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Exploring seasonal variation in the faecal glucocorticoid concentrations of African elephants (*Loxodonta africana*) living in a drought-prone, anthropogenic landscape

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ABSTRACT

Context. The wide-ranging movement of African elephants (*Loxodonta africana*) is largely driven by the spatio-temporal distribution of water and forage, and often leads to their travelling outside of formally protected areas. With an increase in drier periods predicted across Africa due to climate change, it is critically important to understand how elephants physiologically respond to alterations in the availability and distribution of resources. **Aims.** We assessed variation in the adrenal activity of elephants living in Kenya's Tsavo East National Park between wet and dry seasons, as well as among individuals found in Tsavo East National Park and privately protected Rukinga Wildlife Sanctuary (part of the Kasigau REDD+ wildlife corridor) in the dry season, when the area experiences an influx of elephants in search of alternative resources. **Methods.** We opportunistically collected fresh elephant faecal samples across the two seasons and locations for analysis of faecal glucocorticoid metabolite (fGCM, a proxy for stress) and nitrogen (Nf, an indirect measure of diet quality) concentrations. The Normalised Difference Vegetation Index (NDVI) was employed as an additional indicator of habitat quality. **Key results.** In Tsavo East N.P. Nf and NDVI were both significantly lower during the dry season, indicating poorer habitat quality compared with the wet season. Although elephant fGCM concentrations tended to be higher in the dry season than the wet, the differences were not significant. There was no difference between elephant fGCMs measured in Tsavo East N.P. and Rukinga W.S. during the dry season, nor in habitat quality between the two locations. **Conclusions.** Elephants living in Tsavo may be physiologically unaffected by (or adapt to) typical seasonal changes in habitat quality that could lead to nutritional stress; however, whether this is the case during extended periods of severe drought requires further investigation. Rukinga W.S. provides a safe haven of sufficient habitat quality for elephants searching for alternative resources during this period. **Implications.** Extended dry periods are likely to become increasingly common in semiarid savannahs, and implications for wildlife must be closely monitored. Privately protected land outside formally protected areas plays an important role in conservation efforts, which should be considered when making land management plans.

Keywords: African elephants, anthropogenic, conservation, endocrine analysis, forage quality, glucocorticoids, habitat, herbivore nutrition, seasonal, stress.

Introduction

With the rising occurrence of extreme weather such as droughts (Dai 2013; Cook *et al.* 2014; Masih *et al.* 2014), it is increasingly important to understand how the spatio-temporal distribution of resources affects the physiological stress response of wildlife. Herbivore nutrition is largely dependent on forage quality, which along with growth, is positively associated with the amount of rainfall (Marshal *et al.* 2005). Elevated stress levels have been observed in a number of wildlife species in response to declining forage quality during dry periods, including koalas (*Phascolarctos cinereus*) (Davies *et al.* 2014), spider monkeys (*Ateles geoffroyi yucatanensis)* (Rangel-Negrín *et al.* 2009) and Greater rhea (*Rhea americana*) (Lèche *et al.* 2014). If individuals are unable to respond appropriately, repeated exposure to stressful stimuli can lead to chronic levels of stress, which may have major implications for reproductive function, growth and immunity (Wingfield and Sapolsky 2003; Romero 2004; Rasmussen *et al.* 2008; Koren *et al.* 2012).

The African elephant *(Loxodonta africana*) is a wide-ranging herbivore whose movement is largely driven by the need to find water and forage (Birkett *et al.* 2012; Wall *et al.* 2013; Tshipa *et al.* 2017). These mega-herbivores require adequate nutrition for growth, reproduction, energy metabolism and immunity (McCullagh 1969; Obanda *et al.* 2011; Ishiguro *et al.* 2018). Meeting these nutritional requirements becomes more challenging during the dry season, when the quality of forage often declines (Codron *et al.* 2007; Kohi *et al.* 2011; Kos *et al.* 2012). Accordingly, the body condition scores of African elephants have been reported to be lower in the dry than in the wet season (Foley *et al.* 2001), and Asian elephants (*Elephas maximus*) with low body condition scores are observed more frequently in drier periods (Pokharel *et al.* 2017). Dry seasons of poor forage quality may ultimately result in elephants becoming nutritionally stressed.

Glucocorticoids form the basis of the neuroendocrine stress response in animals (Keay *et al.* 2006), thus faecal glucocorticoid metabolites (fGCMs) are commonly used as a proxy for physiological stress. Although primarily adaptive, allowing an individual to respond to a potential stressor, elevated fGCM concentrations may disrupt the reproductive tactics of male elephants, through suppressing the occurrence of musth signals (Rasmussen *et al.* 2008). In addition, female elephants born during months when their mother exhibits increased stress concentrations experience faster reproductive senescence and reduced lifetime reproductive success (Mumby *et al.* 2015). Despite African elephant fGCMs being negatively correlated with habitat quality (Oduor *et al.* 2020), and Asian elephants with the lowest body condition scores displaying the highest fGCM concentrations (Pokharel *et al.* 2017), previous investigation has produced mixed results regarding whether elephants exhibit seasonal variation in hormone levels (Foley *et al.* 2001; Viljoen *et al.* 2008; Woolley *et al.* 2009). Such inconsistencies are to be expected, as different ecosystems vary in local environmental conditions, which will inevitably influence the extent to which elephants are impacted.

During dry periods, elephants face a key trade-off between resting to save energy and walking in search of forage and water (Mramba *et al.* 2019). Approximately 70% of African elephant range lies outside of formally protected areas (Blanc *et al.* 2007), thus the search for permanent water and adequate forage during the dry season frequently results in elephants having to navigate unprotected areas where human disturbance is high (Cook *et al.* 2015; Evans *et al.* 2020). This can often lead to human–elephant conflict, particularly in the form of crop-raiding, which presents a risk to their survival (Obanda *et al.* 2008; Mijele *et al.* 2013). Land outside formally protected areas is therefore critically important for the conservation of elephants living in anthropogenic landscapes (Ahlering *et al.* 2013; Goswami *et al.* 2014; Ihwagi *et al.* 2015), and includes areas that offer informal protection for wildlife such as community-based conservancies and wildlife sanctuaries managed by private conservation bodies, as well as wildlife corridors that facilitate the safe passage of elephants between these areas (Songhurst *et al.* 2016; Adams *et al.* 2017; Talukdar *et al.* 2020).

In order to be suitable for use, these areas of land must mitigate any potential elevation in the stress response of elephants as a result of moving through areas of increased human activities. Several previous studies have found elephants to exhibit heightened stress levels in response to various forms of anthropogenic disturbance (Ahlering *et al.* 2011; Tingvold *et al.* 2013; Hunninck *et al.* 2018; Szott *et al.* 2020), but this is not the case in all contexts (Munshi-South *et al.* 2008; Ahlering *et al.* 2013; Pokharel *et al.* 2019). Land outside formally protected areas should also provide elephants with habitat of sufficient quality to meet their nutritional requirements throughout the year.

We explored seasonal variation in the faecal glucocorticoid response (as a proxy for physiological stress) of African elephants living in Kenya's Tsavo ecosystem, a drought-prone, anthropogenic landscape home to the country's largest population of elephants (Ngene *et al.* 2017). Habitat quality was additionally assessed through analysis of nitrogen (N) in faeces (total N of faeces, Nf) and the Normalised Difference Vegetation Index (NDVI). Nf is employed as an indirect measure of diet quality in herbivores (Leslie *et al.* 2008; Gil-Jiménez *et al.* 2015) because nitrogen provides an estimate of protein, which is limiting in herbivores (White 1993). NDVI is a widely used indicator of vegetation greenness (Pettorelli *et al.* 2005).

Tsavo East and Tsavo West National Parks are separated by the Taita Taveta County, which is primarily comprised of small-scale farming communities and privately owned ranches, of which several are managed for conservation purposes. This includes the privately protected Rukinga Wildlife Sanctuary, part of the Kasigau REDD + (an abbreviation for 'reducing emissions from deforestation and forest degradation') Wildlife Corridor running centrally through Taita Taveta connecting Tsavo East and Tsavo West National Parks (Freund and Bird 2013). Rukinga had been grazed to dust by cattle before being converted to a wildlife sanctuary in 1997 by Wildlife Works (Wildlife Works 2021). During the dry season when water and forage become limited inside the national parks, Rukinga W.S. sees an influx of elephants probably in search of the permanent water sources the sanctuary provides (McKnight 2004; Williams *et al.* 2018). Crop-raiding is frequently reported in nearby farming communities (Kagwa 2011; Von Hagen 2018). We also investigated the habitat quality and fGCMs of elephants found in Rukinga W.S. during the dry season, to enable insight into the area's suitability for use by elephants when travelling outside the national parks. Therefore, while other studies exploring fGCMs in elephants often focus on temporal or spatial variation, our research combined both in order to provide a more holistic picture of their physiological response when searching for resources.

We hypothesised that: (1) elephants in Tsavo East N.P. would exhibit elevated levels of glucocorticoids during the dry season in compared with the wet season – this was based on the prediction that there would be a marked decline in habitat quality (as indicated by Nf and NDVI) from wet to dry seasons in Tsavo East N.P.; and (2) Elephants in Rukinga W.S. would exhibit increased glucocorticoid concentrations compared with elephants in Tsavo East N.P. during the same (dry) season, potentially resulting from Rukinga's closer proximity to farming communities and other human-related disturbances. We predicted that with improved management, the habitat quality of Rukinga W.S. would be comparable to that of Tsavo East N.P. during the same (dry) season, supporting Rukinga in providing sufficient forage resources for elephants when travelling outside the national park. Findings will ultimately contribute to our understanding of how seasonal variation in habitat quality may influence the stress response of elephants living in Tsavo, an already drought-prone ecosystem that is increasingly vulnerable to climate change.

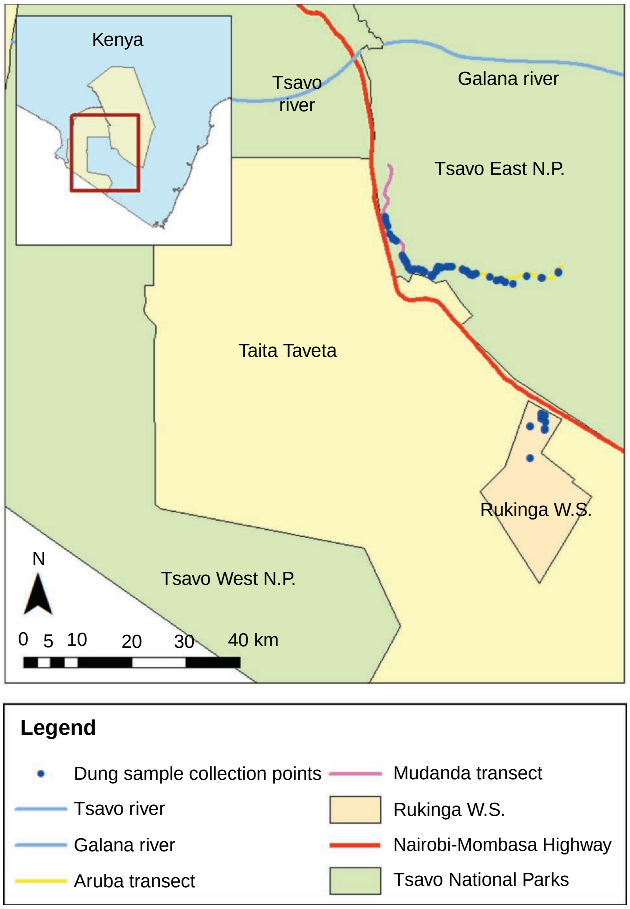

Fig. 1. Map of the study area. Tsavo East and West N.P.s are separated by the Taita Taveta County (largely comprised of agricultural areas and ranches); Rukinga W.S. provides refuge for wildlife outside the national parks.

Materials and methods

Study area

The Tsavo ecosystem covers approximately 42 000 km^2 in south-eastern Kenya (Lat: 2°57′59.99″S, Long: 38°27′59.99E) (Fig. 1). Tsavo East and Tsavo West National Parks occupy an area of 21 000 km^2 (Smith and Kasiki 2000) and are separated by the Taita Taveta County (17 084 km^2), which acts as a vital corridor and dispersal area for wildlife travelling between the two national parks (Smith and Kasiki 2000; McKnight 2004). In 2015 the County's human population was estimated at ~329 000, with the most common livelihood being small-scale farming (MoALF 2016). As of the most recent aerial census count, Tsavo is also home to ~12 866 elephants (Ngene *et al.* 2017), the largest population of elephants in Kenya.

Taita Taveta largely comprises agricultural areas, as well as human settlements, private ranches and conservancies. This includes the Rukinga Wildlife Sanctuary (323 km^2), part of the Kasigau REDD+ Wildlife Corridor running centrally through Taita Taveta (Freund and Bird 2013). Rukinga was run as a commercial cattle ranch up until 1997, when it was converted to a wildlife sanctuary by Wildlife Works Inc. The area was barren of wildlife; cattle had grazed the land to dust, there was heavy poaching, and charcoal burning was widespread (Wildlife Works 2021). Wildlife Works now protects Rukinga with their own rangers, who work alongside the Kenya Wildlife Service (KWS). With the environment restored and able to provide wildlife with a source of quality habitat (including forage for herbivores), the sanctuary is currently home to over 300 bird species, 20 bat species and 50 large mammal species, including elephants (Freund and Bird 2013). Human–elephant conflict occurs throughout Taita Taveta (Smith and Kasiki 2000; King *et al.* 2017; Troup *et al.* 2020), including in the agricultural communities in close proximity to Rukinga (Kagwa 2011; Von Hagen 2018). The sanctuary primarily consists of wooded bushland, with small pockets of grassland and shrubland (Mutiti *et al.* 2017). Several community owned ranches/sanctuaries provide a permanent water supply for wildlife during the dry season, including Rukinga W.S.

Tsavo National Park consists of semiarid savannah, with the primary vegetation type being remnants of the formerly extensive *Acacia commiphora* woodlands, which have been heavily modified by elephants (Cobb 1976). The parks are now dominated by dry grassland and shrubland, with some areas of wet grassland, forest and bushland. A short, heavy wet season occurs from November to December, followed by the long, weaker rains from March to May (Omondi *et al.* 2008). Spatial and temporal patterns of rainfall are unpredictable, ranging from 250 to 700 mm with an average of 550 mm annually (van Wijngaarden 1985; Ngene *et al.* 2014). There are two permanent rivers in the Tsavo ecosystem (the Galana and Tsavo Rivers, both of which are located in the Tsavo National Parks), in addition to several seasonal rivers (including the Tiva and Voi rivers) (van Wijngaarden 1985).

Elephant faecal sample collection

We collected fresh elephant faecal samples in Tsavo East N.P. from 1 April to 29 May (long wet season) and 2 August to 30 September (long dry season) 2018. Additional fresh faecal samples were collected from 1 August to 29 September 2018 in Rukinga W.S., because the sanctuary experiences a marked increase in elephants during the long dry season. Elephant faecal samples were not collected during the wet season in Rukinga because of the lower elephant numbers, making it very difficult to locate fresh (within a few hours of defecation) samples. We initially identified seasons based on the general classification of seasons for Tsavo (Tyrell and Coe 1974; Leuthold and Leuthold 1978), and then slightly adjusted our classifications based on daily rainfall records. Daily rainfall records collected by the Tsavo East Research Centre staff (captured from a rain gauge located at the Research Centre, inside the Tsavo East N.P. Voi Entrance) were averaged with those collected by Save the Elephants' Elephants and Bees Project (captured from a rain gauge at the Projects Research Centre, located in the small-scale farming community of Sagalla, adjacent to Tsavo East N.P. and approximately 20 km north-west of Rukinga W.S.). Wet and dry seasons were then defined following the methods of Rasmussen *et al.* (2006), based on the amount of precipitation required to bring about a vegetative response. In total, 98 elephant faecal samples were collected: 41 during the long wet season in Tsavo East N.P., 30 during the long dry season in Tsavo East N.P., and 27 during the long dry season in Rukinga W.S.

In Tsavo East N.P., dung piles were located by following two main roads (Mudanda and Aruba transects) that had been identified by local rangers or research assistants as regularly utilised by large numbers of elephants (see Fig. 1). We visited Tsavo East N.P. every 3–4 days, alternating our search of the two transects each sampling day. In Rukinga W.S., dung piles were located opportunistically, often near dams where elephants were known to frequent. We visited Rukinga every 4 days, searching several areas known to be commonly utilised by elephants on a rotational basis. All faecal samples were collected from unknown individuals, and it was rare that we had the opportunity to see the elephant whose dung we collected. It was therefore not possible to identify the exact age of the elephant whose dung we sampled. Dung <12 cm in boli diameter was not collected to avoid sampling of young elephants, which may vary in their stress response compared with older elephants (see Woolley *et al.* (2009)). Fresh faecal samples were selected in order to avoid those where hormones had already started to degrade (Wong *et al.* 2016). The majority of samples were estimated to be collected <3 h after defecation, as indicated by their moist interior, strong odour, and unhardened outer mucus layer (hardening of the mucus layer occurs quickly from exposure to the sun, causing it to dry out). Due to our relatively small sample size (an average of 33 across each location and season), in addition to the large population of elephants in Tsavo, we considered each faecal sample to be independent. Over 6000 elephants are estimated to be distributed in the southern area of Tsavo East N.P. (Ngene *et al.* 2017) (where our transects identified by research staff as heavily utilised by elephants were centrally located), thus the probability of sampling the same elephant more than once was very small. Similarly, the Kasigau Wildlife Corridor (which includes Rukinga W.S.) is utilised by ~2000 individuals (Wildlife Works 2021), and re-sightings of the same individuals in Rukinga W.S. have been reported to be relatively low (McKnight 2004). Further, due to our sampling design, a maximum of two faecal samples were collected every 6–8 days along the same transect/in the same area of both locations, ensuring that samples from the same area were not collected closely in time.

We obtained two samples from the inner core of each dung pile. Because hormones do not distribute evenly, we collected small sections of dung from several areas of several boli from each separate dung pile; our final samples were a mixture of these small sections. The first sample was collected for hormone analysis, specifically glucocorticoid metabolites (fGCMs), in addition to progestogen and testosterone metabolites. Faecal progestogen and testosterone were analysed in order to use as controls for reproductive status, due to the sex of elephants being unknown. Glucocorticoids have previously been demonstrated to correlate with reproductive state in both sexes of African and Asian elephants, increasing during pregnancy (Foley *et al.* 2001; Kajaysry and Nokkaew 2014) and the oestrous cycle in females (Fanson *et al.* 2014; Glaeser *et al.* 2020; Edwards and Brown unpubl. data), as well as during musth in bulls (Brown *et al.* 2007; Chave *et al.* 2019). Therefore, quantifying these hormone metabolites in addition to fGCM enabled us to control for any potential differences in fGCM that may have been related to reproductive status.

The second faecal sample (~10–20 g DM) was used for an analysis of total N of faeces (Nf), a proxy for diet quality in herbivores (Leslie *et al.* 2008). We used Nf as an indicator of habitat quality between seasons (wet vs dry), because nutritional stress was considered a likely reason for potential seasonal differences in fGCM levels, as has been shown in several previous studies (Rangel-Negrín *et al.* 2009; Davies *et al.* 2014; Lèche *et al.* 2014). If locational variation (between Tsavo East N.P. and Rukinga W.S.) in fGCMs was found, we considered this likely to be due to differences in proximity to human disturbance. However, following years of degradation before being taken over by Wildlife Works, we also considered it pertinent to assess Rukinga's habitat quality. Samples collected for Nf analysis were thoroughly mixed, spread to 1-cm thickness across a piece of A4 paper, and then air-dried out of direct sunlight before being transferred into paper bags.

Due to the absence of sample freezing facilities at our remote field site, we extracted and stored 1.00-g faecal samples for hormone analysis following the protocol described by Edwards *et al.* (2014), with some small modifications. Firstly, we used Whatman® syringe filters to separate the extract from the faecal material, followed by an additional 1 mL 90% MeOH to rinse the filter. We then added 6.75 mL dH_2O to the faecal extract, to achieve the required 40% MeOH concentration for loading into the C8 SPE cartridges. Samples were stored upright in an airtight container at ambient temperature for up to 6 months, then exported to the Smithsonian Conservation Biology Institute (SCBI) in the U.S.A. At the SCBI, hormone metabolites were eluted from the SPE cartridges using 5 mL 100% MeOH, evaporated to dryness, and re-suspended in 1-mL phosphate buffer (39 mM NaH_2PO_4, 61 mM Na_2HPO_4, 0.15 mM NaCl; pH 7.0). Extracts were stored frozen at −20°C until hormone analysis, described below.

Endocrine analyses

fGCMs were measured using a double antibody enzyme immunoassay (EIA) incorporating a secondary goat-anti rabbit IgG antibody and polyclonal rabbit anti-corticosterone antibody (CJM006, C. Munro) adapted from Watson *et al.* (2013) and previously described by Edwards *et al.* (2019). The immunoassay was validated biochemically for measuring GCs in faecal extracts through parallelism ($y = 1.214x + 1.921$, $R^2 = 0.983$, $F_{1,7} = 406.405$, $P < 0.001$) and matrix interference assessment ($y = 1.263x - 36.805$, $R^2 = 0.972$, $F_{1,4} = 140.149$, $P < 0.001$). This assay has previously been biologically validated for measuring adrenocortical activity in African elephants following an adrenocorticotrophic hormone (ACTH) challenge (Santymire *et al.* 2012).

Faecal progestogen metabolites were measured using a double antibody EIA incorporating a secondary goat-anti mouse IgG antibody and monoclonal mouse anti-progesterone antibody (CL425, C. Munro) adapted from Munro and Stabenfeldt (1984) and previously described by Glaeser *et al.* (2020). The immunoassay was validated biochemically for measuring progestogens in faecal extracts through parallelism ($y = 1.175x - 15.039$, $R^2 = 0.946$, $F_{1,6} = 105.474$, $P < 0.001$) and matrix interference assessment ($y = 1.071x - 6.067$, $R^2 = 0.990$, $F_{1,4} = 402.391$, $P < 0.001$). This assay has previously been biologically validated through demonstration of increased progestagen metabolite concentrations during the oestrous cycle and pregnancy in female African elephants (Graham *et al.* 2001; Freeman *et al.* 2011).

Faecal androgen metabolites were measured using a double antibody EIA incorporating a secondary goat-anti rabbit IgG antibody and polyclonal rabbit anti-testosterone antibody (R156/7, C. Munro) adapted from Munro and Stabenfeldt (1984). The cross-reactivities of the R156/7 antibody have been reported elsewhere (DeCatanzaro *et al.* 2003). This EIA follows the protocol for fGCMs as described in Edwards *et al.* (2019) with the following exceptions: testosterone standards ranged from 0.047 to 12 ng mL^{-1}; working dilutions of testosterone-HRP (25 μL; 1:50 000; C. Munro) and primary anti-testosterone antibody (25 μL; R156/7 1:50 000); and incubation of chromogenic substrate for 10 min. The immunoassay was validated biochemically for measuring androgens in faecal extracts through parallelism ($y = 1.576x - 9.275$, $R^2 = 0.998$, $F_{1,5} = 2797.389$, $P < 0.001$) and matrix interference assessment ($y = 1.160x - 13.609$, $R^2 = 0.992$, $F_{1,4} = 488.900$, $P < 0.001$). Faecal extracts were diluted 1:10, 1:50–1:300, and 1:20–1:100 for analysis of glucocorticoid, progestagen and androgen metabolites, respectively. Inter and intra-assay coefficients of variation (CVs) for all EIAs were maintained below 15% and 10%, respectively.

Assessment of habitat quality: total N of elephant faeces

Near infrared spectroscopy (NIRS)

Near Infrared Spectroscopy (NIRS) was used to estimate the total N concentration of elephant faeces (Nf), which involves irradiating samples with near infrared light in order to obtain their near infrared reflectance spectra. The relationship between these spectra and the spectra of a subset of the samples that have been measured for nutritional traits can be used to predict the same traits in additional unknown samples (Foley *et al.* 1998; Rothman *et al.* 2009).

Collection of NIR spectra

All 98 dried elephant faecal samples were transported to Crop Nutrition Laboratory Services Ltd. in Nairobi, where they were ground to pass a 1.0-mm sieve (Foss Tecator Cyclotec centrifugal mill). The spectra of each sample between 800 nm and 2500 nm in duplicate was taken with a Bruker MPA Fourier Transform near-infrared reflectance (NIR) spectrometer. All spectral data were converted from wavenumbers to wavelengths in MATLAB R2016b using spline interpolation. Following methods described in Au *et al.* (2020), spectra were then cropped from 1102 nm to 2498 nm with 2-nm intervals, and the data were subjected to standard statistical pre-treatments to reduce baseline variation and/

or noise; specifically, taking a first-derivative with a gap window of 7, second order polynomial and standard normal variate. The average spectra of each duplicate pair was then calculated in R version 3.5.1 (R Core Team 2019).

Calibration, model selection and validation, and chemical analysis

Nf values predicted for the 98 elephant faecal samples collected in this study represented a subsample of the Nf values predicted for a larger nutritional study of elephant diet quality in Tsavo, carried out between April 2017 and September 2018 (G. Troup, unpubl. data). Nf concentrations in the larger study were predicted from statistical models, using a subset of samples that best represented the spectral variation within the dataset (i.e. calibration samples). We identified these calibration samples using a Kennard Stone algorithm with Mahalanobis distance metric, applied from the R package *prospectr* (Stevens and Ramirez-Lopez 2013). Sixty-four out of the entire dataset of 464 samples were analysed for Nf using the Dumas method (Etheridge *et al.* 1998; Marco *et al.* 2002) in a Leco TruSpec CHN elemental analyser (Leco St. Joseph, Michigan).

Partial least squares (PLS) regression was used to model the relationship between NIR spectra and nutritional traits in MATLAB 2016b. Due to laboratory constraints, there were a limited number of samples chemically analysed; therefore, we allocated all of these samples to calibration. Recent research has suggested that introducing nested structure of a dataset during cross-validation can provide information about the predictive performance of a model (Au *et al.* 2020). We developed 30 PLS models, with the number of latent factors ranging from 1 to 30, and identified a satisfactory number of latent factors to build a single prediction model using 'leave-season/year-out' cross-validation. This cross-validation technique involves temporarily removing samples from a given year and season, developing a PLS model using the remaining samples, and then testing the model with the excluded samples. These steps were repeated until all samples were tested, and their fitted values were compared to their reference chemistry value. We selected the optimal PLS model by balancing the lowest root mean squared error of cross-validation (RMSECV) and coefficient of determination (R^2) with the fewest number of latent factors. Our optimal PLS model was developed with six latent factors; the root mean squared error of cross validation (RMSECV) was 0.32% Nf, and the coefficient of determination (R^2) was 0.58. This final PLS model was used to predict Nf of all samples in the dataset, and the predicted data were used for statistical analysis.

Assessment of habitat quality – NDVI

The Normalised Difference Vegetation Index (NDVI), a remotely sensed indicator of vegetation productivity (Pettorelli *et al.* 2005), was employed as an additional indicator of habitat quality between seasons (wet vs dry) and locations (Tsavo East N.P. vs Rukinga W.S.). Ninety-eight individual 250-m 16-day composite MODIS NDVI values corresponding to each faecal sample (based on date and GPS location) were extracted using NASA's EarthData AppEEARS software (AppEEARS 2019) and data (Didan 2015*a*, 2015*b*). Aqua (MYD) and Terra (MOD) values were averaged for final calculations of the individual NDVI values corresponding to each of the 98 faecal samples.

Statistical analysis

Generalised Linear Mixed Models (GLMMs) were created using the *lme4* package (Bates *et al.* 2015) in R version 3.5.1 (R Core Team 2019). All models were separately tested for spatial autocorrelation using the Moran's *I* test from the *ape* package (Paradis and Schliep 2019), which is reported only where significant. We created a GLMM using fGCM concentration as a (continuous) response variable in order to assess differences in elephant fGCM concentration between seasons (wet, dry) and locations (Tsavo East N.P., Rukinga W.S.). We applied a $\log_{10}$ transformation to this data in order to meet statistical normality requirements (Munshi-South *et al.* 2008; Tingvold *et al.* 2013; Hunninck *et al.* 2018). Given that the sex of elephants was unknown, faecal progestogen and androgen metabolite concentrations were included as covariates to control for reproductive status. Area (transect or more specific location) was used as a single random effect.

In order to assess habitat quality between seasons and locations *a posteriori*, we created two separate GLMMs; one using Nf as a (continuous) response variable, and one using NDVI as a (continuous) response variable. Both models included season (wet, dry) and location (Tsavo East N.P., Rukinga W.S.) as fixed effects, and area as a single random effect. We applied a square root transformation to our Nf data in order to meet statistical normality requirements.

Results

Comparison of habitat quality between seasons (wet vs dry) and locations (Tsavo East N.P. vs Rukinga W.S.)

Nf in samples collected during the wet season in Tsavo East N.P. was significantly higher than for those collected in the dry season ($t = 16.13$, $P \leq 0.01$, Fig. 2*a*). There was no significant difference in Nf collected from elephants in Tsavo East N.P. and Rukinga W.S. during the dry season ($t = -0.19$, $P = 0.87$, Fig. 2*a*). NDVI values in Tsavo East N.P. were significantly higher during the wet season than dry season ($t = 16.45$, $P \leq 0.01$, Fig. 2*b*), and there was no significant difference in NDVI between Tsavo East N.P. and Rukinga W.S. ($t = -0.44$, $P = 0.69$, Fig. 2*b*). There was significant spatial autocorrelation in this model (Moran's *I* test observed = 0.14, s.d. = 0.06, $P = 0.02$), but this was not considered to affect our analysis heavily because we were interested in analysing broad differences in NDVI between seasons and locations. A summary of statistics for habitat quality across seasons and locations is provided in Table 1.

Comparison of elephant fGCMs between seasons (wet vs dry) and locations (Tsavo East N.P. vs Rukinga W.S.)

The fGCM concentrations of samples collected during the dry season in Tsavo East N.P. were higher than those collected during the wet season, although the difference was marginally non-significant ($t = -1.86$, $P = 0.06$, Fig. 3). There was no significant difference in the fGCM concentration of samples collected in Tsavo East N.P. and Rukinga W.S. during the dry season ($t = -0.37$, $P = 0.72$, Fig. 3). There was a significant effect of faecal androgen ($t = 5.86$, $P < 0.01$) and faecal progestogen ($t = 3.31$, $P < 0.01$) on fGCM concentrations (Table 2). A summary of statistics for fGCM concentrations across seasons and locations is provided in Table 2. A summary of untransformed fGCM, androgen and progestogen concentrations across seasons and locations is provided in Table 3.

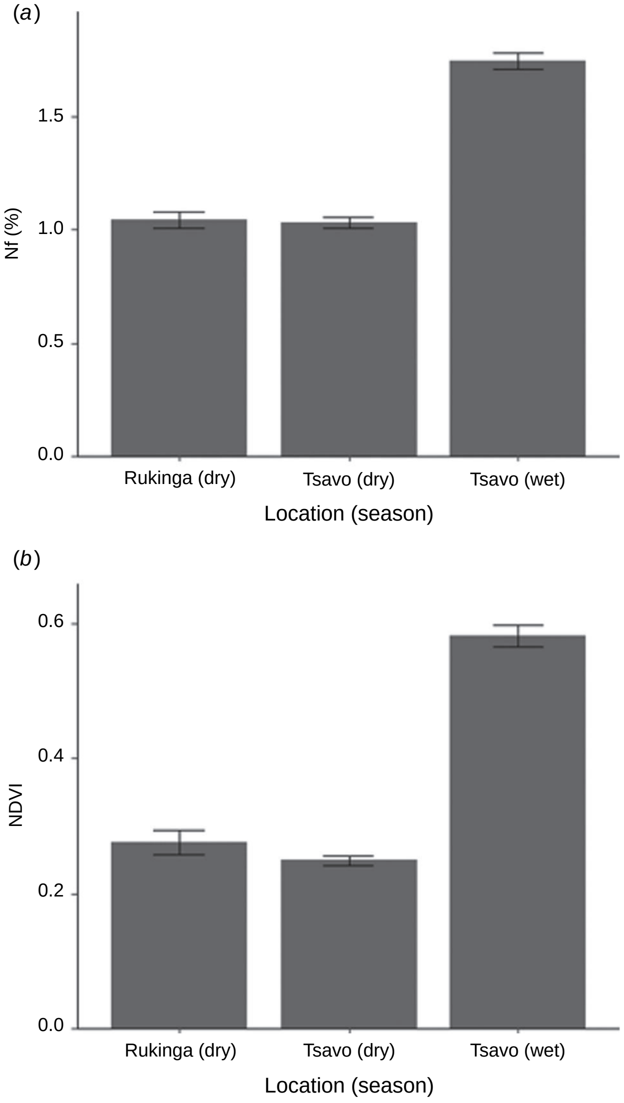

Fig. 2. Means ± s.e. of untransformed habitat quality data for Tsavo East N.P. during the wet ($N = 41$) and dry ($N = 30$) seasons, and Rukinga W.S. during the dry season ($N = 27$). (*a*) Nf, and (*b*) NDVI.

Discussion

This study assessed seasonal variation in the faecal glucocorticoid metabolite concentrations of African elephants

Table 1. Effect of season and location on habitat quality (Nf and NDVI) based on GLMMs.

sqrt(Nf) ~ season × location + (1|area)					
Fixed	**Estimate**	**s.e.**	**d.f.**	***t*-value**	***P*-value**
Intercept – Rukinga (dry)	1.02	0.02	2.31	46.20	<0.01
Season – wet	0.31	0.02	82.52	16.13	<0.01
Location – Tsavo	0.00	0.04	1.33	−0.19	0.87
NDVI ~ season × location + (1|area)					
Fixed	**Estimate**	**s.e.**	**d.f.**	***t*-value**	***P*-value**
Intercept – Rukinga (dry)	0.27	0.02	6.32	10.81	<0.01
Season – wet	0.33	0.02	90.20	16.45	<0.01
Location – Tsavo	−0.02	0.04	3.81	−0.44	0.69

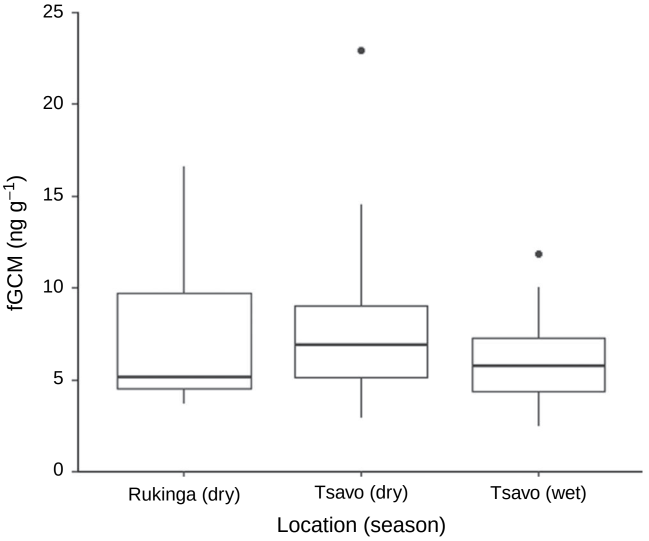

Fig. 3. Boxplots of untransformed fGCM data for elephants from Tsavo East N.P. during the wet ($N = 41$) and dry ($N = 30$) seasons, and Rukinga W.S. during the dry season ($N = 27$). The lower and upper limits of the boxplots represent the 25th and 75th percentiles, the centre line is the median value, the ends of the vertical lines indicate the minimum and maximum values, and the circles outside represent potential outliers.

Table 2. Effect of season and location on fGCM concentrations based on a GLMM.

$\text{Log}_{10}(\text{fGCM}) \sim \text{season} \times \text{location} + \text{fAnd} + \text{fProg} + (1|\text{area})$

Fixed	Estimate	s.e.	d.f.	t-value	*P*-value
Intercept – Rukinga (dry)	0.74	0.06	6.00	12.43	<0.01
Season – wet	−0.06	0.03	86.84	−1.86	0.06
Location – Tsavo	−0.04	0.10	3.86	−0.37	0.72
fProg	0.00	0.00	88.90	3.31	<0.01
fAnd	0.00	0.00	88.24	5.86	<0.01

Table 3. Means ± s.e. of untransformed fGCM, androgen and progestogen concentrations in Tsavo East N.P. during the dry (*N* = 30) and wet (*N* = 41) seasons, and in Rukinga W.S. during the dry season (*N* = 27).

Location – season	fGCM (ng g^{-1})	Androgen (ng g^{-1})	Progestogen (ng g^{-1})
Tsavo East N.P. – wet season	6.10 ± 0.35	12.60 ± 3.28	40.58 ± 7.29
Tsavo East N.P. – dry season	7.54 ± 0.74	11.01 ± 1.59	52.58 ± 11.26
Rukinga W.S. – dry season	7.52 ± 0.79	12.16 ± 2.65	18.54 ± 3.23

living in Kenya's drought-prone, anthropogenic landscape of Tsavo, and whether any observed differences may correspond to changes in habitat quality. We found limited support for our first hypothesis (H1), because there was a marginally non-significant difference in elephant fGCM concentrations between dry and wet seasons in Tsavo East N.P., although habitat quality (as indicated by Nf and NDVI) was significantly poorer during the dry season. The data did not support our second hypothesis (H2), because elephants sampled within privately protected Rukinga W.S. during the dry season exhibited no significant difference in fGCMs when compared with those from Tsavo East N.P. However, there was also no difference in habitat quality (as indicated by Nf and NDVI) between the two locations. In contrast to most other studies exploring the stress response of elephants, by analysing both temporal and spatial variation in fGCMs we provide a more holistic picture of the physiological response of these mega-herbivores as they search for resources. Here we discuss how elephants living in Tsavo physiologically respond to decreasing habitat quality in drier periods, and in what way land outside of formally protected areas can benefit elephants during these seasons.

Our assessment of Nf and NDVI in Tsavo East N.P. indicates that habitat quality during the dry season is inferior to the wet season. Specifically, we found that elephant Nf is significantly lower in the dry season, consistent with several other studies assessing seasonal variation in African elephant diet quality (Codron *et al.* 2006; Woolley *et al.* 2009; Turner *et al.* 2013). We also observed NDVI to be significantly lower during the dry season than the wet season. NDVI is widely used to describe patterns in the habitat selection, seasonal movements and distribution of elephants throughout Africa (Selier *et al.* 2015; Gara *et al.* 2016; Purdon *et al.* 2018). Poor nutrition can impact the health of wildlife in a number of ways, including decreasing immunity, and ultimately limiting the chance of survival (reviewed by Acevedo-Whitehouse and Duffus (2009)).

Although differences in fGCM concentrations between wet and dry seasons did not quite reach significance, we did find a tendency for higher fGCM concentrations during the dry season in Tsavo East N.P., when habitat quality was poorer. Several studies have reported elephants to exhibit higher fGCM concentrations during the dry season (Foley *et al.* 2001; Viljoen *et al.* 2008), but this difference is not always apparent (Woolley *et al.* 2009). Inconsistencies between study populations can be influenced by factors such as age and sex, or attributed to the level of habitat quality change between seasons (Woolley *et al.* 2009). Our results support those of Woolley *et al.* (2009), who reported an overall 20% decrease in African elephant diet quality (Nf) from wet to dry seasons in South Africa's Pilanesberg N.P., but no seasonal difference in the stress hormone concentration of adults. Habitat quality decreased more between dry and wet seasons in our study (~42% reduction in Nf and ~% 57 reduction in NDVI) in comparison with Woolley *et al.* (2009), which may explain why seasonal variation in our fGCM concentrations were closer to significance.

The dry season conditions that presented during our study period were typical of bi-annual dry season conditions in the ecosystem, thus our results may suggest that elephants living in Tsavo are physiologically unaffected by typical seasonal changes in habitat quality. Alternatively, they may be physiologically affected, yet are able to adapt to such changes. We state these possibilities cautiously due to our single year of data collection, and note that both of these possibilities rely on the assumption that the dry season conditions observed during our study period were adequately limited to potentially cause elephants' substantial nutritional stress. Research by Rasmussen *et al.* (2006) implies that following the peak in NDVI in a given wet season, sufficient forage should be available for elephants up to 3 months into the proceeding dry season. After finding the highest mortality rate of elephants in Tsavo to occur at low NDVI values and around permanent water, Wato *et al.* (2016) therefore strongly suggested that this elephant population would starve to death when dry season conditions extend beyond 3 months, as a result of depleting all available forage resources near permanent water. While our study focused on the quality (not quantity) of habitat available to elephants, our dry season sampling took place more than 3 months into the dry season. Coupled with the suggestion made by Wato *et al.* (2016), our results regarding Nf and NDVI infer that the dry season conditions observed during our study period would have been sufficiently limited to potentially cause elephants to experience considerable nutritional stress.

Africa is predicted to experience more severe drought periods in the future (Masih *et al.* 2014; Gan *et al.* 2016), and understanding how elephants will respond to these conditions is essential for their long-term survival. Tsavo has historically experienced low and unpredictable rainfall (Ottichilo 1987), and much of the ecosystem falls under a 'drought-prone' zone (Corfield 1973; Leuthold and Sale 1973). Given that elephants tended to exhibit higher (although not significantly) fGCMs during the typical dry season sampled, it is possible that extreme drought conditions could cause elephants to experience chronic stress. Future research across multiple years is required to determine how these mega-herbivores may physiologically respond to increasingly severe drought periods caused by climate change, noting that age and sex classes could be affected differently. This should not only involve the inclusion of a time period when conditions are representative of extreme (consistent, multi-year) drought, but should also include multiple wet seasons for comparison.

African elephant movement is largely driven by the need for water and forage (Birkett *et al.* 2012; Wall *et al.* 2013; Tshipa *et al.* 2017). This can motivate elephants to leave the protection of national parks in search of alternative resources (Osborn 2003; Cook *et al.* 2015; Branco *et al.* 2019), particularly during dry periods. In Tsavo, Rukinga W.S. experiences an influx of elephants during the dry season. While the sanctuary is privately protected, it is unfenced and surrounded by human disturbances that could potentially cause elephants to exhibit an elevated stress response. In order to be beneficial for their use, Rukinga must provide elephants with a safe refuge when navigating through farming areas and human settlements. Elephants that move into the area from Tsavo East N.P. are required to cross the Nairobi–Mombasa Highway, which is heavy in commercial truck traffic. Previous research has shown that elephants may increase their speed when crossing unprotected roads (Blake *et al.* 2008), suggesting this to be a potentially stressful experience.

Elephants residing in Rukinga or travelling to the area from the N.P. may have also actively chosen to engage in crop-raiding nearby farming communities (such as neighbouring Sisenyi (Von Hagen 2018) and others in Kasigua (Kagwa 2011)), before returning to the sanctuary for water and safe refuge. In support of this theory, elephant dung piles containing crops have been found in Rukinga (G. Troup, unpubl. data). Crop-raiding presents a significant risk to the survival of elephants through injury from farmers (Obanda *et al.* 2008; Mijele *et al.* 2013), and may be related to increased stress in elephants (Ahlering *et al.* 2011). Our finding that there is no difference in the fGCM concentration of elephants from Rukinga W.S. and Tsavo East N.P. suggests that these potential disturbances resulting from being in closer proximity to humans when moving in and around Rukinga are not sufficient to alter fGCMs on a chronic level.

Although previously mismanaged and grazed to dust, our results additionally show that improved management by Wildlife Works has resulted in Rukinga exhibiting comparable habitat quality (as indicated by Nf and NDVI) to that of Tsavo East N.P. This is also important for ensuring Rukinga's benefit for use by elephants, and combined with our finding of no locational difference in fGCMs, provides support for the suitability of Rukinga W.S. in offering a safe refuge of sufficient habitat quality for elephants when moving outside the N.P. Our results are consistent with the conclusion made by Williams *et al.* (2018) that the Kasigau Wildlife Corridor (which includes Rukinga) is a key corridor and habitat for elephants in Tsavo; however, we are cautious in our support and highlight the limitation of only including one (dry) season in our sampling. As the surrounding land continues to be cleared and converted to agriculture (Gathongo 2012; Pearlman 2014), safe refuges outside the national parks will become increasingly important for the conservation of wildlife in the Tsavo ecosystem. This is especially true for wide-ranging species such as elephants, particularly during dry periods when they may be more likely to move closer to areas of human disturbance in search of alternative resources such as water and forage.

As previously mentioned, the results of this study would be greatly strengthened by future research across multiple years (including multiple wet and dry seasons), which would limit any element of speculation regarding the possible explanations for our seasonal fGCM results in Tsavo East N.P., and potential conservation benefit of Rukinga W.S. This would also be beneficial for investigation into how elephants living in Tsavo may physiologically respond to increasingly severe drought periods caused by climate change. Although they were only included in our study to control for potential correlations between hormones, we observed a significant effect of androgen and progestogen on fGCM concentrations. Further detailed demographic information on the age and sex composition of the Tsavo elephant population would be necessary to discuss these results without conjecture, and may prove interesting to explore in the future. In addition, future studies could assess potential differences in the stress response of elephants found in neighbouring agricultural communities compared with those from protected areas such as the Tsavo National Parks and/or Rukinga W.S., providing insight into how they may adapt to expanding 'high conflict' zones in this anthropogenic landscape.

Conclusion

With semiarid ecosystems such as Tsavo often experiencing low and unpredictable rainfall, it is important to understand how keystone species, such as the African elephant, will respond to increasing climate pressures. We found habitat quality (as indicated by NDVI and Nf) to be significantly poorer during the dry season, which corresponded with only marginally higher fGCM concentrations. These findings indicate that elephants living in Tsavo may be physiologically unaffected by (or adapt to) typical seasonal changes in habitat quality that could lead to nutritional stress, but further investigation is required to determine how elephants would respond to more extreme drought conditions driven by climate change. We also observed no difference in fGCM concentrations between elephants from Tsavo East N.P. and privately protected Rukinga W.S. (part of the Kasigau REDD + corridor connecting Tsavo East and Tsavo West N.P.), as well as comparable habitat quality between the two locations. This suggests that Rukinga plays an important role in conservation efforts by providing elephants with a safe refuge of sufficient forage quality when travelling outside the Tsavo National Parks.

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Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

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