Exploration of Hair Cortisol Concentrations and Implications of Stress in Hunting Dogs on the Bosawas Biosphere Reserve, Nicaragua

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Margo

Abstract

This thesis examines the relationships between physiological factors of hair color, sex, nutritional health, and body size (mass) with measures of hair cortisol in domesticated Nicaraguan hunting dogs on the Bosawas Biosphere Reserve. Prior research on cortisol in dogs has been relatively limited to small, homogenous population samples under restricted environmental conditions. This leaves a gap in current knowledge, and a need for larger-scale studies to assess whether current understandings of how cortisol acts in dogs is applicable to dogs within dynamic and heterogeneous environments. The population of hunting dogs examined in this study live in an unpredictable environment in which disease, malnutrition, and mortality rates are high. Physical examinations were performed on each dog by a veterinarian during which a body condition score (BCS) was assigned, and measurements of size were taken, and medical and familial history were recorded. Hair samples were also taken to determine basal hair cortisol concentrations (HCC), and fur color was noted as light, dark, or agouti/mixed. Results showed that light HCC was on average significantly higher than dark and mixed HCC ($p<.001$). In addition, BCS scores and chest width were found to be negative effect on HCC ($p<.001$). In contrast with findings from prior studies, female dogs had significantly higher average cortisol levels than males ($p=.016$), suggesting sex differences in cortisol are present in domesticated dogs. The results of this study suggest a relationship between cortisol levels and BCS and body size, confirming previous findings and adding to the current understanding of physiological and nutritional variables of stress within dogs under varying environmental conditions. The results of this study are also relevant to current discussions within biology, ecology, and biological anthropology regarding the physiological effects of cortisol and evolutionary fitness.

Introduction

Humans and domesticated dogs have an extensive and intimate history wherein people have long relied on their canine companions for food, protection, and companionship. The dynamic complexity and nature of this relationship has sparked much interest among academic, social and biological scientific fields of study (Morey, 2005; Skoglund et al., 2015; Fiorello et al., 2017; Stahl, 2013). Dogs possess a unique ability to recognize and interpret both physical and verbal cues from humans, and respond accordingly, which sets them apart from other animals (Hare and Tomasello, 2005; Gracsi et al., 2004). Historically, much of the interest has revolved around the impact of dogs on humans, but more recently the focus has shifted to humans' effects on dogs. For example, it has been found that just like their human counterparts, dogs also become less stressed and benefit when receiving physical affection from a person (Coppola et al., 2005). This is one of a growing number of studies focusing on various physiological and environmental factors that affect behavior and development in our canine counterparts. Understanding how dogs adapt and respond to human demands, as well as environmental and physiological variables, provides insight into the affiliation between humans and dogs and inform larger debates within the fields of evolutionary biology, ecology and biological anthropology regarding theories of domestication and the importance of canines as tools for survival and success, as well as the evolutionary impact this relationship between humans and dogs has had in shaping both species.

Studies on the impact of environmental factors such as home life, seasonality, weather, and human-dog interactions on dogs have been conducted through cortisol analysis (Beerda et al., 1998; Dreschel and Granger, 2005; Roth et al., 2016). Research has also been conducted in dogs to assess the effect of certain activities such as playing and socializing on cortisol levels, and further research has explored the link between cortisol and lifestyles in working (i.e. hunting) and companion dogs (Sandri et al., 2015; Roth et al., 2016).

Cortisol is a glucocorticoid hormone responsible for many regulatory functions in the body, such as sleep/wake cycles and metabolism (Selye, 2002). As such, it has been linked with physical or perceived environmental stressors and analysis of cortisol is often used when assessing the effects of potential stressors (which may range in degree of severity) such as malnutrition, illness, and physical or psychological trauma. Cortisol can be obtained from many sources for short term analysis including saliva, blood and feces, however hair provides both a noninvasive and reliable method for measuring time-averaged cortisol levels, as the hormone is incorporated into the hair shaft during hair growth (Bryan et al., 2013). Hair cortisol analysis has been conducted with humans, as well as a number of both wild and domesticated animals, including dogs to assess cortisol variation in relation to stress, growth, and development (Vincent and Michell, 1992; Beerda et al., 1998; Stephen and Ledger, 2007; Sandri et al., 2015; Bryan et al., 2013; Macbeth et al., 2010; Bechshøft et al., 2013; Terwissen et al., 2013). While hair cortisol analysis has been validated in domestic dogs, much of the literature pertaining to this research focuses on dogs who are bred and used solely for companionship, with less research having been conducted on dogs working in different capacities. Furthermore, the dogs used in these studies have come from high income countries, and presumably live relatively healthy and predictable lives which is not necessarily representative of all dog populations globally.

In this thesis, I will be presenting results from hair cortisol analysis of hunting dogs from the indigenous Miskito and Mayangnas communities living on the Bosawas Biosphere reserve of

Nicaragua. This population (N=455) provides a unique opportunity to inform and expand current literature on hair cortisol analysis in dogs and stress energetics. Current literature on the subject of stress in dogs is based on studies with small sample sizes, in highly regulated clinical and laboratory settings in North America or Europe. Furthermore, the dogs included in these studies are primarily purebred. This has left a gap in knowledge as to how domesticated dogs respond to stress under the normal conditions of life, as well as what variability there may be in the energetics of stress between different populations. In contrast with the sample populations of current studies on stress and cortisol levels in domestic dogs, the dogs at Bosawas live in highly unpredictable environments, where rates of disease, malnutrition, and injury contribute to high mortality (Fiorello et al., 2017). These dogs live far different lives from those living with owners in high income countries and are not purebred.

I examine hair cortisol levels from the hunting dogs at Bosawas to 1) validate existing hair cortisol analytical methodologies and known associations from studies of purebred dogs, and 2) provide further understanding of the interactions between physiological factors of stress in a population of dogs within the dynamic environmental context of daily life. The research presented in this thesis applies to larger discussions within biology and anthropology concerning the evolution and biological effects of canid domestication, as well as the physiological response elicited by cortisol in cortisol-dominant animals, including humans.

In addition, the indigenous communities at Bosawas display a heavy reliance on dogs for hunting, which is unusual in the Neotropic region of the Americas (Koster, 2008a). This thesis could further provide important insight into the stress response of dogs in these indigenous communities to the human demand of hunting by their owners. This study may improve our understanding of how we might better facilitate this relationship for both humans and dogs. For example, in working dogs which are used for numerous vital tasks such as guarding, search and rescue, or hunting, knowing what makes some dogs better than others at their tasks, and how to improve their ability, could be extremely valuable.

In chapter 1, I will discuss the physiology of cortisol and its relation to stress and review the published and current literature on cortisol studies in dogs. In chapter 2, I review the history of the domesticated dog in the Americas and evaluate current literature on the history and culture of the Mayangnas and Miskitos peoples and their hunting dogs living on the Bosawas Biosphere Reserve. The end of chapter 2 also presents the hypotheses that I will test in this thesis. Chapters 3 and 4 detail the materials and methods, and the results of my research, respectively. I discuss these results in chapter 5 and conclude the thesis in chapter 6.

Chapter 1: The Physiology of Cortisol and Stress:

The word “stress” is used colloquially to reference a broad range of experiences and challenges that an individual might face. For the purposes of this study, the term “stress” will refer to the body’s physiological response to a demand, either perceived or real, which may alter basal homeostasis (Fink, 2009). “Stressors” are those variables which elicit a stress response from an individual, and thus may be environmental (e.g. weather or comfort and predictability of surroundings), physiological (e.g. nutrition, disease, or physical labor) or psychosocial (e.g. relationships with others). Although stress is most often associated with negative experiences or interactions, the physiological response to stress is one that may be employed by the body under any stimulus which requires action, such as during exercise or socializing with others.

1.1. The HPA Axis

In mammals, the physiological response to stress is produced by multiple nervous and endocrine system interactions more generally referred to as the stress system. A central component of this system is the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is vital to maintaining many of the body’s day to day and long-term functions, as well as in responding to external stimuli such as stress through the production and regulation of glucocorticoids. The anterior hypothalamus secretes corticotropin releasing factor (CRF), which in turn stimulates the secretion of adrenocorticotropic hormone (ACTH) in the pituitary gland. ACTH is transported by the bloodstream to the adrenal glands, where the end-products, glucocorticoids and mineralocorticoids, are released (Selye, 2002 and Charmandari et al., 2005). Glucocorticoids are a group of biochemical actors which include the corticosteroid hormones cortisol and cortisone, with cortisol being the primary hormone. The primary function of cortisol is to promote gluconeogenesis, a process in the liver by which glucose is generated from the breakdown of proteins for energy metabolism by the brain and body during activity. Cortisol is also involved in the breakdown of fats during lipolysis (Selye, 2002). A diagram depicting this process, and the specific subsequent physiological changes produced is included in Figure 1.1.

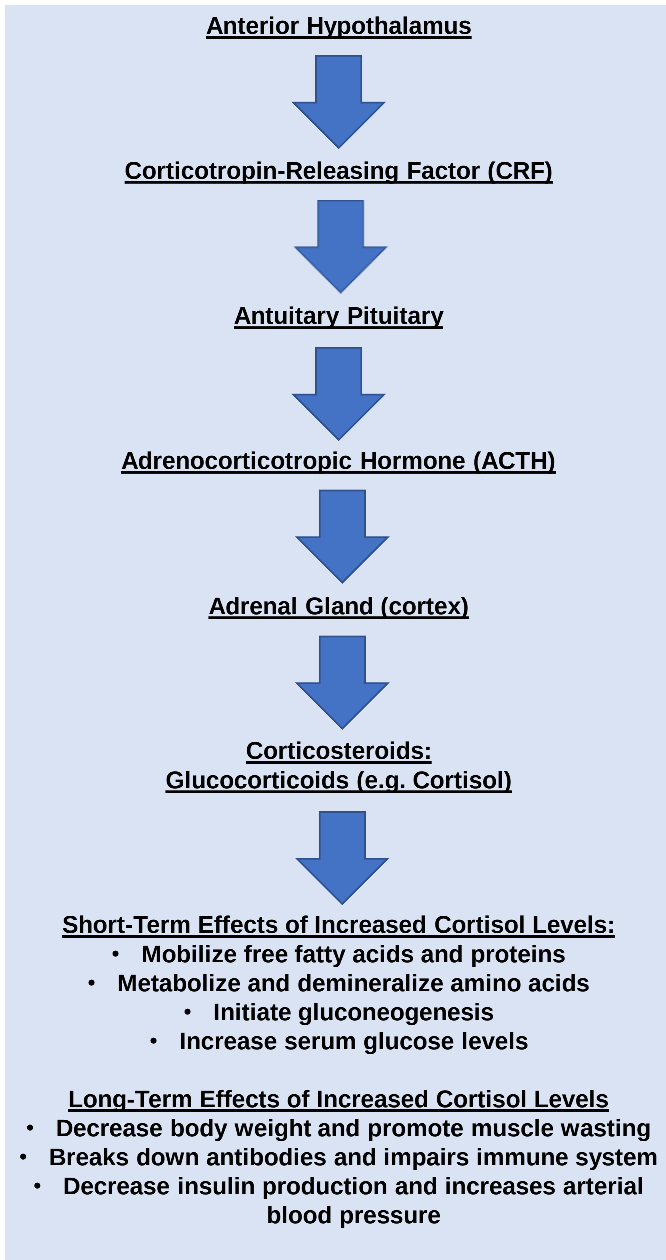


Figure 1.1. Adapted from Selye, 2002; Outline of the neuroendocrine pathway through which cortisol acts upon the body and the short and long-term effects of elevated cortisol levels.

While cortisol is most often synonymous with stress, it is more accurately thought of as a regulatory factor of the body—a product created by the neuroendocrine pathways of the HPA axis to prepare the body for changes to homeostasis. The HPA axis is the system by which regulation of metabolic and cardiovascular processes are performed within the body, and therefore plays a crucial role in maintaining basal homeostasis. Part of the HPA axis processes are subserved by cortisol, which mobilizes energy (i.e. nutrients and oxygen) to active organs and tissues within the body (Fink, 2009). This process occurs within a pattern where the secretion of CRF by the hypothalamus follows a pulsatile rhythm, which remains highly consistent in the absence of stress. The amplitude of CRF pulses increases early in the morning, subsequently leading to an increase in ACTH and cortisol production known as the diurnal cortisol pattern, wherein the body's cortisol production peaks during the early morning and continues to fall throughout the day. This daily cycle is necessary for the regulation of metabolism, immune system function, growth and reproduction, and proper sleep/wake cycles (Chrousos, 2009).

HPA axis activity and cortisol production not only vary throughout the day but follow ontogenetic patterns as well. Binz et al. (2017) found that cortisol levels vary significantly by age in humans, where toddlers showed higher cortisol values than both adolescents and adults while adolescents had the lowest cortisol values of all three groups. Elevated cortisol during early childhood correlates with accelerated growth patterns during this time, particularly in the skeletal muscle tissues and brain. Cortisol levels decrease throughout childhood and into adolescence before increasing again during adulthood. This increase is thought to be the result of increasing cortisol resistance over time. There is also a sizable literature on non-human primates regarding developmental and physiological effects of cortisol. Like humans, other primate species have shown the same developmental cortisol patterning (Fourie and Bernstein, 2011 and Laudenslager et al., 2012). In dogs, further assessment of cortisol levels by age has yielded limited agreement with the findings of Binz et al. (2017) that cortisol decreases with age (Sandri et al., 2015). However, a similar study in another non-primate species, the Canadian Lynx, found no correlation between age and cortisol, demonstrating an area for continued research into cortisol and development in non-primate mammals (Terwissen et al., 2013).

In addition to age, size and sex have also been shown to correlate with cortisol levels. Sandri et al. (2015) found that salivary cortisol concentrations in dogs were inversely related to size. Haase et al. (2016) confirmed that this trend appears to hold across cortisol-dominant mammal species. Size, defined as body mass, and cortisol have both been related to metabolic rate in mammals. Cortisol plays a regulatory role in metabolic rate, which also decreases with mass (Haase et al., 2017). In addition, a link between cortisol levels and size may be explained by the glucocorticoid's suppressive effect on growth hormones, a concept explored at greater length below. Evidence of sex-based differences in cortisol concentrations has been noted in humans, where males had higher cortisol levels on average than females (Binze et al., 2017). Studies of non-human primates have also revealed sex-based differences in cortisol. In vervets, this pattern was noted to differ between males and females, with males exhibiting lower cortisol levels than females with the onset of puberty (Laudenslager et al., 2012). This suggests that sex differences are linked with cortisol levels in some primate species. However, these findings have not been replicated in dogs, and multiple studies on dogs have failed to find any significant differences between individual cortisol levels by age or sex (Stephen and Ledger, 2006; Sandri et al., 2015).

The effects of the HPA axis and cortisol are widespread throughout the body, and consequently, any disruption of the HPA axis or cortisol production can lead to significant health issues. When stressed or disturbed by changes in activity or feeding/sleeping schedules, the diurnal pulsatile release of ACTH and cortisol can be disrupted. Exposure to stress causes the amplitude and frequency of the pulsatile release of CRF to increase in intensity, leading to a rise in ACTH and cortisol levels in the body (Charmandari et al., 2005). When responding to stress, the body releases a mix of hormones whose effects vary in onset and duration. Some last only a matter of seconds, as is the case for epinephrine or norepinephrine. However, the metabolic reaction rates of ACTH and cortisol generated by the HPA axis can last minutes, hours, days, or even weeks. Thus, the prolonged effects of stress are attributed to these longer acting hormones (Selye, 2002). The effects of long-term stress can be detrimental to the body. Elevated basal cortisol levels have been found to degrade white blood cells and weaken the immune system, as well as decrease muscle mass through the breakdown of muscle tissue during gluconeogenesis. Increased cortisol levels have also been linked with high blood pressure, and developmental growth disruption (Chrousos, 2009; Fink, 2009; Selye, 2002).

As a regulatory hormone, cortisol levels vary to meet the changing demands of the body. The sections below review the published literature on environmental and physiological factors which affect cortisol levels in mammals, and the related outcomes.

1.2. Physiological Factors of Stress

a. Nutritional Stress:

Prolonged periods of stress and nutritional deficiency produce permanent changes in glucose-insulin sensitivity. Cortisol mobilizes energy stores within the body through gluconeogenesis, turning proteins into glucose to be consumed by active tissues. During stress, cortisol and blood glucose levels increase, allowing the body to respond quickly to sustained activity levels. If the stress becomes chronic, elevated blood-glucose levels caused by cortisol can lead to insulin resistance and increased risk of developing type 2 diabetes, as well as hypertension (Charmandari et al., 2005; Chrousos, 2009; Fink, 2009). Further research has linked the restriction of caloric intake to a significant rise in cortisol levels (Tomiyama et al., 2010). Continually elevated cortisol levels have also been associated with weight gain and obesity. Studies examining the relationship between abdominal fat and cortisol levels in women and peripubertal girls found that increased cortisol levels positively correlated with measurements of abdominal fat (Donoho et al., 2011 and Moyer et al., 1994).

Research also suggests that stress influences appetite regulation. During acute stress, the body mobilizes energy stores to be diverted to those tissues that are more active under stress, namely the skeletal muscles and brain, while other bodily functions which are immediately non-essential, such as digestion, are suspended (Schneiderman et al., 2005). The body works to inhibit hunger during stress through the same hormonal cascade carried out by the HPA axis. The hypothalamus stimulates the secretion of CRF under stress, which inhibits gastric acid secretion and emptying. CRF has also been implicated in the stimulation of colonic motility, meaning the body freezes digestive processes while preparing the bowels to void themselves in preparation for flight. In addition, the secretion of ACTH has been found to counteract the effects of ghrelin, an appetite stimulant primarily produced by the stomach (Chrousos, 2007; Schneiderman et al., 2005; Tache and Bonaz, 2007). The pathophysiology of cortisol and its effects on appetite and gastrointestinal motility have been studied in several mammal species, including humans (Charmandri et al., 2005), rats (Tache and Bonaz, 2007) and dogs (Bueno and Fioramonti, 1986).

b. Growth, Development, and Reproductive Stress:

The interactions between the HPA axis and the reproductive and growth axes have been extensively studied. When experiencing stress, the body diverts energy and nutrients away from all processes that are not immediately essential, including reproduction and growth (Schneiderman et al., 2005). The HPA axis acts on the reproductive axis by various components. CRH is a direct suppressant of the gonadotropin-releasing hormone (GnRH) neuron, and peripheral elevation of glucocorticoids also contributes to inhibition of the GnRH neuron. CRH also indirectly inhibits gonadic (reproductive) function through interference with endocrine cells, known as pituitary gonadotrophs, which produce hormones that affect the gonads (Charmandari et al. 2005 and Chrousos, 2007). Suppression of reproductive functions by chronic HPA activity presents in low testosterone in males, and amenorrhea in females (Chrousos, 2007). It is important to also note that the relationship between CRF and the reproductive axis is bidirectional, as estrogen levels increase the production of CRF via estrogen-response elements on the promotor region of the CRF gene. This likely explains the increase in cortisol seen in females during pregnancy (Chramandari et al., 2005). Studies on several mammalian species including humans, rats, and sheep have shown that cortisol levels increase throughout pregnancy and are a facilitating factor in the onset of labor (Hasiec and Misztal, 2018). In addition, cortisol levels continue to remain elevated during lactation as a result of the continued high energetic demand on the mother to supply her offspring with nutrition (Hasiec and Misztal, 2018).

As with the reproductive axis, the growth hormone (GH) – insulin-like growth factor

(IGF) axis is inhibited at many levels by the HPA axis during stress. Extended periods of HPA activation suppresses GH production, and glucocorticoids induce the inhibition of IGF-I. In addition, CRF indirectly cause the inhibition of GH secretion through inducing a rise in somatostatin levels (Charmandari et al., 2005). Diminished GH response and low IGF-I concentrations related to stress have been noted in studies of children (Skuse et al., 1996) and nervous pointer dogs (Uhde et al., 1992). Other developmental consequences of a hyperactive stress system include delayed puberty and growth, cardiovascular disease, and osteoporosis (Charmandari et al., 2005; Chrousos, 2007 and 2009; Schneiderman et al., 2005).

c. Immunological stress:

When responding to stress, the immune system is activated, and immune cells (macrophages and natural killer cells) leave the lymphatic tissue and enter the bloodstream, which raises immune cell levels temporarily in a process known as leukocytosis (Schneiderman et al., 2005). During leukocytosis, immune cells then migrate into areas most likely to be damaged during physical danger. However, when stress becomes chronic, elevated glucocorticoid levels within the body begin to have an adverse effect on the immune system and can slow the healing process of injuries or wounds. Increased levels of anti-inflammatory corticosteroids in the blood work to suppress the immune system and pro-inflammatory cytokines, weakening the body's ability to respond and defend against disease infection. While cortisol works as an immunosuppressant, it can also cause chronic inflammation. It has been suggested that with chronic stress, immune cells become resistant to cortisol through down-regulation of number of expressed cortisol receptors. Without the ability of cortisol to control the inflammatory response, stress continues to promote indefinite inflammation (Schneiderman et al., 2005).

1.3. Environmental Factors of Stress

d. Psychosocial Stress:

Psychosocial stress has been a growing field of study and has been associated with numerous health conditions. Until recently, much of the research into psychosocial environmental factors of stress has focused on humans. However, growing concern for animal wellfare has prompted interest in the environmental factors of stress in non-human animals, particularly our canine companions. In dogs, housing context plays an important role in cortisol levels. Predictability and safety of the environment are inextricably linked to the body's regulation of homeostasis and cortisol levels. Social and spatial restrictions significantly impact cortisol levels in dogs (Stephen and Ledger, 2006; Sandri et al., 2015). Dogs living in shelters, or that are privately owned and kenneled have shown higher levels of cortisol than those living in a home without space restrictions (Coppola et al., 2005; Beerda et al., 1998). In addition, home life in dogs has been shown to significantly affect cortisol levels. In a study conducted by Dreschel and Granger (2005), dogs living in single-dog households have lower basal cortisol levels than those in multiple-dog homes. However, when both groups were exposed to a stressor, the dogs from multi-dog homes did not show as great a change in cortisol levels as did those from single-dog homes. This would suggest that the dogs in multi-dog households possess a greater resistance to stress due to decreased sensitivity than those in only-dog households.

In addition to home environment, lifestyle impacts cortisol levels in dogs. In a study of the long-term cortisol response to stress, Roth et al. (2016) analyzed hair from 59 German shepherds. The findings concluded that competition dogs had higher cortisol levels than both working and companion dogs. In another study of working dogs, avalanche rescue dogs showed increased fecal cortisol levels in relationship to training and exercise-related activities (Slotta-Bachmayr and Schwarzenberger, 2007).

Another important psychosocial factor implicated in canine cortisol levels is human interaction. Activities with humans such as playing, grooming, petting and reviewing commands reduced cortisol levels significantly in shelter dogs (Coppola et al., 2005). Roth et al. (2016) found that positive interactions between dogs and their owners, as in during play or rewarding good behavior with a treat, correlated with decreased cortisol levels in the dogs. Human demands as related to lifestyle have also been implicated in affecting dog cortisol levels. Roth et al. (2016) points out that competition and working dogs are required to train and exercise more than is expected of those who are companions. An additional possibility for increased cortisol levels in working and competition dogs as proposed by Roth et al. (2016) is that these dogs may be selected by humans for such lifestyle purposes based on certain physiological or behavioral traits associated with high cortisol levels. While these studies on psychosocial environmental stressors in dogs provide valuable insight, all studies were conducted in at least semi-clinical settings with healthy dogs who have access to consistent veterinary care. There is a gap in knowledge as to how such factors may interact with cortisol levels in a population of dogs outside such settings, and without ready access to proper health care. In addition, current studies show a focus on purebred dogs which may impact results. Since the dogs at Bosawas are overwhelmingly of mixed-breed, they may provide important findings for breed-based differences in cortisol levels.

e. Climatic and Weather-Related Variation in Cortisol Levels:

Cortisol levels have been found to fluctuate with seasonal weather changes in humans and dogs (Roth et al., 2016 and Hadlow et al., 2014). In both studies, the highest cortisol levels occurred during the winter months, and the lowest during the summer. This fluctuation in cortisol is strongly believed to be connected to changes in the number of daylight hours that occur throughout the year. However, these changes are only noted in more seasonal regions of the world, and both the human and dog studies were conducted in the Northern hemisphere. Another proposed explanation by Roth et al. (2016) for the seasonal and climatic variations in cortisol levels in dogs was related to the coinciding changes in activity which occurred with the seasons. Roth et al. (2016) surmised that the observed changes in cortisol concentrations of competition dogs may be related to training and exercise schedules based around the seasonal schedule of competitions.

1.4. Hair Cortisol:

Cortisol is an ideal indicator of stress and overall health widely used in research because of its integral role in many regulatory functions of the body. The numerous physiological pathways cortisol acts upon give it a systemic presence within the body, making it possible to isolate and extract from a number of bodily products. Methods have been established to measure cortisol levels in samples including urine, feces, blood, saliva, and hair, validated in a number of mammals including humans (Binze et al., 2017 and Persson et al., 2008), rodents (Hayssen et al., 2002), domestic cats (Accorsi et al., 2007) and dogs (Beerda et al., 1998; Bryan et al., 2013; Sandri et al., 2015; Stephen and Ledger, 2007; Vincent and Michell, 1992).

As cortisol acts upon both short- and long-term processes within the body, the biological medium of sampling choice for cortisol concentrations is also important. Blood and saliva provide an immediate reading of cortisol levels in the minutes after stressing up to 1-2 hours (Vincent and Michell, 2002). However, restraints are often needed for use of both methodologies on animals, which is stressful for the individual and may cause an increase in cortisol levels. Feces and urine reveal cortisol concentrations over a short period of time which varies depending upon excretory patterns of the animal. In addition, urine samples can be difficult to obtain (Stephen and Ledger, 2006), and cortisol may not be evenly distributed in fecal matter (Hayssen et al., 2002), making both methodologies potentially unreliable.

Recognition of the advantages of using hair cortisol has been increasing, as this non-invasive method allows for the study of animals where other methods may prove unfeasible. For example, hair cortisol levels have been used to assess the impacts of anthropogenic climate and environmental changes on wildlife populations, particularly in nonhuman primates (Fourie and Bernstein, 2011). Research of hair cortisol in orangutans (Carlitz et al., 2014) and baboons (Fourie et al., 2015) has contributed to a growing understanding of how human interaction impacts other primate species. Studies have also been conducted on other wildlife species, such as grizzly bears (Macbeth et al., 2010), polar bears (Bechshøft et al., 2013), and Canadian lynx (Terwissen et al., 2013).

Hair is the only non-invasive and reliable way to obtain time-averaged basal cortisol levels, and concentrations of cortisol found in hair have been shown to correlate significantly to those obtained from feces (Accorsi et al., 2007) and saliva (Bryan et al., 2013). Cortisol is integrated into the hair shaft during hair growth through the capillaries that feed the hair follicle, and adjacent sebaceous and eccrine glands (Bryan et al., 2013). The hair shaft records chronological changes in cortisol levels over the length of its growth period and can be used to assess basal cortisol levels as well as elevated levels induced by chronic stress spanning months or years. Hair growth is controlled by a number of exogenous and endogenous factors, but the growth cycles of all hair can be broken into 3 phases. The anagen phase consists of active growth, followed by catagen, which is a short transition phase, before the hair enters a period of resting known as telogen, and finally, exogen, where the hair is shed to form a new hair follicle (Kligman, 1959). The length of each hair growth phase varies by species. In dogs, hair growth cycles have been found to last 3-4 months, and to be affected by temperature and photoperiod based on seasons (Diaz et al., 2004). A similar study on hair growth in dogs was also conducted on dogs living in the tropical climate of Brazil. This study concluded that the length of anagen and telogen phases was still affected by seasonal temperature variations, with the highest levels of hairs in the telogen phase during the summer months, and higher levels of hairs in anagen during the winter months (Favarato and Conceição, 2007).

Due to wide interspecies variation in patterning of hair growth and cortisol expression, the validation of hair as a method of obtaining cortisol concentrations must be performed for every species of interest. The measurement of hair cortisol in domesticated dogs has been validated by numerous studies (Bennett and Hayssen, 2010; Bryan et al., 2013; Roth et al., 2016). Within the process of validating hair, potential confounding variables, such as hair color, must be considered. According to Bennett and Hayssen (2010), in domesticated dogs, sable fur had significantly lower concentrations of cortisol when compared to hair that was yellow, white, or red in coloration. In addition, agouti hair did not significantly differ from either the dark or light coat coloring. The reasons for differences in cortisol levels of differently pigmented hair is not clearly understood, however the literature provides two hypotheses. First, since glucocorticoids are involved in the inhibition of hair growth during stress as well as melanocyte differentiation and development, differences in cortisol levels between hair pigmentation types may be due to differences in control mechanisms (Botchkarev, 2003; Slomski et al., 2004). The second hypothesis is that lighter hair has less pigmentation, therefore allowing for more room in the hair matrices for cortisol to be stored than in darker melanin-rich hair (Russell, 1948). This suggests that fur color may be a significant factor to account for while analyzing differences in cortisol levels of dogs.

Chapter 2: The Domesticated Dog in the Americas

The domesticated dog (*Canis lupus familiaris*) has long been a topic of scientific interest, sparking continued debate in the ongoing effort to reconstruct the process of domestication (Morey, 2005). Current genetic evidence suggests the domesticated dog is descended from wolves with origins somewhere in Europe or Asia (Savolainen et al., 2002; Skoglund et al., 2015). Additionally, the discovery of archaeological remains of dogs reflect ritualistic burial practices dating as far back as 14,000 years (Morey, 2005). Despite contention surrounding the exact timing and circumstances of domestication, the dog has been widely established as the most ancient domestic animal and is unique in its relationship to humans as the only domesticate present in ancient human societies on every continent (Clutton-Brock, 1995). Because of the established presence of dogs throughout human history, it comes as no surprise that with this relationship dogs have evolved a unique ability to read and interpret human social and physical cues (Gracsi et al., 2004; Hare and Tomasello, 2005). The social and physical skills of domesticated canines have proved useful for humans in a number of ways, including transportation, protection, herding, and perhaps most importantly, in hunting (Koster, 2009).

2.1. Archeological Evidence of Dogs in the Americas

Dogs have been, and continue to be, present in subsistence hunter societies spanning a wide range of environmental and geographical settings, across both the Old and New World. In the Americas, pre-Columbian archaeological and historical data provide the earliest evidence of dogs dating back 10,000—8500 years ago (van Asch et al., 2002; Leonard et al., 2013). It has been widely theorized that the domesticated dog was first introduced to the Americas from the Old World by migrating south Siberian peoples who first arrived following the deglaciation of the pacific coastal corridor some 15,000 years ago (van Asch et al., 2013; Morey, 2005; Leonard et al., 2002). As Paleo-Indian populations continued to migrate so did their canine domesticates, subsequently proliferating across Central and South America. These dogs were then widely replaced by a new wave of domesticated dogs brought by European colonists in the post-Columbian era (Leonard et al., 2013). Consequently, the modern domesticated dogs found across North, South, and Central America are descendants of the interbreeding between ancient native and European dog populations (van Asch et al., 2002; Leonard et al., 2013).

Evidence of domesticated dogs has been found in North and South America predating written history (Koster, 2009). Yet in the lowland Neotropics of central America, the historical presence of dogs is more obscure. Evidence of dogs predating history in the Neotropics is scarce, and it appears many Amazonian societies did not have dogs until the historical period following contact with European explorers (Koster, 2009). The relative recency of dogs to the region has not gone unnoticed and has generated interest among anthropologists as to why this might be. Suggested explanations include that indigenous populations may have simply declined to adopt dogs when presented with the opportunity, as the dogs may not have been proficient enough at hunting the local prey and the cost of resources in caring for them too great. Instead, it has been suggested that local communities opted for other hunting technologies such as firearms which were also introduced at the time (Koster, 2009). However, this is refuted by research on the hunting ability of dogs in neotropical settings which finds their success rates comparable to those of hunting with rifles, making them a valuable tool (Koster, 2007). Another more likely explanation proposes that the high mortality rates which are common in neotropical environments may have prevented their diffusion into the region (Koster, 2009; Stahl, 2013). Although domesticated dogs are a relatively more recent introduction to the Neotropics than elsewhere in the Americas, their presence is now widespread among Amazonian indigenous communities (Koster, 2009). This has attracted attention from the archaeological and anthropological communities, as well as from the fields of genetic and ecological research, whose focus centers on the potential impacts of domestic dogs on wildlife management and conservation efforts (Koster, 2007). Thus, this thesis will contribute valuable knowledge towards a greater interdisciplinary understanding of the interactions and relationships between humans and dogs in the lowland Neotropics of Central America.

2.2. Ethnographic Description and History of the Mayangna/Miskito Communities at Bosawas

This study focuses on the hunting dog population within the indigenous Mayangna and Miskito communities living on the Bosawas Biosphere Reserve. Located in the northern region of Nicaragua, Bosawas was created by presidential decree in 1991 before being designated as a UNESCO biosphere reserve in 1997 and is part of the largest intact tract of lowland tropical rainforest north of the Amazon (Stocks, 2003). According to the Nicaraguan government, the reasons for creating the reserve were to conserve wildlife and protect the natural resources and cultural heritage of the Mayangna and Miskito peoples inhabiting the region (Stocks, 2003). The Mayangna and Miskito are two of Nicaragua's predominant indigenous ethnic groups. Historically, both communities remained isolated from outside influence, settling in the Bosawas region to avoid interaction with others (Stocks, 2003). The Mayangna and Miskito have similar traditional subsistence practices, relying on swidden horticulture for most of their nutritional needs. They are also known to keep farm domesticates such as chickens, cattle, and pigs, but a majority rely on hunting and fishing for their primary source of protein (Koster, 2007).

2.3. Hunting with Dogs in Miskito and Mayangna Communities

The sample population of hunting dogs included in this study come from Miskito and Mayangna communities located in the Bocay River Valley watershed (See Figure 2.1). The Bocay River is a part of a larger interconnected system of rivers, including the Coco River and the smaller Lakus and Amaka Rivers, which form a natural border between Bosawas and Honduras. These rivers are home to a handful of Mayangna and Miskito villages, which are characterized by a heavy reliance on dogs for hunting. Ethnographic research conducted on the Miskito and Mayangna villages of Arang Dak and Suma Pipi reported that approximately 85% of the harvested animals are captured with the help of dogs (Koster, 2008b), which is unique among indigenous lowland neotropical societies. Further research shows that hunting at Arang Dak and Suma Pipi are almost exclusively male activities, however women will use dugout canoes to opportunistically pursue deer and other animals that have been chased into the river by dogs (Koster, 2012). The primary tools used for hunting are dogs and rifles, although hand held weapons such as axes, machetes, or lances are also common (Koster, 2008a). The number of dogs on an expedition varies, depending on how many dogs a hunter owns that are able to hunt (Koster, 2009). During hunting trips, dogs will disperse in search of game, barking to alert their owners and other dogs once they detect prey. Once the dogs have cornered the animal, usually in a hollow tree or underground burrow, the hunter will block the exit with sticks before cutting or digging their way in until they can reach and dispatch their prey with lances or machetes. In the event that the pursued animal flees into water and attempts to swim away, hunters will follow and attack with a wide variety of hand held or projectile weapons (Koster, 2008a). Dogs are usually brought along on hunting forays, which are often opportunistic and rarely planned for the pursuit of a specific type of game. Except for a few species, dogs are generally beneficial in hunting most game on the reserve. Dogs are not useful when hunting monkeys or birds as the prey are often alerted to the hunters' presence by the noise of the dogs before they can get close (Koster, 2008b). The types of prey most commonly harvested with the use of dogs are agouti, armadillo, paca, iguana, and collared peccary. Dogs are also used in the pursuit of larger prey such as deer or tapir, but such events are comparably rarer (Koster, 2008b).

Figure 2.1. Map of Nicaragua with Bocay River watershed outlined in red.

Households acquire dogs in multiple ways, including the breeding of their current dogs. Puppies may also be given as gifts, often between related households. In addition, both adult dogs and puppies may be bought or sold, and a dog with a reputation for being particularly good at hunting can fetch a high price (Koster, 2012). There is little by way of formal training for hunting dogs. Instead, owners will bring them along on hunting excursions once they are old enough in the hopes that they will learn from watching and imitating the other dogs. A dog's skill level is assessed on the size of their prey, and thus dogs that are capable of taking down larger prey like collared peccary or tapirs are considered better hunters than those that catch only smaller prey like pacas or agouti. Further study of the hunting ability of dogs at Arang Dak and Suma Pipi found that older dogs and male dogs are associated with greater harvests of game. This may be due to increased levels of experience from time spent hunting, or to larger size and ability to take down bigger prey (Koster, 2012).

2.4. Description of the Hunting Dog Population at Bosawas

Dogs are fed portions of the same foods prepared for the household, however they will commonly seek to scavenge around the community for additional food. Milk may also be purchased for puppies which have been separated from their mothers (Koster, 2009). Both the purchase and sharing of household food resources with dogs suggests the importance of these animals within the family. However, further evaluation of the health status of dogs at Bosawas has indicated both malnutrition and dehydration are common among the population (Fiorello et al., 2017). While the consumption of dogs has been documented in other neotropical societies, there is no evidence that the Mayangna and Miskito view dogs as a food source (Koster, 2012).

In addition to malnourishment, common sources of mortality in adult dogs are injuries incurred while hunting from jaguar attacks and snakebites (Koster, 2012; Fiorello et al., 2017). A high prevalence of disease within the dogs of the communities also contributes to the mortality rates seen in the population. One study found evidence of canine distemper, canine parvovirus, *Rickettsia reckettsii*, and *Leptospira* spp. in over 50% of the dog population at Bosawas (Fiorello et al., 2017). Life expectancy for hunting dogs at Bosawas is short, due to the high risk of illness or injury, and the oldest dogs observed have been 6-8 years of age (Koster, 2007). Figure 2.2: A-C below depict the state of the dogs at Bosawas.

A: 

B: 

C: 

Figure 2.2. A, B, and C: Hunting dogs from Bosawas, Nicaragua.

2.5. Hypotheses

The importance of dogs in hunting makes them invaluable assets to the Miskito and Mayagna communities at Bosawas, and this relationship is potentially negatively impacted by the poor health of the dogs. Shorter lifespans and increased likelihood of illness and injury may significantly diminish a dog's productivity while hunting, thus increasing the risk to investments made by the owners in raising and providing for the animals. Cortisol offers another approach to help fully understand how these dogs respond and adapt to challenges in their environments. In addition, analyzing the cortisol levels in this population of dogs provides a valuable opportunity to test currently published findings on the energetics of stress in dogs in an environment that is not highly controlled and with a much larger and unlimited population. According to the findings of Bennett and Hayssen (2010), I hypothesize that: 1) HCCs in dogs with light coat colors will be significantly higher than those of dogs with dark or sable coats, while HHCs in dogs with agouti hair coloring should not be significantly different from either group, and 2) I hypothesize that I should see no significant differences in HCC between male and female dogs at Bosawas, given that these differences have not been found in previous studies (Sandri et al., 2015; Stephen and Ledger, 2006).

Given the patterns of covariation in metabolic rate, and cortisol levels that have been previously demonstrated in dogs, I hypothesize that: 3) the size of dogs will be negatively correlated with HCC, and specifically that 4) measures of chest breadth and circumference will be more closely related to HCC than height or head width, given the former measurements reflection of overall mass (a marker of current energetic condition) as well as prior skeletal growth.

In addition to size and metabolic rate, nutritional health is also closely related to cortisol levels. Based on the literature published about the hunting dogs living in the Mayangna and Miskito communities at Bosawas, the nutritional status of the dogs is likely poor. According to veterinarians, a body condition score (BCS) of 4 to 5 is ideal, while anything below is considered underweight, and anything above is overweight or obese (Karr-Lilienthal, 2013). The majority of dogs in the Bosawas population are scored under 5, therefore, I hypothesize that 5) the nutritional status of the dogs as described by their BCS score will be a better predictor of HCC than size, and that BCS and HCC will show an inverse relationship.

Chapter 3: Materials and Methods

3.1. Study Site

This study was conducted in three predominantly Mayangna communities located in the Bocay River watershed on the Northern border of the Bosawas Biosphere Reserve (Figure 3.1). The town of Amak, located between the Bocay River and Amaka River tributary (N14.065417, W85.142233), Ahsa Was (N14.144835, W85.23464302049297) and Pulu Was (N14.225, W85.12057256608806) (Koster 2019, personal communication; Fiorello et al., 2017). In this remote region the villages are extremely isolated, making travel by water the only feasible way to reach them (Koster and Tankersley, 2012; Fiorello et al., 2017).

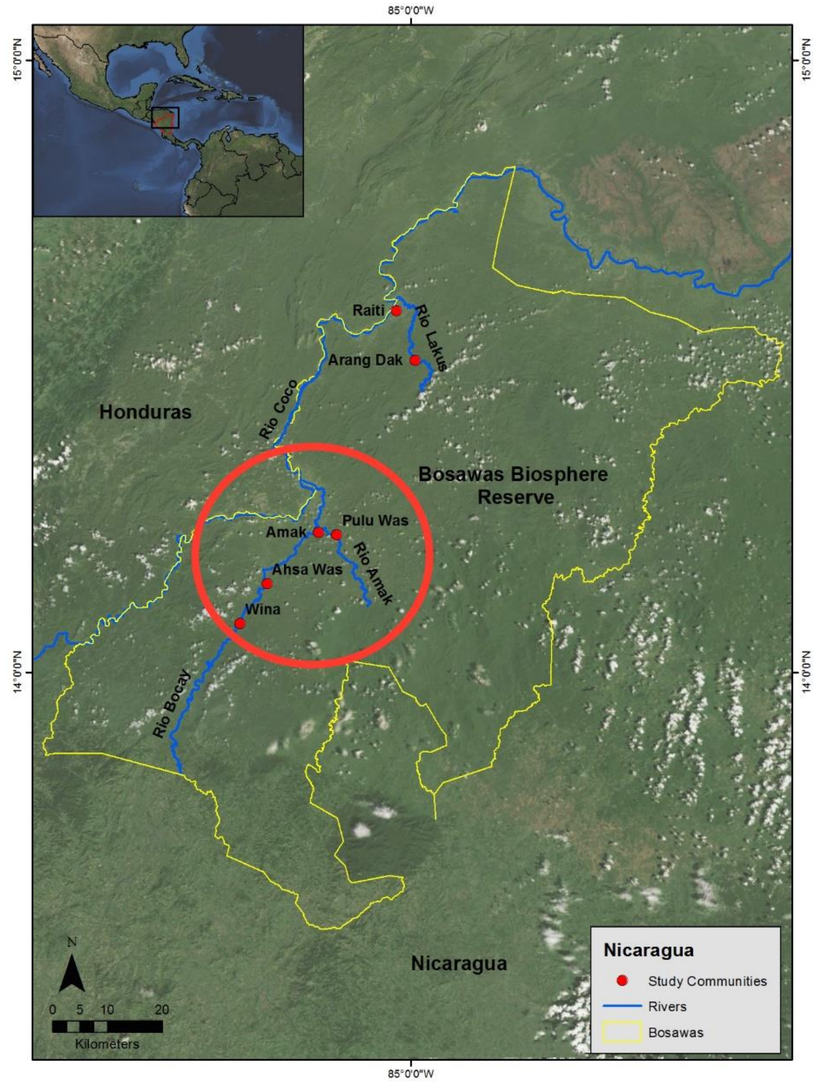


Figure 3.1. Map of the Bosawas Biosphere Reserve with Mayangna and Miskito communities; Amak, Pulu Was, and Ahsa Was are circled in red.

3.2. Dog Recruitment

This study is a part of a larger ongoing project conducted by Dr. Jeremy Koster, Associate Professor of Anthropology at the University of Cincinnati, Ohio. His work on hunting dog populations at Arang Dak and Suma Pipi and the Bocay River watershed has included a multidisciplinary collaboration with a team of veterinary students led by Professor of Veterinary

Medicine Dr. Maris Brenn-White DVM, MPVM from the University of California (UC), Davis. The teams which visited Amak, Pulawas, and Ahsa-was were comprised of veterinarians from UC Davis, a local assistant to aid in interpreting and communication, and sometimes Dr. Koster and/or Dr. Brenn-White. Voluntary participation and consent were obtained verbally from community members who were willing to have their dogs examined by the veterinarians. Some modest compensation was provided for the time owners dedicated to bringing their dogs for examination.

3.3. Physical Examinations and Sample Collection

A small group of veterinary students examined each dog, collecting and recording information as specified by a form. Information recorded on the form includes general physical and behavioral attributes, vital measurements, sex, and appearance of health including any medical problems present. To determine the size of the dogs, measurements (cm) were obtained for height, body length, chest-width, chest-girth, and head-width. In assessing the nutritional status of each dog, a numerical value was assigned using the Body Condition System (BCS) scale of 1 to 9. An image of the form used for conducting exams and recording information on the dogs, as well as an infographic of the BCS used is included in Appendix A. A scale for measuring weight was not available. Instead, body measurements (cm) were obtained to determine size (Table 1).

Table 1: Measurements used to determine size of dogs

	Chest Girth	Head Width	Body Length	Chest Width	Height
Point of measurement	At widest point	At widest point	From head to base of tail	At widest point	At shoulder blades

Hair samples were also obtained during examination by cutting a small chunk of fur from the coat using scissors or shears. However, the location from which the hair sample was taken was not specified on the form, and so was likely not standardized. The owner of each dog was also interviewed to obtain information on the age and medical, familial, and general life history of the dog. Another form was used to record this information, which is included for reference in Appendix A. Table 2 shows the compiled demographic data used to analyze the HCC results.

Table 2: Demographic data with assigned abbreviations and values.

Body Condition Score (BCS)	Dog ID	Dog Record ID	Visit date	Sex	Age	Fur color
1-9	From 1 to 577	From 4000 through 6200	Month/day/year	Male (M) or Female (F)	In years or months	Mixed or agouti (M); Dark or sable (D); Light—yellow, red, or white (L)

Exams in which hair samples were collected from the dogs were conducted annually during the months of July and August from 2014 through 2016. During this time, approximately 750 hair samples were collected, and a total of 580 dogs from Amak, Pula-was, and Ahsa-was participated in the study. Due to errors in labeling of hair samples, insufficient sample weights, and sample loss during processing, seventy-six samples (~10%) were unusable (final study

N=672). Of the total sample population of dogs, 213 were female and 240 were male. Ages as reported by owners ranged from 0.08 to 13 years, with a mean of 2.86 years (*SD*=2.17). When possible, the team attempted to examine and collect hair samples from the same dogs during each of their visits, thus providing a set of longitudinal data on these subjects (repeat sampling population *N*=396). However, it was not always possible to include the same dogs all three years as some were no longer present. This may have been due to dogs being lost, deceased, or traded away between visits. Another reason may have been misidentification of individuals by the veterinarians during examinations. The resulting dataset presents a mixture of varying data points collected on each participating dog in the sample population. During each exam, dogs were assigned a 4-digit identification number called a “Dog Record ID”. A new Dog Record ID number was assigned to the dog with each subsequent exam they participated in and written in connection to their name. These data were then transformed when entered into the master data set and the dog’s name was replaced with another number labeled “Dog ID” to keep the Dog Record ID numbers linked with the appropriate individuals they were assigned to.

3.4. Dog Hair Cortisol Processing

Upon collection, each hair sample was placed in a paper envelope labeled with the Dog Record ID number and date of exam. The samples were then shipped to the laboratory of Dr. Robin Bernstein at the University of Colorado, Boulder for processing and analysis. I processed all hair samples and Dr. Bernstein analyzed the samples. Samples ranging from 10 to 30 mg of hair were weighed and placed into 2mL Eppendorf tubes. Weights were recorded along with the corresponding ID number and date, as well as the fur color. As noted in Table 2, fur color was denoted as either “D,” “M,” or “L” and categories were defined according to the parameters used by Bennett and Hayssen (2010). Each hair sample was then washed a minimum of three times with 1.5 mL isopropanol to ensure all dirt, dead skin, and sebum were removed. Samples were left to air dry under a fume hood for approximately 2 days to ensure complete evaporation of the isopropanol. The hair was ground by placing a stainless steel or tungsten carbide ball in each tube and using a Ball Mill at 25 Hz for 10 minutes. Samples where hair was not sufficiently ground were placed back on the machine and ground for an additional 5 minutes. After grinding, 1 mL of methanol was added, and samples were vortexed before being incubated on a shaker plate (~100 rpm) over night. The following day, samples were centrifuged for 12 minutes at 2500 rpm. 850 uL of supernatant was extracted and transferred to a clean Eppendorf tube. The supernatant was then evaporated using a Microvap nitrogen evaporator (Organomation, Berlin MA)—a machine with a heated block at around 63C and drying needles which blow nitrogen gas down over the supernatant to speed up the drying process. Samples were placed on the Microvap for ~16 minutes. If at the end of the drying time some liquid was still present, samples were left for a few more minutes. Samples were then reconstituted using 0.2 mL EIA buffer solution and stored at -20C until processing.

Hair cortisol concentrations were quantified using enzyme-linked-immunosorbent assays (ELISA- Salimetrics, Carlsbad CA), validated for use in measuring cortisol in dog hair (data not shown). Results were converted from ng/mL to ng/mg for statistical analysis.

3.5. Statistics

Data Treatment: HCC and size measurements were $\log_{10}$ transformed to correct for non-normal distribution, as well as to account for differences in measurement units (e.g. cm, ng/mg, etc.). To determine the overall size of individual dogs, the geometric mean of the combined height, body length, chest width, chest girth, and head width measurement data was calculated.

BCS data were analyzed as ordinal and continuous to assess whether data classification would affect the results. No significant difference was observed between the two, and BCS was treated as a continuous variable and $\log_{10}$ transformed to normalize the data distribution.

Statistics: Data analyses and statistical testing were all performed using JMP Pro 14. All factors which may have had a possible effect on HCC values were identified as sex, age, fur color, BCS score, and size measurements (height, chest width/girth, body length, etc.). Due to the large amount of error in age reporting by owners, as well as the abundance of confounding variables, age as an effect on HCC was not possible to determine in this study and so was not used in further data analyses.

Differences in HCC values by sex and fur color were tested for significance using ANOVA and T-tests. For all ANOVA and T-tests, Dog ID was weighted as a uniform random variable to control for repeat measurements taken from some of the dogs. Mixed models were employed to evaluate the relationship between HCC and BCS scores, as well as the aforementioned size measurements to determine the strength of the relationship between all variables and HCC. HCC and Measurements of size were also cross-referenced with sex of the individual dogs using ANOVA.

The relationships between hair cortisol levels, fur color, BCS and size were analyzed using 2-way ANOVA and multiple linear regression. Dog ID was used as a random effect variable to control for repeat measurements within the data set. Fur color and BCS were entered as fixed effects, and geometric mean (GM) size was included as a covariate. Macros were set to factorial to degree (2) to include both main effects and interactions between effects within the models.

The strength of the relationships between HCC and individual size measurements (height, body length, head width, and chest girth and width) were examined using bivariate fit plots. Dog ID was included as a random uniform weight variable to account for repeat measures.

Chapter 4: Results

4.1. Cortisol and Fur Color

The relationship between fur color and hair cortisol concentration (HCC) was tested using ANOVA, which indicated a significant difference ($F(2, 667)=20.88$, $p<.001$) in cortisol concentrations was present between light (L), mixed/agouti (M), and dark (D). Post hoc testing using all pairs Tukey-Kramer yielded a significant difference in cortisol levels between light ($M=20.30$, $SD=13.17$) and dark ($M=16.66$, $SD=7.42$) ($t(667)=2.35$, $p<.001$), and light and mixed/agouti ($M=16.31$, $SD=4.99$) ($t(667)= 2.35$, $p<.001$) fur color (See Figure 4.1). There was no significant difference in HCC detected between D and M fur coloration (See Figure 4.2). A statistical summary table of HCC means, std deviations, and ranges for each fur color is included in appendix B.

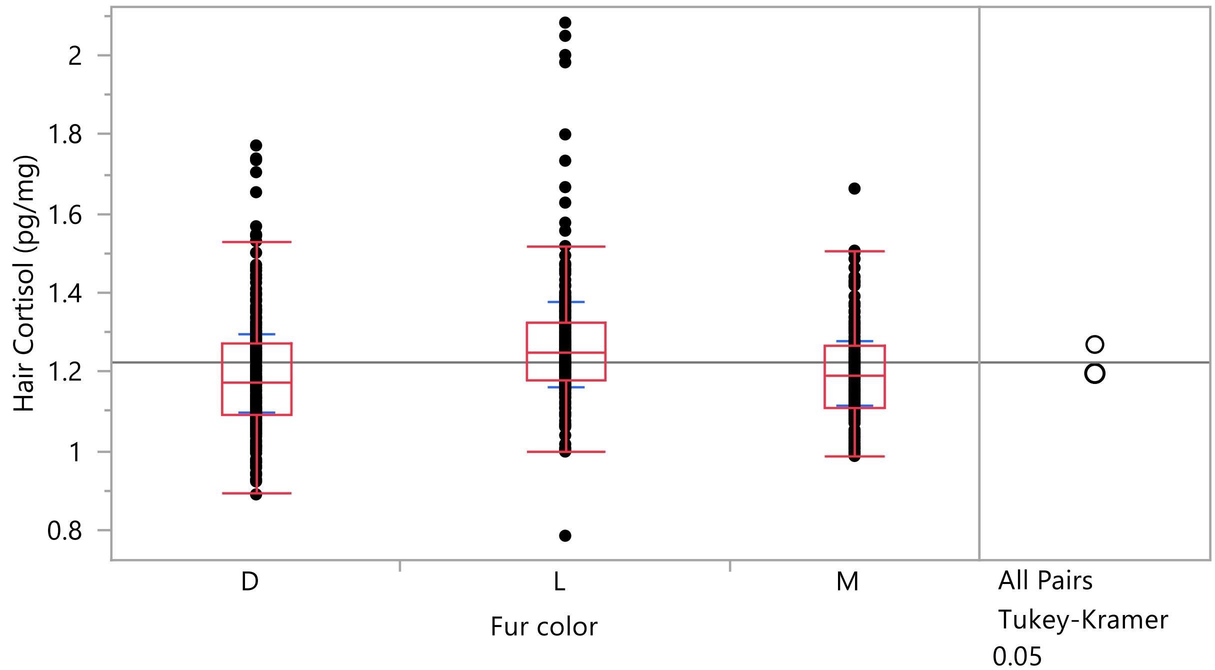


Figure 4.1. Scatter plot with box plot overlay of HCC in dark, light, and mixed fur color.

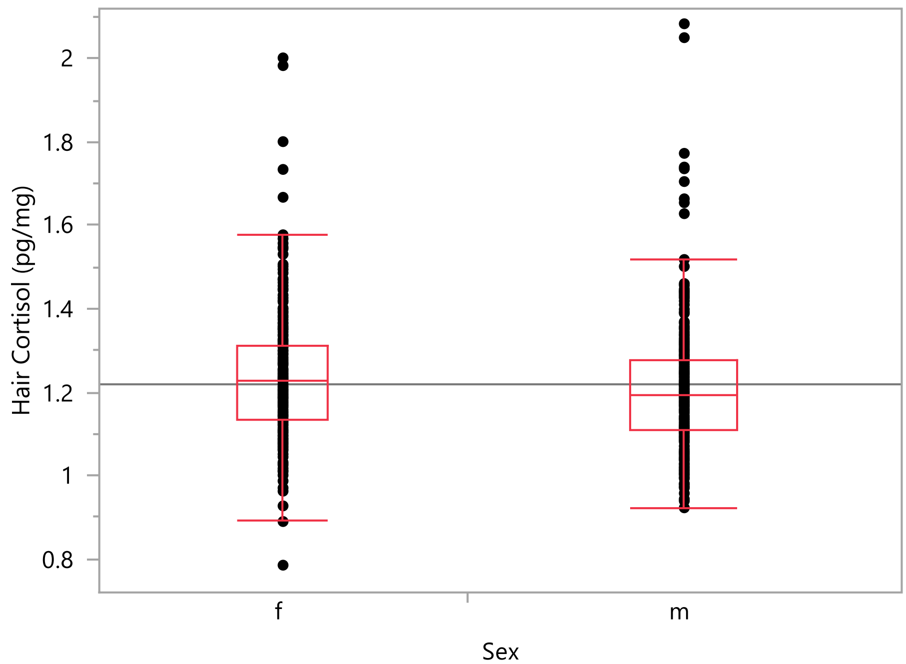
Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
L	D	0.0733128	0.0130478	0.042664	0.1039615	<.0001*	
L	M	0.0730740	0.0135872	0.041158	0.1049897	<.0001*	
M	D	0.0002388	0.0139802	-0.032600	0.0330775	0.9998	

Figure 4.2. Post hoc testing results for HCC differences between dark, light, and mixed fur color.

4.2. Hair Cortisol and Sex

Pooled t-Test of HCC between male and female dogs showed a significant difference between the sexes ($t(668)= -2.6$, $p=.0047$). On average, female dogs had higher HCC than males ($M=18.43$, $SD=9.17$ vs. $M=17.34$ $SD=10.04$) (See Figure 4.3). A statistical summary table of HCC means, std deviations, and ranges for males and females is included in appendix B.

To investigate whether differences in HCC levels between males and females was its potentially be related to size, ANOVA was run on GM size by sex (See Figure 4.4). The results of the analysis indicated that males ($M=24.41$, $SD=2.62$) were significantly larger than females ($M= 22.42$, $SD=2.4$) ($F(1, 575) = 48.58$, $p<.001$).



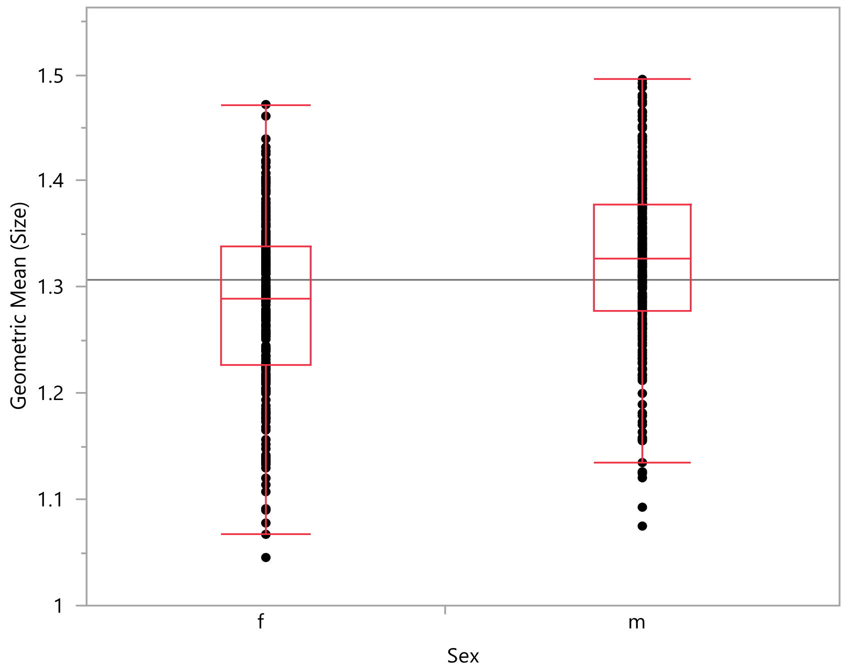
Pooled t Test

m-f

Assuming equal variances

Difference	-0.03044	**t Ratio**	-2.60479
Std Err Dif	0.01169	**DF**	668
Upper CL Dif	-0.00749	**Prob > |t|**	0.0094*
Lower CL Dif	-0.05339	**Prob > t**	0.9953
Confidence	0.95	**Prob < t**	0.0047*

Figure 4.3. Scatter plot with box plot overlay and statistical comparison table of HCC in male and female dogs



Pooled t Test

m-f

Assuming equal variances

Difference	0.044792	**t Ratio**	6.969745
Std Err Dif	0.006427	**DF**	575
Upper CL Dif	0.057414	**Prob > |t|**	<.0001*
Lower CL Dif	0.032169	**Prob > t**	<.0001*
Confidence	0.95	**Prob < t**	1.0000

Figure 4.4. Scatter plot with box plot overlay and statistical comparison table of GM size by sex

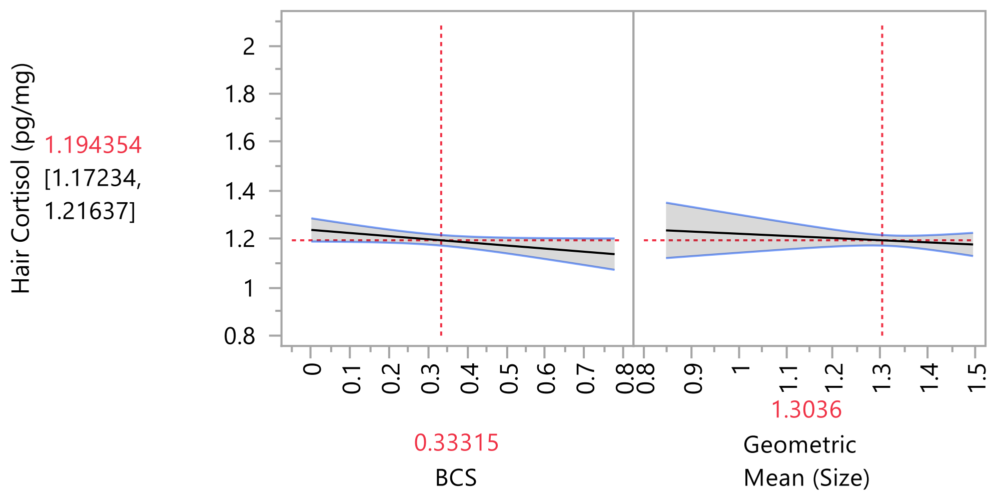
4.3. Hair Cortisol, BCS, and Size

2-way ANOVA and multiple linear regression were run using BCS and fur color as main effects, and GM size as a covariate. Models including interactions between BCS, fur color, and GM size were also run. ANOVA results found that BCS and fur color had a significant effect on HCC (($F(1, 560.8)=13.77$, $p<.001$) and ($F(2, 512)=12.79$, $p<.001$), but GM size did not. In addition, none of the variables were indicated as having an effect on one another (See Figure 4.5). BCS and GM size were both negatively correlated with HCC (See Figure 4.6 A).

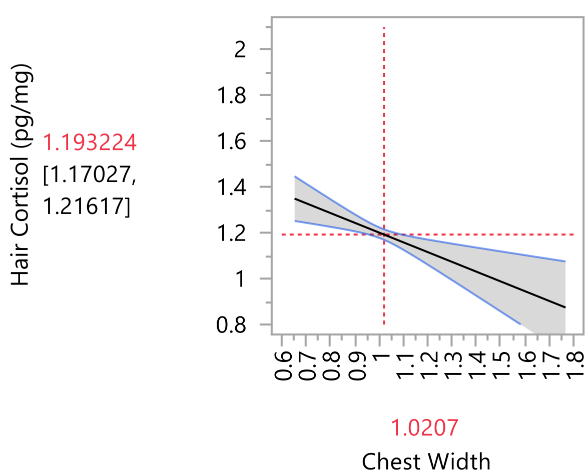
Individual measurements of size were also analyzed to assess the comparative strength of relation with HCC. Height ($F(1, 580)=5.21$, $p=.023$), and chest girth ($F(1, 578)=9.7$, $p<.001$) and width ($F(1, 579)=6.12$, $p=.014$) were found to be most strongly correlated with HCC. These 3 measurements were then added to the multiple linear regression model above to explore potential interactions with variables of BCS and fur color. Results indicated that chest width had a significant effect on HCC ($F(1, 549.1)=31.55$, $p<.001$), while chest girth and height did not. Like BCS and GM size, chest width was negatively correlated with HCC (See Figure 4.6 B).

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Fur color	2	2	512	12.7872	<.0001*
Log10[BCS]	1	1	560.8	13.7732	0.0002*
Geometric Mean[...10[ChestWidth]]	1	1	526.7	1.4665	0.2264
Fur color*Log10[BCS]	2	2	560.1	0.2438	0.7837
Fur color*Geometric Mean[...10[ChestWidth]]	2	2	556.7	0.0118	0.9883
Log10[BCS]*Geometric Mean[...10[ChestWidth]]	1	1	559.9	0.0657	0.7978

Figure 4.5: Effects Tests ANOVA results for BCS, GM size, and fur color on HCC



A:



B:

Figure 4.6. Graph of HCC trends with shaded confidence intervals: *(A)* HCC by BCS and GM size; *(B)* HCC by Chest Width.

Chapter 5: Discussion

5.1. Fur Color and Hair Cortisol

The first purpose of this study was to evaluate the findings of previous studies on factors which may affect cortisol levels within hair. Based on the findings of Bennett and Hayssen (2010), as well as current theories as to the mechanisms behind cortisol integration into the hair follicle, we expected to see differences in cortisol levels between light (L) and dark (D) hair, but not in relation to agouti/mixed (M) hair. The results of our study on the dogs at Bosawas did yield a significant difference between light and dark fur color, supporting our hypothesis and the previous findings of Bennett and Hayssen (2010). However, our results also indicated a significant difference between cortisol in light fur and agouti/mixed fur, although there was no detected difference between dark and agouti fur color. Light fur had the highest average cortisol levels (M=20.30, SD=13.17), while dark (M=16.66, SD=7.42) and agouti/mixed (M=16.31, SD=4.99) were lower. These findings that lighter hair possesses higher HCC could support the theory that, because lighter hair has less melanin, the hair shaft matrices offer more room to store cortisol than those of darker hairs possessing more melanin. However, this theory does not take into account the fact that cortisol is a fat-soluble molecule, while melanin is water-soluble, meaning that they would not be stored within the same location matrices of the hair. Another potential theory has suggested genetic pleiotropy in which control for hair pigmentation and structure of hair shaft matrices is regulated by a single gene. This may explain the differences in cortisol levels and fur color more thoroughly than the aforementioned theory, however more research as to the specifics of this relationship are required.

This study also has implications for hair cortisol research beyond domesticated canines. Studies of hair color and cortisol concentrations in humans have reported that darker hair has higher levels of cortisol than lighter hair (Binz et al., 2017). The findings from our study are in direct conflict with this publication, which may point to additional confounding variables affecting the levels of cortisol within hair. Studies of hair cortisol in humans have also been interested in exploring the links between chronic socioeconomic inequality often defined by race. Increasing attention has been paid within the fields of genetics and epidemiology to the impacts of historical racism on the health of contemporary peoples of color. It may be that the prejudice and persecution still faced by people of color today has a greater effect on HCC levels than the hair color itself. It may also point to a potential difference in genetic mechanisms which control for hair color and cortisol retainment between humans and domesticated canines. Identifying further confounding variables and mechanisms at play within the issue of hair color and cortisol retention is an important point for further research moving forward.

5.2. Hair Cortisol and Sex

Another aim of this study was to further evaluate the relationship between cortisol and sex in domestic canids. Literature on the subject of sex differences in cortisol levels have reported varying results by species. As noted in the background, there is a large amount of published research regarding humans and other primates which suggests that cortisol differences in hair do indeed exist between the sexes. However, studies of other cortisol-dominant mammal species did not find such results, and the literature on the subject in domesticated dogs has been contentious at best. Due to the lack of consensus, and no strong findings of a relationship between hair cortisol differences by sex in dogs, I hypothesized that there would be no significant difference in our study of the dogs at Bosawas. However, our results did yield a significant difference in HCC between male (M) and female (F) dogs, and this hypothesis turned out to be unsupported by our findings. Female dogs at Bosawas had higher average HCC levels than males (*M*=18.43, *SD*=9.17 vs. *M*=17.35, *SD*=10.04). After transforming the data using Log_{10} to normalize the distribution, this difference was shown to be significant. One potential explanation for this difference in HCC between males and females could be due to sexual dimorphism. Current research on the energetics of stress and cortisol have found that metabolic rates of cortisol are affected by size, where smaller animals have higher levels of cortisol than larger animals. As female dogs tend to be smaller than males, the observed differences in HCC between sexes may be due to metabolic and size variations. To test this, HCC and size (geometric mean of size measurements taken from the dogs) were analyzed in relation to sex. The results of the ANOVA indicated that on average, males (*M*=24.41, *SD*=2.62) were significantly larger than females (*M*= 22.42, *SD*=2.4) ($F(1, 670) = 0.016$).

Another potential reason for the observed differences between male and female HCC could be reproductive. Energetic stress on females during pregnancy is high and continues to be elevated during lactation due to the continued nutritional demand from offspring. The length of gestation in female dogs is approximately 63 days, and lactation between 1-2 months on average (Fontaine, 2012). This extended period of hypercortisolism would likely be reflected within the hair and could account for the differences seen in cortisol levels between male and female dogs at Bosawas. Data were collected on reproductive history of the female dogs at Bosawas, including the date of last birth and litter size. However, these owner-reported data were largely incomplete, leaving room for significant error. Due to data discrepancies, analysis of cortisol levels in female dogs in relation to pregnancy, lactation, and litter size was not conducted. Yet, further research into this relationship could prove useful in the future. The sub-population of dogs from this study which were resampled each year would be especially useful for this inquiry. If hair was resampled from the same site, the new regrowth each year following the initial fur sampling would provide information on cortisol levels from a clear measure of time. If any of the resampled dogs had been pregnant during the year between sample collections, the HCC results should reflect that.

5.3. Relationship of BCS and Size with Hair Cortisol

The third aim of this study was to examine previous findings regarding the energetics of stress in cortisol-dominant animals. Prior literature has noted covariation between size and cortisol levels where cortisol levels decrease as size increases (Charmandari et al. 2005; Chrousos, 2007; Haase et al., 2017). Therefore, we hypothesized that the geometric mean of size (GM size) measurements taken from the dogs at Bosawas would have a negative relationship with HCC. Our results indicated that HCC was negatively impacted by size, which supported our hypothesis and is concurrent with previous findings. However, an important caveat to these assessments is the non-standardization of body region for hair sampling. Previous research has shown that cortisol levels can vary significantly among regions of the body, making it important to establish a standard methodology for collection (Terwissen et al., 2013). Therefore, the results of our analyses may be affected by the lack of control in HCC variation by body region.

While overall size may be a reliable predictor of cortisol levels in healthy animals, in populations which are chronically malnourished, size may not be as strong a predictor. Thus, individual size measurements in relation to HCC were also compared with GM size to evaluate the strength of the relationship between overall size and HCC. Because of high levels of malnutrition at Bosawas, we hypothesized that the individual size measurements of chest width and chest girth would be more significant predictors of cortisol levels than GM size due to their potential to be most affected by the body's fat storage patterns. The results of our data analysis did indicate that chest width ($F(1, 549.1)=31.55$, $p<.001$) had a significant effect on HCC levels, whereas GM size did not appear to significantly effect on HCC. Chest width was also negatively related with HCC, and although insignificant, so was GM mean. This suggests measurements of chest width may serve as a more reliable marker of skeletal development than overall size when nutrition is compromised.

To further examine stress energetics in the dogs at Bosawas, the link between nutritional health and HCC was also investigated. By using the dogs' BCS scores as a proxy for health status, HCC levels were analyzed. It was hypothesized that cortisol levels would be negatively correlated with BCS scores, as cortisol appears to increase with malnutrition. The results of the analysis yielded a significant negative effect of BCS ($F(1, 544.5)= 29.51$, $p<.001$). These findings support our hypothesis, are congruent with those of prior studies and publications on the energetics of stress. Additionally, since the dogs at Bosawas appear chronically undernourished, BCS was also expected to be more strongly related to HCC than measurements of size. Although BCS was more significant than GM size, chest width appeared to be equally statistically significant with BCS (both $p<.001$). The results indicate that BCS possesses a significant inverse relationship with HCC, suggesting the variable may be a reliable way to measure cortisol. However, further analysis of BCS and comparison with other measures of nutrition are needed to assess the relative level of significance to nutritional stress. As a definitive link between BCS and nutrition has not been made, these results may not be the strongest assessment of HCC and nutritional status. Perhaps analysis of nutritional stress using measurements of weight by overall size measurements could prove more significantly related to outcome of HCC. In addition to BCS, chest width was also shown to have a significant negative effect on HCC. Since overall size was not found to be a significant variable in HCC, measurements of chest width may prove more effective in nutritionally compromised populations for use in assessing HCC outcomes. Furthermore, if chest width is a stronger indicator of the effect of mass on HCC than GM size, the experimental process can be greatly simplified and expedited by collecting only measurements for chest width.

5.4 Comparison of HCC from Dogs at Bosawas with Prior Study Populations

To explore how cortisol levels may relate to environment, our results from the dogs living at Bosawas were compared to those from previous studies which took place in Switzerland and Italy. Table 3 below reports the summary statistics from two prior studies (Bennett and Hayssen, 2010 and Roth et al., 2016) on hair cortisol in dogs and compares them with those from our study.

Table 3: Comparison of HCC results from the dogs at Bosawas and prior studies

Study	**N**	**Range (pg/mg)**	**Std Dev. (pg/mg)**	**Mean (pg/mg)**
Bennett and Hayssen (2010)	47	4.56-27.09	+/- 5.45	12.63
Roth et al. (2106)	94	~6-54	(N/A)	~15
Bosawas Population	670	6.1-121.2	+/-9.63	17.88

As the table above illustrates, the means of the three studies did not differ by much, and all remained between 10-20 pg/mg of hair cortisol. The similarity in HCC results between the dogs at Bosawas and those from the other studies could be due to chronic stress experienced by the Bosawas population. Under extended stress, adrenal fatigue occurs, and the body cannot continue to produce high levels of cortisol. This makes it difficult to use basal-cortisol levels as an indicator when assessing differences between healthy and chronically stressed populations. In addition, during periods of chronic hypocortisolism, the body's response to a stressor is greatly reduced. Because of this, it may be more valuable to study the short-term cortisol changes in response to a stressor when assessing overall differences between populations. In such studies, populations under higher stress on average would likely show less reactivity to a stressor than a population which does not experience chronic stress.

The findings of this study provide further evidence supporting the link between average cortisol levels within the body and variables related to size and nutritional health. The relationship between lower BCS scores and GM size and increased HCC levels suggests that cortisol interacts with both growth and nutritional factors within the body. This finding has implications for further research regarding human domestication of dogs, and selective breeding. Understanding the possible impacts of human selection for size in dogs may produce a better understanding of the physiological processes at work, and the ways in which these changes increase the fitness of both the dog, and by extension humans. Furthermore, this research may be able to apply more widely to our understanding of the function and impact of cortisol within cortisol-dominant animals.

Finally, it would be interesting to further study whether seasonal patterns in activity or weather have an effect on the dogs at Bosawas and compare the results with prior findings on such research. Roth et al. (2016) concluded that hair cortisol patterns of domestic dogs in Switzerland reflect seasonal changes. This is also supported by theories discussed in the literature review above in which both cortisol levels and hair growth patterns fluctuate in response to changes in daylight. The dogs at Bosawas are likely not experiencing such high variations in daylight length or temperatures as those living in more temperate zones. However, cortisol levels may be affected by activity patterns in hunting which could fluctuate based on the dry or rainy season. The hair samples used for our study were taken during the months of June and July, which are during the dry season. If the dogs at Bosawas were to be resampled for hair during the rainy season, it would be interesting to compare the results with those from the dry season to see whether they differ. In addition, by combining HCC with detailed ethnographic data on patterns in hunting frequency and success rates, a potential connection between the two variables could be explored.

Chapter 6: Conclusion

The results of this study suggest that hair color can significantly impact cortisol concentration results and should be considered a variable when conducting any study which uses hair as a medium for extracting cortisol.   Although lack of standardization in body region sampling of hair may have affected the results outcome, significant relationships between physiological variables were still apparent. These findings support those of prior research on hair cortisol in dogs and may provide a basis for further exploration of the effects of hair pigmentation on cortisol in other animal species. The significance of color in cortisol concentrations of hair makes it an important variable to consider controlling for in future studies. Furthermore, the findings of this study support previous relationships between size, nutritional health and cortisol levels found in smaller, more limited studies of dogs. The corroboration of findings on HCC in dogs from those of Bennett and Hayssen (2010) which used purebred study subjects also suggests that the differences between breed, and significant differences in environmental factors do not significantly alter the corollary relationship between cortisol and hair color. However, our findings did indicate a relationship between cortisol and sex which was previously unclear in other studies. This may be explained by the body size differences between the sexes, as males were significantly larger than females.   The relationship could also point to certain developmental and reproductive differences that are produced by environmental factors which are absent in studies that take place within a limited and clinical setting. Since it was not possible to control for pregnant or lactating females within this study, our results may not account for such differences within the population.

Further research regarding the impact of environmental factors of hunting and human demand placed on the dogs at Bosawas could shed more light on the variables affecting cortisol production, as well as the relationship between hunting and cortisol levels. It would be interesting to see whether there are any selective pressures of hunting which impact cortisol levels in the dogs. In addition, analysis of HCC and birth/pregnancy history of the female dogs at Bosawas could offer an explanation for the observed sex differences in cortisol. It would also be interesting to evaluate the presence of chronic disease and severe injury in relation to HCC within the dog population at Bosawas to better understand how immunological factors may be impacted by stress.

Appendix A: Demographic Data Collection Forms

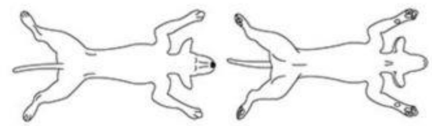
PHYSICAL EXAM RECORD: Dog name: ________________Owner name: _________________ Dog ID: ________________

Examined by:________________ **Date**:__________ ☐ Photo taken **Sex:** Male / Female **Age:** __________
Behavior: ☐ Gentle ☐ Social ☐ Fearful ☐ Aggressive **Mentation**: ☐ BAR ☐ QAR Other:__________
Pulse: ________bpm **RR:** ________bpm **MM:** ☐ Pink ☐ Pale ☐ Moist ☐ Tacky **CRT:** ☐ < 2 secs Other: _______

BCS (1-9): _______ **Hydration**: ☐ Adequate ☐ Marginal (5% dehydration) ☐ Severe (>5% dehydration)

General:
Lameness: ☐ 0 (None) ☐ 1 (Inconsistent) ☐ 2 (Consistent) ☐ 3 (Severe)
Respiratory status: ☐ Normal ☐ Increased ☐ Panting
Lymph nodes: ☐ Normal ☐ Enlarged: Submandibular/Prescapular/Axillary/Inguinal/Popliteal

Integument: M: mass T: tick B: barrier compromise
Ectoparasites: ☐ Fleas ☐ Ticks No: 1-3/4-6/7-10/ >10 ☐ Other
Coat cover: ☐ Full ☐ Patchy alopecia ☐ Severe alopecia
Coat quality: ☐ Shiny ☐ Dull
Masses: ☐ Absent ☐ Present Describe:
Footpads: ☐ Intact ☐ Compromised ☐ Hardened
Barrier compromise: Focal—Diffuse Healing—Non-healing Non-infected—Infected
Notes:



Eyes/Ears/Nose/Throat: ☐ Normal
Eyes: ☐ Clear ☐ Cloudy ☐ Hyperemic ☐ Discharge: Mucoid—Mucopurulent and Mild—Copious
Dentition: ☐ Normal (some calculus) ☐ Missing ☐ Broken☐ Severe calculus ☐ Gingivitis
Notes:

Cardiovascular/Respiratory:
Murmur: ☐ Absent ☐ Present Type/Grade: ________________________
Rhythm: ☐ NSR/SA ☐ Arrhythmia Type: ______________________________
Pulse: ☐ Strong/Synch/Symm ☐ Other: _____________________________
Respiratory: ☐ Clear ☐ Upper airway ☐ Crackles ☐ Wheezes ☐ Inspiratory—Expiratory

GI/Urinary tract:
Abdominal Palpation: ☐ Normal ☐ Abnormal ☐ TTTP
Notes:

Reproduction:
Female Pregnancy: ☐ Pregnant ☐ In Heat ☐ Lactating Notes:____________________
Vulva: ☐ Normal ☐ Abnormal ☐ Discharge: Serous—Sanguinous—Mucopurulent
Male Prepuce: ☐ Normal ☐ Abnormal ☐ Discharge: Serous—Sanguinous—Mucopurulent
Testes: ☐ Normal/Symmetrical ☐ Abnormal
Both TVT: ☐ Present ☐ Absent

Sample/Dx checklist:
☐ Whole blood ☐ Serum ☐ Blood smear ☐ Urine: Cysto—Free catch ☐ in media ☐ in PBS

☐ Feces ☐ Ectos: Ticks/Fleas ☐ Nasal/conjunctival swab ☐ Hair

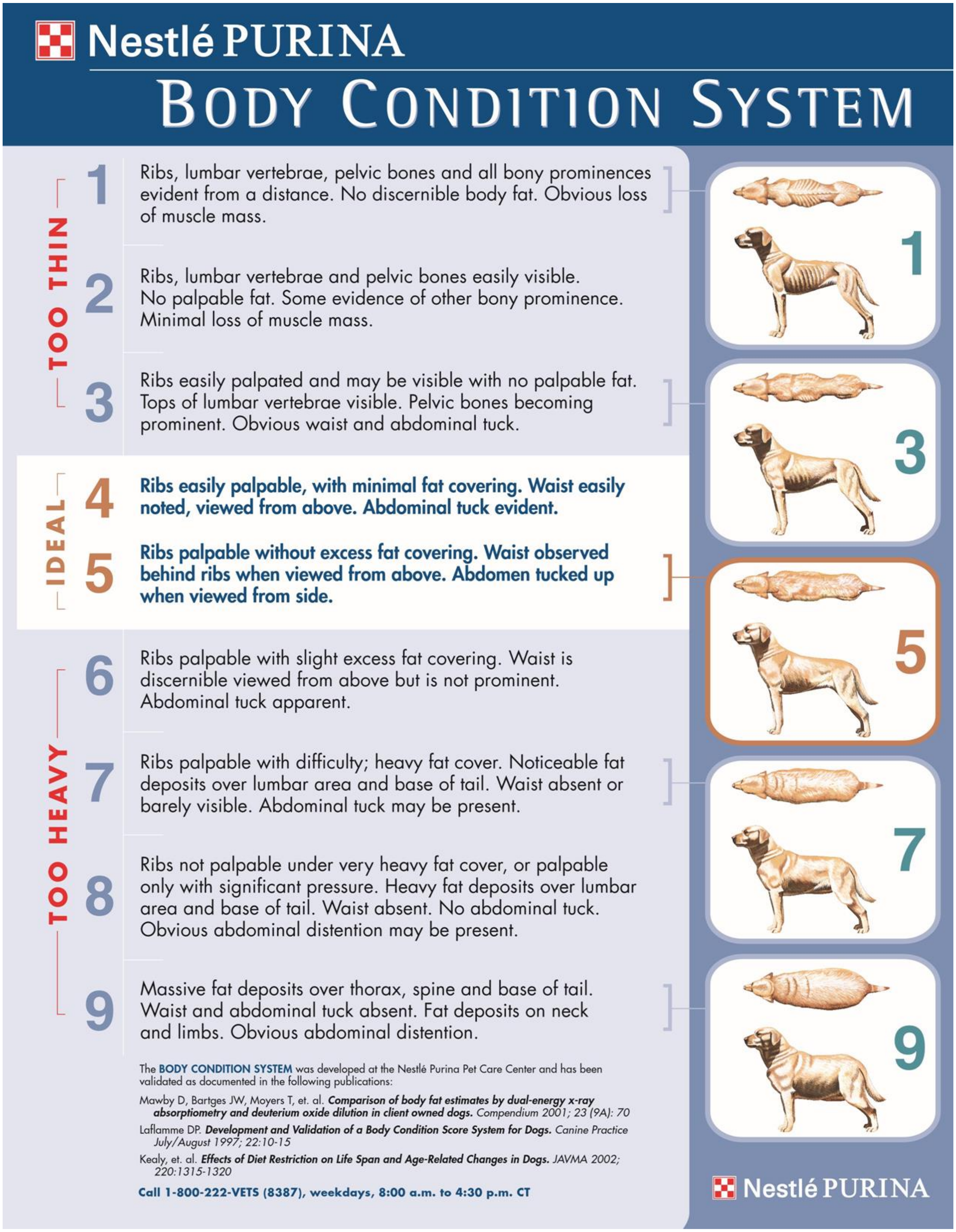
☐ 4dxSnap Test: *Anaplasma*–HW–*Ehrlichia*–*Borrelia*–All Negative ☐ Not done PCV:_________TP:__________

Height: _________ **Head Width:** _________ **Rear dewclaws:** Y N

Snout length: _________ **Body length:** _________ **Ears:** Floppy Folded Erect

Chest Girth: _________ **Chest Width:** _________ **Tail:** Straight Curly

A1: Physical exam form used by veterinarians to assess and record information on dogs



A2: BCS chart used by veterinarians to assign scores to dogs during examinations el ______ de __________________ 2015

Dueño de la casa: ____________________________

Persona entrevistada: _________________________

Pueblo y sector: ____________________________

¿Cuántas personas viven en la casa? __________

¿Tamaño del campo de arroz el año pasado? _______

¿Cuántas libras de semillas se plantaron? _________

¿Cuántos quintales de arroz se cosecharon? _______

¿Desde julio, había perros muertos o cambiados a otra gente? Explica aquí:

vacas: _________

cerdos: _________

armas: ________

mascaras: ________

achas: ________

focos: ________

hamacas: ________

teléfonos celulares: ________

radios: ________

televisiones: _______

plantas: _________

motosierras: ________

motores: ________

GPS:

Nombre y código: ___________________,__________ **Dueño del perro:** ____________________________

¿Cómo se adquirió? Criado por la casa Regalado de ________________ Comprado de ________________por ____C$

¿Cuándo se adquirió? __________________________

¿Dónde nació? _______________________________

Edad: ______________ **Sexo:** M F

Madre: ________________ de quien?______________

Padre: _________________ de quien?______________

¿Qué mata? (marque todo lo que este perro mata **<u>solo</u>)**

malaka wiya ukmik mulukus pamka

Ya sea un éxito o no, hizo una gira este sábado? ______

¿Qué mató este sábado? _______________________

Si no el sábado, cuándo fue la última vez que este perro mató algo? ____________________________

¿Qué fue? ____________________________

¿En los últimos seis meses, ¿cuántas veces se recibió?

Oxi: ____ Ivermectina: _____

¿Cuándo fue la última vez que recibió una medicina?

____

¿Cuándo estaba ella en celo la última vez? __________

¿Cuándo fue el último parto? ____________________

¿Cuántos perritos nacieron? ________________

¿Cuántos murieron jóvenes? _______________

¿Estaba examinado por los veterinarios en 2014? _____

Código de 2014: _________

Nombre y código: ___________________,__________ **Dueño del perro:** ____________________________

¿Cómo se adquirió? Criado por la casa Regalado de ________________ Comprado de ________________por ____C$

¿Cuándo se adquirió? __________________________

¿Dónde nació? _______________________________

Edad: ______________ **Sexo:** M F

Madre: ________________ de quien?______________

Padre: _________________ de quien?______________

¿Qué mata? (marque todo lo que este perro mata **<u>solo</u>)**

malaka wiya ukmik mulukus pamka

Ya sea un éxito o no, hizo una gira este sábado? ______

¿Qué mató este sábado? _______________________

Si no el sábado, cuándo fue la última vez que este perro mató algo? ____________________________

¿Qué fue? ____________________________

¿En los últimos seis meses, ¿cuántas veces se recibió?

Oxi: ____ Ivermectina: _____

¿Cuándo fue la última vez que recibió una medicina?

____

¿Cuándo estaba ella en celo la última vez? __________

¿Cuándo fue el último parto? ____________________

¿Cuántos perritos nacieron? ________________

¿Cuántos murieron jóvenes? _______________

¿Estaba examinado por los veterinarios en 2014? _____

Código de 2014: _________

A3: Form used by the research team to record other demographic information regarding household and dog history.

Appendix B: Further Data Analysis Results and Figures

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
D	241	16.655136	7.4189479	0.4778964	15.713729	17.596543
L	243	20.296054	13.165988	0.8445985	18.632351	21.959757
M	185	16.31102	4.9906131	0.3669172	15.587115	17.034926

B1: Summary statistics for HCC by fur color

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
f	313	18.434695	9.1679791	0.5182048	17.415077	19.454313
m	357	17.348201	10.038591	0.5312986	16.303323	18.39308

B2: Summary statistics for HCC by sex

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