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# STRESSED SNAKES: INVESTIGATING A POSSIBLE LINK BETWEEN BASELINE LEVELS OF CORTICOSTERONE AND BODY CONDITION IN THE WESTERN RATTLESNAKE (CROTALUS OREGANUS)

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**B.Sc. Honours thesis – Biology** 





# STRESSED SNAKES: INVESTIGATING A POSSIBLE LINK BETWEEN BASELINE LEVELS OF CORTICOSTERONE AND BODY CONDITION IN THE WESTERN RATTLESNAKE (*CROTALUS OREGANUS*).

by

# COLE RICHARD HOOPER

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#### ABSTRACT

Western Rattlesnakes (Crotalus oreganus) are vital members of ecological communities in the arid regions of southern British Columbia. These areas are ecologically unique compared to the rest of BC, but they are facing higher levels of agricultural and urban development. Therefore, it is critical for us to understand how exactly the conversion of natural habitat to a landscape dominated by vineyards, orchards and resorts will impact wildlife. So far Rattlesnakes have been able to persist in areas with anthropogenic disturbance, but previous research has shown that there may be hidden effects on the health of individuals. Rattlesnakes near Osoyoos, BC living in areas with abundant human disturbance exhibit relatively poor body condition when compared to those living in undisturbed, natural habitats. Considering the process whereby stress reduces body condition via tissue catabolism, I developed a prediction. I hypothesized that habitat disturbance in Osoyoos is leading Western rattlesnakes (*Crotalus oreganus*) to exhibit higher levels of baseline stress, causing a reduction in their body condition. Throughout the summer of 2018, I collected sixty-eight blood samples from rattlesnakes within the Osoyoos population and analyzed the baseline corticosterone (stress hormone) content using enzyme-linked immunoassay kits. I found no difference in baseline corticosterone levels between snakes living in natural habitats and those in disturbed habitats. In addition I found no overarching relationship between baseline concentrations of corticosterone and body condition among all the sampled rattlesnakes. While these results do not agree with our original hypotheses, they still provide an important insight into the stress ecology of the Western Rattlesnake in BC. This work will serve as an important baseline for future stress related research on the Osoyoos Rattlesnake population.

Thesis Co-Supervisor: Professor Dr. Karl Larsen

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### INTRODUCTION

Twenty-first century wildlife conservation is facing more scrutiny than ever, driving increased pressure on wildlife researchers to identify the causal mechanisms behind conservation issues to legislators, courts and land managers. One approach has been conservation physiology, an emerging discipline (Wikelski and Cooke, 2006) that uses physiological tools and techniques to look at the causal relationships affecting organisms as they respond to changing environments and human disturbance. Environmental disturbances of any kind, natural or anthropogenic, are major selective forces (Wingfield and Romero 2011). As a result, animals have evolved a range of responses to these stressors that are defined as adverse stimuli that invoke a stress response, the cascading physiological and behavioural response by the individual.

The study of stress is one of the largest areas of research within conservation physiology. Acutely, the stress response is a vital physiological process that enables organisms to overcome immediate environmental challenges and alterations (Baker et al. 2013). There are two main physiological branches in the response to stress: (1) stimulation of the sympathetic nervous system (SNS) releases catecholamines, and (2) stimulation of the hypothalamic-pituitary-adrenal axis (HPA) works to restore homeostasis via a process known as allostasis (Sheriff et al. 2011). Through allostasis the physiological systems of the body revert back to the normal dynamic consistency of homeostasis (McEwen and Wingfield, 2003). McEwen and Wingfield developed a model conceptualizing stress, made up of three key concepts: (i) "Allostasis is achieving stability through change.", (ii) allostatic loads represent "the result of the daily and seasonal routines organisms have to obtain food and survive and extra energy needed to migrate, molt, breed, etc." and it is tolerated up to a limit, and (iii) allostatic overload, where the organism can no longer cope with its condition, it is unable to exceed its energetic demands. Under natural conditions it would be normal for an individual to experience allostatic overload; at that point the stress response would then act to mobilize energy reserves or change behaviour so that the animal returns to a regular allostatic load. However, under certain conditions the stress response can become chronically activated (Baker et al. 2013) and the individual becomes stuck in a state of allostatic overload. This is an issue as the stress response evolved to solve temporary issues and is not meant to be activated in the long term. This chronic condition can lead to additional problems.

The benefits and detriments of stress are directly tied to the physiological components that the response is derived from, namely the stimulation of the SNS and HPA axis. The response from the SNS is almost instantaneous after interaction with a stressor, and induces the secretion of norepinephrine and epinephrine (Reeder and Kramer, 2005). Together these neurohormones increase heart rate, redirect blood flow and make energy stores available by stimulating glycogenlolysis and lipolysis. Stimulation of the HPA axis occurs simultaneously with the SNS, but through a signalling cascade that eventually signals the production of glucocorticoids (GCs). The primary GC produced varies depending on the taxa of interest and may be either cortisol or corticosterone. During acute secretion GCs increase available energy through gluconeogenesis and decrease glucose use, insulin sensitivity, and protein and fat metabolism; all these functions help the organism counter a specific stressor. In the short-term, elevated levels of GCs are critical in aiding an animal's escape from a lifethreatening situation (Wingfield et al. 1998). However, if the stressor somehow becomes chronic, prolonging the stress response, deleterious effects start to accumulate; these include neuronal cell death, hyperglycemia, insulin resistance, muscle and bone atrophy, hypertension, growth inhibition, and even immune system collapse. This highlights the need to balance focus, between the positive and negative effects of stress.

For example, the implications that elevated levels of stress hormones may have on reproductive function may be overlooked. While this relationship can be highly variable between species there is a general pattern. There has been an historic view that any stress is detrimental towards reproduction but moderately elevated levels of GCs may be positively associated with reproduction through the mobilization of energy stores (Moore and Jessop, 2003). While some recent evidence notes the benefits, the historic point of view is well supported with an abundance of evidence showing a link between highly elevated levels of GCs and the suppression of reproductive behaviours and even total inhibition of reproduction. Just as any other life history trait, we must consider both the costs and benefits associated with the stress response.

Previous research has shown that the intensity and duration of the stress response is directly linked to the general health of the animal (Boonstra et al. 1998), making it a valuable metric of study. In quantifying stress, researchers mainly examine GC concentrations because they persists for minutes to hours (Wingfield and Cooke 2006), unlike the SNS response hormones. An important considerations in study design is the origin of the sample. It is critical to understand exactly how stress is linked to the sample type that you have chosen, as the relationship between the material and stress will vary. Blood plasma (or serum) has been the sample type of choice for most stress research for some time now. The GC concentration in blood plasma reveals the current physiological state of the organism, i.e. what the current stress state of the organism is at that moment in time (Sheriff et al. 2011). It is understood that there are three components that may influence circulating GC content: (1) endogenous cycles (circadian rhythms, seasonal variation), (2) prior acute environmental stressors (actual or perceived; predator encounters, extreme weather, etc) and (3) chronic stressors (predators, environmental conditions, etc). These factors must be kept in mind when concentrations of GC are being examined in any analyses. Glucocorticoids in the blood

are metabolized in the liver, and then make their way into either the urine through the kidneys, or the gut through the bile ducts. Afterwards the gluccocorticoid metabolites (GCMs) do not follow a straightforward path: they may be reabsorbed by the bloodstream within the gut, and undergo hepatic degradation (Klasing, 2005) or their structure may be altered by the intestinal microflora (Eriksson and Gustafsson, 1970; Sadoul and Geffroy, 2019). Both of these processes would then alter the ultimate GC content of the excreta. While blood plasma provides information about the current circulating concentration of GCs, it remains important to capture a longer term picture of the stress the animal is experiencing.

Measuring chronic or long term stress can be accomplished using structures such as hair or feathers because they have slow growth rates and incorporate GCs into their structure during development. Thus, GCs accumulate over time in these tissues and provide information about stress from the chronic perspective. Along with hair and feathers it is possible to use other components of the integumentary system to understand chronic stress, such as finger or toe nails (Frugé et al. 2018). In using these materials, it is important to understand their exact growth rates, so that the time scale of the GC accumulation can be accurately identified (Sheriff et al. 2011). Currently these methods are still somewhat exploratory because the exact process by which GCs are incorporated into these structures is not fully understood. Blood supply during formation is thought to be the main source of GCs in integumentary structures, but they could also be absorbed from the GC-containing secretions from a multitude of glands. Sample selection and collection is only the first step of stress related study.

Following collection of tissues containing GCs, the next challenge becomes the quantification of GC concentration within these various samples. Immunoassays are the primary approach to quantifying GCs. The two most common versions utilized for this purpose are radio-immunoassays (RAI) and enzyme immunoassays (EIA or ELISA). These two approaches are similar in the fact that they are both sensitive, competitive binding assays, both use an antibody specifically targeted against the GC. Radioimmunoassays use a radioactive isotope that generates a radioactive signal indicative of the GC concentration. Alternatively, the EIA method uses an enzyme to create a colorimetric signal that allows for quantification of GC concentration. In order to read either of these signals, expensive equipment is required, including a scintillation or gamma reader to register the radioactive signal of the RIA and/or a microplate reader to read the optical densities of the colorimetric signal of the EIA. An increase in the commercial availability of these assays in recent years has greatly enhanced the quality and quantity of stress research on wildlife across the globe.

The vast majority of work on wildlife stress has focused on a small range of taxa. Typically, researchers target the primary GC produced by their study species, as this is the most biologically active. In general the primary GC is different between taxa: for mammals, and fish it is cortisol, for amphibians, reptiles, and birds, corticosterone is most abundant, but there can be intrataxonomic variation as well. (Sheriff et al. 2011). There is a vast body of literature regarding cortisol based studies, since cortisol is the primary GC produced in humans. Researchers have examined a wide range of subtopics relating to cortisol in humans, including: the effects cortisol has on various components of the metabolism (Brillon et al. 1995; Khani and Tayek, 2001), the link between cortisol and memory (Newcomer et al. 1999), how cortisol may influence development (Goodyer et al. 2001), understanding the heritability of factors controlling cortisol production (Ising and Holsboer, 1996), even the sociological context of stress and cortisol production (Taylor, 2012), and this is only to name a few of the major avenues of study relating to cortisol in humans. While much of the existing research on stress has been conducted historically with humans in mind, the interest surrounding stress in animals is growing. We do already have a fairly complex understanding of stress in some laboratory animals, such as muroid rodents and primates, but our knowledge for most wildlife species is minimal at best (Reeder and Kramer, 2005).

In mammals there has been several key factors identified as determinants in GC secretion. Coe and Levine (1995) revealed how the activity of the HPA axis and thus the concentration of GCs varies with circadian and circannual rhythms with respect to regulating energy balance as it relates to environmental conditions. This kind of temporal variation is linked to stress in more ways than one, as other work has shown the impact of seasonal variation of both biotic and abiotic factors greatly alters adrenocortical activity (Boonstra et al. 2001). The cyclical activity of the HPA axis is most notable in species at high latitudes, as these areas have the greatest disparity in environmental conditions and resources between different times of year (Gustafason and Belt, 1981). While these temporal differences in GC secretion are generally shared across most animals, there is significant variation between species, and even intraspecific sex differences that should be considered (Reeder and Kramer, 2005). With a growing understanding of the processes underlying stress and the stress response, research is moving towards more functional conservation based questions. The extensive human influence on the planet is behind a lot of these questions, such as how pollution might be altering the stress response of mammals (Oskam et al. 2005), how high levels of tourism may increase the stress of local mammals (Zwijacz-Kozica et al. 2013), and the link between habitat disturbance and increased cortisol levels in other mammalian species (Jaimez et al. 2012). Many of the same results seen for mammalian species have also been described in the other primary cortisol producing group, the fishes.

Many researchers have examined cortisol in fishes, likely due to the commercial value of fisheries. Within this context many of the research questions relate to how fishery practices or conditions could influence cortisol levels in fish, such as overcrowding, confinement, and handling (Montero et al. 1999; Pankhurst and Sharples, 1992; Strange and Schreck, 1978), all of which were found to increase the stress levels of fish, potentially impacting tissue quality. While an abundance of funding from fishing industries supports a great deal of this direct fishery related research, there is other work looking at cortisol in natural populations of fish. The conservation theme is carried over here, especially in the context of anthropogenic factors relating to stress, including increases in cortisol secretion in response to ship noise (Wysocki et al. 2006), impaired or reduced cortisol production in fish exposed to environmental pollutants (Hontella et al. 1992), and how habitat degradation may lead to increased cortisol levels (Hasler et al. 2015). Similar to mammals, temporal variation has also been noted as a factor in altering cortisol production, specifically in terms of seasonal and daily timing (Thorpe et al. 1987), as well as points in the reproductive cycle (Wingfield and Grimm, 1977). Most of the research on stress within the different taxonomic groups follows similar overarching themes, but the extent of more specific questions varies a great deal. This variation in specific questions is sometimes linked to the actual GC that is being examined.

Unlike the mammals and the fishes, corticosterone, not cortisol is the primary biologically active GC amongst bird species (Sheriff et al. 2011). Although the specific GC is different, the causes and ultimate effects of stress do not change much despite the active molecule being different. Just like the other taxa, many avian studies have examined the link between temporal variation and corticosterone secretion (Romero et al. 1998; Romero and Remage-Healey, 2000). Other environmental factors that can activate a stress response have also been identified and these include, but are not limited to harsh weather (Romero et al. 2000), urbanization (Fokidis et al. 2009), and even habitat quality (Marra and Holberton, 1998). Extensive work has even shown that corticosterone may play some role in influencing the life history, as species with higher

levels of stress induced corticosterone were found to have higher annual survival rates (Hau et al. 2010). Two specific studies conducted on birds were part of the foundation for this Honours thesis. The first found that baseline corticosterone levels rose in association with a decline in body condition throughout the breeding season in Black-legged kittiwakes (*Rissa tridactyla*)(Kitaysky et al. 1999). The other showed that corticosterone secretion in American redstarts (*Setophaga ruticilla*) differed between habitat types, and this effect was compounded in the spring when the habitat types differed the most in quality (Mara and Holberton, 1998). These studies are especially relevant since birds and herpetiles both produce the same main GC.

Amphibians and reptiles are the other vertebrate groups that also primarily secrete corticosterone (Sheriff et al. 2011). As mentioned the foundation for our understanding of stress in wildlife has been built on the study of endotherms, like mammals and birds (Claunch et al. 2017). In general, much less research has examined stress ecology in amphibians and reptiles, possibly as a result of the relatively slow or variable metabolisms of ectotherms such as amphibians and reptiles. The rates of physiological processes and reactions are dictated by body temperature, so when body temperature is variable it becomes difficult to interpret physiology. Some of the work that has been done has actually identified differences between amphibians and reptiles, in terms of their corticosterone secretion. Amphibians appear to be more sensitive to certain geographic variation, their baseline levels of corticosterone are related positively to latitude, and negatively to elevation (Eikenaar et al. 2012). The reptiles exhibit neither of these relationships. While some differences are apparent, many of the underlying factors influencing stress in the other groups are shared, including seasonal and daily variation (Pancak and Taylor, 1983; Tyrrell and Cree, 1998), environmental conditions (Cash and Holberton, 2005), and habitat quality (Janin et al. 2011). Recently Lind et al. (2018) has compiled and listed a number of studies looking at the relationship between

body condition and corticosterone in other species of snakes. From studies in that list, no consistent pattern has been discovered, with results varying between a negative relationship (Moore et al. 2000: Palacios et al. 2012) and no relationship (Holding et al. 2014; Lind and Beaupre, 2015; Lutterschmidt et al. 2009) between corticosterone and body condition. Several interesting observations can be made when examining the differences in the studies on that list; the only other study on a fellow northern species, the Common garter snake (*Thamnonphis sirtalis*) found no significant relationship between body condition and corticosterone (Dayger et al. 2013). In addition the only studies comparing body condition and corticosterone in the *Crotalus* genus twice found that there was no significant relationship between the two variables in Timber rattlesnakes (*Crotalus horridus*) (Lind and Beaupre, 2015; Lutterschmidt et al. 2009). A comparison between body condition and corticosterone is notably absent for *Crotalus oreganus*. This leads in to my study species, the northern most population of the Western rattlesnake (*Crotalus oreganus*).

The Western Rattlesnake (*Crotalus oreganus*) in British Columbia is inhabiting the northern-most limit of its range in North America. As ectotherms their daily and seasonal activity is dictated by the weather (Gregory, 2007). In Canada the winter weather is far too cold to permit above ground activity, so snakes hibernate for a large portion of the year. By limiting the time available for growth, courting and mating, the Canadian climate has serious effects on the population ecology and life-histories of these animals. In addition to the natural challenges imposed by life at higher latitudes, various anthropogenic threats are placing additional pressure on these species. Lesbarrères et al. (2014) identified the main anthropogenic impacts facing northern herpetiles as habitat loss and fragmentation, roads, pesticides and other contamination, infectious diseases and climate change. Among these habitat loss and fragmentation have been considered the most serious threats to all herpetofauna in northern

environments. This is especially prevalent in British Columbia where the Western Rattlesnake (*Crotalus oreganus*) occupies arid grasslands and riparian areas, as these land types are the most desirable for agricultural and urban development (COSEWIC, 2015). As of 2005, approximately 16.1% of all grassland habitat in BC has been lost, but this effect is even more pronounced in the Okanagan where sub-regions have seen amounts of land conversion as high as 20-28% percent.

My study population occured at the southernmost tip of the Okanagan valley, adjacent to the American border near the town of Osoyoos, BC (49°01′56″N 119°28′05″W). The study site itself is located on the east side of Osoyoos Lake, on Osoyoos Indian band land, an extremely hot and arid area. This site offered a contrasting environment where I was able to compare snakes from the same population that were living in either disturbed or undisturbed habitats. The property contained a section of extensive natural shrub steppe habitat known as the north desert, and an area of high anthropogenic disturbance in the southern portion. The disturbed resort area had a variety of different disturbance regimes. The complex included a golf course, hotel, condominiums, winery, interpretive centre, campground and several roads. In addition, several key components of the resort were surrounded by a thin gauge wire fence, built to exclude snakes from these areas. This in itself was an interesting component of the overall anthropogenic disturbance in the area, since this physical barrier blocked the normal migration route of snakes in the area (Maida, 2018).



Figure 1. Map of the Osoyoos Indian Reserve (OIR) study site, with a legend denoting the different disturbance regimes in the area.

The site is the subject of a long-running snake research program (>12 yrs) that provides the background for this thesis. Lomas et al. (2015) examined the effects of

anthropogenic disturbance on this population. They used a disturbance ranking system that separated snakes into disturbance categories based on the straight-line distance to the nearest disturbance. Western rattlesnakes (*Crotalus oreganus*) in the higher disturbance categories (closer distance to disturbance) displayed a lower body condition than those in the lower disturbance categories. I used a simplified version of this system where I separated snake captures into three primary areas: (1) the north desert, where the distance to disturbance is always far (capture location is at the minimum 250 meters to the nearest disturbance) (2) the snake fence, where the distance to disturbance is short but variable (capture location between 0-150m to disturbance). Data from small mammal monitoring in the area found that there was not a significant difference in small mammal numbers or densities between the disturbed and undisturbed habitats (Maida, 2018). If there is no implicit difference in food availability, then examining other factors causing this reduction in body condition remains an important area of research.

I proposed that physiological stress may be playing an underlying role in influencing body condition in snakes occupying the Osoyoos habitat, similar to that reported elsewhere. Given the mixed habitat quality of our site, we can draw comparisons to the Mara and Holberton study (1998) where they found that lower quality habitats were associated with higher levels of corticosterone. Then considering the results of Lomas et al. (2015), we can speculate that stress may be the cause based on the studies that have demonstrated this effect (Kitaysky et al. 1999; Moore et al. 2000; Palacios et al. 2012). Elevated levels of stress hormones are known to cause tissue catabolism that ultimately reduces energy reserves (Sheriff et al. 2011). Following the work by Lomas et al. (2015) I investigated this potential mechanism within the Osoyoos snake population. I hypothesized that habitat disturbance in Osoyoos is leading Western rattlesnakes (*Crotalus oreganus*) to exhibit higher levels of baseline stress, causing a reduction in their body condition. I predicted that snakes living in the undisturbed north desert area would exhibit relatively low baseline levels of corticosterone. The resort and snake fence areas both represent disturbed habitats, but due to the physical interaction between snakes and the fence I expect them to show the highest levels of baseline corticosterone. Snakes at the resort do not face any physical barriers, but instead a variety of indirect forms of disturbance so I expect their baseline corticosterone secretion to lie between the undisturbed habitat snakes and those living along the snake fence. I also predict there will be a negative relationship between baseline corticosterone and body condition across sites, among all sampled individuals.

### **METHODS**

This project was conducted under federal and provincial permits that allowed capturing and handling of the targeted wildlife species. Protocols for handling were approved by an animal care committee from Thompson Rivers University. All data used in this thesis were collected during July and August of 2018, as these were the months when the Western rattlesnakes have settled into their summer foraging habitat. All snakes were found through walking surveys, or through visitation of known congregation sites. I conducted these surveys in the three primary disturbance areas which I described before: the north desert, the snake fence and the resort. I avoided the hottest times of day when snakes were hiding, surveying in the morning (06:00-12:00) and in the late evening (19:00-24:00). As soon as a snake was encountered, a timer was used to ensure blood samples were obtained within 5 minutes of the initial encounter, to prevent corticosterone concentration from being influenced by handling (Schuett et al. 2004). Tongs were used to maneuver the anterior portion of the snake into an acrylic

tube. Following immobilization I palpated the snakes to ensure no gravid females were used for blood samples. At least 0.5 mL of blood was drawn from the caudal vein with a 1mL syringes (Terumo Medical Corporation®, Japan) with 27G x 1/2" gauge needles. The blood was transferred to Vacutainer<sup>™</sup> (Becton Dickinson, United States) tubes coated in lithium heparin to prevent clotting. These tubes were labelled and stored in a portable insulated lunch kit with an icepack. Then within 4 hours the samples were transferred to 1.5mL microcentrifuge tubes (Fisher Scientific, United States), and centrifuged at 6000 rpm for 10 minutes (VWR Galaxy mini centrifuge, VWR International, United States). Afterwards the blood plasma supernatant was removed and transferred to a new microcentrifuge tube (Fisher Scientific, United States) and frozen. At the end of the field season, blood samples were transported on ice to a -80°C freezer in the laboratory at Thompson Rivers University, where they remained until further analysis.

In addition to blood sampling, I also recorded the GPS coordinates (±5m), disturbance category, snout-to-vent length (SVL), weight, and sex of each captured animal. The most proximal segment of the rattle was painted light blue to ensure individuals were not resampled. I also recorded any anecdotal evidence that may have influenced corticosterone levels such as the snake having been substantially disturbed prior to capture.

I used corticosterone ELISA kits (ENZO Biochem Inc., United States) to assay stress levels in the blood samples collected from the snakes. Samples were thawed before analysis, then duplicate 100µL plasma samples were pipetted into assay wells. After this 50 µL of both the alkaline phosphatase conjugate and sheep polyclonal antibody were added to the wells. I prepared the standardized wells by completing serial dilutions using a solution with a known corticosterone concentration. The plate was then incubated at room temperature on a shaker (500 rpm) for 2 hours. The wells were subsequently washed by emptying the contents of the wells, and then adding 400  $\mu$ L of wash solution. This process was completed three times. After the final wash, all remaining moisture was removed from the wells and 200  $\mu$ L of pNpp substrate solution was added to every well, before the last incubation phase at room temperature for 1 hour, without shaking. Following this, 50  $\mu$ L of the stop solution was added to every well and the plate was read immediately using a microplate reader at both 406nm and 621nm. To validate samples between kits, I analyzed 5 samples with both kits and determined the coefficient of variation between the kits. In addition, 5 more samples had widely varying results within their replicates in the first kit, so they were repeated on the second to obtain a more consistent result. To derive the concentration of corticosterone in the samples, a standard curve was generated using the standard solutions with known concentrations of corticosterone. Using the equation of the line for these curves and the percentage bound (a function of optical density), I derived the associated concentration of corticosterone in pg/mL for each sample.

### Statistical analyses

Following Lomas et al. (2015) body condition was derived from a regression between log<sub>10</sub>-transformed SVL and mass and the residual values were calculated; this method is considered appropriate for deriving a measurement of body condition in snakes based on general size (Reist, 1985).

All statistics were performed in Minitab (Version 18.1). All data were first tested for normality using the Kolmogorov-Smirnov. I compared body condition index between our 3 primary sites with a one-way ANOVA test. I then repeated this test to compare the concentration of corticosterone between the sites. Tukey's honestly significant difference test was used post-hoc for detecting differences between groups. Finally I built a linear regression to examine the relationship between LOG BCI and the concentration of corticosterone (pg/mL).

### RESULTS

In total 68 blood samples were collected, with 32 from snakes in the resort area, 26 from snakes in the north desert and 10 from snakes located at the snake fence. I found no significant difference between the mean body condition index values of snakes captured at the 3 different sites. (F=1.60 df=2 df=65 P=0.08, Figure 2). Similarly there was no difference in the mean concentration of corticosterone in blood samples between the 3 different sites (F=1.98 df=2 df=65 P=0.15, Figure 3). The linear regression between corticosterone concentration and LOG BCI showed a very weak relationship (F=1.11 df=1, P=0.30 R<sup>2</sup>=0.017, Figure 4).



Figure 2. Boxplot comparing the body condition of snakes captured at the three different sites. No significant difference in body condition was detected between these groups (F=1.60 df=2 df=65 P=0.08).



Figure 3. Boxplot of corticosterone concentrations (pg/mL) in blood samples collected from snakes within the 3 different sites. No difference in corticosterone concentration was seen between these groups (F=1.98 df=2 df=65 P=0.15).



Figure 4. Linear regression between plasma corticosterone concentration (pg/mL) and LOG body condition (F=1.11 df=1, P=0.30 R<sup>2</sup>=0.017).

#### DISCUSSION

Overall there were no trends that matched my predictions. My results comparing BCI between snakes occupying different portions of the landscape contrasted strongly with those reported earlier by Lomas et al. (2015) in that there were no apparent differences between snakes in the different disturbance regimes. There was no difference in baseline concentrations of corticosterone between the blood samples collected from snakes at the different sites. It appears that increased habitat disturbance in Osoyoos is not leading the Western Rattlesnake population (*Crotalus oreganus*) to exhibit higher levels of baseline stress, causing a reduction in their body condition. I attempted (post-hoc) to determine if there was an underlying relationship between baseline corticosterone and body condition, independent of site, but again there was no detectable link between them.

While my hypotheses and predictions were not supported, the results are not unlike those found in the literature. I based my hypothesis upon research that showed a clear negative relationship between corticosterone concentrations and body condition (Kitaysky et al. 1999; Moore et al. 2000; Palacios et al. 2012), yet other work has shown no relationship whatsoever between these metrics (Dayger et al. 2013; Lind and Beaupre, 2015; Lutterschmidt et al. 2009). The body condition index reflects the amount of stored energy an animal has available, or in other words, it is the physiological energy balance of the individual and the allocation of that energy. This is a critical internal process that must result in a favourable distribution of energy to life-history processes such as foraging, reproduction, and predator avoidance or else there may be significant fitness losses (Dayger et al. 2013). Since one of the primary functions of GCs is to mobilize energy stores, a negative relationship often exists between circulating GC concentrations and body condition (Kitaysky et al. 1999; Moore et al. 2000; Palacios et al. 2012). However in our case, and in others no relationship between the two variables was found (Dayger et al. 2013; Lind and Beaupre, 2015; Lutterschmidt et al. 2009). This suggests the presence of this relationship is contextual, and varies depending on the environmental conditions and life-history. Previous research on the Common Garter Snake (*Thamnophis sirtalis*) has come to similarly-conflicting conclusions about the relationship between corticosterone and body condition. A study of the species in Oregon (Lat 45°N) found a negative correlation between body condition and concentrations of corticosterone (Moore et al. 2000). Conversely, a parallel study in Manitoba at a higher latitude (51°) found no significant relationship (Dayger et al. 2013). Although more comparative data are needed, there may be life-history elements that northern populations specifically balance off energetically, resulting in little relationship between body condition and corticosterone.

The mean baseline corticosterone levels I recorded in this study (=57405 pg/mL) were approximately five times higher than those detected in a similar study of timber rattlers (*C. horridus*) in Pennsylvania (Lutterschmidt et al. 2009). This further suggests that underlying differences in baseline GC levels may exist between populations and species. It also adds credibility to the argument that northern populations of snakes may be less likely to exhibit a relationship between corticosterone and body condition. Since the season for breeding and reproduction is condensed by the climate (Gregory, 2007) there may be higher levels of corticosterone secretion by all individuals independent of site. This higher level of corticosterone secretion could facilitate more rapid reproductive behaviour and the mobilization of the necessary energy stores to accomplish this. In order to validate this claim a much broader data set would be required to make such a wide comparison between northern and southern populations. Although it could be a plausible explanation, there are other components that should be considered when interpreting these results.

The discrepancy in my results compared to those of other studies may be due to the fact I was unable to control for all the factors that determine GC content. There are 3 main elements influencing circulating GC content: (1) Endogenous cycles (Circadian rhythms, seasonal variation), (2) Prior acute environmental stressors (actual or perceived; predator encounters, extreme weather, etc.) and (3) Chronic stressors (predators, environmental conditions, etc) (Sheriff et al. 2011). My hypothesis and predictions were based on the third element, in that I suggested that long-term poor environmental conditions due to human disturbance would elevate baseline corticosterone concentrations. However, controlling the 1<sup>st</sup> and 2<sup>nd</sup> elements is not always possible. It is difficult to know the individual experiences of these animals and as a result other acute environmental stressors (unknown to us) may be influencing the results. In addition other endogenous factors and cycles, including sex (Honman et al. 2003), age (Reichert et al. 2012), reproductive stage (Rubenstein and Wikelski, 2005), and time of year or day (Pancak and Taylor, 1983; Tyrrell and Cree, 1998) also may be affecting GC levels.

Endogenous cycles in particular may have had a profound effect on my results. Western Rattlesnakes are cryptic and difficult to locate, even when relatively abundant. Thus, a blood sample was taken every time an individual was encountered, provided they met the base criteria. This meant that blood samples were taken from individuals at different times of year, varying times during the day, and under different weather conditions and then all incorporated into the same model. One such factor that I did not account for was the reproductive stage any particular individual was in. Given that among other species, peak corticosterone secretion generally occurs during the mating season (Dayger et al. 2013), levels in Western Rattlesnakes should peak during late July or early August (COSEWIC, 2015). This window in time coincided with the latter part of my blood sample collection period. This implies all sampled rattlesnakes captured

during this period may have been exhibiting higher than normal levels of baseline corticosterone. In turn, the similarity between mean baseline corticosterone concentrations I detected may be due to the coincidental reproductive stage all individuals were experiencing. Moreover, corticosterone secretion must be relatively similar in all individuals at certain points of the breeding season because there will be a specific range of GC concentrations that allows mating to occur (Dayger et al. 2018). While it is important to consider these endogenous factors, there are ways to enhance the complexity of my methods to avoid these issues to some degree.

A more parsimonious explanation for my results is that baseline stress alone is not the most revealing metric and it is unsuitable to address questions around comparing stress between habitats. It is possible that the snakes in anthropogenically disturbed habitats are facing greater environmental stressors, without exhibiting higher levels of baseline corticosterone due to a difference in the sensitivity of the HPA axis (Dayger et al. 2013). In addition, the work of Sapolsky et al. (1984), found that sensitivity to stress could be altered not only within the HPA axis, but at the cellular level as well. Sustained elevated levels of circulating corticosterone will result in a reduction in the number of cytosolic corticosterone receptors within the targeted cells. Both of these physiological mechanisms work to mitigate the negative effects of chronic stress, cells dampen their receptivity to corticosterone to buffer the higher concentrations of corticosterone; effectively reducing the link between corticosterone and any physical effects such as reduced body condition. Rich and Romero (2005) have detailed the downstream effects of acute stress response suppression: European starlings exposed to chronic stress were found to downregulate corticosterone secretion upon stimulation from acute stressors. While this effect may be present in the Western rattlesnake, it cannot be examined if the data consists of baseline concentrations of corticosterone alone.

I have identified a number of issues with my protocols and these could be addressed in future work on stress in the Osoyoos Rattlesnakes if more elaborate methods are used, ones that can decouple the effects of chronic and acute stress. One approach could be a capture stress protocol (Pakkala et al. 2013), a strategy developed to detail the magnitude of the acute stress response. Another option could be to utilize more experimental techniques such as using clipped scutes or entire sheds (Berkens et al. 2013) as measures of chronic stress. Lastly, it should become a focus to maximize the potential sample size. My sample (N=68) was relatively small compared to those in the Lomas et al (2015) study, where data was collected over multiple years (N=623).

While I did not detect differences in corticosterone levels between snakes occupying different habitats, my work still serves as an important baseline for future study. Conservation physiology strives to uncover the underlying mechanisms behind conservation issues, in part so that law and policy makers can be adequately informed so that proper protections can be implemented. While I was unable to support my hypothesis, I have discovered several interesting components about the stress physiology and ecology of the Western rattlesnake in southern British Columbia. This information could lead to more directed questions about the real implications of human disturbance on the stress response of the Western rattlesnake and the downstream effects. Stress ecology is an important component of conservation biology that must be understood in order to effectively protect species from the negative effects of human development.

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