DISSERTATION

USE OF TELEMETERED HEART RATE AS AN INDICATOR OF ACUTE STRESS IN BIGHORN SHEEP (*OVIS CANADENSIS*) EWES

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY MARGARET A. WILD ENTITLED "USE OF TELEMETERED HEART RATE AS AN INDICATOR OF ACUTE STRESS IN BIGHORN SHEEP (*OVIS CANADENSIS*) EWES" BE ACCEPTED AS FULLING PART OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

USE OF TELEMETERED HEART RATE AS AN INDICATOR OF ACUTE STRESS IN BIGHORN SHEEP (OVIS CANADENSIS) EWES

Increased exposure of bighorn sheep (*Ovis canadensis*) to human-associated disturbance is a primary concern of resource managers. However, quantitative methods to assess a free-living animal's physiological response to acute environmental stressors are limited. I developed a technique for discrete long-term, long-range monitoring of heart rate in captive Rocky Mountain bighorn sheep (*O. c. canadensis*) and determined the usefulness of this technique as an indicator of stress. To monitor heart rate, I surgically implanted telemetry transmitters on the dorsolateral thorax of 15 captive adult bighorn sheep ewes. Twelve of 15 transmitters remained functional ≥1 yr. Heart rate data collected from the transmitters, using a telemetry receiver/datalogger, accurately reflected heart rate as measured with electrocardiogram tracings. Line of sight signal range was at least 800 m in 95% (37/39) of collections made from standing ewes, while data could be collected reliably (74%; 29/39) to 600 m from bedded ewes.

I examined the effects of controlled stressors on the relationship between telemetered heart rate and serum cortisol in these bighorn sheep. Ewes responded to graded stressors with a predictable rise in serum cortisol that was related to increased heart rate, but in many cases no overt behavioral responses were observed. Serum cortisol levels tended to increase linearly with heart rate for all ewes, although the magnitude of response varied with reproductive status ($p < 0.001$). Serum cortisol concentrations increased 0.11 ng/mL (SE = 0.017) for each 1 bpm in response heart rate in non-pregnant ewes and 0.09 ng/mL (SE = 0.023) for ewes in early pregnancy. In contrast, serum cortisol response of ewes in late pregnancy more than doubled to 0.23 ng/mL (SE = 0.030) for each 1 bpm in response heart rate.

I investigated the daily and seasonal variation in heart rate and serum cortisol in adult bighorn sheep ewes. I collected telemetered heart rate at about 1-min intervals, and blood for serum cortisol analysis at 2-hr intervals over a 24 hr period on the spring and fall equinox and summer and winter solstice, 1996. Heart rate varied by month of sampling ($p = 0.007$) with generally lower values in March and December. Daily variation was also observed with higher ($p \leq 0.009$) heart rates during light than darkness. No daily ($p = 0.63$) or seasonal variation ($p = 0.06$) in serum cortisol was observed. Daily and seasonal variation in heart rate but not serum cortisol concentrations suggest that the predictive value of heart rate as an indicator of change in serum cortisol concentrations will vary slightly with time of day and season.

I conclude that remotely monitored heart rate can be used as a predictor of change in serum cortisol levels for ewes of known reproductive status and, when used in conjunction with overt behavioral observations, can provide resource managers with an improved assessment of acute stress in bighorn sheep.

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Though the observed actions of men hide their real thoughts and feelings,

these are revealed by the observations of their hearts

Gantt 1960

TABLE OF CONTENTS

CHAPTER 1. INTRODUCTION 1
LITERATURE CITED 18

CHAPTER 2. SURGICAL IMPLANTATION AND EVALUATION OF HEART RATE TRANSMITTERS IN CAPTIVE BIGHORN SHEEP 28
ABSTRACT 28
INTRODUCTION 29
MATERIALS AND METHODS 30
Surgical technique 30
Evaluation of transmitters 33
RESULTS 34
Surgical technique 34
Evaluation of transmitters 36
DISCUSSION 36
LITERATURE CITED 41

CHAPTER 3. HEART RATE AS A PREDICTOR OF ACUTE STRESS IN BIGHORN SHEEP (Ovis canadensis) EWES 48
ABSTRACT 48
INTRODUCTION 49
METHODS 52
Animals 52
Experimental protocol 52
RESULTS 56
DISCUSSION 59
REFERENCES 65

CHAPTER 4. DAILY AND SEASONAL RHYTHMS IN HEART RATE AND SERUM CORTISOL IN BIGHORN SHEEP (Ovis canadesis) EWES 76
ABSTRACT 76
INTRODUCTION 77
METHODS 79
Animals 79
Experimental protocol 79
RESULTS 81
DISCUSSION 83
REFERENCES 88

CHAPTER 1

INTRODUCTION

Wildlands in western North America provide a diversity of recreational opportunities for outdoor enthusiasts. For most, these recreational pursuits are enhanced by the presence of wildlife in their natural habitat. Natural resource management agencies attempt to provide both satisfying recreational experiences for people and protection for wildlife populations; however, implementation of these objectives may conflict. While managers must maintain a sensitivity to the potentially deleterious effects of human disturbance on wildlife, overly conservative management may limit human enjoyment and appreciation of the wildlife resources. To make wise decisions on recreational land use, managers need an understanding of the effects that human disturbance may have on wildlife and also a method to determine when these disturbances are occurring.

Here, I will review potential impacts of human disturbance on wildlife, particularly the potentially deleterious effects of the stress response. Bighorn sheep (*Ovis canadensis*) are highly charismatic animals that have been reported to be sensitive to the negative impacts of stress (Spraker et al. 1984). Methods to monitor human activities that do and do not induce stress in bighorn sheep are needed to formulate appropriate management plans. Therefore, I will also review methods used to estimate stress in ungulates and propose experimental investigations to develop techniques to monitor and predict stress levels in bighorn sheep.

Effects of Human Activities on Wildlife

Although wildlife may be subjected to a variety of disturbances, anthropogenic disturbance is often of primary concern to managers. While not all human activities have negative impacts on wildlife, some human-induced disturbances adversely affect animals directly via acute mortality or indirectly through changes in behavior or physiology. For most disturbances, the simplest and frequently the most economical response for the animal is to alter its behavior. However, behavioral changes alone may not return the animal to homeostasis and physiological responses may be required as well.

Behavioral Responses.-- The effects of human activities on behavioral responses of wildlife have been categorized as attraction, habituation, or avoidance (Knight and Cole 1991). Attraction and avoidance are responses to positive rewards and negative stimuli, respectively. While attraction can be a positive experience for both wildlife and recreationists, it can have negative impacts as well. In some cases attraction can lead to unnatural congregation of animals that increases the potential for disease transmission, alteration of movement and migration patterns, and at the extreme even alter ecosystems (Knight and Cole 1991). Avoidance can result in displacement, increased energy expenditure, decreased energy acquisition, and decreased productivity (Knight and Cole 1991). Habituation occurs when animals stop responding to repeated activities that are not accompanied by positive or negative reinforcement (Knight and Gutzwiller 1995). Habituation is a response to activities that are biologically meaningless to an animal (Eibl-Eibesfeldt 1970), that is, have no behavioral or physiological impact on an animal. Behavioral responses are commonly used as indicators of habituation; however, various stimuli elicit physiological changes in the absence of overt behavioral responses (MacArthur et al. 1982, Kreeger et al. 1989, Baldock and Sibly 1990). In these cases, although an animal does not respond behaviorally to a stimulus, it may not be habituated to that stimulus. For example, bighorn sheep that do not abandon an area that is encroached upon by human disturbance may be habituated to predictable human activities (Geist 1975, Papouchis et al. 2001). However, conservative management recommendations are often made (e.g., by the Colorado Division of Wildlife, U. S. National Park Service) based on the premise that an area is not abandoned because of the strong fidelity that bighorn sheep show to traditional ranges, regardless of changes in suitability of those ranges (Geist 1971). That is, bighorn sheep may endure increased levels of stress rather than expand to new ranges to avoid the disturbance. Such situations require additional data, such as physiological responses, to explain observed behavior and determine what impact, if any, the stimulus exerts.

Physiological responses.-- The physiological response of an animal to disturbance has been termed "stress", although this term has been used so broadly that it is difficult to clearly define. Selye (1950) first developed a comprehensive biological theory of stress. He proposed a nonspecific response to stress in which the body (specifically the adrenal glands) reacted similarly to all types of stressors. Moberg (2000) proposed a contemporary model of stress in which he recognized that not all stress was bad. Understanding these models of stress is necessary to appreciate the need to monitor and manage stress in animals.

Models of Stress

Selye's Model.-- Selye (1950) described a nonspecific response to noxious stimuli that he termed the General Adaptive Syndrome (GAS). His model consisted of 3 stages in which an animal: (1) responds to the stressor with an alarm or emergency reaction, (2) biologically copes with the physiological demands of the stress, and (3) if the stress continues, experiences exhaustion of the biological defense system. Selye's concept of a nonspecific stress response was eventually rejected as an over-simplification of a complex interaction of responses; however, he made several crucial contributions to the understanding of stress biology. These contributions include identification of the importance of the neuroendocrine system in the stress response, recognition that diverse stimuli evoke biological responses and that these responses, although helpful in maintaining an animal's homeostasis, have the potential to result in a pathologic state (Moberg 1985).

Contemporary Stress Model.-- Moberg (2000) proposed a more contemporary model of stress. He defined stress as the biological response elicited when an individual perceives a threat to its homeostasis. Distress occurs when the stress response truly threatens the animal's well being. Moberg's (2000) model is divided into 3 general stages similar to those of Selye's (1950): (1) recognition of a stressor, (2) biological defense against the stressor, and (3) consequences of the stress response. The stress response begins with the central nervous system (CNS) perceiving a potential threat to homeostasis. The CNS reacts with some combination of 4 general biological defense responses: the behavioral response, the autonomic nervous system response, the neuroendocrine response, or the immune response (Moberg 2000).

Behavioral responses to stressors are dependent on the species, type of stressor, and context. Although there are no general behavioral stress responses, one common dichotomy for behavioral responses is "take it" or "leave it" in which animals endure a stressor or adjust behavior to modify the impacts (Wingfield and Ramenofsky 1999). Either response can have biological costs and the motivational and neurophysiological bases must be determined to understand the behavior.

The autonomic nervous system response mobilizes the body's resources for "fight or flight" (Cannon 1929) in which heart rate and cardiac output are increased, glucose is redistributed from skin and viscera to the central nervous system and muscles, bronchi are dilated, lymphocytes and blood-coagulating factors are released, glucagon is released from the pancreas, and other various biological systems are affected (Moberg 1985). These responses are of relatively short duration and affect very specific biological systems.

In contrast, hormones secreted from the hypothalamic-pituitary neuroendocrine system have a broad, longer lasting effect on the body. Stress can effect secretion of pituitary hormones and function of the hypothalamic-pituitary-adrenal (HPA), somatotrophic, lactotrophic, gonadotrophic, and thyrotrophic axes (reviewed by Matteri et al. 2000). In general, these responses tend to inhibit non-essential functions such as growth and reproduction in favor of maintenance and survival and have been implicated in failed reproduction (Moberg 1987, Rivier 1995), altered metabolism (Elasser et al. 2000), and altered immune function (Blecha 2000).

The best known, most consistent of these responses to stress is activation of the HPA axis. In this response, an external stimulus that is perceived as a stressor activates the sympathetic division of the nervous system and stimulates release of corticotrophinreleasing hormone (CRH) and vasopressin from the hypothalamus. Sympathetic stimulation also initiates release of catecholamines from the adrenal medulla. Catecholamines act on various target organs and tissues (including an influence on heart rate). CRH and vasopressin stimulate the anterior pituitary to release adrenocorticotropic hormone (ACTH). ACTH causes the adrenal cortex to increase secretion of glucocorticoids that act on a variety of target tissues and organs to maintain homeostasis. In most mammals, including bighorn sheep, cortisol is the primary glucocorticoid. The HPA axis response to stress is controlled by a negative feedback system in which glucocorticoids inhibit response at the level of the brain and pituitary (reviewed by Matteri et al. 2000).

In addition to the behavioral, autonomic, and neuroendocrine responses, Moberg (2000) includes immune response to a perceived stressor as one of the general biological defense responses. This is in contrast to the traditional view of modulation of immune function only by other stress-responsive systems, particularly the HPA axis. Alterations in immune function have been linked to disturbances in the HPA axis and hypothalamic autonomic nervous system; however, many physiological and behavioral factors likely interact to influence host immunity (Blecha 2000). Regardless of the pathway, altered immune function is one of the most widely studied consequences of the stress response.

Prolonged or repeated exposure to stressors can lead to excessive stimulation of the neuroendocrine system (reviewed by Stephens 1980, Roth 1985) and direct modulation of the immune system (Moberg 2000) with resultant immune compromise. Although glucocorticoids have been implicated in the impairment of humoral immune function under some conditions (Gwazdauskas et al. 1978), it appears that the effects of glucocorticoids are more pronounced on neutrophil function (Roth et al. 1982) and cell-mediated immune response (Muscoplat et al. 1975, Westley and Kelley 1984, Coppinger et al. 1991, Hanlon et al. 1995) in domestic ungulates. While many studies have shown that an increase in glucocorticoids compromises immune function (Coppinger et al. 1991, Minton et al. 1992, Hanlon et al. 1995), other studies have shown no effect (Minton et al. 1995). These equivocal results have raised questions regarding the importance of cortisol on immune function. It is likely that in addition to the cortisol profile, the immune response is also dependent on other factors such as CRH, ACTH, and β-endorphin that may be more difficult to modulate under artificial laboratory conditions. Further, recent work by Rhind et al. (1999) has shown that the magnitude, duration, and timing of changes in cortisol also determine affects on immune function. While elevated cortisol levels *per se* did not have a significant inhibitory effect on immune function, large, relatively infrequent increases in cortisol compromised cell-mediated immune function in domestic sheep. This finding likely explains the failure of some investigators (e.g., Minton et al. 1995) to demonstrate a consistent relationship between cortisol and immune suppression.

Stress and Bighorn Sheep

Disease susceptibility.-- Some wildlife species may be more adversely affected than others by human-induced disturbances. Immunosuppressive effects of distress have been implicated in the bovine shipping fever complex (a respiratory disease complex of domestic cattle; reviewed by Confer et al. 1988) and may be an important component of the bighorn pneumonia complex (Hudson 1973, Spraker et al. 1984). Potts (1937) first diagnosed Pasteurella-associated respiratory disease in bighorn sheep in 1935, although earlier outbreaks likely occurred (Rush 1927, Buechner 1960, Post 1962). Respiratory disease remains the most serious health problem affecting survival and population dynamics of bighorn sheep in western North America (Onderka and Wishart 1984, Hobbs and Miller 1992). *Pasteurella haemolytica* has been identified as a significant component of this disease complex that causes all-age die-offs and subsequent poor lamb recruitment (Spraker et al. 1984, reviewed by Hobbs and Miller 1992). *P. haemolytica* has recently been reclassified to *Mannheimia haemolytica* (formerly *P. haemolytica* biotype A) and *P. trehalosi* (formerly *P. haemolytica* biotype T). A non-hemolytic *P. trehalosi* is generally considered the "bighorn pasteurella" (Onderka and Wishart 1988) and can be isolated from most clinically healthy bighorn sheep (Wild and Miller 1991). However, the bacterium can also serve as a primary pathogen (Miller et al. 1991a) or opportunistic pathogen acting with predisposing disease agents. Predisposing factors of *P. trehalosi* infection may include respiratory viral infections (Parks et al. 1972, Clark et al. 1985, Spraker et al. 1986), lungworm (Forrester 1971, Spraker and Hibler 1982), mineral deficiencies (Schwantje 1986), or stress (Spraker et al. 1984). Although stress-induced respiratory disease in bighorn sheep has been difficult to reproduce experimentally (Miller et al. 1991a), environmental disturbances have sometimes preceded, and been implicated in the occurrence of, pneumonia epizootics in bighorn sheep (Feurerstein et al. 1980, Spraker et al. 1984, Festa-Bianchet 1988, Ryder et al. 1992). The mechanism by which stress influences the frequency and severity of such epizootics is unclear; however, in addition to data on immune suppression extrapolated from domestic ruminants (Roth 1985, Blecha 2000), *in vitro* experiments support cortiosteroid-induced impairment of lymphocyte reactivity (Hudson 1973) and increased susceptibility of bighorn neutrophils to *Pasteurella haemolytica* leukotoxin (Kraabel and Miller 1997).

Effects on management.-- A survey of residents of Denver, Colorado (Manfredo and Larson 1993) found that bighorn sheep were among the most important species for wildlife viewing recreation. However, due to the belief by wildlife managers that human disturbance to bighorn sheep will lead to distress and outbreaks of respiratory disease, restrictive limitations are often placed on recreationists. These restrictions may limit the satisfaction of some groups of wildlife viewers, but are deemed necessary for the welfare of the bighorn sheep. If wildlife managers were able to determine which human activities did and did not induce distress in bighorn sheep, management plans could be developed that would maximize benign recreational activities and manage those activities that caused disturbance. This science-based decision-making could improve bighorn management and may provide increased recreational opportunities for outdoor enthusiasts. However, implementation of this management approach would require improved methods for monitoring and quantifying the effects of disturbance on bighorn sheep. The purpose of this dissertation is to address this management need.

Monitoring Disturbance in Bighorn Sheep

Potential techniques.—Stress is commonly measured in domestic and wild ruminants using blood cortisol levels; however, blood cortisol reflects only the status of an animal at a point in time that is likely influenced by the invasive sampling technique itself (Thurley and McNatty 1973, Spraker et al. 1984). Techniques for remote collection of blood samples from captive red deer (*Cervus elaphus*) have been developed (Bubenik and Bartos 1993, Ingram et al. 1994, Cook et al. 2000) but are not known to be available in the United States. Additionally, although these devices have been used in red deer ranging in pens, the feasibility for long term use in free-ranging wildlife is unknown. Measurement of salivary cortisol has been performed in domestic ungulates (Fell and Shutt 1986) but use in free-ranging wildlife is not feasible. Fecal and urine cortisol (Miller et al. 1991b) provide promising methods for noninvasive monitoring of free-ranging wildlife; however, these techniques can be difficult to use for monitoring individual animals, especially in their response to specific events. Behavioral responses alone are inadequate indicators of excitation in bighorn sheep (MacArthur et al. 1982, Stemp 1982) and other species (Kreeger et al. 1989, Baldock and Sibly 1990) because various stimuli, including disturbance by humans, elicit physiological changes even in the absence of overt behavioral responses.

Remotely monitored heart rate has been used to assess disturbance in bighorn sheep (MacArthur et al. 1979, Weisenberger et al. 1996, Krausman et al. 1998) although the relationship between heart rate and other indicators of stress are unclear. Harlow et al. (1987a, 1987b) suggested that heart rate had potential for reliably predicting cortisol levels but noted that additional research would be needed before heart rate could be used to predict blood cortisol levels. Further, an improved method for monitoring heart rate in free-ranging bighorn sheep would be needed.

Heart rate telemetry.--Various techniques have been used to remotely monitor heart rate. Backpack harnesses (MacArthur et al. 1979, Stemp 1982) and repeater collars (Cupal et al. 1974) were among the first heart rate telemetry systems used in wild ungulates. Surgical implantation of transmitters has been performed but has been accompanied by morbidity and mortality from complications when the transmitter was expelled from the body following tissue necrosis (Coates et al. 1990), herniated through the ventral body wall, or engulfed by the rumen (Wallace et al. 1992). Further mortality has been associated with anesthetic death (Wallace et al. 1992). Although signal strength and battery life expectancy of transmitters has improved markedly from early technology (Skutt et al. 1973; Cupal et al. 1974), reliability continues to be a significant problem with heart rate telemetry systems. Lead breakage frequently caused early failure of external systems (MacArthur et al. 1979) and implanted systems (Wallace et al. 1992) alike. Leakage of body fluids into implanted transmitters has been a further complication leading to early failure (Wallace et al. 1992).

To be successfully applied to monitoring of free-ranging bighorn sheep in a wildlife recreation area, a telemetry system would need to be reliable, function over a long range (> 1 km) and over a long time period (> 1 yr), be safe and humane, and not detract from the aesthetic value of the animal. None of the previously developed techniques (MacArthur et al. 1979, Stemp 1982, Bunch et al. 1989, Coates et al. 1990, Wallace et al. 1992) meet these requirements. Therefore, development of a new heart rate telemetry technique is needed.

Correlation Between Heart Rate and Serum Cortisol

Considerable research on the utility of heart rate for predicting metabolic rate and energy use of wild ungulates was performed in the 1970s (Johnson and Gessaman 1973, Holter et al. 1976, Moen 1978, Nilssen et al. 1984, Renecker and Hudson 1985). Heart rate has also been investigated as an indicator of disturbance and stress in domestic (Stephens and Toner 1975, Harlow et al. 1987a, Hargreaves and Hutson 1990) and wild (Freddy 1977, MacArthur et al. 1979, MacArthur et al. 1982, Espmark and Langvatn 1985, Harlow 1987b, Cassirer 1990, Chabot 1991, Weisenberger et al. 1996, Krausman et al. 1998) ungulates. Although heart rate has been commonly used as an indicator of stress in wild ungulates, only Harlow et al. (1987b) and Chabot (1991) attempted to correlate heart rate to other physiological indices. Chabot (1991) used heart rate to study increased metabolic rate associated with disturbance. Harlow et al. (1987b) used heart rate as a predictor of stress by demonstrating a linear relationship between heart rate and blood cortisol for 2 individual bighorn sheep. However, Harlow et al. (1987b) noted that due to individual variation, a larger sample size was needed to determine if a single regression equation of predictive value could be determined for bighorn sheep. Additional research would also be needed to further confirm the relationship between heart rate and blood cortisol in bighorn sheep under varying circumstances. Although heart rate and cortisol likely respond similarly to stress, they are not inextricably linked and may respond independently to other effectors.

Heart rate responses are of short duration and in response to increases in secretion of catecholamines following autonomic stimulation. Cortisol has a broader, longer lasting effect on the body and is secreted in response to stimulation of the hypothalamic-pituitary neuroendocrine system (Moberg 2000). The use of heart rate as a predictor of blood cortisol response requires the assumption that although the variables are in different pathways of the biological response, they will retain their correlation in different situations and with different stressors. This assumption is likely generally true for response of bighorn sheep to similar categories of stressors (e.g., audiovisual stressors), however, under some situations, the functional relationship between the variables may change. One physiological response pathway may become sensitized while the other remains neutral or becomes desensitized. In pigs, cortisol responses gradually diminished over time with repeated exposure to isolation stress but heart rate responses remained unchanged (Schrader and Ladewig 1999). Other factors influencing an animal's physiological state may also influence the relationship between heart rate and serum cortisol and need to be considered when using heart rate as an indicator of cortisol levels.

Other Factors Influencing Heart Rate and Serum Cortisol

Recognizing the influence of modifiers on the relationship between heart rate and cortisol is essential for accurately predicting blood cortisol levels based on heart rate data and for understanding the inherent variation in responses. Individual variation in sex, age, genetics, physical condition, and previous experience are unavoidable variables when studying animals in populations. Even so, Harlow (1987a) demonstrated a good correlation between heart rate and plasma cortisol values in domestic sheep exposed to graded stressors in a laboratory setting. Other effectors of heart rate and blood cortisol that should be considered when monitoring free-ranging animals over extended periods include activity level, reproductive status, and prandial, daily, and seasonal cycles.

Exercise.-- Activity status influences heart rate and blood cortisol levels because exercise increases the physiological demands on an individual. A positive relationship between heart rate and activity level has been demonstrated in bighorn sheep (MacArthur et al. 1979). Heart rate increased linearly with running speed in deer (Mautz and Fair 1980) and reindeer (Nilssen et al. 1984). Alexander et al. (1991) demonstrated that exercise resulted in a rise in plasma cortisol level in horses. However, rise in cortisol was not accompanied by increasing CRH therefore suggesting that increased cortisol during exercise is not directly related to the HPA pathway but to an alternate pathway, likely via arginine vasopressin (AVP) (Alexander et al. 1991). When using heart rate to predict blood cortisol values, exercise-induced changes in heart rate that are not associated with psychological stress (for example when an animal walks or runs to a preferred feeding location) could overestimate the predicted stress response. This would be most marked with strenuous exertion and likely of little consequence during mild activity. MacArthur et al. (1982) reported that during sampling periods the likelihood of heart rate increasing >12 bpm in an undisturbed bighorn sheep while lying, standing, or foraging was <5%. Therefore, collection of heart rate data for cortisol predictions are best made from animals during times of low activity. Although exercise limits the value of heart rate in predicting disturbance, in many cases exercise is induced by a disturbance. If an animal moves away from an activity, that activity can be classified as a disturbance based on behavioral response alone.

Reproduction.-- The metabolic costs of reproduction have been studied in both domestic animals and wild ruminants and have been shown to increase significantly during the last trimester of gestation and early lactation (Brockway et al. 1963, Robbins 1983). Baseline cortisol levels increase markedly in humans (Demey-Ponsart et al. 1982) and domestic ewes during late pregnancy (Bell et al. 1991, Keller-Wood 1998). In pregnant, domestic ewes there is an increase in basal ACTH and a doubling of cortisol (Keller-Wood 1996) that does not correlate with concentrations of gonadal steroids (Bell et al. 1991). Pregnancy in domestic ewes apparently resets the pituitary-adrenal axis and basal cortisol concentration required for normalization of basal ACTH concentration (Keller-Wood 1998). Elevation in baseline cortisol of pregnant, domestic ewes may be essential for normal fetal development and homeostasis (Keller-Wood 1998). The maternal adrenal gland is the primary source of cortisol and is responsible for up to 98% of the circulating fetal cortisol before the fetal adrenal gland becomes fully functional at 135 days of a 145 day gestation (Wood 1996).

Feeding.-- Prandial cycles may influence heart rate and cortisol levels as well. In fasted cattle, decreased ruminoreticular fill results in a reflex slowing of heart rate due primarily to increased parasympathetic tone (Clabough and Swanson 1989). Meal feeding was suggested to be a significant signal in the daily release of cortisol in domestic sheep (Holley et al. 1975). In several studies of domestic sheep fed once daily, concentrations of cortisol were highest at the time of food presentation (Holley et al. 1975, Slater and Mellor 1981, Simonetta et al. 1991).

Daily and seasonal rhythms.-- Daily and seasonal rhythms of metabolic rate and other physiological factors have been reported for a number of wild and domestic ungulates; however, demonstration of these cycles has often been difficult to consistently reproduce. A daily cycle for plasma cortisol level has been reported in cattle (Fulkerson et al. 1980), domestic sheep (McNatty et al. 1972), and desert bighorn sheep (*O. canadensis cremnobates*) (Turner 1984). This cycle is suggested to be due to an inherent adrenocortical rhythm (Demura et al. 1966, Turner 1984); however, detection of the cycle may be confounded due to effects of feeding (Holley et al. 1975, Simonetta et al. 1991), changes in visibility and mobility associated with light and dark periods (Turner 1984), or handling procedures. A daily rhythm in plasma cortisol concentration was observed in pregnant domestic ewes fed once daily but not in ewes fed at frequent intervals throughout the day (Simonetta et al. 1991). Although cortisol data presented by Turner (1984) suggests a daily rhythm with peak during the hours of darkness, the reported plasma cortisol values are markedly higher (range about 23-40 ng/mL) than those observed in tractable Rocky Mountain bighorn sheep (*O. c. canadensis*) in Colorado (about 1-20 ng/mL) (Miller 1988). Miller (1988) noted that in tractable bighorn sheep plasma cortisol values rose not only with handling for blood collection but also with the anticipation of venipuncture. These observations underscore the importance of experimental conditions on resultant data and its interpretation. No daily rhythm in cortisol has been documented in studies on dogs (Thun et al. 1990), white-tailed deer (*Odocoileus virginianus*) (Bubenik et al. 1983), or Eld's deer (*Cervus eldi thamin*) (Monfort et al. 1993). Daily variation in heart rate has been reported in bighorn sheep (MacArthur et al. 1979) and dogs (Pouchelon et al. 2000) but not foxes (*Vulpes vulpes*) (Kreeger et al. 1989). Daily cycles in heart rate may correlate with activity cycles rather than inherent rhythms.

A seasonal cycle, with summer peak and winter decline, in heart rate has been demonstrated in many ungulate species and parallels the cycle of basal metabolic rate that is determined by daylength (Moen 1978, Chappel and Hudson 1980, Baldock et al. 1988, Françoise Domingue et al. 1992). Alternatively, Mautz et al. (1992) suggest that the appearance of a seasonal cycle is due to sampling artifact, particularly influences of temperatures outside the thermal neutral zone and differences in activity level. Reports describing seasonal rhythms in cortisol secretion are also equivocal. No distinct seasonal differences in cortisol secretion have been observed in domestic rams (Kennaway et al. 1981) or Eld's deer (Monfort et al. 1993), but were reported in a group of 4 white-tailed deer (Bubenik and Leatherland 1984).

Experimental Investigation

Despite inherent individual animal variations and potential environmental influences, use of heart rate as an indicator of stress in bighorn sheep shows promise. Collection of data from a large sample size of physiologically similar animals (e.g., non-pregnant adult ewes) may allow a general regression to be determined so that heart rate can be used to predict serum cortisol. The impact of some of the most significant environmental influences can be measured and correction factors applied to the regression as appropriate. Here, I describe research to investigate the utility of heart rate data collected via telemetry systems to predict cortisol levels in captive Rocky Mountain bighorn sheep. The research provides a novel and effective means for monitoring stress in bighorn sheep, and other species given establishment of species-specific regressions of serum cortisol to heart rate. In future work, the monitoring technique and the predictive models developed here could be applied to study the effects of disturbance on free-ranging bighorn sheep. The objectives of this research are:

1. To develop a safe, reliable, and discrete system to remotely monitor heart rate in bighorn sheep over an extended time period.

2. To determine the correlation between heart rate and serum cortisol levels in adult, female bighorn sheep exposed to graded stressors under controlled conditions.
3. To understand the effects of reproductive status and daily and seasonal cycles on the heart rate-serum cortisol correlation.

Working hypotheses of this research are:

1. A strong linear correlation will exist between heart rate and serum cortisol level in ewes and this correlation can be used to predict serum cortisol given heart rate.
2. The relationship between heart rate and serum cortisol will change with reproductive status of the animal.
3. Heart rate will exhibit daily variation that may not parallel the daily rhythm in serum cortisol concentrations.
4. Seasonal heart rate and cortisol levels are cyclic in phase with basal metabolic rate, being greater in summer during periods of increased feed intake and activity and lower in winter.

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CHAPTER 2

SURGICAL IMPLANTATION AND EVALUATION OF HEART RATE TRANSMITTERS IN CAPTIVE BIGHORN SHEEP[1]

ABSTRACT: A surgical approach was developed for implantation of transmitters to monitor heart rate of bighorn sheep (*Ovis canadensis*) with an objective of discrete long-term long-range data collection. We surgically implanted Telonics model HR400 transmitters on the dorsolateral thorax of 15 captive adult bighorn sheep ewes in April-May and October-November 1995. No complications or marked impairment of function were associated with the surgery; however, a transmitter was passively expelled from one ewe 19.5 mo post-implantation. Twelve of 15 transmitters remained functional ≥1 yr, while three failed 3.5 to 4.5 mo following implantation. Heart rate data collected from the transmitters using a Lotek SRX_400 telemetry receiver/datalogger equipped with W9 EVENT_LOG accurately reflected heart rate as measured with electrocardiogram tracings. Line of sight signal range was at least 800 m in 95% (37/39) of collections made from standing ewes, while data could be collected reliably (74%; 29/39) to 600 m from bedded ewes. When a reliable long-lasting inconspicuous telemetry system is required, we believe that this approach holds promise for success in free-ranging as well as captive ungulates.

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INTRODUCTION

Remote collection of physiological data using telemetry can be a useful tool to wildlife biologists because it allows normal physiological responses to be measured without interference from physical or chemical restraint. Heart rate data have been collected remotely from wildlife to: determine baseline heart rate and associated natural variations (Moen, 1978; Kreeger et al., 1989), study and predict metabolic rate and relative energy expenditure (Holter et al., 1976; Nilssen et al., 1984; Chabot, 1991), and monitor disturbance and stress (MacArthur et al., 1982; Kreeger et al., 1990b; Weisenberger et al., 1996).

In our studies, we required a telemetry system to monitor heart rate as an indicator of disturbance in bighorn sheep (*Ovis canadensis*) at wildlife viewing areas. This application required a system that was inconspicuous and would function reliably over a long range (>0.5 km) for an extended period (≥1 yr). Implantable telemetry systems to monitor physiologic parameters have been reported for use in a variety of wildlife species (Skutt et al., 1973; Philo et al., 1981; Renecker and Hudson, 1985; Kreeger et al., 1989; Cassirer, 1990; Coates et al., 1990; Kreeger et al. 1990a; Wallace et al., 1992), but no system fully met our criteria for wild ruminants. Heart rate telemetry systems in ruminants have generally been short-lived due to battery depletion, transmitter or electrode failure, or expulsion from the body. Although a relatively successful system adapted from Bunch et al. (1989) using intra-abdominal implantation was used in bighorn sheep and mule deer by Wallace et al. (1992), problems associated with intra-abdominal placement and transmitter failure were reported. Further, signal range of the intra-abdominally placed transmitters was ≤0.5 km on flat terrain. Here, we tested an alternative approach by surgically implanting the Telonics model HR400 heart rate transmitter (Telonics, Inc., Mesa, Arizona, USA) on the dorsolateral thorax of captive bighorn sheep. We evaluated 1) the surgical procedure by monitoring morbidity and mortality in implanted bighorn sheep and 2) transmitter function by measuring accuracy, longevity, and signal range from standing and bedded animals.

MATERIALS AND METHODS

Surgical technique

We implanted heart rate transmitters into 15 tractable bighorn sheep ewes held at the Colorado Division of Wildlife's Foothills Wildlife Research Facility, Fort Collins, Colorado (USA; 40°35'N, 105°10'W). Groups of ewes were maintained in about 3-ha pastures and received ad libitum quantities of grass/alfalfa hay, water, trace mineral block, and about 500 g/animal/day of a pelleted supplement (Baker and Hobbs, 1985). Surgeries were performed during April-May (n = 5) and October-November (n = 10) 1995. Ewes ranged in age from 1.5 to 13 yr and in body mass from 55 to 82 kg.

Ewes were isolated and fasted for about 48 hr prior to surgery. We induced anesthesia with intravenous (IV) administration of 1.4 mg/kg ketamine HCl (Ketaset, Fort Dodge Laboratories, Fort Dodge, Iowa, USA) and 0.15 mg/kg xylazine (Rompun, Miles Inc., Shawnee Mission, Kansas, USA). Ewes were intubated with an 11 mm endotracheal tube and surgical anesthesia was maintained using a rebreathing circuit with isoflurane (AErrane, Anaquest, Madison, Wisconsin, USA) at 1.0-2.0% concentrations in oxygen flows of 1-2 l/min. A 1.1 mm x 5.1 cm indwelling intravenous catheter (Deseret Medical

Inc., Sandy, Utah, USA) was placed for fluid administration. Normal saline solution (0.9% sodium chloride; Baxter Healthcare Corp., Deerfield, Illinois, USA) was administered at a rate of 200 ml/hr. We monitored anesthetic depth by continuous visual inspection of mucous membrane color, jaw tone, respiratory rate, and pulse rate. Patient monitoring was assisted electronically with pulse oximetry (Nellcor Model 10, Nellcor Inc., Haywood, California, USA), end tidal CO_2 (Vet/Cap 7000 capnometer, Sensor Devices, Inc., Waukesha, Wisconsin, USA), electrocardiogram (ECG; model 420; Mennen Medical Inc., Clarence, New York, USA), and temperature probe (model 870, Datascope Corp., Paramus, New Jersey, USA).

Anesthetized ewes were prepared for surgery by clipping hair on the left lateral thorax from the scapula to the last rib. They were then carried to a designated surgery area, placed in right lateral recumbency, and the surgical site prepared using a standard surgical scrub. Using aseptic technique we implanted Telonics model HR400 heart rate transmitters (Telonics, Inc.), which had been previously sterilized using the Sterrad system (Sterrad 100, Advanced Sterilization Products, Irvine, California, USA), on the dorsolateral thorax. The transmitters were 11 cm length x 3.3 cm diameter, and weighed 120 g. Positive and negative leads were 15 and 50 cm in length, respectively. We first made a 15 cm paramedian skin incision 12 cm lateral to the dorsal midline and just caudal to the scapular cartilage. We elevated fat and made a 10 cm incision parallel to the fibers in the *latissimus dorsi* muscle. Deep fascia was incised parallel to the ventral lateral border of the *longissimus dorsi* muscle to allow for its elevation. The transmitter was placed, with leads exiting caudally, in the naturally occurring shelf between the ribs and *longissimus dorsi* muscle (Fig. 2-1). Initially (April-May 1995), no procedure was used to prevent the transmitter from migrating craniad under the scapular cartilage over time; however, in later surgeries (October-November 1995) we placed one or two far-far near-near sutures (2-0 Maxon; Davis & Geck Monofil Inc., Manati, Puerto Rico, USA) in the split fascia cranial to the transmitter to deter migration. Two to four far-far near-near sutures (0 Maxon; Davis & Geck Monofil Inc.) were used to reattach the split fascial plane over the transmitter.

With the transmitter secured, the electrodes were then placed. The positive electrode was sutured to muscle fascia just ventral to the transmitter. The electrode was first tacked to the fascia, then surrounded by fascia using a staple suture. Excess lead wire was looped and tacked to the fascia using simple interrupted sutures. The negative electrode was threaded through a trocar (6 mm outer diameter x 36 cm long) that had been passed subcutaneously from the primary incision to a 4 cm incision on the ventral lateral thorax at the level of the antebrachiohumoral joint. The electrode and excess lead wire was sutured to superficial muscle as previously described for the positive electrode. Function and accuracy of the transmitter was confirmed by comparing ECG output to signal received using a Lotek model SRX_400 receiver/datalogger (Lotek Engineering Inc., Newmarket, Ontario, Canada). Incisions in the muscle layer were closed using a cruciate pattern, while the subcutaneous and skin layers were closed with simple continuous patterns (2-0 Maxon; Davis & Geck Monofil Inc.). Ceftiofur sodium (2.5 mg/kg IV; Naxcel, The Upjohn Company, Kalamazoo, Michigan, USA) was administered perioperatively. Phenylbutazone (4 mg/kg; Butatabs-E, Burns Veterinary Supply, Rockville Centre, New York, USA) was administered orally when the ewes had partially recovered from anesthesia.

Evaluation of transmitters

We monitored transmitter function at least once every 2 wk from the time of implantation until failure. We also developed a data acquisition system to collect and store large volumes of heart rate data using the Lotek receiver/datalogger with standard 128K byte data memory, equipped with EVENT_LOG (version 2.5x, W9; Lotek Engineering Inc.), and with a standard H antenna (Telonics, Inc., model RA-2A). The W9 EVENT_LOG package converts interpulse period of received signals to beats per minute (bpm). We programmed the package to determine heart rate based on sets of eight pulses before scanning to the next transmitter frequency. Data was downloaded in ASCII format via RS-232 serial port to a MS-DOS compatible computer.

We performed a calibration trial in July 1995 to determine the accuracy of the data acquisition system. Bighorn sheep implanted with heart rate transmitters (n = 5) were held in a 0.75 x 2 m stall while we elicited a range of heart rate responses (low, medium, high). Data were collected for three independent 1-min intervals from each animal under each of three heart rate responses. We initially sedated ewes lightly with about 0.15 mg/kg xylazine (Miles Inc.) IV and attached leads via clips to obtain a lead II ECG tracing. We then simultaneously collected heart rate data using ECG tracings on strip print-outs and using the data acquisition system. After collection of data under a period of low heart rate, heart rate was increased slightly by administering atropine sulfate (0.04 mg/kg IV; Vedco, Inc., St. Joseph, Missouri, USA). Heart rate was then further increased by administering yohimbine HCl (0.15 mg/kg IV; Wildlife Pharmaceuticals, Inc., Fort Collins, Colorado, USA) with or without 0.8 mg/kg ketamine (Fort Dodge Laboratories) IV and a mild human disturbance applied. We determined heart rate from the ECG by manually counting QRS complexes on the strip print-out and from the data acquisition system by calculating harmonic mean of heart rate data during the 1-min periods (Chabot et al., 1991). We analyzed data from this calibration trial using the method of least squares with the SAS statistical package (SAS Institute, Inc., 1988).

We also assessed the line of sight range of the signal from transmitters to the receiver on relatively level terrain. Bighorn sheep (n = 5) were sedated with ketamine and xylazine as described for surgical induction. Sedation was required so that the ewes could be placed consistently in the same location in a bedded or standing position with the right side of body facing the receiver. Data were collected during 1-min sampling periods from distances of 20, 100, 200, 300, 400, 500, 600, 700, and 800 m. Tests at distances >800 m were not attempted due to marked changes in topography of the land at our site. Two independent collections were made from each animal in each body position (bedded or standing) during September and November 1995; March and May 1996. We analyzed data using GENMOD and ANOVA procedures of the SAS statistical package (SAS Institute, Inc., 1988).

RESULTS

Surgical technique

No serious complications occurred during the surgical procedure. All parameters monitored remained within acceptable limits except for a period of decreased respiration rate lasting about 30 min in two bighorn sheep and associated mild arrhythmia in one. Intermittent positive pressure ventilation was applied during these periods at a rate of 8 breaths/min until respiration rate returned to within normal limits. Mean surgery time was 54 min. Time to standing recovery was about 30 min in these tractable bighorn sheep.

Ewes were more sedentary for about 4 to 5 days following surgery, but no complications or impairment in function were observed. Two bighorn sheep received one additional dose of phenylbutazone about 2 mo post-operatively to decrease acute onset of moderate lameness. Lameness was associated with extension of the left forelimb and was apparently caused by the transmitter. Seromas associated with the transmitter occurred in two bighorn sheep, one about 2 mo post-operatively and the other about 6 mo post-operatively. Palpation indicated that the transmitter had migrated slightly craniad and partially under the scapula in the first ewe. Both seromas resolved without treatment. A transmitter was passively expelled from one ewe 19.5 mo post-implantation when a 2.5 cm diameter muscle/skin defect formed at the caudal end of the transmitter. No clinical signs of infection or pain associated lameness were noted in the ewe. The ewe was sedated for examination using ketamine and xylazine as described for surgical induction. The transmitter was removed by clipping the leads just below skin level. The defect was flushed with dilute betadine solution (The Purdue Frederick Company, Norwalk, Connecticut, USA) and closed using 2-0 Maxon (Davis & Geck Monofil Inc.) in cruciate and simple interrupted suture patterns. Prophylactic antibiotics (22,000 U/kg subcutaneous penicillin G benzathine and penicillin G procaine, Durvet, Inc., Blue

Springs, Missouri, USA) and analgesics (4 mg/kg phenylbutazone per os, Burns Veterinary Supply) were administered. The ewe recovered without further complications.

Evaluation of transmitters

Twelve of 15 transmitters (80%) remained functional for at least 1 yr (Fig. 2-2). Three transmitters failed 3.5 to 4.5 mo following implantation. Failure was characterized by lack of signal transmission. Transmitters were not retrieved to determine cause of failure. The calibration trial indicated a strong, positive linear relationship ($r^2 = 0.98$; $\delta_{y/x} = 4.20$; $\underline{P} < 0.001$) between heart rate as indicated by the ECG tracing and heart rate determined using the data acquisition system (Fig. 2-3). In assessing line of sight signal range, we performed two independent collections from each of five bighorn sheep in two body positions over four sampling periods between September 1995 and May 1996, except that the transmitter of one ewe failed prior to the second collection in May. At our study site, line of sight signal range from standing ewes was at least 800 m in 95% of the collections (37/39; Fig. 2-4). Although signal range decreased ($\underline{P} < 0.001$) when ewes were bedded, signals were recorded from 74% (29/39) of the collections at 600 m (Fig. 2-4). Transmitter function varied ($\underline{P} < 0.001$) between individual ewes.

DISCUSSION

We developed a safe and reliable technique for remotely monitoring heart rate in captive bighorn sheep. The Telonics model HR400 heart rate transmitter was successfully implanted in 15 captive, adult bighorn sheep. With the exception of one transmitter that was passively expelled after 19.5 mo, we observed no complications or impairment of function that would likely have affected survivability in free-ranging bighorn sheep. We attribute much of this success to the design of the Telonics transmitter, optimal anesthetic regimen, strict aseptic technique, and site of implantation. The dorsolateral thorax proved to be an ideal site for implantation from the perspective of both surgical technique and transmitter function. By placing the transmitter in a natural depression and covering it with superficial musculature, as well as subcutaneous tissue and skin, pressure on the incision line was minimized and dehiscence was avoided. Complications associated with intraperitoneal placement (Wallace et al., 1992) were also avoided and transmitter function was enhanced because the dorsal location likely increased signal range of the transmitter. Signals from temperature-sensitive transmitters were received for markedly greater distances when implanted on the back of the neck than when placed intra-abdominally in grizzly bears (Philo et al., 1981). The strong signal emitted from these transmitters should allow data to be collected at ranges that do not alter behavior or physiological response of animals under study. Additionally, because the lateral thorax is an area of minimal movement, interference from spurious signals (Jacobsen et al., 1981) is minimized.

We believe that this technique could be applied to other species of ungulates as well; however, a minimum body size for successful implantation of a transmitter this size likely exists. In this study, all ewes were ≥55 kg. In smaller animals, the shelf between the ribs and *longissimus dorsi* muscle may not be large enough to accommodate placement of the transmitter without pressure on the overlying tissues. Although all elements of a surgical procedure are important, the most critical aspects for success using this procedure appear to be strict aseptic technique and positioning of the transmitter well caudal to the scapular cartilage. Ample space should be allowed to accommodate range of motion of the scapula without interference with the transmitter. Addition of sutures to close the split fascia cranial to the transmitter in later surgeries apparently aided in preventing migration of the transmitter toward, or under, the scapular cartilage. This cranial migration was the most likely cause of the eventual expulsion of the transmitter from one ewe. The cranial end of the transmitter under the scapular cartilage likely served to increase the motion and angle of the transmitter (deeper at the cranial end under the scapular and more superficial at the caudal end). Based on the location of the muscle/skin defect, it appeared that pressure and mechanical trauma exerted by the caudal end of the transmitter was responsible for the tissue damage and consequent expulsion. In addition to the cranially placed stop sutures, the expulsion also may have been prevented by anchoring the transmitter to the deep fascia and *longissimus dorsi* muscle using a nylon surgical mesh. Alternatively, this complication could be avoided by surgically removing transmitters upon their failure.

Transmitters remained functional for an extended period. Because our intent was not to perform multiple surgeries on an animal, we were unable to determine cause of transmitter failures. We assume that the cause of three early failures most likely was due to leakage of body fluid into the transmitter because the units simply quit producing a signal. Transmission of a mortality signal, as would occur with lead breakage or inability to detect electrical activity of the heart, was not observed with any transmitter failure. We suspect that subsequent failures were due to battery depletion. Calculated battery life was 18.5 mo at 60 bpm (B. Burger, pers. commun.); however, because a bighorn sheep's heart rate is frequently >60 bpm, expected battery life is shorter.

Unfortunately, it is difficult to compare longevity of transmitters in this study with others because results of long-term evaluation have rarely been reported. Longevity of intra-abdominal heart rate transmitters was noted to be about 40 days in fox (Kreeger et al., 1989) and ≥180 days in wolves (Kreeger et al., 1990a). Heart rate transmitters implanted subcutaneously in a domestic and a bighorn sheep functioned 30 and 14 days, respectively, prior to expulsion following tissue necrosis (Coates et al., 1990). Transmitters implanted subcutaneously on the neck of elk functioned 5 to 9 mo ($\chi = 7$ mo; Cassirer, 1990). Bunch et al. (1989) did not report longevity of transmitters place intra-abdominally in two bighorn sheep; however, Wallace et al. (1992) used a similar technique to ours with good, although variable, success. In that study, seven transmitters failed due to lead breakage or disconnection ≤38 days post-implantation, seven transmitters short-circuited after seepage of body fluid between 126 and 509 days post-implantation, nine were functional when they were removed from live or dead animals 90 to 153 days post-implantation, and two were functioning at study termination 328 and 456 days post-implantation (Wallace et al., 1992). Because all of the transmitters in the current study continued to function >110 days post-implantation and 80% functioned to meet our criterion of ≥1 yr (Fig. 2-2), we believe that this approach may be more reliable for long-term monitoring when compared to other alternatives currently available.

Implantation of HR400 transmitters using this surgical approach met our criteria for success. The reliability and longevity of the Telonics transmitter implanted on the lateral thorax appeared to be an improvement over previously reported telemetry systems used in ruminants. The system did not detract from the aesthetic value of the animal because the implanted transmitter was not visible following regrowth of hair on the skin. Although a transmitter was expelled from one ewe, overall the technique was safe and humane to experimental animals. We believe that this technique could be successfully used in a field application; however, transportation of free-ranging animals to a base camp with aseptic surgical facilities would be required. Wallace et al. (1992) had limited success in capturing, transporting, and surgically placing transmitters intra-abdominally in free-ranging bighorn sheep. Although two of eight animals in that study died during or immediately following transport, modifications were made to the handling protocol to improve success over the period of animals captures. This finding highlights the importance of safe capture (Kock et al., 1987), transport, and anesthetic protocols in addition to use of a tested surgical technique for transmitter implantation. Although less invasive methods for monitoring heart rate in wild ruminants may be appropriate in some situations, when a reliable, relatively long-lasting inconspicuous telemetry system is required, we believe that this approach holds promise for success in free-ranging as well as captive animals.

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Fig. 2-1. Placement of heart rate transmitter relative to deep musculature of the lateral thorax (adapted from Pasquini, 1982) of a bighorn sheep. The transmitter was placed, with leads exiting caudally, in the naturally occurring shelf between the ribs and *longissimus dorsi* muscle.

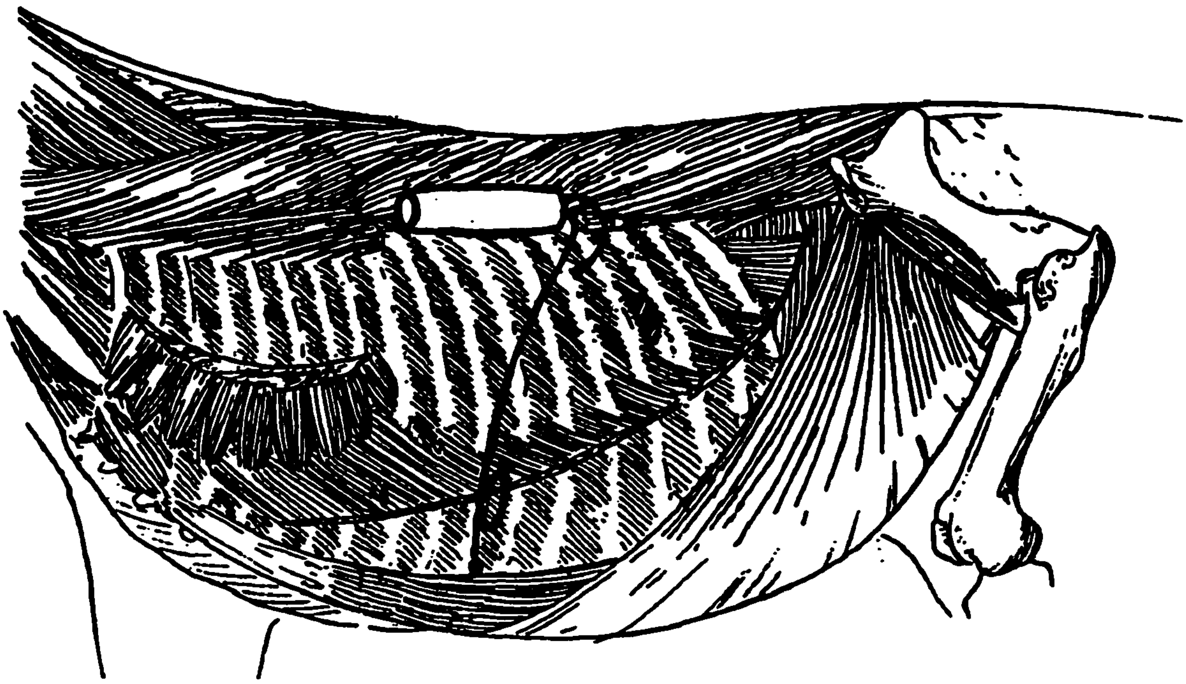


Fig. 2-2. Functional duration of heart rate transmitters surgically implanted into captive bighorn sheep (n = 15).

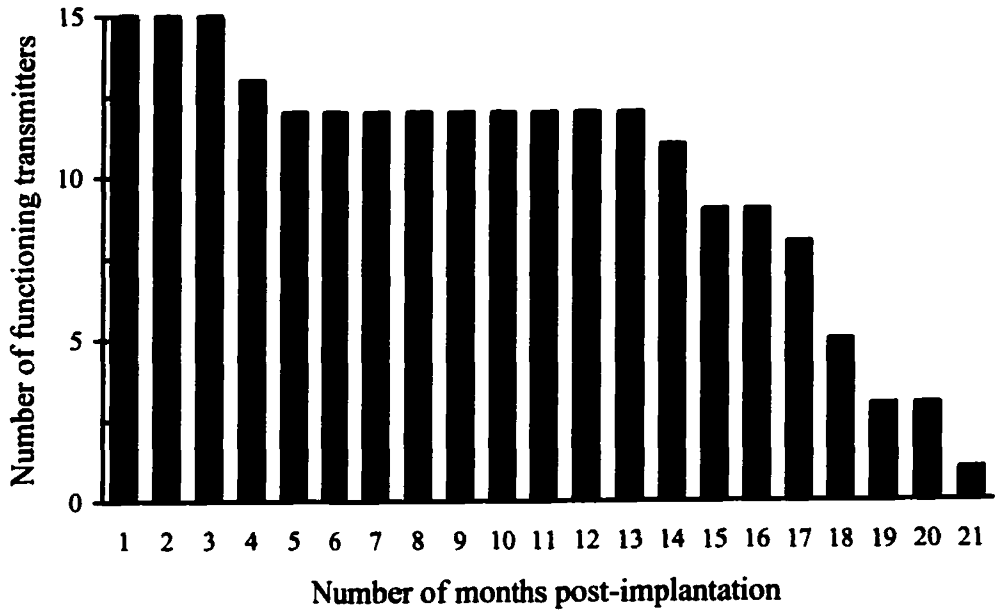


Fig. 2-3. Correlation between heart rate data collected using the data acquisition system and heart rate determined using an ECG tracing was high ($r^2 = 0.98$; $\delta_{y/x} = 4.20$) for bighorn sheep ($n = 5$) sampled over a range of heart rate responses.

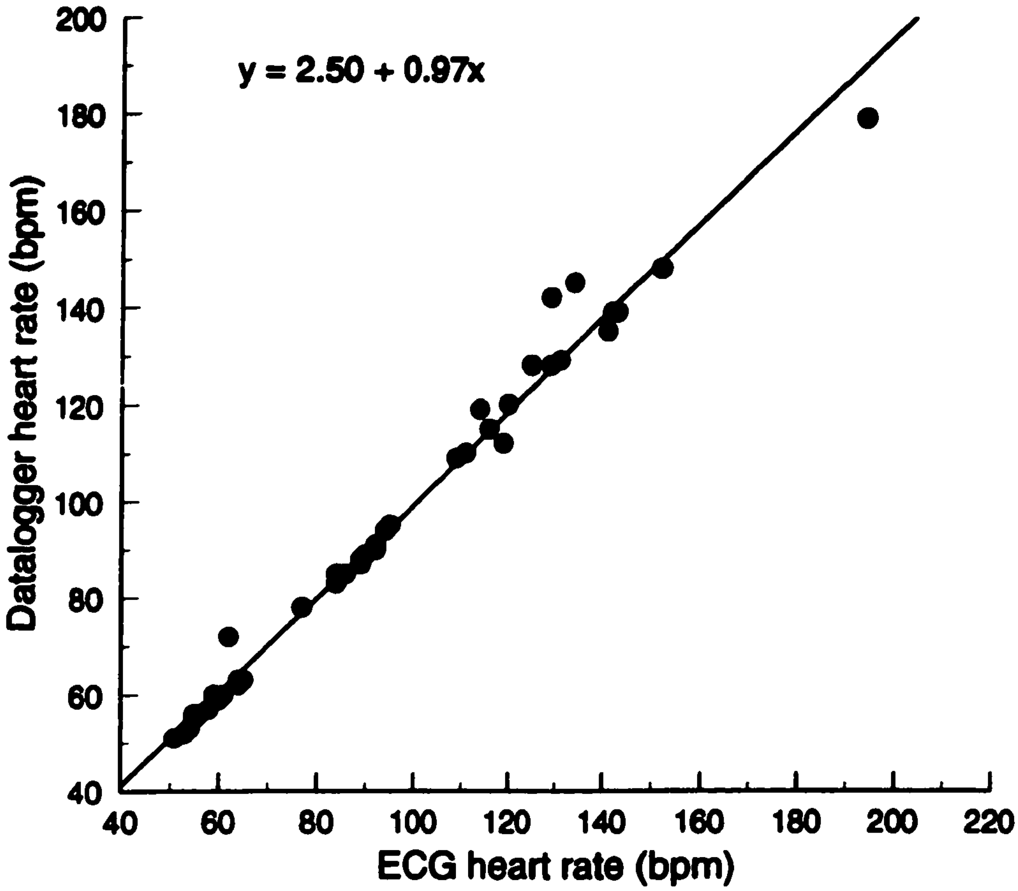
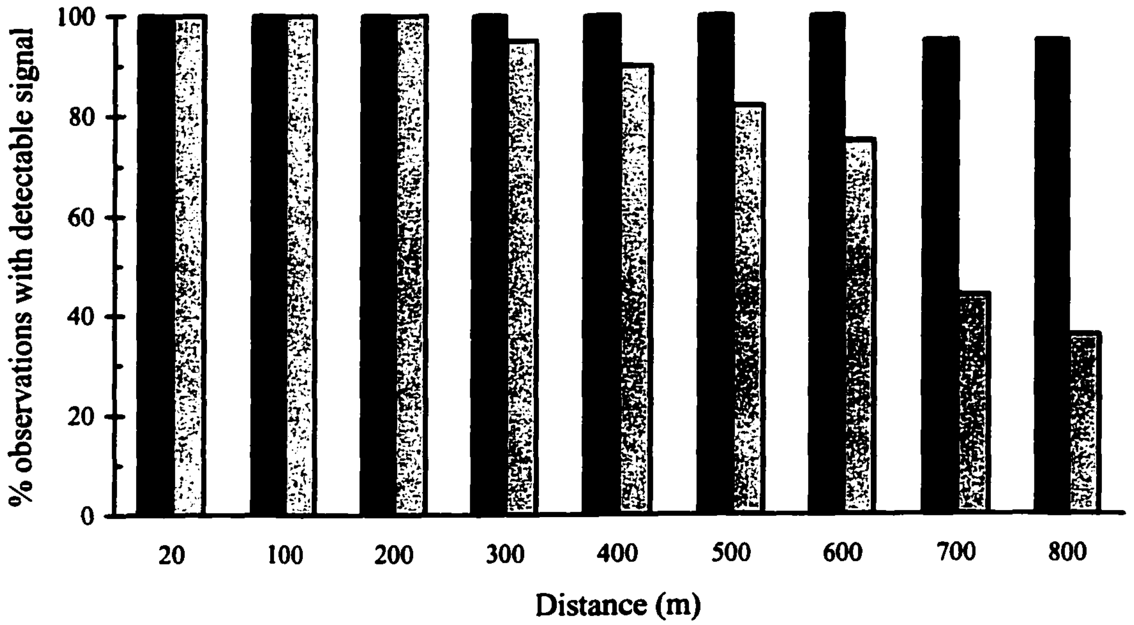


Fig. 2-4. Percent of observations with a detectable telemetry signal (n = 39 observations from each distance) collected from bighorn sheep at line of sight distances 20 to 800 m, September 1995 to May 1996. A solid bar indicates standing position and a stippled bar indicates bedded position.



CHAPTER 3

HEART RATE AS A PREDICTOR OF ACUTE STRESS IN BIGHORN SHEEP (*Ovis canadensis*) EWES [1]

Abstract: Increased exposure of bighorn sheep (*Ovis canadensis*) to human-associated disturbance is a primary concern of resource managers. However, quantitative methods to assess a free-living animal's physiological response to acute environmental stressors are limited. We examined the effects of controlled stressors on the relationship between telemetered heart rate and serum cortisol in 15 captive female Rocky Mountain bighorn sheep (*O. c. canadensis*). Ewes responded to graded stressor with a predictable rise in peak serum cortisol that was related to increased heart rate, but in many cases no overt behavioral responses were observed. Serum cortisol levels tended to increase linearly with heart rate for all ewes, although the magnitude of response varied with reproductive status ($p < 0.001$). Serum cortisol concentrations increased 0.11 ng/mL (SE = 0.017) for each 1 bpm in response heart rate in non-pregnant ewes and 0.09 ng/mL (SE = 0.023) for ewes in early pregnancy. In contrast, serum cortisol response of ewes in late pregnancy more than doubled to 0.23 ng/mL (SE = 0.030) for each 1 bpm in response heart rate. We conclude that remotely monitored heart rate can be used as a predictor of change in serum cortisol levels in bighorn sheep ewes of known reproductive status and, when used in conjunction with overt behavioral observations, can provide an improved assessment of acute stress.

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[1][1]This chapter will be submitted to Canadian Journal of Zoology as Wild, M. A., Baker, D. L., and Bowden, D. C. "Heart rate as a predictor of acute stress in bighorn sheep ewes".

Introduction

Wildlands in western North America provide a diversity of recreational opportunities for outdoor enthusiasts. For most, these recreational pursuits are enhanced by the presence of wildlife in their natural habitats. This has created a dilemma for resource management agencies that seek to provide both a satisfying recreational experience for people and protection for wildlife populations. Accomplishing this dual goal requires knowledge of the sensitivity of wildlife to human disturbances and new techniques for guiding the behavior of recreationists.

Human-induced disturbances may adversely affect animals directly via acute mortality or indirectly through changes in behavior or physiology. Behavioral changes can result in displacement, increased energy expenditure, decreased energy acquisition, and decreased productivity (Knight and Cole 1991). Although an animal's acute physiologic response to the stress of disturbance is a protective means to deal with a perceived change in homeostasis, prolonged or repeated exposure to stressors can lead to excessive stimulation of the endocrine system (reviewed by Stephens 1980; Roth 1985) and direct modulation of the immune system (Moberg 2000) with resultant immune compromise. Rhind et al. (1999) have shown that large, relatively infrequent increases in cortisol compromise cell-mediated immune function in domestic sheep.

Some wildlife species may be adversely affected more than others by human-induced disturbances. Wildlife managers are often concerned about the effects of disturbance on bighorn sheep (Hicks and Elder 1979; Krausman et al. 1998; Ostovar 1998; Papouchis et al. 2001). This is due in part, to the belief that bighorn sheep exhibit strong fidelity to traditional ranges, regardless of changes in suitability of those ranges (Geist 1971). Bighorn sheep may endure increased levels of stress from disturbance rather than expand to new ranges, or even flee in some cases, to avoid it. Immunosuppressive effects of distress have been implicated in the bovine shipping fever complex (reviewed by Confer et al. 1988) and may be an important component of the bighorn sheep pneumonia complex (Hudson 1973; Spraker et al. 1984) that impairs population performance (Buechner 1960; Spraker and Hibler 1982; Thorne 1982; Onderka and Wishart 1984). On the other hand, bighorn sheep may habituate to predictable human activity and be unaffected by recreationists (Geist 1975; Papouchis et al. 2001).

If wildlife managers were able to determine which human activities did and did not induce distress in bighorn sheep, management plans could be developed to maximize benign recreational activities and manage those activities that cause disturbance. However, no reliable, long-term, noninvasive technique is available to provide this quantitative indicator of stress in free-ranging wildlife. Serum cortisol levels are commonly measured and are considered the "gold standard" for stress determination but reflect only the status of an animal at a point in time that is likely influenced by the invasive sampling technique itself. Fecal and urine cortisol (Miller et al. 1991) provide promising methods for noninvasive monitoring; however, these techniques can be difficult to use for monitoring individual free-ranging bighorn sheep, especially in their response to specific events. Behavioral responses alone are inadequate indicators of excitation in bighorn sheep (MacArthur et al. 1982; Stemp 1982) and other species

(Kreeger et al. 1989; Baldock and Sibly 1990) because various stimuli, including disturbance by humans, elicit physiological changes even in the absence of overt behavioral responses. Remotely monitored heart rate has been used to assess disturbance of bighorn sheep (MacArthur et al. 1979; Krausman et al. 1998) although the relationship between heart rate and other indicators of stress are unclear.

Harlow et al. (1987a; 1987b) showed a strong linear relationship between heart rate and blood cortisol in a group of domestic sheep and 2 individual bighorn sheep. However, Harlow et al. (1987b) noted that due to individual variation, a larger sample would be needed to determine whether a regression equation of predictive value could be obtained for bighorn sheep or if individual variation would preclude applicability of heart rate as a predictor of cortisol.

The objectives of our study were to: (1) further investigate the relationship between heart rate and serum cortisol, specifically to determine the functional relationship between heart rate and serum cortisol level in adult, female bighorn sheep exposed to graded stressors; and (2) develop a model that could be used to predict the physiological effect of disturbance on free-ranging bighorn sheep using remotely monitored heart rate.

Heart rate changes likely correspond to metabolic and pituitary-adrenal axis responses, including serum cortisol levels, in response to audiovisual stressors (Harlow et al. 1987a; Harlow et al. 1987b; Chabot et al. 1991). Therefore, we hypothesized that a strong linear correlation would exist and that this correlation could be used to predict serum cortisol given heart rate data. However, we also hypothesized that the relationship between heart rate and serum cortisol would change with physiological status of the animal, particularly during late pregnancy when baseline cortisol levels increase as previously documented in domestic ewes (Keller-Wood 1998). To test these hypotheses, we determined the relationship between heart rate and serum cortisol in non-pregnant ewes and ewes in early and late stages of pregnancy.

Methods

Animals

Female bighorn sheep used in these experiments were all tractable adults (body mass range 55.5 - 85 kg) born in captivity and housed at the Colorado Division of Wildlife's (CDOW) Foothills Wildlife Research Facility, Fort Collins, Colorado, USA. Prior to these experiments, we trained ewes to repeated handling, blood sampling techniques, holding pens, and individual stalls. Between experiments ewes were housed in 3-ha pastures and received *ad libitum* quantities of grass/alfalfa hay, water, trace mineral block, and high energy supplement (0.5 kg/animal/day; Baker et al. 1998). We performed all experiments in accordance with the principles and guidelines of the Canadian Council on Animal Care and United States Animal Welfare Act under the review of the CDOW and Colorado State University Institutional Animal Care and Use Committees.

Experimental Protocol

We examined the relationship between heart rate and serum cortisol levels in 3 separate experiments. We conducted experiment 1 with non-pregnant ewes ($n = 15$) during January to March 1996. We conducted experiments 2 and 3 using a subset ($n = 7$) of these ewes in early pregnancy during January to February 1997 and late pregnancy during March to April 1997, respectively. Each experiment consisted of 5 trials in which we simultaneously measured heart rate and serum cortisol levels of ewes exposed for 3 min to 1 of 4 treatments that differed in severity.

Heart rate.--An evaluation of the heart rate telemetry system and associated surgical implantation procedure have been previously described (Wild et al. 1998). Briefly, with ewes under gas anesthesia we aseptically implanted Telonics model HR400 heart rate transmitters (Telonics Inc., Mesa, Ariz., USA) on the dorsolateral thorax. We placed the transmitter in the naturally occurring shelf between the ribs and *longissimus dorsi* muscle, and closed muscle, subcutaneous, and skin layers over the transmitter. Surgery was performed at least 2 mo prior to beginning experiment 1.

During experimental periods, heart rate data were collected continuously using a Lotek SRX_400 telemetry receiver/datalogger equipped with EVENT_LOG (version 2.5x, W9; Lotek Engineering Inc., Newmarket, Ont., Canada) which converted interpulse period of received signals to beats per minute (bpm). We programmed the receiver to determine heart rate based on sets of 8 pulses before scanning to the next transmitter frequency. We downloaded heart rate data from the datalogger to an MS-DOS compatible computer. Heart rate (bpm) for a specified period was determined by calculating the harmonic mean of raw heart rate data. Preliminary analyses suggested that the heart rate response elicited by acute stressors was best represented by the harmonic mean heart rate for the initial 5 min of the trial (3 min stressor period and 2 min following; HR5). Therefore, HR5 was used in analyses as a measure of response heart rate.

Serum cortisol. -- Approximately 16 hours before exposure to a controlled stressor, we sedated each ewe with 130 mg ketamine (Ketaset, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa, USA) and 13 mg xylazine (TranquiVed, Vedco, Inc., St. Joseph, Mo., USA) intravenously (IV) and placed an indwelling jugular catheter (16 gauge x 14 cm Abbocath-T, Abbott Hospitals, Inc., North Chicago, Ill., USA). The catheter was sutured to the skin and bandaging was applied to cover the catheter. Sedation was reversed with 15 mg yohimbine (Antagonil, Wildlife Pharmaceuticals, Fort Collins, Colo., USA) IV. Ewes were placed in holding pens (5 x 9 m) overnight and provided hay and water. The following day, we moved ewes to individual metal screen stalls (2.3 x 1.1 m). We attached catheters to 2 m of sterile tubing 1.5-2.5 h prior to start of the trial so that blood samples could be collected without disturbance to the bighorn sheep. We collected a 6 mL blood sample via the tubing 5 min before (pre-treatment; CORT_5), and 10, 15, 20, 30, 40, 60, 80, 100, 120, and 180 min after treatment application. We flushed the tubing with 20 mL heparinized saline after each use and cleared tubing of saline prior to sample collections. Following each trial, intravenous catheters were removed and 125 mg ceftiofur (Naxcel, The Upjohn Company, Kalamazoo, Mich., USA) was administered IV prophylactically. Serum was harvested from blood samples within 24 h of collection and stored at -20° C. Serum cortisol levels were determined using radioimmunoassay (Miller et al. 1991).

Prior to experiments 2 and 3, we modified our sampling protocol in two ways. One, we used extended use polyurethane intravenous catheters (16 gauge x 13.4 cm Milacath, Mila International, Inc., Covington, Ky., USA) that were placed once, flushed with heparinized saline through a high flow extension set with reflux valve (Mila International, Inc.) every 48 h, and removed at the end of the 5-wk experimental period. And two, the period of data collection was decreased to 80 min post-treatment based on the rapid return to baseline values observed in experiment 1.

Preliminary analyses, and previous work by other investigators, suggested that the peak cortisol elicited by an acute stressor most frequently occurred 15 min post-stressor application (CORT15). Therefore, CORT15 was used in analyses as a measure of peak cortisol response.

Acute stressors. – We exposed ewes, in sets of 3 assigned at random for each trial, to a single acute stressor and collected data between 0900 and 1300 h. Our interest was not the reaction to a particular stressor, but to elicit a wide range of heart rate and serum cortisol responses. Initially, we performed a control trial with no stimulus applied. Subsequently, we performed trials exposing ewes to a mild stressor (technician appearing suddenly and pacing in front of the stalls), a moderate stressor (technician appearing suddenly and pacing and waving a flag in front of the stalls), and a severe stressor (technician on a motorcycle appearing suddenly and driving in front of the stalls). Finally, we repeated the control trial to test for sensitization or habituation to our experimental conditions. Each stressor was applied for a 3-min period commencing at study time 0. Ewes were used on no more than one trial in a 3-day period.

Statistical Analysis. -- We used ANOVA and multiple regression techniques to determine the variables that significantly ($p < 0.05$) influenced the cortisol response (CORT15) of ewes. For each ewe that completed all trials for a given experiment, a multiple regression of CORT15 on initial cortisol value (CORT_5) was run. Using Proc

GLM in SAS (SAS Institute Inc. 1988) and data from ewes common to each of the 3 experiments, we tested for significant differences in regression coefficients for HR5 among experiments and animals. Because a significant difference in HR5 coefficients among experiments was found, we also performed analyses for each experiment separately.

Simple linear regressions of CORT15 on HR5 were run for each experiment. We also used simple linear regression to calculate a regression equation describing the linear relationship between change in cortisol (CORT15 – CORT_5) and HR5. Least squares means were used to test for differences between initial and final control trials conducted in 1996 to determine if sensitization or habituation had occurred or if there was a seasonal effect.

Results

All bighorn sheep remained healthy during the study periods; however, one ewe died about 1 mo following experiment 1. Necropsy findings indicated a pheochromocytoma (adrenal medulla tumor) with diffuse metastasis. Data from this ewe were not outliers in the data set and were included in analyses. Heart rate transmitters failed in 3 ewes 3.5 to 4.5 mo post-implantation, but remained functional $\geq$ 1 yr in remaining ewes (Wild et al. 1998). As a result of transmitter failure during the study period, samples sizes were reduced to 12 for experiment 1, and 6 for experiments 2 and 3.

All ewes diagnosed pregnant using pregnancy-specific protein B (Drew et al. 2001) and having functional heart rate transmitters in January 1997 were selected for use in experiments 2 and 3. All of these ewes subsequently produced healthy lambs between 29

April and 30 May 1997. Based on back calculation from lambing date using a 180 d gestation, ewes were in the first half of gestation (from 37-64 d and 68-98 d of gestation at the initiation and completion, respectively) during experiment 2. Ewes were in the last half of gestation (from 107-138 d and 134-162 d at the initiation and completion, respectively) during experiment 3.

Bighorn sheep exhibited a wide range of behaviors during pre-treatment, treatment, and post-treatment periods of the trials. Activities included standing quietly, standing watching, pacing, climbing, head butting, rubbing, feeding, and lying bedded. Activity levels generally declined with time during the pre-treatment period. We observed varying responses to treatment with the graded stressors. Behavioral responses included standing alert, moving from front to back of stall, circling, climbing, trembling, and panting. Each of the stressors elicited a range of behavioral responses from mild to marked, varying with the individual.

Inter-assay variation for serum cortisol analysis was within standard and acceptable laboratory limits. Coefficients of variation were 9.3% and 5.2% for samples collected in 1996 and 1997, respectively.

We did not detect sensitization or habituation to our experimental conditions. No differences were observed between baseline data collected during the first (January) and last (March) trial of experiment 1. During the January and March control trials, mean baseline heart rate was 62 and 63 bpm ($F = 4.04$, $df = 11$, $p = 0.57$), CORT_5 was 8.6 and 6.8 ng/mL ($F = 0.52$, $df = 11$, $p = 0.46$), and CORT15 was 7.9 and 8.2 ng/mL ($F = 0.73$, $df = 11$, $p = 0.88$), respectively.

Heart rate increased immediately upon presentation of the stressor, while serum cortisol lagged with a peak response most frequently 15 min post-stressor initiation (Fig. 3-1). Harmonic mean heart rate during the first minute of stressor application ranged from baseline to 237 bpm. Heart rate returned to baseline within minutes after removal of the stressor (Fig. 3-2). Cortisol responses ranged from unchanged to 26.5 ng/mL based on individual, stressor, and experiment. By 30 min post-stressor initiation, cortisol had usually returned to near baseline levels (Fig. 3-3). We did not observe a plateau in the cortisol response curve. Increased heart rate was accompanied by increased cortisol regardless of initial cortisol value with the exception of one ewe with a high initial cortisol. This positive linear response can be visualized in plots of change in cortisol on HR5 for ewes in each reproductive stage (Fig. 3-4).

In multiple regression analyses using data only from ewes common to each of the 3 experiments (n = 6), we found a significant difference in HR5 coefficients among experiments. Therefore, a single predictive model for ewes in all reproductive stages could not be determined and analyses were performed separately for each experiment.

For experiment 1, data were available from 12 non-pregnant ewes. No significant differences were found among the regression coefficients for HR5. The best predictive model (r^2 = 0.78, standard deviation = 3.08) allowed intercepts to vary by animal and used a CORT_5 coefficient of 0.393 (SE = 0.113) and a HR5 coefficient of 0.111 (SE = 0.013). For ewes in early pregnancy (experiment 2) only HR5 was a significant variable in the model (r^2 = 0.33). For ewes in late pregnancy (experiment 3) HR5 and CORT_5 contributed significantly to the best model (r^2 = 0.81) for cortisol response.

In a field application only heart rate response would be known. Because multiple regression analyses showed no significant difference in HR5 coefficients among individual ewes within a specific reproductive stage (i.e., experiment), we used HR5 as a predictor of change in cortisol. The change in cortisol associated with a given response heart rate was predicted using the slope of the simple linear regression line of CORT15 on HR5 for each experiment (Table 3-1). A positive linear relationship was observed between HR5 and CORT15 for ewes in all experiments, although the magnitude of response varied with reproductive status of the ewes ($p = 0.0001$). Cortisol response in late pregnancy ewes was about twice that of non-pregnant or early pregnancy ewes (Table 3-1).

Discussion

Bighorn sheep responded to acute stressors with a predictable rise in serum cortisol that was related to increasing heart rate. Although more severe behavioral responses were often associated with high heart rate and cortisol levels, in some cases bighorn sheep stood motionless, watching alertly while they experienced extreme physiologic responses as evidenced by increased heart rate and serum cortisol levels. This finding supports conclusions by other investigators (MacArthur et al. 1982; Stemp 1982) that behavioral response is not always an accurate indicator of acute stress in bighorn sheep. If an animal moves away from a stimulus it can clearly be classified as a disturbance. The difficulty in evaluating the impact of an environmental stressor on an animal occurs when there is no overt behavioral response. Our results suggest that heart rate can be used in addition to behavioral studies as a predictor of physiological response to acute disturbances.

We hypothesized that a strong linear correlation between change in heart rate and cortisol in animals of similar reproductive status exposed to acute stressors would allow us to generate a general regression equation to predict serum cortisol values given heart rate data alone. This hypothesis was only partially supported by our experiments. Contrary to our hypothesis, heart rate was not found to be a useful direct predictor of serum cortisol level in these experiments. As suggested by Harlow et al. (1987b), high individual animal variability prevented formulation of a single regression equation to predict cortisol given heart rate. However, the individual animal variation within an experiment was primarily due to initial cortisol value; the rate of increase in cortisol in relation to response heart rate did not differ among individuals of a given reproductive class. Therefore, a single rate coefficient may be useful in predicting the increase in cortisol for any given initial cortisol level for ewes in a specific reproductive class. The increase in cortisol following an acute stressor can be predicted by multiplying the response heart rate (HR5) by 0.11, 0.09, or 0.23 if the ewe is non-pregnant, early pregnant, or late pregnant, respectively (Table 3-1).

It is understandable that individuals may begin their response to a stressor with varying cortisol values. In our experiments, ewes sometimes had pre-treatment cortisol levels that were higher than expected baseline levels. In humans, cortisol is secreted episodically (Hellman et al. 1970). Normal pulsitile secretion of cortisol by the adrenal glands may explain, in part, the observed variation in baseline cortisol values. Unintended disturbance may have also contributed. Regardless, when the cortisol response to a stressor was added to a high baseline cortisol level, the resulting value was higher than predicted by heart rate (Fig. 3-1). Because of this variation in initial cortisol values, change in cortisol was a more useful measure in the study of response to acute stressors.

Within the range of heart rate and cortisol data collected, no plateau was observed in the cortisol response curve. Regardless if the initial cortisol value was high or low, heart rate increase was followed by cortisol increase. Therefore, as Harlow et al. (1987b) noted, the cortisol response to multiple disturbances in a day can be additive. However, because cortisol levels return to baseline about 30 min after an acute stressor, to have an additive effect, acute stressors would have to occur in relatively rapid succession.

We found the response heart rate measured during the 3-min stressor and for the 2 following min (HR5) to be the best predictor of change in cortisol. HR5 was biologically important because this time frame included the degree of heart rate response to the 3-min stressor and also an indication of the rate of return to baseline. Although this time period would likely vary under non-controlled conditions, it provides a basis for predicting cortisol response to acute stressors. Further, HR5 could be used in field experiments to predict cortisol response to controlled application of acute stressors that resemble specific types of human-induced disturbances.

Similar to the findings of Harlow et al. (1987b), who used 2 bighorn sheep, we saw a positive linear relationship between heart rate and serum cortisol response over a range of values for ewes in all experiments; however, as we had hypothesized, the slope of the lines (i.e., rate of increase), varied with reproductive status of the ewes. Therefore, a separate model was required for each reproductive stage. For non-pregnant ewes and ewes in early pregnancy, rate of change in cortisol was similar. Each 1 bpm in response heart rate was accompanied by a 0.11 ng/mL (SE = 0.017) increase in serum cortisol in non-pregnant ewes, while ewes in early pregnancy responded with a 0.09 ng/mL (SE = 0.023) increase in serum cortisol (Table 3-1). During late pregnancy the coefficient used to predict change in serum cortisol more than doubled to 0.23 ng/mL (SE = 0.030; Table 3-1).

The increase in cortisol that we observed in late pregnancy is similar to that observed in humans (Demey-Ponsart et al. 1982) and domestic sheep (Bell et al. 1991). In pregnant, domestic ewes there is an increase in basal ACTH and cortisol that does not correlate with concentrations of gonadal steroids (Bell et al. 1991). Pregnancy in domestic ewes apparently resets the pituitary-adrenal axis and basal cortisol concentration required for normalization of basal ACTH concentration (Keller-Wood 1998). Elevation in baseline cortisol in pregnant ruminants may be essential for normal fetal development and homeostasis (Keller-Wood 1998).

Animals may react to audiovisual stressors with a biological response that includes a combination of behavioral, autonomic, neuroendocrine, or immunological responses (Moberg 2000). Heart rate responses are of short duration and in response to autonomic stimulation. Cortisol has a broader, longer lasting effect on the body and is secreted in response to stimulation of the hypothalamic-pituitary neuroendocrine system. When we use heart rate as a predictor of serum cortisol response, we make the assumption that although the variables are in different pathways of the biological response, they will retain their correlation in different situations and with different stressors. This assumption is likely generally true for response of bighorn sheep to similar categories of stressors (e.g., audiovisual stressors), however, under some situations, the functional relationship between the variables may change. One physiological response pathway may become sensitized while the other remains neutral or becomes desensitized. In pigs, cortisol responses gradually diminished over time with repeated exposure to isolation stress but heart rate responses remained unchanged (Schrader and Ladewig 1999). Although we were unable to detect sensitization or desensitization of heart rate or serum cortisol responses to the novel stressors used in this experiment, this possibility should be considered in application of the technique over extended time periods with repeated stressors.

We believe that the functional relationship observed between heart rate and serum cortisol in tractable bighorn sheep in this study is the best available representation for free-ranging bighorn sheep. Although tractable and wild bighorn sheep may differ in the degree of response to particular acute stressors, the relationship between the heart rate and cortisol response is likely similar. Monfort et al. (1993) warn that physical restraint or anesthesia can lead to erroneous conclusions regarding adrenal function. Given currently available technology in North America, it is not possible to remotely collect repeated blood samples from wild bighorn sheep without inducing stress and therefore biasing cortisol results. Therefore, tractable bighorn sheep are currently the best available model for predicting adrenal function in wild bighorn sheep.

Stress is a part of life and animals have evolved to cope with the stresses in their lives. One difficulty of using cortisol response (measured or predicted) as an indicator of stress is that we cannot differentiate between non-threatening stress and distress. For example, stallions secrete similar amounts of cortisol when restrained, exercised, or permitted to mate with a mare (Colborn et al. 1991). Contrary to Selye's (1950) prediction, there is no non-specific stress response that applies to all stressors; however, we believe that a combination of behavioral monitoring and prediction of change in cortisol based on heart rate response are important measures to assess the impact of acute audiovisual stressors on bighorn sheep.

If remotely monitored heart rate data are collected, our models can be applied to predict the physiological effects, or lack thereof, of applied disturbances. Although the ultimate effects of these physiological impacts remain largely unknown, our models provide a quantitative measure that may in future work be related to animal well-being, health, and resultant acceptable and unacceptable management practices. Future work should test the applicability of this heart rate collection technique on free-ranging bighorn sheep in areas of human activity. Conservative management practices, particularly in areas where behavioral responses by bighorn sheep do not suggest human disturbance, should be tested using methods that include heart rate monitoring to predict cortisol response. These studies will help determine whether well planned, predictable forms of human recreation may be increased without jeopardizing bighorn sheep populations or if conservative management practices are scientifically warranted.

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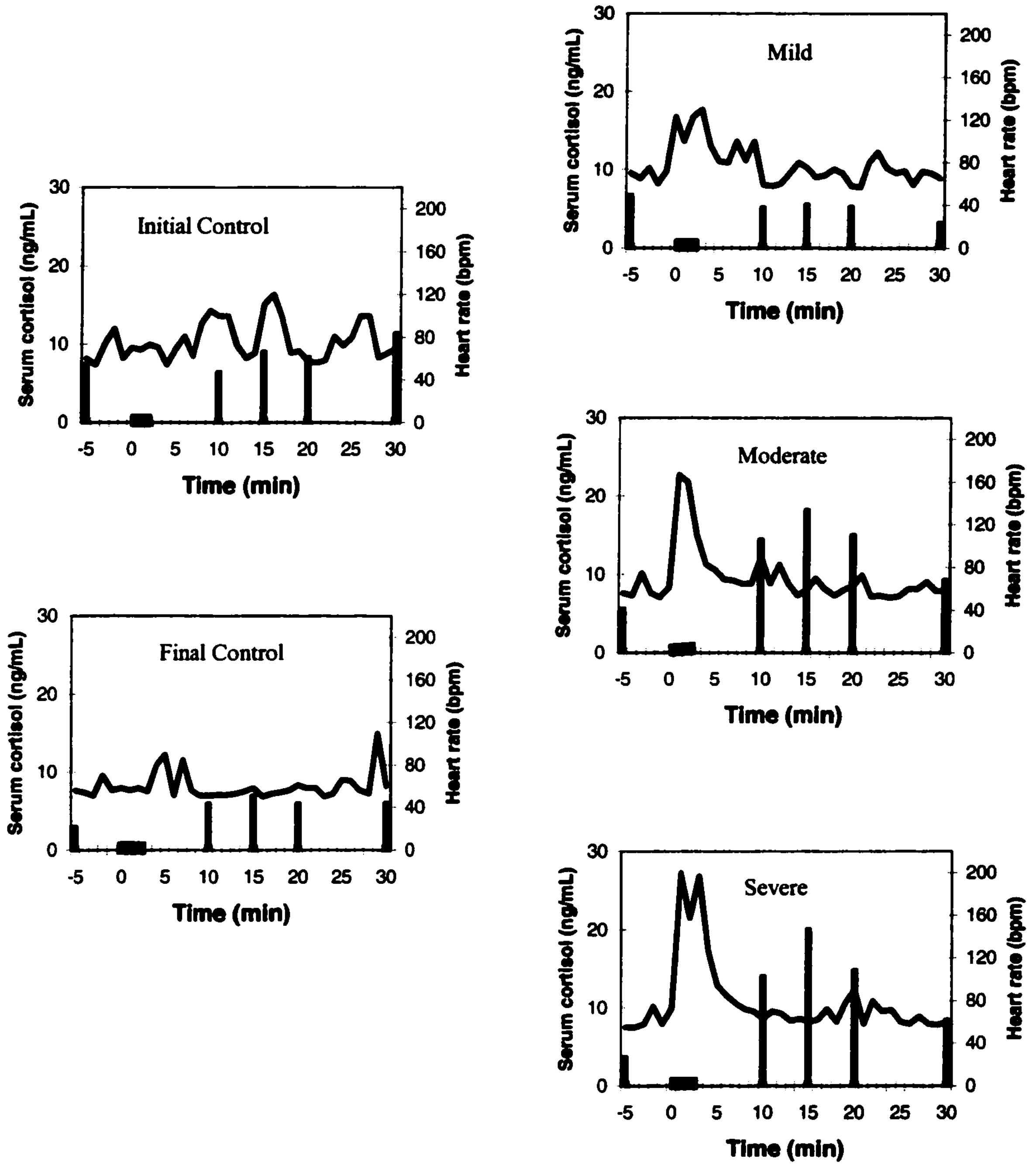
Table 3-1. Slope of simple linear regression line of peak serum cortisol (CORT15; ng/mL) on response heart rate (HR5; bpm) in bighorn sheep ewes exposed to acute stressors.

Experiment	Pregnancy Status	n	Slope[a]	SE	r^2
1	Non-pregnant	12	0.11	0.017	0.45
2	Early pregnancy	6	0.09	0.023	0.33
3	Late pregnancy	4	0.23	0.030	0.76

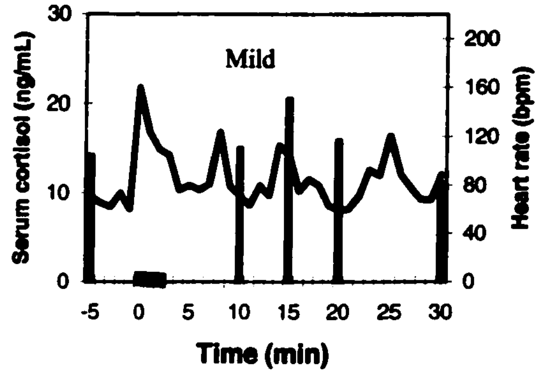
[a]Rate of increase (ng/mL) in cortisol per 1 bpm in response heart rate.

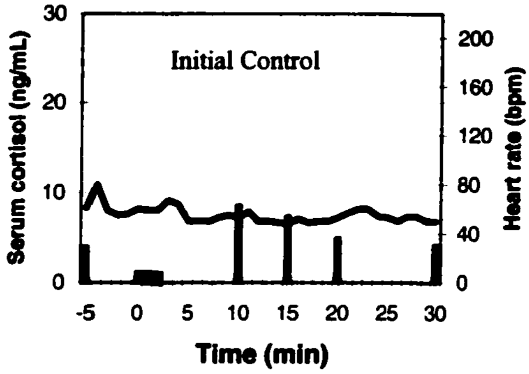
Fig. 3-1. Heart rate and serum cortisol from two representative ewes (EWE1 with low baseline cortisol and EWE 2 with an elevated baseline cortisol) during initial (January) and final (March) control trials and in response to mild, moderate, and severe stressors applied at time 0. Ewes responded to stressors with an immediate heart rate increase and with a rise in serum cortisol that peaked 15 min post-stressor application.

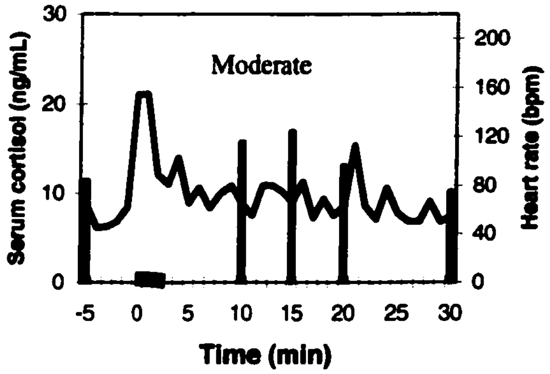
EWE 1

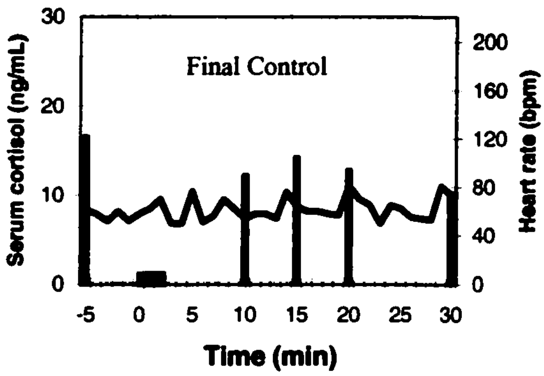


EWE 2


Mild
Serum cortisol (ng/mL)
Heart rate (bpm)
Time (min)
30
20
10
0
200
160
120
80
40
0
-5 0 5 10 15 20 25 30


Initial Control
Serum cortisol (ng/mL)
Heart rate (bpm)
Time (min)
30
20
10
0
200
160
120
80
40
0
-5 0 5 10 15 20 25 30


Moderate
Serum cortisol (ng/mL)
Heart rate (bpm)
Time (min)
30
20
10
0
200
160
120
80
40
0
-5 0 5 10 15 20 25 30


Final Control
Serum cortisol (ng/mL)
Heart rate (bpm)
Time (min)
30
20
10
0
200
160
120
80
40
0
-5 0 5 10 15 20 25 30

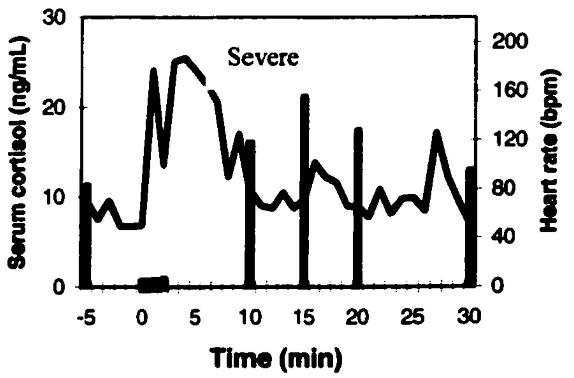

Severe
Serum cortisol (ng/mL)
Heart rate (bpm)
Time (min)
30
20
10
0
200
160
120
80
40
0
-5 0 5 10 15 20 25 30

Fig. 3-2. Harmonic mean heart rates (bpm) of non-pregnant bighorn sheep ewes prior to (-5 to -1 min), during (0 to 2 min; as indicated by bar), and following (3 to 9 min) application of a heavy stressor. Unique symbols indicate individual animals.

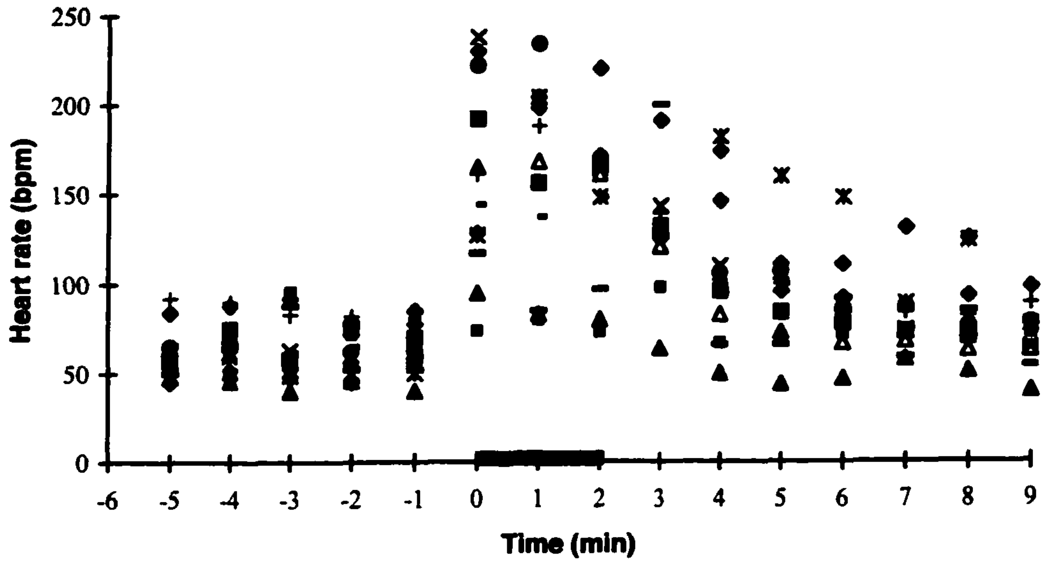


Fig. 3-3. Cortisol response of non-pregnant bighorn sheep ewes exposed to a heavy stressor for 3 min commencing at time 0 (as indicated by bar). Unique symbols and line markings indicate individual animals.

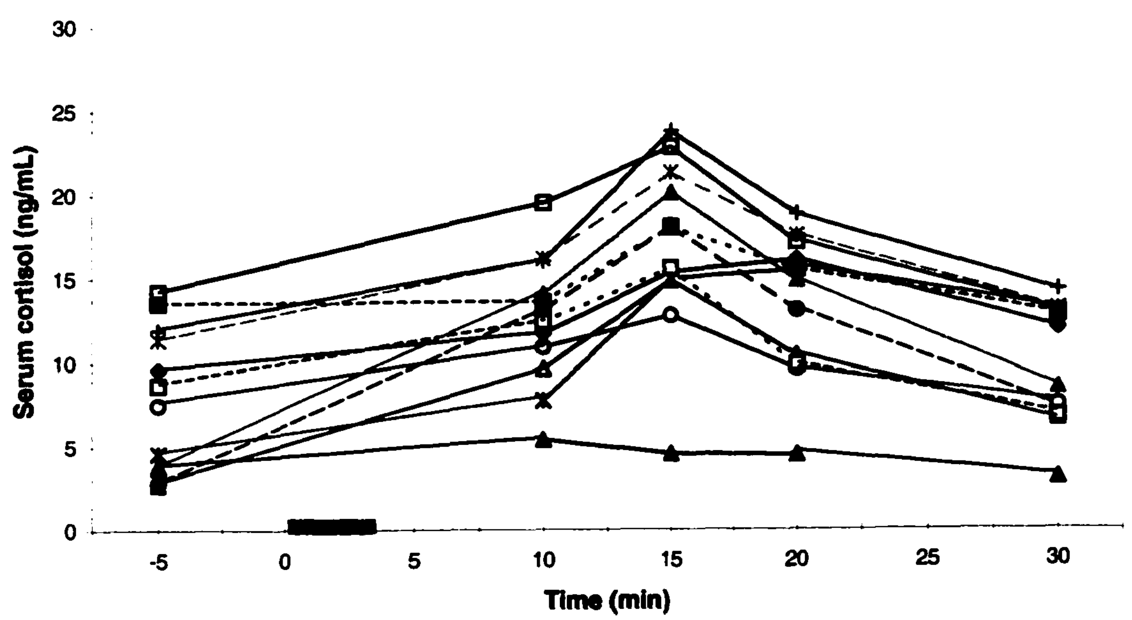
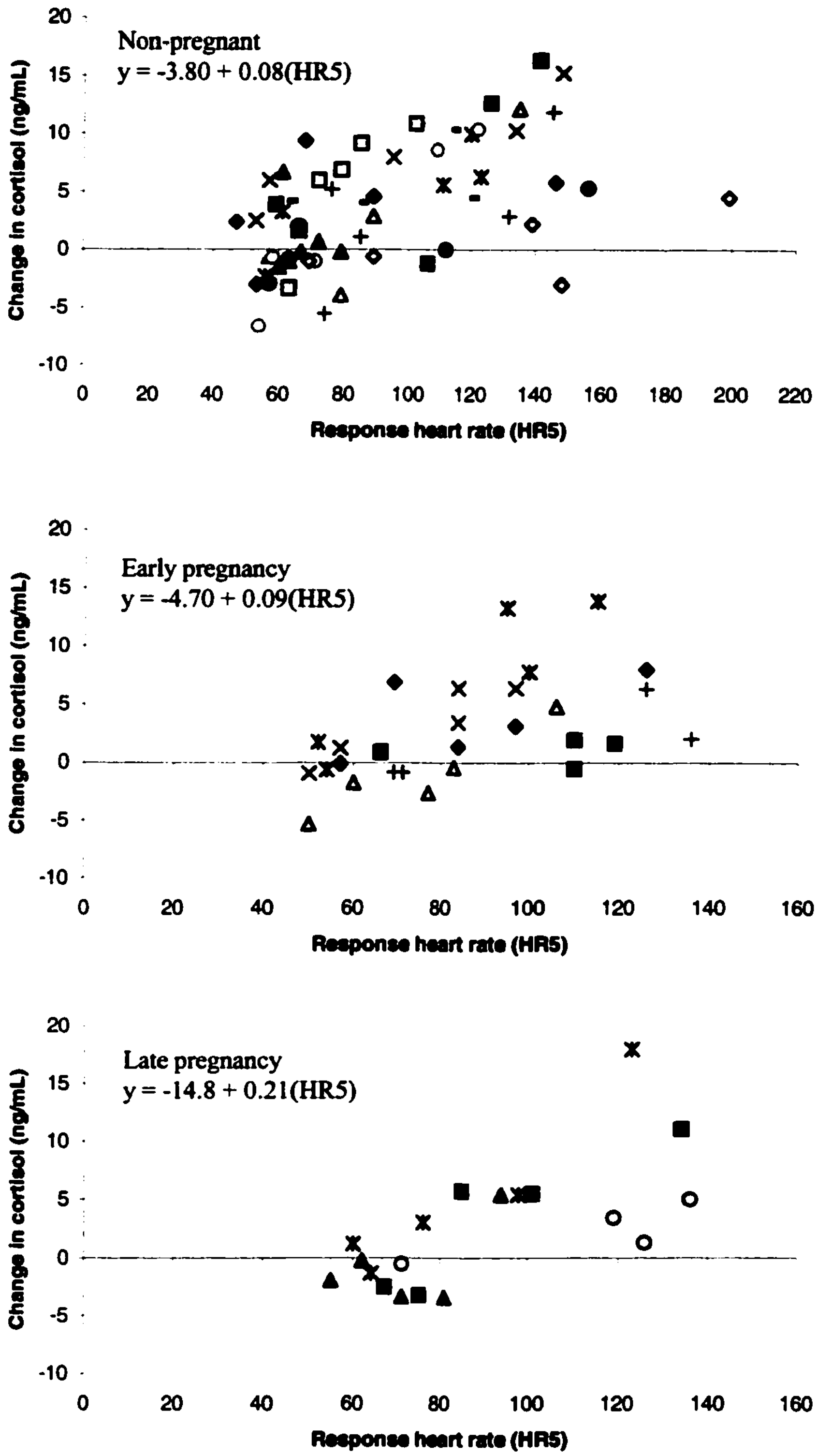


Fig. 3-4. Regardless of reproductive stage, bighorn sheep ewes responded with a positive linear relationship in a comparison of the change in cortisol (CORT15 – CORT_5) with heart rate (HR5). Unique symbols indicate individual animals.



CHAPTER 4

DAILY AND SEASONAL VARIATION IN HEART RATE AND SERUM CORTISOL CONCENTRATIONS IN CAPTIVE BIGHORN SHEEP (*Ovis canadensis*) EWES [1]

Abstract: Heart rate has been suggested as a predictor of blood cortisol values and acute stress in bighorn sheep (*Ovis canadensis*). However, daily and seasonal variation in heart rate and serum cortisol may influence the functional relationship between these variables and may affect the predictive value of heart rate as an indicator of cortisol values. The purpose of this study was to investigate the daily and seasonal variation in heart rate and serum cortisol in adult bighorn sheep ewes. Telemetered heart rate was collected at about 1-min intervals and blood for serum cortisol analysis at 2-h intervals over a 24-h period on the spring and fall equinox and summer and winter solstice, 1996. Heart rate varied by month of sampling ($p = 0.007$) with generally lower values in March and December. Daily variation was also observed with higher ($p \leq 0.009$) heart rates during light than darkness. Overall, no significant daily ($p = 0.63$) or seasonal variation ($p = 0.06$) in serum cortisol was observed. However, using pair-wise comparisons, September values tended to be higher than other months sampled and varied with time of day ($p = 0.04$). Daily and seasonal variation in heart rate but not serum cortisol concentrations suggest that the predictive value of heart rate as an indicator of serum cortisol concentrations will vary slightly with time of day and season.

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[1] Formatted for submission to Canadian Journal of Zoology.

Introduction

Use of telemetered heart rate to predict cortisol response to acute stressors is a promising tool for monitoring disturbance in bighorn sheep (*Ovis canadensis*) (Harlow et al. 1987; Chapter 3). Disturbance-induced changes in heart rate are of short duration and occur in response to increased secretion of catecholamines following autonomic stimulation. Cortisol has a broader, longer lasting effect on the body and is secreted in response to a stressor's stimulation of the hypothalamic-pituitary neuroendocrine system (Moberg 2000). The use of heart rate as a predictor of blood cortisol response requires the assumption that although these variables are in different pathways of the biological response, they will retain their functional relationship in different situations and with different stressors. This assumption is likely generally true for response of bighorn sheep to similar categories of stressors, but under some situations, such as exercise (Alexander et al. 1991) and reproductive status (Chapter 3), the functional relationship between the variables may change. Strong daily and seasonal cycles in heart rate and/or serum cortisol secretion could also influence this relationship. These changes in the functional relationship could influence the accuracy of heart rate as a predictor of serum cortisol response.

Cortisol is secreted episodically to maintain homeostasis (Hellman et al. 1970) and additionally in response to stressors (Moberg 2000). In humans, a strong daily cycle in cortisol secretion has been described (Hellman et al. 1970). Daily and seasonal cycles of metabolic rate and other physiological factors have been reported for a number of wild and domestic ungulates; however, results are often conflicting. A daily cycle for plasma cortisol level has been reported in cattle (Fulkerson et al. 1980), domestic sheep (McNatty et al. 1972), and desert bighorn sheep (*O. canadensis cremnobates*) (Turner 1984). This cycle is suggested to be due to inherent adrenocortical rhythm (Demura et al. 1966; Turner 1984); however, detection of the cycle may be an artifact of feeding (Holley et al. 1975; Simonetta et al. 1991), changes in visibility and mobility associated with light and dark periods (Turner 1984), or handling procedures. Other studies have shown no daily rhythm of cortisol in domestic sheep (Slater and Mellor 1981; Bell et al. 1991; Simonetta et al. 1991), dogs (Thun et al. 1990), white-tailed deer (*Odocoileus virginianus*) (Bubenik et al. 1983), or Eld's deer (*Cervus eldi thamin*) (Monfort et al. 1993). Gardiner and Hall (1997) observed daily variation in wild but not captive harbor seals (*Phoca vitulina*).

Daily variation in heart rate has been reported in bighorn sheep (MacArthur et al. 1979) and dogs (Pouchelon et al. 2000) but not foxes (*Vulpes vulpes*) (Kreeger et al. 1989). Diurnal cycles in heart rate may correlate with activity cycles rather than inherent rhythms.

A seasonal cycle, with summer peak and winter decline, in heart rate has been demonstrated in many ungulate species and parallels the cycle of basal metabolic rate that is believed to be determined by daylength (Moen 1978; Chappel and Hudson 1980; Baldock et al. 1988; Françoise Domingue et al. 1992). However, Mautz et al. (1992) suggest that the appearance of a seasonal cycle is due to sampling artifact, particularly influences of temperatures outside the thermal neutral zone and differences in activity level.

Reports describing seasonal rhythms in cortisol secretion are also equivocal. No distinct seasonal differences in cortisol secretion have been observed in domestic rams (Kennaway et al. 1981) or Eld's deer (Monfort et al. 1993), but were reported in a group of

4 white-tailed deer (Bubenik and Leatherland 1984).

The objective of this study was to investigate daily and seasonal variation in heart rate and serum cortisol in captive adult female bighorn sheep. This information is useful in understanding basic physiology of bighorn sheep and would be useful when investigating the utility of heart rate as an indicator of serum cortisol response (Harlow et al. 1987; Chapter 3). I hypothesize that daily and seasonal variation in heart rate and serum cortisol will be observed in bighorn sheep.

Methods

Animals

Female bighorn sheep used in these studies were all tractable adults (ages 2-11 years) born in captivity and housed at the Colorado Division of Wildlife's (CDOW) Foothills Wildlife Research Facility, Fort Collins, Colorado, USA. Prior to these studies, sheep were trained to repeated handling, blood sampling techniques, holding pens, and individual stalls. Between experiments ewes were housed in 3-ha pastures and received *ad libitum* quantities of grass/alfalfa hay, water, trace mineral block, and high energy supplement (0.5 kg/animal/day; Baker et al. 1998). All experiments were performed in accordance with the principles and guidelines of the Canadian Council on Animal Care and United States Animal Welfare Act under the review of the CDOW and Colorado State University Institutional Animal Care and Use Committees.

Experimental Protocol

I examined the daily rhythm of heart rate and serum cortisol in bighorn sheep (n = 6) at 4 periods during 1996: spring and fall equinox and summer and winter solstice. Collections were made over a 24 h period beginning at 0900 to 1000. Minimum and maximum ambient temperature during each trial was recorded. Ewes were non-pregnant, non-lactating for all collections with the exception of winter solstice when ewes were in the first trimester of pregnancy.

Heart rate.-- Heart rate data were collected remotely from implanted heart rate transmitters (Wild et al. 1998). Heart rate data were collected at about 1-min intervals using a Lotek SRX_400 telemetry receiver/datalogger equipped with EVENT_LOG (version 2.5x, W9; Lotek Engineering Inc., Newmarket, Ont., Canada) which converted interpulse period of received signals to beats per minute (bpm). The package was programmed to determine heart rate based on sets of 8 pulses before scanning to the next transmitter frequency. For statistical analyses, heart rate data were grouped into 12 2-h intervals for consistency with serum cortisol collections.

Serum cortisol. -- Approximately 16 hours before studies, each ewe was sedated with 130 mg ketamine (Ketaset, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa, USA) and 13 mg xylazine (TranquiVed, Vedco, Inc., St. Joseph, Mo., USA) intravenously (IV). An indwelling catheter (16 gauge x 14 cm Abbocath-T, Abbott Hospitals, Inc., North Chicago, Ill., USA) was placed in the jugular vein and sutured to the skin. Bandaging was applied to cover the catheter hub. Sedation was reversed with 15 mg yohimbine (Antagonil, Wildlife Pharmaceuticals, Fort Collins, Colo., USA) IV. Ewes were placed in holding pens (5 x 9 m) overnight and the following day were moved to individual metal screen stalls (2.3 x 1.1 m) for collections. Hay and water were provided *ad libitum*. Ewes were allowed a 1.5 h adjustment period in the collection stalls prior to the first sample collection. Six mL blood was collected through the catheter via a high flow extension set with reflux valve (Mila International, Inc., Covington, Ky., USA) every 2 h for 24 h. Physical restraint of bighorn sheep was not required for blood collections. Following each 24 hr trial, intravenous catheters were removed and 125 mg ceftiofur (Naxcel, The Upjohn Company, Kalamazoo, Mich., USA) was administered IV prophylactically. Serum was harvested from blood samples within 24 h of collection and stored at -20° C. Serum cortisol levels were determined using radioimmunoassay (Miller et al. 1991).

Statistical Analysis. – Preliminary searches for daily rhythmic components in the dataset were performed using the Lomb-Scargle method (Ruf 1999). Statistical analyses were made using SAS (SAS Institute Inc. 1988). Harmonic mean heart rate of each animal for each 2-h sampling period was determined. Because heart rate was influenced by our blood sampling technique, prior to calculating the harmonic mean I trimmed from the dataset heart rate collected 3 min before through 3 min after completion of each blood sample collection. Two-hour sampling periods were further categorized into light or dark cycle based on ambient conditions. Regression and ANOVA were used to identify within day and seasonal differences in heart rate and in serum cortisol levels. Least squares means were used as estimates of heart rate and serum cortisol levels.

Results

All bighorn sheep remained healthy during the study period. A heart rate transmitter in one ewe failed following the fall equinox collection therefore sample size was reduced to 5 for the winter solstice collection. Based on back calculation from lambing date using a

180 d gestation, ewes were in the first trimester of pregnancy (from 19-51 d) during the winter solstice collection. Ambient temperature range for the 24-hr sampling period in March, June, September, and December was 2-16° C, 11-35° C , 7-18° C, and –7-4 ° C, respectively.

Using the Lomb-Scargle analysis, no daily rhythmicity was detected except that of increased heart rate at 2-h intervals related to disturbance during blood sampling. However, standard statistical analyses indicated some differences in daily and seasonal heart rate and serum cortisol data.

Heart rate varied by month of sampling ($p = 0.007$) with generally lower values in March and December (Table 4-1). Heart rate also varied ($p \leq 0.002$) by time of day. Heart rate decreased 0.7 to 1.1 bpm/2 h interval over the 24-h collection period. Mean heart rate was higher ($p \leq 0.009$) by 4.6 to 10.8 bpm during ambient light than during darkness.

Overall, serum cortisol values did not vary ($p = 0.06$) by month of sampling. However, using pair-wise comparisons cortisol concentrations were lower ($p = 0.01$) in June than in September (Table 4-1). Serum cortisol values varied with time of day for collections made in September ($p = 0.003$) but not other months ($p \geq 0.42$) (Fig. 4-2). Cortisol values decreased 0.38 ng/mL per hour during the 24-h collection period in September. Similarly, only September cortisol values differed between ambient light and darkness ($p = 0.04$) (Table 4-1). Overall mean cortisol values did not differ ($p = 0.63$) during light and darkness (8.0 and 8.3 ng/mL, respectively); however, a month x light phase interaction was observed ($p = 0.05$).

Discussion

Clear inherent daily and seasonal rhythms in heart rate and serum cortisol levels in bighorn sheep were not apparent using the Lomb-Scargle technique; however, applicability of the technique was limited due to lack of continuous data and collections over only a single 24-h period during each month. Using standard statistical analyses, the data suggest rhythmicity in heart rate of bighorn sheep.

As hypothesized, daily and seasonal variation in heart rate was observed. Heart rate varied with month ($p = 0.007$) and was generally lower in December and March. These findings correspond to seasonal variation with a nadir in heart rate during winter months that has been suggested for bighorn sheep (MacArthur et al. 1979) and described in white-tailed deer (Moen 1978) and domestic sheep (Baldock et al. 1988). Seasonal variation in heart rate of bighorn sheep in this study (Table 4-1) and white-tailed deer (Moen 1978) was markedly less than the 46 bpm between June and December reported for domestic sheep (Baldock et al. 1988). This discrepancy may be due in part to comparison of data collected from sedentary bighorn sheep and deer with domestic sheep that were moving freely and exhibiting a variety of activities in yards.

Reproductive status and associated metabolic demands may affect heart rate late, but not early, in pregnancy (Baldock et al. 1988; Chapter 3). Therefore, heart rate of ewes in this study was likely not influenced by reproductive status. Seasonal change in heart rate of domestic sheep was most closely correlated with daylength and, to a lesser degree, ambient temperature (Baldock et al. 1988). However, seasonal cycles in voluntary feed intake that may influence metabolic rate, and therefore heart rate that occur in wild ruminants apparently do not occur in domestic sheep (Arnold 1985). Because feeding levels influence metabolic rate, seasonal metabolic cycles may shadow energy supply, being lower in winter when feed intake is voluntarily reduced (Hudson and Christopherson 1985). Alternatively, a lower nutritional demand resulting from lower basal metabolic rate may drive seasonal feed intake cycles. Seasonal variation in feed intake may have influenced metabolic cycles and therefore heart rate in this study. However, because captive bighorn sheep with continuous access to high quality forage may not reduce their voluntary intake in winter to the same extent as wild bighorn sheep (D. L. Baker, unpub. data), nutritionally-influenced seasonal metabolic cycles may have been blunted.

Although it has not been determined if energy supply or demand drives the seasonal cycle, most research supports the contention that a seasonal metabolic cycle occurs. However, Mautz et al. (1992) reported that seasonal variation in metabolic rate of white-tailed deer was an artifact of sampling animals maintained outside of their thermal neutral zone in summer. Lower critical temperature (T_{lc}) of bighorn sheep has not been reported, but T_{lc} of fasted white-tailed deer in spring, summer, and winter have been reported to be 10° C, 26° C, and -2° C, respectively (Mautz et al. 1992). Chappel and Hudson (1980) noted that T_{lc} appears considerably lower in bighorn sheep than other species of similar size and that temperatures as low as –20° C were within the thermal neutral zone in winter. Therefore, during these collections bighorn sheep were likely not altering metabolic rate due to, and measured physiologic parameters were likely not influenced by, ambient temperature.

Heart rate of bighorn sheep was lower during darkness than light. Similar temporal variation in 3 free-ranging bighorn sheep has been reported with the nadir occurring between 0400 and 0800 and the peak between 1200 and 1600 (MacArthur et al. 1979). Differences between light phases may have been related to inherent rhythmicity but could also be explained by variation in activity levels. Although activity budgets were not recorded in this study, subjective assessments indicated that bighorn sheep were less active and more likely to be bedded during periods of darkness. The difference in heart rate of 4.6 to 10.8 bpm that I observed between light phases are similar to differences observed for bedded vs. standing ungulates. Heart rates were 5 to 14 bpm lower for bedded vs. standing bighorn sheep (MacAuthur et al. 1979), domestic sheep (Baldock et al. 1988), and white-tailed deer (Moen 1978). Further, MacArthur et al. (1979) noted that although night time activity of bighorn sheep was low, when bighorn sheep were observed moving at night heart rate was significantly higher than similar movements made during the day.

Contrary to my hypothesis, no daily or seasonal rhythm was apparent in serum cortisol concentration of bighorn sheep in this study. Turner (1984) reported a daily pattern in cortisol levels of desert bighorn sheep (*O. c. cremnobates*). Cortisol values of captive and wild bighorn sheep in that study were substantially higher than observed here. Mean cortisol levels reported by Turner (1984) for captive and wild bighorn sheep were 33 and 49 ng/mL, respectively, while in this study mean cortisol of tractable captive bighorn sheep was 8 ng/mL. Harlow et al. (1987) reported a similar discrepancy between cortisol values of resting tame bighorn sheep and restrained wild bighorn sheep (6 vs. 25

ng/mL, respectively). Harlow et al. (1987) demonstrated that low values of tame bighorn sheep were not due to adrenal exhaustion. Similarly, bighorn sheep used on this study had strong cortisol responses to acute stressors in a previous study (Chapter 3) with no evidence of adrenal exhaustion.

The lower cortisol levels reported here are likely representative of, or at least more closely approximate, baseline values. Although details of physical restraint and anesthesia required to obtain blood samples from desert bighorn sheep were not reported (Turner 1984), it is likely that collection methods were stressful and therefore influenced cortisol values. It seems unlikely that an inherent daily cortisol pattern would be apparent through these stress-induced increases in cortisol. Because bighorn sheep are primarily diurnal, the response to a stressful situation may be exaggerated in darkness. MacArthur et al. (1979) noted increased heart rate in bighorn sheep active during night and a similar response that mimicked a daily pattern may have occurred during sampling of the desert bighorn sheep. Higher drug dosages may also have been required during periods of darkness in the study by Turner (1984). Xylazine alone or in combination with other anesthetics is commonly used in bighorn sheep. In white-tailed deer (Chao et al. 1984) and Eld's deer (Monfort et al. 1993) xylazine was implicated in raising cortisol values. Our results support conclusions by Monfort et al. (1993) that the effects of physical restraint and anesthesia on cortisol may lead to erroneous conclusions regarding adrenal status.

Overall, no seasonal variation in cortisol values was observed in this study. These findings are consistent with most studies of domestic sheep (Slater and Mellor 1981; Bell et al. 1991; Simonetta et al. 1991). Cortisol values were highest in September, although these values differed significantly only from June cortisol values (Table 4-1). Sampling during more than one 24-h period for each season would have helped determine if seasonal variation actually occurred. Monfort et al. (1993) point out the potential for error in interpretation of cortisol data that is collected infrequently.

Generally higher heart rates were also observed in September (Table 4-1). If heart rate and cortisol values were in fact higher in September, this could have been a result of inherent seasonal cycles or environmental disturbances. High winds occurred during the morning of the September collection but no other environmental disturbances were recorded. Reproductive status in September could also have accounted for differences. Although collections were not made during the peak of the breeding season (1 Nov to 1 Dec), ewes exhibit silent estrus in October (Wild and Drew, unpub. data) and potentially in late September. However, no differences in serum cortisol were observed in cycling vs. non-cycling domestic sheep (Bell et al. 1991), so this explanation seems unlikely.

Because daily and seasonal variation occur in heart rate but not serum cortisol concentration, the functional relationship between these variables will differ based upon when samples are collected. However, changes in heart rate are minimal and will likely not significantly alter the serum cortisol response predicted by heart rate.

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Table 4-1. Least squares means heart rate and serum cortisol concentration from light and dark periods of 24 h collections of bighorn sheep ewes in March, June, September, and December 1996.

			Heart Rate		Serum Cortisol	
Time of Day	Month	n	Mean	SE	Mean	SE
Light	Mar	6	54.5 [abc]	3.9	7.4 [a]	1.2
	Jun	6	60.8 [a]	3.9	5.7 [b]	1.2
	Sep	6	64.2 [b]	3.9	11.5 [abc]	1.2
	Dec	5	60.6 [c]	4.0	7.3 [c]	1.3
Dark	Mar	6	49.9 [a]	3.9	9.8	1.3
	Jun	6	54.4 [b]	3.9	6.9	1.4
	Sep	6	59.4 [abc]	3.9	9.0	1.3
	Dec	5	49.7 [c]	4.0	7.5	1.4
Daily Mean	Mar	6	53.8 [ab]	4.0	8.3	1.2
	Jun	6	59.1 [a]	4.0	6.1 [a]	1.2
	Sep	6	62.6 [bc]	4.0	11.1 [a]	1.2
	Dec	5	55.6 [c]	4.1	7.3	1.3

Note: Within time of day/month combinations, column means with the same letter differ ($P < 0.05$) using ANOVA.

Fig. 4-1 Harmonic mean heart rate determined for 2-h sampling intervals for bighorn sheep ewes in March, June, September, and December. Interval 0 is centered on 2100 h. Bar indicates periods of darkness. Unique markers represent individual ewes.

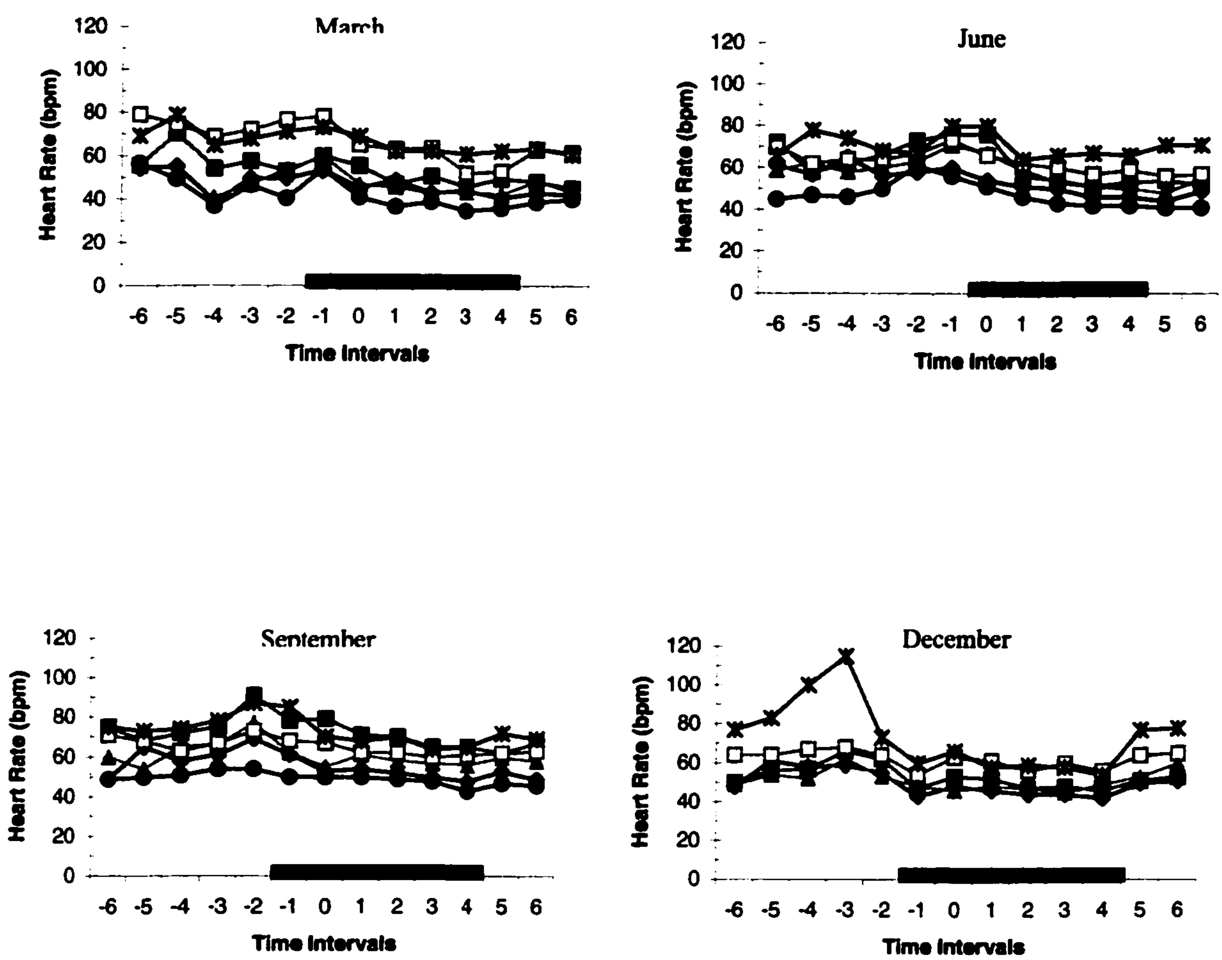

Fig. 4-2. Serum cortisol values of bighorn sheep ewes in March, June, September, and December. Bar indicates periods of darkness. Unique markers represent individual ewes.