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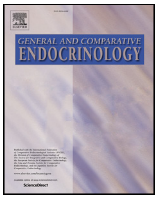

Glucocorticoid and triiodothyronine concentrations do not correlate with behavior in vicuñas (*Vicugna vicugna*)

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ABSTRACT

State-dependent foraging theory posits that animals should make foraging decisions based on energetic condition, where animals with fewer energetic reserves prioritize foraging over other behaviors, including antipredator behaviors. However, few studies have investigated these trade-offs at an individual level in wild, free-ranging animals. We investigated the relationships between internal condition and behavior in a wild mammal, the vicuña (*Vicugna vicugna*), which makes state-dependent decisions about the use of two habitats with different characteristics that contribute to their internal condition. Using non-invasively collected fecal samples, we measured glucocorticoid metabolites (GCMs) and thyroid hormones (THs) as indicators of combined stress (predation and nutritional), and just nutritional stress, respectively. We video recorded 20-minute behavioral observations and focused on behaviors which often demand a trade-off between energy acquisition and antipredator behaviors—vigilance and foraging. We found differences in expression of these behaviors between the two sites but found no relationships between physiological parameters (GCMs and THs) and behavior (vigilance and foraging) at either site. We suggest that state-dependent foraging may be difficult to observe in large mammals under baseline conditions and that GCMs and THs may be insensitive to small changes in stress stimuli at this scale, and where these wild animals have the entire suite of behavioral responses available to them.

1. Introduction

Food consumption provides the energy essential for reproduction, influencing subsequent individual fitness and population dynamics (Parker et al., 2009; Pedersen and Greives, 2008; Taylor et al., 2005). Simultaneously, animals must balance other needs, such as engaging in antipredator behaviors, which can themselves be energetically expensive (Bourdeau et al., 2016; Persons et al., 2002; Van Buskirk, 2000). For example, animals are particularly susceptible to predation when foraging (Stephens et al., 2007), leading to trade-offs between foraging and antipredator behaviors (Houston et al., 1993; Lima, 1998; Lima and Dill, 1990). Therefore, animals in poor energetic condition or that have high energetic demands may be required to take risks to reach a minimum energy requirement for daily maintenance and future reproduction (Beale and Monaghan, 2004; Lima and Bednekoff, 1999; Wirsing et al., 2007; Ydenberg et al., 2007).

State-dependent foraging theory addresses the relationship between internal condition and behavior, suggesting that when internal condition is low, animals should accept more risk in order to obtain the required daily energy at the cost of other behaviors, including vigilance and refuge use (Mangel and Clark, 1986; McNamara and Houston, 1996). One potential indicator of an animal's state is hormone concentrations, specifically, glucocorticoids (GCs). An animal's behavior can influence GCs, for example by changing their exposure risk and energy intake (Angelier et al., 2007; Jesmer et al., 2017). GCs can reciprocally alter behavior, for example by increase foraging activity through mediation of metabolism, the inhibition of glucose uptake, lipolysis, and glucose activation (Sapolsky et al., 2000). In the laboratory, experimentally elevated GC concentrations can increase foraging (Astheimer et al., 1992; Hamelink et al., 1994; King et al., 1992). In wild animals, higher baseline concentrations of GCs can increase foraging behavior (Chmura et al., 2016; Dallman et al., 1993; Landys-Ciannelli et al., 2002), and GC concentrations decrease as individuals find food (Angelier et al., 2007). Conversely, GCs may induce anxiety and fearful behavior through changes in brain morphology (Harris and Carr, 2016; Korte, 2001; Mitra and Sapolsky, 2008). Elevated GCs have been positively linked to expression of antipredator behaviors such as alarm-calling (Blumstein et al., 2006), anxiety and fearful behavior (Korte, 2001; Thaker et al., 2009), and increased vigilance (Thaker et al., 2009; Voellmy et al., 2014). Thus, evidence from experimental

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manipulations suggests that high concentrations of GCs may increase both foraging and antipredator behavior, yet these two types of behaviors are often considered trade-offs against one another in optimal foraging theory (Brown, 1999; Brown et al., 1999).

Many stimuli can elicit elevated GC concentrations including predation risk and food restriction. In unmanipulated, observational studies of wild animals, the relative contributions of food and predation stress to GC concentrations cannot be distinguished based on GC values alone. Recent work has highlighted the need for physiological measures capable of distinguishing between such environmental stressors, and thyroid hormones have been offered as one possibility (Wasser et al., 2010). Specifically, triiodothyronine (T3) has recently been used effectively to represent energetic condition in a variety of species (Ayres et al., 2012; Vynne et al., 2014; Wasser et al., 2010). In addition to elevating GC concentrations, nutritional deficits generally decrease T3 concentrations in humans and some other animals (Douyon and Schteingart, 2002; McDaniel and Samuels, 1997; Schew et al., 1996). T3 is important in regulating thermoregulation and metabolism and is secreted via the hypothalamic-pituitary-thyroidal (HPT) axis (Douyon and Schteingart, 2002). This hormone decreases during food restriction, lowering basal metabolic rate and resting energy expenditure (Harvey and Klandorf, 1983; Kitaysky et al., 2005; Klandorf et al., 1981; Rosen and Kumagai, 2008). Although we currently have no estimates of how quickly T3 changes in response to food availability in vicuñas (*Vicugna vicugna*), serum T3 levels change substantially over the first 90 days of birth. In other herbivorous mammals, fecal T3 rates can decrease over 70% within five days (Wasser et al., 2010). By using GCs in tandem with T3, we may be able to estimate the relative importance of multiple stressors simultaneously. For example, limited food resources should decrease T3 and increase GC concentrations, whereas fear should increase GC concentrations but not alter T3 concentrations (Ayres et al., 2012; Wasser et al., 2011, 2010).

Our objective in this study was to test state-dependent foraging theory in wild, unmanipulated animals in the high Andes Mountains in San Guillermo National Park (SGNP), San Juan Province, Argentina. At ~3400 m above sea level, this region is considered a desert, receiving less than 240 mm of precipitation annually (Salvioli, 2007). The park is pristine, with few anthropogenic disturbances, such as roads, novel noise, tourism, and habitat degradation or modification to account for as sources of stress. Here, puma (*Puma concolor*) predation accounts for approximately 90% of all vicuña mortality; pumas are the only predator of adult vicuñas, and predation risk is higher for juveniles than adults, and similar for males and females (Donadio et al., 2012). Vicuñas are the main prey item of pumas in SGNP (Donadio and Buskirk, 2016). The most recent estimate of vicuña density in SGNP (2008–2009) is 9.5–12.7 vicuñas/km^2 (Donadio et al., 2012).

There are three habitat types in SGNP (Fig. 1). First, canyons are valleys (10–300 m wide) between hills, and are edged by steep, loose rubble, and rocky outcroppings. They have low forage quality largely consisting of sporadic shrubby cover (for details see Donadio and Buskirk, 2016). Vicuñas in canyons experience approximately 90% more predation than expected based on spatial extent alone. Canyon habitat occupies approximately 15% of the park so, assuming predation was distributed uniformly, we would expect 15% of all predation events to occur within canyon habitat; but, in fact, 28% of all predation events (23 of 82 carcasses found) occurred in the canyon habitat (Donadio and Buskirk, 2016). Second, meadows are flat areas where vegetation mainly consists of tall, dense grasses (Donadio and Buskirk, 2016). Vicuñas in meadows experience approximately 480% more predation than expected given their spatial extent; meadows occupy approximately 4% of the park but experience 20% of all predation events (16 of 82 carcasses; Donadio and Buskirk, 2016). This high predation rate appears to be a function of both vicuña density (which is ~5× higher in meadows than canyons; Donadio and Buskirk, 2016) and perhaps the ambush-style hunting mode of pumas, which is facilitated in meadow habitat by the dense vegetation cover. Third, the most extensive habitat type in SGNP is plains, visually similar to flat, gravel parking lots. In this habitat type, puma predation occurs 30% less than would be expected given their spatial extent; plains occupy 81% of the park but host only 51% of all predation events (43 of 82 carcasses; Donadio and Buskirk, 2016). Thus, these three habitats may vary inversely in predation risk and food abundance, with meadows having the highest relative predation and the highest forage availability, and plains having the lowest relative predation and lowest forage availability, with canyons falling between these two.

Pumas are ambush predators, and as such, should elicit a stronger behavioral response than would cursorial predators such as wolves (Preisser et al., 2007; Thaker et al., 2011). Previous work in SGNP suggests that vicuñas make state-dependent habitat selection decisions. Specifically, the percent of fat found in femur bone marrow of vicuña carcasses tended to be lower in canyon and meadow (higher risk, higher reward) habitats compared to plain habitats (safer, lower reward; Donadio et al., 2012) indicating animals in poor nutritional condition may be actively selecting these riskier but more food-rich habitats. Further, vicuña carcasses found in canyons and meadows were often in poorer condition than those in plains (as estimated by visual inspection of color and consistency of bone marrow; Donadio et al., 2012). These data suggest that vicuñas may be making trade-offs to facilitate increasing their body condition through foraging at the cost of increasing predation risk. Lastly, previous behavioral observations indicated that

Fig. 1. Behavioral observation sites. Left: Quebrada San Guillermo (QSG) meadow surrounded by the hills of a canyon. Right: Vega de los Leones (VL) meadow surrounded by flat, gravel plains.

vicuñas in plains foraged more than those in canyons and meadows (Donadio and Buskirk, 2016), supporting an assumption that vicuñas may be perceiving that risk is lower in these plains habitats. Here, we expanded on previous studies of the state-dependent decision making of vicuñas by exploring relationship between internal condition and behavior between two sites.

Specifically, we hypothesized that internal condition (concentrations of GCs and T3) caused by stimuli such as nutritional deficits and perceived predation risk should influence foraging and vigilance. Both physiological parameters were assessed non-invasively by measuring GC metabolites (GCMs) and T3 in fecal samples, and matched to behavioral observations. Previous work (Arias et al., 2013) found that GCM concentrations in vicuñas peaked one day after the onset of an acute stressor, so the measured hormone concentrations should reflect the physiological state over the previous ~24 h. We predicted that 1) an animal with elevated T3 would forage less relative to an individual with low T3, 2) an animal with elevated GCs would be more vigilant relative to an animal with low GCs, and 3) that GCs and T3 would be negatively related within an individual, in concordance with the biomedical literature (Douyon and Schteingart, 2002; Rosen and Kumagai, 2008; Walpita et al., 2007). This study thus links unmanipulated physiology and behavior at an individual level in a wild animal, with important implications for conservation physiology.

2. Methods

2.1. Study animal and study site

Vicuñas are medium-sized ungulates (45–55 kg in the region of this study) that inhabit high altitudes in South America. The smallest members of the Camelidae family, vicuñas are relatives of llamas (*Lama glama*), alpacas (*Vicugna pacos*), and guanacos (*Lama guanicoe*). Family groups consist of a single dominant and territorial male, and several females and yearling offspring. Family groups are commonly isolated, although they occasionally mix into groups of over 50 (CEP, *personal obs.*). Sexual dimorphism is not present in this species, with the exception of genitalia which cannot be observed from afar, and behavior, where males tend to be more alert or defend territory. Therefore, this study was only conducted on known females.

Critically, vicuñas may use each of the three available habitat types (canyon, meadows, plains) during daily migrations. They ascend into higher elevation plains before dusk, presumably because the risk of predation from crepuscular pumas is lower (Koford, 1957). After dawn, they may descend into any of these habitat types, where they forage during the daylight hours. We conducted behavioral observations on vicuñas at two sites within SGNP separated by approximately 10 km: Quebrada San Guillermo (QSG) and the Vega de los Leones (VL). Data from GPS collars deployed on vicuñas in this population indicate that there is no movement of collared adult females between these sites. Observations were collected within meadows at both sites, but QSG is located at the base of a canyon (Fig. 1) while VL is surrounded by plains. Vicuñas from both sites thus use all three habitat types, but possibly at different frequencies (e.g., in QSG they may spend more time in the adjacent canyon whereas in VL they may spend more time in the surrounding plains), and thus may be exposed to differences in predation risk, food availability, which would be reflected in physiology (e.g. GCs and T3) and behavior (e.g., the trade-off between foraging and vigilance).

2.2. Capture and tagging

We captured and tagged only adults (determined by size), and equipped with them with either a VHF or GPS collars and unique ear tags. Captures were made in both 2014 and 2015, and thus not all animals are present in each year (Table S1). Behavioral observations were only conducted on females. In total, 24 animals were captured and used for behavioral observations at VL, and 7 animals were captured and used for observations at QSG.

2.3. Behavioral analyses

At least two days passed between capture and behavioral observations and sample collection began on collared animals (mean = 127 days; range = 2–716 days). Fecal GCMs peak ~24 h after exposure to an acute stressor in vicuñas (Arias et al. 2013), and so vicuñas should have recovered from the stress of handling before behavioral observations were conducted. There was no difference in the behavioral response to researcher presence of collared animals and animals within the same family group that were uncollared (Donadio, *unpublished*).

We obtained 20-minute video-recordings of behavior of tagged and collared individuals in late fall and early winter (April-June), 2014, 2015, and 2016, when most females are pregnant and in their second trimester of pregnancy (Koford, 1957). A previously captured vicuña was located with telemetry, observed from a distance of 50–300 m for 10 min to allow her to acclimate to our presence, and then her behavior was video recorded from this same distance. Most individuals were observed multiple times per year (mean 4, range 1–7 observations/individual/year), and were observed in one, two, or three of these years, when relocated via telemetry, until they died of natural causes (predation or illness; Table S1). Fifteen of the 31 individuals were observed over multiple years (Table S1). Whether or not an individual was sampled in each year (2014–2016) depended on the year and date they were captured and died (Table S1). All behavioral observations were conducted between 10:00 am and 5:30 pm.

In total, we collected 203 full-length videos (4060 min). Any video less than 20 min long, due to an animal being lost amongst a larger herd or migrating to higher altitudes, were discarded ($n = 43$). Twenty minutes has previously been determined as sufficient for detecting differences in behavior in other ungulates (Laundré et al., 2001). Although travel time through different habitats and time spent previously in different habitats could affect both behavior and physiology, at the time of observations all individuals were either at QSG or VL. Food abundance and the likelihood of being predated upon were assumed to be a function of the site in which the animal was located during focal observations.

Behavioral videos were analyzed using JWatcher software (v1.0) by a single investigator (CEP). We measured the total percentage of time of the 20-minute video observation that an individual spent being vigilant, foraging, engaging in other behaviors, or out of sight. Vigilance was defined as an animal having its head up and looking around, and was not mutually exclusive of chewing, but the individual was not actively engaged in foraging. Foraging was defined as an animal having its head down, either actively foraging or searching for forage. Because group size can affect behavior, we measured group size by determining the number of animals that stayed in close proximity to each other (~50 m) and moved together. We also recorded wind speed and time of day at the point of each observation, Julian date of the observation, and year for inclusion in analyses, as these may affect behavior.

2.4. Fecal sample analyses

Once a behavioral observation was completed, we waited for the focal individual to defecate. We then moved towards the sample slowly but immediately, because we only gathered fecal samples which we were confident matched our focal individual, which required it being warm and moist relative to the surrounding samples. Due to the harsh climate, samples cooled and the exterior dried within minutes. If the observer could not identify the fecal sample (it had cooled), or another individual was observed to have recently defecated in that area (resulting in two warm, moist samples), no sample was collected. The entire sample (all pellets in the fecal mass) was collected immediately into a plastic clinical urine jars, placed on ice in the field, and stored at −20 °C upon returning to the field station. All fecal samples were collected between 10:45 am and 6:00 pm.

Fecal GCM concentrations were measured with an 11-oxoetiocholanolone enzyme immunoassay (Palme and Möstl, 1997) validated for this species (Arias et al., 2013). Briefly, all pellets in the feces sample were lyophilized for 48 h and ground to a fine powder with mortar and pestle. 0.500 ± 0.050 g of this powder was extracted for 5 min on a vortexer with 5 mL of 80% methanol, and centrifuged (2500*g*; 30 min). The supernatant was then diluted (1:10) with an inhouse assay buffer (composed of Trishydroxaminomethane, NaCl, bovine serum albumin, and Tween 80) before being assayed. The fecal GCM EIA inter-assay variation was 4.4%, and intra-assay variation was 5.0%. Assay sensitivity was 2.0 ng/g .

Fecal T3 was measured with L-triiodothyronine ^{125}I- RIAs (06B254216; MP Biomedicals, Orangeburg, NY) following Wasser et al. (2010) and manufacturer instructions. Briefly, fecal samples were lyophilized and homogenized with a mortar and pestle. We then combined 0.1 g ± 0.050 g of ground sample and 15 mL 70% ethanol, vortexed this on a multi-tube vortexer for 30 min at 1 pulse/s, and then centrifuged the tubes at 2200 rpm for 20 min. The supernatant was then decanted, and the fecal powder was extracted a second time using the same extraction protocol. The supernatants from both extractions were then combined prior to assay. The fecal T3 RIA inter-assay CV was 5.8% and intra-assay variation was 7.71%, and assay sensitivity was 6.7 ng dL^{-1}.

A total of 181 fecal samples were collected, but those with coefficients of variation (CVs) over 10% were omitted from analyses. Here, we analyzed multiple fecal samples (up to 9; collected during the initial captures and after subsequent behavioral observations) and video observations (up to 17; see Table S1) from the same individual, collected over up to three years to determine if hormone concentrations and behavior were related (*n* of fecal GCM samples = 139, *n* of T3 = 120).

2.5. Statistical analyses

2.5.1. Assumption of hormone concentration consistency within an individual over ~2 weeks

Others have collected a single fecal sample and compared resulting physiological metrics to behavioral observations of the same individual conducted over the two subsequent weeks, on the assumption that the fecal physiology at sampling reflected that during the later behavioral observation (Chmura et al., 2016; Mateo, 2007). We tested this assumption for our study species by statistically analyzing whether the physiological measures obtained from (2–6) fecal samples of a single individual were consistent across a two-week time span. We used linear mixed effects models to test if changes in hormone concentrations increased with time among samples. Hormone concentrations (fecal GCMs and T3) were response variables in separate models, the time difference between samples (in days) as the covariate, and vicuña identity (ID) and year were added random effects. We found that the time between samplings had no effect on T3 concentrations ($t = -1.445$, $df = 26$, $p = 0.16$) or GCM concentrations ($t = 1.067$, $df = 45$, $p = 0.292$), indicating that hormone concentrations remained relatively consistent within an individual within an approximately 2-week sampling period within a single year. Therefore, we filled (using the 'fill' function in R package tidyr) the dataset for behavioral observations for which we did not collect associated fecal samples from that individual on that day, using the mean hormone concentrations or that individual in that year (range 0.92–17 days apart, mean 2.3 days apart). In other words, for days on which fecal samples were collected, we used the measured hormone values. For days on which a fecal sample was not able to be collected, we used data created by taking the means of other physiological data within that two-week period. Using our full dataset of 207 behavioral observations rather than limiting this to only 53 matching behavioral and physiological samples (matching dataset) allowed us to increases our sample size substantially. Further, we explored the same analyses with the matching dataset, and found results consistent with the non-matching dataset, as presented below.

2.5.2. Statistical analyses of hormonal and behavioral data

To determine if there were relationships between hormone concentrations and behavioral observations, we used the penalized quasi-likelihood (PQL; function 'glmmPQL' in package MASS) method of analysis due to working with proportional data (family = quasibinomial) and unbalanced design (more observations were made at VL than at QSG). Model parameters were selected using backwards step selection and were retained where $p < 0.10$. The proportion of time a vicuña spent vigilant and feeding were response variables for separate analyses. GCMs and T3 were our independent variables of interest, and wind speed, Julian date, time of day of the observation, and group size were covariates. Fixed factors were site and year. All samples were included (rather than just an average value for each individual), and individual was included as a random slope effect in all models to account for repeated samples from a single individual. We retained variables of interest (GCMs and T3) in all models.

We examined the relationship between GCMs and T3 using PQL linear models; since all samples were included in this analysis, individual identity was included as a random slope effect to account for multiple samples per individual. We examined the relationships between GCMs and site, and T3 and site using a separate glmmPQL models, with the physiological measurement (GCM or T3) as the response variables, site as the fixed factor, and since there were multiple samples per individual, individual was a random effect. Model plots of fitted vs. residual values were used to explore model assumptions, which were met. Collinearity was checked using the autocorrelation output from the summary() function of the model, and this assumption was also met (all $p < 0.338$). All analyses were performed in R Studio (v. 3.4.1; R Core Team 2017). Data are presented as means ± 1 standard error (SE).

2.6. Animal ethics statement

All protocols were approved by The Pennsylvania State University Institutional Animal Care and Use Committee under protocol #45139. Samples were imported under U.S. Fish and Wildlife Service permitting for threatened animals, with Federal Fish and Wildlife Permit #MA70993B-2. Research permits (# DCM 455 and subsequent renewals) were issued by the Argentinean National Park Administration.

3. Results

Overall, we conducted 36 behavioral observations of 7 individuals at QSG and 167 observations of 24 individuals at VL. Vicuñas in plains were extremely vigilant, and would either not allow us to approach (i.e. started moving away immediately, and therefore we were affecting their behavior and also could not complete a full 20-minute observations) or became constantly vigilant (again, we were affecting their behavior). Therefore, we did not include behavioral observations from the plains. In contrast, animals in meadows and canyons resumed foraging and natural behaviors within our 10-minute acclimation period (and often immediately). Vicuñas at QSG were much more difficult to locate with telemetry than those at VL, due to their location within a canyon, and we were constrained by road access to all habitats, reducing our sample sizes at QSG.

3.1. Behavior

Animals spent between 0 and 98% of the time foraging (mean 59%, range 0–94%) or being vigilant (mean 30%, range 0–98%). The remaining 7% of the time was spent walking (mean 2%, range 0–14%), laying down (mean 4%, range 0–100%), running (mean 0.2%, range

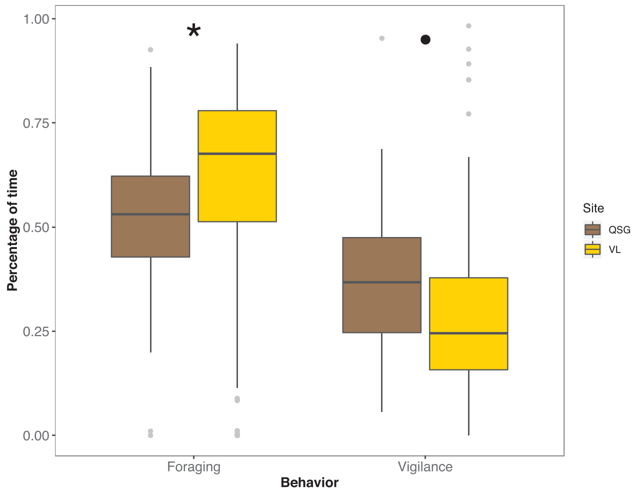

Fig. 2. The percentage of time that vicuñas spent foraging and vigilant in Quebrada San Guillermo (QSG) and Vega de los Leones (VL), San Guillermo National Park, Argentina. Boxes represent first to third quartiles, horizontal bars indicated medians, error bars are 1.5× the interquartile range (IQR), and grey points are outliers, defined as $> 1.5 \times$ IQR. Data were obtained from 203 video-observations of 31individuals. * indicates $p = 0.049$, • indicates $p = 0.052$.

0–2%), nursing (mean 0.3%, range 0–10%), and engaging in other behaviors such as scratching (mean 0.3%, range 0–3%). Since these behaviors were rare and our research question was focused on understanding the role of external and internal conditions in the trade-offs between foraging and vigilance, we did not analyze these other behaviors. There were no effects of year, group size, wind speed, time of day, or Julian date on the proportion of time spent foraging (all $t < -1.45$, all $p > 0.253$). Vicuñas spent approximately 10% more time foraging at VL than QSG (61 ± 2% vs 52 ± 4%, respectively; estimate = 0.367, SE = 0.189, t = 1.985, p = 0.049, $R^2 = 0.022$; Fig. 2) during the 20-minute observations. There was no effect of group size, time of day, or Julian date of the proportion of time spent vigilant (all $t < 1.148$, all $p > 0.253$). Vicuñas were about 11% more vigilant at QSG than at VL (38 ± 3% and 28 ± 1%, respectively; estimate = −0.369, SE = 0.189, t = 1.961, p = 0.052, $R^2 = 0.028$; Fig. 2).

3.2. Relationships between behavior and hormones: Foraging

The final model examining the relationship between the proportion of the time spent foraging and fecal GCMs included only fecal GCMs (the variable of interest, which the model was forced to retain) and habitat (model $R^2 = 0.027$). However, there was no significant relationship between the proportion of time vicuñas spent foraging and their fecal GCM concentrations (estimate −0.003; SE 0.007, t = −0.436, p = 0.663, $R^2 = 0.001$). Likewise, the final model examining the relationship between the proportion of time spent foraging and fecal T3 concentrations included only fecal T3 (the variable of interest, which the model was forced to retain) and habitat (model $R^2 = 0.027$). However, there was no significant relationship between the proportion of time vicuñas spent foraging and their fecal T3 concentrations (estimate −0.0001; SE = 0.002, t = −0.510, p = 0.652, $R^2 = 0.002$). Because we found significant differences in foraging between sites, we analyzed each site separately with respect to the relationships between hormones and foraging. We found no relationships between fecal GCMs and foraging in either QSG (estimate = 0.013, SE = 0.016, t = 0.767, p = 0.453, $R^2 = 0.056$) or VL (estimate = −0.006, SE = 0.008, t = −0.79, p = 0.431, $R^2 = 0.005$). We also found no relationships between fecal T3 and foraging at either QSG (estimate = −0.003, SE = 0.007, p = 0.647, $R^2 = 0.012$) or VL (estimate = -0.0007, SE = 0.002, t = -0.350, p = 0.727, $R^2 = 0.001$).

3.3. Relationships between behavior and hormones: Vigilance

The final model examining the relationship between the proportion of the time spent vigilant and fecal GCMs included only fecal GCMs (the variable of interest, which the model was forced to retain), wind speed (estimate = −0.009, SE = 0.004, t = −1.955, p = 0.052, $R^2 = 0.028$), and habitat (estimate = −0.513, SE = 0.0211, t = −2.458, p = 0.016, $R^2 = 0.048$). However, there was no significant relationship between the proportion of time vicuñas spent vigilant and their fecal GCM concentrations (estimate −0.002, SE 0.007, t = −0.295, p = 0.769, $R^2 = 0.001$).

Likewise, the final model examining the relationship between the proportion of time spent vigilant and fecal T3 concentrations included only fecal T3 (the variable of interest, which the model was forced to retain), wind speed (estimate = −0.011, SE = 0.005, t = −2.184, p = 0.031, $R^2 = 0.041$) and habitat (estimate = −0.503, SE = 0.250, t = −2.018, p = 0.046, $R^2 = 0.040$). However, there was no significant relationship between the proportion of time vicuñas spent vigilant and their fecal T3 concentrations (estimate −0.0001; SE = 0.002, t = −0.510, p = 0.652, $R^2 = 0.018$). Because we found significant differences in behavior between sites, we analyzed each site separately with respect to the relationships between hormones and vigilance. We found no relationships between fecal GCMs and vigilance in either QSG (estimate = −0.004, SE = 0.007, t = −0.575, p = 0.5661, $R^2 = 0.002$) or VL (estimate = −0.006, SE = 0.008, t = −0.79, p = 0.431, $R^2 = 0.005$). We also found no relationships between fecal T3 and vigilance in either QSG (estimate = 0.004, SE = 0.006, t = 0.591, p = 0.564, $R^2 = 0.020$) or VL (estimate = 0.003, SE = 0.002, t = 1.127, p = 0.222, $R^2 = 0.019$).

3.4. Hormones

There was no difference in GCM concentrations (estimate = −4.21, SE = 2.88, t = −1.461, p = 0.146, $R^2 = 0.029$) between sites. Fecal GCM concentrations in QSG ranged from 7.76 to 61.0 ng g^{-1}, with mean concentrations of 23.3 ± 2.2 ng g^{-1}. Fecal GCM concentrations in VL ranged from 2.1 to 49.6 ng g^{-1}, and mean with mean concentrations were 20.1 ± 0.8 ng g^{-1}.

There was no difference in T3 concentrations (estimate = −4.523, SE = 8.109, t = −0.574, p = 0.567, $R^2 = 0.005$) between sites. Fecal T3 concentrations in QSG ranged from 17.1 to 160 ng g^{-1} in QSG, and mean fecal T3 concentrations were 78.4 ± 6.4 ng g^{-1}. Fecal T3 concentration in VL ranged from 16.0 to 246, and mean T3 concentrations were 87.2 ± 3.8 ng g^{-1}.

There was no relationship between GCMs and T3 within individuals (estimate = −0.317, SE = 0.282, t = −1.125, p = 0.263, $R^2 = 0.013$).

4. Discussion

In this study, we found that neither GCM nor T3 concentrations were significantly correlated with vicuña foraging or vigilance behavior, the two most common behaviors exhibited by these animals. Vicuñas tended to be more vigilant and spend less time foraging at QSG than at VL, but there were no significant differences in either GCM or T3 concentrations between the two sites. GCM and T3 concentrations were not correlated within an individual.

There are, however, a few caveats that need to be considered as we interpret our findings. First, our estimates of “habitat riskiness” (i.e. predation risk) are based on carcass data, which is a result of only successful predation attempts. Secondly, despite the T3 protocol used in this study having been validated for a wide variety of species (bird, mammals; Wasser et al., 2010), it has not been validated for vicuñas and may not accurately reflect their nutritional reserves. It is also possible that despite twenty-minute observations previously being determined as sufficient to detect differences in behavior in other ungulates (Laundré et al., 2001), it may not have been long enough to accurately capture behavior of individual vicuñas, though most individuals had at least 4 observations each year. Further, our R^2 values were very small, suggesting that other variables not measured in this study may be driving hormone concentrations and behavior more strongly that habitat riskiness or food availability. Factors such as social interactions, climate variables over the past 24 h, or reproductive status could be contributing to hormone concentrations or behavior. As with many studies of wild animals, not all of these variables were able to be assessed but should be considered in a future study.

The evidence presented here suggests that neither GCM nor T3 biomarkers may be reliable in predicting behavior in wild, largely anthropogenically undisturbed populations of vicuñas, that should have been exhibiting baseline physiological conditions. Although vicuñas at both canyons and meadows were found to be in worse condition than in plains as assessed in carcasses killed by pumas (via bone marrow), suggesting they behave in a state-dependent context by selecting riskier, higher reward habitats, we found no evidence for state dependent behavior based on activity budgets. This lack of relationship between hormones and behavior could be driven by vicuñas' baseline energetic condition at the time of sampling. For instance, state-dependent behavioral switches from antipredator behaviors to foraging in animals are often observed only under energetically constrained conditions (Astheimer et al., 1992; Gray et al., 1990; Landys et al., 2006; Santana et al., 1995; Tempel et al., 1992). Furthermore, under baseline conditions, GCs may not be sufficiently responsive to reflect subtle changes in body condition (Sorenson et al., 2017).

State-dependent foraging theory was largely developed for small animals with high metabolic rates (Caraco et al., 1990; Carter et al., 2016; Croy and Hughes, 1991), with one assumption being that the animal would starve if it did not obtain sufficient forage daily (Gilby and Wrangham, 2007; Stephens, 1981). State-dependent foraging theory holds for several small species, such as willow tits (*Parus montanus*), for which 90% of their field metabolic rate is attributed to maintenance (thermoregulation and maintenance metabolism; Moreno et al., 1988). However, this theory may not extend well to animals who are unlikely to reach starvation within short periods of time (Mirza et al., 2006; Stephens, 1981). For example, large animals may not adhere as strictly to state-dependent foraging as small animals due to generally lower metabolic rates, which reduce energy requirements and increase time to starvation (Brown et al., 2004). Thus, detectable state-dependent behavior may be less prevalent in large animals under baseline conditions, such as our vicuñas in this system.

Other studies of state-dependent decision-making in large, wild animals generally occur at long temporal scales, or address state-dependent decisions across a broad spatial landscape (Long et al., 2014; Montgomery et al., 2013). For instance, Monteith et al., (2011) describe the effects of nutritional condition of mule deer (*Odocolileus hemionus*) on biannual migration, reporting that deer in good nutritional condition delayed their autumn migration compared to animals in poor nutritional condition. Studies taken at these large scales provide a foundation on which to base the context of state-dependent decision making, but crucial too, is to understand if and how these decisions are made at short-timescales, which may influence a more persistent nutritional condition. Although we found no relationships between short-term activity budgets and hormone concentrations in this study, vicuñas may be making state-dependent decisions at larger spatial and temporal scales, including habitat selection. The recent deployment of GPS collars in SGNP will help us address this possibility. Crucial to recognize in this study, too, is that the free-living vicuñas in this study have an entire suite of behavioral responses available to them to regulate their physiological condition, in contrast to experimental settings where animals' behavioral (e.g. activity budget, habitat selection) responses may be constrained (Calisi and Bentley, 2009; Dickens and Romero, 2013; Ricklefs and Wikelski, 2002).

Contrary to our expectations based on biomedical literature that GCM and T3 concentrations would be negatively correlated within an individual (Douyon and Schteingart, 2002; Hunt et al., 2012; Welcker et al., 2015), we found no such relationship between these two measures. In contrast to our understanding of the relationships between GCs and T3 concentrations in the biomedical literature (Degroot and Hoye, 1976; Engler et al., 1977; McDaniel and Samuels, 1997; Shimokaze et al., 2012), the relationships between GCs and T3 are not well-understood and are complex in many wild animals (Jeanniard du Dot et al., 2009; Jesmer et al., 2017). For instance, in Hawaiian monk seals (*Monachus schauinslandi*), GCs and T3 were positively correlated at four study sites, but not in two others (Gobush et al., 2014). Others have found even more complex relationships between these biomarkers (Ayres et al., 2012; Hayward et al., 2011). For example, Keogh et al. (2013) found variable relationships between GCs and T3 among years, but that both biomarkers decreased as the elapsed time since exposure to a stressor increased. Our study adds to this literature, but there is clearly much we still need to learn about interactions between these two hormones in wild animals.

We observed differences in behavior between sites, where vicuñas in QSG foraged less and tended to be more vigilant than animals at VL. This was an interesting result, given that food availability is lower in QSL than VL and so we would have predicted that animals in QSL would have to forage more to obtain the same amount of food. However, if animals selected QSG because based on its lower predation risk compared to VL, rather than avoiding it due to its lower food availability, it might indicate that they have adequate energetic reserves and do not *have* to take the risk in order to forage, and they can devote more time to antipredator behaviors (vigilance). However, this was not reflected by the hormone data— individuals in QSG did not have lower GCs or higher T3 than those in VL. In contrast, animals in VL may have selected the habitat specifically because it was high in forage availability, even though predation risk is higher, because they have to meet energetic minimums for current maintenance and future reproduction. In this study, behaviorally-mediated physiology may make sense in the predator-prey context that chronic stress in the face of high predation is likely maladaptive. This was similarly seen in green sea turtles (*Chelonia mydas*) where individuals in very good condition (compared to good, fair, or poor condition) tended to use safer edge seagrass habitats rather than risky interior habitats where forage availability was high, forgoing foraging for increased safety (Wirsing et al., 2007). Likewise, in response to predator cues, axolotls (*Ambystoma mexicanum*) increased refuge use and decreased activity, leading to a decline in prey capture efficiency by predators (Alcaraz et al., 2015). By only utilizing risky habitats when body condition is relatively poor and sufficient forage cannot be obtained in safe habitats, predation risk can be reduced across an individual's lifetime.

5. Conclusion

Both behavior (Carney and Sydeman, 1999; Donadio and Buskirk, 2006; Satterthwaite and Mangel, 2012) and physiology (Ahlering et al., 2013; Madliger and Love, 2016) have been used in conservation to understand potential population stressors or environmental health. Here, although we found differences in behavior between sites, we found no differences in physiology nor a link between physiology and behavior. Wildlife and conservation managers are increasingly searching for techniques to monitor population health within a single generation, as opposed to previous approaches that track changes in demography (Sorenson et al., 2017). Biomonitoring tools including glucocorticoids and thyroid hormones have been suggested as a potentially strong approach to identify changes in conservation-related variables (Busch and Hayward, 2009; Cooke and O'Connor, 2010; Madliger et al., 2016; Wikelski and Cooke, 2006). This work adds to a growing body of evidence suggesting that even when applied properly (e.g., GCMs are validated: Palme, 2019), these markers are not simple to interpret and so are unlikely to provide an easy monitoring tool for conservation biologists. Field studies across taxa are needed to understand the information provided by glucocorticoids, thyroid hormones, and their interactions in wild animals, before they can be accurately used as biomarkers of psychological and nutritional stress (Boonstra, 2013; Otovic and Hutchinson, 2015). There is a need for an integrated understanding of how animals respond to their habitats through landscape-level movements, behavior, and physiology, especially in the face of environmental change and anthropogenic disturbance (Cooke and O'Connor, 2010; Ellis et al., 2012; Németh et al., 2013).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2019.113299.

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