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ORIGINAL ARTICLE

Human Disturbance and the Physiological Response of Elk in Eastern Washington

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Abstract

Stress hormone measures have proven useful for assessing effects of human disturbance on wildlife populations. However, most studies are of short duration or limited geographic scope (i.e., without spatial replication), leading to concerns about confounding effects of biotic conditions. Previous research correlated fecal glucocorticoid metabolites (FGMs) of elk (*Cervus elaphus*) with human disturbance, but this factor also co-varied with seasonal climatic conditions, making it difficult to make broader inference regarding the role of human disturbance. In this study we attempted to simultaneously evaluate the effects of climatic conditions and human disturbance by comparing the year-round physiological stress response of elk to varying levels of human disturbance at three study sites in south-central Washington State. FGMs were consistently elevated throughout the year at the study site receiving the greatest amount of human disturbance. We observed support for a positive effect of precipitation and increasing temperature on FGMs at the low-disturbance site, but less support for importance of climatic variables in explaining FGMs at the high-disturbance sites – suggesting that climatic variables were likely of secondary importance compared to anthropogenic stressors in elk at those sites. Collectively, while we were unable to disentangle the effects of site-specific stressors, our findings suggest that in this environment, humans were a dominant stressor influencing FGM levels. Therefore, interpreting results of physiological studies requires that researchers account for a broad combination of biotic and abiotic stressors at a particular study location. We particularly encourage future investigators to account for the potential confounding effect of human disturbance that could override other stressors.

Introduction

Human disturbance associated with road development and activity has been linked to a variety of negative effects on some wildlife, ranging from population declines and loss of genetic diversity [1, 2], to altered spatial ecology [3, 4] and physiology [5, 6, 7, 8]. However, the impacts of human disturbance can be difficult to monitor, and detecting negative effects such as loss of genetic diversity or population declines often requires long-term research [9]. Increasingly, to quantify such insidious effects on wildlife, stress hormone measures are being used as an efficient, non-invasive metric for detecting the impacts of roads and other human disturbances on wildlife physiology [8, 10, 11].

Many factors affect physiological stress responses in wildlife, which has led to calls for caution when incorporating study results into conservation planning [12]. For large herbivores in particular, seasonal availability of water and forage vary greatly in both temperate and tropical climates, resulting in seasonal differences in basal fecal glucocorticoid metabolite concentrations (FGMs) [5, 13, 14, 15, 16]. In the western United States, most field studies of elk (*Cervus elaphus*) physiology have only sampled during certain months or seasons [e.g., 6, 17] or are constrained within a single site where human disturbance co-varies with climatic conditions [e.g., 5], making it difficult to account for confounding effects of climate without multiple studies and spatial replication. For example, Millspaugh et al. [5] observed distinct elevations in FGMs associated with road activity and high temperatures; however, peaks in tourism disturbance and temperatures co-occurred in the summer, making it difficult to discriminate the relative importance of tourism vs. temperature.

In this study, we sampled fecal material to determine FGMs in elk at three study sites with differing degrees of human disturbance throughout the year to evaluate the relationship between human disturbance and seasonal climatic conditions (i.e., temperature and rainfall). Specifically, we tested the hypotheses that (1) FGMs would be highest at sites with high levels of human disturbance, and (2) that human disturbance was an overriding factor influencing the ability to detect patterns in FGMs based on climatic conditions.

Methods

Study area

We selected three study sites in south-central Washington State based on *a priori* knowledge that each site contained established elk populations as well as known differences in human disturbance (Fig. 1). The Arid Lands Ecology Reserve (ALE) is an approximately 31,200 ha reserve located in relatively low elevation (163 m) sagebrush-steppe habitat that is typically flat, dry, and hot in the summer (19.38 cm total annual precipitation and 29.22 ℃ average maximum daily summer temperature during our 1-year study), and receives little snow in winter and has mild spring and fall temperatures [18]. Despite the occurrence of agricultural practices outside of the reserve, we classified this site as low human disturbance because elk typically resided within the protected boundary of ALE that was managed as an ecological reserve

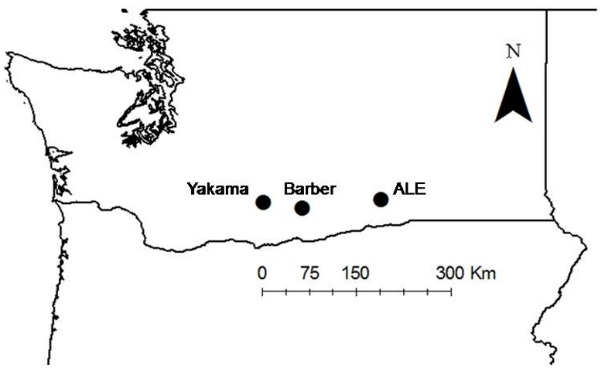

Fig. 1: Location of the Arid Lands Ecology Reserve (ALE), Barber Flats (Barber) and Yakama study sites within south-central Washington, USA.

closed to public entry and received no vehicle traffic on most days and only a few research/management related vehicles each week. In addition, during our sampling, limited harvest occurred for animals that ventured outside of the reserve and there has been no hunting disturbance within the reserve since the 1970's [19, 20, 21].

The other two study sites were within the Yakama Nation Reservation and contained elk that were exposed to high human disturbance levels, but that were separated by 75 km and experienced different climates (Fig. 1). Elk sampled at these two study areas range over a landscape that encompasses 200,000 ha of the Yakama Reservation, approximately 300,000 ha of federal land within the Gifford Pinchot and Wenatchee National Forests, and some 50,000 ha managed by Boise Cascade Corporation or the Washington Department of Natural Resources [22]. Collectively, approximately 310,000 ha of federal, tribal, and private land were intensively managed for commercial timber production, and 98,000 ha were administratively designated as wilderness, primitive area, or alpine reserve. Additionally, limited timber harvest was practiced on 72,000 ha to enhance specific noncommercial management objectives [22]. The winter range for this population was located entirely within the Yakama Reservation [23]. This area in particular contained an extensive road network, but only one road that accessed a series of elk traps was plowed during winter, and access to this road was controlled via a locked gate from 15 November to 1 April.

Of these latter two study sites, Barber Flats was located at a moderate elevation (499 m) and was classified as riparian oak woodland habitat. Climatic conditions at this site consisted of moderate summer temperatures (24.56 cm total annual precipitation and 27.17 ℃ average maximum daily summer temperature during our 1-year study). The site was a tribal wildlife refuge with a gated road that received light research/management traffic in the winter, and modest to high traffic (~10 vehicles per day) during the other seasons. Hunting was not permitted within the Barber Flats site.

The third study site (hereafter referred to as Yakama) was within the Yakama Nation Reservation 150 km away from the ALE site and experienced different climate and human disturbance levels (Fig. 1). Yakama was managed as a mixed conifer forest at relatively high elevation (1004-1163 m). This study site received four times more precipitation and was typically colder (81.8 cm total annual precipitation and 21.15℃ average maximum daily summer temperature during our 1-year study) than the ALE site and accumulated deeper snow during winter [23, 24]. The site received frequent traffic from forest management and tribal recreational/cultural activities were high throughout the year [23]. In addition, hunting was permitted for antlered elk on the Yakama site year-round for the ~9,000 tribal members and antlerless deer and elk hunting occurred only during fall (September 1 – December 31) [22].

Fecal sample collection and analysis

We collected fecal samples from the three study areas from May 1999 to June 2000. We attempted to revisit the ALE and Yakama sites every 3-4 weeks throughout the year to collect fresh elk fecal samples. We visited the Barber Flats site during September and October (i.e., 2 sampling occasions) only. While collection of samples would ideally be linked to individual animals thus allowing us to account for potential sex- or individual-specific intrinsic factors that can influence FGM levels, such as reproductive state [25, 26], we were unable to link fecal samples with individual animals due to reclusive elk behavior and logistical limitations. Rather, we visited areas known to support predictable elk use and randomly walked these areas, searching for fresh (i.e, yet to undergo desiccation or decomposition and <24 hrs old) fecal pellet groups. We collected a few pellets from each group sampled and bagged them (i.e., each sample bag represented a single pellet group). After collection, we homogenized and then immediately froze all elk fecal samples [27, 5].

Techniques for quantifying glucocorticoid metabolites from fecal samples were detailed by Millspaugh et al. [5], and validated in captive elk [27], but briefly consisted of the following steps. Once shipped to the University of Missouri laboratory, samples were thawed, freeze-dried, and sifted through a stainless steel mesh. Dried feces (0.2 g) were then placed in a test tube with 2.0 ml of 90% methanol, vortexed for 30 min, then centrifuged at 2,200 rpm for 20 min at which point the supernatant was saved until assayed [5]. Assays were conducted using 125-I corticosterone radioimmunoassay kits (ICN #07-120103, MP Biomedicals, Solon, OH). Assay accuracy and precision was confirmed by conducting standard assay validations, including assessment of parallelism, recovery of exogenous analyte, intra- and inter-assay precision and assay sensitivity [28, 29]. Interassay variation for four assays was 5.3% and average intra-assay variation was 3.1%.

Data Analysis

To test our first hypothesis, that elk at sites that received a high level of human disturbance exhibited increased FGMs, we first used a mixed model analysis of variance (SAS PROC MIXED [30]) to test for significant difference between FGMs in elk at high compared to low disturbance sites. Within our model, we treated site as a random effect and day as a repeated effect, and set significance levels at $p = 0.05$.

To test our second hypothesis, that human disturbance was an overriding factor influencing the ability to detect patterns in FGMs based on climatic conditions, we used an information theoretic model selection framework to evaluate competing *a priori* hypotheses regarding climatic factors that might influence elk FGMs across our three study sites. We hypothesized that high temperatures would result in increased FGM concentrations [5], and included a measure of the average and maximum daily temperature from the nearest weather station on the day before sample collection (to account for lag time in fecal sample reflecting physiological state of the individual [27, 5]. We hypothesized that in our relatively arid study system, low availability of water (particularly during hot summer months) could act as a stressor and result in elevated FGMs [15]. To evaluate the hypothesized negative effect of increased water availability on FGMs, we estimated the total amount of precipitation at each collection site during the month a sample was collected based on historic climatic models developed by the PRISM research group at Oregon State University (http://www.prism.oregonstate.edu/). Finally, we also evaluated support for a series of more complex models that contained different combinations of the hypothesized effects of human disturbance and temperature and precipitation (Tables 1 and 2).

Table 1: Support for models used to predict fecal glucocorticoid metabolite concentrations (FGMs) in elk (*Cervus elaphus*) at the low elevation, low human disturbance Arid Lands Ecology Reserve study site in south-central Washington, USA during 1999-2000. Support for each model in explaining the FGM concentrations in elk was based on Akaike's Information Criterion for small sample sizes (AICc). Variable *Precip* stands for the total precipitation for month sampled. Variables *Mtemp* and *Temp* stand for maximum temperature (℃) of day prior to when sample was collected and the average temperature (℃) of the day prior to when sample was collected, respectively.

MODEL	log (l)	K	AIC$_c$	ΔAIC$_c$	AIC$_c$ weight	σ^2_{model} [a]	Absolute variation explained
Precip*Mtemp	984.15	6	996.15	0.00	0.5452	87.8	24.70
Global	980.80	8	996.80	0.65	0.3945	86.7	25.62
Precip*Temp	988.58	6	1000.58	4.43	0.0596	92.1	21.00
Mtemp	1001.98	4	1009.98	13.83	0.0005	105.8	9.24
Temp	1005.75	4	1013.75	17.59	0.0001	111.4	4.47
Intercept only	1009.93	3	1015.93	19.78	0.0000	116.6	0.00
Precip	1009.14	4	1017.14	20.99	0.0000	117.2	-0.53

[a] σ^2_{model} = covariance parameter estimate

Table 2: Support for models used to predict fecal glucocorticoid metabolite concentrations (FGMs) in elk (*Cervus elaphus*) at the high human disturbance Barber Flats and Yakama study sites in south-central Washington, USA during 1999-2000. Support for each model in explaining the FGM concentrations in elk was based on Akaike's Information Criterion for small sample sizes (AICc). Variable *Precip* stands for the total precipitation for month sampled. Variables *Mtemp* and *Temp* stand for maximum temperature (℃) of day prior to when sample was collected and the average temperature (℃) of the day prior to when sample was collected, respectively.

MODEL	log (l)	K	AIC$_c$	ΔAIC$_c$	AIC$_c$ weight	σ^2_{model} [a]	Absolute variation explained
Precip*Temp	825.36	6	837.36	0.00	0.3283	185.5	5.36
Global	822.07	8	838.07	0.71	0.2298	181.9	7.20
Intercept only	832.42	3	838.42	1.06	0.1929	196.0	0.00
Temp	832.21	4	840.21	2.86	0.0787	196.4	-0.19
Mtemp	832.30	4	840.30	2.94	0.0753	193.7	1.19
Precip	832.39	4	840.39	3.03	0.0721	197.4	-0.72
Precip*Mtemp	830.67	6	842.67	5.32	0.0230	190.4	2.87

[a] σ^2_{model} = covariance parameter estimate

To comparatively evaluate the influence of climatic variables between sites, we fit models to data collected from both high disturbance sites and the single low disturbance site separately. Because multiple samples were collected on a given day, we used linear mixed models to evaluate support for each of our hypothesized models with a random effect of site and a repeated effect of sampling day. We first fit a global model using restricted maximum likelihood (REML) to select the appropriate covariance structure [29]. To evaluate the assumption of independence, we evaluated both unstructured and compound symmetry covariance structures. In addition, because data collected on consecutive days might be more correlated than on nonconsecutive days, we evaluated an autoregressive structure [27]. We ranked models using Akaike's information criteria corrected for small sample size (AIC_c) and found the greatest support for an autoregressive structure, which we then used in subsequent modeling. Second, because models cannot be compared using REML in an AIC_c framework [31], we used a maximum likelihood approach to rank models based on ΔAIC_c values [32].

We evaluated model performance by calculating the percent of variation in FGM concentrations explained by each model [34]. We used the maximum likelihood covariance parameter estimate for the absolute variation explained for each model, where:

$$\%\text{ variation explained} = \left| \left(\frac{\sigma^2_{\text{process}} - \sigma^2_{\text{residual}}}{\sigma^2_{\text{process}}} \right) x\ 100 \right|$$

and σ^2 *process* = variance component estimate for the intercept-only model, and the σ^2 *residual* = variance component estimate for the model in question [34].

Results

We collected and assayed 234 fecal samples from elk (*n* = 136 ALE, *n* = 22 Barber Flats, *n* = 76 Yakama) during 13 months of sampling. The ALE site was sampled during all months of the year (with the exception of July), and on average we collected 12.4 samples/month (SE = 0.68). The Yakama site was sampled during most months (with the exception of March, April, September and December), on average collecting 6.9 samples/month (SE=2.13). The Barber Flats site was only sampled during September (*n* = 10) and October (*n* = 12).

In support of our first hypothesis, we found that FGMs were consistently elevated at the high disturbance sites compared to the low disturbance site ($F_{1, 230} = 153.80$, $P < 0.0001$). On average, FGMs of elk sampled at the sites receiving high human activity (Barber Flats and Yakama) were over twice as high as those sampled at the low human disturbance site (ALE) (Fig. 2). Monthly differences in stress hormone concentrations were most evident at the low-disturbance site (ALE), where FGMs were on average 50-70% higher during the spring months (March 16-May 31), compared to the other seasons (i.e., summer [June 1-August 31], fall [September 1-December 15], winter [December 16-March 15]; Fig. 2). In contrast, at the high-disturbance Yakama site we observed the highest level of FGMs during November (Fig. 2).

At the low-disturbance site we observed a relatively low amount of model uncertainty, with support for an interaction between precipitation (for the month when sample was collected) and maximum temperature (on the day prior to fecal sample collection) as our top-ranked model used to explain variation in FGMs (Table 1). All models containing the interaction of precipitation and temperature variables explained >21% of observed variation compared to those without an interactive effect that explained <9%. The parameter coefficient for the interaction was positive (Fig. 4),

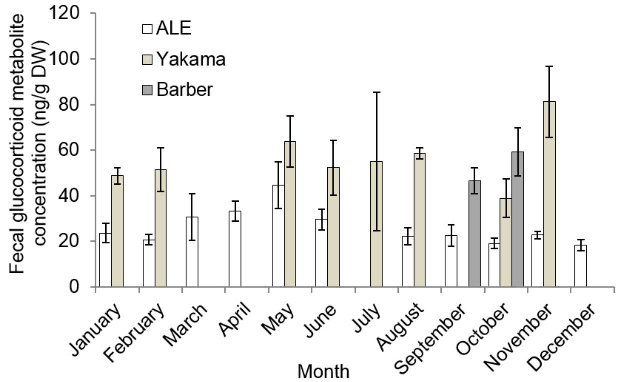

Fig. 2: Mean (with 95% confidence intervals) fecal glucocorticoid metabolite concentrations in elk (*Cervus elaphus*) across seasons sampled between May 1999 and June 2000 in south-central Washington at sites receiving high or low levels of human disturbance.

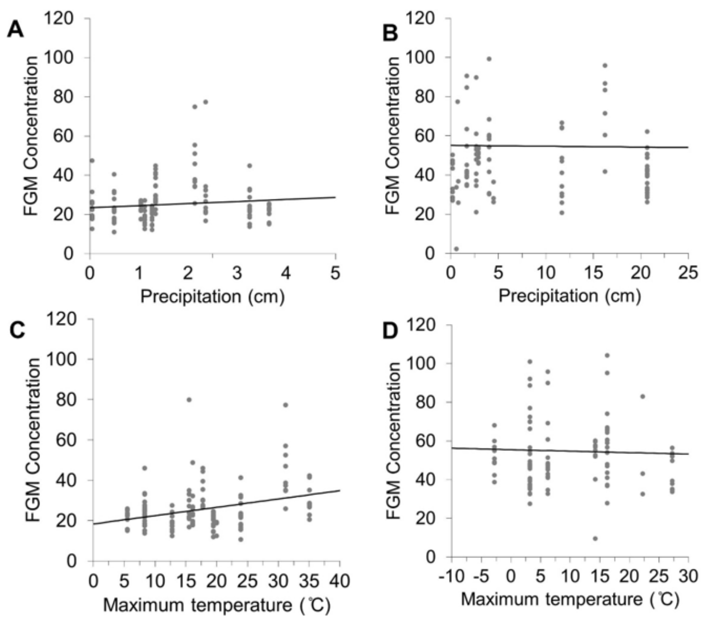

Fig. 3: Elk (*Cervus elaphus*) fecal glucocorticoid metabolite (FGM) concentrations (ng/g) plotted as a function of monthly total precipitation and maximum daily temperature (on day before sample was collected) for elk surveyed at a site that received a low level of human disturbance (**A, C**) and sites receiving a high level of human disturbance (**B, D**) in south-central Washington, USA. Solid lines represent predicted linear fit based on observed values.

where we found that elk FGMs at the low-disturbance site were predicted to increased 5% for every 1-cm increase in monthly precipitation, and 11% for every 5℃ increase in maximum temperature on the day prior to sampling (Figs. 3 and 4).

By contrast, at the high-disturbances sites, we observed a relatively high amount of model uncertainty (Table 2). While an interactive effect of precipitation and temperature was retained within the top model, this model explained a relatively low 5% of variation and performed only slightly better than the intercept-only model

(Table 2). Further, we observed considerable variability in the overall influence of climatic variables compared to the low-disturbance site (Figure 3). Overall, in support of our second hypothesis, climatic conditions were a poorer predictor of observed elk FGMs at the high-disturbance site.

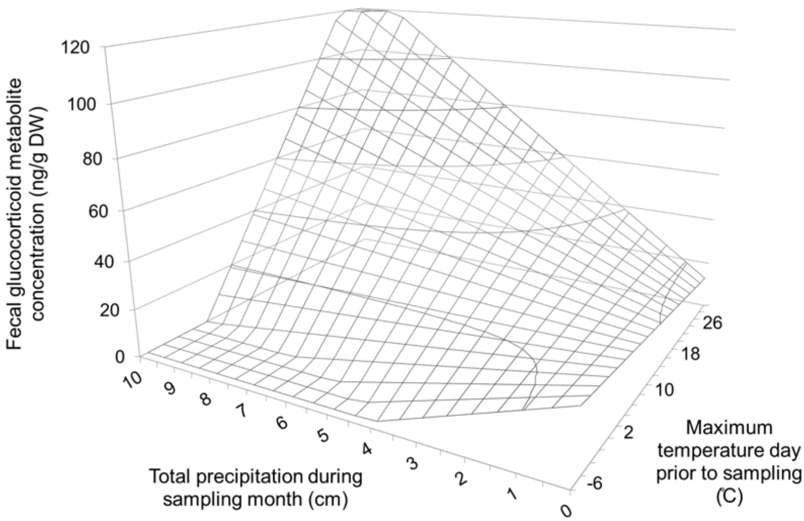

Fig. 4: Predicted relationship between elk (*Cervus elaphus*) fecal glucocorticoid metabolite (FGM) concentrations (ng/g) and monthly total precipitation and maximum daily temperature (on day before sample was collected) for elk surveyed at a site that received a low level of human disturbance in south-central Washington, USA. Predicted values are based on parameter coefficients from most-supported model (see Table 1).

Discussion

Although we were unable to definitively tease apart the effects of site-specific stressors (e.g., road activity, hunting disturbance, predation risk, etc.), our study suggests that persistent human disturbance was correlated with an elevated stress response that largely overrode “natural” climatic or seasonal patterns in FGMs in elk. Our results support previous work that found heightened stress responses in elk during periods of high human disturbance [5, 6], and that trends in elk demography and distribution also were correlated with certain human activities [35, 36, 37, 38]. In addition, previous studies that assessed year-round patterns in elk FGMs identified distinct peaks in FGMs corresponding to summer periods of high temperature and tourism activity [5] or cool temperatures during winter months [13, 16]. We found that clear monthly differences and comparatively distinct effects of climate on FGMs were only evident at the low-disturbance site. This result suggests that climatic effects were of lesser importance in explaining observed variability in comparison to site-specific levels of human disturbance.

Elk at the low-disturbance site exhibited distinct climatic trends in FGMs that were likely a result of extremely high temperatures and arid climatic conditions of that site. In arid environments, increases in FGMs are generally associated with increases in maximum and average temperatures during dry, hot months for a variety of large herbivores [15, 38, 39], including elk [5]. During the summer months, the low elevation ALE site is one of the hottest sites where free-ranging elk exist, and the population exhibits an inverted fat cycle where they gain condition in winter and lose condition in summer [40]. Accordingly, we observed a slight increase in FGM levels in elk during periods of extreme high temperatures (Fig. 3). However, the reason behind the distinct peak in FGM levels in the spring season (i.e., March, April and May; Fig. 2) when the ALE population was likely in peak physiological condition is unclear. Elevated FGM levels during March sampling were likely due in large part due to a capture event that occurred on March 20, 2000 [20]. This event involved helicopter tranquilization and fitting 24 individuals with radio-tracking collars, and likely initiated a stress response that persisted at least through sampling that occurred on March 30, 2000. However, the reason for persistent elevation of FGM levels during April, May, and June sampling (Fig. 2) is not as evident, but a number of potential explanations exist. First, altered gestation and parturition have been associated with elevated FGM concentrations in elk [17], and the altered physiology of adults and early parturition of calves by this population compared to surrounding populations [see 40] could result in a May peak in FGM levels. Second, due to heightened risk of predation on neonates during these spring months, increased vigilance by adult cow elk could be associated with peaks in FGMs [41]. Third, changes in foraging behavior could be linked to fluctuations in glucocorticoid secretion [42]. Finally, because forage water content and nutrient composition can influence FGM level in herbivores [43, 44], rapid spring growth of grasses in this typically arid environment could have resulted in dietary switching that influenced FGM concentrations within feces [25].

A modest peak in elk FGMs at the high-disturbance sites during November was likely primarily influenced by seasonal patterns of migratory behavior or human disturbance, rather than site-specific climatic conditions. At the high human disturbance sites we observed consistently elevated FGMs with little variation explained by climatic factors (Fig. 3). Despite elk at these sites being exposed to cooler temperatures than the low elevation (ALE) site and thus more likely exposed to winter thermal challenge and reduced nutritional intake during periods of severe winter weather [14, 16], FGMs were not consistently elevated during the coldest winter months of January and February. The peak in FGMs we observed in November (two times higher than values in October) at the Yakama site was potentially a result of onset of fall hunting pressure and the initiation of elk migration to winter range [23].

In addition to the role of human disturbance and climatic variables that we hypothesized were the predominant stressors in our study system, the relatively low percentage of variation explained by our top predictive models suggests additional factors could have contributed to observed elk FGMs that require further investigation. We particularly encourage future research into a variety of site- and population- specific factors such as population density [45, 46] and predation risk [47] that are known to influence FGMs in wildlife and could impact observed FGMs in elk during our study area. While Creel et al. [48] failed to find a positive correlation between glucocorticoids and predation risk in elk residing in the Yellowstone ecosystem, vigilance behavior has been shown to be correlated with FGMs in other large herbivore populations [41]. Therefore, future investigations into potential for variations in predation risk between study systems should be considered. In addition, site-specific differences in diet and forage water content could influence elk FGMs [39, 25] and require further investigation. Finally, sex- and individual-specific factors can influence and explain variation in stress hormone levels [25, 26]. Therefore, we encourage future research to focus on collecting more detailed information on the individuals being sampled so that those intrinsic factors can be incorporated into subsequent evaluations.

Regardless of the proximate cause of elevated FGMs, the consistent elevation in FGMs we observed at the high disturbance sites should be of management concern and be a topic of further investigation due to the potential for chronic stress [49]. In particular, we suggest further research to determine the relationship between chronic stress and elk demography, particularly of adult females [50]. Further, physiological surveys should be coupled with behavioral (in addition to demographic) monitoring to determine the potential effects of chronic stress on wild populations and potential ways to mitigate its occurrence [e.g., 51]. For example, the extent to which elk avoid roads has been found to be dependent on the amount of cover that limits visibility of roadways by the elk [52, 53]. Further research on the relationship between physiological state and behavior could provide management alternatives that minimize exposure to anthropogenic stressors spatially and/or temporally.

Collectively, our study provides further evidence of the importance of human disturbance as a primary source of stress in wildlife populations, and suggests that researchers should be cautious in interpreting the relative importance of stressors when site-specific effects of stressors might override normal patterns. Similar to a variety of studies of large herbivores [e.g., 14, 15], we documented seasonal trends in FGMs that likely varied based on climatic conditions at the site where human disturbance was relatively low. However, when acute human disturbance events exist such as capture for radio-tracking studies, distinct spikes in FGM levels can occur. Further, where human disturbance is consistently high, similar to studies of other large herbivores [e.g., 38, 39], our results demonstrated that high levels of human disturbance can be an overriding stressor impacting observed FGMs. In other populations or species, predation could be the overriding stressor [48, 54]. It is important to consider the potential for overriding stressors that affects the relative importance of other environmental stressors commonly evaluated in physiological studies of wildlife.

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