Considerations of context and scale when using fecal glucocorticoids to indicate stress in large mammals: a study of wild American plains bison

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Non-invasive measures of the stress response are used to understand the impacts of natural and anthropogenic disturbances on wild animals. They can, however, be challenging to interpret without additional contextual information and specifics of the animals in question. Here, we used fecal samples collected from the Henry Mountains bison herd in Utah to measure the glucocorticoid hormone corticosterone (CORT), which is indicative of stress. We compared site-specific measures of fecal CORT concentration with measures of covariates related to geography (elevation, slope, aspect, distance to roads, distance to water, food quality, habitat type, season), bison physiology (body condition, parasite load, sex), and human activity (traffic volume at multiple time scales, hunting seasons). Our aim was to determine whether an unexpected habitat selection pattern could be a response to human disturbance, and thus whether ecological covariates could explain variations in fecal CORT concentration in free-ranging bison. No meaningful relationships were found for any of the covariates included in the study. At least some of those covariates should be related to the stress state of the herd, but in large and highly mobile species such as bison there is a scale mismatch between the physiological stress response of an animal and the spatiotemporal distribution of fresh feces left on the landscape. We offer our assessment of fecal CORT in bison as a case study demonstrating the utility and complications associated with using fecal indicators of stress in wildlife populations.

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Considerations of context and scale when using fecal glucocorticoids to indicate stress in large mammals: a study of wild American plains bison

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Abstract

Non-invasive measures of the stress response are used to understand the impacts of natural and anthropogenic disturbances on wild animals. They can, however, be challenging to interpret without additional contextual information and specifics of the animals in question. Here, we used fecal samples collected from the Henry Mountains bison herd in Utah to measure the glucocorticoid hormone corticosterone (CORT), which is indicative of stress. We compared site-specific measures of fecal CORT concentration with measures of covariates related to geography (elevation, slope, aspect, distance to roads, distance to water, food quality, habitat type, season), bison physiology (body condition, parasite load, sex), and human activity (traffic volume at multiple time scales, hunting seasons). Our aim was to determine whether an unexpected habitat selection pattern could be a response to human disturbance, and thus whether ecological covariates could explain variations in fecal CORT concentration in free-ranging bison. No meaningful relationships were found for any of the covariates included in the study. At least some of those covariates should be related to the stress state of the herd, but in large and highly mobile species such as bison there is a scale mismatch between the physiological stress response of an animal and the spatiotemporal distribution of fresh feces left on the landscape. We offer our assessment of fecal CORT in bison as a case study demonstrating the utility and complications associated with using fecal indicators of stress in wildlife populations.

Key Words: *Bison bison*, fecal CORT, habitat selection, physiological ecology, anthropogenic disturbance, stress, wildlife, hunting

Introduction

Conservation biologists, ecologists, and wildlife managers are concerned about the impacts of natural and anthropogenic disturbances on wild animals, especially in species with high conservation value (Frid & Dill, 2002; Russell et al., 2009). Such disturbances, which vary in scale and intensity, commonly include vehicle traffic, hunting, natural disasters, and habitat treatments. The impacts could be measured in terms of range shifts or changes in habitat use, but ultimately the key response variable is fitness (Gill, Norris & Sutherland, 2001; Acevedo-Whitehouse & Duffus, 2009). It is difficult, however, to directly measure fitness in wild populations. Instead, the stress response, which is assumed to be related to fitness, is increasingly being used to quantify the effects of disturbances on focal animal populations. The particular appeal of studying the stress response is that sampling can be non-invasive, thereby avoiding the problem of behavioral responses to researchers being confounded with those to the disturbance stimuli in question.

Since the employment of field endocrinology nearly 40 years ago, glucocorticoids (GCs) have been used increasingly as indicators of generalized stress at the population level (Wingfield & Farner, 1976; Marra & Holberton, 1998; Romero & Wikelski, 2001; Cabezas et al., 2007; Jaatinen et al., 2013). These hormones, such as cortisol and corticosterone, are regulated by the hypothalamic-pituitary-adrenal axis and mobilize energy in an organism during stressful events (Wingfield, John & Romero, 2001). During acute stress, GCs are generally considered beneficial to the organism by enhancing physiological functions such as immunity and memory development (Sapolsky, Romero & Munck, 2000). However, organisms experiencing chronic stress often have depressed immune systems, decreased body condition, and reduced reproduction because GCs mobilize energy away from non-essential processes to support the central nervous system (Sapolsky, Romero & Munck, 2000; Dhabhar, 2009).

Because of the importance of GCs in regulating multiple physiological systems, researchers have developed minimally invasive procedures for testing GC levels (Walker, Boersma & Wingfield, 2005; Kersey & Dehnhard, 2014). One of the least invasive procedures uses fecal samples (Tempel & Gutiérrez, 2004; Goymann, 2005), which reflect the composite GC deposition and excretion over a defined period of time (Tempel & Gutiérrez, 2004; Goymann, 2005). That can be informative for certain study purposes, but interpreting a fecal GC level as a stress indicator can be challenging in the absence of additional knowledge about the individual, such as body condition, sex, diet, and age (Von der Ohe & Servheen, 2002; Goymann, 2005). Additionally, using GCs as indicators of stress can lead to conflicting results when the measured GC levels are positively, negatively, or not correlated with typical measures of fitness (Bonier et al., 2009b; Dickens & Romero, 2013). It appears that the interpretation of these measures is context-dependent and thus other metrics, such as immune function, must also be taken into account (Jaatinen et al., 2013; Schoech et al., 2013).

The field of stress physiology has been searching for correlates of the energetic and stress state of animal groups to better interpret up-scaled ecological patterns (Jessop, Woodford & Symonds, 2013). Research thus far has focused more on physiological than environmental covariates, such as habitat quality for example (Breuner, Delehanty & Boonstra, 2013; Schoech et al., 2013). In our study of a wild herd of American plains bison (*Bison bison*), we hoped to determine if environmental covariates could explain variations in the fecal concentrations of corticosterone (CORT), thus elucidating spatiotemporal variations in the stress-state of the herd. Our study population, in the Henry Mountains (HM) of southern Utah (Fig. 1), was established in the early 1940s with 20 animals (15 females, 5 males) translocated from Yellowstone National Park (Popov & Low, 1950; Nelson, 1965) and now numbers ~325 adults (post-hunt). These bison are unique in that they range freely on public land where they comingle with cattle and are legally hunted, but are disease-free and genetically pure (Ranglack et al. 2015a), making them extremely valuable to bison conservation (Sanderson et al., 2008). The HM bison have, however, become a focus of contention due to their summer use of low-elevation habitats (Vuren, 2001; Ranglack & du Toit, 2015a) that are considered key winter range for cattle, leading to intense conflict with local ranchers, especially during dry years (Ranglack & du Toit, 2015b).

This pattern of habitat use is counterintuitive. We would expect bison to prefer the high-elevation areas of the HM during the hot summer months when lower ambient temperatures, shade from tree cover, proximity to water, and availability of green grass should be attractive habitat attributes. However, the mule deer (*Odocoileus hemionus*) population in the HM, which is renowned for its exceptional trophy quality, keeps to the high-elevation habitats in the summer, to which hunters and recreationists are consequently drawn to scout for upcoming hunts or photographic opportunities. The bison are also hunted and so they are extremely wary of human presence. We thus hypothesize that bison spatial ecology is driven by stress-avoidance behavior in response to human disturbance. To test this, we analyzed site-specific fecal CORT concentrations for comparison with a suite of physiological and environmental covariates that, we assumed, could influence a stress response at the herd level.

Methods

Study Area

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The Henry Mountains (HM) study area (Fig. 1) in south-central Utah [38°5’ N, 100°50’W] includes arid, semi-arid, and alpine habitats. Bison and cattle are the only large grazers in the region. A small herd (~20 animals) of elk (*Cervus canadensis*) is present, though the Utah Division of Wildlife Resources actively manages against elk by issuing hunting permits. Mule deer are common on the HM, but their preference for forbs implies negligible levels of competition with the grazers (van Vuren and Bray 1983). Black-tailed jackrabbits (*Lepus californicus*) and desert cottontail (*Sylvilagus audubonii*) are common in the low- and mid-elevations and have significant impacts on forage availability for bison and cattle (Ranglack, Durham & du Toit, 2015). Mountain lions (*Puma concolor*) and coyotes (*Canis latrans*) utilize the study area, but their populations are controlled by government and private entities. Detailed descriptions of the study area can be found in Nelson (1965) and van Vuren and Bray (1986).

Sample Collection

Satellite-download GPS telemetry collars and traditional VHF radio-collars were deployed on 63 female and 19 male bison in the HM area in January 2011. Effort was taken to ensure that the collars were distributed representatively among groups throughout the HM area. From May 2012 to April 2013, both types of telemetry (VHF and GPS) were used to locate bison without visibility bias between open versus closed habitat types, with effort taken to balance observations among all habitat types to the extent possible. Observations were primarily collected during the summer months (May – August), with opportunistic observations throughout the remainder of the seasonal cycle depending on accessibility. Direct observation of bison proved difficult in the winter months as the bison tended to use a large roadless area with extremely rough topography that made access prohibitively difficult.

Adult female body condition (BC) was scored between 1 and 5, with 1 being poor condition and 5 being excellent, following the visual condition scoring scale used for African buffalo (*Syncerus caffer*) (Prins, 1996). BC was then averaged to derive one score for the herd at that time and place. The habitat the bison were occupying was classified into one of 12 habitat types (alpine meadow, aspen woodland, barren ground, recently burned, chaining, coniferous woodland, grass-shrub mix, grassland, oakbrush, piñon-juniper woodland, riparian, shrubland). In addition, the location of each observation was marked using GPS and, through the use of a GIS, elevation, slope, aspect, distance to roads, and distance to water were associated with each observation. Digital elevation models and the locations of roads and water sources were obtained from the Utah Automated Geographic Reference Center, the Bureau of Land Management, and the Utah Division of Wildlife Resources, all at the 30 x 30 m scale. We recorded Euclidean distance (km) to roads and water sources for each pixel, together with aspect and slope from the digital elevation model in ArcGIS. Aspect was then reclassified for analysis as a categorical variable with eight levels (N, NE, E, SE, S, SW, W, and NW). Human activity was also indexed through the use of several traffic monitors placed strategically around the HM area by the local BLM office. These data were summarized to derive the average number of crossings per monitor per day.

Fecal samples were collected from fresh dung pats after each focal bison group had departed from the area in which it had been observed. When possible, individuals were monitored to allow for the collection of known-sex samples, though this proved to be difficult. Approximately five fecal samples were collected from each group, depending on the size of the group, along a transect perpendicular to the movement of the bison group to avoid sampling the same individual twice. Each fecal sample was homogenized and divided into sub-samples for analysis of total nitrogen (N) and carbon (C) content (g kg^{-1} dry feces), endoparasite load using a modified McMaster technique (Zajac & Conboy, 2006), and CORT steroid analysis. The fecal N, C, and CORT sub-samples were frozen within three hours of collection, whereas the endoparasite load sub-sample was refrigerated until analysis could be completed in the field, generally within five hours of collection, to prevent the degradation of helminth eggs. Fecal N (assayed by the Utah State University Analytical Laboratory) was used as an index of diet quality as it represents dietary crude protein for grazing ungulates (Leslie Jr & Starkey, 1987). As endoparasite load was only used as an adjunct to tracking bison condition, total egg counts were performed without noting endoparasite species.

All sampling was conducted in accordance to Utah State University Institutional Animal Care and Use Committee approved protocol #1452 and the Utah Division of Wildlife Resources Certificate of Registrations for Banding, Collection, Depredation, and Salvage permit #6BANC8393.

Fecal CORT Extraction

Phosphate buffer solution was mixed at room temperature in equal volume with methanol and added 0.5 mL of this solution to each scintillation vial with the 0.5 g of homogenized fecal sample. Each sample was vortexed and then placed onto a shaker for approximately 16 hours at 200 RPM. After shaking, we allowed the solution to settle for one hour. From the top of the supernatant, 50 uL was decanted into 12 x 75 mm polypropylene tubes and centrifuged for one hour at 4,000 RPM. The centrifuged supernatant was decanted into then frozen at -80º C until the radioimmunoassay was initiated. The solution and fecal material left behind in the scintillation tubes were dried overnight in a vented 40º C oven. The dried material was cooled to room temperature and weighed in order to determine the dry weight of the remaining fecal sample when calculating hormone concentrations (Shideler et al., 1994; Bauman & Hardin, 1998).

Radioimmunoassay

Corticosterone concentrations were determined using radioimmunoassay (ImmuChem™ Double Antibody RIA kit, MP Biomedicals, Orangeburg, NY), following the manufacturer's instructions, with the exception of halving the amounts of reagents used per sample. Briefly, a standard curve was created with the PBS-methanol solution and provided standards. Samples were added to 10 x 75 mm glass tubes, diluted 1:5 using supplied steroid dilutant. ^{125}I and anti-corticosterone antibody were added to each tube and incubated for two hours at room temperature. A precipitant solution was then added, the samples were vortexed, and centrifuged for 15 minutes at 2400 RPM. The tubes were decanted and blotted to remove as much liquid as possible. Each sample was read on a gamma counter for one minute. Samples were run on a single assay and intra-assay variation was 9.5%. Validations were conducted to test for non-specific binding, linearity, and interference. Serially diluted samples had average parallelism of 0.9969 and spiked samples had a recovery of 81.5%

Statistical Analysis

We analyzed the relationship between fecal CORT concentrations and our various covariates in univariate models using linear regression for continuous variables (elevation, slope, distance to road, distance to water, fecal N, fecal C, parasite load, body condition, date, and traffic) and ANOVA for categorical variables (sex, season, aspect, and vegetation type), using R version 3.0.2. (2013), with an alpha level of 0.05. Fecal CORT concentrations were log transformed for analysis to meet assumptions of normality. Date was converted to a continuous variable to examine the effects of both deer and bison hunts, as well as the rut, on CORT levels. As such, date indicated the number of days since the beginning of either deer or bison hunts, or the mid-point of the rut. In this way, we would expect to see a spike in CORT, followed by a decline. Due to the relative scarcity of some habitat types and the difficulty in obtaining observations during the winter season, vegetation types were collapsed into 3 categories: open (alpine meadow, grass-shrub mix, grassland, riparian, shrubland), closed (aspen woodland, coniferous woodland, oakbrush, coniferous woodland), and disturbed (burned ~10 years prior to this study and chaining). Season was classified as early (January – June) and late (July – December). This timing reflects an observed change in bison habitat use and behavior that occurs during the mid-summer in preparation for the rutting season (July – August).

Results

A total of 147 fecal samples were used in the analyses. No significant ($p<0.05$) relationships were found between the logarithm of fecal corticosterone concentration and slope, distance to water, fecal N, fecal C, parasite load, date, vehicle traffic on the day of sample collection, vehicle traffic during the week prior to sample collection, and vehicle traffic during the month prior to sample collection, or the number of days since deer or bison hunting began or the mid-point of the rutting season. Significant ($p<0.05$), but positive, relationships were detected for elevation, distance to road, and body condition, though the adjusted R^2 values were low (0.073, 0.028, and 0.078 respectively), indicating that the relationships had little explanatory power and were likely significant only because of the large sample sizes used in the analysis (Fig. 2 and 3). The

ANOVA found no significant differences (*p*<0.05) in log corticosterone concentration across sex, season, aspect, or vegetation type (Fig. 4).

Discussion

Our findings show that for most of the covariates we measured, there was no relationship with fecal CORT concentration. Those covariates that did show significant relationships (elevation, distance to road, body condition) provided little explanatory power and had positive slopes, which is paradoxical to our predictions. If fecal CORT concentration is an index of overall stress, as is generally assumed, then higher values should indicate higher stress. Yet the weak positive relationship with distance to road contradicts the expectation that bison are wary of human presence and should thus be less stressed in areas further from roads. However, it is possible that animals near roads get stressed by traffic and move rapidly away in response, then defecate in locations remote from roads and thereby produce fecal samples with unexpectedly high CORT concentrations for those locations. Alternatively, perhaps the configuration of the road network prevented an adequate high-low gradient of disturbance to detect a road effect on fecal CORT. That is, however, unlikely because the HM is classified as one of the most "roadless" BLM areas in the western USA (Dickson, Zachmann & Albano, 2014). Then, the weak positive relationship of fecal corticosterone with body condition is also counterintuitive because animals in better overall body condition should be less stressed (Breuner, Delehanty & Boonstra, 2013).

As GCs have become more and more commonly used as indicators of stress at the population level, it has also become evident that the functional complexity of these hormones makes it difficult to generalize that animals in stressful environments will reliably display elevated concentrations of GCs (Dickens & Romero, 2013). This is likely because the production of GCs is highly context-specific (Sapolsky, Romero & Munck, 2000).

Although we extensively measured environmental covariates for each site- and time-specific measure of fecal CORT concentration, we were unable to associate individual animals with most of the samples. Sex, age, body condition, and reproductive status each influence CORT production (Von der Ohe & Servheen, 2002). A significant spike in fecal cortisol in bison bulls during (compared to before) and after the rut, has been documented (Mooring et al., 2006), but with marked individuals in a focal-animal sampling scheme. As our samples were non-individualized and mainly of unknown sex, all the fecal CORT concentrations could indicate was a general herd-level stress response, and the sampled individuals may or may not have been impacted by the stressor. Additionally, the body condition score was consistently high at the herd level, but there were individual variations that complicate the application of a herd-level body condition score to an individual fecal CORT concentration.

Furthermore, individuals experiencing chronic stress might exhibit relatively low CORT levels due to sensitization, adaptation, or downregulation of the HPA axis to the stressor (Von der Ohe & Servheen, 2002; Rich & Romero, 2005). It might be that differences in CORT baseline level between groups and/or populations are less significant than differences in the magnitude of response to an acute stressor (Romero, Dickens & Cyr, 2009; Neuman-Lee et al., 2015). For example, Galapagos marine iguanas (*Amblyrhynchus cristatus*) from areas of high tourist visitation had baseline levels of corticosterone similar to those from non-tourist areas, but after a 30-minute stressor the tourist-exposed iguanas had much higher levels than the others, and then only during non-breeding seasons (French et al., 2010).

Finally, we measured total CORT in fecal samples but other factors, such as corticosteroid binding globulins, can play an important role in GC metabolism and excretion (Breuner, Delehanty & Boonstra, 2013). Nevertheless, the measurement of total CORT has been defended as an adequate index of stress when other metrics, such as immune function, are also taken into account (Schoech et al., 2013). We measured endoparasite loads from fecal egg counts assuming that chronic stress may suppress immune function and thereby allow an increase in parasite load (Dhabhar & McEwen, 1997; Dhabhar, 2009), but we found no relationship between fecal CORT concentration and parasite load.

Our study indicates the potential challenges in measuring fecal CORT in situ with large animals in wild populations. Whereas fecal CORT is increasingly used as an indicator of the health and energetic status of populations, the context- and scale-dependent nature of fecal CORT can confound applications at the population level and landscape scale (Breuner, Patterson & Hahn, 2008; Bonier et al., 2009a,b; Dickens & Romero, 2013). Even with an extensive suite of environmental covariates we failed to find any explanatory power in herd-level fecal CORT as a determinant of habitat selection by bison. We conclude that, in large and highly mobile species such as bison, there is a scale mismatch between the physiological stress response of an animal and the spatiotemporal distribution of fresh feces left on the landscape. As researchers continue to measure fecal CORT concentration as an indicator of stress in wild animals, we urge that all conclusions be narrowly drawn and physiological correlates be taken into account at the individual level.

Acknowledgements

Field and logistical assistance was provided by Erika Cologgi, David Cook, Sue Fivecoat, Kent Hersey, David Koons, Wade Paskett, and Jake Thurston.

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Figure Captions

Fig. 1. The location of the Henry Mountains in the state of Utah.

Fig. 2. The relationship between the logarithm of fecal corticosterone concentration (CORT) and various environmental and physiological covariates. Where present, lines indicate significant (p<0.05) relationships with the shaded area indicating the associated 95% confidence interval. All covariates are measured at the individual level, except for body condition, which was measured at the group level.

Fig. 3. The relationship between the logarithm of fecal corticosterone concentration (CORT) in bison and date, where the light shaded areas indicated periods of deer hunting, and the dark shaded areas indicate periods of bison hunting. The peak of the bison rutting period corresponds roughly with the beginning of the first deer hunting season.

Fig. 4. The relationship between the logarithm of fecal corticosterone concentration (CORT) and various categorical covariates. Box plot shows median, quartiles, and 1.5x interquartile range. Points show outliers beyond 1.5x interquartile range.

Figure 1(on next page)

The location of the Henry Mountains in the state of Utah.

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Figure 2(on next page)

The relationship between the logarithm of fecal corticosterone concentration (CORT) and various environmental and physiological covariates.

Where present, lines indicate significant ($p<0.05$) relationships with the shaded area indicating the associated 95% confidence interval. All covariates are measured at the individual level, except for body condition, which was measured at the group level.

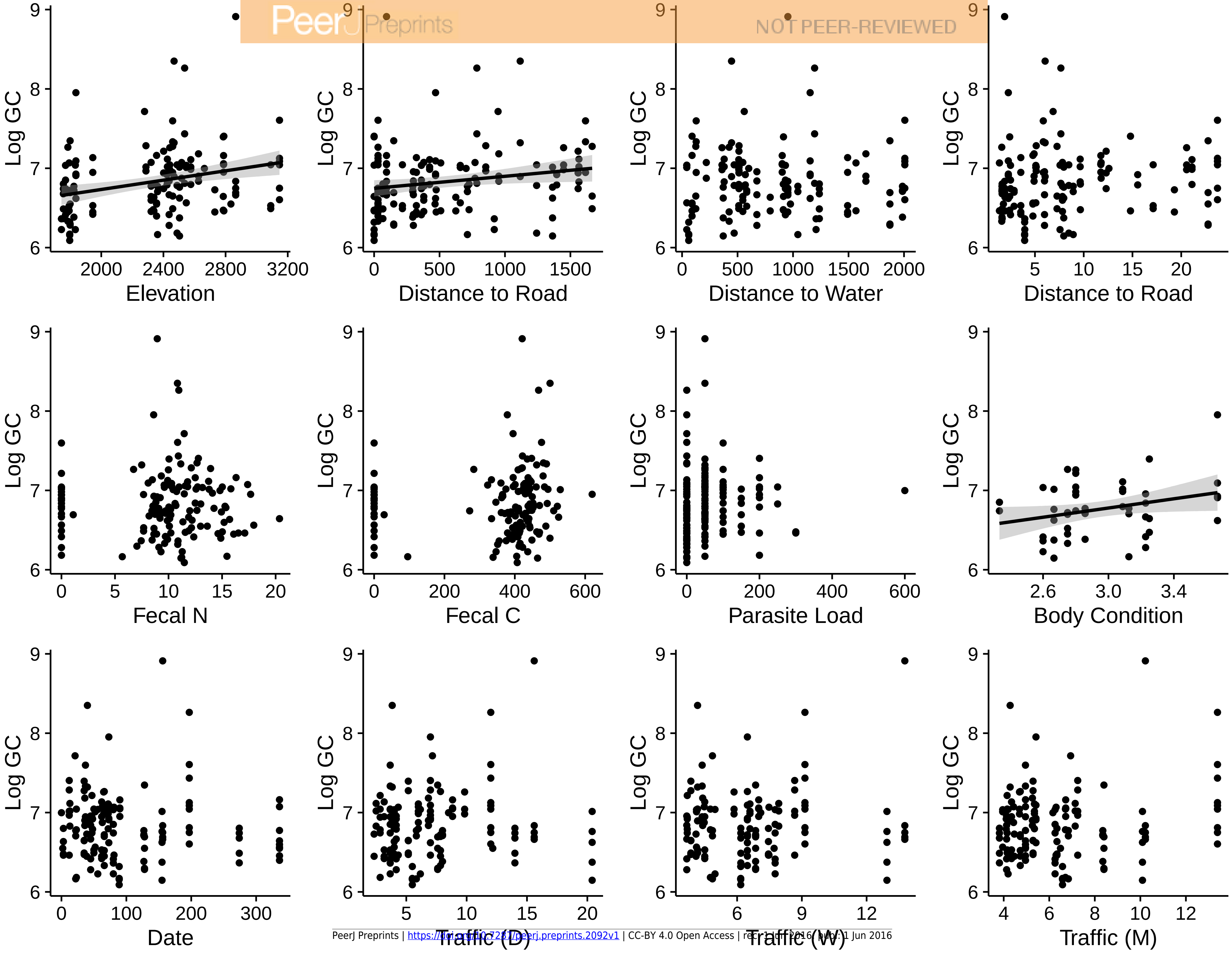
Log GC
Elevation
Distance to Road
Distance to Water
Distance to Road
Fecal N
Fecal C
Parasite Load
Body Condition
Date
Traffic (D)
Traffic (W)
Traffic (M)

Figure 3(on next page)

The relationship between the logarithm of fecal corticosterone concentration (CORT) in bison and date.

The light shaded areas indicated periods of deer hunting and the dark shaded areas indicate periods of bison hunting. The peak of the bison rutting period corresponds roughly with the beginning of the first deer hunting season.

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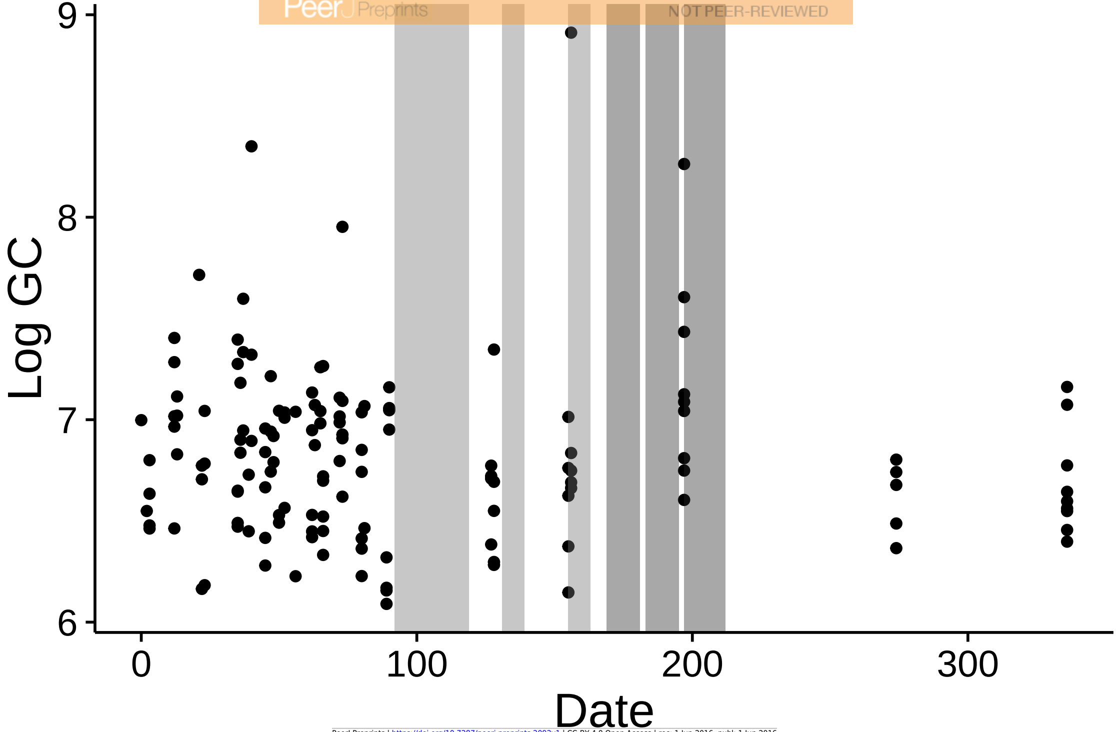
Log GC
9
8
7
6
0
100
200
300
Date

Figure 4 (on next page)

The relationship between the logarithm of fecal corticosterone concentration (CORT) and various categorical covariates.

Box plots show median, quartiles, and 1.5x interquartile range. Points show outliers beyond 1.5x interquartile range.

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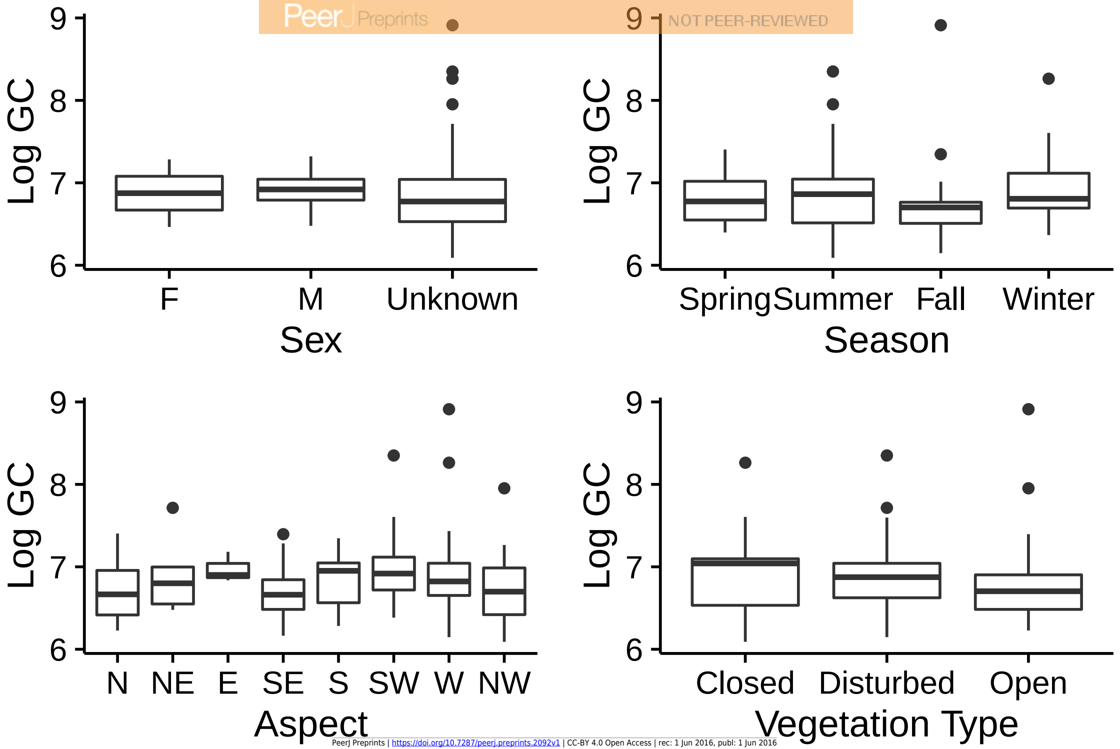
9
8
7
6
Log GC
F
M
Unknown
Sex
Spring
Summer
Fall
Winter
Season
N
NE
E
SE
S
SW
W
NW
Aspect
Closed
Disturbed
Open
Vegetation Type