

Research paper

Stress as a facilitator? Territorial male impala have higher glucocorticoid levels than bachelors

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ABSTRACT

Territoriality is a common behavioural adaptation, widespread among ungulates. 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, on average, higher FGM concentrations than bachelors. Increased food quality did not affect FGM levels in territorial males, but decreased FGM levels in bachelors by 78%. Greater population density increased FGM levels by 47%, 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 repercussions of being territorial, our findings support the hypothesis that elevated GC levels may be beneficial and act as a facilitator of a male's reproductive potential. The elevated GC levels may increase the ability of territorial males to maintain a territory by increasing energy mobilisation and metabolic rate, ultimately increasing their reproductive fitness. Appreciating that long-term increases in GC levels are not simply costly but may have an adaptive, potentially facilitating role in an animal's life history is key to understanding HPA-axis reactivity and its potential in eco-physiological studies.

1. Introduction

Sociality is a common adaptation in animals, as it can yield significant benefits including increased resistance to pathogens (Mooring and Hart, 1992), decreased *per capita* predation risk and increased access to mates (Creel et al., 2013). However, these benefits often come at a cost of, for example, increased resource and mate competition (Krause and Ruxton, 2002). Furthermore, social interactions between conspecifics can result in unpredictable or uncontrollable situations, such as agonistic conflicts or dominance contests, potentially causing adverse effects for the individuals involved. These unpredictable interactions require a rapid mobilization of energy stores and can be potent stressors (Creel, 2001). 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, for the first time in a wild African ungulate, the hypothesis that territoriality increases GC secretion. 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 activating 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 – e.g. increase glucose levels (Exton, 1979), increased heart and respiratory rate (Sapolsky et al., 2000), and suppressed digestion (Caso et al., 2008) – allowing animals to better mitigate various stressors, such as predation risk, food deprivation, and dominance-subordinate interactions (Clinchy et al., 2013; Creel et al., 2013; Dantzer

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et al., 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 and Hayward, 2009; Sapolsky et al., 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; MacDougall-Shackleton et al., 2019; Sheriff et al., 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 (Palme, 2019; Sheriff et al., 2011).

Territoriality is a widespread breeding tactic in ungulates (Bowyer et al., 2020; Corlatti et al., 2012; Neff and Svensson, 2013). It is almost exclusively a male trait, and territorial males gain a temporary 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 (or a group of individuals) defends against, excludes, or shows dominance over competitors; excluding them from resources and indicating ownership over an area (Maher and Lott, 2000; Owen-Smith, 1977). In polygynous species it is common that territories are established to include specific resources (e.g. food, water, or particular types of habitat), which attract females and thus grant the territorial male increased mating opportunities (Bowyer et al., 2020) – though sometimes it is the size of the territory rather than the resources therein that females prefer (Vanpé et al., 2009).

Bowyer et al. (2020) review various factors affecting the costs of maintaining control over a territory, given that excluding competitors and herding females can be an energetically demanding occupation (Corlatti et al., 2013; Maher and Lott, 2000). Here, we elaborate on three factors: 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 or heterogeneous food availability (i.e. energy deficiency), males might choose to not exhibit territoriality at all (Maher and Lott, 2000). Second, the cost of maintaining a territory has been shown to increase with increasing population density, as more rivals need to be contended with (i.e. male-male interactions) and the frequency of agonistic behaviours increases (Creel et al., 2013). Males may cease territorial behaviour when the cost of male-male interactions exceeds the cost of resource defence (Gosling, 1986). Third, herd size, or an increasing number of females within a defended group (i.e. male-female interactions), is expected to increase the energetic cost of maintaining a territory, given the increased herding and exclusion behaviours needed (Bowyer et al., 2020; Jarman, 1979), as was found in red deer (*Cervus elaphus*; Pavitt et al., 2015).

Impala are an excellent species to investigate how territoriality GC levels are related across different contexts. They are a gregarious and polygynous antelope, common in eastern and southern Africa (Jarman, 1974). Group sizes vary considerably, with groups of bachelors – i.e. adult males currently not holding a territory – reaching up to 60 individuals, and female herds (i.e. associated with one territorial male, consisting mainly of adult females, but also juvenile males and females, and calves) ranging from a few to over 100 individuals (Owen-Smith, 1977; Setsaas et al., 2018). Their population distribution 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/km² (average: 30 ind./km²; Averbek, 2001; Rosenblatt et al., 2019). Agonistic interactions between territorial males and rivals (i.e., bachelor males) 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 males spend a significant amount of time and energy on mate-defence (though female herds enter and leave a territory at will; Jarman, 1974). In fact, the energy spent on territorial behaviours is so great, about 70% of the territorial males can only hold a territory for

three months or less, after which they are exhausted and replaced by a rival (Jarman, 1979; Oliver et al., 2007). Impala also show pronounced variation in FGM levels in relation to anthropogenic and environmental disturbances. For example, impala have higher FGM levels in response to human disturbances, such as traffic and roads (Lunde et al., 2016), and livestock and human population density (Hunninck et al., 2020), but not in response to the presence of predators (Chizzola et al., 2018). Impala have also been shown to strongly decrease FGM concentrations in response to increasing forage quality (Hunninck et al., 2020). However, there is little information on how social behaviours such as territoriality and FGM levels are related in this species.

Here, we tested the hypothesis that territoriality affects GC levels 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; 2) as food quality decreased, FGM levels would increase in territorial males more so than bachelor males, given the energetic costs of defending a territory; 3) 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, 4) 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 (ca. 27,000 km²) has similar day and night cycles throughout the year. However, the area experiences high geographic and temporal 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, sexually dimorphic – i.e. males have lyre shaped horns – 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 km², increasing only slightly in the dry season (Averbek, 2001). 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; Jarman and Jarman, 1973). Grassy vegetation is considerably more palatable than browse, and impala therefore prefer to forage on grasses. Only when these are unavailable do impala include browse in their diet (Codron et al., 2007; Jarman and Jarman, 1973). 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; Oliver, 2005).

Territorial impala vary in their degree of sociality. Contrary to southern Africa populations, in equatorial areas, the breeding season extends practically year-round, yet individual male impala can only maintain a territory for around 1–3 months (Oliver et al., 2007). Territorial males are either solitary or associated with a female herd (consisting mainly of adult females, but also juveniles). The majority of males in a population, however, do not hold a territory and are referred to as bachelors, which aggregate in bachelor groups. These bachelor groups 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). Impala herds are relatively stable, though they can change over weeks or months. Therefore, in the field, the status of a male is easily determined by the group it is associated with; i.e., territorial if it associated with females and young or bachelor if it is associated with other males.

2.2. Collection and analysis of faecal samples

To assess GC levels in impala we measured FGM concentrations (Palme, 2019). FGMs reflect the biologically active free plasma GCs (Sheriff et al., 2010), and, importantly, sample collection can be completely non-invasive. Additionally, FGMs are an integrative measure of plasma GCs (± 2 h in impala), representing an average value rather than a point value of glucocorticoid levels, FGM concentrations can offer insights into an animal's longer-term physiological status (Palme, 2019). In impala, faecal samples give an indication of the plasma GC levels an individual has experienced over the previous 24 – 48 h (Chizzola et al., 2018), allowing for a more relevant proxy of an individual's GC concentration compared to other matrices such as blood (Sheriff et al., 2011).

We collected 139 samples in both wet and dry seasons from different territorial and bachelor male impala. Since a male's status is relatively stable and easily determined by its associated group type, we could be sure the collected faecal sample was associated to either a territorial or bachelor male. When the focal male was observed to defecate, a picture was taken of the position of the impala in the landscape, the time of defecation was noted, and the distance to the individual was measured with a range finder from the car (typically within 80 m from the car). When the herd had moved on (within 45 min), one person walked towards the sample with the range finder and measured the distance back to the car to until this was equal to the sample distance measured earlier. This method, described by Lunde et al. (2016), rendered it very easy to collect a fresh faecal sample of the specific individual that had been observed defecating. We prevented pseudo-replication by not sampling one individual or one group more than once and as such avoided problems with anonymous sampling (Corlatti, 2018). We sometimes sampled more than one individual from one group but accounted for this in the statistical analysis. For each faecal sample that was collected, we recorded male status (i.e. territorial or bachelor), group size, and GPS location. Samples were placed on ice within 45 min and stored at -20°C within 12 h of defecation, until further analysis.

Faecal samples were defrosted at room temperature for 30 min and homogenized by hand for 5 min. A subsample (mean \pm SD = 0.52 ± 0.017 g) of homogenized faeces were mixed with 5 ml of 80% methanol and vortexed for 1 min (Palme et al., 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 5 β -3 α -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.

To assess a possible effect of water content on our FGM estimates, (Palme et al., 2013), e.g. because of different diets (Morrow et al., 2001), we calculated water content of a subset of our samples ($N = 301$, including samples from female impala not included in this study) by weighing a part of the faecal sample before and after oven-drying at 60°C for 24 h. We then calculated FGM estimates based on both wet and dry weight. The correlation between wet weight and dry weight FGM values was 99.7% ($p < 0.0001$). We therefore conclude that in our dataset, water content did not significantly affect FGM estimates.

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., 2020). 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 spatio-temporal 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/appears/>), 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. With increasing population density, the number of potential rival bachelor males increases, and as such, population density represents a proxy for the frequency of interactions of male-male interactions, or the intensity of competition with rivals (Bowyer et al., 2020).

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.

With increasing population density, both male and female population density increases. However, herd size was not correlated with population density ($r = 0.26$, $p = 0.0025$). Population density and herd size thus represents different dynamics within impala populations.

2.5. Statistical analyses

To test hypotheses 1 (i.e. that territorial males would have higher FGM levels than bachelor males), we constructed a linear mixed multiple regression model (*lmer* function, *lme4* package v.1.1–17 in R; Bates, Mächler, Bolker, & Walker, 2015), with FGMs as the response variable and status (territorial or bachelor) in interaction with NDVI and population density as predictors. This model was also used to test hypotheses 2 (i.e. that as food quality decreased, FGM levels would increase in territorial males more so than bachelor males), and 3 (i.e. as population density increased FGM levels would increase in territorial males, more so than in bachelor males). To test hypothesis 4 (i.e. as herd size increased FGM levels would increase in territorial males), we created a similar univariate mixed model with herd size as predictor, but only including data of territorial males ($N = 42$). FGM values were log-transformed to obtain normal distribution of model residuals.

Seasonal differences in water and fiber content of the diet could bias FGM measurements, as these can affect gut transit time and thus the quantity of glucocorticoid metabolites excreted in the faeces (Morrow

Table 1

Model estimates from the two mixed effects models explaining the variation in faecal glucocorticoid metabolite (FGM) concentrations in impala. See text for further details.

MODEL 1: $\log(\text{FGM}) \sim \text{Status}^a (\text{NDVI} + \text{Pop. Density}) + 1 \text{Location} / \text{Group ID}$						
Fixed	Estimate	SE	df	t-value	P-value	
(Intercept)	7.35	0.62	77.33	11.793	< 0.001	***
Status - Territorial	-1.43	0.94	104.45	-1.516	0.132	
NDVI	-5.83	1.51	77.66	-3.865	< 0.001	***
Pop. Density	0.01	0.00	23.49	1.826	0.081	.
Status - Territorial : NDVI	4.76	2.37	100.03	2.008	0.047	*
Status - Territorial : Pop. Density	0.00	0.01	104.30	0.255	0.799	
Random	Variance	SD				
Group ID : Location	0.36	0.60				
Location	0.00	0.06				
Residual	0.22	0.47				
MODEL 2^a: $\log(\text{FGM}) \sim \text{Group Size} + 1 \text{Location} / \text{Group ID}$						
Fixed	Estimate	SE	df	t-value	P-value	
(Intercept)	5.78	0.26	25.79	22.12	< 0.001	***
Group Size	0.00	0.01	34.10	0.17	0.863	
Random	Variance	SD				
Group ID : Location	< 0.001	< 0.001				
Location	0.35	0.59				
Residual	0.72					

^a Only including territorial males; Significance codes: P < 0.001 ***; 0.001–0.01 **; 0.01–0.05 *; 0.05–0.1.

et al., 2002). However, we found no effect of season (i.e. wet: November – April, dry: May – October) on FGM concentrations in male impala ($p = 0.554$), and thus conclude that water and fiber content in food did not affect FGM levels in impala in our study. With only two categories, it did not capture the temporal variation in FGM levels appropriately; FGM levels were only significantly lower in August compared to other months. Similarly, NDVI was not significantly different between dry and wet season ($p = 0.894$). Due to its limited temporal detail and relevance to the study, season was not included in the analyses. Group ID (i.e. unique per group) nested within sampling location were included as random effects in all models, correcting for differences between groups and sample location (Table 1). Residuals were visually checked for normality and heteroskedasticity, and multicollinearity of predictors was assessed. No heteroskedasticity was found, residuals were normally distributed, and 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 and 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 significantly higher FGM levels than bachelor males, but only when food quality, estimated by NDVI, was high (Table 1; Fig. 1A).

NDVI did not affect FGM concentration in territorial males, though it significantly altered FGM concentrations in bachelors (Table 1; Fig. 1A). In bachelor males, FGM concentrations increased on average by 78% as NDVI values decreased from the highest (0.52) to lowest (0.26) values. Furthermore, at low NDVI values, predictive of poor food quality, FGM concentrations were not different between territorial and bachelor males (NDVI = 0.26; mean [95% confidence intervals]: territorial = 386 ng/g [280 – 584 ng/g], bachelor = 447 ng/g [308 – 647 ng/g]); and only at high NDVI values, predictive of good food quality, did territorial males have higher FGM levels than bachelor males (territorial = 293 ng/g [155 – 435 ng/g], bachelor = 99 ng/g [61 – 161 ng/g]).

Increasing population density tended to increase FGM concentrations for both territorial and bachelor males (Table 1; Fig. 1B), such that

FGM concentrations increased on average by 47% between lowest (0.09) and highest (80.53) population density, but this effect was not different between territorial or bachelor males (Table 1). Herd size did not affect FGM levels in territorial males (Table 1; Fig. 1C).

The main model, which included the interaction between status and NDVI and population density, explained a considerable amount of variation in FGM levels (marginal $R^2 = 25\%$). Including the random effects (i.e. conditional R^2), this model explained approximately 72% of the variation in the response variable.

4. Discussion

We found that, on average, territorial male impala tended to have higher FGM concentration than their bachelor counterparts (Fig. 1A, 1B). This supports our hypothesis and corroborates previous findings that in certain species, territoriality comes with both reproductive benefits, and high GC levels – also referred to as the ‘stress of dominance’ hypothesis (Creel et al., 2013). For example, Corlatti et al. (2012) found that territorial male chamois (*Rupicapra rupicapra*) sharply increased FGM levels during the rut. Tending bison (*Bison bison*), those that were herding females, 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 comes with significant fitness costs (Sapolsky et al., 2000) – as exemplified by the relatively short 1–3 month tenure time of male territorial impala (Oliver et al., 2007) – we suggest that this upregulation of the HPA axis is not pathological in nature and is therefore not maladaptive, as described in detailed by Boonstra (2013). Rather, it is an adaptive mechanism that, by increasing energy mobilisation and metabolic rate, may increase the ability of territorial males to maintain a territory. By having nearly exclusive mating rights with females within a territory, territorial males gain a considerable reproductive advantage, ultimately increasing their reproductive fitness. We suggest that these reproductive benefits of territoriality outweigh the fitness repercussions of long-term elevated GC concentrations, and results in a net 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 (Fig. 1A). Our NDVI estimates were associated with a specific faecal sample from either a

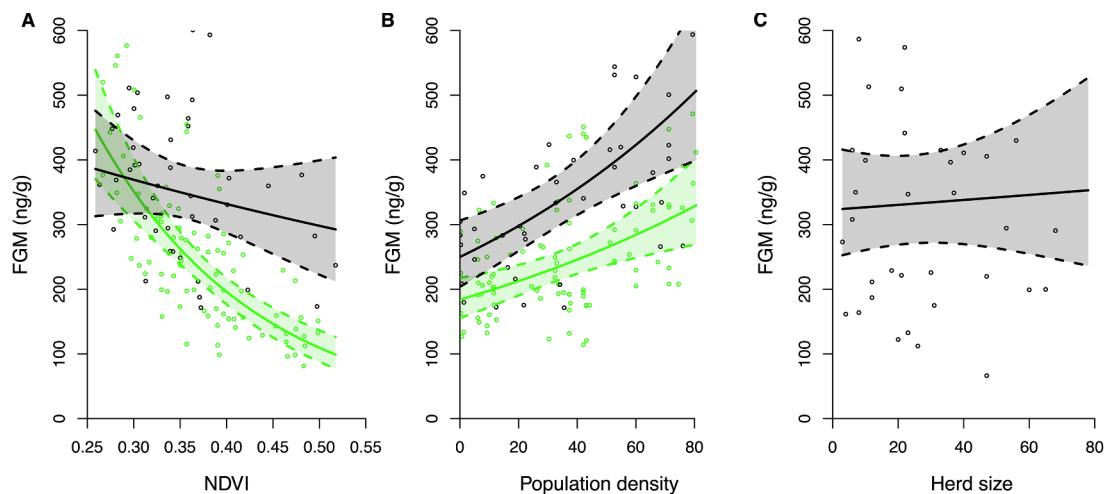


Fig. 1. Influence of environmental context on relationships between territoriality and glucocorticoid levels in impala. Faecal glucocorticoid metabolite (FGM) levels (ng/g) of free-living territorial (black) and bachelor (green) male impala, from the Serengeti Ecosystem. A) Normalized Difference Vegetation Index (NDVI; measure for food quality), B) Population density (in individuals / km²), and C) Herd size. Shaded areas and dashed lines represent standard errors. Points are the back-transformed adjusted response variable (i.e. values take the full model into account).

territorial or bachelor male, and so it provided a measure of what the NDVI was for a male at a certain time and place. This estimate was then related to their FGM levels, offering an indication of the stress levels an impala experiences over the previous 24 – 48 h (Chizzola et al., 2018).

At low NDVI, indicative of poor food quality, 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 and Lott, 2000). Although territorial and bachelor males reside in relatively close proximity, in polygynous species like impala, territories are often established in areas with certain resources (e.g. water or forage; Bowyer et al., 2020), thus, territorial males could have potentially secured areas with higher local food resources and therefore could limit the consequences of low food conditions.

At high NDVI levels, indicative of high food quality, territorial males had greater FGM levels 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., 2020). 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 (Mooring et al., 1996; Oliver et al., 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, therefore, 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 FGM concentrations in male impala tended to increase with increasing population density, but that this positive effect was the same for territorial and bachelor males (Fig. 1B). 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 females a male may need to defend, did not affect FGM levels in territorial male impala (Fig. 1C). 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 (Oliver et al., 2007), female herds move freely between territories and males are often unsuccessful in preventing females 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 increased resistance to pathogens (Mooring and Hart, 1992), decreased *per capita* predation risk and

increased access to mates (Creel et al., 2013). We hypothesized that territorial males would have higher levels of glucocorticoids compared to their bachelor conspecifics, as predicted by the stress of dominance hypothesis. While we did find that on average territorial male impala tended to have higher FGM levels compared to bachelors, variation in FGM levels was largely driven by an interaction with food quality; territorial males had high FGM levels regardless of food quality, while FGM levels in their bachelor counterparts decreased with increasing food quality. Elevated GC levels are often argued as a costly repercussion of being territorial; indeed increases in GC hormones and subsequent energy expenditure are costly and cannot be sustained indefinitely – in impala, males eventually lose their territories after around 1–3 months. However, we find 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. Therefore, we suggest that an increase in GC might be a proactive, adaptive mechanism, enhancing a male's ability to defend and maintain a territory during their tenure by increasing energy mobilization and metabolic rate, thereby increasing a male's reproductive potential – though alternate mechanisms may be at play in species where dominant individuals have lower GC levels. However, to test this hypothesis, further study of this system, with a larger sample size and repeated sampling of both aspiring and territorial males, together with measurements of tenure time and fitness variables are needed. 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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CRedit authorship contribution statement

L. Hunnink: Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Investigation. **R. Palme:** Methodology, Writing - review & editing. **M.J. Sheriff:** Conceptualization, Methodology, Supervision, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ygcen.2020.113553>.

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