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Thesis for the degree of Philosophiae Doctor

Trondheim, May 2020

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How to write an acknowledgment worthy of the those who have stood by me, indulged in my questionable sense of humour, and endured me during my numerous ups-and-downs of this PhD. There really isn't any text, any poetic phrasing, that I could come up with, that would do justice to the immense gratitude I feel for my people. But here's my attempt anyway.

First and foremost, I would like to thank the Department of Biology, NTNU, for giving me the opportunity of a lifetime to work as an academic scholar, allowing me to travel the world, and finish this PhD with at least some sanity to spare. Of course, my PhD position would never have existed in the first place without my supervisor, Prof. Eivin Røskaft and his AfricanBioServices project. I'm thankful for his support throughout the PhD, for letting me roam free, and for not escalating my often irrational lines of thought. Thank you Eivin for letting me see and live the Serengeti experience; truly a debt I cannot hope to ever repay. Thanks to my tireless co-supervisors, Dr. Craig Ryan Jackson and Dr. Roel May, who, apart from shaping my scientific papers, also shaped my scientific thinking. Thank you for the guidance and experience!

Separate paragraphs for people on different continents. Michael, the first time we communicated was as messy as it was desperate from my side. Thank you so much for the exposure, ideas, beers, comments, and more. But mostly, thank you for making me see this PhD through to the end, and for making me believe in science. Here's to many more collaborations in the future!

Poa kichizi kama ndizi! I made it! A huge thanks to my Tanzania colleagues, without whom I would have barely seen any 'samples' happening. Dr. Robert, Onesmo, Juma, Noel, Ali, and many more, this PhD would not have been possible without your extensive and tireless help both in the field and with the administration. Ahsante sana! I hope to soon be back in that most wonderful country of yours. Immense thanks, of course, to TAWIRI and TANAPA for allowing me to conduct my research. Tanzania for life!

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Summary

Anthropogenic activities are causing biodiversity loss at an unprecedented rate and attempts to ameliorate our impacts have largely failed. This thesis aimed to contribute to our understanding of how anthropogenic activities affect wildlife populations, using physiological and behavioural indices as a window into the status of a wild ungulate population. We used various remotely-sensed proxies of both direct human disturbance (i.e. land use area, settlement density, and distance from a protected area) and environmental conditions (i.e. forage quality, temperature, and rainfall) to test how these disturbances affected the physiological state (i.e. glucocorticoid and thyroid hormone concentrations), behaviour, and demography of impala populations in the Serengeti ecosystem, Tanzania. We found that impala showed significant variation in hormone concentrations in response to both direct human disturbances and changes in environmental conditions. However, hormone concentrations were much more strongly affected by variation in the environment, such as forage quality and temperature, than by direct human disturbances. Impala populations in land use areas which allow more exploitation showed behavioural and demographical changes that could lower population persistence. These results suggest that, although human activities do have a significant impact, with climate change becoming increasingly pervasive, climate and land use change are likely to be the biggest long-term threats to impala populations in the Serengeti ecosystem. Therefore, we suggest that managers focus on conserving larger natural systems, well beyond the boundaries of few strictly protected areas. Additionally, this thesis shows the advantages of combining both behavioural, physiological, and remote-sensing methods to obtain a more complete picture of the threats facing wildlife populations, disentangling their responses to disturbances, and the fitness implications of those responses. However, one should always consider the physiological, ecological, and evolutionary context of the biological methods used to inform conservation research. It is my hope that future work will recognise this complexity and design studies and interpret results with this in mind.

Contents

List of Papers

I) **Hunninck L.,** May R., Jackson C. R., Palme, R., Røskaft, E., Sheriff, M.J., 2020. Consequences of climate-induced vegetation changes exceed those of human disturbance for wild impala in the Serengeti ecosystem. *Conservation Physiology*, *8*(1), 1–14

II) **Hunninck L.,** Jackson C. R., May R., Røskaft, E., Sheriff, M.J., Triiodothyronine (T3) levels fluctuate in response to thermoregulation rather than nutritional state in a wild tropical ungulate. *Submitted to Conservation Physiology*.

III) **Hunninck L.,** Palme, R., Sheriff, M.J. Glucocorticoids may act as a proactive facilitator rather than a reactive cost of territoriality in male impala. *In Review in General and Comparative Endocrinology*.

IV) Setsaas T., **Hunninck, L.**, Jackson, C. R., May, R., & Røskaft, E. (2018). The impacts of human disturbances on the behaviour and population structure of impala (*Aepyceros melampus*) in the Serengeti ecosystem, Tanzania. *Global Ecology and Conservation*, *16*, 1–10.

Declaration of Contributions

ER received funding through a project developed by ER, CJ, RM, and collaborators, which enabled this PhD. LH collected all faecal samples in the field during 2016-2018, with help from TAWIRI staff.

Paper I: Ideas were developed and planned by LH in collaboration with CJ, RM, and ER. The laboratory analyses were performed by RP. LH conducted the statistical analyses under supervision of RM and CJ. LH wrote the manuscript with input from all co-authors.

Paper II: Ideas were developed and planned by LH, CJ, and MJS. The laboratory analyses were performed by LH. LH conducted the statistical analyses. LH wrote the manuscript with input from all co-authors.

Paper III: Ideas were developed and planned by LH and MJS. The laboratory analyses were performed by RP. LH conducted the statistical analyses. LH wrote the manuscript with input from all co-authors.

Paper IV: Ideas were developed and planned by ER, RM, CJ, TS, and LH. Fieldwork was conducted by TS, LH, and field assistants. TS conducted the statistical analyses under supervision of RM and LH. TS wrote the manuscript with input from all co-authors.

Introduction

A world in turmoil

Biodiversity and population densities are declining at an unprecedented rate, human activities are the cause, and attempts to ameliorate our impacts have largely failed (Pimm *et al.*, 2014; Ceballos *et al.*, 2017). With the sixth mass extinction being well underway, our world is indeed in turmoil (Barnosky *et al.*, 2011). However, conservationists have employed numerous strategies to limit the decline of natural diversity, including but not limited to: gazetting of protected areas, performing diversity and population censuses, understanding animal behaviour, and unravelling physiological mechanisms driving individual fitness (Pullin, 2002; Caro, 2005; Madliger *et al.*, 2016).

At its core, this thesis aims to contribute to our understanding of how anthropogenic activities affect the natural world around us, and if and how potential disturbances need to be dealt with. To do so, we used physiological and behavioural indices as a window into a wild ungulate population status and studied the effect of human and environmental perturbations. This thesis is the record of how and why we studied the impacts of anthropogenic activities on the physiology and behaviour of the impala (*Aepyceros melampus*) in the Serengeti ecosystem, Tanzania.

Anthropogenic disturbances

To be able to understand population declines, conservationists must first understand the threats to those populations. Oftentimes, these threats are a direct or indirect consequence of anthropogenic activities. Coined the ‘Evil Quartet’ and later updated to the ‘Evil Sextet’, human-induced disturbances have been recognised to be ubiquitous, various, and pervasive (Brook *et al.*, 2008). Further, these disturbances are sometimes difficult to measure. For example, while short-term and intense land use impacts such as deforestation might be easier to quantify, long-term and gradual disturbances such as pollution and climate change have eluded scientists for decades (Lindenmayer *et al.*, 2010). Introduced here are those direct and indirect human disturbances that we predicted to affect impala populations in the Serengeti ecosystem. Direct disturbances were contributed to different land use practices, illegal activities, and traditional livestock herding, while indirect disturbances are mainly mediated by climate change, including changes in vegetation, temperature, and rainfall. Many other human activities, such as tourism and agriculture, are likely also perceived as disturbances to impala, but have not been studied in this thesis (Laurance *et al.*, 2014; Fitzgerald and Stronza, 2016).

Protected areas have long been a cornerstone of conservation efforts (Thirgood *et al.*, 2004; Lindsey *et al.*, 2018). These areas are endowed with certain protection measures, aimed to preserve and protect their cultural or natural value. Various levels of protection now exist, from complete exclusion of human presence to multiple-use-areas, where the extraction of natural resources is allowed but controlled (Caro and Davenport, 2016). However, the effectiveness of strictly protected areas has been challenged (Craigie *et al.*, 2010; Hoole and Berkes, 2010; Habel *et al.*, 2015). Indeed, the so-called fortress-and-fence approach, which resulted in the expulsion of local peoples, and with it their traditional and spiritual connection to their land, has in many places been replaced by community-based conservation schemes, with varying success (Kideghesho *et al.*, 2007; Popova, 2014). Multiple use areas too have their share of problems, with human-wildlife conflicts being of prime concern. Depredation of livestock, damage to property, competition with wildlife, and even human injuries are a sometimes inevitable outcome of living in close proximity with wildlife (Dickman *et al.*, 2014; Redpath *et al.*, 2015).

Moreover, many protected and multiple use areas and their wildlife populations suffer from illegal activities, such as poaching, illegal grazing, firewood collection, and other forms of natural resource extraction (Knapp, 2012; Lindsey *et al.*, 2013; Ogutu *et al.*, 2016). These activities often concentrate around the borders of protected areas, as the human population density there is often disproportionately high (Denninger Snyder *et al.*, 2019; Veldhuis *et al.*, 2019). This clustering is caused by both pushing and pulling effects: the previous expulsion of people from an area often meant these people moved to the nearest legal place to settle (i.e. outside of the protected area), and secondly, tourism activities create jobs and opportunities that attract people to these areas (Estes *et al.*, 2012). This phenomenon can potentially create a hard border between protected and unprotected land, aggravating the edge effect and deteriorating the value of the protected area towards its borders (Hansen and DeFries, 2007; Veldhuis *et al.*, 2019).

Especially in eastern Africa, traditional livestock herding has been shown to have far reaching effects on the landscape (Homewood *et al.*, 2001). Focused around settlements termed 'boma', which are semi-permanent enclosures where pastoralists keep their cattle overnight and from which they walk them to nutritious pastures during the day, this way of livestock herding can lead to high stocking densities of herbivores and lead to significant competition with wildlife populations (Prins, 2000). This induced competition may result in the displacement of wildlife populations (Young *et al.*, 2005), decreases in the available forage near bomas (Riginos *et al.*, 2012), and forced adjustments of wildlife behaviour, increasing the energetic cost of foraging (Odadi *et al.*, 2011). However, due to their ephemeral nature, bomas might also increase soil nutrients through overnight deposition of dung, potentially facilitating future plant growth (Riginos *et al.*, 2012).

As one of the most pervasive human-induced global disturbances, climate change is already affecting nearly every habitat on the planet (Scheffers *et al.*, 2016; Segan *et al.*, 2016). Many species are shifting their ranges in response to a changing environment, disrupting established species interactions (Diamond, 2018). Furthermore, some protected areas that might have been able to maintain their natural value, are now strongly affected, unable to remain refugia for biodiversity (Thuiller *et al.*, 2006).

Changing weather patterns result in changes in vegetation structure and states, resulting in a whole range of cascade effects (Midgley and Bond, 2015). Droughts and other inclement weather events are predicted to become more frequent and intense in eastern Africa (Boko *et al.*, 2007; Niang *et al.*, 2014). This will likely have significant effects on shifts in vegetation states, and thus food availability (Pettorelli *et al.*, 2005), potentially leading to reduced nutritional status in ungulates, which could result in significant population declines (Oliver *et al.*, 2013).

Though climate change has direct effects on forage availability in the landscape, land cover change due to human activities is also a major contributor to the reduction in natural habitat and forage quality. Furthermore, interactive effects of land cover change often facilitates the effects of climate change, inducing a synergistic effect on forage availability (Martin *et al.*, 2015).

Incremental changes in temperatures can manifest in substantial hindrances to natural populations, often resulting in range shifts, disrupting community structure (Pecl *et al.*, 2017). However, aggravated seasonal fluctuations or extreme, unpredictable weather events, such as floods and droughts, can also have significant effects on population resilience (Oliver *et al.*, 2013). These more acute environmental perturbations require physiological and behavioural adjustments to deal with these stressors (Shrestha *et al.*, 2014; Tomasi *et al.*, 2019). The physiological mechanisms behind thermoregulation can be energetically costly and can result in a significant selection pressure on individuals (Silva, 2006).

Climate change is likely to significantly alter regional temporal and spatial precipitation patterns in eastern Africa (Moore *et al.*, 2015). This is exacerbated through human activities resulting in extensive land use change, such as agriculture (Mendelsohn and Dinar, 2009). Furthermore, grass growth is mainly regulated by soil fertility and rainfall (Bartzke *et al.*, 2018). Given the strong seasonality that is characteristic to eastern Africa, grasses in these savanna ecosystems periodically dry and become less nutritious for herbivores (Hopcraft *et al.*, 2010). The seasonal fluctuations in rainfall in eastern Africa, and thus vegetation, are predicted to become more extreme with increasingly severe climate change (Dore, 2005; Sinclair *et al.*, 2007; Midgley and Bond, 2015), potentially resulting in prolonged drought (Dai, 2011; Kotir, 2011).

Animal responses to disturbances

The pervasiveness of many anthropogenic disturbances has elicited a plethora of physiological, behavioural, and demographical responses in disturbed animals (Frid and Dill, 2002; Madliger *et al.*, 2018). Understanding the potential effects of these disturbances on natural populations is essential if we are to efficiently and effectively halt their declines and prevent species extinctions.

Opening up an entirely new approach to studying the effects of anthropogenic disturbances on wildlife populations, the fairly new discipline of conservation physiology aims to develop and apply a physiological toolbox to conservation issues (Wikelski and Cooke, 2006; Cooke *et al.*, 2013). Endocrinological markers – hormones – have been a versatile and important part of that toolbox (Madliger *et al.*, 2018). Indeed, the use of such hormones in ecological studies has increased considerably over the past decades (Palme, 2019).

Glucocorticoids (GCs) in particular have been studied intensively due to their responsiveness to various physiological stressors, such as anthropogenic disturbances, environmental variation, and social interactions (Sheriff *et al.*, 2011). The primary physiological function of GCs is energy mobilization, which generates glucose from non-carbohydrate substrates such as proteins, lipids, and fatty acids – a process called gluconeogenesis (Exton, 1979). GCs influence many bodily functions, however, affecting growth, immune function, reproduction, and regulating anabolic and catabolic processes (Busch and Hayward, 2009). Their responsiveness to human disturbances was shown in a classic study on the effect of snowmobile activity on the GC response in wolves (*Canis lupus*) and elk (*Cervus elaphus*). This study found that glucocorticoid concentrations significantly increased in both species with increasing exposure to snowmobiles (Creel *et al.*, 2002). In general, GCs are higher in animals residing in human dominated areas (Viljoen *et al.*, 2008; Creel *et al.*, 2013; Hunninck *et al.*, 2017). GC levels were also found to be higher in relation to vehicle traffic in moose (Creel *et al.*, 2002) and impala (Lunde *et al.*, 2016). Taken together, GCs have been used extensively as a measure for physiological stress, especially in relation to human disturbances (Palme, 2019).

Thyroid hormones (THs), produced by the thyroid gland and regulated through the hypothalamic-pituitary-thyroidal (HPT) axis, also have far reaching effects in the body – reviewed by Behringer et al. (2018). Their main functions are the regulation of metabolic rate and thermogenesis, and these hormones directly impact important metabolically active tissues such as brown adipose tissue or brown fat (essential for thermoregulation) and skeletal muscles (López *et al.*, 2013). TH concentrations have been shown to drop considerably when animals are starving or hibernating (Azizi *et al.*, 1979), strongly reducing basal metabolic rate and therefore saving energy. Experimental studies of the role of THs have focused on nutritional deficits, showing that THs decrease dramatically when animals are food deprived (Schaebs *et al.*, 2016), and thus, conversely, high TH levels indicate a high food intake and thus better nutritional condition. THs have also been reported to affect male and female reproduction, pregnancy, growth, aging, and thermoregulation (Bowers *et al.*, 2013; Behringer *et al.*, 2018). Given this, THs are uniquely suited to study an animal's nutritional stress.

Behavioural responses can be pivotal in an animal's ability to cope with anthropogenic disturbances, including a rapidly changing climate (Frid and Dill, 2002; Buchholz *et al.*, 2019; but see Goldenberg *et al.*, 2017). These responses, though sometimes seemingly benign, can have considerable consequences and affect individual fitness (Lind and Cresswell, 2005; Laundre *et al.*, 2010).

Various changes in behaviour in response to human activities have been observed in animals, including changing activity patterns, adjusting foraging behaviour, and altering species interactions (Wronski *et al.*, 2015; Johnson *et al.*, 2017). For example, animals often tend to avoid interactions with humans by either changing their habitat use, or by changing to a more nocturnal activity pattern (Crosmary *et al.*, 2012; Szott *et al.*, 2019). Species that are forced to interact frequently with humans have been found to become significantly more wary of humans, spending more time and energy on behaviours enhancing their vigilance and showing longer flight distances (Stankowich and Blumstein, 2005; Ciuti *et al.*, 2012). Indeed, conservationists have used vigilance rates and flight behaviour as indicators of human disturbances for decades (Caro, 2005; Stankowich, 2008).

Other human activities, such as trophy hunting and poaching, have direct population consequences through induced changes in demography (Milner *et al.*, 2007). Both tourism-based and illegal hunting tend to have a male bias in their offtake due to differences in body traits and behaviour between sexes (Coltman *et al.*, 2003; Holmern *et al.*, 2006). This can have repercussions for population persistence (Ginsberg and Milner-Gulland, 1994). Human disturbances have also been shown to decrease recruitment rates, measured as number of juveniles per adult female, affecting population viability (Gaillard *et al.*, 1998).

Impala

Aepyceros melampus melampus
Lichtenstein 1812

Impala are a medium-sized antelope common in eastern and southern African savanna ecosystems (IUCN SSC Antelope Specialist Group, 2016). They are a gregarious and polygynous antelope (Jarman, 1974) and group sizes vary considerably, with female herds (i.e. juveniles, adult females, territorial male) ranging from a few to over 100 individuals, and bachelor groups (i.e. adult and subadult males) reaching up to 60 individuals (Owen-Smith, 1977). Impala are non-migratory herbivores with small home ranges typically between 5-10 km^2, increasing only slightly in the dry season (Averbeck, 2001). They are mixed feeders, i.e. though they prefer more nutritious and palatable grassy vegetation, they include more browse in their diet when the grasses seasonally dry (Jarman and Jarman, 1973; Wronski, 2002).

They are often found on the edge of open savanna as their preferred habitat is open woodland (Ford *et al.*, 2014). Their habitat requirements result in a clumped and irregular, but locally abundant distribution (Averbeck, 2001). Due to their ability to consume both grasses and browse, their local densities can reach up to 600 individuals/km^2 (Averbeck, 2001; Rosenblatt *et al.*, 2019). In the Serengeti ecosystem, located in northern Tanzania, they are found throughout the system, with exception for the short grass plains (**Figure 1**).

In East Africa, impala males are territorial year round (Oliver, 2005) and male-male aggression likely elicits a stress response (e.g. Corlatti, 2018). Territorial impala vary in their degree of sociality. Territorial males are either solitary or associated with a female herd. The majority of males in a population, however, do not hold a territory and are referred to as bachelors, which often aggregate in bachelor herds. These bachelor herds consist of both aspiring males (i.e., those that have not previously had a territory) and defeated males (i.e., those that previously held a territory but have been defeated by a competitor; Jarman, 1979). Agonistic interactions between territorial males and rivals often result in highly ritualized, complex displays, which can include vocalizations, chasing, and physical confrontations, but rarely result in injuries (Owen-Smith, 1977).

Picture 1. Female impala in the Serengeti National Park, Tanzania. *© L. Hunninck*

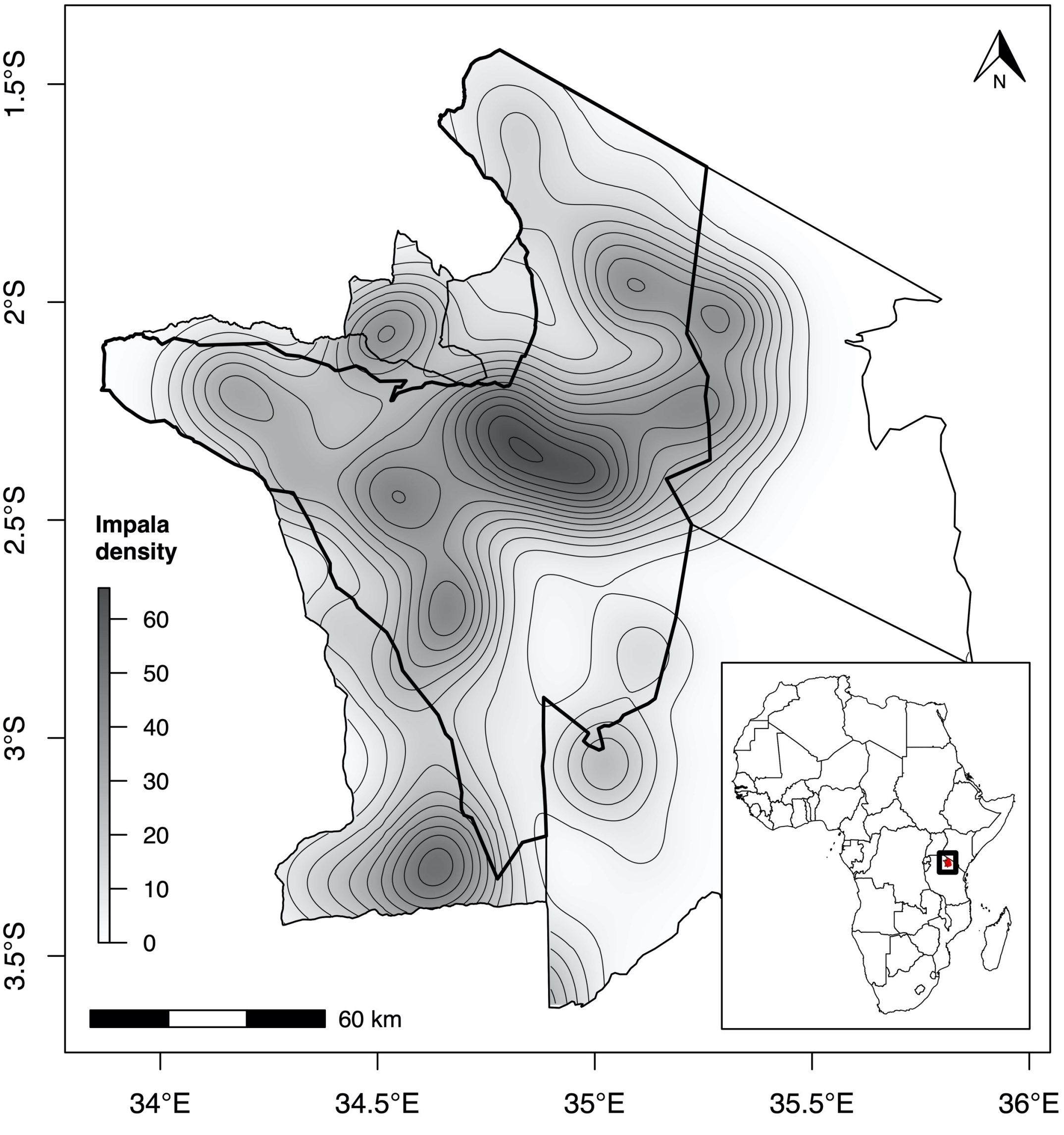

Figure 1. Population density of the impala in the Serengeti ecosystem. Kernel density estimation of the population density of impala in the Serengeti ecosystem in individuals per km^2; data from aerial census (TAWIRI, 2010). Inset shows the location of the Serengeti ecosystem in northern Tanzania.

Aims

The aim of this thesis was to elucidate whether and how anthropogenic disturbances, both directly and indirectly, affect the physiology, behaviour, and demography of the impala, a common antelope in the Serengeti ecosystem. Impala responses to direct human disturbances were studied in relation to i) management area, ii) settlement density, and iii) distance to park boundary. Responses to indirect human disturbances – which are affected by both climate and land use change – were studied in relation to i) forage quality, ii) temperature, and iii) rainfall.

Specific aims

PAPER I : Study the combined effects of reduced forage quality and human disturbance on glucocorticoid concentrations in wild impala

PAPER II : Disentangle the effects of nutritional and physiological stress in relation to anthropogenic disturbances in wild impala

PAPER III : Investigate how territoriality affects glucocorticoid levels in male impala, and how this relationship is influenced by their environmental context

PAPER IV : Study how anthropogenic disturbances affect the behaviour and demography of wild impala populations

General methods

Study area

The world-renowned Serengeti ecosystem (±27,000 km^2) is one of the largest protected areas in Sub-Saharan Africa. At its core is the Serengeti National Park (SNP), designated in 1951 specifically to protect and conserve the second largest mammal migration in the wild: the annual blue wildebeest (*Connochaetes taurinus*) migration. This core area is surrounded by six buffer areas with different management strategies and human land use (**Figure 3A**): Ngorongoro Conservation Area (NCA), Grumeti Game Reserves (GGR), Ikorongo Game Reserve (IGR), Maswa Game reserve (MGR), Ikona Wildlife Management Area (IWMA), and Loliondo Game Controlled Area (LGCA). As a National Park, SNP benefits from a high level of protection and extractive activities such as hunting and livestock grazing are strictly prohibited. Tourism, traffic, and illegal activities (e.g. poaching) are considered the main human disturbances in the park, as settlements are not allowed (Loibooki *et al.*, 2002; Nyahongo *et al.*, 2005; Lindsey *et al.*, 2013). Game Reserves allow licensed hunting and tourism, but no settlements or agropastoralism. IWMA and LGCA allow settlements, licensed hunting in designated hunting blocks, and agropastoralism.

Faecal sample collection and analysis

We measured faecal GC and TH hormone metabolite levels in impala (hereafter referred to as FGM and FTM, respectively). These faecal metabolites reflect the biologically active free plasma GCs and THs (Sheriff *et al.*, 2010), and sample collection is both non-invasive and cost-effective (Sheriff *et al.*, 2011; Madliger *et al.*, 2018). Using faecal samples, our measure of FGM and FTM represent an integrative measure of their plasma concentrations, representing an average value rather than a point value of stress hormone levels (Palme, 2019).

639 samples were collected from individual impala (499 females, 140 males) across five collection periods, spanning four years (2012, 2016, 2017, and 2018) in both wet and dry seasons (**Figure 2A-C**). Faecal samples were only collected when they could be linked to a specific

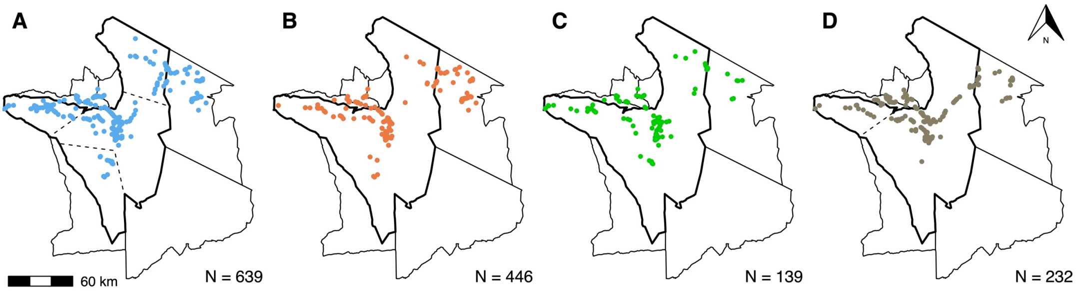

Figure 2. Collection location of samples. Collection locations of each sample (faecal and behavioural) used in this thesis. (A) Paper I, (B) Paper II, (C) Paper III, (D) Paper IV. Number of samples used in each of the four papers is shown (N).

individual. This, in addition to collecting relevant metadata, is important to control for confounding factors. More details on sample and collection are presented in papers I and II.

FGM levels were analysed using a group specific enzyme immunoassay (EIA) according to Palme (2005) and Touma and Palme (2005) by the Palme Lab at the University of Veterinary Medicine, Vienna, Austria; while FTM levels were analysed using a radioimmunoassay (RIA) measuring the concentration of total triiodothyronine (T3) metabolites at the University of Massachusetts Dartmouth facilities. For more detail on the extraction and laboratory analyses, see papers I (FGM) and II (FTM).

Anthropogenic disturbances

To obtain relevant, spatially explicit proxies for anthropogenic disturbances, we considered the following indices: i) management area, ii) settlement density, and iii) distance to SNP border.

Different management areas with different human activities and disturbances make up the Serengeti ecosystem (**Figure 3A**). In Paper I, we distinguished seven areas, four in SNP (i.e. central, cSNP; west, wSNP; north, nSNP; and south, sSNP), GGR and IGR (GIGR), IWMA and LGCA. In Paper IV we recognized five areas, two in SNP (wSNP and cSNP), GGR, IWMA and LGCA.

Settlement density was based on aerial census data provided by the Tanzanian Wildlife Institute on the location of included bomas (i.e. semi-permanent enclosures used by pastoralists to protect their livestock), thatch roof huts, and iron sheet huts/houses in and around most of the Serengeti ecosystem (**Figure 3B**; TAWIRI, 2016). We applied a Kernel density estimation (KDE) to the data to obtain a relative settlement density score. The specific settlement density score for each faecal sample was extracted after scaling the data (mean ± SD ≈ 0 ± 1).

Distance to SNP border was calculated as the shortest Euclidean distance from the GPS location of each datapoint to the nearest park boundary (mean ± SD = 15.0 ± 9.7 km; **Figure 3C**).

While these human disturbance proxies do not vary temporally, the inter-annual variation in these stressors is unlikely to have changed dramatically other than having become more intense. Therefore, we believe that these proxies present a highly relevant insight into spatially explicit patterns of human disturbance.

Environmental variables

To assess spatial and temporal variation in environmental variables, we collected three different remotely sensed variables: i) forage quality, ii) temperature, and iii) rainfall. Data were retrieved from the online Application for Extracting and Exploring Analysis Ready Samples (AppEEARS), courtesy of the NASA and ClimateSERV.

Forage quality was approximated by using the normalized difference vegetation index (NDVI) data (NASA MODIS; Didan, 2015; **Figure 3D**). NDVI is a measure of primary productivity or greenness of vegetation cover calculated from the amount of red and near-infrared light reflected from the Earth's surface (Pettorelli et al., 2005). NDVI is a commonly used metric for estimating changes in primary production, but care should be taken with the interpretation of their results (Pettorelli et al., 2011). We performed several analyses to validate the use of NDVI as a proxy for forage quality for impala in our study system (see paper I). Lastly, the data was manipulated by i) filtering out cloud obstructed pixels using the accompanying pixel reliability dataset, and ii) using a Savitzky-Golay smoothing filter to account for empty data points and outliers.

Temperature was approximated by using the Land Surface Temperature (LST) data (**Figure 3E**). To obtain a better representation of relevant variation in temperature, we calculated the average temperature over a 7-day period prior to sample collection for each sample, specific to its location, which made sure we had no empty values in our data. Prior to its use, we again performed several analyses to validate the use of the manipulated and transformed LST data as a proxy for ambient temperature (see paper II).

Rainfall data were retrieved from the CHIRPS dataset (Funk et al., 2015; **Figure 3F**). To obtain a relevant estimate of rainfall for our samples, we calculated the cumulative rainfall over a 7-day period prior to sample collection for each sample. Due to many datapoints with no rainfall, we categorized the data in three levels: "No rainfall", "Low rainfall", and "High rainfall". Once all data was collected and manipulated, we extracted the pixel value closest in time and space to each faecal sample for each variable. Though the data has varying spatial and temporal resolution, since impala have small home ranges and limited movement, we found our data to provide a reasonable representation of the environment utilized by the sampled impala over the past week.

Behavioural and demographical data

We observed 232 impala groups, and recorded age and sex of every individual in the group, giving us an estimate of number of calves, juveniles, subadults and adults of both males and females in the different management areas (**Figure 2D**). Of those 232 groups, we observed 102 individuals in 44 groups and recorded their behaviour at 1-minute fixed intervals for a total of 30 min per individual. This gave us an estimate of time spent on a certain behaviour. We recognized 10 mutually exclusive behaviours: resting, grooming, ruminating, moving, rutting, grazing, browsing, fleeing, watching the observer, and vigilance. For more details on this data collection, see paper IV.

Statistical analyses

In papers I, II, and III, we applied linear multiple mixed effects models with hormone concentration as the response variable. We *log*-transformed the response variable to obtain normal distribution of model residuals. Residuals were visually checked for normality and heteroskedasticity, and multicollinearity was assessed with a generalized variation inflation factor (GVIF) analysis, which is a measure of the harm done by collinearity among predictors (Fox and Weisberg, 2011).

In paper IV, we used different tests depending on the analysis and the type of data, but they included Pearson's Chi-square tests, Welch Two Sample t-tests, and generalized linear mixed models. We used Akaike's Information Criterion corrected for small sample sizes (AICc) to select the best fitting model to the data (Burnham and Anderson, 2002).

More information on the details of the statistical analyses can be found in each respective paper. All statistical analyses were performed in *R*, v.3.5.0 (RCoreTeam, 2018), using RStudio v.1.1.453 (RStudio, 2016).

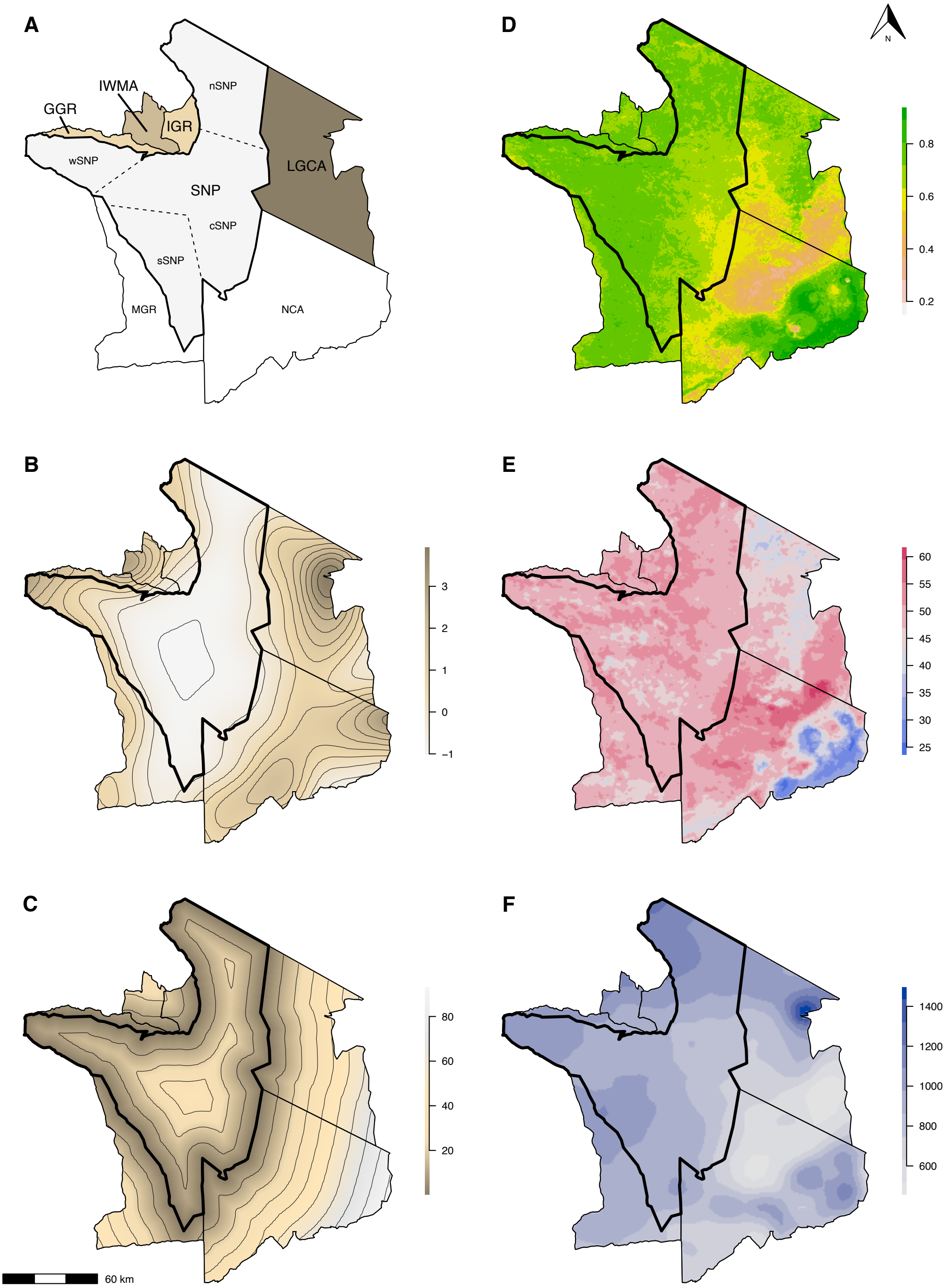

Figure 3. Overview of human activities and environmental properties of the Serengeti ecosystem. Human disturbances A-B-C. Darker colours represent increasing expected disturbances from human activities. (A) Management and study areas. (B) Estimated settlement density. (C) Distance from the Serengeti National Park border (in km). Environmental variables D-E-F. (D) Maximum annual Normalised Difference Vegetation Index (NDVI). (E) Maximum annual Land Surface Temperature (LST; in °C). (F) Mean annual rainfall (in mm). See text for more details.

Main Results and Discussion

The results of this thesis show that anthropogenic disturbances can have measurable effects on both animal physiology and behaviour. Indeed, impala showed significant variation in hormone concentrations in response to both direct human disturbances and changes in the environment, which is affected by both climate and land cover change. However, we found that direct human disturbances were not the main driver of change of impala's physiological state. Instead, hormone concentrations were primarily affected by variation in the environment, such as forage quality and temperature. These results suggest that, although direct human disturbances do have a significant impact, climate and land use changes might be the biggest threats to impala populations in the Serengeti ecosystem. Impala populations in land use areas which allow more exploitation did show behavioural and demographical changes that imply lower population persistence. Together, our results show that the various anthropogenic perturbations in the environment can have considerable effect on wildlife populations in the Serengeti ecosystem, potentially reducing individual fitness and population viability.

In paper I, we investigated the differential importance of human disturbances and forage quality on glucocorticoid (FGM) hormone concentrations in impala. We predicted that FGM concentrations would be mainly affected by the three spatially explicit proxies of human disturbances that we include in this study: management area, settlement density, and distance to SNP border. We also predicted that FGM concentrations would increase with decreasing forage quality, measured as lower NDVI values.

Human population densities tend to be highest closer to national park borders, and though this does not indubitably lead to increased disturbance, land use change and illegal activities (such as poaching and illegal grazing) tend to concentrate around the park boundaries (Estes *et al.*, 2012). Veldhuis *et al.* (2019) showed that intrusions of human activities into SNP are indeed concentrated at its borders, displacing wildlife and potentially affecting ecosystem functioning, resulting in a 'squeezing' effect of the Serengeti. We found that FGM concentrations significantly increased with increasing settlement density and increasing proximity to the SNP border. Settlement density is a measure for both human- and livestock-related disturbances, and as such this result was expected, since FGM levels have previously been shown to increase with increasing human disturbance in impala and other ungulates (Lunde *et al.*, 2016; Formenti *et al.*, 2018). Additionally, competition with livestock is predicted to be highest near bomas, hence contributing to high FGM concentrations. FGM concentrations were highest at the SNP border which supports the hypothesis that illegal activities concentrate around protected area boundaries.

FGM concentrations increased significantly with decreasing forage quality, and forage quality was the most influential predictor of FGM concentrations (**Figure 4A**). Previously, Stabach *et al.* (2015) found that blue wildebeest had significantly lower FGM concentrations when in areas of new forage growth (estimated with NDVI). However, FGM concentrations in these wildebeest also increased with increasing human disturbances. Similarly, Pokharel *et al.* (2018) found that Asian elephants (*Elephas maximus*) outside of a protected area had, unexpectedly, lower FGM concentrations than those inside the protected area. They attributed this result to higher forage quality (approximated with NDVI) in those areas due to agricultural activity. The authors stated that such high-quality forage could in fact be seen as a 'pacifier' against disturbances from human activities.

Summary. It is perhaps not surprising that forage quality is an important predictor of FTM levels in an ungulate. However, though certain human activities undoubtedly have negative consequences for wildlife populations, our results suggest that reducing the negative effects of poor forage quality would be most effective in lowering FGM concentrations in impala. A such, conservation management of impala should focus on ensuring high forage quality through, for example, drought mitigation, habitat protection, and sustainable land use, if they are to protect and conserve wild ungulates populations (Ogutu *et al.*, 2009).

In paper II, we studied how human and environmental disturbances affect the nutritional status of impala. We used thyroid hormones (FTM) as an endocrinological marker for nutritional status, and predicted that FTM concentrations would be mainly influenced by, and increase with, i) increasing forage quality, ii) and decreasing livestock disturbance, and we predicted that iii) low FTM concentrations, indicative of low nutritional status, would result in high FGM concentrations.

FTM concentrations were not directly affected by forage quality, but when accounting for temperature (see below), FTM concentrations decreased with increasing forage quality. Though this result was unexpected, we propose two non-exclusive mechanisms that could explain this result: i) impala could employ an energy-saving mechanism when food quality is high, storing fat reserves (Dunham and Murray,

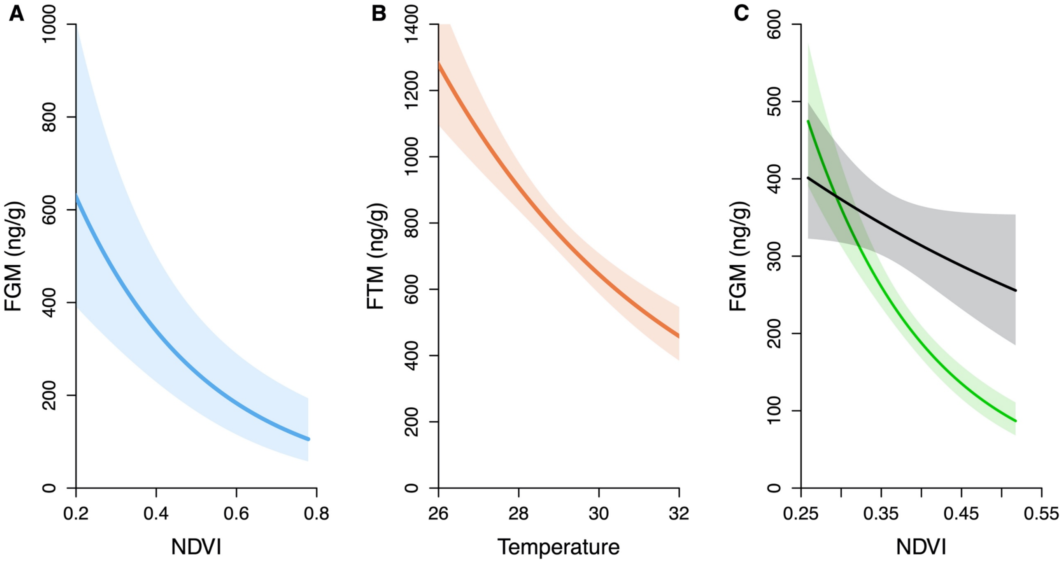

Figure 4. Most important results from papers I, II, and III. Most influential predictors explaining the variation in hormone metabolite levels in impala. (A) the effect of Normalised Difference Vegetation Index on faecal glucocorticoid metabolite (FGM) levels; Paper I. (B) the effect of Temperature of faecal total triiodothyronine metabolite (FTM) levels; Paper II. (C) the effect of NDVI on FGM levels in territorial (black) and bachelor (green) male impala. See text and respective papers for more information.

1982), or ii) thyroid hormones could increase in response to increased food quantity intake due to low quality forage (Behringer *et al.*, 2018). If impala were to lower FTM concentrations, their metabolic rate would decrease which would allow them to store excess energy. Further, impala consume higher quantities of low-quality forage to compensate for the lack of high-quality forage (Wronski, 2002), and therefore, increased metabolic rate could be required to digest this higher quantity of food.

Strikingly, temperature was the most influential predictor of FTM levels, and FTM levels increased as ambient temperature decreased (**Figure 4B**). Thyroid hormones are crucially important in thermogenesis (Dauncey, 1990), which would explain the increased FTM concentrations in response to cold stress. Tropical ungulates have limited thermal insulation, allowing them to better cope with extreme heat, but making them vulnerable to lower temperature (Owen-Smith, 1997), potentially explaining the importance of temperature in explaining FTM variations in impala (Silva, 2006). Lastly, FTM concentrations tended to increase with FGM concentrations. Since we show that FTM levels are mainly associated with thermoregulation in impala, and thermoregulation is an energetically costly process, higher FGM levels could be expected as this facilitates the mobilisation of the required energy (Exton, 1979).

Summary. These results indicate that FTM concentrations in wild animals may only indicate poor nutritional status in the most extreme cases, and that seasonal changes in temperature may be a greater driver of FTM levels. Thus, thyroid hormones, which are primarily responsible for regulating metabolic rate, may be better indicators of how animals metabolically respond to environmental factors.

In paper III, we investigated FGM concentrations in relation to territoriality in impala, and how this might be influenced by environmental context. We predicted that territorial males would have higher FGM concentrations, especially so when i) food quality is low, ii) population density is high, and iii) herd size large.

We found that territorial males had higher FGM concentrations than their bachelor counterparts. This relationship has been found in many species before, and has generally be thought of as a cost of territoriality (Creel, 2001).

FGM levels did not respond to changes in forage quality in territorial males, but decreased with increasing forage quality in bachelor males, indicating there was potentially no extra cost to lower food quality and no benefit to higher food quality (**Figure 4C**). We propose 3 non-exclusive reasons to explain this result: i) territorial males may not be able to take advantage of the increase in food quality because they spend significantly less time feeding (Oliver *et al.*, 2007), ii) increased food quality might increase the competitive ability of bachelor males (Jarman, 1979), and iii) territorial males need to mobilize sufficient energy to be able to perform these behaviours, which may be facilitated by an increased FGM concentrations (Exton, 1979). Population density and herd size did not affect FGM concentrations in territorial males differently than they did bachelors, indicating FGM concentrations are not simply a cost to territoriality, as we would expect greater costs of increased population density and herd size.

Summary. Here, we propose that elevated FGM concentrations in territorial males enhances their ability to defend and maintain a territory – and hence increase their reproductive success – by increasing energy mobilization and metabolic rate. While increases in FGM cannot be sustained indefinitely, we suggest this is a proactive mechanism and not simply a reactive cost to being territorial.

In paper IV, we studied how different land use practices affect the behaviour and demography of impala populations in the Serengeti ecosystem. We predicted that impala in the least protected area (i.e. LGCA) would spend more time on costly behaviours (negatively affecting their fitness), and that impala populations would have a more female-skewed sex ratio and lower recruitment rates than impala populations in the national park.

Impala in less protected areas spent less time on restorative behaviours, potentially contributing to reduced individual fitness. We also found that impala synchronized their behaviour, especially vigilance, which is a costly behaviour potentially facilitating more efficient anti-predator strategies (King and Cowlishaw, 2009).

In less protected areas, impala populations had a more female-skewed sex ratio and had lower reproductive and recruitment rates (**Figure 5**). These results could be caused by a myriad of potentially human-induced disturbances in these areas, including legal and illegal hunting, which tend to target male impala (Holmern *et al.*, 2006; Milner *et al.*, 2007). Human disturbance may force animals to prioritise evading human disturbances, negatively affecting resource acquisition, and indirectly reducing survival and reproductive success.

Summary. Our results indicate that human disturbances affect both behaviour and demography of impala populations, potentially reducing population persistence in the areas with lower protection status. Continued monitoring of impala behaviour and demography is needed to better understand the implications and long-term effects of different human activities.

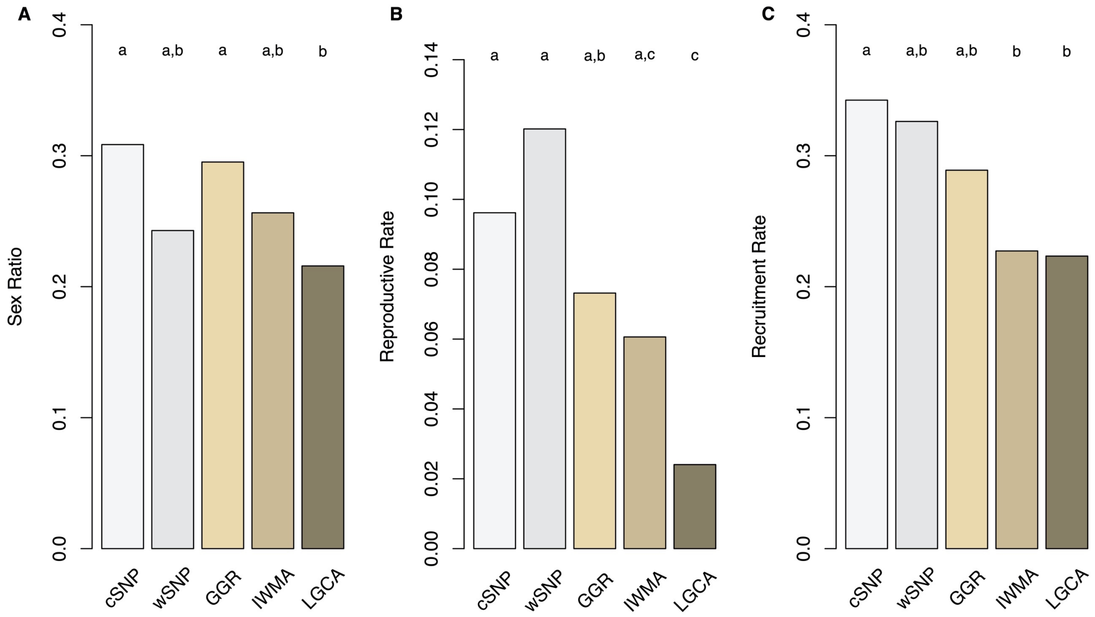

Figure 5. Demographical differences between impala populations in different areas in the Serengeti ecosystem. (A) Sex ratio calculated as proportion of total observed number of males over total number of females per area. (B) Reproductive rate calculated as total number of calves over the total number of adult females per area. (C) Recruitment rate calculated as total number of juveniles over the total number of adult females per area. Darker colours represent increasing expected disturbances from human activities (See Figure 3A).

Synthesis and perspectives

The two main findings of this thesis are that, first, anthropogenic disturbances do affect both the physiology and behaviour of impala, though it is environmental fluctuations – aggravated by both climate and land use change – that have the largest effect, not direct human activities. Secondly, endocrinological markers should be interpreted within their ecological and evolutionary context, as they facilitate life-history events, increasing fitness, and are not just a reactive cost.

An ecological story

Importance of the physical environment

Strikingly, in both papers I and II it is the physical environment, more than any other factor, that determines fluctuations in hormone concentrations in impala. Paper I showed that rather than direct human disturbances, it is forage quality that determines FGM concentrations, and, similarly, in Paper II it was temperature that determined FTM concentrations, not human-induced disturbances.

These extensive and pervasive perturbations in their physical environment force impala to employ energetically costly physiological mechanisms to cope with and adapt to these changes. Indeed, these increases in hormone concentrations – leading to an increase in metabolic rate and energy mobilization – can have potentially important repercussions for the impala's individual fitness and can have downstream effect on population persistence (Sapolsky *et al.*, 2000).

For example, paper III shows that territoriality – which is an essential reproductive strategy in impala – is enabled and facilitated by increased energy mobilization. However, this increased energy use cannot be sustained indefinitely. Thus, the ability to maintain a territory is likely to be negatively affected by worsening environmental conditions which impede energy intake, therefore lowering individual reproductive success.

That adaptations in response to human activities can be costly is the key finding of IV, this time from a behavioural perspective. Diverting energy to behaviours as a response to anthropogenic disturbances (e.g. vigilance), and therefore away from reproductive activities, growth and/or immunocompetence, can lower both the impala's reproductive success and survival rates (Frid and Dill, 2002).

The observed lower reproductive and recruitment rates in the least protected study areas LGCA (paper IV) were attributed to both direct mortality as a result of human activities and indirect effects of human-induced disturbance. Both behavioural (paper I) and physiological (papers I and II) responses to human disturbance are costly, and as such, could very well contribute to the observed reduction in vital rates.

Conservation implications

With climate change becoming increasingly pervasive, environmental fluctuations in temperature and rainfall, amplified by increasing land use change, are predicted to become worse (Niang *et al.*, 2014; Moore *et al.*, 2015). It is important to note that direct human disturbances such as illegal killing of wildlife and agropastoralism do have a significant impact on impala, yet these disturbances are often limited in either space or time and are not necessarily continuously pervasive. Climate and land use changes, however, are likely to be more deleterious to wildlife population in the long term.

Our results indicate that managers should therefore shift their focus from fortress-and-fence conservation to conserving whole natural systems at large, also beyond the boundaries of few well-defined strictly protected areas. Climate change has global effects, but even land use changes far outside of a protected area can significantly affect its ecosystem functioning (Veldhuis *et al.*, 2019).

The use of endocrinological markers together with population censuses can provide a powerful tool for conservationists to determine population status and trends, but care should be taken with the interpretation of variations in physiological indices.

Rethinking the physiological toolbox

Recap: the underlying physiology

To fully understand the implications of these results, it is important to recognise the physiological function these hormones fulfil. Though glucocorticoids affect a range of bodily functions such as anabolic and catabolic processes, their main function in the body is the regulation of energy mobilization through a process called gluconeogenesis (Exton, 1979). In fact, in ruminants like impala, this process is responsible for producing up to 80% of the required glucose (Young, 1977). This allows animals to divert energy away from processes non-essential for short-term survival, and towards enhanced recovery and preparation for a repeated stressor. Thyroid hormones, on the other hand, are fundamentally important in regulating both metabolic rate and thermogenesis (Silva, 1995). They also affect longevity and reproductive success. Both of these hormones are instrumental in regulating an animal's energy balance, and fluctuations in hormone concentrations within or between individuals should be interpreted with careful consideration of their physiological context.

Proactive facilitation vs. reactive cost

In paper III, we posit that the increase in GC in territorial males is facilitating a male impala's ability to become and remain territorial, thereby increasing their competitive capacity and thus increasing their reproductive potential. Indeed, we suggest that an increase in glucocorticoid concentrations should not be seen as a reactive cost, but rather a proactive facilitator of territoriality. As we explain in paper I, an increase in glucocorticoids is an adaptive mechanism, and although a long-term elevation in glucocorticoids in the body can have negative consequences on survival, it is important to consider the ecological and evolutionary context of these endocrinological pathways before condemning glucocorticoids to the negatively connotated 'stress' hormones (Boonstra, 2013). The evolved pathways regulating both glucocorticoids and thyroid hormones, and their effects in the body, have evolved specifically because it is adaptive: regulating the production and excretion of these hormones within the body allows animals to better deal with both unexpected and predictable perturbations in their environment. In light of this, Boonstra (2013) argues that there is no such 'maladaptive chronic stress' that results in pathology, but that, although there might be fitness costs to prolonged exposure to elevated glucocorticoid concentrations, chronic stress is both beneficial and does not fail to sustain reproductive success and survival. In the previous section, the energetic cost these physiological changes can entail were discussed, and it was concluded that with increasingly challenging perturbations in the environment, impala's ultimate fitness could ultimately be negatively affected. However, considering the evolutionary context of the endocrinological pathways, we could argue that the regulation of these hormones in response to environmental changes – seen in papers I and II – is not a direct and reactive cost but a proactive, necessary adaptation to better cope with these perturbations, increasing their overall reproductive success and fitness, as compared to the alternative where chronic stress response would be absent.

Additionally, especially in regard to conservation physiology, it is necessary to consider the limitations of endocrinological markers to accurately identify individuals in poor physiological condition. Baseline or threshold levels of these hormones differ considerably between species and even between conspecifics. Without these, inferences on potential population persistence implications should not be made lightly. Another major drawback in this field is the lack of comparability between studies. Only recently have potential methods been proposed to evaluate variations in hormone levels in such a way that the interpretation of the result can be compared within and across species (MacLarnon *et al.*, 2014), a vital step for the future of this field.

Future prospects

The field of conservation biology is ever evolving and branching out to different disciplines to obtain, among other, tools to better inform conservation management. Combining both behavioural and physiological methods to obtain a more complete picture of the threats faced by wildlife, disentangling their responses to disturbances, and their fitness implications, has yielded promising results already. Indeed, the physiological toolbox has proven to be versatile and informative, for those creative enough to develop endocrinological tools (Hunt *et al.*, 2013). However, with an increasing number of papers being published (Palme, 2019), we should not lose sight of the ecological and evolutionary context of the underlying physiology (Boonstra, 2013). It is my hope that future studies will recognise this complexity and design studies and interpret their results with this in mind.

Furthermore, this PhD thesis has heavily relied on the use of remotely sensed data to estimate the spatially and temporally specific environment of wild animals. In my view, this wealth of different data has not been fully recognised by ecologists and conservationists. Though these proxies have their limitations, their global availability, ease of use, and the potential to retroactively link with individual datapoints can offer valuable insights into animal behaviour and even physiology, as demonstrated here. Perhaps with increasing awareness of these different datatypes and their advantages (and drawbacks), more researchers will recognise their potential and we will see more studies exploiting the plethora of opportunities these data offer.

Picture 2. Solitary male impala in Loliondo Game Controlled Area, Tanzania. *© L. Hunninck*

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PAPER I

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SOCIETY FOR EXPERIMENTAL BIOLOGY

Research article

Consequences of climate-induced vegetation changes exceed those of human disturbance for wild impala in the Serengeti ecosystem

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In East Africa, climate change is predicted to reduce vegetation quality, and pervasive human disturbance has already resulted in significant declines in biodiversity. We studied the combined effects of reduced forage quality and human disturbance on faecal glucocorticoid metabolite (FGM) concentrations. We predicted that decreasing nutritional quality and increasing human disturbance would have an additive positive effect on FGM levels in wild impala (*Aepyceros melampus*). Employing a space-for-time approach, we used normalized difference vegetation index (NDVI) as a measure of forage quality, combined with spatially explicit proxies of human disturbance across areas of different protection management strategies in the Serengeti ecosystem. We collected 639 faecal samples, spread over 4 years, including both wet and dry seasons. Impala FGM levels increased significantly with declining NDVI and, to a lesser extent, with increasing proxies for human disturbance. However, we found no interaction between the two, such that impala had elevated FGM levels with low NDVI and low FGM levels with high NDVI regardless of human disturbance levels. This implies that impala will have high FGM levels if forage quality is poor, even with significant protection and reduced human disturbance. Understanding how animals respond to and cope with changes in forage quality and human land use across different protected areas is important for conservationists and managers to better protect species at risk and predict population viability.

Key words: conservation, cortisol, forage quality, NDVI, protected areas, stress, ungulate

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Introduction

Global biodiversity is in decline, caused primarily by anthropogenically induced changes in climate and land use (Pimm *et al.*, 2014; Johnson *et al.*, 2017). Anthropogenic disturbances now significantly impact nearly every habitat on Earth, and human-induced rapid environmental changes are forcing many species to either adapt at an unprecedented pace or perish (Sievers *et al.*, 2018). Organisms may adapt to these disturbances through behavioural, physiological and/or

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morphological mechanisms (Sih *et al.*, 2011). While certain species thrive in these new, human-altered environments, most species face population declines, with some researchers predicting that a large proportion of the Earth's biodiversity will be extinct by 2100 (Stork, 2010; IPBES, 2018). In East Africa, climate change is severely altering weather patterns and could significantly reduce forage quality (Boko *et al.*, 2007; Niang *et al.*, 2014). Furthermore, exceedingly pervasive human land use and land cover change, mainly due to agricultural expansion, is considerably changing and reducing the region's natural habitat (Willcock *et al.*, 2016). The reduction in forage quality through the combined effect of climate-induced and human land cover change poses a significant threat to the region's biodiversity (Midgley and Bond, 2015; Segan *et al.*, 2016). In the Serengeti-Mara ecosystem, wildlife populations have declined dramatically, especially in areas with high human disturbance (Ogutu *et al.*, 2009; Veldhuis *et al.*, 2019). Although protection measures have been implemented, including the creation of protected areas such as Serengeti National Park (SNP), understanding how animals respond to and cope with declines in vegetation quality and increased human land use across areas with different protection strategies is important for conservationists and managers to better protect species at risk and predict population viability.

Ungulate populations are to a large extent regulated by forage quality (Hopcraft *et al.*, 2010). In the east African savanna, grass growth is mainly regulated by soil fertility and rainfall (Bartzke *et al.*, 2018) and is characterized by strong seasonality. Grasses in these savanna ecosystems periodically dry and become less nutritious for herbivores (Codron *et al.*, 2007). Animals can adapt to decreased forage quality by either migrating to better grazing patches or adjusting their diet (Hopcraft *et al.*, 2010). For example, the Serengeti ecosystem is able to sustain more than one million blue wildebeest (*Connochaetes taurinus*) because most migrate between the northern and southern part of the ecosystem, drawn to fresh pastures which appear after the first rains (McNaughton and Banyikwa, 1995; Hopcraft *et al.*, 2013). Impala (*Aepyceros melampus*), on the other hand, are sedentary and thus must forage on a mixed diet, preferring nutritious grasses but needing to include more browse in their diet as grasses dry out (Jarman and Jarman, 1973; Dunham, 1982). The seasonal fluctuations in rainfall in eastern Africa, and thus forage quality, are predicted to become more extreme with increasingly severe climate change (Dore, 2005; Sinclair *et al.*, 2007; Midgley and Bond, 2015), potentially resulting in prolonged drought periods (Dai, 2011; Kotir, 2011) and significant reductions in nutritious grasses across savanna habitat (Stevens *et al.*, 2016).

As a proxy of spatiotemporal variability in forage quality, we used the normalized difference vegetation index (NDVI; NASA MODIS; Didan, 2015). NDVI is a measure of primary productivity or greenness of vegetation cover calculated from the amount of red and near-infrared light reflected from the Earth's surface (Pettorelli *et al.*, 2005). NDVI is a commonly used metric for changes in primary production, though care should be taken with the interpretation of the results (Pettorelli *et al.*, 2011). For example, changes in plant species composition or habitat structure can significantly affect the interpretation of NDVI values in space. As such, it is advised to only compare changes within the same habitat, and not between different ecosystems (Pettorelli *et al.*, 2005). Here, we employ NDVI within areas specific to our study species in the African savanna ecosystem, mainly consisting of a particular grassland and woodland mosaic.

In African savanna ecosystems, ungulates also face increasing pressure from anthropogenic disturbances. Some human activities, such as infrastructure and tourism, invoke a multitude of behavioural responses which can sometimes be so pervasive they impact population viability (Frid and Dill, 2002; Szott *et al.*, 2019). For example, African elephants (*Loxodonta africana*) and impala adjust their diurnal activity or movement patterns to limit exposure to these human activities (Wronski *et al.*, 2015; Gaynor *et al.*, 2018). Additionally, several studies have shown that animals in strictly protected areas such as national parks have lower glucocorticoid (GC) levels than their conspecifics in less protected areas, implying that GC levels might be a good indicator for protection level of an area (Ahlering *et al.*, 2011; Spercoski *et al.*, 2012; Huninck *et al.*, 2017). Human-induced changes in land use and cover have also contributed considerably to the degradation of natural grasslands in east Africa, particularly in areas with high agricultural and pastoral activities (Laurance *et al.*, 2014), resulting in a decline in overall vegetation productivity (Landmann and Dubovyk, 2014). Growing livestock numbers increase resource competition with wild ungulate populations and result in habitat modifications (Prins, 2000; Young *et al.*, 2005; but see Schuette *et al.*, 2016). Together, reduced forage quality combined with changes in human land use are predicted to pose the biggest threat to wildlife in eastern Africa, and a better understanding of their impact on animal populations is needed (Vié *et al.*, 2009; Niang *et al.*, 2014).

The physiological stress response is an essential part of vertebrates' ability to cope with and respond to challenges in their environment (Boonstra, 2013). One part of this response is through the activation of the hypothalamic–pituitary–adrenal (HPA) axis and subsequent secretion of GCs into the blood stream (Romero, 2004). Although GCs affect a range of bodily functions, their primary role is energy mobilization (Strack *et al.*, 1995). Upregulating the secretion of GCs allows animals to mobilize the energy needed—even at the cost of tissue mass—to facilitate the required physiological and behavioural responses needed for organisms to mitigate a stressor (Romero and Wingfield, 2015). These temporary changes allow organisms to better deal with adverse situations (i.e. stressors; MacDougall-Shackleton *et al.*, 2019), such as increased predation pressure or food deprivation (Sheriff *et al.*, 2011a; Dantzer *et al.*, 2014), by, among other, increasing energy availability for muscles, and suppress anabolic processes non-essential for short-term survival such as growth, reproduction and digestion. The adaptive value of this energy mobilization under threat helps by both diverting energy where it is needed while enhancing recovery and preparation for a repeated stressor (Sapolsky *et al.*, 2000). However, if the stressor is frequently recurring or constant over a longer time span (i.e. chronic stressor), this adaptive stress response can result in adverse effects for the organism, such as suppressed growth, lower immune function, increased energy expenditure, and potentially reduced reproduction and survival (Busch and Hayward, 2009; Romero and Wingfield, 2015). Thus, the measurement of GCs may provide a robust assessment of animals' overall health, their ability to cope with changes within their environment, and the potential fitness consequences of their responses (Sheriff *et al.*, 2011a; Dantzer *et al.*, 2014).

In this study, we tested the hypothesis that decreased forage quality and increased anthropogenic land use would significantly increase GC levels in wild impala within the Serengeti ecosystem. Impala are a common herbivore in this system and, due to their small home ranges, high local abundance and non-migratory behaviour, are an ideal model species to study the effect of spatially explicit disturbances on an animal's adrenocortical activity. To test our hypothesis, we used NDVI and spatially explicit proxies of human disturbance across areas of different protection management strategies, including SNP (see Methods). This allowed us to study the interactive effects between forage quality and human disturbances on faecal glucocorticoid metabolite (FGM) levels of a wild ungulate. Specifically, we predicted that impala would have significantly higher FGM levels (i) in areas with reduced forage quality as measured by lower NDVI scores, (ii) in areas with greater human disturbance, measured as settlement density, and especially (iii) in areas with reduced forage quality and high human disturbance. We also predicted that the protection status of an area would influence impala FGM levels, such that (i) impala in adjacent areas but near SNP would have lower FGM levels that those further away and (ii) impala in areas with greater protection status would have lower FGM levels.

Methods

Study area and species

The Serengeti ecosystem (±27 000 km^2) experiences high geographic variability in rainfall, from around 450 mm in the southeast to > 1400 mm in the north; rainfall comes in two separate wet seasons (March–May and November–December). The ecosystem consists of seven areas with different management strategies and human land use; our study was limited to five of these areas (Fig. 1); SNP, Grumeti and Ikorongo Game Reserves (GIGR), Ikona Wildlife Management Area (IWMA) and Loliondo Game Controlled Area (LGCA). Of these, SNP has the highest levels of protection and extractive activities such as hunting and livestock grazing are strictly prohibited. Tourism, traffic and illegal activities such as poaching (i.e. illegal bushmeat hunting) are considered the main human disturbances in the park, as settlements are not allowed (Nyahongo *et al.*, 2005). We distinguished four subareas within SNP because of their differences in intensity of human activities: central (cSNP; high tourism, low poaching), west (wSNP; high poaching, medium tourism), north (nSNP; low tourism, low poaching) and south (sSNP; medium tourism, medium poaching) (Loibooki *et al.*, 2002; Lindsey *et al.*, 2013). GIGR is our medium protected area; it allows licensed hunting and tourism, but no settlements or agropastoralism. IWMA and LGCA have the lowest protection; they allow settlements, licensed hunting in designated hunting blocks and agropastoralism. The cumulative effect of different human disturbances is particularly difficult to estimate and compare; however, we expect LGCA to have the highest level of human disturbance, followed by IWMA, GIGR and lastly the areas inside SNP. SNP has comparatively low human disturbance (although the number of tourists is increasing), and this was expected to be similar in cSNP, sSNP and nSNP but higher in wSNP, due to potentially higher poaching levels.

Impala are a medium-sized antelope species common in eastern and southern African savanna ecosystems (IUCN SSC Antelope Specialist Group, 2016). Impala are non-migratory herbivores with small home ranges typically between 5 and 10 km^2, increasing only slightly in the dry season (Averbeck, 2001). They are often found on the edge of open savanna as their preferred habitat is open woodland (Ford *et al.*, 2014). Their habitat requirements result in impala having a clumped and irregular distribution, but locally abundant (Averbeck, 2001). In East Africa, impala males are territorial year round (Oliver, 2005) and male–male aggression likely elicits a stress response (e.g. Corlatti, 2018).

Collection and analysis of faecal samples

To assess GC levels in impala, we measured FGMs. FGMs reflect the biologically active free plasma GCs (Sheriff *et al.*, 2010), and sample collection is non-invasive (Sheriff *et al.*, 2011a; Madliger *et al.*, 2018). FGMs are an integrative measure of plasma GCs (±2 h in impala), representing an average value rather than a point value of GC levels (Palme, 2019).

We collected 639 samples from individual adult impala (499 females, 140 male) across five collection periods, spanning 4 years (2012, 2016, 2017 and 2018) in both wet and dry seasons (Supplementary Table S1). When a suitable individual was seen defecating, a picture was taken and the distance to the individual was recorded with a range finder. This method allowed us to easily identify the specific sample (Lunde *et al.*, 2016). The sample was not collected when two or more samples were close to each other (within 1 m). For each faecal sample that was collected, we recorded the sex of the individual from whom the sample came (adult males have horns), and the size and type (family [one territorial male,

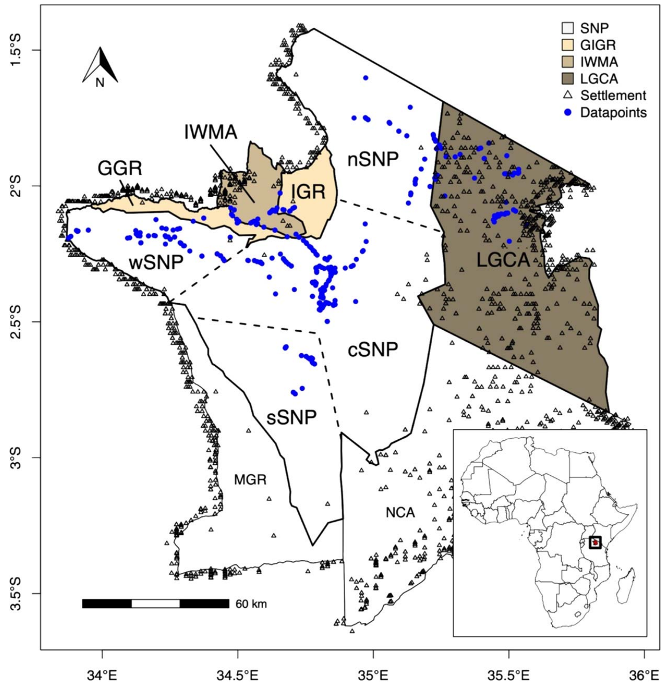

Figure 1: The Serengeti ecosystem. Map of the Serengeti ecosystem, consisting of seven areas with different management strategies and different human land uses. Serengeti National Park (SNP) is subdivided in four areas by dashed lines (see Methods). Areas with darker fills are areas with higher predicted intensities of human disturbance. Locations where samples were collected are in solid (blue) circles, while settlement locations are represented as open triangles

females and juveniles], bachelor [only adult and subadult males], or mixed [when family herds mixed with bachelor herds]) of social group. We also took a GPS location of the collection site and habitat and noted the time of day. Habitat was categorized into four different types; grassland (grass dominated with $< 2\%$ tree canopy), savanna (grassland with $< 20\%$ tree cover), woodland ($>20\%$ tree cover, defined as trees > 6 m with canopy cover 20% or higher) and bushland (dense woody vegetation < 6 m in height with $> 20\%$ bush canopy). We could sample individuals from multiple groups from a single location in a single day; however, we did not return to the same location within a collection period to avoid potential pseudo-replication, i.e. resampling the same individual. Samples were collected within 60 min of defecation (mean ± SD = 28 ± 14 min) and immediately placed on ice and, within 12 h of defecation, stored at −20°C until further analysis.

Analysis of FGMs

FGMs were analyzed using a group specific enzyme immunoassay (EIA) according to Palme (2005) and Touma and Palme (2005). Briefly, faecal samples were defrosted at room temperature for 30 min and homogenized by hand for 5 min. A portion of 0.52 ± 0.023 g (mean ± SD) of homogenized faeces were mixed with 5 ml of 80% methanol and vortexed for 1 min. Samples were then centrifuged for 20 min at 2500 *g*, and 0.5 ml of supernatant was removed. Samples were then placed in a fume hood for up to 48 h to allow methanol to evaporate. Samples were then sealed and stored at −20°C until shipment and analysis at the University of Veterinary Medicine, Vienna, Austria. FGMs were measured with an 11-oxoetiocholanolone EIA, first described by Möstl *et al.* (2002) which measures metabolites with a 5β-3α-ol-11-one structure. This EIA has been specifically validated for impala (Chizzola *et al.*, 2018). Intra-assay variations of high- and low-value quality controls were 5.27 and 5.76%, respectively, and inter-assay coefficients of variation of high- and low-value quality controls were 10.39 and 12.15%, respectively.

Collection of remote sensed NDVI data

The data were retrieved from the online Application for Extracting and Exploring Analysis Ready Samples

(AppEEARS), courtesy of NASA (https://lpdaacsvc.cr.usgs.gov/appeears/). Using the pixel reliability dataset that accompanies the NDVI data, pixels containing clouds were filtered out. NDVI data was adjusted to account for empty data points and outliers using a Savitzky–Golay smoothing filter.

NDVI measurements should only be compared within the same habitat, and not between different ecosystems (Pettorelli *et al.*, 2005). However, impala are most often found in very similar habitat, regardless of area, preferring semi-open to bushy savanna and rarely venturing far from the cover of woody vegetation (Jarman and Jarman, 1973; Ford *et al.*, 2014). To assess habitat differences in sample locations, we used remotely sensed data on woody cover (MOD44B MODIS/Terra; Dimiceli *et al.*, 2015). As expected, woody cover percentage at sample locations was low (mean ± SD = 5.6% ± 3.2; $N = 693$) and adjusting NDVI for % woody cover did not affect model estimates (see Supplementary Information S1). Therefore, although NDVI measures greenness of vegetation of both woody and non-woody plants, in this data set, variation in NDVI is mostly due to variation in grassy vegetation. Thus, here, NDVI correlates positively with the abundance of grassy vegetation, and since grassy vegetation is considerably more palatable than browse and therefore preferred by impala (Jarman and Jarman, 1973; Codron *et al.*, 2007), NDVI represents an unbiased proxy for forage quality for impala (Pettorelli *et al.*, 2011).

For each of the faecal samples collected, we extracted the closest NDVI value in space (250 m MODIS pixel resolution) and time (8-day interval). Thus, we acquired an NDVI score specific to our faecal sample with regards to location and time of collection. Considering the limited movement of impala, impala equipped with a GPS collar moved on average 262 m in 3 h (SD = 247, $N = 212\,000$; *unpublished data*) away from their initial location; this NDVI score provides a reasonable representation of the environment utilized by the sampled impala over the past week (well within the integrated hormone levels experienced by each individual).

Collection of remote sensed rainfall data

To estimate the potential effect of rainfall on FGM concentrations in impala, we collected data from the CHIRPS dataset (Funk *et al.*, 2015). This dataset has a temporal resolution of 1 day and a spatial resolution of 5 km resolution; data was downloaded from ClimateSERV (https://climateserv.servirglobal.net/). We calculated the cumulative rainfall over a 7-day period prior to sample collection for each sample (t0 to t-7, with t0 = time of sample collection), specific to its location. The rainfall data was zero-inflated as many samples were collected in the dry season and was therefore converted in a categorical variable with three levels: 'No rainfall' (Rainfall = 0; $N = 287$), 'Low rainfall' ($0 < \text{Rainfall} \leq 12$; $N = 151$) and 'High rainfall' ($\text{Rainfall} > 12$; $N = 201$). The third quantile of the rainfall data was 12 mm and was therefore chosen as a threshold.

Human disturbance

The Tanzanian Wildlife Institute provided data on settlement locations, which included bomas (i.e. used by pastoralists to protect their livestock), thatch roof huts and iron sheet huts/houses in and around most of the Serengeti ecosystem (Fig. 1; TAWIRI, 2016). Some settlements are located within the national park which is not allowed but does happen (Fig. 1). However, since a Kernel density estimation (KDE) was applied to the data, isolated points had little effect on the overall settlement density score. The specific settlement density score for each faecal sample was extracted and, after scaling the data (mean ± SD ≈ 0 ± 1), used for analyses. Distances to SNP boundary were calculated as the shortest Euclidean distance from the GPS location of each data point to the nearest park boundary (mean ± SD = 15.0 ± 9.7 km). Seven areas, four in SNP (i.e. cSNP, wSNP, nSNP and sSNP), GIGR, IWMA and LGCA, with different human activities and disturbances were recognized in this study (Fig. 1; shapefiles available on https://www.protectedplanet.net/). Accurate data for relevant human disturbance proxies are particularly difficult to come by, especially in high temporal and spatial resolution. Here, our human disturbance proxies do not have temporal variation; however, the inter-annual variation in these stressors is unlikely to have changed dramatically other than having become more intense. Therefore, we believe that these proxies still present a highly relevant insight in spatially explicit patterns of human disturbance on FGM levels.

Statistical analyses

We constructed multiple linear mixed models using the *lmer* function of the *lme4* package v.1.1–17 in R (Bates *et al.*, 2015). The response variable, FGM, was log-transformed to obtain normal distribution of model residuals. The following fixed predictors were all included in the basic model: (i) NDVI as measure for forage quality, and (ii) settlement density, distance to SNP border and land use area as measure for human disturbance. Lastly, time of day was included in the model as a fixed predictor because research has shown that it is important to either account for time-of-day in the study design (i.e. collect samples at similar times of the day) or control for this confounder by including it as a predictor of FGMs (Palme, 2019; *see* Supplementary Information S2). We used a quadratic function to model time of day and distance to SNP border; a decision supported by model selection criteria (ΔAICc < 2; Akaike information criterion adjusted for small sample sizes; Burnham and Anderson, 2002). Random effects included group number nested within sampling location, and collection period as a crossed random effect. This way, we accounted for differences between groups, spatial location and collection period (Table 1).

Several potential confounding factors were identified (but see Supplementary Information S2): sex, group size and type, the interaction between sex and group type, distance to the nearest road, habitat and rainfall. By comparing AICc values, we determined which of these confounding factors,

Table 1: Model estimates from the final mixed effects model explaining the variation in faecal glucocorticoid metabolite concentrations in impala. See text for further details

Fixed effects	Estimate	SE	df	*t* value	*P* value	
(Intercept)	7.27	0.32	19.37	22.89	<0.001	***
NDVI	−3.08	0.63	155.34	−4.85	<0.001	***
Settlement density	0.33	0.10	37.91	3.26	0.002	**
Distance to SNP (lin.)	−1.31	3.19	17.66	−0.41	0.686	
Distance to SNP (qua.)	−5.94	1.75	18.15	−3.40	0.003	**
Land use area						
wSNP	−0.21	0.21	25.04	−0.99	0.332	
nSNP	−0.60	0.21	18.56	−2.83	0.011	*
sSNP	0.48	0.27	12.87	1.79	0.097	.
GIGR	−0.59	0.28	25.84	−2.14	0.042	*
IWMA	−0.15	0.29	20.89	−0.52	0.607	
LGCA	−0.65	0.28	37.43	−2.37	0.023	*
Time-of-day (lin.)	−1.11	0.98	304.71	−1.13	0.260	
Time-of-day (qua.)	2.10	0.97	299.35	2.16	0.032	*
Rainfall						
Low	−0.06	0.14	254.26	−0.44	0.663	
High	−0.25	0.11	304.35	−2.38	0.018	*
Random effects	Variance	SD				
Group ID: location	0.24	0.49				
Location	0.03	0.19				
Sampling period	0.16	0.40				
Residual	0.28	0.53				

*Significance codes: $P < 0.001$ ***; 0.001–0.01 **; 0.01–0.05 *; 0.05–0.1.*

when added to the basic model, significantly improved the variation explained by the model (ΔAICc < 2); only rainfall significantly improved the model and was therefore included in the final model. Residuals were visually checked for normality and heteroskedasticity, and a multicollinearity was assessed with a generalized variation inflation factor (GVIF) analysis, which is a measure of the harm done by collinearity among predictors (Fox and Weisberg, 2011). No heteroskedasticity was found, and residuals were normally distributed; GVIF values corrected for the degrees of freedom ($GVIF^{[1/(2*df)]}$) were all lower than 1.8 (*vif* function of the *car* package v.3.0-0 in R (Fox and Weisberg, 2011)), which is well below the conservative threshold of 3 (Zuur *et al.*, 2010). The correlation matrix of fixed predictors is presented in Supplementary Table S2.

To test for the interactive effect of forage quality and human disturbance on FGM levels in impala, we added an interaction term to the final model between NDVI and settlement density (now called 'interaction model'). We compared AICc values of both models to determine whether the addition of the interaction would improve the fit of the model.

All statistical analyses were performed in the statistical program *R*, v.3.5.0 (RCoreTeam, 2018), using RStudio v.1.1.453 (RStudio, 2016). Back-transformed model estimates are shown in all figures; plots illustrate adjusted response values, which show the relationship between the fitted response and a single predictor, with the other predictors averaged out. The *Y*-axis in the figures are truncated at 1000 ng/g to aid the presentation of results.

Results

Our final model explained a large proportion of the variation in impala FGM concentrations (*conditional* $R^2 = 72.0\%$; Nakagawa & Schielzeth 2013); the main predictors in the model (i.e. fixed effects: NDVI, Settlement density,

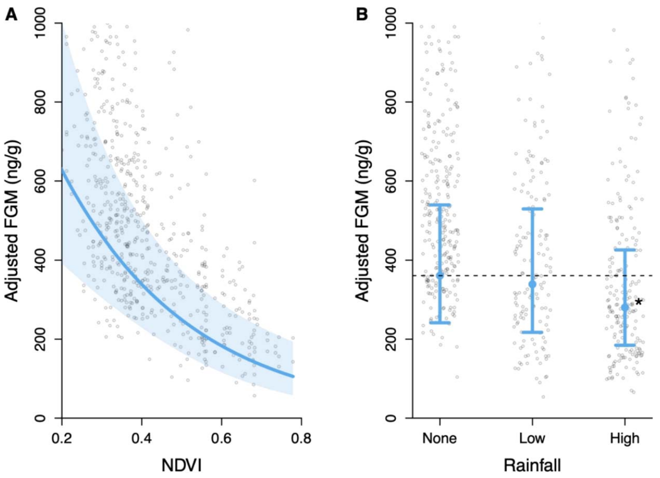

Figure 2: Changes in impala FGM concentrations due to environmental factors. The effect (blue line) of (**A**) the normalized difference vegetation index (NDVI), and (**B**) rainfall on impala faecal glucocorticoid metabolite (FGM) concentrations. Adjusted response values are represented as points; 95% confidence interval is the shaded blue area. On panel B, star denotes significant difference from no rainfall category (dashed line; $P < 0.05$).

Distance to SNP, Land use area, Rainfall and Time-of-day) explained (*marginal* R^2) 28.3% of FGM concentration variation.

We found that impala had significantly higher FGM levels in areas with lower NDVI scores (Table 1), such that mean FGM levels increased from 106 ng/g (95% confidence interval (CI) = 58–194 ng/g) at the highest NDVI values to 632 ng/g (CI = 394–1015 ng/g) at the lowest NDVI values (Fig. 2A). Rainfall (range: 0–27.5 ml) had a significant negative effect (Table 1) and mean FGM were highest (361 ng/g, CI = 241–539 ng/g) with no rainfall, and lowest (280 ng/g, CI = 185–426 ng/g) with relatively high rainfall (mean ± SE = 17 ± 0.37 mL; Fig. 2B).

FGM levels were significantly higher in areas with greater settlement density (Table 1), such that mean FGM levels increased from 252 ng/g (CI = 167–380 ng/g) at lowest settlement density to 1050 ng/g (CI = 456–2413 ng/g) at highest settlement density (Fig. 3A). Furthermore, we found that impala had significantly higher hormone levels at the border of the SNP (330 ng/g, CI = 222–491 ng/g; Table 1), while hormone levels decreased as distance to border increased whether inside or outside of the park (Fig. 3B). Management strategies across the region did not influence impala FGM levels as predicted (Fig. 3C; Table 1). Based on the management strategies, impala FGM concentrations in cSNP, sSNP and nSNP were expected to be similar, but lower than wSNP. Higher FGM values were expected in GIGR followed by IWMA and lastly LGCA. However, impala in sSNP tended to have the highest FGM levels (676 ng/g, CI = 340–1342 ng/g), followed equally (i.e. no significant difference these areas) by impala living in cSNP, wSNP and IWMA (m_{cSNP} = 418 ng/g, CI_{cSNP} = 263–664 ng/g). Impala in LGCA, GIGR and nSNP had the lowest FGM levels (m_{LGCA} = 218 ng/g, CI_{LGCA} = 128–371 ng/g; Table 1).

Impala mean FGM levels were significantly higher at dawn (6 am; 572 ng/g, CI = 333–983 ng/g) and dusk (6 pm; 413 ng/g, CI = 257–665 ng/g) and lowest at noon (1 pm; 323 ng/g, CI = 218–479 ng/g; Table 1). However, we accounted for this variation in our analysis and thus, these findings do not confound our results. Additionally, although FGM levels were significantly higher in territorial males compared to bachelors, adding this as a separate variable in the basic model did not improve the model fit and was therefore excluded.

Importantly, since the interaction model had a ΔAICc value of 1.03 compared to the final model and adhering to the principle of parsimony, this means that the addition of the interaction term did not significantly improve the amount of variation in FGM explained by the model. We therefore conclude that there was no support for an interaction between NDVI and settlement density in our data (Fig. 4). The most influential predictor was NDVI, regardless of human disturbance levels; NDVI alone explained as much as 20% of the variation in impala FGM concentrations.

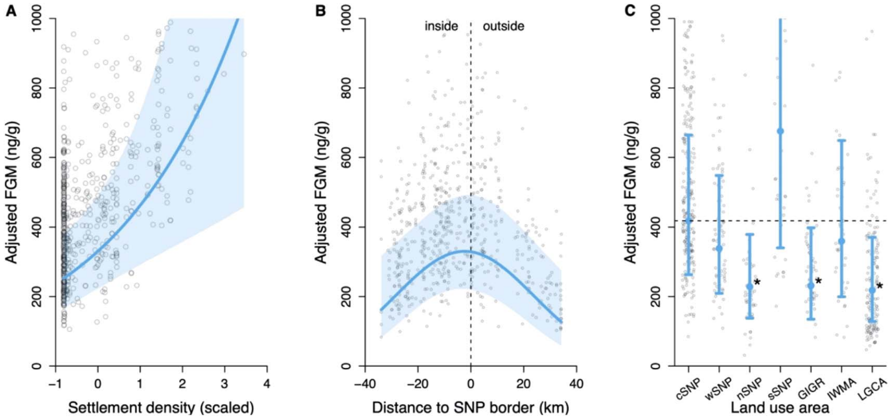

Figure 3: Changes in impala FGM concentrations due to environmental factors. The effect (blue line) of (**A**) Settlement density (kernel density estimate; scaled), (**B**) shortest Euclidean distance to nearest boundary of Serengeti National Park (SNP; dashed line) in kilometre and (**C**) land use area on impala faecal glucocorticoid metabolite (FGM) concentration. Adjusted response values are represented as points; 95% confidence interval is the shaded blue area or the error bars. On panel C, stars denote significant difference from cSNP (dashed line; $P < 0.05$).

Discussion

We tested the hypothesis that forage quality and anthropogenic land use would significantly affect FGM levels in wild impala. As predicted, impala experiencing lower forage quality had elevated FGM levels. Impala FGM concentrations increased with heightened levels of human disturbance, but levels differed unexpectedly in areas with different management regimes. There was no interaction between NDVI and settlement density, and our results show that NDVI was the most important factor predicting FGM levels in impala, regardless of human disturbance.

Forage quality

We found that impala FGM levels significantly increased with decreasing NDVI (Figs. 2A and 4). The Serengeti ecosystem is a semi-arid savanna habitat (Sinclair *et al.*, 2008), and the nutrient-rich grassy vegetation recedes drastically during the dry season, forcing impala to include more browse in their diet. This corroborates previous findings that GC concentrations correlate negatively with food abundance (Busch and Hayward, 2009). To our knowledge, NDVI has only twice been used as a proxy for forage quality in relation to FGMs in wild ungulates. Stabach *et al.* (2015) found a strong negative relation between the change in NDVI over 2 weeks and FGMs in blue wildebeest, indicating that nutrient poor dry or senescent grass may lead to higher FGM concentrations in wildebeest. FGM levels of Asian elephants (*Elephas maximus*) were found to negatively correlate with NDVI values (Pokharel *et al.*, 2018). Similarly, even when controlling for the effect of predation pressure, song sparrows (*Melospiza melodia*) were found to have significantly higher GC levels when experiencing low food abundance (Clinchy *et al.*, 2004). Thus, we expect that it is a shift to a less nutrient-rich diet when NDVI is low that results in greater FGM levels for impala.

We also found that impala FGM levels were significantly higher when there had been no rainfall in the past week, compared to when there was relatively high rainfall (Fig. 2B). Droughts are associated with reduced forage quality for impala, as grassy vegetation recedes drastically during extended period of no rainfall. That impala are sensitive to climatic conditions, having the greatest FGM levels in areas with poor vegetation and drought like conditions, was expected. In red deer (*Cervus elaphus*), variation in FGMs was better explained when including stochastic weather events, such as flash floods, indicating that such weather events might be relevant environmental stressors (Corlatti *et al.*, 2011).

Climate change is predicted to have severe effects in eastern Africa, with higher temperatures and increased variability in rainfall potentially leading to increased number of inclement weather events and seasonal declines in abundance of nutrient-rich grasses (Niang *et al.*, 2014). We found that impala experienced elevated FGM levels when forage quality was low, and when rainfall was absent, and therefore FGM levels are likely to further increase in the future. Additionally, since forage quality is an important predictor of reproductive success (Parker *et al.*, 2009), a decline in green, nutrient-rich vegetation through both climate and human land use change is likely to impact population persistence of impala and other herbivores, especially exclusive grazers who cannot shift their diet to include more browse (Parker *et al.*, 2009).

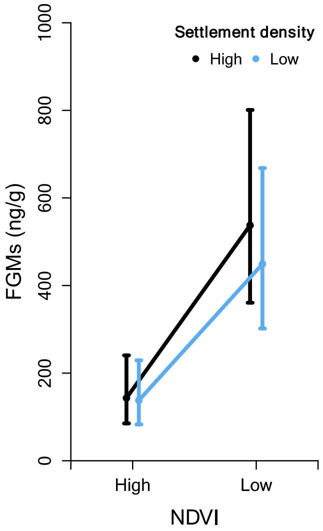

Figure 4: Interaction between NDVI and settlement density on impala FGM concentrations. The effect of high and low normalized difference vegetation index (NDVI) values on impala faecal glucocorticoid metabolite (FGM) concentrations when in high (black line) and low (light coloured line) settlement density (SD). High values are those higher than third quantile, and low values are those lower than first quantile. Includes 176 data points: 45 high SD and NDVI; 34 high SD, low NDVI; 40 low SD and high NDVI; 53 low SD and NDVI. Error bars show standard error of the estimate.

Human disturbance

Impala FGM concentrations increased with increasing settlement density (Fig. 3A). Increasing human density is associated with both direct human–wildlife conflicts and indirect human effects such as increased competition with livestock. For example, impala may adjust their daily activity in areas with higher human disturbance, reducing daytime activity, increasing afternoon activity and omitting their midday rest (Wronski *et al.*, 2015). Time spent vigilant, which is considered a costly behaviour, increases in impala and other ungulates in relation to human disturbances (Caro, 2005; Setsaas *et al.*, 2018). Similarly, GC concentrations can increase in ungulates due to human-related disturbances such as infrastructure and traffic (Creel *et al.*, 2002; Formenti *et al.*, 2018), and livestock and human presence (Stabach *et al.*, 2015). Lunde *et al.* (2016) found that impala in the Serengeti ecosystem had elevated FGM concentrations in relation to increased road type and traffic.

Furthermore, in areas with higher livestock densities, impala and livestock are likely competing for limited resources, especially during the dry season, adjusting their behaviour and thus increasing the energetic cost to obtain nutritious forage (Odadi *et al.*, 2011). Cattle in particular have been shown to suppress wildlife populations (Riginos *et al.*, 2012). We suggest that this increased habitat and forage competition with livestock, together with increased interactions with humans, results in an increased energy expenditure to obtain sufficient resources, and thus increased FGM concentrations in impala.

Impala FGM concentrations significantly increased with increasing proximity to the SNP border, regardless of whether impala were inside or outside of the park (Fig. 3B). We expected FGM levels to be lowest inside the park and increase with increasing distance from the park boundary. African elephants exhibited elevated FGM levels outside of protected areas, compared to inside (Tingvold *et al.*, 2013; Hunninck *et al.*, 2017), and lions (*Panthera leo*) had lower FGM concentrations when residing inside a conservation area, compared to those in a buffer zone with human settlements (Creel *et al.*, 2013). SNP has a rapidly growing human population density just outside of its borders (Estes *et al.*, 2012). The phenomenon of higher population density around protected areas is not unique to SNP; in fact, this pattern is evident in most countries in Africa and South America (Wittemyer *et al.*, 2008). Though this does not indubitably lead to increased disturbance in the surrounding natural areas, when combined with greater poverty near the park, land conversion and illegal activities (such as poaching and illegal grazing) tend to concentrate around the park boundaries (Estes *et al.*, 2012). Furthermore, Veldhuis *et al.* (2019) showed that intrusions of human activities into SNP are also concentrated at its borders. These intrusions can have far-reaching effects in the Serengeti ecosystem, such as displacing wildlife and reducing soil carbon storage. Our results indicate that the concentration of human activities and disturbances around the park boundaries, coined the 'Serengeti squeeze', could result in elevated FGM concentrations in impala living closer to the park boundary (Veldhuis *et al.*, 2019).

Contrary to our predictions, impala in most study areas with higher protection and reduced human land use practices did not have lower FGM levels. We observed large variation in impala FGM concentrations within the national park, with nSNP having significantly lower FGM levels and impala in sSNP tending to have higher FGM levels to those in cSNP (Fig. 3C). This variation within the park could be partly due to varying levels of illegal poaching in SNP; however, recent studies are lacking to confirm this. Strikingly, impala in LGCA and GIGR, where they are arguably most affected by human disturbance, had significantly lower FGM levels than those in cSNP. Comparing GC levels in populations between management areas has given counterintuitive results before, indicating that the relationship between human activities and FGM levels in wild populations are not straightforward. African elephants living on communal lands where human activities and livestock are present did not show elevated FGM levels compared to those in protected areas (Ahlering *et al.*, 2013). Similarly, forest elephants (*Loxodonta cyclotis*) were found to have lower FGM concentrations outside of protected areas (Munshi-South *et al.*, 2008). Indeed, below, we discuss two mechanisms by which human activities could lower FGM levels in impala.

Using coarse-scale artificial spatial categorizations such as 'inside vs outside a protected area', however, might not fully represent the variation in FGM levels. Combining with or using instead relevant spatially explicit proxies of human disturbance, such as settlement density and proximity to protected area boundary, could perhaps provide better insight in FGM variation. Although environmental proxies such as NDVI are globally available at a high spatial and temporal resolution, this is often not the case for proxies of human disturbance. Especially for studies covering a large temporal and spatial extent such as presented here, accurate data on human disturbance is usually not available. The proxies of human disturbance presented in this study lack temporal resolution; however, they are unlikely to vary considerably within and between years; for example, impala residing in areas with high settlement density are likely to experience human disturbance throughout the year.

Can human protection offset human disturbance?

We found that NDVI was a clear driver of FGM levels in impala, explaining 20% of the variation in FGM levels (while the full model explained 28%). Although the effect was comparatively weak, human disturbance did significantly increase FGM levels in impala. We found no evidence of an interaction between NDVI and human disturbance, however, suggesting that the effects of human disturbance might be masked by the more important stressor of low forage quality (Fig. 4). Taken together, our results indicate that impala will have higher FGM levels when lacking nutritious vegetation even when in areas without any human disturbance. In other words, impala residing in human disturbed areas *with* plenty of nutritious forage will exhibit lower FGM levels than those impala in protected areas *without* good quality forage. Pokharel *et al.* (2018) found that crop-raiding Asian elephants, which are predicted to have higher FGM levels due to their increased interaction with humans (*see* Ahlering *et al.*, 2011), actually had lower FGM levels than elephants in the protected area. They found that crop-raiding elephants utilized more nutritious food sources, shown in part by higher NDVI values of the human-dominated areas. They conclude that improved diet could potentially function as a 'pacifier' against human-induced stress. Compared to SNP, mean NDVI in LGCA and GIGR was indeed significantly higher (Supplementary Fig. S1). These differences in NDVI could perhaps partly explain our results (see Supplementary Information S3).

Additionally, compared to SNP, surrounding areas such as LGCA also have considerably lower densities of large predators (*personal communication*). Studies have shown that GC levels can increase with higher perceived predation pressure (Clinchy *et al.*, 2013). Increased predation risk was also shown to considerably increase FGM concentrations in snowshoe hares, regardless of season and even during low predator density and low food quality (Sheriff *et al.*, 2011b). On the other hand, Chizzola *et al.* (2018) did not find a significant difference in FGM levels of impala and blue wildebeest living in areas with or without lions. Similarly, plains zebra (*Equus quagga*) living with lions did not have significantly higher FGM levels (Périquet *et al.*, 2017). Clearly, more studies are needed to disentangle the effect of predation risk on FGM (Boonstra, 2013). However, since large carnivores are abundant in the Serengeti—the park boasts one of the largest populations of lion (Swanson *et al.*, 2014)—and these predators are largely absent in human-dominated areas such as LGCA; this disparity could partly explain why impala in LGCA had lower FGM levels than those in cSNP. However, although human disturbance may influence FGM levels on an immediate level—perhaps functioning as a 'human-shield' by reducing predator density (Berger, 2007)—we propose that in the long term, the effect of forage quality far outweighs such disturbance for ungulates in the Serengeti ecosystem.

Conclusion

Here we show how the interaction between proxies of environmental and anthropogenic factors affects FGM levels in a wild ungulate. Our results demonstrate the importance of forage quality in determining FGM levels in impala, much more so than human disturbance. The proxies of human disturbance used in this study, however, did elicit higher FGM levels in impala. Climate change is predicted to increase the frequency of extreme weather events, potentially leading greater seasonal fluctuations forage quality. Though certain human activities undoubtedly have negative consequences for wildlife populations in protected areas such as in the Serengeti ecosystem, our results suggest that management should focus on ensuring forage quality through drought mitigation, habitat protection and sustainable land use, if they are to protect and conserve wild ungulates populations.

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Supplementary material

Supplementary material is available at *Conservation Physiology* online.

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Supplementary Information

Table S1. Number of samples collected in each collection period (CP) and in each study area. Numbers in brackets are those collected in the rainy season.

CP \ Area	cSNP	wSNP	nSNP	sSNP	GIGR	IWMA	LGCA	*total*
2012	80	34	26	17	18	1	15	191
2016	45(6)	8	6	0	18	14	51(12)	142(18)
2017	90(54)	22	0	0	12	6	16	214(54)
2018	38(12)	17	13	15	4	19	54(50)	160(62)
total	253(72)	81	45	32	52	40	136(62)	639(134)

S1. Calculation of percent woody cover and adjusting NDVI estimates

The data on percent woody cover (WC) were retrieved from the online Application for Extracting and Exploring Analysis Ready Samples (AppEEARS), courtesy of the NASA (https://lpdaacsvc.cr.usgs.gov/appeears/). These data are annual estimates of percent woody cover of the study are (Dimiceli et al., 2015). We extracted WC estimates of sample locations from the data specific to the year of collection (i.e. 2012, 2016, 2017, or 2018). WC estimates at sample locations were low (mean ± SD = 5.6% ± 3.2; N = 693). Even so, we created a separate variable with adjusted NDVI values by reducing the original NDVI estimate by a percentage equal to the WC. For example, if a datapoint had an NDVI = 0.2 and a WC = 10%, the adjusted NDVI = 0.18. Comparing the two full models including either NDVI or adjusted NDVI revealed no significant improvement of the model fit (AICc = 1340.191 and 1340.667, resp.).

S2. Additional information on potential confounders

Due to the fact that GCs are metabolized in the gut, factors affecting their metabolism could also affect FGM concentrations without reflecting a change in plasma GCs (Goymann, 2012). For example, it is possible that due to their diet change in the dry season (Wronski, 2002), impala ingest more fiber which could slow digestion, thus increasing GC metabolism and hence affecting FGM concentrations (Dantzer et al., 2011). In addition, impala tend to ingest more forage in the dry season than in the rainy season (Jarman & Jarman, 1973), potentially lowering FGM levels in the feces. However, these findings are ambiguous at most and are unlikely to affect our interpretation of the results as these effects, if present, would affect impala in all study areas similarly. The sample was collected for analysis if it had not been urinated on and less than 1h had passed before we were able to retrieve it (if for example we had to wait for the group to move). Fecal pellets from different parts of the pile were collected to account for any potential variation in hormone metabolite concentrations.

Studies show that in order to correctly interpret variations in FGM concentrations, it is important to either account for potential confounders in the study design, or by including such factors as predictors explaining FGM concentrations. Here, we included time-of-day as fixed predictor, since, although FGM concentrations reflect a cumulative measure of plasma GC over a certain timeframe, they have been shown to significantly change with an animal's diurnal activity, increasing FGM in dawn and dusk (Baker, Gobush, & Vynne, 2013; Palme, 2019). Our results corroborate this as FGM levels increased significantly in early and late hours of the day.

However, certain confounders are more difficult to account for to quantify than others. By including only adult females that were not visibly pregnant, we attempted to minimize the effect of reproductive status in impala, which can significantly affect GC concentration in plasma and feces (Dantzer, Fletcher, Boonstra, & Sheriff, 2014; Edwards & Boonstra, 2018). We cannot exclude, however, the possibility that certain impala were pregnant. In fact, it is likely that many of the individuals we sampled were at varying stages of their pregnancy, since in the subtropical Serengeti region, there is no well-defined breeding season and impala give birth throughout the year (Jarman & Jarman, 1973; Schenkel, 1965). However, this variation should be similar throughout the different years and areas, so failing to properly account for reproductive status is, other than introducing error or random variation, unlikely to affect our interpretation of the results.

S3. NDVI differences between study areas

To test whether the unexpected result, that impala in more human disturbed areas showed lower stress levels, could be partly explained by a difference in forage availability (as per Pokharel *et al.* 2018), we modelled the variation in NDVI by land use area. We used a linear mixed effect model with NDVI as response variable, land use area and season (Wet – Dry) as fixed effects, and collection period (CP) as random effects to obtain normally distributed residuals that showed no pattern in the residual pot. The model had a marginal R^2 of 7.8%, indicating that the combination of land use area and season did not explain much variation in NDVI. Compared to cSNP, mean NDVI in LGCA and GIGR was significantly higher; NDVI was also significantly higher in nSNP and sSNP compared to cSNP (Fig. S1).

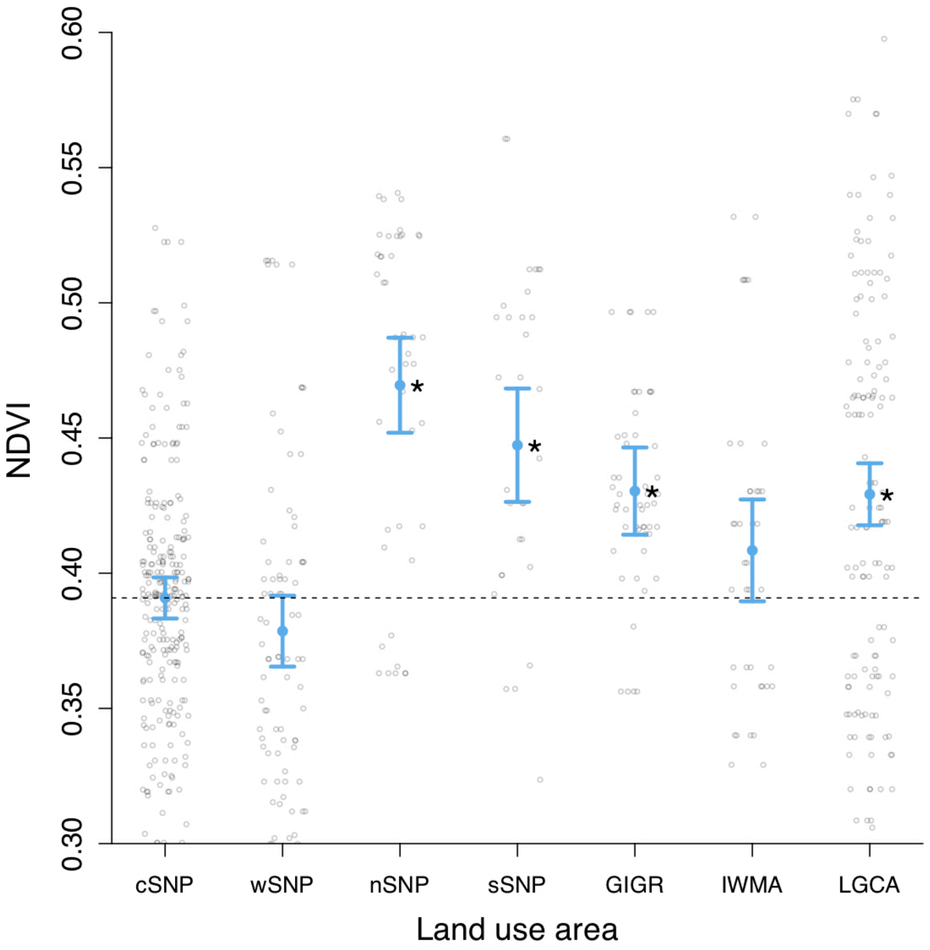

***Fig. S1 Mean NDVI in different land use areas**. The effect of land use area on normalized difference vegetation index (NDVI) values. Dashed line indicates reference (i.e. cSNP). Error bars show standard error of the estimate, star indicates significant difference (p < 0.001).*

Table S2 Correlation matrix of fixed effects in final model.

		(Intercept)	NDVI	Settlement density	Land use area					Distance to SNP (lin.)	Distance to SNP (qua.)	Time-of-day (lin.)	Time-of-day (qua.)	Rainfall
					wSNP	nSNP	sSNP	GIGR	IWMA	LGCA				Low
NDVI		-0.709												
Settlement density		0.055	0.101											
Land use area	wSNP	-0.23	0.011	-0.456										
	nSNP	-0.052	-0.24	-0.154	0.345									
	sSNP	0.046	-0.167	-0.067	0.06	0.115								
	GIGR	-0.13	-0.163	-0.227	0.418	0.426	-0.026							
	IWMA	-0.17	-0.048	-0.005	0.325	0.353	-0.041	0.457						
	LGCA	-0.127	-0.257	-0.265	0.419	0.548	0.038	0.613	0.495					
Distance to SNP (lin.)		0.082	0.012	-0.492	0.001	-0.215	0.266	-0.388	-0.445	-0.487				
Distance to SNP (qua.)		-0.11	0.134	-0.217	0.32	0.09	-0.357	0.213	0.208	-0.013	-0.003			
Time-of-day (lin.)		0.066	-0.031	0.031	-0.047	-0.04	-0.041	0.026	-0.024	-0.033	-0.044	0.028		
Time-of-day (qua.)		0.036	-0.136	-0.075	0.12	0.048	0.124	0.162	0.048	0.168	-0.064	-0.04	0.016	
Rainfall	Low	-0.087	-0.056	0.027	-0.108	0.015	-0.012	-0.061	-0.138	0.09	0.107	-0.081	-0.123	-0.009	
	High	-0.083	-0.109	-0.139	0.03	0.113	0.068	0.114	0.02	0.101	0.145	-0.117	-0.064	0.038	0.444

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PAPER II

TITLE PAGE

Article Title: Triiodothyronine (T3) levels fluctuate in response to thermoregulation rather than nutritional status in a wild tropical ungulate

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Lay summary:
Thyroid hormones (THs), traditionally regarded as indicator of nutritional status, were mainly driven by variation in ambient temperature, and were negatively related to food quality. THs may be better indicators of metabolic responses to environmental changes and only in extreme cases indicate nutritional status in wild animals.

Key words: validation; thyroid hormones; stress; glucocorticoid; impala; Serengeti

Abstract

Animals can employ a range of physiological mechanisms in response to unpredictable changes within their environment, such as changes in food availability and human disturbances. For example, impala exhibit higher fecal glucocorticoid metabolites (FGM) levels – indicative of physiological stress – in response to low food quality and higher human disturbance. In this study, we measured fecal triiodothyronine (T3) metabolite (FTM) levels in 446 wild impala to test the hypothesis that environmental and human disturbances would affect their nutritional status. We predicted that individuals would have lower FTM levels, indicative of poor nutritional status, 1) when food quality was poor, and 2) in areas of high human disturbance (due to competition with livestock). Examining both FGM and FTM levels together, we predicted that individuals with high FGM levels would have low FTM levels. Since T3 drives thermoregulation, we included ambient temperature as a co-variate in our analyses. Surprisingly, we found that temperature was the most important predictor of FTM – FTM levels decreased by 70% from lowest to highest temperatures – and food quality and human disturbance only influenced FTM levels when accounting for temperature. FTM levels tended to increase with increasing FGM levels. Our results suggest that food quality and availability may only partially influence FTM levels, and that fluctuations in temperature may be a greater driver of FTM levels in wild impala. Given that thyroid hormones are primarily responsible for regulating metabolic rate, they may be better indicators of how wild animals metabolically and energetically respond to environmental factors and only indicate poor nutritional status in the most extreme cases.

Introduction

Anthropogenic disturbances are greatly affecting wild animal populations both through direct human-wildlife interactions and through human- and climate-induced changes in food availability (Midgley and Bond, 2015; Segan et al., 2016). For example, in East Africa, climate change is severely altering weather patterns and reducing forage quality (Boko et al., 2007; Niang et al., 2014; Pettorelli et al., 2005). Wildlife populations have already declined dramatically in the region, particularly in areas with high human disturbance (Ogutu et al., 2009; Veldhuis et al., 2019). While some animals may cope with and adapt to these changes via behavioral and physiological mechanisms (Sih et al., 2011), many populations face extinction. One of the primary physiological indicators used to understand the impact of environmental stressors on animal populations are glucocorticoid hormones (GCs; also termed 'physiological stress hormones'), given their direct relationship to individual fitness (Bonier et al., 2009; Breuner et al., 2008; Dantzer et al., 2014; Sheriff et al., 2011). For example, high GCs were found to predict lower survival rates in both ring-tailed lemurs (*Lemur catta*; Pride, 2005) and cliff swallows (*Petrochelidon pyrrhonota*; Breuner et al., 2008; but see Brown et al., 2005). Thus, understanding the physiological response of animals may provide key insights into how environmental stressors can alter individual fitness and population dynamics. Here, we investigated how food availability and human disturbance alters thyroid hormone levels – a different, yet equally important and understudied hormone – in a free-living tropical ungulate in the Serengeti ecosystem.

Thyroid hormones (THs) are produced as the end product of the activation of the hypothalamic-pituitary-thyroidal (HPT) axis (reviewed in Behringer et al., 2018). Their main function relates to the regulation of metabolic rates and as such they are important in metabolically active tissues such as brown adipose tissue and skeletal muscles (López et al., 2013). Studies have shown that when animals are food-deprived, such as during starvation or hibernation, thyroid hormone levels are significantly lower and this acts as an mechanism to reduce metabolic rate and save energy (Behringer et al., 2018). For example, yellow-breasted capuchins (*Sapajus xanthosternos*) were found to have lower FTM levels when energy intake was low (Schaebs et al., 2016), and TH levels in American black bears (*Ursus americanus*) decreased considerably and consistently during hibernation (Azizi et al., 1979). Studies have also shown that low levels of THs are associated with reduced reproductive success (Torre et al., 2014) and increased longevity (Bowers et al., 2013). THs have therefore been proposed as a good indicator of the nutritional status of an animal related to their overall fitness potential, with higher levels associated with better condition and higher fitness (Behringer et al., 2018). However, most studies on thyroid hormone physiology in mammals have been done in captivity, and studies on wild populations are lacking (Behringer et al., 2018).

In this study, we used a wild impala (*Aepyceros melampus*) population in the Serengeti ecosystem to investigate the influence of food availability and human disturbance on TH levels. Impala are an ideal species for this study given their small home ranges (typically between 5-10 km^2; Averbeck et al., 2010), high local abundance (Ford et al., 2014), and non-migratory behavior (Jarman, 1979). This allows us to compare individuals experiencing vastly different, spatially and temporally explicit variation in food quality and human disturbance. Working in the same ecosystem, we previously found that impala had greater GC levels when food quality was low and in areas with high human disturbance (Hunninck et al., 2020). Similarly, wildebeest (*Connochaetes taurinus*) GC levels decreased considerably in response to new plant growth (Stabach et al., 2015) and other studies have shown that animals often have higher GC levels when in areas with higher human disturbances (Ahlering et al., 2011; Creel et al., 2002; Hunninck et al., 2017). While human disturbance may directly influence GC levels in wild animals, it may also indirectly influence GC levels by reducing forage quality and availability. In the Serengeti ecosystem Masai pastoralists keep their cattle overnight in semi-permanent enclosures (‘boma’) from which they walk them to nutritious pastures during the day. Livestock herding has been shown to result in competition between wildlife and livestock (Prins, 2000; Young et al., 2005), and livestock grazing significantly decreases the available forage near such bomas (Riginos et al., 2012). Because THs do not respond to direct human disturbance, they (alongside GC levels) may provide important insights to how animals are impacted by food availability and human disturbance (Behringer et al., 2018).

We measured fecal thyroid hormone metabolite (FTM) levels to test the hypothesis that changes in food quality and anthropogenic disturbances would affect the nutritional status of wild impala living in the Serengeti ecosystem. Specifically, we predicted that individuals would have low FTM levels 1) when food quality was poor, and 2) in areas of high boma density, because higher boma density results in higher foraging competition and less forage availability. We also examined the relationship between FTM levels and FGM levels, and predicted that individuals with lower FTM levels, indicative of nutritional stress, would have higher FGM levels. We have previously shown that FGM levels increase with decreasing food quality (Hunninck et al., 2020). Low FTM levels would indicate a poor nutritional status of impala and possibly reduced fitness (Behringer et al., 2018). Prior to our use for this study, we also provide the first validation of this method for use in wild impala.

Methods

Study system and species

The Serengeti ecosystem (± 25 000 km^2) is characterized by strong geographic and seasonal variation in rainfall, varying from 450 mm rainfall in the southeast to over 1400 mm in the north (Ogutu et al., 2014). Mean annual variation in temperatures ranges from 15°C to 28°C (range = 10°C - 35°C; Seronera weather station; data courtesy of TAWIRI). The ecosystem consists of a core area, Serengeti National Park (SNP), and 6 adjacent buffer areas that have varying levels of protection status. Our study area was limited to SNP and the buffer area Loliondo Game Controlled Area (LGCA) to the east, which, contrary to SNP, allows the building of temporary and permanent settlement, agricultural activities, regulated hunting, and traditional livestock herding or pastoralism.

Impala are a medium-sized, gregarious ungulate common in Eastern and Southern Africa (IUCN SSC Antelope Specialist Group, 2016). They are usually found in distinct groups: female herds (consisting of a territorial male, females and juveniles), bachelor herds (consisting of adult and sub-adult non-territorial males), and solitary males. Impala are mixed feeders, preferring high quality forage (i.e. nutritious, more palatable green grasses) when available, but including higher quantities of low quality forage (i.e. browse) into their diet when grassy vegetation becomes dry

(Codron et al., 2007; Jarman and Jarman, 1973; Wronski, 2002). Impala are constricted to relatively small home ranges throughout the year, typically between 5-10 km^2 (Averbeck, 2001). Though impala can be locally abundant, their specific habitat requirements – preferring the edge of open savanna and open woodland – result in a patchy distribution (Ford et al., 2014).

Collection of fecal samples

We collected 446 fecal samples from individual adult impala over three years (2016, 2017, and 2018) in both wet and dry seasons. We only collected fecal samples from individuals that were visually checked to have a good body condition (e.g. no injuries, not pregnant, no ribs showing). A detailed description of fecal sample collection can be found in Hunninck *et al.* (2020). In brief, we recorded the sex of the individual, and the herd size and type (family or bachelor herd). We noted GPS location and the time of collection. We prevented pseudo-replication (i.e. sampling the same individual more than once) by not returning to the same location within a collection period. Samples were immediately placed on ice and, within 12h of defecation, stored at -20°C until further analysis.

Analyses of fecal samples and validation of radioimmunoassay

In order to correctly interpret the variations in hormone concentrations, it is essential to validate the method used to measure hormone metabolite concentrations in feces in one's study species (Touma and Palme, 2005). The hormone extraction method was based on (Palme, 2005) and detailed in (Hunninck et al., 2020). Briefly, fecal samples were defrosted at room temperature (30 min) and homogenized. Around 0.5 ± 0.001g feces were mixed with 5 ml of 80% methanol, vortexed (1 min), and then centrifuged (20 min; 2500 *g*). We evaporated 0.5 ml of supernatant. To determine FTM levels, we used ^{125}I Total Triiodothyronine (T3) radioimmunoassay (RIA) kits (Catalogue No.06B254216, MP Biomedicals, Costa Mesa, CA). We adhered to the manufacturer's protocol, with the exception that we halved the volume of all reagents to ensure FTM levels were detectable by the assay. We validated the procedure through the use of standard assay validations of parallelism and precision. Parallelism analysis tests whether the assay maintains linearity under dilution (Andreasson et al., 2015). Precision was determined via intra-assay variation calculated by averaging coefficients of variation of all samples, and via inter-assay variation calculated by calculating the coefficient of variation of measured FTM of one standard between assays. Cross-reactivity of the primary antibody, as reported by the manufacturer, is 100% with L-triiodothyronine (T3), 0.18% with L-thyroxine (T4), 0.44% with 3,5-diiodothyronine (T2), 0.01% with 3,3',5'-triiodothyronine (rT3), and <0.01% for 3,5-diiodotyrosine, phenylbutazone, sodium salicylate, diphenylhydantoin, and dicumerol.

Environmental variables

Vegetation and temperature data were retrieved from the online Application for Extracting and Exploring Analysis Ready Samples (*AppEEARS*), courtesy of NASA (https://lpdaacsvc.cr.usgs.gov/appeears/). We collected data on normalized difference vegetation index (NDVI – a proxy for forage quality; MOD13Q1 & MYD13Q1 MODIS/Terra & Aqua; temporal resolution (TR) = every 8 days, spatial resolution (SR) = 250m; Didan, 2015) and percent woody cover (WC; MOD44B MODIS/Terra; TR = yearly, SR = 250m; Dimiceli et al., 2015), and land surface temperature (LST; MOD11A1 MODIS/Terra; TR = daily, SR = 1000m; Wan Z., 2015). Using the pixel reliability dataset that accompanies the NDVI dataset, pixels containing clouds were filtered out, and data were adjusted to account for empty data points using a Savitzky-Golay smoothing filter. In order to obtain an NDVI value specific to each of our fecal samples with regards to location and time of collection, we extracted the closest value in space and time (Hunninck et al., 2020). To obtain a better representation of relevant variation in temperature, we calculated the average temperature over a 7-day period prior to sample collection for each sample, specific to its location, which made sure we had no empty values in our data. Impala have small home ranges and limited movement – impala equipped with a GPS collar moved on average 262m in 3 hours (SD = 247, N = 212000; *unpublished data*) away from their initial location. Therefore, our data (NDVI and Temperature) provides a reasonable representation of the environment utilized by the sampled impala over the past week (previously used in Hunninck et al., 2020).

We performed several analyses to validate the use of LST as a proxy for ambient temperature in our system. First, as LST is significantly affected by variation in land cover (i.e. certain surfaces heat up more than other), we analyzed the proportion of woody cover in the areas where we collected samples. Mean woody cover was negligibly low (mean ± SD = 5 ± 3.2, N = 446), and no human made structures were present at our sample locations. Therefore, variation in land cover between sampling locations did not affect our LST measurements. Secondly, we correlated our weekly average LST estimates with concurrent weekly averages of maximum air temperature measured at the Seronera weather station (located in central SNP). Though this correlation was 0.66 (N = 88), we believe LST can sufficiently approximate a relevant measure of ambient temperature. Lastly, since NDVI measurements are used in the calculation of LST, we first correlated NDVI and LST measures from all cells in the study system on one day (r = -0.50), and secondly calculated the difference in LST given an NDVI of 0 and 1 (i.e. min and max values of NDVI) while keeping surface reflectance constant (the only other input variable to calculate LST). This resulted in only a 0.01°C difference in LST, showing that NDVI has a negligible impact on LST estimation. We transformed the LST values based on maximum air temperatures from Seronera to better represent temperature range experienced by impala. Taken together, these analyses show that LST is a useful and relevant proxy for ambient temperature in our study system, and that NDVI and LST are practically independent and can be used as separate variables in regression models (Hunninck et al., 2020).

Statistics

We used multiple linear mixed-effects regression models (*lmer* function of the *lme4* package v.1.1-17; Bates *et al.*, 2015) with FTM as response variable. The response variable was log-transformed to obtain normal distribution of model residuals. For our initial model, we included 1) NDVI (i.e., forage quality in the landscape), 2) boma density (i.e. human and livestock disturbance; None, Low, High), and 3) FGM as main predictors. We also constructed a second model which added temperature to the initial model (i.e. proxy for ambient temperature, based on maximum daily air temperature and LST) to control for the potential confounding effect related to the involvement of T3 in thermoregulation (i.e. 'final model'). We included group ID as a random effect to control for differences among groups; differences among sampling locations did not explain any additional variation and was therefore not included in the model. Residuals were visually checked for normality and heteroskedasticity, and multicollinearity was assessed with a generalized variation inflation factor (GVIF) analysis, which is a measure of the harm done by collinearity among predictors (Fox and Weisberg, 2011). No heteroskedasticity was found, and residuals were normally distributed; GVIF values corrected for the degrees of freedom (*GVIF^[1/(2*df)]*) were all lower than 1.23 (*vif* function of the *car* package v.3.0-0 in R (Fox and Weisberg, 2011)), which is well below the conservative threshold of 3 (Zuur et al., 2010). All statistical analyses were performed in the statistical program *R*, v.3.5.0 (RCoreTeam, 2018), using RStudio v.1.1.453 (RStudio, 2016).

Results

Validation of FTM assay

Serial dilutions of fecal FTM extracts resulted in displacement curves that were parallel to the standard T3 curve (Quotient of slopes = 0.97). Inter-assay variation was 5% and average intra-assay variation was 4% ± 2 (mean ± SD, N = 499).

Model results

In the initial model, none of the predictors (i.e. NDVI, boma density, and *log*(FGM)) significantly affected the variation in FTM levels ($p > 0.09$; Table 1).

However, in the final model which included temperature as a fixed effect, we found that impala had significantly higher FTM levels in areas with lower NDVI values, such that mean FTM levels increased by 44% from 566 ng/g (95% confidence interval (CI) = 456 - 703 ng/g) at the highest NDVI values to 1018 ng/g (CI = 876 - 1183 ng/g) at the lowest NDVI values (Table 1; Figure 1A). Impala mean FTM levels where significantly higher (20%) in areas without the presence of bomas (869 ng/g; CI = 786 - 960 ng/g) compared to areas with high boma density (691 ng/g; CI = 599 - 797 ng/g; Table 1; Figure 1B). FTM levels tended to increase with increasing FGM levels, such that mean FTM levels increased by 28% from 674 ng/g (CI = 562 - 808 ng/g) at the lowest FGM levels to 935 ng/g (CI = 772 - 1133 ng/g) at the highest FGM levels (Table 1). FTM levels increased significantly with decreasing Temperature, such that mean FTM levels increased by 70% from 414 ng/g (CI = 338 - 507 ng/g) at the highest temperature levels to 1358 ng/g (CI = 1147 - 1609 ng/g) at the lowest temperature (Table 1; Figure 1C).

Importantly, temperature was the most influential predictor in the final model (Table 1), having an effect size approximately twice as big as NDVI (Table 1). The final model explained 40% of the variation in impala FTM levels (*conditional* R^2); fixed effects (i.e. *marginal* R^2) explained 16% of the variation (Nakagawa and Schielzeth, 2013).

Discussion

We investigated the relationship between FTM level and changes in food quality and anthropogenic disturbances in wild impala. We hypothesized that FTM, a proxy for nutritional status, would be influenced by variation in food quality. However, we found that food quality, human disturbance, and FGM levels did not directly influence FTM levels. Only when we accounted for variation in ambient temperature did we find an effect of food quality and human disturbance on FTM levels. Furthermore, we found that ambient temperature was the most important predictor of FTM levels; an unexpected result. This implies that thermoregulation, and not nutritional stress, foremost drives FTM levels in free-living impala.

The effects of food quality on FTM levels

We found that FTM levels were not directly affected by NDVI, a measure for food quality in the landscape. THs have been studied extensively in relation to the nutritional status of animals, and many studies have found that FTM levels decrease significantly when animals experience low food availability, such as during starvation or fasting (Behringer et al., 2018). For example, howler monkeys (*Alouatta palliata*) and yellow-breasted capuchins were found to have lower FTM levels when energy intake was low (Dias et al., 2017; Schaebs et al., 2016), and European black bears (*Ursus arctus arctus*) were found to have significantly lower thyroid hormone levels both during times of reduced food and during hibernation (Tomasi et al., 1998). In these situations, low FTM values are indicative of an energy saving mechanism, as this would result in a slower metabolism. In our study, we found that FTM levels were only related to NDVI when we accounted for ambient temperature, but the relationship was negative such that impala had high FTM levels when food quality was low. This could be explained by two non-exclusive mechanisms. First, impala may employ an energy saving mechanism when food quality is high. Impala have been shown to store fat reserves during the wet season when food quality is high, thus having low THs at this time period would increase their ability to store energy. Stored energy can then be mobilized during energetically challenging situations such as pregnancy or rut to maintain energy balance (Dunham and Murray, 1982). Secondly, impala may increase THs when food quality is low. Unlike large bodied ungulates in artic regions, tropical ungulates often lack extensive subcutaneous fat reserves (but instead rely in part on kidney fat) as these may reduce heat tolerance (Owen-Smith, 1997). To maintain energy balance when food quality is low, these ungulates increase foraging time to maximize forage intake (i.e., quantity; Jarman and Jarman, 1973). For example, Owen-Smith (1997) found that kudu (*Trugelaphus strepsiceros*) had similar energy intake rates throughout the year, even when forage quality decreased drastically during the dry periods. Kudu maintained their nutritional condition instead by increasing their foraging time and thus food intake, thereby compensating for the lack of forage quality by increasing forage quantity intake. Impala were similarly observed to increase time spent feeding during the dry season (Oliver et al., 2007; Wronski, 2002), and to increase their rumen volume to accommodate the increased forage intake at this time (Lane et al., 2014). Therefore, FTM levels might not reflect nutritional state *per se*, but rather be an adaptive response to changing nutritional value of their food: being lower when food quality is high as an energy saving mechanism, but needing to upregulate THs, and thus metabolic rate, when food quality is low to accommodate for increased food intake.

Although we did not find any evidence that lower forage quality resulted in lower FTM levels as would be expected in poor condition individuals, we did not sample any individuals that were visibly in poor body condition (i.e. ribs showing). Thus, we cannot rule out the possibility that low FTM levels may reflect nutritional deficiencies, particularly in animals where reduced food quality is not accompanied by an increase in food intake; our results suggest that FTM levels may reflect nutrient deficiencies only when an individual’s body condition deteriorates past a certain threshold (i.e., when they are starving).

The effects of human disturbance on FTM levels

FTM levels were not affected by boma density in the initial model, and only when we accounted for temperature did we find that FTM levels were lower in areas with higher boma density. Boma density results in lower forage availability and quality because of both foraging competition with and depletion of resources by livestock (Riginos et al., 2012). Evidence for competition between livestock and wildlife has been found before (Prins, 2000), for example, zebra (*Equus* spp.) were found to be 46% more abundant in areas where cattle was excluded (Young et al., 2005). The impact of human activities is not always straightforward, however, and human presence may also benefit wild animals. For example, agricultural crops – though it brings about human presence, pollution, and replaces natural habitat – can benefit wildlife populations by being nutritionally attractive, providing both high amounts of energy and minerals, as was found in crop raiding Asian elephants (*Elephas maximus*; Pokharel et al., 2018). Previously, we found impala had higher GC levels in areas with higher boma density, however, this result was negated if areas with high boma density also had high forage availability (Hunninck et al., 2020). Our results here further suggest that human disturbance may influence impala by reducing their forage availability and quality. While impala normally increase forage intake in food poor areas, human disturbance may limit this behavioral response, and thus the reduction in FTM in relation to boma density may act as an energy saving mechanism. Further studies are needed to tease apart the behavioral and physiological aspects of human disturbance on wild impala.

The relation between FTM and FGM levels

At the individual level, FTM levels tended to be higher when they had higher FGM levels. We predicted that individuals with low FTM levels would have greater FGM levels, indicative of poor nutritional status. Laboratory-based studies have shown that the activation of the hypothalamic-pituitary-adrenal (HPA) axis – responsible for the production of GCs – decreased TH levels in the body (Charmandari et al., 2005). Yet, few studies in wild animals have investigated both THs and GCs, and the relationship between THs and GCs is still unclear in free-living animals. In a declining population of free-living Hawaiian monk seals (*Neomonachus schauinslandi*) FGM levels were positively correlated with FTM levels, indicating that the seals had sufficient foraging opportunities to sustain basic functions (i.e., high FTM), but prey may be stressful to acquire, hence eliciting high FGM levels (Gobush et al., 2014). Similarly, there was a positive relationship between FTM and FGM in wild moose (*Alces alces*), with high FGM levels perhaps due to increased exposure to predators when moose increased foraging, thus increasing FTM levels (Jesmer et al., 2017). If animals increase their foraging behavior and food intake when food quality or availability is low, as impala do (Wronski, 2002), they may also increase their TH levels as we argue above. Increased foraging behavior may result in greater GC levels if it exposes animals to greater threats (e.g., risk of predation; Clinchy et al., 2013; Sheriff et al., 2011) or if animals require greater energy mobilization due to the increased foraging activity (Birt-Friesen et al., 1989; Jesmer et al., 2017). Clearly, much work is needed to better understand the relationship between GCs and THs in wild animals.

The effects of ambient temperature on FTM levels

We found that temperature was the most influential predictor of FTM levels, whereby FTM levels increased as ambient temperature decreased. Although THs are crucially important in thermogenesis and thermoregulation in homeotherms (Silva, 2006), we did not expect this to be the main driver of FTM variation in a tropical ungulate. THs, especially T3, are essential for facultative thermogenesis – the main mechanism extensively used by endotherms to maintain homeothermy – and it has been shown that they increase in concentration in response to cold temperatures. For example, barbary macaques (*Macaca sylvanus*) were found to have significantly higher TH levels when ambient temperatures decreased (Cristóbal-Azkarate et al., 2016), and burros (*Equus asinus*) and llamas (*Lama glama*) similarly increased TH levels in response to cold temperatures (El-Nouty et al., 1978).

The main mechanism by which THs regulate thermogenesis is by regulating brown adipose tissue (BAT) or by alternative forms of (non-shivering) facultative thermogenesis. These mechanisms are also important in tropical ungulates, which lack extensive subcutaneous fat reserves (Dunham and Murray, 1982). Since such fat reserves are particularly good insulators, they would make maintaining homeothermy a costly process for tropical ungulates, which often experience extreme heat (Owen-Smith, 1997). Indeed, impala showed signs of heat stress at ambient temperatures between 35-50°C (Maloiy and Hopcraft, 1971), negatively affecting their diurnal activity (Shrestha et al., 2014). However, lacking subcutaneous fat reserves can result in a higher vulnerability to even modest decreases in ambient temperature, and thus a strong effect of temperature on FTM (Mitchell et al., 2018). This vulnerability of tropical ungulates could explain why the importance of this thermoregulatory function of THs has not been described before in other free-living ungulates.

The importance of THs for thermoregulation could also explain the positive relationship between FTM and FGM levels. When ambient temperatures drop, mammals must generate heat through thermogenesis to maintain homeothermy, an energetically costly process mainly regulated by THs (Silva, 2006). Since GCs are critically important for gluconeogenesis (Exton, 1979) – a mechanism through which animals, especially ruminants (Young, 1977), mobilize energy – an increase in GCs, resulting in increased energy mobilization, might be necessary to facilitate the energy requirements for thermoregulation. Thus, if THs are primarily responsible for thermoregulation, this could in part explain the positive correlation between FTM and FGM levels found here and in other studies.

Conclusion

Understanding how environmental factors drive FTM levels in wild animals is important given the link between thyroid hormones and fitness (Behringer et al., 2018). Contrary to previous findings, we found that FTM levels increased, rather than decreased, with lower food quality and suggest this may be due to increased food intake seen in impala in response to lower food quality. As expected, we found lower FTM levels in impala living in areas with high boma density, likely due to competition with livestock; impala may not be able to compensate for the reduction in food availability by increasing foraging activity due to human activity and in such cases may need to lower THs. This suggestion is supported by our previous findings that impala have greater FGM levels in areas with higher boma density, but that such an effect is negated in areas with high food quality (Hunninck et al., 2020). Lastly, we found that ambient temperature was the most important factor driving FTM levels in wild impala. We suggest that thermoregulation may be a critical aspect of TH regulation and that THs may only provide an indication of nutritional status in extreme cases (e.g., starvation). Overall, we propose that in wild animals, TH levels should be considered as a metabolic or energetic regulator, and that variation in TH levels should be interpreted in relation to the energetic needs and state of an animal, rather than a strict indicator of nutritional status of an individual. Clearly, more studies on wild populations are needed to elucidate how THs facilitate energy regulation in response to rapid environmental change and human disturbances.

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Tables

Table 1. Model estimates from both initial and final mixed-effects models, explaining the variation in fecal total triiodothyronine (T3) metabolite (FTM) levels in impala. Scaled estimates represent relative effect size of predictors, allowing comparison among predictors within a model. Reference level for predictor Boma is 'None'. See text for further details.

Initial model: *log(FTM) ~ NDVI + log(FGM) + Boma density + (1|Group)*

Fixed effects	Est. ± SD	Scaled Est. ± SD	df	t-value	p-value	
(Intercept)	6.25 ± 0.29	6.68 ± 0.06	396.96	21.67	< 0.001	***
NDVI	0.08 ± 0.29	0.01 ± 0.04	137.01	0.28	0.782	
log(FGM)	0.07 ± 0.04	0.05 ± 0.03	440.81	1.68	0.093	.
Boma density – Low	-0.05 ± 0.1	-0.05 ± 0.1	130.47	-0.49	0.627	
Boma density – High	-0.05 ± 0.1	-0.05 ± 0.1	126.89	-0.5	0.619	
Random effects	**Variance**	**Std.Dev.**				
Group	0.16	0.4				
Residual	0.21	0.46				

Final model: *log(FTM) ~* ***Temperature*** *+ NDVI + log(FGM) + Boma density + (1|Group)*

Fixed effects	Est. ± SD	Scaled Est. ± SD	df	t-value	p-value	
(Intercept)	13.89 ± 1.16	6.75 ± 0.05	113.88	12	< 0.001	***
Temperature	-0.26 ± 0.04	-0.28 ± 0.04	108.3	-6.79	< 0.001	***
NDVI	-1.01 ± 0.29	-0.14 ± 0.04	114.58	-3.43	< 0.001	***
log(FGM)	0.07 ± 0.04	0.05 ± 0.03	414.48	1.85	0.065	.
Boma density – Low	-0.07 ± 0.08	-0.07 ± 0.08	115.14	-0.81	0.422	
Boma density – High	-0.23 ± 0.09	-0.23 ± 0.09	108.93	-2.59	0.011	*
Random effects	**Variance**	**Std.Dev.**				
Group	0.09	0.29				
Residual	0.22	0.47				

*Significance codes: P < 0.001 ***; 0.001–0.01 **; 0.01–0.05 *; 0.05–0.1 .*

Figures

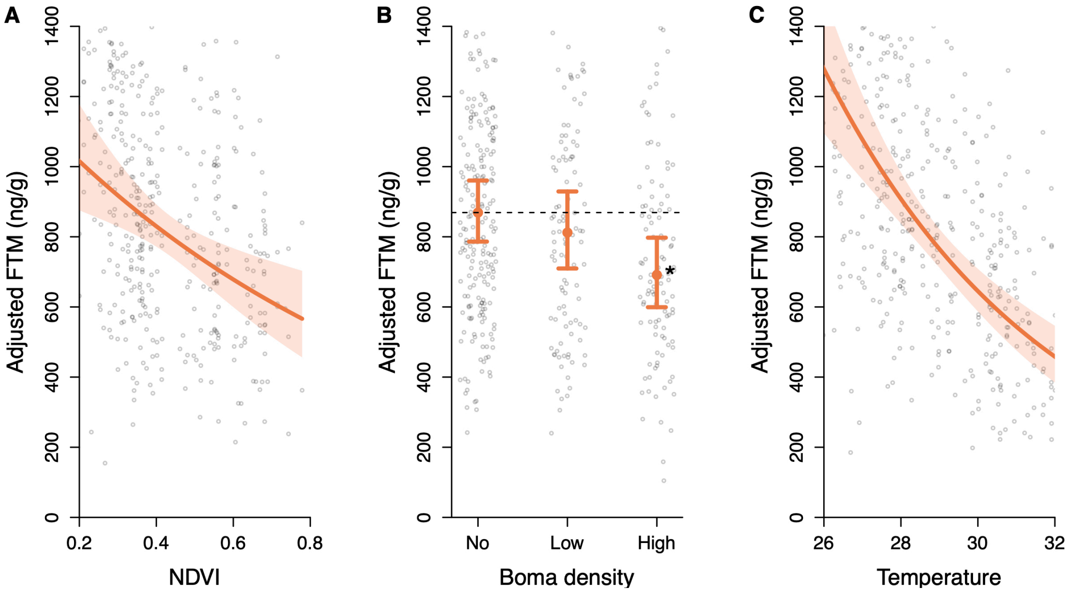

Figure 1. Final model effects on impala fecal total triiodothyronine (T3) metabolite (FTM) levels. The effect (red line) of (A) NDVI (Normalized Difference Vegetation Index; proxy for forage quality), (B) Boma density (categorized based on Kernel Density estimate of boma locations), and (C) Temperature (based on LST estimation) on impala FTM levels. Adjusted response values are represented as points; 95% confidence interval is the shaded red area or the error bars. On panel B, star denotes significant difference from reference level (dashed line; $P < 0.05$)

PAPER III

Title page

Manuscript title: Glucocorticoids may act as a proactive facilitator rather than a reactive cost of territoriality in male impala

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Highlights

- Territoriality is a common adaptation to minimize mating competition
- Territorial males had elevated GC levels, regardless of food availability
- Population density and herd size did not drive GC levels in territorial males
- GC could act as a facilitator for territoriality, increasing fitness

Abstract

Territoriality is a common behavioural adaptation, widespread among ungulate species that have intense competition for mating. Here, we tested the hypothesis that territorial individuals have higher glucocorticoid concentrations than non-territorial bachelors, in wild impala (*Aepyceros melampus*) in the Serengeti ecosystem. We also investigated how the relationship between territoriality and glucocorticoid levels is influenced by environmental context, specifically, food quality, population density (i.e., territory defence intensity), and herd size (i.e., mate defence effort). We collected 139 faecal samples over 4 years and analysed these for faecal glucocorticoid metabolites (FGMs). We used Normalised Difference Vegetation Index (NDVI) as a proxy for food quality, and population density was based on aerial surveys. Territorial males had significantly higher FGM concentrations than bachelors. Increased food quality did not affect FGM levels in territorial males, but decreased FGM levels in bachelors by 82%. Increased population density elevated FGM levels by 64%, but this effect was not different between territorial and bachelor males. Herd size did not affect FGM levels in territorial males. While elevated GC levels are often suggested to be a cost of being territorial, we argue these elevated GC levels may act as a facilitator and increase the ability of territorial males to maintain a territory by increasing energy mobilisation and metabolic rate, ultimately increasing their reproductive fitness. Appreciating that GCs are not simply costly but may have an adaptive, potentially facilitating role in an animal's life history is key if we are to understand HPA-axis reactivity and its potential in eco-physiological studies.

Keywords

Cortisol, sociality, rut, ungulate, Normalized Difference Vegetation Index, population density

1. Introduction

Sociality is a common adaptation in animals, as it can yield significant benefits including decreased *per capita* predation risk and knowledge sharing (Creel, Dantzer, Goymann, & Rubenstein, 2013). However, these benefits often come at a cost of, for example, increased resource and mate competition (Krause & Ruxton, 2002). Furthermore, social interactions between conspecifics can result in unpredictable or uncontrollable situations potentially causing adverse effects for the individuals involved. The unpredictable nature of these interactions can be potent stressors (Creel, 2001), and many studies have shown such interactions to consistently increase glucocorticoid (GC) secretion (reviewed in Creel et al., 2013). For example, territoriality and the associated antagonistic interactions between competitors have been shown to significantly increase GC concentrations in animals (Creel et al., 2013). Here, we test the hypothesis that territoriality increases GC secretion for the first time in a wild African ungulate. Additionally, we investigate how the relationship between territoriality and GC levels is influenced by environmental context, including the effects of food quality and population density.

Animals cope with and respond to unpredictable challenges in their environment (i.e. stressors) partly by activation of their hypothalamic-pituitary-adrenal (HPA) axis, which in turn releases glucocorticoid hormones (Boonstra, 2013). This adaptive physiological mechanism, and resulting changes in GC concentrations, affects a range of bodily functions allowing animals to better mitigate various stressors, such as predation risk, food deprivation, and dominance-subordinate interactions (Clinchy, Sheriff, & Zanette, 2013; Creel et al., 2013; Dantzer, Fletcher, Boonstra, & Sheriff, 2014). When the stressor is chronic (i.e., frequently recurring or constant over a longer time span), however, this adaptive response can result in adverse effects for the organism, such as suspended growth, lower immune function, increased energy expenditure, and potentially reduced reproduction and survival (Busch & Hayward, 2009; Sapolsky, Romero, & Munck, 2000). While measuring GCs does not equate to measuring ‘stress’, it may provide a robust assessment of animals’ overall health and their ability to cope with changes within their environment (Dantzer et al., 2014; Sheriff, Dantzer, Delehanty, Palme, & Boonstra, 2011). The quantification of GC levels is commonly done by measuring GC metabolite concentrations in faeces (faecal GC metabolites; FGMs); this is a fast, accurate, and non-invasive approach (Rupert Palme, 2019; Sheriff et al., 2011).

Territoriality is a common behavioural adaptation, also termed an alternative mating tactic, widespread among ungulate species that have intense competition for mating (Corlatti et al., 2012; Neff & Svensson, 2013; Owen-Smith, 1977). It is almost exclusively a male trait, and territorial males gain a temporal reproductive advantage by having nearly exclusive mating rights to females in their territory. Here, we define a territory as: a fixed area from which an individual defends against, excludes, or shows dominance over competitors; excluding them from resources and indicating ownership over an area (Maher & Lott, 2000; Owen-Smith, 1977). Territories are often established in areas that are resource rich, attracting females and thus granting the territorial male increased mating opportunities (Emlen & Oring, 1977) – though sometimes it is the size of the territory rather than the resources therein that females prefer (Vanpé et al., 2009).

There are three main drivers that can influence the costs of maintaining control over a territory, given that excluding competitors and herding females can be an energetically demanding occupation (Maher & Lott, 2000). First, food availability has been shown to be a major factor determining the intensity and occurrence of territorial behaviours (Carpenter, 1987); in environments with low food availability (i.e. energy deficiency), males might choose to not exhibit territoriality at all (Maher & Lott, 2000). In impala (*Aepyceros melampus*), about 70% of the territorial males can only hold a territory for less than three months, after which they are exhausted and replaced by a bachelor male (M. V. Jarman, 1979; Colin Malcolm Oliver, 2005). Second, the cost of maintaining a territory has been shown to increase with increasing population density, as more rivals need to be contended with and the frequency of agonistic behaviours increases (Corlatti, Caroli, Pietrocini, & Lovari, 2013;

Creel et al., 2013). Third, increasing the number of females within a defended group is expected to increase the energetic cost of maintaining a territory, given the increased herding and exclusion behaviours needed (M. V. Jarman, 1979), as was found in red deer (*Cervus elaphus*; Pavitt et al., 2015). As such, we expect these factors to be the main predictors of GC levels in relation to territoriality in impala.

Impala are an excellent species to investigate how territoriality influences GC levels across different contexts. They are a gregarious and polygynous antelope in eastern and southern Africa (P. J. Jarman, 1974). Group sizes vary considerably, with bachelor herds reaching up to 60 individuals, and female herds (i.e. juveniles and adult females) ranging from a few to over 100 individuals (Owen-Smith, 1977). Their population density can be irregular and clumped, but due to their ability to consume both grasses and browse, their local densities can reach up to 600 individuals/km^2 (Averbeck, 2001; Rosenblatt et al., 2019). Territorial impala vary in their degree of sociality. Contrary to southern Africa populations, in equatorial areas such as our study area, the Serengeti ecosystem in Tanzania, impala males tend to be territorial year-round, and are thus practically non-social most of the year. Territorial males are either solitary or associated with a female herd. The majority of males in a population, however, do not hold a territory and are referred to as bachelors, which often aggregate in bachelor herds. These bachelor herds consist of both aspiring males (i.e., those that have not previously had a territory) and defeated males (i.e., those that previously held a territory but have been defeated by a competitor; Jarman, 1979). Agonistic interactions between territorial males and rivals often result in highly ritualized, complex displays, which can include vocalizations, chasing, and physical confrontations, but rarely result in injuries (Owen-Smith, 1977). In addition to these interactions, territorial impala spend a significant amount of time and energy on mate-defence (though female herds enter and leave a territory at will; Jarman, 1974). When nutritious grasses recede during the dry season and males switch to foraging on less nutritious browse, energetic costs of maintaining a territory may greatly increase (Fairall, 1972; Colin Malcolm Oliver, 2005).

Here, we tested the hypothesis that territorial individuals have significantly higher GC levels than bachelors, in free-living impala in the Serengeti ecosystem. We also investigated how this relationship between territoriality and GC levels is influenced by environmental context, specifically, food quality, population density, and herd size. We predicted that 1) territorial males would have higher FGM levels than bachelor males. Furthermore, we predicted that 2a) as food quality decreased, FGM levels would increase in territorial males more so than bachelor males, given the energetic costs of defending a territory; 2b) as population density increased FGM levels would increase in territorial males, more so than in bachelor males, given the increased frequency of agonistic behaviours territorial males would face; and lastly, 2c) as herd size increased FGM levels would increase in territorial males, given the increased costs related to mate-defence and herding behaviours.

2. Methods

2.1. Study site and species

Located at equatorial latitudes, the Serengeti ecosystem (±27,000km^2) has similar day and night cycles throughout the year. However, the area experiences high geographic variability in rainfall (between 450 mm and 1400 mm) resulting in a dry season (June-October) and a wet season (November-May).

Impala are a medium-sized antelope species common in eastern and southern African savanna ecosystems (IUCN SSC Antelope Specialist Group, 2016). Impala are non-migratory herbivores with small home ranges typically between 5-10 km^2, increasing only slightly in the dry season (Averbeck, 2001). Grassy vegetation is considerably more palatable than browse, and therefore impala prefer to forage on grasses. Only when these are unavailable do impala include browse in their diet (Codron, Lee-Thorp, Sponheimer, & Codron, 2007; M. V. Jarman & Jarman, 1973). Impala prefer semi-open to bushy savanna with similar density of woody cover, and are often found on the edge of open and closed savanna (Ford et al., 2014; M. V. Jarman & Jarman, 1973).

2.2. Collection and analysis of faecal samples

To assess GC levels in impala we measured FGM concentrations (Rupert Palme, 2019). FGM reflect the biologically active free plasma GCs (Sheriff, Krebs, & Boonstra, 2010), and, importantly, sample collection is completely non-invasive. FGM are an integrative measure of plasma GCs (± 2 h in impala), representing an average value rather than a point value of glucocorticoid levels (Rupert Palme, 2019).

We collected 139 samples from individual male impala in both wet and dry seasons. For each faecal sample that was collected, we recorded group size and type (female herd [territorial males and females] or bachelor group [aspiring and defeated males]), and GPS location. Samples were immediately placed on ice and, within 12 h of defecation, stored at -20°C until further analysis.

Faecal samples were defrosted at room temperature for 30 min and homogenized by hand for 5 min. A subsample (mean ± SD = 0.52 ± 0.017g) of homogenized faeces were mixed with 5 ml of 80% methanol and vortexed for 1 min (R. Palme, Touma, Arias, Dominchin, & Lepschy, 2013). Samples were then centrifuged for 20 min at 2500 *g* and 0.5 ml of the supernatant was removed. We used a fume hood for up to 48 h to evaporate the extract, and subsequently sealed and stored the tubes at -20°C until shipment and analysis at the University of Veterinary Medicine, Vienna, Austria. Faecal GC metabolites were measured with an 11-oxoetiocholanolone EIA, first described by Möstl et al. (2002), and specifically validated for impala (Chizzola et al., 2018), which measures metabolites with a 5β-3α-ol-11-one structure. Intra-assay variation of high- and low-value quality controls were 5.3 and 5.8%, respectively, and inter-assay coefficient of variation of high- and low-value quality controls were 10.4 and 12.2%, respectively.

2.3. Forage quality

We used the normalized difference vegetation index (NDVI) data (NASA MODIS; Didan, 2015) as a proxy of spatiotemporal variability in forage quality, as we have done previously (Hunninck et al. *in review*). NDVI is a measure of primary productivity and measures ‘greenness’ of land cover (Pettorelli et al., 2005). In a strongly seasonal area such as the Serengeti ecosystem, NDVI is especially useful to study spatiotemporal variation in grassy vegetation, which is more palatable and nutritious than browse and thus the preferred food source for impala (Pettorelli et al., 2005). However, NDVI measurements should only be compared between areas with similar woody cover and habitat structure (Pettorelli et al., 2011). Therefore, we adjusted our NDVI measure by correcting for percent woody cover (MOD44B MODIS/Terra; Dimiceli et al., 2015). NDVI data was retrieved from the online Application for Extracting and Exploring Analysis Ready Samples (AppEEARS), courtesy of the NASA (https://lpdaacsvc.cr.usgs.gov/appeears/), and is freely available with global coverage. The raw data was adjusted for cloud cover and irregularities using a Savitzky-Golay smoothing filter. We extracted the closest NDVI value in space (250 m MODIS pixel resolution) and time (8-day interval) for each faecal sample in the dataset, and as such, acquired an NDVI score specific to our faecal sample with regards to location and time of collection. Considering the limited movement of impala, this NDVI score provides a reasonable representation of the environment utilized by the sampled impala over the past week. Together, this means that in this study, NDVI is an unbiased proxy of forage quality for impala (i.e. grassy vegetation; Pettorelli et al., 2011).

2.4. Population Density and Herd size

Data for population density was provided by the Tanzanian Wildlife Research Institute (TAWIRI). Locations and number of impala were recorded during an aerial survey (TAWIRI, 2010). We used the *density* function in the *stats* package in R to obtain a kernel density estimate and extracted local density of impala or each faecal sample. Herd size was estimated by counting every individual within 50 m of each other. Since impala mostly form tight groups, nearly all individuals are always in sight.

We aged and sexed every individual in the herd. Herd size represents a measure for mate-defence effort as with increasing number of females in a herd (Pavitt et al., 2015), the frequency of chases and male herding effort should increase.

2.5. Statistical analyses

To test our first prediction, we used a univariate linear mixed model structure (*lmer* function, *lme4* package v.1.1-17 in R; Bates, Mächler, Bolker, & Walker, 2015). The response variable for this basic model, FGM, was log-transformed to obtain normal distribution of model residuals, and group type was the only predictor variable. Group ID (i.e. unique per group) nested within sampling location were the random effects in all models, correcting for differences between groups and sample location (Table 1). Season had no effect on FGM concentrations in male impala ($t = 0.593$, $p = 0.554$), and was therefore not included in the analyses.

To test our secondary predictions, we updated the basic model to create two new models, one including an interaction between group type and NDVI, and the other an interaction between group type and population density. Lastly, we created a univariate mixed model with herd size as predictor, but only including data of territorial males ($N = 42$). Residuals were visually checked for normality and heteroskedasticity, and multicollinearity was assessed with a generalized variation inflation factor (GVIF) analysis. No heteroskedasticity was found, and residuals were normally distributed; GVIF values corrected for the degrees of freedom (*GVIF^[1/(2*df)]*) were all below 2 (*vif* function of the *car* package v.3.0-0 in R (Fox & Weisberg, 2011)) and therefore multicollinearity among predictors did not affect model estimates. Model fit was also assessed by calculating marginal (i.e. variation explained by fixed predictors only) and conditional (i.e. variation explained by both fixed and random effects) R^2 values (Nakagawa & Schielzeth, 2013). Model estimates were back-transformed to present relationships between response and predictors in the figures. All statistical analyses were performed in the statistical program *R*, v.3.5.0 (RCoreTeam, 2018), using RStudio v.1.1.453 (RStudio, 2016).

3. Results

We found that territorial males had 39% higher FGM levels than bachelor males (t = 2.132, df = 97.7, p = 0.036, N = 139; Figure 1).

NDVI, representative of food quality, did not affect FGM concentration in territorial males (t = -0.944, df = 105.8, p = 0.347); however, it significantly altered FGM concentrations in bachelors (t = -4.425, df = 79.1, p < 0.001; Figure 2A). In bachelor males, FGM concentrations increased on average by 82% as NDVI values decreased from the highest (0.52) to lowest (0.26) values. Furthermore, at low NDVI values (NDVI = 0.30), predictive of poor food quality, FGM concentrations were not different between territorial and bachelor males (95% confidence intervals: territorial = 373 ng/g [271 – 513 ng/g], bachelor = 361 ng/g [271 – 481 ng/g]); and only at high NDVI values (NDVI = 0.5), predictive of good food quality, did territorial males have higher FGM levels than bachelor males (territorial = 263 ng/g [146 – 474 ng/g], bachelor = 96 ng/g [62 – 149 ng/g]).

Increasing population density significantly increased FGM concentrations for both territorial and bachelor males (t = 2.840, df = 19.2, p = 0.010; Figure 2B), such that FGM concentrations increased on average by 64% between lowest (0.09) and highest (80.53) population density, but this effect was not different between territorial or bachelor males (t = -0.375, df = 97.6, p = 0.709). Herd size did not affect FGM levels (t = 0.174, df = 34.1, p = 0.863, N = 42) in territorial males (Figure 2C).

The models including the interaction between group type and NDVI and population density explained a considerable amount of variation in FGM levels (marginal R^2 = 19% and 15%, resp.). Including the random effects (i.e. conditional R^2), these models explained approximately 70% of the variation in the response variable.

4. Discussion

We found that territorial male impala had higher FGM concentration than their bachelor counterparts (Figure 1). This supports our hypothesis and corroborates previous findings that in certain species, territoriality comes with both reproductive benefits, and high GC levels (Creel et al., 2013). For example, Corlatti *et al.* (2012) found that territorial male chamois (*Rupicapra rupicapra*) sharply increased FGM levels during the rut. Territorial bison (*Bison bison*) were also found to have highest FGM levels during rut (Mooring et al., 2006). These results show that, when animals are territorial, they considerably upregulate their secretion of GC hormones. Though this upregulation may come with significant fitness consequences over time (Sapolsky et al., 2000), here, we argue that this upregulation of the HPA axis is not a cost of territoriality but rather an adaptive mechanism allowing for the energy mobilization necessary to uphold a territory. By having nearly exclusive mating rights with females within a territory, territorial males gain a considerable reproductive advantage. Building on the Cort-Adaptation hypothesis (Bonier, Martin, Moore, & Wingfield, 2009), we argue that this reproductive advantage warrants a temporary elevation of circulation GCs and results in a fitness increase for territorial individuals.

4.1. Food Quality

Overall, we found that NDVI did not alter FGM levels in territorial males, but did alter FGM levels in bachelor males (Figure 2A). At low NDVI, indicative of poor food quality, we found that territorial and bachelor males both had relatively high FGM levels. We predicted that territorial males should have higher FGM levels compared to bachelors at low NDVI, due to the energetic costs associated with maintaining a territory (Maher & Lott, 2000). Although territorial and bachelor males reside in relatively close proximity, territories are established in resource rich areas (Emlen & Oring, 1977), thus, territorial males could have potentially secured areas with higher local food resources and therefore could limit the consequences of low food conditions.

As NDVI increased, indicative of increased food quality, we found that FGM levels in territorial males did not change, while levels in bachelor males decreased; resulting in territorial males having greater FGM levels at high NDVI than bachelor males. Previously, we found that as food quality increased, FGM levels in free-living impala decreased, and that NDVI was more important in explaining FGM levels than human disturbance (Hunninck et al. *submitted*). FGM levels may not decline in territorial males with high food quality for three non-exclusive reasons. First, because males are defending a territory and herding females, they may not be able to take advantage of the absolute increase in food quality. Indeed, in impala it has been shown that territorial males spend significantly less time feeding and significantly more time chasing and roaring, compared to bachelors (C. M. Oliver, Skinner, & Van Der Merwe, 2007). Second, an increase in food quality across the landscape may increase the competitive ability of bachelor males, since they *can* increase their energy intake. As such, territorial males may need to engage in more agonistic interactions (i.e., territory defence) with bachelor males. In rainbow trout (*Oncorhynchus mykiss*), Toobaie and Grant (2013) found that the intensity of territoriality increases monotonically with increasing food resources (until maximum naturally occurring food abundance), due to a higher incursion rate by rivals. Therefore, as food abundance increases and more bachelor males challenge territorial males, FGM levels of territorial males remain high due to increased frequency of agonistic interactions. Lastly, as mentioned before, territoriality comes with a range of energetically demanding behaviours such as mate-defence, agonistic interactions, and mating (Owen-Smith, 1977). Males need to mobilize sufficient energy to be able to perform these behaviours, regardless of the energy available in the environment (i.e. forage). Elevated GC levels – and hence FGM concentrations – may facilitate increased energy mobilization and expenditure in territorial males, and, thus, remain elevated regardless of food quality.

4.2. Conspecific Population Density

We found that increasing population density elevated FGM concentrations in male impala, but that this effect did not differ between territorial and bachelor males (Figure 2B). We expected FGM levels to increase with increasing population density but for this effect to be greater in territorial males than bachelor males, given the expected increase in competitive and agonistic interactions between the two (e.g., increased territorial invasions by bachelors). This positive relationship between population density and the activity of the HPA axis is a well-known phenomenon (Creel et al., 2013). For example, Dettmer et al. (2014) found that increased population density was directly related to an increase in FGM concentrations in rhesus monkeys (*Macaca mulatta*). Thus, while increased population density in our system likely increases competitive and agonistic behaviours between territorial males and bachelors, it also seems to increase those among bachelor males themselves, resulting in a similar increase in FGM levels among all males.

Herd size, reflecting the number of potential mates, did not affect FGM levels in territorial male impala (Figure 2C). We hypothesized that with increasing herd size, mate-defence effort and therefore GC concentrations would increase. This was found in red deer, where FGM concentrations increased with increasing harem size in territorial stags (Pavitt et al., 2015). However, although territorial impala engage in extensive mate-defence (C. M. Oliver et al., 2007), female herds move freely between territories and males are often unsuccessful in preventing herds from leaving. Mate-defence, then, although a common behaviour for males, might not require as much energy as territory defence, and the frequency of agonistic interactions with rivals may be unrelated to the number of females in a herd. Behavioural observations of interactions with conspecifics could aid in elucidating the importance of mate-defence and herd size on the related costs of territoriality in impala.

5. Conclusion

Sociality in animals has evolved due to the many benefits it provides to individual fitness, such as reducing per capita predation risk and knowledge sharing. We hypothesized that territorial males will express higher levels of stress hormones than their conspecifics. In support of this hypothesis, we found that territorial male impala have higher FGM levels than their bachelor counterparts. While elevated GC levels are often argued as a cost to being territorial, we put forth the argument that elevated GC levels in territorial males increases their ability to defend and maintain a territory during their tenure by increasing energy mobilization and metabolic rate, thereby facilitating increased activity and behaviour. While increases in GC hormones and subsequent energy expenditure clearly cannot be sustained indefinitely, and males eventually will lose their territories, we suggest this is a proactive mechanism and not simply a reactive cost to being territorial. This is supported by our findings that (i) food quality did not alter FGM levels in territorial males – indicating there was potentially no cost to lower food quality or benefit to higher quality in terms of FGM levels – while in bachelor males, FGM levels decreased as food quality increased; and (ii) there was no difference in the effects of population density on territorial or bachelor males – if FGM levels were simply a cost to defending a territory, we would expect greater effects of population density. Recognizing the adaptive value of glucocorticoids, and their facilitating role in an animal's life history, is key if we are to fully understand how the HPA-axis activity influences the function and fitness of free-living animals.

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Figures (print in colour)

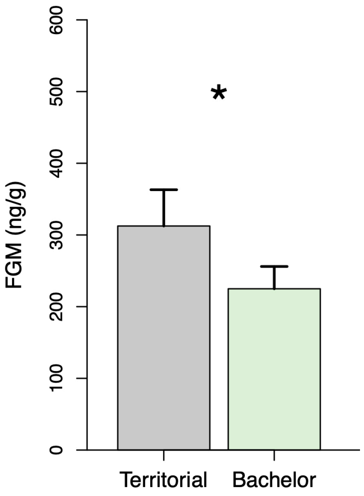

Figure 1. Glucocorticoid levels in territorial and bachelor impala. Faecal glucocorticoid metabolite (FGM) levels (ng/g; mean ± SE) in free-living male impala from the Serengeti Ecosystem. Asterisk denotes significant difference.

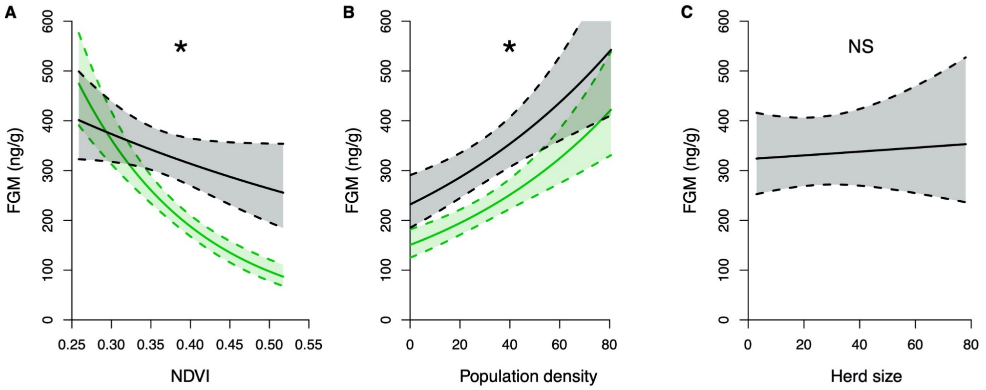

Figure 2. Influence of environmental context on relationships between territoriality and glucocorticoid levels in impala. Faecal glucocorticoid metabolite (FGM) levels (ng/g) of free-living territorial (black) and bachelor (green) male impala, from the Serengeti Ecosystem. A) Normalized Difference Vegetation Index (NDVI; measure for food quality) B) Population density C) Herd size. Shaded areas represent standard errors; asterisk represent overall significance; NS = non-significant.

PAPER IV

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Original Research Article

The impacts of human disturbances on the behaviour and population structure of impala (*Aepyceros melampus*) in the Serengeti ecosystem, Tanzania

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Serengeti ecosystem

ABSTRACT

Human activities can lead to various changes in wildlife populations' behaviour and dynamics. Understanding specific responses to disturbances can help conservation management better protect wildlife populations to coexist with local communities. Here, we studied the response of a model ungulate species, the impala (*Aepyceros melampus*), to different types of human activities in the Serengeti ecosystem. Impala were studied in central Serengeti National Park (CSNP), an area with relatively low human disturbance, and, with increasing levels of human disturbance, in western Serengeti National Park (WSNP), Grumeti Game Reserve (GGR), Ikona Wildlife Management Area (IWMA), and Loliondo Game Controlled Area (LGCA); pastoralism and/or hunting are the main disturbances in these areas. We hypothesised that in the exploited GGR, IWMA and LGCA areas, 1) impala would spend more time on costly behaviours, 2) sex ratios would be more female skewed, and 3) group sizes, reproduction and recruitment ratios would be lower compared to CSNP. We found that impala spent less time resting in IWMA and LGCA, as well as a significantly lower proportion of males, and lower reproduction and recruitment ratios in LGCA. Impala synchronized their behaviour, increasing their social cohesion potentially resulting in more efficient anti-predator strategies. Human activities induced a behavioural response in impala, indicating that in less regulated areas where pastoralism and hunting are allowed, these disturbances can potentially affect impala population persistence. These results highlight the importance of protected areas for wildlife populations, and the need for continuous monitoring of the impala and other ungulate populations in less regulated areas, as the underlying anthropogenic disturbances are likely impacting diverse species.

1. Introduction

Human activities may lead to behavioural changes in individuals, and ultimately affect the demography and the social organization of a species (Stankowich, 2008). Disturbance caused by human activities can alter animals' perception of

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humans as potential predators; the response to disturbance can in this context be studied in the same way as the response to predation risk (Frid and Dill, 2002; Stankowich, 2008). Changes in behaviour caused by anthropogenic disturbance may force animals to allocate more time to costly behaviours, such as vigilance or increased movement activity, to decrease their predation risk. These come at the expense of restorative behaviours, such as foraging or reproduction (Houston et al., 1993). Animals adapt to this cost-benefit trade-off by varying certain aspects of their behaviour to control both the rate of energetic gain and risk of predation (Kie, 1999; Lima and Dill, 1990). A high proportion of time spent vigilant can result in reduced predation risk, but also a lower rate of energetic gain. Habitat preference can also be affected, as an animal's energetic gain and risk of predation may change depending on the distance to cover at which it feeds. This cost-benefit trade-off is an important predictor of individual fitness and may ultimately affect population dynamics (Frid and Dill, 2002).

Vigilance is considered a costly behaviour and usually refers to scanning or watching the surroundings, and is related to the probability that an animal will detect a potential threat (Illius and Fitzgibbon, 1994). Various factors may influence the proportion of time spent vigilant, including spatial and temporal patterns in predation risk (Hunter and Skinner, 1998), time-of-day and habitat structure (van der Meer et al., 2012), position within group (Beauchamp, 2007; Matson et al., 2005), presence of conspecifics (Périquet et al., 2010), and group size (Beauchamp, 2017; Lima, 1995). With increasing group size, *per capita* vigilance is predicted to be reduced while maintaining an equal overall level of vigilance in the group, resulting in a reduced trade-off between time spent vigilant and feeding.

Synchronization of behaviour can benefit individuals and groups in several ways, among other by decreasing the predation pressure on offspring, increasing the effectiveness of anti-predator strategies, and social cohesion (Duranton and Gaunet, 2016). Behavioural synchronization in the form of activity synchrony is defined as exhibiting the same behaviour at the same time (Duranton and Gaunet, 2016), either at the group level (Conradt, 1998; Ruckstuhl, 1999) or between close neighbours (May et al., 2008; Rands et al., 2014).

Diverse forms of human disturbance may not only alter ungulate behaviour, but also affect a species' demography. Social ungulates living in open habitats form larger groups than those living in closed habitats, a relationship related primarily to predator avoidance strategies (Kie, 1999). Aggregation can benefit prey animals in reducing the predation risk in many ways, such as by increasing the chances of predator detection (i.e. the many-eyes hypothesis; Pulliam, 1973), dilute predator encounter possibility and increase predator confusion (Vine, 1971), reducing *per capita* predation risk (i.e. selfish-herd hypothesis; Hamilton, 1971), and through the risk-allocation hypothesis (Lima and Bednekoff, 1999). However, aggregation should only occur when it increases individual fitness; ungulates have been observed to live in smaller groups in areas with high human disturbance, indicating that a lower group size can be preferred under such conditions (Averbeck et al., 2010; Fischer and Linsenmair, 2007; Manor and Saltz, 2003).

Wildlife hunting, especially for trophies, is often very selective for males and can skew the sex ratio in ungulate populations in favour of females due to differences in body traits and behaviour between the sexes (Coltman et al., 2003; Ginsberg and Milner-Gulland, 1994). Poaching might also have a male bias in some species (Holmern et al., 2006). The increased collective vigilance of groups of prey is likely to result in hunters favouring individuals on their own or in small groups. The preference of hunters to stalk solitary individuals may result in increased predation of males, which are more likely to be on their own or in small groups (Fitzgibbon, 1990; Jarman, 1974). Additionally, ungulate populations that experience high hunting activity tend to be more wary and likely to flee than non-hunted populations (Caro, 2005; Stankowich, 2008).

Animals typically respond to human disturbances by expressing anti-predator behaviours (Frid and Dill, 2002). Consequently, ungulate populations could suffer similar negative effects from increased human disturbance as when perceiving higher natural predation pressure. Recruitment rates in elk (*Cervus canadensis*) decreased in Yellowstone National Park due to the indirect effects (i.e. anti-predator behaviour) that emerged after the reintroduction of wolves (*Canis lupus*; Christianson and Creel, 2014; Creel et al., 2007). A decreased reproduction or recruitment ratio measured as number of calves and juveniles per adult female respectively, can be a significant driver of population dynamics. Assessing whether human disturbance is correlated with reduced reproduction ratios improves our understanding of how human disturbance affects these dynamics and allows assessing potential consequences better (Gaillard et al., 1998).

Conservation efforts are vital to mitigate anthropogenic threats to ecosystems, and biodiversity in general. This, however, depends on a thorough understanding of how human activities affect specific species. Examining the behaviour and the demography of social mammals can serve as an important tool in conservation biology (Caro, 2005). Here, we studied the behaviour and demography of impala (*Aepyceros melampus*) in the Serengeti ecosystem to investigate how they are affected by different anthropogenic disturbance. The Serengeti ecosystem, on the border of Tanzania and Kenya, has been subjected to natural and human-induced processes for centuries (Sinclair et al., 2008a). The increase in the number of people living in the areas surrounding the Serengeti National Park (SNP) poses a significant threat to animal populations through various processes such as overgrazing, habitat fragmentation and hunting (Sinclair et al., 2008b). Therefore, we hypothesised that an increasing proportion of time would be spent on costly (i.e. vigilance or moving) compared to restorative (i.e. resting or feeding) behaviours in areas with increasing levels of human disturbance, i.e. those areas surrounding the national park, and the western part of SNP, where illegal hunting is more pervasive (*see Methods*). Observed reproductive and juvenile recruitment ratios were hypothesised to be lower, and group sizes smaller, in these areas due to human disturbance. Additionally, we hypothesised that there would be a more female skewed sex ratio in areas with high hunting pressure.

2. Methods

2.1. Study area

The study was conducted in SNP, Tanzania, and in the immediately adjoining protected areas, between May and July 2016 (Fig. 1). This coincides with the end of the long wet season and the start of the long dry season. Non-consumptive tourism and research are the main human activities in SNP. Besides some infrastructure for management (offices, ranger posts), tourism (information centres, lodges, tented camps) and research purposes; livestock grazing and resource extraction are strictly prohibited (Sinclair et al., 2008a). SNP thus served as a control area in our analyses, given the strict protection and relatively low human disturbance. However, although the SNP is strictly protected, in the western part of the SNP, called 'the western corridor', poaching is still pervasive (Holmern et al., 2006; Kaltenborn et al., 2005; Loibooki et al., 2002). We therefore distinguished between the central SNP (CSNP), and the western SNP (WSNP). In Loliondo Game Controlled Area (LGCA) and Ikona Wildlife Management Area (IWMA), human settlements, livestock grazing, and resource extraction are permitted but regulated. In the Grumeti Game Reserve (GGR) activities related to both consumptive and non-consumptive tourism are permitted and regulated, but settlements and agro-pastoralism are prohibited (Sinclair et al., 2008a). Additionally, hunting licenses are granted in the hunting season (1st July − 31st December) in both GGR and LGCA, where impala is an important game species (Thirgood et al., 2008), though especially in the last few years, hunting in GGR has been minimal. The estimated abundance of impala in the Serengeti ecosystem is around 75,000 (SE 9000) individuals (TAWIRI, 2010), and is widely distributed throughout wooded grassland and open woodlands.

In order to assess the potential effects of different human activities on the impala, the study area was divided into the above-mentioned five areas (Fig. 1). Relative to CSNP (the control area), we expected impala in the less-protected areas, along the area-specific disturbance-gradient of WSNP, GGR, IWMA and LGCA, to show an increasing response in terms of behaviour and demography.

2.2. Data collection

Existing roads inside and outside SNP were used to locate impala groups ($n = 232$), in addition to cross-country driving where roads were unavailable. As soon as an impala group was located, the car was stopped and the GPS location and number of individuals in the group were recorded along with their respective sex and age (when possible). Individuals were considered a group if they were within 15 m of their nearest neighbour, and other family groups had to be at least 50 m away. Age was classified into three categories: adult, juvenile and calf, based on horn length and shape, and body proportions (Brooks, 1978; Simpson, 1971). Age category and sex were recorded as unknown if it was not possible to record for the given individual in the group (e.g. sex of calves).

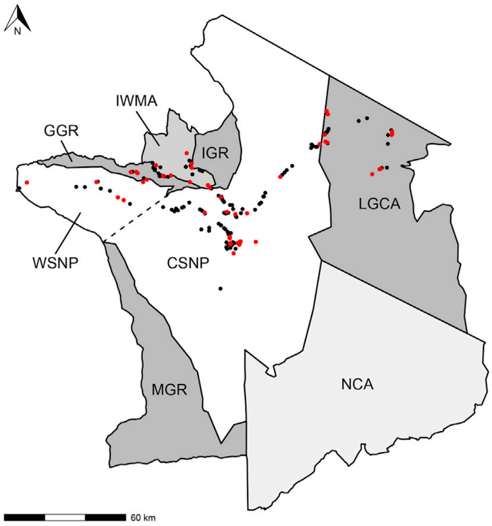

Fig. 1. The Serengeti ecosystem, with central and western Serengeti National Park (CSNP and WSNP, resp.), and immediately adjoining Grumeti (GGR), Ikorongo (IGR) and Maswa (MGR) Game Reserves, Loliondo Game Controlled Area (LGCA), Ikona Wildlife Management area (IWMA), and Ngorongoro Conservation Area (NCA). Black dots indicate locations where only demography data was collected, while red dots indicate location where both behaviour and demography data was collected. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Impala have small home ranges and groups usually stay in the same area, however to control for pseudo-replication (e.g. neighbouring female and bachelor groups, resampling of groups over time) we clustered the observed groups which were co-located within 1 km of each other (rendering 27 unique clusters).

Behavioural data were collected by observing 102 focal individuals within 44 groups. Observations were made from a Toyota Land Cruiser, which in areas of thick vegetation, had to be moved frequently during data collection due to the restricted visibility. No behavioural data were collected until the impala were accustomed to our presence (i.e. impala resumed original behaviour and did not actively watch the observers), which usually took less than 10 min, to avoid influence of observer presence (Kiffner et al., 2014). Habitat of the observed group was categorized into four different types; grassland (grass dominated with <2% tree canopy), savannah (grassland with 2–20% tree cover), woodland (>20% tree cover, defined as trees > 6 m with canopy cover 20% or higher) and bushland (dense woody vegetation <6 m in height with 2–20% bush canopy). Data were recorded between 7:00 a.m. and 7:00 p.m., covering the whole period of daylight. Individual behaviour was estimated using focal animal sampling with instantaneous sampling and a fixed-interval time-point recording rule (Altmann, 1973; Martin and Bateson, 2007). The fixed-point interval was one minute, and a total of 30 min of observation was spent on each focal individual. Individual behaviour was recorded as ten mutually exclusive behavioural types: resting (laying down or standing without ruminating), grooming (including allogrooming and scratching), ruminating (ears and eyes relaxed, chewing but not foraging), feeding (grazing and browsing), moving (moving for a minimum of four consecutive steps), vigilance (head high, ears forward and not moving, eyes wide open), watching the observer (i.e. vigilance towards observer), fleeing, and rutting (exclusively male behaviour; any territorial behaviour). In the analyses, we recognised five distinct behaviours and analysed them separately: vigilant (i.e. vigilance, watching the observer, and fleeing), moving, feeding, resting (i.e. grooming, ruminating and resting), and rutting. Two researchers observed two adult individuals per group each, simultaneously (i.e. four individuals were observed at one time). This allowed us to assess synchrony: per group, the territorial male or a random female was selected as focal individual for each observer, and both the focal animal's behaviour and that of its neighbour to the right was noted at the abovementioned intervals. Synchrony was then calculated as the proportion of times the focal individual's behaviour was the same as its neighbour's. Observer skill and bias were considered and minimised through trials before data collection. Only family herds were considered, and only adult individuals were observed; to examine differences between sexes, both the territorial male (if present or visible for observation) and a randomly chosen female were observed. Data were discarded if fewer than 20 recordings were collected per focal individual, e.g. when the animal moved out of sight ($n = 16$).

2.3. Data analyses

First, effects of areas with different land use regimes on group demography were assessed. Demographic variables included group size, reproductive ratio, juvenile recruitment ratio, and sex ratio. Reproductive ratio was calculated as the number of calves per adult female (calf:adult female ratio), while juvenile recruitment ratio was calculated as the number of juveniles per adult female (juvenile:adult female ratio). To test for overall differences in sex ratio, calf:adult female ratio, and juvenile:adult female ratio between areas, a Pearson's Chi-squared test of independence and a Chi-squared post-hoc test with pairwise comparisons were applied. Thereafter, generalized linear mixed models (GLMM) were used to relate the demographic variables at group level to assess differences between areas with different land use regimes. GLMM modelling was performed in R version 3.4.2 using the *glmer* function of the *lme4* package (Bates et al., 2014). Sex ratio could however not be assessed on group level due to sexually differentiated group compositions (i.e. family versus bachelor herds). Prior to analyses, the fit of each intercept model using a Poisson (R package *lme4*, function *glmer*) or negative binomial distribution (R package *MASS*, function *glmer.nb* (Venables and Ripley, 2003)) was compared. Further analyses were based on the better fitting distribution. Reproductive ratio and juvenile recruitment ratio used, respectively, number of calves and juveniles as response variable and included an offset on log-transformed number of adult females. We corrected for potential spatial and temporal variation by including Julian day and cluster as random effects. We used Akaike's Information Criterion corrected for small sample sizes (AICc) to select the best fitting model to the data (Burnham and Anderson, 2002). Of the competing models ($\Delta\text{AICc} \leq 2$), the most parsimonious model was preferred and discussed here. All models used in the analyses were first compared to their respective intercept model and only accepted when the ΔAICc value of the latter was larger than 2; model residuals were visually checked to be normally distributed. Mixed effect models were compared to their respective intercept models by analysis of variance (ANOVA; Table S1).

To test if impala synchronized their behaviour with their neighbour, we tested whether the observed proportion of synchrony was significantly different than the proportion of synchrony that would be expected by chance, given the observed frequency of behaviours in our data. We created a variable with random distribution of behaviour types, but in line with the observed frequencies of those behaviours, to obtain a synchrony measure for this test data. After aggregating the data per individual, yielding a mean ratio of synchrony, we then applied a Welch Two Sample *t*-test to compare average ratio of synchrony between observed and test data. This was done for moving, feeding, resting, and vigilance behaviour and for overall synchrony.

We applied generalized linear mixed models (GLMM) to relate the proportion of time performing different behaviours (resting, feeding, moving, vigilant, rutting) to explanatory variables. Number of scans spent on specific behaviours versus other behaviours was fitted with a two-column variable with binomial distribution. To correct for any temporal differences, we included Julian day as a random effect. Similarly, cluster and group ID were included as random effects to account for between- and within-group differences. To assess which covariates affected behaviour, potential differential factors and strategies were modelled separately and thereafter combined. While impala require to take account of differential factors such as sex, time-of-day (categorized as morning 6am-11am, noon 11am-4pm, and evening 4pm-7pm) or land use area (non-adjustable); they may be able to adjust their strategy optimizing their preferred behaviour (adjustable). Potential strategies (and related covariates) include: encounter dilution and confusion effects (group size; Vine, 1971), the pass-along effect of vigilance (synchrony; Beauchamp, 2007), risk reduction through social cohesion (synchrony; Conradt and Roper, 2000), the selfish-herd hypothesis (position within the group; Hamilton, 1971), and the risk-allocation hypothesis (habitat; Lima and Bednekoff, 1999). We compared single-parameter models including one of each of the covariates mentioned above as fixed effect to the intercept model. Based on the best single-parameter models, additive models were constructed, and compared to the single-parameter models to identify the covariates in the best model (Table S2). All covariates were retained when ΔAICc remained <2. Prior to analyses collinearity among the covariates was tested using the *pcor* function of the R package *ppcor* (Kim, 2015). Only sex and synchrony were significantly correlated with each other ($r_s = -0.367$, $t = -3.504$, $p = 0.001$).

3. Results

Overall, there was a significant difference in sex ratio between areas with different land use (Table 1). The sex ratio was significantly more female skewed in LGCA compared to CSNP and GGR (χ^2 post-hoc test: respectively $p < 0.001$ and $p = 0.002$). The sex ratio did not differ significantly among any of the other areas. Overall, a significantly lower calf:adult female ratio was found in LGCA compared to the other areas (Table 1). At group level, reproductive ratio (Table S1) was significantly lower in LGCA relative to CSNP ($p = 0.003$) and WSNP ($p = 0.037$). Overall, juvenile:adult female ratio was also significantly lower in LGCA compared to the other areas (Table 1). At group level, juvenile recruitment ratio was, however, not significantly affected by area (Table S1). Group size, family herd size and bachelor herd size were not significantly different among the five areas (Tables 1 and 2).

Impala synchronized their behaviour significantly more than would be expected by chance (Table 2). All behaviours apart from feeding were significantly synchronized, especially vigilance and moving (Table 2).

Impala behaviour was significantly affected by the proportion of synchrony with neighbouring females, in addition to either sex or land use area; all the best models included synchrony (Table S2). Resting was affected by land use area, sex and synchrony (respectively $F = 4.046$, $F = 0.122$ and $F = 11.928$; Table S2). Impala rested significantly less in LGCA (odds ratio (OR) [± 95% C.I.]: 0.308 [0.144–0.658]) and IWMA (OR: 0.208 [0.077–0.564]) compared to CSNP (OR: 1.588 [0.642–3.930]); respectively: $z = -3.041$, $p = 0.018$ and $z = -3.087$, $p = 0.016$). There tended to be less resting in IWMA compared to GRR (OR: 0.837 [0.341–2.054]; $z = -2.415$, $p = 0.105$); resting in WSNP did not differ significantly from the other areas (OR: 0.910 [0.221–3.738]). Males and females rested for equal amounts of time (OR: 1.040 [0.811–1.333], $z = -0.307$, $p = 0.759$). Resting decreased with increasing synchrony (OR: 0.155 [0.041–0.586], $z = -2.750$, $p = 0.006$).

Feeding differed by sex and increased with synchrony (Table S2). Males tended to spend a slightly higher proportion of their time feeding (OR: 1.023 [0.814–1.286], $F = 0.039$). More time was spent feeding when synchronized (OR: 23.966 [6.886–83.407], $F = 32.631$). The low effect of sex in the additive model indicates that this was mainly due to differences in synchrony between sexes. Moving tended to be negatively associated with synchronization (OR: 0.347 [0.084–1.437],

Table 1
Sex ratio (male:female), mean group size per herd type (±SE), calf:adult female (C:AF) ratio, and juvenile:adult female (J:AF) ratio of impala groups in the different study areas ($n =$ number of individuals for all measurement except for group size where $n =$ observations). χ^2 is given for the overall test.

Area	Sex ratio	n	Group size	n	Family herd size	n	Bachelor herd size	n	C:AF ratio	n	J: AF ratio	n
χ^2	30.928^*		2.294^{ns}		4.733^{ns}		0.645^{ns}		33.088^*		20.533^*	
CSNP	$1: 2.24^a$	2470	22.77 (±2.32)	115	39.20 (±3.61)	55	7.70 (±0.98)	60	0.10^a	148:1539	0.34^a	527:1539
LGCA	$1: 3.63^b$	820	18.13 (±2.77)	46	25.86 (±3.79)	28	6.11 (±1.58)	18	0.02^b	14:582	0.22^b	130:582
GGR	$1: 2.39^b$	830	27.16 (±5.03)	32	40.42 (±6.91)	19	7.77 (±1.87)	13	$0.07^{a,b}$	39:533	0.29^a	154:53
WSNP	$1: 3.12^a$	350	21.00 (±5.29)	18	33.50 (±7.39)	10	5.38 (±1.58)	8	0.12^b	28:233	0.33^a	76:233
IWMA	$1: 2.90^a$	390	19.33 (±3.32)	21	29.00 (±3.75)	12	6.44 (±1.49)	9	$0.06^{a,b}$	16:264	0.23^a	60:264

Alphabetical superscript denotes significant differences between groups; $^*p < 0.001$, ^{ns} $p > 0.05$.

Table 2
Observed and randomly generated synchrony ratios (SR), and frequencies of the different impala behaviours.

	Observed		Test data		t-statistic	df	p-value
	SR	Freq.	SR	Freq.			
Feeding	0.53	0.46	0.48	0.47	1.439	184.24	0.152
Vigilant	0.33	0.05	0.07	0.05	4.120	77.10	<0.001
Resting	0.38	0.33	0.29	0.31	2.614	168.39	0.010
Moving	0.40	0.16	0.16	0.17	6.062	158.90	<0.001
Total	0.51		0.34		9.249	181.20	<0.001

F = 7.028) and males moved more than females (OR: 1.331 [1.002–1.769], F = 3.855). The proportion of time spent vigilant varied by sex and proportion of synchrony (the latter only slightly: ΔAICc = 1.1; respectively F = 18.946 and F = 3.271; Table S2). Males were less vigilant relative to females (OR: 0.267 [0.148–0.483]), however vigilance decreased with increasing synchrony (OR: 0.014 [0.001–0.157]). Rutting in males was slightly affected by group size (ΔAICc = 1.6): rutting behaviour increased with group size (OR: 1.031 [0.998–1.065], F = 3.192). Given the importance of synchrony, the potential differentiating effect of sex, time-of-day and land use area on the proportion of time spent synchronized were further assessed. While sex affected synchrony, time-of-day and land use area did not (ΔAICc from the intercept model (AICc = 471.8): 14.2, −3.5 and −8.0, respectively). Males were less synchronized compared to females (OR: 0.693 [0.581–0.827], *F* = 16.648).

4. Discussion

In the face of rapid human-induced changes, it is necessary to understand how the diverse threats and pressures impact wildlife populations' behaviour and demography. Our results show that human activities in less regulated areas significantly reduce the time impala spent resting, exacerbate a female skewed sex-ratio, and reduce observed reproductive and recruitment ratios; group size did not differ between study areas. Impala synchronized their behaviour, increasing social cohesion. Contrasting the different management areas regarding their level of human disturbance indicated that behaviour and demography in impala was mostly affected in areas where land-use practice including livestock grazing and agriculture is allowed (IWMA and LGCA). Relative to the control area in the central part of SNP, potential indirect disturbances (e.g. bushmeat hunting or livestock incursions) seemed not to affect impalas significantly (WSNP and GGR).

4.1. Behavioural responses to disturbance

Negative interactions with humans are known to have significant impacts on ungulate behaviour, especially those associated with perceived predation risk such as vigilance and flight (Frid and Dill, 2002; Stankowich, 2008). Flight distances have also been reported to increase in ungulates exposed to disturbing human activities (Stankowich, 2008). By spending time and resources on these ‘costly behaviours’, less time and energy are spent on restorative behaviours (e.g. foraging and resting), potentially contributing to reduced individual fitness. These behaviours can thus be used as indicators to study the level of perceived human disturbances, and in extension, management effectiveness (but see Goldenberg et al., 2017). Our analyses indicate impala reduce time spent resting in areas with higher levels of human disturbance, as was observed in IWMA and especially in LGCA.

Although CSNP has been well protected for a prolonged period, the intensity of threats in areas such as GGR have, until fairly recently, been far greater. The implementation of effective conservation management, aimed at reducing anthropogenic threats to wildlife populations can, however, positively influence animal behaviour. The Grumeti Area, and GGR in particular, has undergone drastic changes in conservation management over the past decade, with a heightened focus on non-consumptive tourism. This has led to an increased employment of local people in tourist facilities, and poachers have been trained and employed as rangers. This, together with increased efforts in law enforcement, has reduced poaching intensity (Goodman, 2014). Prior to the implementation of these conservation measures, the impala behaviour and demographics (discussed below) reflected exposure to anthropogenic impacts (Setsaas et al., 2007). However, our results suggest that these measures have positively influenced impala behaviour in this area in terms of, amongst other, vigilance. Impala in GGR did not spend a higher proportion of time on vigilant behaviour compared to the control area, CSNP, or any of the other study areas.

Males spent a higher proportion of time on moving and feeding, and a lower proportion of time on vigilance compared to females. Since most males observed in this study were territorial (as opposed to bachelor), this result was expected. Territorial males generally invest more time and energy in reproductive behaviours such as courtship of females, competitive interactions with rival males, and maintaining their territory, at the expense of for instance vigilance (Jarman and Jarman, 1973). Increased feeding time might be necessitated by the energetic cost of extensive rutting behaviour (Oliver, 2002).

Predation risk strongly influences vigilance behaviour in impala (Hunter and Skinner, 1998; van der Meer et al., 2015). Impala are readily preyed upon by carnivores such as lions (*Panthera leo*), leopards (*Panthera pardus*), spotted hyena (*Crocuta crocuta*) and African wild dogs (*Lycaon pictus*) (Hayward and Kerley, 2008). As spending time vigilant is costly to the animal, prey species have developed various anti-predation strategies. Our analyses indicated that impala behaviour was synchronized, which can facilitate more efficient anti-predator strategies. Earlier studies have shown that behavioural synchrony may enhance spatial cohesion (King and Cowlishaw, 2009; Pays et al., 2007) and stimulate social cohesion through sexual segregation (Conradt, 1998; Conradt and Roper, 2000). Impala form sexually segregated groups of family herds with females, a dominant male and their offspring and nearby bachelor herds. Reasons for this sexual segregation may be caused by reproductive cycles but also each group having a ‘common fate’ regarding the need for similar behavioural activities (Murray, 1981). Although carnivores are present in all our study areas, the recent decline in other wild prey species in LGCA (*unpublished data*) may have increased predation pressure on impala. This could be exacerbated by the fact that impala are non-migratory (available to resident predator populations year-round), and LGCA does not attract many migratory species (relatively small seasonal influx of alternative prey species to dilute the predation pressure on impala). Additionally, impala young are particularly vulnerable to predation, and in combination these factors may partially explain the area's low recruitment ratios.

4.2. Demographic perturbations

The effects of licensed hunting are not limited to behavioural changes but can also have significant effects on population dynamics (Ginsberg and Milner-Gulland, 1994; Milner et al., 2007; Palazy et al., 2012). Trophy hunting in particular often targets large, breeding males. This phenotype-based selective hunting can reduce genetic variability and skew sex ratios (Coltman et al., 2003; Harris et al., 2013). Here, the sex ratio of impala populations was found to be skewed towards females in all the study areas. This was expected, as male antelopes often have a higher mortality rate related to a higher male predation risk due to differences in morphology and behaviour (Jarman and Jarman, 1973). However, the sex ratio in LGCA was found to be significantly more female skewed compared to CSNP and GGR. This may be the result of excessive hunting in this area. The hunting block in LGCA was frequently used by hunters who primarily select for adult males (Holmern et al., 2004; Thirgood et al., 2008). Skewed sex ratios due to hunting activities have also been found in other ungulates (Fischer and Linsenmair, 2007; Marealle et al., 2010; Milner-Gulland et al., 2003). Additionally, Holmern et al. (2006) found that illegal hunting practises were male biased (but see: Averbeck et al., 2010). Male impala generally have a more risk-prone behaviour during the rutting season, which makes them more vulnerable to hunting activities. The observed female skewed sex ratio might thus be explained by both legal and illegal hunting activities.

The sex ratio in GGR was found to be similar to the control area, CSNP, which is contrary to the findings of Setsaas et al. (2007). These results suggest that illegal hunting is either limited in this area or independent of sex, so that the selective pressure on males is not affecting population demography. As mentioned above, the Grumeti Fund has in the last two decades focused on rehabilitation of the wildlife populations of Grumeti Game Reserve and surrounding areas, and Ikorongo Game Reserve (Goodman, 2014). The increased investment in resource protection is likely to have been beneficial for wildlife populations in these areas, particularly in terms of reduced poaching (Thirgood et al., 2008).

However, our results do suggest that the hunting activities in LGCA might have a significant impact on the sex ratio of the local impala population. Even in harem breeding ungulates such as the impala, a female skewed sex ratio caused by removing breeding males can have significant ripple effects in population dynamics by, among other, lowering reproductive rate, and, in extreme cases, to a population collapse (Milner-Gulland et al., 2003). Although we found significantly lower reproductive ratio and juvenile recruitment ratio in LGCA, this is unlikely to be caused by the observed female skewed sex ratio in this area. For such effects to take place, a far more skewed sex ratio is likely to be needed (Fairall, 1985; Ginsberg and Milner-Gulland, 1994; Swanson et al., 2014).

The most pronounced behavioural and demographic differences were evident in LGCA. Besides hunting, livestock herding and human settlements are the main form of human disturbance in LGCA. High levels of human disturbance may indirectly reduce survival and reproduction by causing animals to divert a large proportion of time and energy away from resource acquisition (Frid and Dill, 2002). Livestock herding or pastoralism may lead to interspecific competition for food and habitat between livestock and wildlife (e.g. Dunham et al., 2003), and behavioural adaptations to reduce interactions with humans, both of which could contribute to a decrease in reproductive effort, and consequently, a lower recruitment (Dunham et al., 2003; Prins, 1992, 2000). Furthermore, human settlements and pastoralism might contribute to habitat alteration and degradation (Ogutu et al., 2009), including edge effects and increased exposure to diseases from livestock (Bengis et al., 2002). Further evidence of indirect effects of human disturbance on wildlife is presented in other experimental studies, having various effects on, among other, reproductive success (Freeman et al., 2014) reproductive rate (Phillips and Alldredge, 2000), activity patterns (Wronski et al., 2015), predation risk (Leblond et al., 2013), space use (Manor and Saltz, 2005), and stress levels (Hunninck et al., 2017).

There was a tendency for family herd size to be smaller in LGCA compared to CSNP, but this was not statistically significant. Impala were expected to have smaller group sizes in areas with higher human disturbance, as has been observed in other studies (Averbeck et al., 2010; Manor and Saltz, 2003). However, contrasting results have also been observed in impala earlier (Setsaas et al., 2007). In a social ungulate species such as the impala, group size can play an important role in group functions (Jarman, 1974), anti-predator behaviour (Creel et al., 2014), and intraspecific competition while foraging (Fritz and Garine-Wichatitsky, 1996). However, in many species, the relationship between group size and vigilance is not straightforward, and is likely influenced by many other factors, obscuring a potential linear relationship between the two (Beauchamp, 2008; but see Beauchamp, 2017). This might explain the contrasting and non-significant results in this and other studies.

5. Conclusion

The growing human population surrounding SNP has increasing impacts on the natural system through land uses such as pastoralism and hunting. Our results indicate that human-induced disturbances have a pronounced effect on the behaviour and demography of impala populations in the Serengeti ecosystem. In accordance with our hypotheses, LGCA, the area with the highest level of human disturbance, has resulted in a more female skewed sex ratio, lower observed reproductive and recruitment rate, and reduced time spent on restorative behaviour (i.e. resting). As these effects could have potentially significant implications for the persistence of impala populations in the area, monitoring of the behaviour and demography of impala is needed to better understand the implications and long-term effects of human-induced disturbance in the Serengeti ecosystem. Though the current study included only impala, it could be expected that the anthropogenic factors underlying the results also affect the behaviour and demographics of other ungulate species in the Serengeti ecosystem. Consequently, the results from our single-species study may be indicative of more pervasive human-induced effects, and these need to be properly understood in order to identify appropriate mitigation measures.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gecco.2018.e00467.

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Physiological and behavioural adaptations of impala to anthropogenic disturbances in the Serengeti ecosystem

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