

Linn Marie Flølo

Behavioural and demographic changes in impala (*Aepyceros melampus*) populations after a decade of changed conservation management

– a case study in the Serengeti Ecosystem, Tanzania

Master's thesis in Biology

Supervisor: Eivin Røskoft

Co-supervisors: Louis Hunninck, Roel May and Craig Jackson

May 2019



Photo: Linn Marie Flølo

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Abstract

In the western Serengeti Ecosystem, significant investment has been made in the conservation and management of the Grumeti area since the early 21st century. By using impala (*Aepyceros melampus*) as a study species, we investigated whether these investments have had quantifiable impacts on wildlife populations in the area by comparing the partially protected areas (PPAs) Grumeti and Ikorongo Game Reserves and Ikona Wildlife Management Area, with the adjacent strictly protected Serengeti National Park. Transects were driven along roads during 2003 and 2018; impalas' flight initiation distance was tested and their density, sex ratio, group size and behaviour recorded. When contrasting the two periods, results showed that impala density and group sizes increased significantly, and the behaviour of impala was less flighty, in the PPAs. Sex ratios had become more female-skewed. The positive changes for impala are likely attributable to effective conservation management, particularly in the PPAs, which formerly experienced high levels of illegal hunting activities. A shift to more female-skewed sex ratios was unexpected and the main reason for this is unknown.

Sammendrag

I det vestlige Serengeti-økosystemet er det gjort betydelige investeringer i bevaring og forvaltning av Grumeti-området siden begynnelsen av det 21. århundre. Ved å bruke impala (*Aepyceros melampus*) som studieart, undersøkte vi om disse investeringene har hatt kvantifiserbare innvirkninger på dyrelivspopulasjoner i området ved å sammenligne de delvis beskyttede områdene (PPAene) Grumeti og Ikorongo Game Reserve og Ikona Wildlife Management Area, med den tilstøtende og strengt beskyttede Serengeti nasjonalpark. Transekter ble kjørt langs veier i løpet av 2003 og 2018; impalaers fluktavstand ble testet og deres populasjonstetthet, kjønnsratio, gruppestørrelser og atferd ble registrert. Når de to periodene ble sammenlignet, viste resultatene at tettheten av impala og gruppestørrelser hadde økt betydelig, og impalaenes atferd hadde blitt mindre flyktig, i PPAene. Kjønnsratioene hadde blitt forskjøvet mot flere hunner. De positive endringene for impala kan sannsynligvis tilskrives effektiv forvaltning, særlig i PPAene, som tidligere har erfart høye nivåer av ulovlig jaktaktivitet. Skiftet til en kjønnsratio mer forskjøvet mot hunner var uventet, og årsaken til dette er ukjent.

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1. Introduction

Around the globe, biodiversity is declining at an unprecedented rate (Butchart et al., 2010; IPBES, 2019). Human activities are the main drivers of this new mass extinction, and our impact on both unprotected and protected natural habitats is increasing (Johnson et al., 2017). Ecosystems are being degraded and natural habitats fragmented or destroyed (Fahrig, 2003; Fischer and Lindenmayer, 2007), and overexploitation, agricultural activity, and urban development have been identified as the major threats to biodiversity today (Maxwell et al., 2016). In response to this dramatic decline in biodiversity, many protected areas (PAs) have successfully been created around the world; about 15.4% of the Earth's land surface is now legally protected (Juffe-Bignoli et al., 2014; Secretariat of the Convention on Biological Diversity, 2014). These areas have been gazetted to protect their biodiversity, maintain functioning ecosystems and ecological processes, and to act as refuges for species that cannot survive in human-dominated areas (Chape et al., 2008; Dudley, 2008). PAs may also be positive for people, by generating revenues (e.g. from tourism) (Dharmaratne et al., 2000; Buckley et al., 2012; Steven et al., 2013), or providing job opportunities and ecosystem services (Wittemyer et al., 2008).

The International Union for the Conservation of Nature (IUCN) defined different categories of protected areas with varying degrees of protection, of which each category allows only certain human activities (Dudley, 2008). National parks, nature reserves, and wilderness areas are strictly protected, allowing no extractive human use to take place. Partially protected areas often allow some form of consumptive use of natural resources within their boundaries, including legal hunting. Even though many PAs have successfully protected their biodiversity (Barnes et al., 2016), this is not the case for all (Juffe-Bignoli et al., 2014). Even in strictly protected areas, wildlife populations can decline: Craigie et al. (2010) found that populations of large mammals, mainly herbivores, have declined with an average of 59% in the past decades in PAs across the African continent. Landscape degradation caused by human activities along the edges of PAs may even effectively "squeeze" wildlife into core protected areas and alter ecosystem dynamics, as has been shown for the Serengeti-Mara Ecosystem (Veldhuis et al., 2019). The Serengeti-Mara Ecosystem has for decades been an excellent model system for the challenges PAs may face. It is an iconic area, centred around the Serengeti National Park (SNP), and known among other things for its great biodiversity and the annual migration of more than a million wildebeest (*Connochaetes taurus*), zebra (*Equus quagga*) and other antelopes. But it is also experiencing immense human pressures, with the human population in the areas surrounding Serengeti-Mara having increased by 2.4% per year on average from 1999 to 2012 (Veldhuis et al., 2019). With growing human populations and increasing pressure on PAs and the biodiversity within, disturbance of wildlife can also be expected to increase.

Therefore, there is a need to investigate the impacts humans have on ecosystems and wildlife populations, especially the long-term impacts. Human activities not only have the potential to impact wildlife directly through population declines, but also through more indirect effects such as changes in behavioural and demographical characteristics, which may affect populations negatively.

When exploited unsustainably, e.g. through hunting, wildlife population densities decline (Milner-Gulland et al., 2001) even in partially protected areas (i.e. where human settlement and certain consumptive use of resources is permitted) and national parks (Caro, 1999b; Fischer and Linsenmair, 2001). Hunting may considerably alter the population dynamics and demographics of wildlife species, for example, by disproportionately selecting males for trophy hunting (Ginsberg and Milner-Gulland, 1994), especially adult males exhibiting the most developed secondary sexual characteristics such as horns or tusks (Johnstone, 1974; Cummings, 1989). However, even passive hunting methods, such as wire snares or pitfall traps, have been shown to have a male bias (Holmern et al., 2006). Female-biased sex ratios have been found for several species in the Serengeti Ecosystem, especially in more exploited areas (Setsaas et al., 2007; Magige, 2008; Ndibalema, 2009; Marealle et al., 2010). Generally, males may be more exposed to exploitation because they exhibit more risky behaviour (FitzGibbon, 1998). Even though hunting regimes biased towards adult males may have adverse effects on population demographics and dynamics (Solberg et al., 2002; Milner et al., 2007), sex ratios are often skewed intentionally towards an excess of females to increase reproduction rates (Ginsberg and Milner-Gulland, 1994; Milner et al., 2007). Theoretically, a more female-biased sex ratio increases the productivity of a hunted population (Catto, 1976; Caughley, 1977; Fairall, 1985), because most harvested game species have a polygynous mating system where one mature male is capable of inseminating many females within a breeding season (Murray, 1982b; Mysterud et al., 2002).

Human disturbances, both lethal and non-lethal, may also have an impact on the behaviour of wild animals, such that the behaviour of, for example, hunted populations may differ from non-hunted populations, and behaviour may differ between hunting and non-hunting seasons within the same populations (Stankowich, 2008). Behaviour is therefore a useful tool for assessing the impacts of human exploitation (Caro, 2005). Certain non-lethal disturbances from human activities, ranging from quiet wildlife photographers to low-flying aircrafts, are seen as analogous to predation risk from natural predators (Frid and Dill, 2002). Non-lethal disturbance stimuli and predation risk create similar trade-offs between avoiding perceived risk and other fitness-enhancing activities such as feeding, parental care or mating (Ydenberg and Dill, 1986). It has been argued that disturbance stimuli can indirectly affect fitness and population dynamics via the energetic and lost opportunity cost of risk avoidance (Frid and Dill, 2002). For example,

Berger et al. (1983) found that pronghorn (*Antilocapra americana*) spent more time being vigilant, experienced higher foraging cost and lower foraging efficiency in a human-disturbed site than in an undisturbed site, which most likely involved increased energy costs (Berger et al., 1983). Human disturbance has also been shown to trigger increased vigilance and decreased foraging in elk (*Cervus canadensis*), effects which exceeded those of natural predators (Ciuti et al., 2012). In general, disturbance stimuli directly affect the flight behaviour, vigilance and habitat selection of animals, and indirectly affect population dynamics and potentially mate acquisition and parental investment (Frid and Dill, 2002). Human disturbance has also been shown to increase stress levels in African wildlife (Tingvold et al., 2013; Lunde et al., 2016; Hunninck et al., 2017).

One potential strategy to mitigate predation risk is to aggregate into larger groups. The “many eyes”-hypothesis (i.e. as group size increases there are more eyes scanning for predators (Lima, 1995)), and dilution effect (i.e. the chance that a given individual is caught by a predator decreases as group size increases (Dehn, 1990)), are usually the explanations for why animals should gather in larger groups when they are under threat. However, even among closely related species (e.g. mule deer (*Odocoileus hemionus*) and white-tailed deer (*Odocoileus virginianus*)), it has been shown that some species adopt this anti-predator behaviour, and some do not (Lingle, 2001). In addition, studies have shown that threats in the form of human disturbance do not necessarily induce aggregation into larger groups for protection (Manor and Saltz, 2003; Gude et al., 2006). Mixed-sex herds of impala (*Aepyceros melampus*) were smaller in unprotected farmland than in the protected Lake Mburo National Park in western Uganda (Averbeck et al., 2010). Similarly, mean group sizes of elephants (*Loxodonta africana*) were significantly larger inside the fully protected Lake Manyara National Park, Tanzania, than at the nearby partially protected Manyara Ranch (Kioko et al., 2013). Furthermore, Setsaas et al. (2007) found no significant differences in impala group size among areas with different human activities within the Serengeti National Park in Tanzania, and some partially protected areas immediately adjoining the park.

In addition to potential effects on group aggregation, ungulates in hunted areas tend to have more significant flight responses and be more wary than ungulates in non-hunted areas (Stankowich, 2008). However, ungulates in areas with frequent non-lethal human contact show weaker flight response compared to those in areas where human contact is rare (Stankowich, 2008). Flight initiation distance (FID), i.e. the distance between a predator or human and a prey animal at which the prey animal initiates flight, should increase with increasing cost of remaining (i.e. risk of capture) and decrease with increasing cost of fleeing (i.e. lost feeding opportunity). FID has often been used as an indicator of disturbance or exploitation levels on animals, as it is a metric for quantifying individuals' fearfulness in given circumstances (Stankowich and Blumstein, 2005;

Stankowich, 2008). In general, animals become warier and show greater FID in areas or times with more hunting pressure (de Boer et al., 2004; Reimers et al., 2009; Muposhi et al., 2016). Both Setsaas (2005) and Hariohay et al. (2018) found that impala fled more often from the vehicle and had a significantly longer FID in partially protected game reserves than in strictly protected national parks, indicating a potential influence of hunting pressure on flight behaviour. This is important, because there is a trade-off between time spent on energetically costly anti-predator behaviour and the time spent feeding, resting or reproducing. Hence, the decision to flee incurs a direct energetic cost while additionally inhibiting the possibility of fitness-enhancing activities such as feeding (Ydenberg and Dill, 1986). In a review by Stankowich and Blumstein (2005), most studies of animal populations hunted by humans found that animals have longer FIDs during months when hunting is permitted. Therefore, in times or places where human presence is typically non-threatening, animals have generally shorter FIDs, although several studies have found weak or no effect of hunting on ungulate flight behaviour (Stankowich, 2008). It is clear that behavioural studies are essential to conservation biology for understanding how exactly human disturbance indirectly affects wildlife through behavioural changes, in addition to more direct impacts on population dynamics and demography (Caro, 2005).

As a response to the degradation of partially protected areas (PPAs) adjacent to the Serengeti National Park; i.e. the Grumeti and Ikorongo Game Reserves and the Ikona Wildlife Management Area, the non-profit Grumeti Fund was established at the start of the 21st century, with the goal of rehabilitating and restoring the area after poaching had considerably reduced its wildlife at the start of the century (Singita, 2017). Since the Fund's establishment, Singita (2017) claims their dedication to anti-poaching, law enforcement, research, community partnerships, education and sustainability has quadrupled the number of wildlife in the area. Research conducted on impala in these PPAs in 2003, at the time of establishment of the Grumeti Fund, enables the investigation of changes in behaviour and demography of a local wild ungulate population over time.

This study aims to determine whether the above-mentioned improvement in management in the PPAs over the past fifteen years has resulted in demographical and behavioural changes in local impala populations. Using data collected in both the PPAs and the fully protected SNP, in both 2003 and 2018, we analyse potential changes in four indicators of human disturbances: density, sex ratio, group size, and flight behaviour. Since conservation management in the PPAs has improved considerably since 2003 through stronger enforcement of laws and regulations and more community involvement (Singita, 2017), we predict that, compared to 2003, impala populations in 2018 will have (1) a higher density; (2) a less female-skewed sex ratio; (3) an increased group size; and (4) less flighty behaviour towards humans in the partially protected areas, and that these indicators will not differ between the SNP and the PPAs.

2. Methods

2.1. Study species

Impala is a medium-sized, sexually dimorphic antelope species (Estes, 2012). Impala are widely distributed throughout eastern and southern Africa (Figure 1). The impala population has been estimated to roughly 43,800 (SE \approx 6,200) individuals in Serengeti National Park (TAWIRI, 2010) and about 13,500 (95% CI \approx 10,700–16,900) individuals in the Ikorongo-Grumeti Reserves complex, which includes the Grumeti and Ikorongo Game Reserves, and Ikona Wildlife Management Area (Goodman, 2013). Impala are usually found in woodland and bushland habitat but are also often found in the interface between woodland/bushland and savannah or grassland (Estes, 2012). Impala are non-migratory, but they will move seasonally between habitats within a home range, in relation to the abundance and availability of resources in the dry and wet season (Jarman and Jarman, 1974). Impala are both grazers and browsers, grazing on a range of grasses and browsing in a range of vegetation types within their home area. The diet changes seasonally, as does their preference for vegetation types (Jarman, 1974). They can also adapt to different habitats by being mainly a grazer in one area and a browser in another (Smithers, 1983). The adaptability in utilizing available food sources enables them to lead a sedentary existence and reach very high population densities in certain areas (Monfort, 1972).

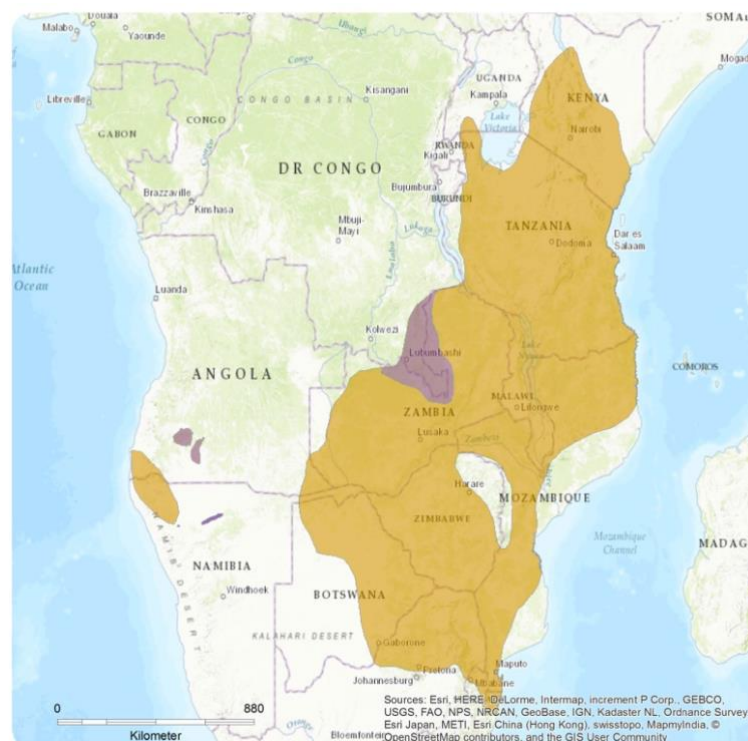


Figure 1. Distribution range of impala in eastern and southern Africa. Taken from IUCN SSC Antelope Specialist Group (2016).

Associated with the behavioural differences between the sexes are secondary physical differences. Sexual dimorphism in weight is evident in impala with adult males weighing 53-76 kg, and adult females 40-53 kg (Estes, 2012). Impala horns are carried only by males and form approximately 2% of their body weight (Jarman, 1974).

Typical group size can vary seasonally and regionally but often ranges between 6 and 60 animals, sometimes as many as 200 (Jarman, 1974). Impala are a polygynous species, and they can be observed in several distinct social groups; female/family herds, bachelor herds, singleton males and mixed herds (Estes, 2012). For at least part of the year, covering the main mating period, a proportion of the adult males hold territory. Non-territorial males and sub-adult males form bachelor herds (Jarman, 1974). Female herds are not confined to single territories but can move freely between male territories within their home ranges (Jarman, 1974; Estes, 2012). The freedom of movement of females is reflected in the nearly random spacing of female impala herds, and the impermanence of their association is shown by the correlation between group size and available food (Jarman, 1974).

Aggression from territorial males may drive other males, not only away from the breeding herds, but often right away from the area occupied by the breeding herds. This may force the other males into marginal habitat where they may suffer relatively higher mortality than the females. Measuring the sex ratio in an area therefore gives a local impression of the results of these processes, rather than an accurate sample of the sex ratio of the whole population (Jarman, 1974).

When a predator is detected, antelope species such as impala may either move slowly away from the predator or carefully pursue it as a group, keeping it in sight (Jarman, 1974). Under attack from larger predators, most of these antelope species run, sometimes in a specialised fashion. The impala are known for their spectacular leaps, and a herd may "explode" in all directions at the moment of attack (Jarman, 1974; Estes, 2012). Impalas also engage in another type of leaping, the motivation and function of which are unclear. It is a gait unique to the impala (Estes, 2012), often referred to as stotting. Females in labour and new-born young depend on concealment for protection from predators. Sick or injured individuals also tend to leave herds and hide in thickets or bushes (Jarman, 1974).

2.2. Study area

This study was conducted in the Serengeti National Park (14,763 km²) in northern Tanzania, and in immediately adjoining partially protected areas; Grumeti Game Reserve (GGR; ca. 410 km²), Ikona Wildlife Management Area (IWMA; ca. 240 km²) and along the border of Ikorongo Game Reserve (IGR; 600 km²) (Figure 2). The study period was from August to December in 2003, which covers the end of the long dry season and the start of the short rains, and from June to July in 2018, which encompasses the start of the long dry season. Mean annual precipitation in the Serengeti Ecosystem varies across a SE-NW gradient; rainfall ranges from 500 mm per year in the southeast, to 1,200 mm per year in the northwest and 1,000 mm per year in the far west (Sinclair et al., 2008; Metzger et al., 2015). Temperatures are higher during the wet season (November-June), while June and July are generally the coolest months (Metzger et al., 2015).

In the SNP, no settlements (except tourist lodges, park headquarters, staff villages and ranger posts), hunting, cultivation, livestock, beekeeping, fishing or timber extraction are permitted (Holmern, 2007). Several tourist lodges with adjoining staff villages are spread throughout the park, but the central area around Seronera experiences the highest human activity. Illegal hunting is widespread along the western border of the SNP, GGR and IGR, but not very common along the eastern border (Holmern et al., 2004). Hence, the most severely affected areas from illegal hunting inside the SNP are in the north-west and part of the Western Corridor (Campbell and Hofer, 1995). The illegal hunting is largely done on foot which is least conspicuous to patrolling rangers, and the preferred hunting method is to set wire snares or dig pitfall traps (Arcese et al., 1995; Holmern et al., 2006). The same legal restrictions as in the SNP apply for the Grumeti and Ikorongo Game Reserves, except that licensed tourist hunting and game cropping are allowed in the hunting season (Holmern, 2007). In Ikona Wildlife Management Area, settlement, beekeeping, some cultivation and cattle grazing, firewood collection and game cropping are allowed (Holmern, 2007). Licensed tourist and resident hunting is allowed in the hunting season, i.e. from July 1st to December 31st (URT, 1974). Most of the legal hunting is conducted from vehicles (Holmern et al., 2004), and both tourist and resident hunters are only allowed to shoot male animals (URT, 2015). The partially protected areas are also subject to a high illegal hunting pressure, which is conducted both by motorcycle and on foot (Campbell and Hofer, 1995; Holmern et al., 2002).

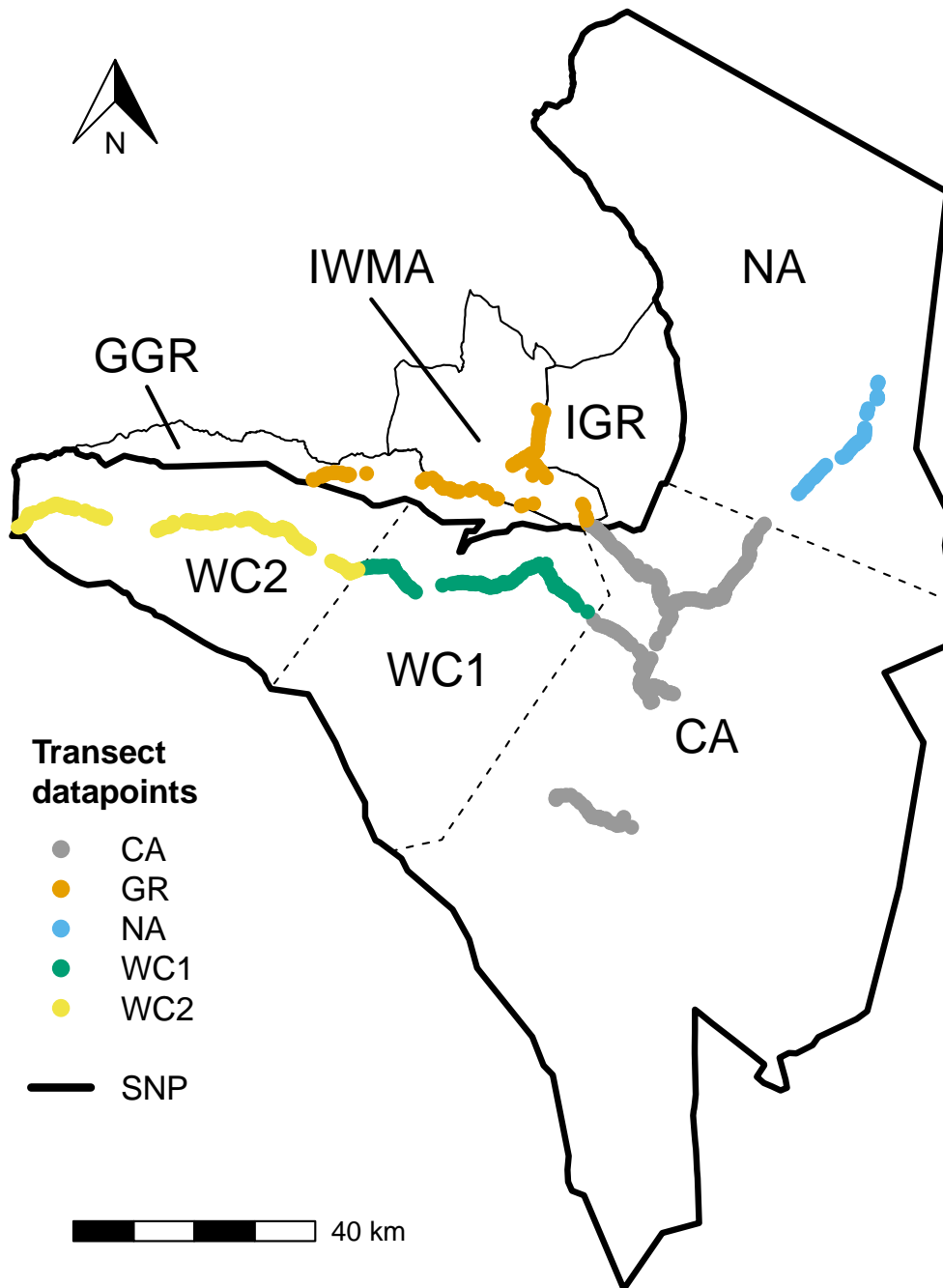


Figure 2. Map of the study area; Serengeti National Park (SNP) and adjoining partially protected areas; Grumeti Game Reserve (GGR), Ikona Wildlife Management Area (IWMA) and Ikorongo Game Reserve (IGR). SNP was divided into four areas for this study; Central Area (CA), Western Corridor 1 (WC1), Western Corridor 2 (WC2) and Northern Area (NA). The coloured data points show where transects were driven within each area. The partially protected areas were pooled together into one area, Game Reserves (GR), in the analyses due to low sample sizes. The thick border denotes the SNP, thin borders the partially protected areas, and dashed lines divide the study areas within the SNP.

2.3. Data collection

The study area was divided into five parts (Figure 2). The SNP was divided into four areas; Central Area (CA), Northern Area (NA), Western Corridor 1 (WC1) and Western Corridor 2 (WC2), in order to separate areas within the park with different human activity. The partially protected areas Grumeti Game Reserve, Ikona Wildlife Management Area and Ikorongo Game Reserve were pooled into one area, henceforth called the Game Reserves (GR), due to low sample sizes. In this study, data from two years (2003 and 2018) were compared with each other. The 2003-data were collected by Setsaas et al. (2007). We were provided with these data for use in the analyses. The 2018-data were collected for this study by replicating the methodology of the original 2003-study as closely as possible (see Setsaas, 2005; and Setsaas et al., 2007), to enable comparisons of results. The following two sections describe the data collection methods used, which are similar to the ones used in 2003. Setsaas et al. (2007) conducted trials according to guidelines in Caro (1994). We also did a test study on about five impala groups to try out the methods beforehand and make sure all 2018-data were collected in the same way.

2.3.1. Transects

Distance sampling (Buckland et al., 2001) was used to determine the density of impala. Transects were driven along existing roads between approximately 7:00 am and 7:00 pm, covering eight transects for a total of 3336 km (2003-data: 2050 km, 2018-data: 1286 km; Figure 2). Transects did not overlap, such that no area was covered in more than one transect. The vehicle was driven at approximately 30-40 km/h, and the number of observers in the car was at least two at all times, with two always being the same persons (within years, but the observers were different between years). The transects were driven an equal number of times alternately forwards and backwards in each year, in such a way that the change in animal behaviour during the day could be taken into account (Jarman and Jarman, 1973). This means that each part of a transect was covered in both the morning and evening (Jarman and Jarman, 1973). All transects were "marked" by recording the GPS-position for their start and end points. In addition, the number of kilometres driven for each transect was noted. During each transect we took records of all impala that were seen, regardless of distance from the road. By using the distance sampling approach it is possible to obtain unbiased estimates of animal densities if certain assumptions are met, namely: 1) animals on the transect (i.e. road) are always detected, 2) all animals are detected in their initial locations, and 3) all measurements are correctly recorded (Thomas et al., 2010).

When a singleton or group was spotted, the car was stopped as soon as possible while maintaining the best possible visibility of the animal(s), and the engine was turned off. Then we recorded the GPS-position of the car and the distance to the animal(s) with a

rangefinder. If it was a group of impala and not a singleton, the distance was defined to represent the line from the observers to the estimated middle of the animal group. Then we measured the angle of the road and the angle of the straight line between the observers and the impala, relative to true north, using a regular compass. These measurements allowed the computation of the perpendicular distance from the object (impala) to the transect (road). This basic information was used in the analyses based on the Distance sampling method (Thomas et al., 2010) to fit detection functions that determine the density of impala. The fitted detection function reflects both the decrease in detectability with distance and the lower proportion of animals that are potentially detectable (Buckland et al., 2001).

In addition, we recorded the total number of individuals (group size), group type (bachelor, family, singleton, mixed), number of individuals of each age class (adult, subadult, calf) and sex (male, female), the initial behaviour when discovered (resting, ruminating, feeding, moving, vigilant, watching the observer or fleeing), habitat (woodland, bushland, grassland), grass length (short, long) and grass quality (brown, green-brown, green, burned). If it was not possible to ascertain age and/or sex for all of the animals in the group, it was recorded as unknown for the individuals in question. If it was a cluster with more than one animal, we recorded the initial behaviour of the majority of the animals.

An animal was defined as vigilant when it lifted its head away from the ground and paid attention to its surroundings. The eyes were open wide, and ears pointed in viewing direction. Resting meant laying down or standing resting, feeding meant either grazing or browsing, and an animal was defined as moving if it took more than two steps without foraging and was not actively scanning. 'Watching the observer' meant actively observing (i.e. being vigilant towards) the car/researchers. Fleeing meant running or leaping away (including jumping or stotting). Lastly, the animals could be ruminating: the head was lifted, eyes not focused, ears were backwards or moving, and the animal was sporadically chewing but not feeding.

In the field we noted whether the habitat was 'woodland' (tree canopy), 'bushland' (dense woody/shrubby vegetation) or 'grassland' (virtually no trees/bushes, grass-dominated), and noted the estimated percentage cover of trees and/or bushes. Habitats with 2-20% bush or tree cover were later categorised as bushed or wooded grassland, respectively. Habitats with >20% bush or tree cover remained as bushland or woodland, respectively. Grass-dominated habitats < 2% canopy cover remained as grassland. As per Setsaas et al. (2007), woodland and bushland were considered 'closed' habitat, while wooded/bushed grassland and grassland were considered 'open' habitat.

2.3.2. Flight experiments

Data on FID were collected mainly while doing the transects, meaning that experiments on FID were done mostly on the transect roads. We took records between 7:00 am and 7:00 pm, covering the whole period of daylight; FID experiments were always done by the same test person in each year (Tomas Holmern in 2003 and Linn Marie Flølo in 2018). FID experiments were conducted by walking at a constant pace towards both groups of impala and singletons. When approaching a group of impala, one impala in a central position with respect to the group was chosen as the focal individual to direct our approach. Groups or individuals recognised in areas previously covered were avoided, to avoid sampling the same impala more than once. Before a new experiment was initiated, the new group had to be out of visual contact with the former. Experiments were never carried out if (1) a potential predator was in sight, (2) a tourist car was nearby, (3) it was raining, (4) the test animals could not see the approacher, or (5) if the animals were currently moving off due to reasons other than the car. If the animals fled before the approach could start, they were scored as "reaction to car". The experiments were abandoned if the animals ran due to other reasons than the test person, such as mating/territorial behaviour, influence from other mammal species, being bothered by flies etc. Experiments were discarded if the impala had potentially been influenced by noise from the car door or other approaching vehicles that were not seen when the experiment started, or by other persons. Experiments were never carried out solely towards sub-adults. A note was made if other species were present within approximately 50 m of the impala.

Prior to the start of the approach, the starting distance (STD) and angle to the animal(s) relative to true north were measured using a rangefinder and a compass, respectively. In addition, the impala were scored as either alert or not alert (being alert meant showing vigilance towards us – ears pointed in our direction). After recording these initial observations, the experiment itself was started. For the experiment, the test person carefully opened the car door, and started to walk at a constant speed in a direct line towards the focal animal or group. When the impala fled, the test person stopped walking instantly. If not all the animals in a focal group fled simultaneously, the approach was halted when the majority of the individuals had fled. The test person then measured the distance to the car from where he or she stopped, using a rangefinder. The FID was calculated as the difference between the STD and the distance from the test person to the car when the approach was halted.

2.4. Statistical analyses

The statistical analyses were carried out using RStudio version 1.1.463 (RStudio Team, 2016) with R version 3.5.1 and version 3.5.2 (R Core Team, 2018), and IBM SPSS Statistics version 25 for Mac OS (IBM Corp., 2017). In all cases a significance level of $P < 0.05$ was considered.

2.4.1. Density

Density analyses were conducted using the R package 'unmarked', version 0.12.3 (Fiske and Chandler, 2011). The data from 2018 and 2003 were analysed separately. Both data distributions were right-truncated at 300 metres. No left-truncation was used, even though the number of detections increased slightly away from the transect line. The data were also divided in 15 distance bins of 20 metres each. First, the data were fitted with null models (i.e., ' $\sim 1 \sim 1$ ') to determine the best key detection function, i.e. the half-normal, hazard rate or negative exponential key function. A key function is the known parametric form that best fits the detection curve, prior to any potential adjustments of the function (Buckland, 1992). The hazard rate key function fitted the data best and was consequently used for further analyses of both data sets. Covariate models were fitted to the data, first with covariates for detection, then for abundance. Using the Akaike Information Criterion (AIC), we determined that the best model for both data sets included area as a covariate for both detection and abundance (i.e., ' $\sim \text{area} \sim \text{area}$ '). The model returned estimates of the number of impala groups per km² in each of the five study areas (CA, WC1, WC2, NA and GR). After checking for detection bias due to group size, the number of impala individuals per km² in each of the five areas was estimated by multiplying the density of groups with the mean group size in each area. 95% confidence intervals (CI) for individual densities were obtained using the delta method. Densities were considered to be significantly different if the means of two values did not lie within the 95% CI of each other.

2.4.2. Sex ratio

Differences in sex ratio between years and areas were analysed using Chi-square tests and Chi-square post-hoc tests (Appendix A). Sex ratio was calculated per area as: (total number of males) / (total number of females + total number of males). We first used a Chi-square test on the 2003-data to test for differences in sex ratio between areas, followed by the Chi-square post-hoc function for pairwise comparisons between areas with Bonferroni-correction for multiple testing. We then ran the same analysis on the 2018-data. To compare overall sex ratios between the two years, we ran a Chi-square test on the combined 2003- and 2018-data with year as only predictor. We then conducted separate analyses for each of the five study areas on subsets of the combined data, with year as only predictor.

2.4.3. Group size

Group size was analysed across all group types using a generalised linear model (GLM) due to the non-normal nature of the data. Due to overdispersion in the data, group size was modelled as a negative binomial distribution instead of a Poisson distribution. We analysed the combined data (2003- and 2018-data) using the model structure '*group size* ~ *year* + *area* + *year* × *area*', in order to test for an effect of year and/or area, and the interaction between them, on group size. The fit of the model was validated visually using the diagnostic plots, as well as by plotting the Pearson's residuals against the fitted values for the model, as per Zuur et al. (2009). The overall significance of the predictor variables and the interaction was tested with an 'Analysis of Deviance', which was run using the 'ANOVA()' function in R. Significant differences in group size estimates between years in GR, and between GR and the other four study areas in each year, were then determined from the model's summary output with GR set as the reference level (Appendix B).

2.4.4. Behaviour

Initial behaviour

Differences in initial behaviour between years and inside/outside the SNP were analysed with Chi-square tests in SPSS, with the following cross-tabulations: '*initial behaviour* * *inside/outside*', '*initial behaviour* * *year*', '*initial behaviour* * *year* * *inside/outside*', and '*initial behaviour* * *inside/outside* * *year*'. For each test, the percentages of each behaviour within the columns were obtained, and the Pearson Chi-square value was calculated, as well as degrees of freedom and the *P*-value. The 'Crosstab' procedure in SPSS is used to generate contingency tables, which is a method for quantitatively analysing the relationship between multiple categorical variables.

Flight initiation distance

We first tested whether the presence of other species nearby was important for FID by using ANOVA to compare a negative binomial GLM with only 'other species' as a predictor for FID with the null model. If other species that had been noted were considered to be close enough to potentially influence the flight response of the impala, i.e. within approximately 50 m, it was a 'yes', if not it was a 'no'. The result of the ANOVA was non-significant ($P = 0.28$), and thus this predictor was not included in the final models. We discarded observations where the impala had potentially been influenced by noise from the car door ($n = 4$) or other vehicles approaching ($n = 5$), where the approach was done towards sub-adults ($n = 5$) or duplicates of impala recognized in areas previously covered ($n = 2$), or where impala were most likely influenced by another person ($n = 1$), resulting in a total sample size of $n = 364$ (2003-data: $n = 174$; inside SNP: $n = 148$, outside SNP: $n = 26$. 2018-data: $n = 190$; inside SNP: $n = 159$, outside SNP: $n = 31$).

To test our hypothesis about a change in FID between the two study periods, and specifically that the behaviour would have become less flighty outside the SNP, we wished to test the following basic model: $FID \sim year + area + year \times area$. In addition, as pointed out by Holmern et al. (2016), we needed to include starting distance (STD) and alertness as covariates, because STD limits the potential range of response in FID by the focal animal(s), while animals that are alert prior to the approach will choose to escape earlier. Therefore, the extended model became $FID \sim year + area + STD + alert + year \times area + STD \times area + alert \times STD$. The selected predictor variables are defined as follows: (1) 'STD': starting distance to the animals; (2) 'year': year the data were collected; (3) 'area': inside or outside the SNP; and (4) 'alert': were the animals alert before the approach started (yes or no)? In addition, a few more covariates were selected based on Setsaas et al. (2007), and reviews by Stankowich and Blumstein (2005), and Stankowich (2008), to test if they could further improve the fit of the model. The selected covariates were: (1) vegetation (open or closed structure); (2) group size (number of individuals); (3) group type (single male, bachelor, female, family or mixed); and (4) calf presence (whether calves were present or not).

We first fitted the extended model as a generalised linear mixed model (GLMM) with the five study areas as a random effect, and a Poisson error structure. However, there was some evidence of overdispersion when using a Poisson distribution. Therefore, a GLMM with a negative binomial distribution was fitted instead. The model returned warnings that the model failed to converge, and that the model was nearly unidentifiable with very large eigenvalues. In addition, the random effect had a variance of only 0.001429. Therefore, we decided to remove the random effect and fit the model as a generalised linear model (GLM) instead. Due to overdispersion when using a Poisson error structure, a negative binomial distribution was specified in the final GLMs. We tested five models; the extended model defined above, and four models consisting of the extended model structure and the additive effect of one each of the four covariates. The tested models can be seen in Table 2. The models were ranked according to AICc (Akaike Information Criterion corrected for small sample sizes), using the `model.sel()` function from the 'MuMIn' R package, version 1.42.1 (Bartoń, 2018). Out of the models with $\Delta AICc < 2$, the most parsimonious model was the one with the least degrees of freedom. The most parsimonious model (Table 2) was validated using the diagnostic plots, and by plotting the Pearson's residuals against the fitted values for the model, as per Zuur et al. (2009). No strong patterns were detected, and thus the model was deemed an acceptable fit to the data.

3. Results

3.1. Density

The number of impala individuals per km² (Figure 3) and the number of impala groups per km² (Figure 4) were both significantly higher in 2018 than in 2003 in WC2 and in GR. The density of groups was significantly lower in 2018 than in 2003 in CA, WC1 and NA (Figure 4), while the density of individuals in these areas did not differ between the two periods (Figure 3).

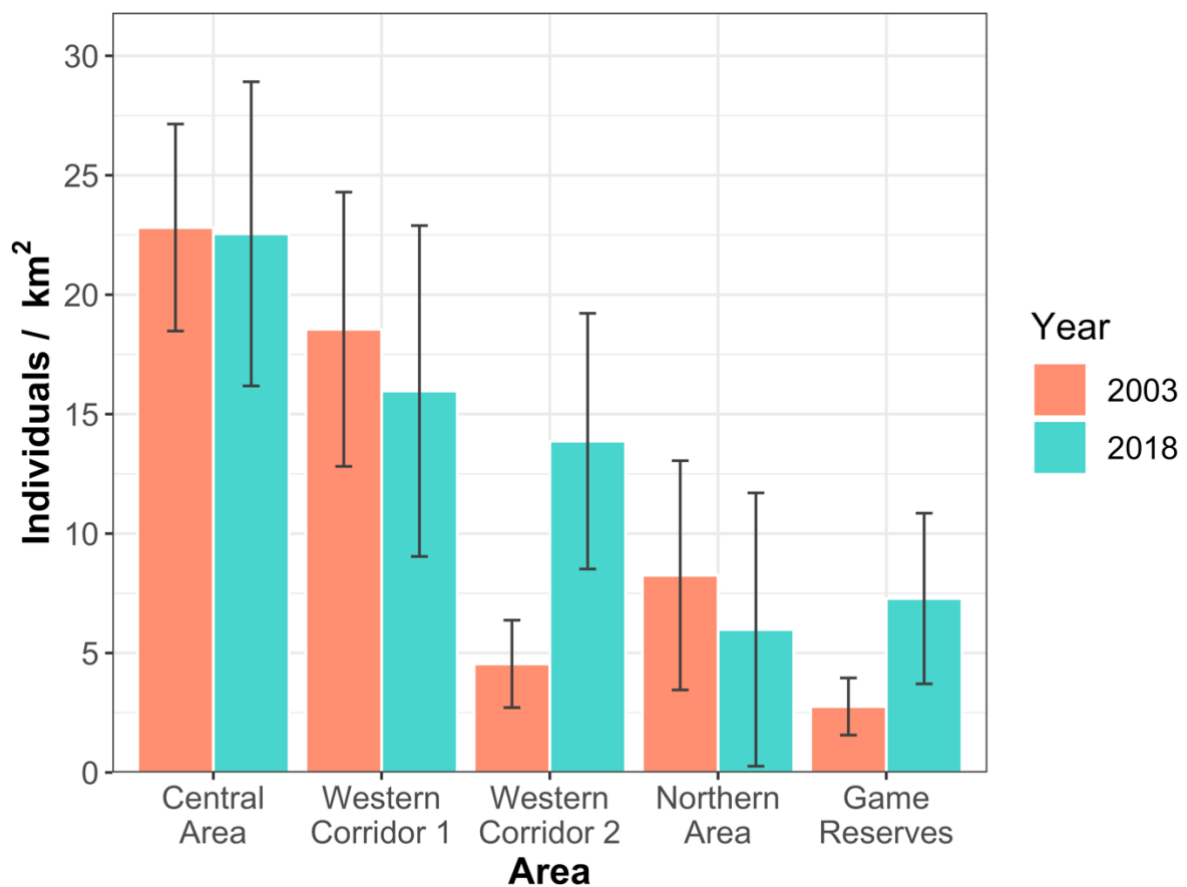


Figure 3. Estimated densities of impala individuals (number of individuals per km²) in each of the five study areas in the Serengeti Ecosystem, for years 2003 and 2018. Error bars represent 95% confidence intervals.

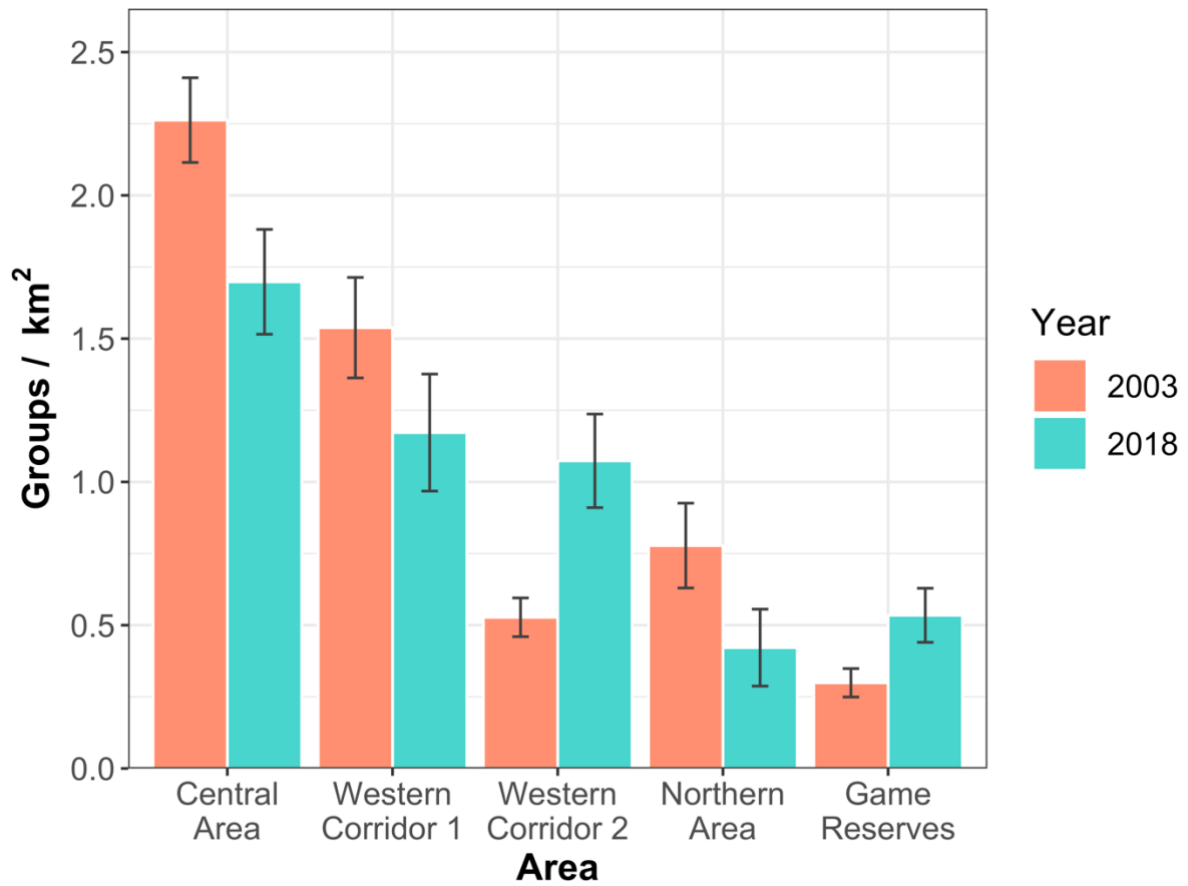


Figure 4. Estimated densities of impala groups (number of groups per km²) in each of the five study areas in the Serengeti Ecosystem, for years 2003 and 2018. Error bars represent 95% confidence intervals.

3.2. Sex ratio

The overall sex ratio was significantly more female-skewed in 2018 than in 2003 ($\chi^2 = 35.1$, $df = 1$, $P < 0.001$; Table 1). A significantly more female-skewed sex ratio in 2018 than in 2003 was found in CA ($\chi^2 = 19.3$, $df = 1$, $P < 0.001$), and in WC2 ($\chi^2 = 6.24$, $df = 1$, $P = 0.013$). The sex ratio was non-significantly more female-skewed in 2018 than in 2003 in GR ($\chi^2 = 2.00$, $df = 1$, $P = 0.158$), and in WC1 ($\chi^2 = 0.30$, $df = 1$, $P = 0.584$). In NA, the sex ratio was significantly less female-skewed in 2018 than in 2003 ($\chi^2 = 4.15$, $df = 1$, $P = 0.042$).

In 2018, a significant difference in sex ratio was found among the five areas ($\chi^2 = 29.9$, $df = 4$, $P < 0.001$; Table 1). The ratio was significantly more female-skewed in GR than in CA, WC1, and NA in 2018 (χ^2 post-hoc tests: $P = 0.0096$, $P = 0.001$, and $P < 0.001$, respectively). The sex ratio was also significantly more female-skewed in WC2 than in WC1 and NA in 2018 (χ^2 post-hoc tests: $P = 0.025$ and $P = 0.006$, respectively).

When analysing the 2003-data, we found a statistically significant difference in sex ratios among the five areas ($\chi^2 = 13.1$, $df = 4$, $P = 0.011$; Table 1). However, in contrast to the results reported by Setsaas et al. (2007), when using a Bonferroni correction of the P -values, the Chi-square post-hoc test revealed no significant differences in any pairwise comparisons of the areas (Appendix C).

Table 1. Sex ratio (males:females) and mean group size \pm standard error (SE) for impala within each of the five study areas, for both year 2003 and 2018. Number of observations (N) is also included. CA = Central Area, WC 1 = Western Corridor 1, WC 2 = Western Corridor 2, NA = Northern Area, and GR = Game Reserves. M = males, and F = females.

Area	N _{sex ratio}		Sex ratio (M:F)		N _{group size}		Mean group size \pm SE	
	2003	2018	2003	2018	2003	2018	2003	2018
CA	391	187	1:1.55	1:1.99	442	187	10.3 \pm 0.7	14.2 \pm 1.3
WC 1	126	68	1:1.65	1:1.75	151	68	12.0 \pm 1.3	13.9 \pm 1.8
WC 2	75	76	1:1.80	1:2.40	92	77	9.6 \pm 1.3	13.4 \pm 1.6
NA	39	35	1:2.01	1:1.41	45	35	11.8 \pm 2.3	8.6 \pm 2.6
GR	54	82	1:2.13	1:2.57	62	83	9.2 \pm 1.3	14.9 \pm 2.5

3.3. Group size

Group size estimates are shown in Table 1 and Figure 5. Overall, the Analysis of Deviance showed that group size differed significantly between years ($P < 0.001$; Appendix B). The effects of area and year \times area were not statistically significant ($P = 0.636$ and $P = 0.139$, respectively). In GR, group size was significantly larger in 2018 than in 2003 ($z = 2.484$, $df = 1$, $P = 0.0130$). In 2003, group size in GR was not significantly different from any of the other areas ($P > 0.1$). In 2018, group size in GR was significantly larger than in NA ($z = -2.452$, $df = 4$, $P = 0.0142$), but did not differ from CA, WC1 or WC2 ($P > 0.1$). However, it should be noted that the sample sizes in GR, WC2 and especially NA were relatively low in both years, and low in WC1 in 2018 (Table 1).

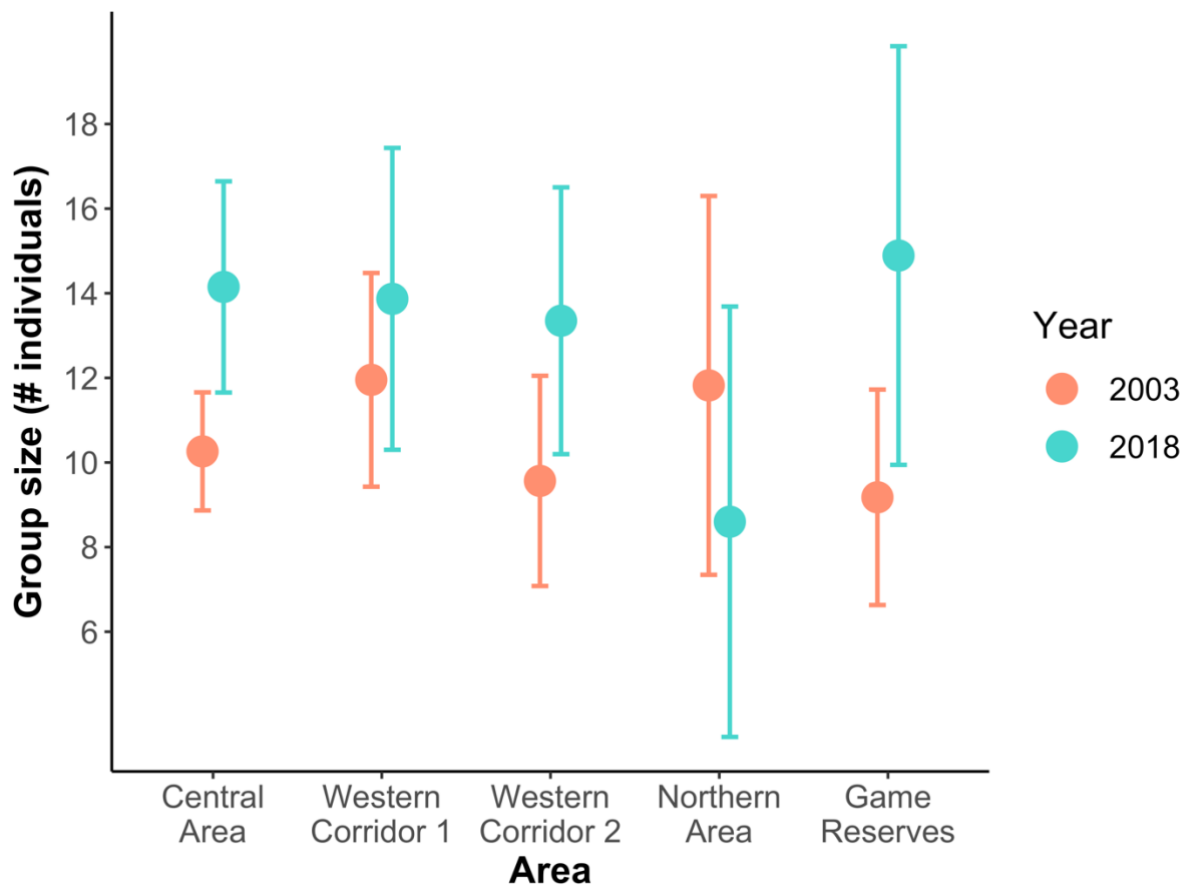


Figure 5. Mean group sizes (dots) with 95% confidence intervals (error bars) in each of the five study areas in the Serengeti Ecosystem in years 2003 and 2018.

3.4. Behaviour

Initial behaviour

There was a significant difference in initial behaviour between inside and outside the SNP, for all data combined ($\chi^2 = 34.4$, $df = 5$, $P < 0.001$). In 2003 there was also a significant difference in initial behaviour between inside and outside the SNP ($\chi^2 = 55.4$, $df = 5$, $P < 0.001$), but there was not in 2018 ($\chi^2 = 5.50$, $df = 5$, $P = 0.358$). Furthermore, there was a significant difference in initial behaviour between 2003 and 2018, for inside and outside the SNP combined ($\chi^2 = 64.8$, $df = 5$, $P < 0.001$). Both inside the SNP ($\chi^2 = 50.8$, $df = 5$, $P < 0.001$), and outside the SNP ($\chi^2 = 32.3$, $df = 5$, $P < 0.001$), there was a significant difference in initial behaviour between 2003 and 2018. In 2003, 39% of the animals outside SNP were fleeing when first discovered, while in 2018, only 6% of the animals were fleeing. In 2003, 6% and 12% of the animals outside the park were resting and feeding, respectively. In 2018, 19% and 34% of the animals outside the park were resting and feeding, respectively (Figure 6).

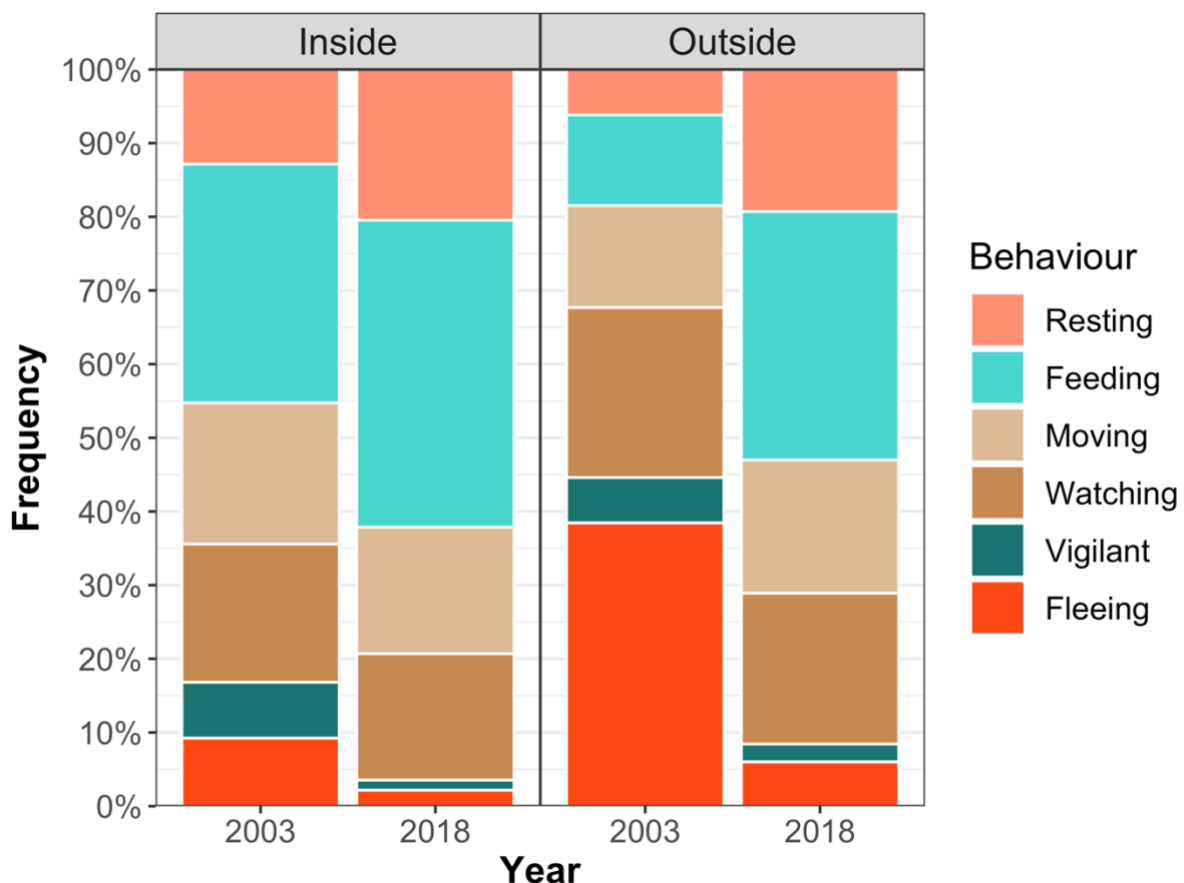


Figure 6. Frequency of the different behaviours observed as soon as impala groups or individuals were discovered, grouped by year (2003 and 2018) and area (inside/outside the SNP). Inside in 2003: $n = 725$, inside in 2018: $n = 367$. Outside in 2003: $n = 65$, outside in 2018: $n = 83$. Watching = watching the observer (see Methods section for definition of the different behaviours).

Flight initiation distance

The most parsimonious GLM for FID, that is, the one with $\Delta AICc < 2$ and least degrees of freedom, included the terms: year, area, STD, alert, year \times area, STD \times area, and alert \times STD (Table 2). The estimates and test statistics for the model coefficients are provided in Table 3. FID was significantly shorter in 2018 than in 2003 and was significantly longer outside the SNP than inside. There was also a significant positive relationship between STD and FID. FID was slightly larger when the impala were alert prior to the approach, but this was not significant. The interaction area \times STD was significant, with a less steep increase in FID with STD outside the SNP than inside. However, at shorter STDs, FID was longer outside the park (Appendix D). The interactions year \times area and alert \times STD were not significant.

Table 2. GLMs tested to answer our hypothesis about flight initiation distance (FID). The models were ranked according to AICc. Df = degrees of freedom, $\Delta AICc$ = the difference in AICc between the given model and the model with the lowest AICc value. ω_i = Akaike's weights, i.e. normalised likelihood of the models. The model with $\Delta AICc < 2$ and the lowest df was the most parsimonious (marked in bold). STD = Starting distance, area = inside/outside Serengeti National Park.

Model	df	AICc	$\Delta AICc$	ω_i
FID ~ year + area + STD + alert + group type + year \times area + STD \times area + alert \times STD	13	3124.8	0.00	0.416
FID ~ year + area + STD + alert + year \times area + STD \times area + alert \times STD	9	3125.9	1.08	0.243
FID ~ year + area + STD + alert + group size + year \times area + STD \times area + alert \times STD	10	3127.0	2.17	0.141
FID ~ year + area + STD + alert + calf presence + year \times area + STD \times area + alert \times STD	10	3127.4	2.58	0.115
FID ~ year + area + STD + alert + vegetation + year \times area + STD \times area + alert \times STD	10	3128.0	3.17	0.085

Table 3. Estimates for the most parsimonious GLM explaining flight initiation distance (FID); 'FID ~ year + area + STD + alert + year × area + STD × area + alert × STD'. Estimates are given on the log scale.

Coefficients	Estimate	SE	z =	P ≤
(Intercept)	3.463	0.043	80.53	< 0.001
year2018	-0.111	0.036	-3.09	0.002
areaOut	0.409	0.100	4.09	< 0.001
STD	0.008	0.000	23.33	< 0.001
alertYes	-0.035	0.070	-0.49	0.622
year2018:areaOut	-0.062	0.081	-0.77	0.439
areaOut:STD	-0.003	0.001	-5.32	< 0.001
STD:alertYes	0.001	0.001	1.72	0.086

4. Discussion

One of the goals of the Grumeti Fund has been to rehabilitate the wildlife populations of the partially protected Grumeti and Ikorongo Game Reserves and the associated Wildlife Management Areas. Over the past ten to fifteen years, the Grumeti Fund has invested significantly into the protection of wildlife in the area, as well as the infrastructure needed to support nature-based tourism and law enforcement (Goodman, 2013). In order to assess what impact the Grumeti Fund has had on wildlife in the area, it is important to understand the impacts that humans have, not only on the abundance of wildlife, but also on the demographic and behavioural characteristics of wildlife populations, and how this in turn may impact the populations in the long-term. The results of this study show that the change to a stricter management in the above-mentioned PPAs has had some beneficial impacts on the impala populations, because the impala population density and group sizes have increased, and impala in the PPAs show reduced flight behaviour towards humans in 2018.

4.1. Density

Both the number of impala individuals per km² and the number of impala groups per km² had increased significantly in GR, which supports our prediction that the density of impala would increase in the partially protected areas. In corroboration with our findings, aerial surveys indicate that the impala population in the Ikorongo-Grumeti Game Reserves complex had nearly doubled from 2003 to 2013, although it appears to have stabilised at around 12,000 individuals (Goodman, 2013).

Although other studies comparing densities of African mammals in a specific area before and after a change in management are rare, many studies have compared densities between protected areas with extractive utilisation and non-extractive utilisation or areas with different exploitation levels (Caro, 1999b; Topp-Jørgensen et al., 2009; Waltert et al., 2009; Wilfred and MacColl, 2016). These studies indicate that mammal population densities are generally higher in less exploited areas than in more exploited areas, which supports our finding that density has increased when exploitation has decreased. Caro et al. (1998) found that across ten census zones in Tanzania, incorporating National Parks, Game Reserves, Game Controlled Areas and Open Areas, poaching was high in areas used by hunters but not patrolled by guards, indicating that on-ground protection is essential for maintaining densities of large mammals, even in legally protected areas. Their results highlight the importance of guards in protected areas for deterring poachers, among other things, even if they receive little financial and material support from the Government (Caro et al., 1998). This is supported by our finding that improved management, including increased law enforcement and reduced poaching, has increased densities of impala.

In CA, WC1 and NA, the density of individuals had not changed significantly, but the density of groups had decreased significantly. Combined with the finding of larger group sizes, this indicates that there were larger but fewer impala groups in these areas in 2018 than in 2003. This could be due to changes in human disturbance, but also other factors such as season and availability of resources, or changes in predator populations. Because the 2003-data and 2018-data were collected during different months of the year (August–December and June–July, respectively), it cannot be ruled out that the observed differences in density of individuals and groups is due to seasonal movements between different habitats within the impala's home ranges (Jarman and Sinclair, 1979). It should also be noted that the density estimates obtained in this study only account for the densities near the roads where transects were driven, as it was not possible to cover more area by driving transects off-road.

4.2. Sex ratio

The sex ratio was female-skewed in all areas in both years, but this is an inherent trait of the social organisation of impala (Jarman, 1974). Aggression from territorial males towards other males may drive the non-territorial males into low-quality marginal habitats where they may suffer relatively higher mortality than females (Jarman, 1974). Measuring the sex ratio in an area can therefore give a local impression of the results of these processes, rather than an accurate sample of the sex ratio of the whole population. However, this study covers a large area within the Serengeti Ecosystem, so there is no reason to believe it does not give a representative sample of the impala sex ratio within the SNP and the partially protected study areas, unless females are more likely to be observed near the roads. However, this seems unlikely, as females are generally more vigilant and tend to flee more readily than males, e.g. due to the presence of young (FitzGibbon, 1998; Stankowich, 2008). The seemingly contradictory results to our predictions regarding density and sex ratio could perhaps be explained by a more female-skewed sex ratio increasing the productivity of the impala populations (Fairall, 1985).

Curiously enough, the sex ratio had become more female-skewed in GR (Table 1), contrary to the expectation that it would be closer to 1:1 following reduced human exploitation. In 2018, the sex ratio was most biased towards females in GR and the Western Corridor, where there is known to have been high illegal hunting pressure before (Holmern et al., 2004). An annual human population growth of >3% in western Serengeti (Estes et al., 2012) (which is higher than the national average (URT, 2003)) could mean that hunting pressure is increasing. A study conducted by Holmern et al. (2006) outside the SNP indicated a male bias in prey choice by bush meat hunters, and even natural predators tended to kill more males than females. The male-bias in kills from human hunting methods could be attributed to sex-specific behaviour of the animals rather than

deliberate choice by the hunters, as there was a tendency for males being more easily caught even with passive hunting methods. Furthermore, legal hunting quotas for tourist and resident hunters around the SNP only allow the shooting of males (URT, 2015), and male-biased hunting regimes have led to severely female-biased sex ratios for many ungulate populations globally (Milner et al., 2007).

Several case studies from Africa have found more female-biased sex ratios in areas more affected by human exploitation, than in areas with less exploitation. For example, Marealle et al. (2010) found that the sex ratio of Maasai giraffe (*Giraffa camelopardalis tippelskirchii*) in SNP was significantly more female-skewed in areas with high risk of illegal hunting compared to low-risk areas, even among new-born calves. Ndibalema (2009) found a more female-biased sex ratio in the resident wildebeest sub-population in the Western Corridor than in the migratory sub-population and argued that the more female-skewed sex ratio in the West could be linked to illegal hunting in this area. However, Hariohay et al. (2018) found no difference in impala sex ratio between Ruaha National Park (strictly protected) and Rungwa Game Reserve (partially protected) in Tanzania, and Caro (1999a) was not able to conclude that the sex ratios of various large mammal species differed between the inside and outside of Katavi National Park in western Tanzania. Furthermore, and contrary to our assumption, Magige (2008) found that the sex ratio of ostriches (*Struthio camelus*) was significantly more female-skewed inside the SNP than in adjacent partially protected areas. A higher level of natural predation on ostriches in the SNP, especially on males, was suggested as an explanation for the observed ostrich sex ratios (Magige, 2008). This introduces the possibility that the more female-skewed sex ratios found in our study are a result of increased natural predation, as it has been shown that males are generally less vigilant and have more risk-prone behaviour than females (FitzGibbon, 1998), which could make them more vulnerable to natural predation. Increased natural predation would also be congruent with the assumption of increased wildlife abundance as a result of improved conservation management in the partially protected areas. However, this cannot directly explain the more female-skewed sex ratio in the SNP.

Clearly, studies show contradictory results of the effects of human exploitation on large herbivore sex ratios, and the increase in female bias in impala populations found in our study could be indicative of different things, increased illegal hunting pressure being one of them. However, changes in natural predator populations, or perhaps increased trophy hunting, would also affect male mortality, and have the potential to skew sex ratios even more towards females. Thus, we cannot conclude on the causes of the observed changes in sex ratios, as we lack the necessary data on the above-mentioned factors. Hence, it is clear that the findings of more female-skewed sex ratios warrant further investigation.

4.3. Group size

We found that group size in the partially protected study area did not differ from any area within the SNP in 2018, except NA. Furthermore, there were no differences in group size between GR and the areas in SNP in 2003. Similarly, Caro (1999a) observed few significant differences in group size inside and outside Katavi National Park, Tanzania, for a range of mammal species, including impala. However, the overall mean group size was larger in 2018 than in 2003. More importantly, the group size had increased significantly in GR.

The increase in group size in this previously overexploited area could be an indication of reduced disturbance from humans. For instance, Hariohay et al. (2018) found larger impala group sizes in a protected area where only non-consumptive activities are permitted; Ruaha National Park, compared to Rungwa Game Reserve (RGR), where the major tourism activity is trophy hunting. They argued that the observed difference in group size might be due to the difference in nature of tourism activities in the two areas, and due to direct effects of hunting on the impala in RGR or behavioural responses of animals selecting safer habitats away from roads (Hariohay et al., 2018). In support of this, Averbeck et al. (2010) found larger group sizes of mixed-sex impala herds inside the strictly protected Lake Mbuho National Park (LMNP) in western Uganda, than in the adjacent unprotected farmland. They argued that the observed decrease in mixed-sex herd size was likely due to female-biased poaching, as poachers around LMNP prefer the hornless females over males as they are less dangerous to handle when caught in nets. This is different to the male-biased hunting occurring in the Serengeti Ecosystem (Holmern et al., 2006), but nonetheless, the intensity of human pursuit in general was seen as the underlying cause for the observed decrease in group size in the more exploited area (Averbeck et al., 2010). Similarly, Manor and Saltz (2003) found that mountain gazelles (*Gazella gazella*) tended to be in smaller groups in open areas with high human disturbance than in open habitat with low human disturbance. Aggregating into larger groups may be advantageous when faced with natural predators, from which there is a chance of escaping. However, being in smaller groups or alone is likely more advantageous for avoiding predators that are too successful, as is the case with human hunters, as small groups or singletons are harder to spot than larger aggregations (Fischer and Linsenmair, 2007).

Impala herds do not have permanent memberships; females especially range at will within their home ranges and position themselves as best they can in relation to resources (Jarman, 1979; Murray, 1982a). The tendency for females to often find themselves in the same group is a product of them sharing similar home ranges or perhaps similar physiological state, as opposed to being dictated by true affinity through kinship. As a result of this, impala groups may regularly merge and split up, and the impermanence of association between individuals is shown by the correlation between group size and

available food (Jarman, 1974). As first argued by Caughley (1964), in species where group sizes depend on animals randomly joining and leaving, an increase in population density will increase the frequency with which groups meet and join each other, and as a direct consequence the mean group size will increase. This is in corroboration with our findings that both impala population density and mean group size had increased in the partially protected study area, and hence, the increase in group size could be a symptom of increased density rather than a direct result of reduced disturbance. Furthermore, impala group sizes might be influenced more by resource availability than by human pressure, because impala group size has been found to be smaller during the dry season than the wet season (Jarman and Jarman, 1979).

As with density, it must be noted that the 2003-data and 2018-data were collected during different times of year, and thus the observed differences in group sizes between the two study periods could be a result of seasonal differences. Furthermore, even though the change in mean group size in CA, WC2 and GR was statistically significant, a change in group size of a few individuals may not mean much biologically, considering how much impala group sizes vary, even the ones observed in this study (i.e. from one individual to more than a hundred). However, the evidence from previous studies corroborate our findings of larger group sizes in times or areas with less human disturbance, in support of the prediction that group sizes would have increased as a result of improved management practices.

Hence, taking into consideration that impala tend to move around and aggregate within their home ranges in accordance with season and available resources, and that the 2003-data were collected largely well into the dry season, and the 2018-data were collected at the start of the dry season, just after the rainy season had ended, it could be that the observed increase in group size is more related to difference in season and food abundance than reduced human disturbance. Even though previous studies have found increased group sizes to be a symptom of less disturbance (see above). Group sizes should therefore be investigated further by collecting data at the same time of year as in 2003, in order to control for possible seasonal effects.

4.4. Behaviour

Initial behaviour

A significant difference in initial behaviour was found between 2003 and 2018, both inside and outside the SNP. Most notably, a much lower proportion of groups encountered outside the park fled when first discovered in 2018 than in 2003, and conversely, a much higher proportion of the groups outside the park were resting or feeding in 2018. This supports the prediction that impala would have become less flighty and nervous in the partially protected areas.

In Rungwa Game Reserve in Tanzania, there was also a significantly larger proportion of impala that were vigilant or fled from the car than there were in the adjacent Ruaha National Park (RNP). The dominant behaviours in RNP were feeding and resting (Hariohay et al., 2018). The difference in behaviour between a National Park and a Game Reserve, as shown both by Hariohay et al. (2018) and for the 2003-data in our study, can be equated to the difference in behaviour outside the SNP between 2003 and 2018, in that the behaviour in 2018 did not differ between outside and inside the SNP. This indicates that the level of protection in the GR is indeed higher in 2018 than it was in 2003, as expected, and is now more similar to the protection level of the SNP.

Caro (1999a) found that mammals in general were significantly more likely to flee from the observer outside the Katavi National Park (KNP) than inside. However, when testing within species, impala were not statistically more likely to flee outside the KNP than inside. For species for which data were available, none fled at significantly greater distances outside the KNP than inside. But he did find that the species that were significantly more likely to flee outside the KNP corresponded to species that were found at lower densities outside the park (Caro, 1999b). Species wary of the vehicle therefore seemed to be those suffering from hunting pressure.

On the other hand, behaviour of impala may differ according to the time of day (Jarman and Jarman, 1973), although transects were covered during the whole period of daylight during both study periods in an effort to control for such behavioural differences. The change in behaviour between the two study periods might also be confounded with the timing of the hunting season. The hunting season is officially from 1st July to 31st December every year (URT, 1974); data for initial behaviour in 2003 were collected between 21st August and 13th December, while the data in 2018 were collected between 10th June and 19th July. Clearly, there is a difference in overlap with the hunting season between the two study periods, and if impala behave differently according to the hunting season, as has been shown for other animals (Stankowich and Blumstein, 2005; Reimers et al., 2009), this could confound the observed differences in behaviour.

Our findings for initial behaviour may therefore give an indication of behavioural differences between the two study periods, especially outside the SNP, but it is questioned how useful the results are considering how the data are collected. The method is to record the observed behaviour of the majority of the animals in a group immediately as the group is spotted. However, the researchers were not the same between years, thus the respective car drivers might have approached the animals differently, and the behaviours might have been observed differently. In addition, by only recording the one behaviour that is observed immediately when an individual or group is discovered, a lot of important nuances may be lost, e.g. if an individual is feeding when first spotted, but starts watching the observer after the car stops, or *vice versa*. Another example would be if a group is encountered

where two behaviours are exhibited by half of the group members each. Then there is no single type of behaviour displayed by the majority of the group.

Flight initiation distance

It was found that the most important variables for explaining FID were year, starting distance and area (inside/outside the SNP). Starting distance did as expected have a highly significant effect on FID. Alertness was also part of the most parsimonious model but did not have a significant effect. These results clearly show that, the year of study and whether experiments were conducted inside or outside the SNP made an important difference in the observed FID. More specifically, FID was significantly shorter in 2018 than in 2003 and had decreased a little bit more outside the park than inside, so that FID was more equal between inside and outside the SNP in 2018, although this interaction effect was not significant (Appendix D). Nevertheless, these findings give support to the prediction that impala would exhibit less flighty behaviour in 2018, especially in the partially protected areas outside the SNP.

In support of the idea that shorter FID is a result of a higher level of protection from human disturbance, ostriches have also been found to have longer FID in partially protected areas outside the SNP than inside the SNP (Magige et al., 2009). Magige et al. (2009) argued that the ostriches might be negatively affected by unregulated legal hunting, which is often conducted from vehicles, rather than illegal bushmeat hunting. Furthermore, impala had significantly longer FID in Rungwa Game Reserve than in Ruaha National Park in Tanzania, and this behaviour might have been affected by game hunting in the RGR (Hariohay et al., 2018). The FID of impala was also significantly higher in the second year in RGR, when Hariohay et al. (2018) collected data at the peak of the hunting season, as opposed to the first year. In the RNP there was no difference in FID between years. Matson et al. (2005) also found that between two areas with differing intensities of hunting in Zimbabwe, impala had significantly greater FID in the area with the greatest hunting intensity of the two, suggesting that impala anti-predator behaviour in these areas might have been affected by the levels of hunting by humans, even though the abundance of natural predators and group sizes were larger in the least hunted area (Matson et al., 2005). In general, ungulate populations that experience higher hunting activity are more wary than less habituated populations (Stankowich, 2008).

The observed overall reduction in FID from 2003 to 2018 could also be a sign of habituation to human presence and disturbance, as the amount of tourist activity in the Serengeti has increased considerably in the past years (the number of vehicles passing through the main gate into SNP in Ngorongoro Conservation Area has increase by about 10% annually (Fyumagwa et al., 2013)), and ungulates in areas with frequent non-lethal contact with humans show reduced flight responses compared to those in areas where

human contact is rare (Stankowich, 2008). In addition, the difference in timing of data collection in relation to the hunting season could be a confounding factor for the observed differences in FID, as it could be for initial behaviour (see discussion above).

5. Conclusion

In this study, we found that the impala population density and mean group size had increased significantly in the partially protected study areas in western Serengeti. In addition, behaviour was significantly less flighty towards an approaching human. These positive changes are likely attributable to more effective conservation management. However, the overall sex ratio had become more female-skewed in 2018, which was contrary to our second prediction.

These results indicate that the increased conservation efforts in the Grumeti Area has had positive effects on the local wildlife, and that the strategy employed by the Grumeti Fund of investing in increased law enforcement and better infrastructure, employing former poachers as rangers and providing benefits to local people, can be a successful one if implemented correctly. This gives hope that improvement of management practices in protected or partially protected areas can indeed facilitate the recovery of previously overexploited wildlife populations. However, managers and conservationists should still make sure to monitor the more subtle, indirect impacts of humans on the demographics and behaviour of wildlife populations, to enable early detection of potential negative trends and thus be able to quickly implement mitigative measures.

Lastly, our results showed a shift to more female-skewed sex ratios. There are various possible explanations for this, such as continued illegal hunting pressure, more intense trophy hunting or even an increase in natural predator populations. But the reasons are ultimately unknown, and further investigation is warranted. Managers and conservationists should therefore draw hope from the positive outcomes seen from the management of the Grumeti Area since the start of the century but should still monitor different demographic and behavioural factors of wildlife populations in order to detect possible detrimental effects that human disturbance might still be having.

6. References

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Appendices

Appendix A: Chi-square post-hoc function

Appendix B: Outputs of the model for group size

Appendix C: Pairwise comparisons of sex ratio between areas in each year

Appendix D: Interaction effects of the model for FID

Appendix A: Chi-square post-hoc function

The Chi-square post-hoc tests that were conducted as part of the sex ratio analyses used the following custom-made function in R:

```
chisq.post.hoc <- function(tbl,test=c("fisher.test"),
popsInRows=TRUE,control=c("fdr","BH","BY","bonferroni","holm","hochberg","hommel"),digits=4,
...) {
  ##### extract correction method
  control <- match.arg(control)
  ##### extract which test (fisher or chi square)
  test = match.fun(test)
  ##### test rows or columns
  if (!popsInRows) tbl <- t(tbl)
  popsNames <- rownames(tbl)
  ##### come up with all possible comparisons
  prs <- combn(1:nrow(tbl),2)
  ##### preallocate
  tests <- ncol(prs)
  pvals <- numeric(tests)
  lbls <- character(tests)
  for (i in 1:tests) {
    pvals[i] <- test(tbl[prs[,i],, ...])$p.value
    lbls[i] <- paste(popsNames[prs[,i]],collapse=" vs. ")
  }
  adj.pvals <- p.adjust(pvals,method=control)
  cat("Adjusted p-values used the",control,"method.\n\n")
  data.frame(comparison=lbls,raw.p=round(pvals,digits),adj.p=round(adj.pvals,digits))
}
```

Appendix B: Outputs of the model for group size

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL			1241	1394.8	
year	1	15.1773	1240	1379.6	9.787e-05 ***
area	4	2.5497	1236	1377.1	0.6358
year:area	4	6.9453	1232	1370.1	0.1388

Figure 7. Output from the Analysis of Deviance conducted on the negative binomial GLM testing the prediction for group size for the combined 2003- and 2018-data; 'group size ~ year + area + year × area'. Terms were added sequentially from first to last. 'year' includes years 2003 and 2018. 'area' includes the study areas Central Area, Western Corridor 1, Western Corridor 2, Northern Area and Game Reserves. Screenshot from RStudio.

Coefficients:					
	Estimate	Std. Error	z value	Pr(> z)	
(Intercept)	2.21675	0.14844	14.934	<2e-16	***
yearFloelo	0.48405	0.19490	2.484	0.0130	*
areaCA	0.11174	0.15842	0.705	0.4806	
areaWC1	0.26429	0.17582	1.503	0.1328	
areaWC2	0.04139	0.19192	0.216	0.8293	
areaN	0.25323	0.22770	1.112	0.2661	
yearFloelo:areaCA	-0.16284	0.21944	-0.742	0.4580	
yearFloelo:areaWC1	-0.33553	0.25771	-1.302	0.1929	
yearFloelo:areaWC2	-0.15062	0.26474	-0.569	0.5694	
yearFloelo:areaN	-0.80227	0.32717	-2.452	0.0142	*

Figure 8. Summary output for the negative binomial GLM testing the prediction for group size for the combined 2003- and 2018-data; 'group size ~ year + area + year × area'. The area GR was set as the reference level. 'yearFloelo' = year 2018. 'areaCA' = Central Area, 'areaWC1' = Western Corridor 1, 'areaWC2' = Western Corridor 2, and 'areaN' = Northern Area. Estimates are given on the log-scale. Screenshot from RStudio.

Appendix C: Pairwise comparisons of sex ratio

Results of Chi-square post-hoc tests used to make pairwise comparisons of sex ratios between the five study areas in year 2003 and 2018 (CA = Central Area, WC1 = Western Corridor 1, WC2 = Western Corridor 2, NA = Northern Area, GR = Game Reserves).

Table 4. Results of Chi-square post-hoc test with Bonferroni correction of P-values comparing sex ratios between areas using only the 2003-data. Both raw and adjusted P-values are reported. Significant P-values are marked in red.

2003-data:	Comparison	Raw p-value	Adj. p-value
1	CA vs. WC1	0.3806	1.0000
2	CA vs. WC2	0.1269	1.0000
3	CA vs. NA	0.0178	0.1782
4	CA vs. GR	0.0058	0.0576
5	WC1 vs. WC2	0.4344	1.0000
6	WC1 vs. NA	0.1037	1.0000
7	WC1 vs. GR	0.0398	0.3975
8	WC2 vs. NA	0.4209	1.0000
9	WC2 vs. GR	0.2380	1.0000
10	NA vs. GR	0.7083	1.0000

Table 5. Results of Chi-square post-hoc test with Bonferroni correction of P-values comparing sex ratios between areas using only the 2018-data. Both raw and adjusted P-values are reported. Significant P-values are marked in red.

2018-data:	Comparison	Raw p-value	Adj. p-value
1	CA vs. WC1	0.1493	1.0000
2	CA vs. WC2	0.0276	0.2759
3	CA vs. NA	0.0145	0.1448
4	CA vs. GR	0.0010	0.0096
5	WC1 vs. WC2	0.0025	0.0247
6	WC1 vs. NA	0.1670	1.0000
7	WC1 vs. GR	0.0001	0.0011
8	WC2 vs. NA	0.0005	0.0055
9	WC2 vs. GR	0.4938	1.0000
10	NA vs. GR	0.0001	0.0007

Appendix D: Interaction effects of the model for FID

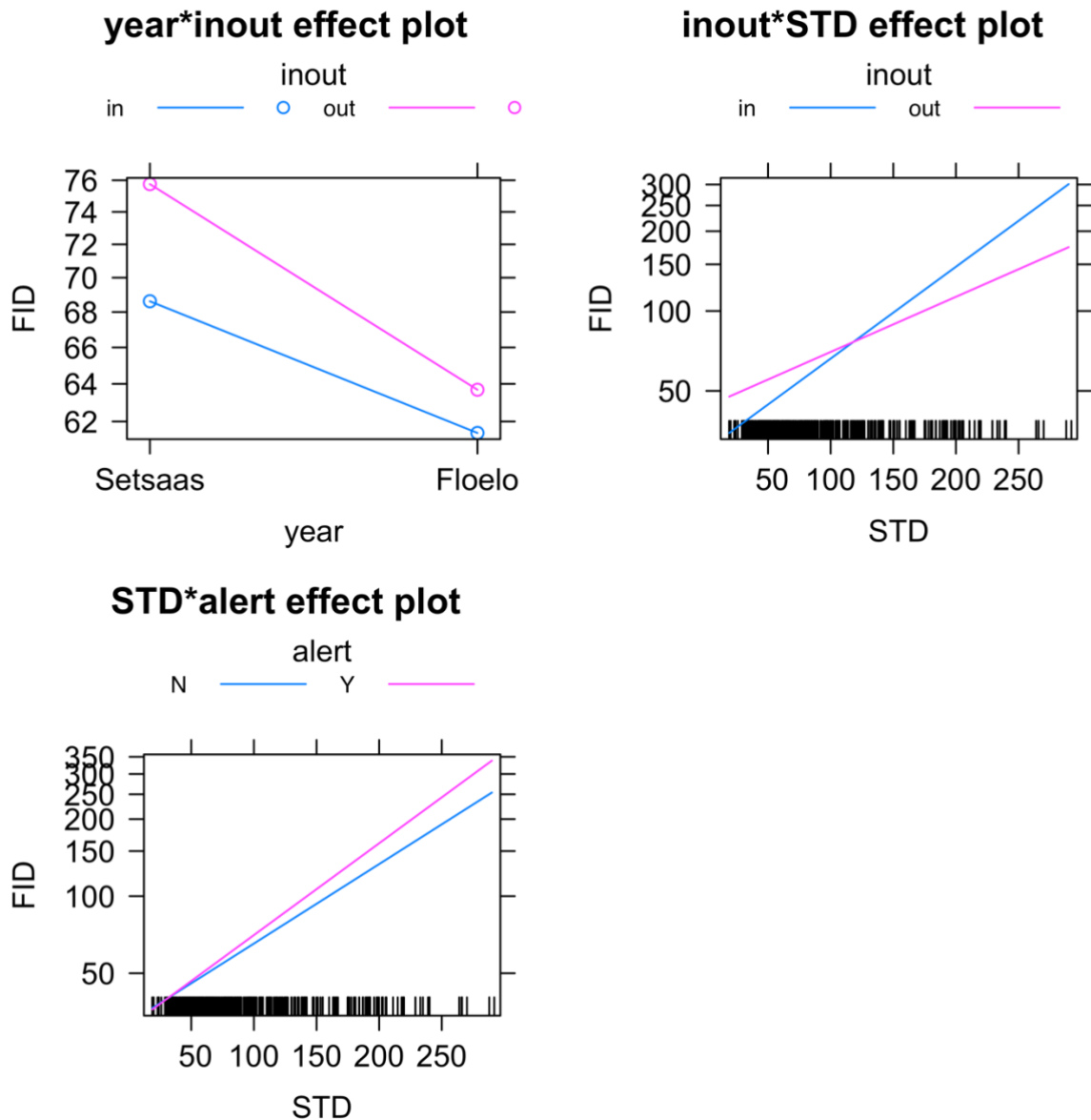


Figure 9. Plot of all interaction effects for the most parsimonious model explaining flight initiation distance (FID); $FID \sim year + area + STD + alert + year \times area + STD \times area + alert \times STD$. Obtained by plotting the `'allEffects()'` function from the `'effects'` R package, version 4.1.0. (Fox, 2003; Fox and Weisberg, 2018). STD = Starting distance. Setsaas = year 2003, Floelo = year 2018. In = inside Serengeti National Park (SNP), out = outside SNP. Alert: N = no, Y = yes. All distance units in metres.

