gartersnakes because the former are dangerously venomous and the latter are not. In Gartersnakes, I expect that larger individuals would release larger volumes of musk than smaller snakes, due to the greater holding capacity of larger scent glands in larger individuals. I assume that a larger volume of musk would be more irritating and more effectively repel a predator, possibly accounting for the size-based variation I observed.

Once released, individuals that were longer, further from cover, or pregnant took significantly more time to flee out of sight than individuals that were smaller, closer to cover, or not pregnant. As for the display of defensive behaviours, relationships between various factors and flight time post handling vary among different snake species; smaller Viperine Snakes (*Natrix mauria*) are more static in the presence of people whereas larger individuals flee more readily, seemingly because smaller snakes are slower and weaker. Smaller snakes are less efficient at delivering oxygen to their tissues than larger snakes (Pough 1977), and therefore have a poorer chance of successful escape from a threat than larger snakes (Hailey & Davies 1986). However, unlike Hailey and Davies (1986), I measured flight post handling. The opposite effect of SVL on flight between Northwestern Gartersnakes and Viperine Snakes may reflect species-specific differences or exemplify handling effects on anti-predator behaviour in snakes.

The distance between a snake and cover had a significantly positive effect on the time it took the snake to flee out of sight. This result is contrary to findings about other animals. When farther from safety (e.g., vegetation in which to hide), animals generally have a greater perception of risk, as demonstrated by their higher tendency to flee earlier (Stankowich & Blumstein 2005). Perhaps this is because the 'safety zone' around an animal was compromised by its capture, thereby invalidating the applicability of this general trend. I suggest that perhaps snakes that were closer to cover when released had a greater chance of reaching safety than individuals that were farther from cover; snakes more removed from cover would need to exert more energy to flee for a larger than a shorter distance to successfully reach safety and thus may have been more cautious in their actions relative to snakes that were closer to a refuge.

Distance to the nearest path also affected the time to flee out of sight in these snakes; snakes that were closer to a path took more time to flee out of sight. Although

not significant, this result merits some discussion ($0.05<p<0.1$). People generally stick to trails when visiting urban parks (Muhly et al. 2011), so proximity to path can be viewed as an area that is concentrated in disturbance level. Northern Watersnakes (*Nerodia sipedon*) are more disturbed when closer to than farther from a path, whereas Common Gartersnakes are not significantly affected either way (Burger 2001). The results of my study suggest that Northwestern Gartersnakes closer to a path were less disturbed than those far away. Snakes that are associated with paths are more likely to encounter a recreationalist and/or pet and thus may have had more experience to learn about how to respond to these disturbances. If encounters with people and/or pets are not threatening, then I expect snakes closer to paths are less likely to flee as readily as snakes farther from paths.

Pregnancy had a significantly positive effect on time to flee out of sight. As discussed above, pregnancy, especially later in the gestation period, makes individuals less mobile, because they are weighed down by developing young, and thus up to 20-30% slower than non-gravid females and males (Shine 1980).

Unlike flight after release, flight upon approach (AD) was not significantly correlated with any independent variable. The inconsistency between results about AD and time to flee out of sight suggests that there may have been a handling effect in Northwestern Gartersnakes. Many field studies assume that interactions between observers and animals do not influence the results (Kerr et al. 2004). However, animals alter their behaviour depending on the type of human interaction (Rodda et al. 1988; Kerr et al. 2004). For instance, handling in Australian Sleepy Lizards (*Tiliqua rugosa*) leads to increased activity compared to pre-handling levels (Kerr et al. 2004).

In addition to the potential for handling effects in these snakes, perhaps AD responses were more variable between individuals than time to flee out of sight because I did not account for the possibility that some snakes detected me before I detected them (Ydenberg & Dill 1986). This was not a problem when measuring time to flee out of sight because every individual had been exposed to a comparable level of handling (i.e., same measurement routine), and was thus responding to a similar level of threat.

Anti-predator behaviours are tightly coupled to potential predation threats (Greene 1979; Gomes et al. 2002). Because I combined small samples from multiple

sites, I could not account for the differences in predator abundances between sites (see CHAPTER 1) and how this may have influenced behaviour. There might well be population differences in AD (Shine et al. 2003). Assuming that the Northwestern Gartersnakes did not move between sites, and thus comprised five distinct populations, perhaps the different disturbance regimes at each site led to differed responses. Geographic variation in defensive behaviours at small scales between snakes with restricted gene flow does exist (Herzog et al. 1989). Intra-population variability in behaviour may be why no significant trends emerged between either AD or some defensive displays and independent variables.

On the other hand, the lack of significant results may reflect habituation in these snakes. Habituation of anti-predator flight responses has been shown in Butler's (*T. butleri*) and Mexican (*T. melanogaster*) Gartersnakes (Herzog et al. 1989). Additionally, adjustments in behaviour when faced with anthropogenic disturbances are common in urban wildlife (Sol et al. 2013). More data for comparison between seasons and sites is required to test the hypothesis that there were no consistent trends in AD of Northwestern Gartersnakes because of habituation.

Northwestern Gartersnakes exhibit a variety of anti-predator behaviours in response to an encounter with a human, relying more on sensory, passive and disruptive than aggressive defensive behaviours. A snake's size, sex/reproductive condition, and cloacal temperature were the most important factors for defensive behaviours in Northwestern Gartersnakes. Stress physiology and defensive behaviours were not correlated in Northwestern Gartersnakes. Perhaps the snakes inhabiting these sites were not stressed enough to warrant behavioural adjustments. Or, perhaps the relationship between stress and behaviour in these snakes was weaker than the influences of other morphological, physiological, and habitat characteristics. This study contributes to growing information about the importance of biotic and abiotic factors to predator-prey interactions (Rapport & Turner 1975).

CHAPTER 5 – CONCLUSIONS AND APPLICATION

Overall it seems as though Northwestern Gartersnake are well adapted to living in urban parks and nature sanctuaries: no individual was chronically stressed (H:L < 2:1 for every individual); and, the lack of significant results for defensive displays during handling indicated possible habituation to humans. I speculate that two major threats to Northwestern Gartersnakes are roads and high abundance of American Crows, which were present at MTP where there were significantly fewer snakes encountered than at the other sites. However, differences in habitat characteristics (e.g., vegetation types and structure) between sites were not controlled for and may be an important factor that affected differences in abundance, physiology, and behaviour of the snakes between sites.

To be more certain that the results of this study reflect the true biology of wild Northwestern Gartersnakes, these populations need to be monitored for more than one field season (Gibbons et al. 2000). It took 10 years to observe a notable detrimental effect of the presence of hikers and fishers on abundance of Wood Turtles (Garber & Burger 1995b), and 20 years to recognize that the loss off bunch grass habitat from grazing decimated the local population of Bunch Grass Lizards (Ballinger & Congdon 1996). If populations are monitored over time, the wildlife habitat can be managed more effectively in an effort to maintain current biodiversity, as well as facilitate a positive and sustainable human-wildlife relationship that contributes to healthy ecosystem functioning (Burger 2001; French et al. 2008). For instance, the Swan Lake/Christmas Hill Nature Sanctuary prohibits dogs, limits recreational activities (e.g., no biking, designated pathways and restricted access to some areas), and does not have any roads that pass through the sites. Managing anthropogenic disturbances likely supports the presence of Gartersnakes at these sites. Generally, the coexistence of wildlife and recreationalists is not possible without some sort of management (Knight & Cole 1995).

Green spaces that support wildlife in urban centres can also be beneficial to recreationists. The presence of natural elements in urban centres (e.g., forests and parks) addresses important non-consumptive human needs (e.g., well-being of urban residents; Chiesura 2004). Also, urban green spaces can provide important environmental services

(e.g., trees purify the air and filter noise and wind) and other economic benefits (e.g., green spaces can increase the attractiveness as a tourist destination; Chiesura 2004). Furthermore, Gartersnakes are not harmful to people and can be beneficial to agriculture as they feed on worms and slugs, especially the invasive Black Slug (Adams et al. 2006).

This study highlights the variability in how reptiles respond to encounters with people, particularly following repeated exposure when in an urbanized landscape. The H:L values and defensive behaviours varied between individuals in terms of differences in their physiology (e.g., sex/reproductive condition), morphology (e.g., body size), and ecology (e.g., degree of exposure to predators and other disturbances; Kerr et al. 2004; Roth & Johnson 2004). These different sources of influence for intra- and inter-specific variability in stress physiology and anti-predator behaviours point to the importance of interdisciplinary studies for understanding how an animal faces urban challenges. Especially in light of the anticipated human population growth that will be concentrated in urban centres (United Nations 2011), accumulating information from many different disciplines is of paramount importance (Magle et al. 2012) to ensure that management decisions are biologically relevant.

LITERATURE CITED

Adams, J.L., Camelio, K.W., Orique, M.J. & Blumstein, D. T. 2006. Does information of predators influence general wariness? *Behavioral Ecology and Sociobiology*, 60: 742–747.

Adams, L., 2005. Urban wildlife ecology and conservation: A brief history of the discipline. *Urban Ecosystems*, 8(2): 139–156.

Alleman, A.R., Jacobson, E.R. & Raskin, R.E., 1999. Morphologic, cytochemical staining, and ultrastructural characteristics of blood cells from Eastern Diamondback Rattlesnakes (*Crotalus adamanteus*). *American Journal of Veterinary Research*, 60(4): 507–514.

Andrén, H., Angelstam, P., Lindström, E. & Widén, P. 1985. Differences in predation pressure in relation to habitat fragmentation: an experiment. *Oikos*, 45(2): 273–277.

Andrews, K.M. & Gibbons, J.W., 2005. How do highways influence snake movement? Behavioral responses to roads and vehicles. *Copeia*, 2005(4): 772–782.

Arnold, S.J. & Bennett, A.F., 1984. Behavioural variation in natural populations. III: Antipredator displays in the garter snake *Thamnophis radix*. *Animal Behaviour*, 32(4): 1108–1118.

Atkins, N., Jones, S.M. & Edwards, A., 2002. Fecal testosterone concentrations may not be useful for monitoring reproductive status in male Blue-Tongued Lizards (*Tiliqua nigrolutea*: Scincidae). *Journal of Herpetology*, 36: 106–109.

Atwell, J.W., Cardoso, G.C., Whittaker, D. J., Campbell-Nelson, S., Robertson, K.W. & Ketterson, E.D. 2012. Boldness behavior and stress physiology in a novel urban environment suggest rapid correlated evolutionary adaptation. *Behavioral Ecology*, 23(5): 960–969.

Bailey, F.C., Cobb, V.A., Rainwater, T.R., Worrall, T. & Klukowski, m. 2009. Adrenocortical effects of human encounters on free-ranging Cottonmouths (*Agkistrodon piscivorus*). *Journal of Herpetology*, 43: 260–266.

Ballinger, R.E. & Congdon, J.D., 1996. Status of the Bunch Grass Lizard, *Sceloporus scalaris*, in the Chiricahua Mountains of southeastern Arizona. *Bulletin of the Maryland Herpetological Society*, 32: 67–69.

Bekoff, M. & Meaney, C.A., 1997. Interactions among dogs, people, and the environment in Boulder, Colorado: A case study. *Anthrozoos: A Multidisciplinary Journal of The Interactions of People & Animals*, 10(1): 23–31.

Blouin-Demers, G. & Weatherhead, P.J., 2001. Thermal ecology of Black Rat Snakes (*Elaphe obsoleta*) in a thermally challenging environment. *Ecology*, 82(11): 3025–3043.

Blumstein, D.T., Anthony, L.L., Harcourt, R. & Ross, G. 2003. Testing a key assumption of wildlife buffer zones: is flight initiation distance a species-specific trait? *Biological Conservation*, 110: 97–100.

Bogert, C.M., 1949. Thermoregulation in reptiles, a factor in evolution. *Evolution*, 3(3): 195–211.

Bounous, D.I., Dotson, T.K., Brooks, R.L. & Ramsay, E.C. 1996. Cytochemical staining and ultrastructural characteristics of peripheral blood leucocytes from the Yellow Rat Snake (*Elaphe obsoleta quadrivitatta*). *Comparative Haematology International*, 6(2): 86–91.

Boyle, S.A. & Samson, F.B., 1985. Effects of nonconsumptive recreation on wildlife: A review. *Wildlife Society Bulletin*, 13(2): 110–116.

Brattstrom, B.H., 1965. Body temperatures of reptiles. *American Midland Naturalist*, 73(2): 376–422.

Breuner, C.W., Delehanty, B. & Boonstra, R., 2013. Evaluating stress in natural populations of vertebrates: total CORT is not good enough. *Functional Ecology*, 27(1): 24–36.

Buehler, D.A., Mersmann, T., Fraser, J.D. & Seegar, J.K.D. 1991. Effects of human activity on Bald Eagle distribution on the northern Chesapeake Bay. *The Journal of Wildlife Management*, 55(2): 282–290.

Bulova, S.J., 1994. Ecological correlates of population and individual variation in antipredator behavior of two species of Desert Lizards. *Copeia*, 1994(4): 980–992.

Burger, J., 2001. The behavioral response of basking Northern Water (*Nerodia sipedon*) and Eastern Garter (*Thamnophis sirtalis*) Snakes to pedestrians in a New Jersey park. *Urban Ecosystems*, 5(2): 119–129.

Burnham, K.P. & Anderson, D.R., 2004. Multimodel inference understanding AIC and BIC in model selection. *Sociological Methods & Research*, 33(2): 261–304.

Bustard, H.R., 1969. Defensive behavior and locomotion of the Pacific Boa, *Candoia aspera*, with a brief review of head concealment in snakes. *Herpetologica*, 25(3): 164–170.

Campbell, T.W. & Ellis, C.K., 2007. *Avian and exotic animal hematology and cytology* 3 edition., Wiley-Blackwell.

Carpenter, C.C., 1952. Comparative ecology of the Common Garter Snake (*Thamnophis s. sirtalis*), the Ribbon Snake (*Thamnophis s. sauritus*), and Butler's Garter Snake (*Thamnophis butleri*) in mixed populations. *Ecological Monographs*, 22(4): 236–258.

Carpenter, C.C., 1977. Variation and evolution of stereotyped behaviour in reptiles. In *Biology of the Reptilia: Ecology and Behaviour A*. Academic Press Inc: 335–404.

Chace, J.F. & Walsh, J.J., 2006. Urban effects on native avifauna: a review. *Landscape and urban planning*, 74(1): 46–69.

Chambers, J.M., Cleveland, W.C., Kleiner, B. & Tukey, P.A. 1983. *Graphical methods for data analysis*, USA: Wadsworth International Group.

Chiesura, A., 2004. The role of urban parks for the sustainable city. *Landscape and Urban Planning*, 68(1): 129–138.

Chiszar, D., Carter, T., Knight, L., Simonsen, L. & Taylor, S. 1976. Investigatory behavior in the plains garter snake (*Thamnophis radix*) and several additional species. *Animal Learning & Behavior*, 4(3): 273–278.

Chiu, K.W., Sham, J.S.K., Maderson, P.F.A. & Zucker, A.H. 1986. Interaction between thermal environments and hormones affecting skin-shedding frequency in the Tokay (*Gekko gecko*) (Gekkonidae, lacertilia). *Comparative Biochemistry and Physiology Part A: Physiology*, 84(2): 345–351.

Chiu, K.W. & Lynn, W.G., 1972. Observations on thyroidal control of sloughing in the Garter Snake, *Thamnophis sirtalis*. *Copeia*, 1972(1): 158–163.

Claver, J.A. & Quaglia, A.I.E., 2009. Comparative morphology, development, and function of blood cells in nonmammalian vertebrates. *Journal of Exotic Pet Medicine*, 18(2): 87–97.

Clinchy, M. et al., 2011. Multiple measures elucidate glucocorticoid responses to environmental variation in predation threat. *Oecologia*, 166(3): 607–614.

Cooke, A.S., 1980. Observations on how close certain passerine species will tolerate an approaching human in rural and suburban areas. *Biological Conservation*, 18(2): 85–88.

Cooper Jr, W.E., 2008. Effect of risk on aspects of escape behavior by a lizard, *Holbrookia propinqua*, in relation to optimal escape theory. *Ethology*, 109(8): 617–626.

Cooper, W.E., 1997. Threat factors affecting antipredatory behavior in the Broad-Headed Skink (*Eumeces laticeps*): repeated approach, change in predator path, and predator's field of view. *Copeia*, 1997(3): 613–619.

Cooper, W.E. & Peréz-Mellado, V., 2004. Tradeoffs between escape behavior and foraging opportunity by the Balearic Lizard (*Podarcis lilfordi*). *Herpetologica*, 60(3): 321–324.

Davis, A.K., 2009. The wildlife leukocytes webpage: the ecologists' source for information about leukocytes of wildlife species.

Davis, A.K. & Maerz, J.C., 2011. Assessing stress levels of captive-reared amphibians with hematological data: implications for conservation initiatives. *Journal of Herpetology*, 45(1): 40–44.

Davis, A.K. & Maerz, J.C., 2008. Comparison of hematological stress indicators in recently captured and captive paedomorphic mole salamanders, *Ambystoma talpoideum*. *Copeia*, 2008(3): 613–617.

Davis, A.K., Maney, D.L. & Maerz, J.C., 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. *Functional Ecology*, 22: 760–772.

Davis, A.K., Ruyle, L.E. & Maerz, J.C., 2011. Effect of trapping method on leukocyte profiles of Black-Chested Spiny-Tailed Iguanas (*Ctenosaura melanosterna*): implications for zoologists in the field. *ISRN Zoology*, 2011: 1–8.

Dearborn, D.C. & Kark, S., 2010. Motivations for conserving urban biodiversity. *Conservation biology: the journal of the Society for Conservation Biology*, 24(2): 432–440.

Dedovic, K., 2009. The brain and the stress axis: the neural correlates of cortisol regulation in response to stress. *NeuroImage*, 47(3): 864–871.

Dirksen, L., Buongermini, E., Strüssmann, C. & Waller, T. 1998. Protective balling-posture behavior of the genus *Eunectes* Wagler, 1830 (Serpentes, Boidae). *Herpetological Natural Hist*ory 6(2): 151–155.

District of Saanich, 2012. District of Saanich, Victoria BC - Online Services - Search Saanich Parks. Available at: http://www.saanich.ca/webapp/parks/parklist.jsp [Accessed July 18, 2013].

Ditchkoff, S.S., Saalfeld, S.T. & Gibson, C.J., 2006. Animal behavior in urban ecosystems: modifications due to human-induced stress. *Urban Ecosystems*, 9: 5–12.

Ditchkoff, S.S. & Wakeling, B.F., 2001. Lizard species distributions and habitat occupation along an urban gradient in Tucson, Arizona, USA. *Biological Conservation*, 97(2): 229–237.

Durner, G.M. & Gates, J.E., 1993. Spatial ecology of Black Rat Snakes on Remington Farms, Maryland. *The Journal of Wildlife Management*, 57(4): 812–826.

Edmunds, M., 1974. *Defence in animals: a survey of anti-predator defences*, Longman.

Endriss, D.A., Hellgren, L.C., Fox, S.F. & Moody, R.W. 2007. Demography of an urban population of the Texas Horned Lizard (*Phrynosoma cornutum*) in central Oklahoma. *Herpetologica*, 63(3): 320–331.

Feldman, A. & Meiri, S., 2013. Length–mass allometry in snakes. *Biological Journal of the Linnean Society*, 108(1): 161–172.

Fernández-Juricic, E., Jimenez, M.D. & Lucas, E., 2001. Alert distance as an alternative measure of bird tolerance to human disturbance: implications for park design. *Environmental Conservation*, 28(3): 263–269.

Fitch, H.S., 1940. A biogeographical study of the *ordinoides artenkreis* of Garter Snakes (genus *Thamnophis*), Berkley and Los Angeles: University of California Press.

Ford, N.B., 1995. Experimental design in studies of snake behavior. *Herpetological Monographs*, 9: 130–139.

French, S.S., Fokidis, H.B. & Moore, M.C., 2008. Variation in stress and innate immunity in the Tree Lizard (*Urosaurus ornatus*) across an urban–rural gradient. *Journal of Comparative Physiology B*, 178: 997–1005.

Frid, A. & Dill, L.M., 2002. Human-caused disturbance stimuli as a form of predation risk. *Conservation Ecology*, 6(1): 11.

Garber, S. D. & Burger, J., 1995. A 20-yr study documenting the relationship between turtle decline and human recreation. *Ecological Applications*, 5(4): 1151–1162.

Garner, T.W.J., Nishimura, D., Antwi, J. & Oliver, N. 2008. Human disturbance influences behaviour and local density of juvenile frogs. *Ethology*, 114(10): 1006–1013.

Gesquiere, L.R., Khan, M., Shek, L., Wango, T.L., Alberts, S.C. & Altmann, J. 2008. Coping with a challenging environment: effects of seasonal variability and reproductive status on glucocorticoid concentrations of female Baboons (*Papio cynocephalus*). *Hormones and Behavior*, 54(3): 410–416.

Gibbons, J.W., Scott, D.E., Ryan, T.J., Buhlmann, A.K., Tuberville, T.D., Metts, B.S., Greene, J.L., Mills, T., Leiden, Y., Poppy, S. & Winnie, C.T. 2000. The global decline of reptiles, déjà vu amphibians. *BioScience*, 50(8): 653–666.

Gill, J.A., Norris, K. & Sutherland, W.J., 2001. Why behavioural responses may not reflect the population consequences of human disturbance. *Biological Conservation*, 97(2): 265–268.

Glaudas, X., 2004. Do Cottonmouths (*Agkistrodon piscivorus*) habituate to human confrontations? *Southeastern Naturalist*, 3(1): 129–138.

Gomes, F.R., Bevier, C.R. & Navas, C.A., 2002. Environmental and physiological factors influence antipredator behavior in *Scinax hiemalis* (Anura: Hylidae). *Copeia*, 2002(4): 994–1005.

Gove, D., 1979. A comparative study of snake and lizard tongue-flicking, with an evolutionary hypothesis. *Zeitschrift für Tierpsychologie*, 51(1): 58–76.

Greenberg, N., 2002. Ethological aspects of stress in a model lizard, *Anolis carolinensis*. *Integrative and Comparative Biology*, 42(3): 526 –540.

Greenberg, N. & Wingfield, J.C., 1987. Stress and reproduction: reciprocal relationships. In D. O. Norris & R. E. Jones, eds. *Hormones and Reproduction in Fishes, Amphibians, and Reptiles*. Springer US: 461–503.

Greene, H.W., 1988. Antipredator mechanisms in reptiles. In *Biology of Reptilia: Ecology B: Defense and Life History*. New York, USA: Alan R. Liss, Inc.: 1–152.

Greene, H.W., 1979. Behavioral convergence in the defensive displays of snakes. *Experientia*, 35(6): 747–748.

Gregory, P.T., 1984a. Correlations between body temperature and environmental factors and their variations with activity in Garter Snakes (*Thamnophis*). *Canadian Journal of Zoology*, 62(11): 2244–2249.

Gregory, P.T., 1978. Feeding habits and diet overlap of three species of Garter Snakes (*Thamnophis*) on Vancouver Island. *Canadian Journal of Zoology*, 56(9): 1967–1974.

Gregory, P.T., 1984b. Habitat, diet, and composition of assemblages of Garter Snakes (*Thamnophis*) at eight sites on Vancouver Island. *Canadian Journal of Zoology*, 62(10): 2013–2022.

Gregory, P.T., 2013. Once bitten, twice shy: does previous experience influence behavioural decisions of snakes in encounters with predators? *Ethology*: 919–925.

Gregory, P.T., Crampton, L.H. & Skebo, K.M., 1999. Conflicts and interactions among reproduction, thermoregulation and feeding in viviparous reptiles: are gravid snakes anorexic? *Journal of Zoology*, 248(2): 231–241.

Gregory, P.T. & Isaac, L.A., 2005. Close encounters of the worst kind: patterns of injury in a population of Grass Snakes (*Natrix natrix*). *The Herpetological Journal*, 15(4): 213–219.

Guyer, C. & Donnelly, M. A., 1990. Length-mass relationships among an assemblage of tropical snakes in Costa Rica. *Journal of Tropical Ecology*, 6(1): 65–76.

Hager, S.B., 2009. Human-related threats to urban raptors. *Journal of Raptor Research*, 43(3): 210–226.

Hailey, A. & Davies, P.M.C., 1986. Effects of size, sex, temperature and condition on activity metabolism and defence behaviour of the Viperine Snake, *Natrix maura*. *Journal of Zoology*, 208(4): 541–558.

Halberkann, J., 1954. Zur hormonalen beeinflussung des hautungszyklus der ringelnatter. *Z. Naturforsch*, 96: 77–80.

Handa, R.J., Burgess, L.H., Kerr, J.E. & O'Keefe, J.A. 1994. Gonadal steroid hormone receptors and sex differences in the hypothalamo-pituitary-adrenal axis. *Hormones and Behavior*, 28(4): 464–476.

Hebard, W.B., 1950. Relationships and variation in the Garter Snakes, Genus, *Thamnophis* of the Puget Sound Region of Washington State. *Herpetologica*, 6(4): 97–101.

Heckrotte, C., 1967. Relations of body temperature, size, and crawling speed of the Common Garter Snake, *Thamnophis s. sirtalis*. *Copeia*, 1967(4): 759–763.

Herzog, H.A., Bowers, B.B. & Burghardt, G.M., 1989. Development of antipredator responses in snakes: IV. Interspecific and intraspecific differences in habituation of defensive behavior. *Developmental Psychobiology*, 22(5): 489–508.

Hoffman, L.H., 1970. Placentation in the Garter Snake, *Thamnophis sirtalis*. *Journal of Morphology*, 131(1): 57–87.

Jemison, S.C., Bishop, L.A., May, P.G. & Farrell, T.M. 1995. The impact of PIT-tags on growth and movement of the Rattlesnake, *Sistrurus miliarius*. *Journal of Herpetology*, 29(1): 129–132.

Kaufman, G.A. & Gibbons, J.W., 1975. Weight-length relationships in thirteen species of snakes in the southeastern United States. *Herpetologica*, 31(1): 31–37.

Keck, M.B., 1994. Test for detrimental effects of PIT tags in neonatal snakes. *Copeia*, 1994(1): 226–228.

Kerr, G.D., Bull, C.M. & Mackay, D., 2004. Human disturbance and stride frequency in the Sleepy Lizard (*Tiliqua rugosa*): Implications for behavioral studies. *Journal of Herpetology*, 38(4): 519–526.

King, R.B., 2002. Family, sex and testosterone effects on Garter Snake behaviour. *Animal Behaviour*, 64(3): 345–359.

King, R.B. & Turmo, J.R., 1997. The effects of ecdysis on feeding frequency and behavior of the Common Garter Snake (*Thamnophis sirtalis*). *Journal of Herpetology*, 31(2): 310–312.

Knight, J., 2009. Making wildlife viewable: habituation and attraction. *Society and Animals*, 17(2): 167–184.

Knight, R.L. & Cole, D.N., 1995. Wildlife responses to recreationists. In *Wildlife and Recreationists: Coexistence Through Management And Research*. Washington, DC, USA: Island Press.

Knight, R.L., Grout, D.J. & Temple, S.A., 1987. Nest-defense behavior of the American Crow in urban and rural areas. *The Condor*, 89(1): 175–177.

Koenig, J., Shine, R. & Shea, G., 2002. The dangers of life in the city: patterns of activity, injury and mortality in suburban lizards (*Tiliqua scincoides*). *Journal of Herpetology*, 36: 62–68.

Kristan, W.B. & Boarman, W.I., 2003. Spatial pattern of risk of Common Raven predation on Desert Tortoises. *Ecology*, 84(9): 2432–2443.

Labra, A. & Leonard, R., 1999. Intraspecific variation in antipredator responses of three species of lizards (*Liolaemus*): possible effects of human presence. *Journal of Herpetology*, 33(3): 441–448.

Landys, M.M., Ramenofsky, M. & Wingfield, J.C., 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *General and Comparative Endocrinology*, 148(2): 132–149.

Layne, J.R. & Ford, N.B., 1984. Flight distance of the Queen Snake, *Regina septemvittata*. *Journal of Herpetology*, 18(4): 496–498.

Lepczyk, C.A., Mertig, A.G. & Liu, J., 2004. Landowners and cat predation across rural-to-urban landscapes. *Biological Conservation*, 115(2): 191–201.

Lima, S.L. & Dill, L.M., 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, 68(4): 619–640.

Lind, A.J., Welsh, H.H. & Tallmon, D.A., 2005. Garter Snake population dynamics from a 16-year study: considerations for ecological monitoring. *Ecological Applications*, 15(1): 294–303.

Linklater, W.L., 2010. Distress-An underutilised concept in conservation and missing from Busch and Hayward (2009). *Biological conservation*, 143(5): 1037–1038.

Love, O.P. & Bird, 2000. Raptors in urban landscapes: a review and future concerns. In *Raptors at risk: proceedings of the Fifth World Conference on Birds of Prey and Owls*. Berlin, Germany: Hancock House and World Working Group on Birds of Prey: 425–434.

Love, O.P., McGowan, P.O. & Sheriff, M.J., 2013. Maternal adversity and ecological stressors in natural populations: the role of stress axis programming in individuals, with implications for populations and communities. *Functional Ecology*, 27(1): 81–92.

Lupien, S.J., McEwen, B.S., Gunnar, M.R. & Heim, C. 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat Rev Neurosci*, 10(6): 434–445.

Maderson, P.F.A., Chiu, K.W. & Phillips, J.G., 1970. Endocrine-epidermal relationships in squamate reptiles. *Mem. Soc. Endocrinol*, 18: 259–284.

Magiakou, M.A., Mastorakos, G., Webster, E. & Chrousos, G.P. 1997. The hypothalamic-pituitary-adrenal axis and the female reproductive system. *Annals of the New York Academy of Sciences*, 816(1): 42–56.

Magle, S.B., Hunt, V.M., Vernon, M. & Crooks, K.R. 2012. Urban wildlife research: past, present, and future. *Biological Conservation*, 155: 23–32.

Maritz, B. & Scott, S.L., 2010. Assessing the threat of avian predation on a small viperid snake. *African Zoology*, 45(2): 309–314.

Marzluff, J.M. & Neatherlin, E., 2006. Corvid response to human settlements and campgrounds: causes, consequences, and challenges for conservation. *Biological Conservation*, 130(2): 301–314.

Matsuda, B., Green, D. & Gregory, P., 2006. *Amphibians & Reptiles of British Columbia*, Victoria, BC, Canada: Royal British Columbia Museum.

Meredith, M. & Burghardt, G.M., 1978. Electrophysiological studies of the tongue and accessory olfactory bulb in Garter Snakes. *Physiology & Behavior*, 21(6): 1001–1008.

Miller, S.G., Knight, R.L. & Miller, C.K., 2001. Wildlife responses to pedestrians and dogs. *Wildlife Society Bulletin*, 29(1): 124–132.

Minton, S.A., 1968. The fate of amphibians and reptiles in a suburban area. *Journal of Herpetology*, 2(3/4): 113–116.

Møller, A.P., 2012. Urban areas as refuges from predators and flight distance of prey. *Behavioral Ecology*, 23(5): 1030–1035.

Mollov, I.A., 2005. A study on the amphibians (Amphibia) and reptiles (Reptilia) from three urban protected areas in the town of Plovdiv (South Bulgaria). *Animalia*, 41: 79–94.

Moore, I.T., Greene, M.J. & Mason, R.T. 2001. Environmental and seasonal adaptations of the adrenocortical and gonadal responses to capture stress in two populations of the male Garter Snake, *Thamnophis sirtalis*. *Journal of Experimental Zoology*, 289(2): 99–108.

Moore, I.T. & Jessop, T.S., 2003. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. *Hormones and Behavior*, 43(1): 39–47.

Moore, I.T., Lemaster, M.P. & Mason, R.T., 2000. Behavioural and hormonal responses to capture stress in the male Red-Sided Garter Snake, *Thamnophis sirtalis parietalis*. *Animal Behaviour*, 59: 529–534.

Moore, I.T. & Mason, R.T., 2001. Behavioral and hormonal responses to corticosterone in the male Red-Sided Garter Snake, *Thamnophis sirtalis parietalis*. *Physiology & Behavior*, 72(5): 669–674.

Muhly, R.B., Semeniuk, C., Massolo, A., Hickman, L. & Musiani, M. 2011. Human activity helps prey in the predator-prey space race. *PLOS One*, 6(3): e17050.

Neill, W.T., 1950. Reptiles and amphibians in urban areas of Georgia. *Herpetologica*, 6(5): 113–116.

Nelson, K.J. & Gregory, P.T., 2000. Activity patterns of Garter Snakes, *Thamnophis sirtalis*, in relation to weather conditions at a fish hatchery on Vancouver Island, British Columbia. *Journal of Herpetology*, 34(1): 32–40.

Parent, C. & Weatherhead, P.J., 2000. Behavioral and life history responses of Eastern Massasauga Rattlesnakes (*Sistrurus catenatus catenatus*) to human disturbance. *Oecologia*, 125(2): 170–178.

Partecke, J., Schwabl, I. & Gwinner, E., 2006. Stress and the city: urbanization and its effects on the stress physiology in European Blackbirds. *Ecology*, 87(8): 1945–1952.

Perpinan, D., Hernandez-Divers, S., McBride, M. & Hernandez-Divers, S. 2006. Comparison of three different techniques to produce blood smears from Green Iguanas, *Iguana iguana*. *Journal of Herpetological Medicine and Surgery*, 16(3): 99–101.

Placyk, J.S. & Burghardt, G.M., 2005. Geographic variation in the frequency of scarring and tail stubs in Eastern Gartersnakes (*Thamnophis s. sirtalis*) from Michigan, USA. *Amphibia Reptilia*, 26(3): 353–358.

Pough, F.H., 1977. Ontogenetic change in blood oxygen capacity and maximum activity in Garter Snakes (*Thamnophis sirtalis*). *Journal of comparative physiology*, 116(3): 337–345.

Preest, M.R., Cree, A. & Tyrrell, C.L., 2005. ACTH-induced stress response during pregnancy in a viviparous gecko: no observed effect on offspring quality. *Journal of Experimental Zoology Part A: Comparative Experimental Biology*, 303A(9): 823–835.

Prior, K.A. & Weatherhead, P.J., 1994. Response of free-ranging Eastern Massasauga Rattlesnakes to human disturbance. *Journal of Herpetology*, 28(2): 255–257.

Raderschall, C.A., Magrath, R.D. & Hemmi, J.M., 2011. Habituation under natural conditions: model predators are distinguished by approach direction. *The Journal of Experimental Biology*, 214(24): 4209–4216.

Rapport, D.J. & Turner, J.E., 1975. Predator-prey interactions in natural communities. *Journal of Theoretical Biology*, 51(1): 169–180.

Reed, S. E. & Merenlender, A. M., 2008. Quiet, nonconsumptive recreation reduces protected area effectiveness. *Conservation Letters*, 1(3): 146–154.

Reed, Sarah E & Merenlender, Adina M, 2011. Effects of management of Domestic Dogs and recreation on carnivores in protected areas in Northern California. *Conservation Biology*, 25(3): 504–513.

Rittenhouse, C.D., Millspaugh, J.J., Washburn, B.E. & Hubbard, M.W. 2005. Effects of radiotransmitters on fecal glucocorticoid metabolite levels of Three-Toed Box Turtles in captivity. *Wildlife Society Bulletin*, 33: 706–713.

Robert, K.A., Vleck, C. & Bronikowski, A.M., 2009. The effects of maternal corticosterone levels on offspring behavior in fast- and slow-growth Garter Snakes (*Thamnophis elegans*). *Hormones and Behavior*, 55(1): 24–32.

Rodda, G.H., Bock, B.C., Burghardt, G.M. & Rand, A.S. 1988. Techniques for identifying individual lizards at a distance reveal influences of handling. *Copeia*, 1988(4): 905–913.

Romero, L.M. & Reed, J.M., 2005. Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology*, 140(1): 73–79.

Romero, L.M. & Wikelski, M., 2002. Exposure to tourism reduces stress-induced corticosterone levels in Galápagos Marine Iguanas. *Biological Conservation*, 108: 371–374.

Romero, M.L. & Butler, L.K., 2007. Endocrinology of stress. *International Journal of Comparative Psychology*, 20(2).

Roth, E.D. & Johnson, J.A., 2004. Size-based variation in antipredator behavior within a snake (*Agkistrodon piscivorus*) population. *Behavioral Ecology*, 15(2): 365–370.

Salakij, C., Salakij, J., Apibal, S., Narkkong, N-A., Chanhome, L. & Rochanapat, N. 2002. Hematology, morphology, cytochemical staining, and ultrastructural characteristics of blood cells in King Cobras (*Ophiophagus hannah*). *Veterinary Clinical Pathology*, 31(3): 116–126.

Santos, X., Feriche, M., Leon, R., Filippakopoulou, A., Vidal-Garcia, M., Llorente, G.A. & Pleguezuelos, J.M. 2011. Tail breakage frequency as an indicator of predation risk for the aquatic snake *Natrix maura*. *Amphibia-Reptilia*, 32: 375–383.

Schall, J.J. & Pianka, E.R., 1980. Evolution of escape behavior diversity. *The American Naturalist*, 115(4): 551–566.

Scheuerlein, A., Van't Hof, T. & Gwinner, E., 2001. Predators as stressors? Physiological and reproductive consequences of predation risk in tropical Stonechats (*Saxicola torquata axillaris*). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268(1476): 1575–1582.

Schoech, S.J., Bowman, R. & Reynolds, S.J., 2004. Corticosterone, nutrition, and timing of reproduction in Florida Scrub-Jays. *Horm Behav*, 46: 121–121.

Schoener, T.W., 1979. Inferring the properties of predation and other injury-producing agents from injury frequencies. *Ecology*, 60(6):1110–1115.

Scudder, R.M. & Burghardt, G.M., 1983. A comparative study of defensive behavior in three sympatric species of Water Snakes (*Nerodia*). *Zeitschrift für Tierpsychologie*, 63(1): 17–26.

Seress, G., Bókony, V., Heszberger, J. & Liker, A. 2011. Response to predation risk in urban and rural House Sparrows. *Ethology*, 117(10): 896–907.

Shine, R., 1980. "Costs" of reproduction in reptiles. *Oecologia*, 46(1): 92–100.

Shine, R., Olsson, M.M., Lemaster, M.P., Moore, I.T. & Mason, R.T. 2000. Effects of sex, body size, temperature, and location on the antipredator tactics of free-ranging Gartersnakes (*Thamnophis sirtalis*, Colubridae). *Behavioral Ecology*, 11(3): 239 –245.

Shine, R., Phillips, B., Waye, H. & Mason, R.T. 2003. Small-scale geographic variation in antipredator tactics of Garter Snakes. *Herpetologica*, 59(3): 333–339.

Shochat, E., Warren, P.S., Faeth, S.H., McIntyre, N.E. & Hope, D. 2006. From patterns to emerging processes in mechanistic urban ecology. *Trends in Ecology & Evolution*, 21(4): 186–191.

Smith, C.F., Schuett, G.W. & Hoss, S.K., 2012. Reproduction in female Copperhead Snakes (*Agkistrodon contortrix*): plasma steroid profiles during gestation and post-birth periods. *Zoological science*, 29(4): 273–279.

Smith, G.R. & Lemos-Espinal, J.A., 2005. Comparative escape behavior of four species of Mexican Phrynosomatid Lizards. *Herpetologica*, 61(3): 225–232.

Sol, D., Lapiedra, O. & González-Lagos, C., 2013. Behavioural adjustments for a life in the city. *Animal Behaviour*, 85(5): 1101–1112.

Stankowich, T. & Blumstein, D.T., 2005. Fear in animals: a meta-analysis and review of risk assessment. *Proceedings: Biological Sciences*, 272(1581): 2627–2634.

Stevenson, R.D., Peterson, C.R. & Tsuji, J.S., 1985. The thermal dependence of locomotion, tongue flicking, digestion, and oxygen consumption in the Wandering Garter Snake. *Physiological Zoology*, 58(1): 46–57.

Stewart, G.R., 1968. Some observations on the natural history of two Oregon Garter Snakes (Genus *Thamnophis*). *Journal of Herpetology*, 2(3/4): 71–86.

Stone, P.A., Snell, H.L. & Snell, H.M., 1994. Behavioral diversity as biological diversity: introduced cats and Lava Lizard wariness. *Conservation Biology*, 8(2): 569–573.

Sullivan, B.K. & Sullivan, O.S., 2012. *Thamnophis elegans* (Terrestrial Gartersnake): defensive behaviour. *Herpetological Review*, 43(1): 153–154.

Sykes IV, J.M. & Klaphake, E., 2008. Reptile Hematology. *Veterinary Clinics of North America: Exotic Animal Practice*, 11(3): 481–500.

Taylor, E. & Denardo, D., 2011. Hormones and reproductive cycles in snakes. In *Hormones and Reproduction of Vertebrates: Reptiles*. San Diego, California: Academic Press.

Thaker, M., Vanak, A.T., Lima, S.L. & Hews, D.K. 2010. Stress and aversive learning in a wild vertebrate: the role of corticosterone in mediating escape from a novel stressor. *The American Naturalist*, 175(1): 50–60.

Thaker, M., Lima, S.L. & Hews, D.K., 2009a. Acute corticosterone elevation enhances antipredator behaviors in male Tree Lizard morphs. *Hormones and Behavior*, 56: 51–57.

Thaker, M., Lima, S.L. & Hews, D.K., 2009b. Alternative antipredator tactics in Tree Lizard morphs: hormonal and behavioural responses to a predator encounter. *Animal Behaviour*, 77(2): 395–401.

Thrall, M.A., Baker, D.C. & Lassen, E.D., 2004. *Veterinary hematology and clinical chemistry*, Wiley-Blackwell.

United Nations, 2011. World urbanization prospects, the 2011 revision. *UN Department of Economic and Social Affairs*.

Vanak, A.T. & Gompper, M.E., 2009. Dogs *Canis familiaris* as carnivores: their role and function in intraguild competition. *Mammal Review*, 39(4): 265–283.

Waye, H.L., 1999. Size and age structure of a population of Western Terrestrial Garter Snakes (*Thamnophis elegans*). *Copeia*, 1999(3): 819–823.

Weatherhead, P.J. & Charland, M.B., 1985. Habitat selection in an Ontario population of the snake, *Elaphe obsoleta*. *Journal of Herpetology*, 19(1): 12–19.

Webb, J.K., Pringle, R.M. & Shine, R., 2009. Intraguild predation, thermoregulation, and microhabitat selection by snakes. *Behavioral Ecology*, 20(2): 271–277.

Webb, J.K. & Whiting, M.J., 2005. Why don't small snakes bask? Juvenile Broad-Headed Snakes trade thermal benefits for safety. *Oikos*, 110(3): 515–522.

Weldon, P.J. & Burghardt, G.M., 1979. The ophiophage defensive response in crotaline snakes: extension to new taxa. *Journal of Chemical Ecology*, 5(1): 141–151.

Werner, P., 2011. The ecology of urban areas and their functions for species diversity. *Landscape and Ecological Engineering*, 7(2): 231–240.

Whittaker, D. & Knight, R.L., 1998. Understanding wildlife responses to humans. *Wildlife Society Bulletin*, 26(2): 312–317.

Whittier, J.M., Mason, R.T. & Crews, D., 1987. Plasma steroid hormone levels of female Red-Sided Garter Snakes, *Thamnophis sirtalis parietalis*: relationship to mating and gestation. *General and Comparative Endocrinology*, 67(1): 33–43.

Wilson, B.S. & Wingfield, J.C., 1992. Correlation between female reproductive condition and plasma corticosterone in the lizard *Uta stansburiana*. *Copeia*, 1992(3): 691–697.

Wingfield, J.C., O'Reilly, K.M. & Astheimer, L.B., 1995. Modulation of the adrenocortical responses to acute stress in arctic birds: a possible ecological basis. *American Zoologist*, 35(3): 285–294.

Wingfield, J.C. & Sapolsky, R.M., 2003. Reproduction and resistance to stress: when and how. *Journal of Neuroendocrinology*, 15(8): 711–724.

Woodley, S.K. & Moore, M.C., 2002. Plasma corticosterone response to an acute stressor varies according to reproductive condition in female Tree Lizards (*Urosaurus ornatus*). *General and Comparative Endocrinology*, 128(2): 143–148.

Yao, M. & Denver, R.J., 2007. Regulation of vertebrate corticotropin-releasing factor genes. *General and Comparative Endocrinology*, 153(1-3): 200–216.

Ydenberg, R.C. & Dill, L.M., 1986. *The economics of fleeing from predators*, Burnaby, BC, Canada: Academic Press, Inc.

Zug, G.R., 1993. Defense and escape. In *Herpetology: an introductory Biology of Amphibians and Reptiles*. London, England: Academic Press, Inc.: 147–161.