The impact of dehorning on the white rhinoceros (*Ceratotherium simum*) and the evaluation of novel anti-poaching tactics

By

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Abstract

The white rhinoceros (*Ceratotherium simum*) is experiencing unsustainable poaching losses fuelled by an increased demand for horn. In an attempt to reduce poaching pressure and faced with rising security costs, increasingly, private and state reserves are dehorning their rhino populations as a management approach. Despite the procedure's wide-scale practice, significant knowledge gaps exist on how dehorning may affect the behaviour or physiology of white rhinos. Given that rhinos require continued protection after dehorning, there is also a need to develop effective, low-cost techniques to aid on-the-ground conservation efforts. This research employed a combination of field studies and laboratory experiments to address this.

To determine whether dehorning affected the social behaviour of white rhinos, behavioural observations were conducted on free-ranging horned and dehorned populations in South Africa. Dehorned individuals exhibited similar agonistic and cohesive social behaviours to horned individuals. However, a rise in the rate of agonistic interactions was detected after a repeat dehorning procedure. The results also provide the first evidence of a non-territorial dominance hierarchy among free-ranging white rhinos, with the lowest ranked individuals shifting in social position after dehorning.

To investigate whether white rhinos exhibited a long-term physiological response to horn removal, changes in adrenal and gonadal steroid levels were monitored in faecal samples. Long-term hormone profiles were not influenced by the number of times a rhino had been dehorned. Furthermore, there was no detectable difference in corticoid concentrations between a dehorned and horned population. Dehorning did not appear to act as a chronic stressor and thus compares favourably to other conservation techniques, such as translocation.

Horn use in a non-social context was examined by camera trapping and behavioural observations. Dehorning did not have a detectable impact on resource access, with no change in digging behaviours observed during geophagy and wallowing, nor on the frequency of horn rubbing behaviours, suggesting limited impact of the procedure on this aspect of the species ecology.

The movement of rhinos from at-risk areas could provide an effective anti-poaching tactic. Evaluation of the efficacy of novel deterrent-based techniques to move rhinos from poaching hotspots into safer areas was undertaken. White rhinos were exposed to acoustic, olfactory and drone-based stimuli and repeatedly fled from low-altitude drone flights and the siren noises. However, olfactory stimuli were ineffective at inciting avoidance behaviour. The drone's long transmission range and capability of pursuit make it most suited to manipulating rhino movement as a means to reduce poaching risk.

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List of Acronyms

ACTH	Adrenocorticotropic hormone
AfRSG	African Rhino Specialist Group
BBS	Batchelder–Bershad–Simpson index
CITES	Convention on International Trade in Endangered Species of Wild Flora & Fauna
CoP	Conference of Parties (CITES)
DEDECT	North West Department of Economic Development, Environment, Conservation and Tourism
ELISA	Enzyme-linked immunosorbent assay
FAM	Faecal androgen metabolites
FCM	Faecal corticoid metabolites
FPM	Faecal progestogen metabolites
ICI	Inter-calving interval
NWPB	North West Parks and Tourism Board
SACAA	South African Civil Aviation Authority
TCM	Traditional Chinese medicine

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Author’s declaration

I declare that the research contained in this thesis, unless otherwise formally indicated within the text, is the original work of the author. The thesis has not been previously submitted to this or any other university for a degree, and does not incorporate any material already submitted for a degree.

Chapter 1 – General introduction

1.1 Introduction

Rhinoceros species require immediate action to save them from unsustainable poaching losses (Haas and Ferreira, 2017; Taylor et al., 2017; Ferreira et al., 2018). A rise in demand for rhino horn in East Asia has resulted in the deaths of over 5,000 rhinos over the last five years (Vigne and Martin, 2018; Emslie et al., 2019; Mokonyane, 2019). Faced with unsustainable security costs, private reserves and national parks in southern Africa are increasingly dehorning their rhinos or translocating them to safer areas in an attempt to stop the slaughter (Rubino and Pienaar, 2018; Emslie et al., 2019). However, in many cases, these interventions are performed as a crisis response and lack more robust biological evaluation (Lindsey and Taylor, 2011; du Toit and Anderson, 2013). Studies into how dehorning affects rhino behaviour or physiology could help aid rhino conservation strategies.

1.2 Rhinoceros population change

There are five extant species within the Rhinocerotidae family; the white rhino (*Ceratotherium simum*) and the black rhino (*Diceros bicornis*) are endemic to Africa, while the Sumatran rhino (*Dicerorhinus sumatrensis*), Javan rhino (*Rhinoceros sondaicus*) and Indian rhino (*Rhinoceros unicornis*) are endemic to Asia (Willerslev et al., 2009). Over the last three centuries all five species have seen their ranges contract and their populations decline due to hunting, habitat loss and population fragmentation (Foose et al., 1997; Emslie and Brooks, 1999; Amin et al., 2006). Several subspecies are now extinct or are threatened with extinction, while others have made a modest recovery (Moodley et al., 2017; Brandt et al., 2018; Emslie et al., 2019). The major historical pattern for all rhino species has been for hunters to deplete one population and then move on to another, progressively extirpating rhinos from across their range (Schafer, 1963; Foose et al., 1997; Emslie and Brooks, 1999; Vigne et al., 2007; Antoine, 2012). The IUCN Red List classifies the Indian rhino as Vulnerable (Talukdar et al., 2008); the black, Sumatran and Javan rhinos as Critically Endangered (van Strien et al., 2008a, 2008b; Emslie, 2012a); and the white rhino as Near Threatened (Emslie 2012b). However, white rhinos experience the greatest absolute losses from poaching (Emslie et al., 2019).

1.2.1 Asian rhino species

All three species of Asian rhino were widely distributed across South and Southeast Asia until at least the middle of the 19th century (Figure 1.1; Foose et al., 1997; Antoine, 2012). By the end of the nineteenth century, hunting and habitat loss had led to their disappearance from across much of their range (Foose et al., 1997; Antoine 2012). Hunting pressure and habitat destruction continue to the present day (Haryono et al., 2016), and demand for their horns remains high (Truong et al., 2016; Taylor et al., 2017).

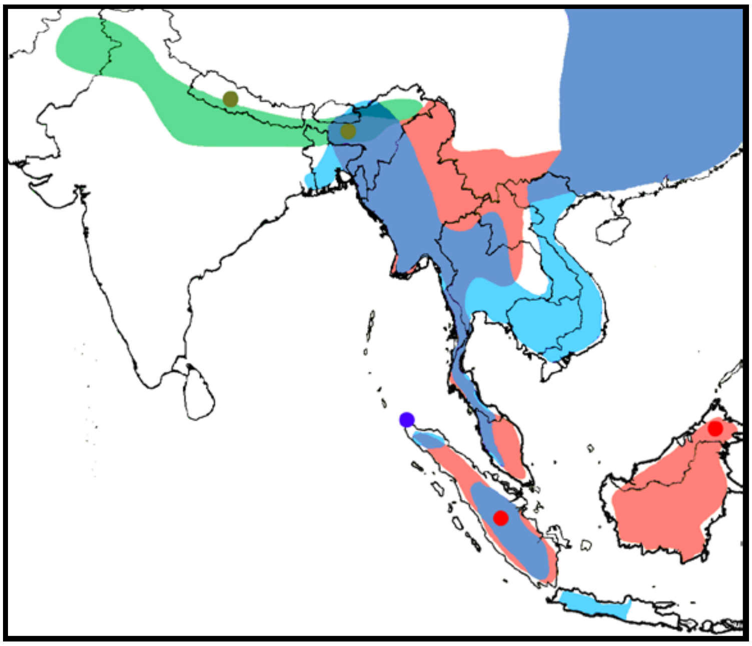

Figure 1.1. Holocene range and current distribution of Asian rhino species. Shading indicates Holocene range (approx. 10,000 to 0 BCE) of the Indian rhino (green), Javan rhino (light blue) and Sumatran rhino (pink); mixed colours indicate species' overlap. Points indicate countries with surviving populations of Indian rhino (dark green), Javan rhino (dark blue) and Sumatran rhino (red) as of 2018. Points are shown within country boundaries and do not indicate exact locations. Holocence range map adapted from Antoine (2012). Current distributions from Emslie et al. (2019).

The Javan rhinoceros, also referred to as the lesser one-horned rhinoceros, is classified into three subspecies (Groves and Leslie, 2011). Two of these subspecies are extinct*: R. s. inermis* was last documented from the Sundurbans in Bangladesh in 1908 and had become extinct by 1925 (Rookmaaker, 1997). While the last surviving individual *of R. s.*

annamiticus was poached in 2010 from Cat Tien National Park in Vietnam (Brook et al., 2012), resulting in the species' extirpation from mainland Asia. All remaining Sumatran rhinos belong to the subspecies *R. s. sondaicus* with the entire global population restricted to Ujung Kulon National Park in Java, Indonesia, where 65 to 68 individuals remain (Emslie et al., 2019). . The park is likely to be at or nearing ecological carrying capacity and the rhino population is unlikely to increase without an intensive management intervention (Haryono et al., 2016). All individuals belong to a single population placing survival at risk from outbreaks of poaching or cattle-mediated disease (Haryono et al., 2016), as well as from major natural disasters such as volcanoes or tsunamis (Setiawan et al., 2018).

The Sumatran rhinoceros, also known as the Asian two-horned rhinoceros, is the smallest of the five extant Rhinocerotidae and more closely related to the extinct woolly rhinoceros (*Coelodonta antiquitatis*) than the other two Asian species (Orlando et al., 2003; Willerslev et al., 2009). The global population may total as few as 40 individuals or as many as 78 (Emslie et al., 2019). The species survived in mainland Asia until the end of the 20th century (Foose et al., 1997), but is now restricted to the islands of Sumatra (subspecies *D. s. sumatrensis*), and Borneo (subspecies *D. s. harrisoni*) (Kretzschmar et al., 2016; Steiner et al., 2018). All remaining sub-populations are at risk from poaching, and their habitat is threatened by the conversion of forest to agricultural plantations, logging, and mining (Miller et al., 2015; Havmøller et al., 2016). All populations are small and fragmented, and without intervention the species' long-term chances of recovery will be hampered by potential inbreeding depression and the Allee effect (Goosens et al., 2013; Miller et al., 2015; Kretzschmar et al., 2016).

The Indian rhinoceros, also known as the greater one-horned rhinoceros, numbered fewer than 200 individuals by the late 19th century (Amin et al., 2006 Cédric et al., 2016). Following effective protection efforts and successful population management, the species had recovered to around 600 to 800 individuals by 1975, and to around 3,558 animals by 2018 (Amin et al., 2006; Emslie et al., 2019). All surviving populations are found within India and Nepal and are at risk from continued poaching and habitat encroachment, including domestic grazing pressure and the illegal burning of grasslands (Amin et al., 2006; Emslie et al., 2016).

1.2.2 African rhino species

The white and black rhino once roamed across much of Africa, occurring in grasslands and savannah woodlands across the southern, eastern, and central parts of the continent (Figure 1.2; Emslie and Brooks, 1999; Rookmaaker and Antoine, 2012). Having once been widespread and common, they are now limited to remnants of their historic range following significant population declines due to human exploitation (Emslie and Brooks, 1999; Moodley et al., 2017). African rhinos are primarily hunted for their horns (MacMillan et al., 2017) but historically they were also exploited as a source of meat (Lang, 1924) and cleared from land destined for human use (Martin and Martin, 1982).

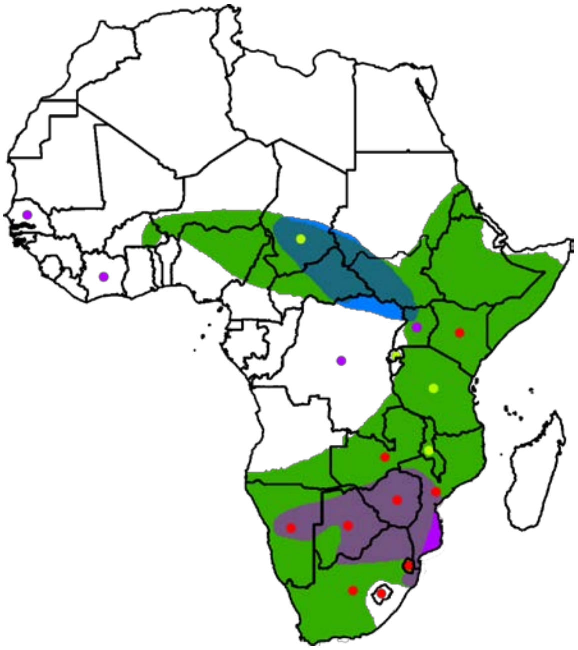

Figure 1.2. Historic range and current distribution of African rhino species. Shading indicates historic range (approx. 1500 CE) of black rhino (green) and of the southern subspecies (purple) and northern subspecies (blue) of white rhino; mixed colours indicate species' overlap. Points indicate countries with surviving, reintroduced or introduced populations of black rhino (green), white rhino (purple) and both species (red) as of 2018. Points are shown within country boundaries and do not indicate exact locations. Historic range maps adapted from Rookmaaker and Antoine (2012). Current distributions from Emslie et al. (2019).

The black rhino can be split into four subspecies: the south-central black rhino (*D. b. minor*), the south-western black rhino, (*D. b. bicornis*), the eastern black rhino (*D. b. michaeli*), and the western black rhino (*D. b. longipes*) which was declared extinct in

2011 (du Toit, 1987; Moodley et al., 2017). Despite continuous hunting pressure, an estimated 100,000 black rhinos survived in Africa until 1960, but by 1970 this had decreased to around 65,000 individuals (Emslie and Brooks, 1999). Over the next 35 years the global population continued to decrease, reaching a low of 2,408 by 1995 - a reduction of 96% (Emslie and Brooks, 1999). As of 2017, the population had recovered to an estimated 5,595 individuals (5,366 to 5,627 individuals at 90% CI) and was increasing by around 2.9% a year (Emslie et al., 2016; Emslie et al., 2019). The largest populations are found in South Africa, Namibia, Kenya, Zimbabwe, and Tanzania (Moodley et al., 2017), with smaller populations occurring in Malawi, Mozambique, Zambia, and Botswana (Emslie et al., 2019). Recent re-introductions have returned them to Rwanda (Gyöngyi and Elmeros, 2017) and Chad (Fearnhead, 2018), although in Chad some of the founding individuals have since died (Emslie et al., 2019).

The white rhino is classified into two subspecies, the southern white (*C. simum simum*) and northern white rhino (*C. simum cottoni*) (Harley et al., 2016). Groves et al. (2011) proposed that the genetic and morphological differences of the northern white rhino qualified it to full species status. Although the two taxa can be clearly differentiated by their dentition and cranial anatomy (Groves et al., 2011), further genetic work indicated sub-species status to be a more appropriate designation, and that taxonomic revision was not required (Emslie and Adcock 2016; Harley et al., 2016; Tunstall et al., 2018). There are few known behavioural differences between the subspecies, but northern white rhino vocalisations can be longer in duration and may differ in spectral frequency to those of southern whites (Cinková and Policht, 2014). The two lineages are estimated to have diverged less than 80,000 years ago (Tunstall et al., 2018) and since recorded history the range of the two subspecies has been separated by around 2000 km (Figure 1.2; Rookmaaker and Antoine, 2012).

At the beginning of the 20th century, the northern white rhino was the more abundant of the two subspecies, numbering between 2000 and 3000 individuals (Harper, 1945). The northern white's range encompassed the Central African Republic, Chad, the Democratic Republic of the Congo (DRC), South Sudan, Sudan, and Uganda (Emslie and Brooks, 1999; Rookmaaker and Antoine, 2012). This encompassed habitat wetter than that occupied by the southern white rhino, covering seasonally flooded savannah intersected by marshes, water courses and swamps (Hillman-Smith, 1982, 1987). It continued to be persecuted throughout the 20th century, with protection hampered by civil war and armed conflict (Emslie and Brooks, 1999, Hillman-Smith and Ndey, 2005). This reduced the global population from around 2,200 individuals in the 1960s to a single population of just 15 individuals by the 1980s, resident to Garamba National Park in the DRC (Figure 1.3; Emslie and Brooks, 1999; Emslie et al., 2016). Conservation efforts managed to increase the population to 30 individuals by 2003, but further poaching reduced this to just four animals the following year (Hillman-Smith and Ndey, 2005). The last sighting of a wild northern white rhino occurred in 2006 and since then extensive foot surveys have confirmed the subspecies extinction in the wild (Emslie, 2012a; Tunstall et al., 2018).

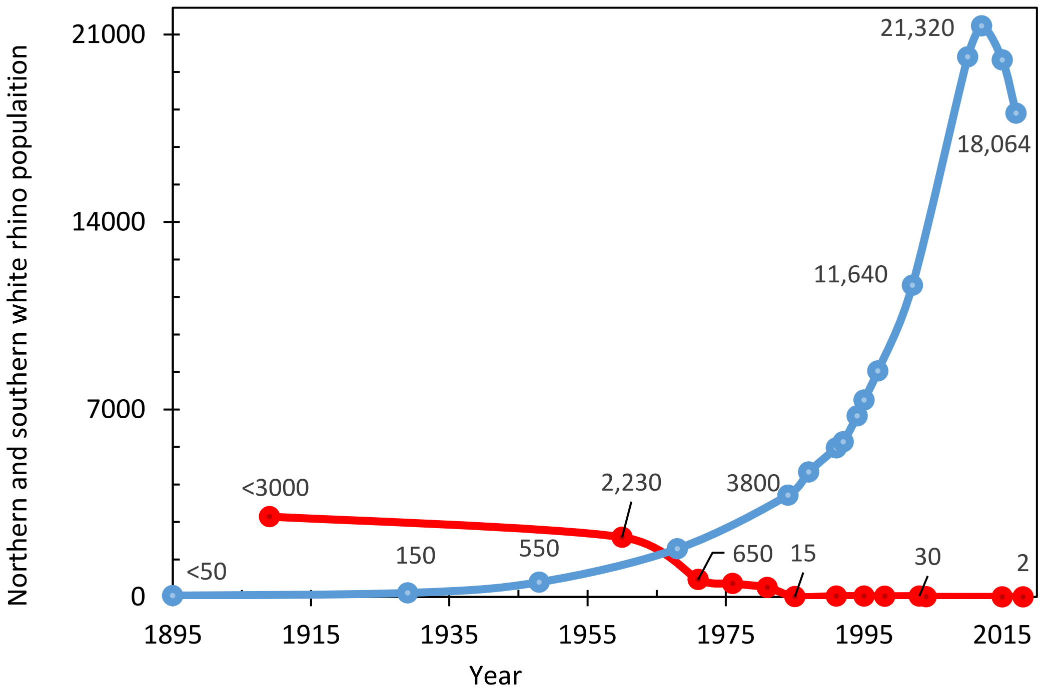

Figure 1.3. Change in northern white (red) and southern (blue) white rhino populations in Africa between 1895 and 2017-2018. Points represent years for which data were available. Labels are shown to aid interpretation. Source: Emslie and Brooks (1999); Hillman-Smith and Ndey (2006); Emslie et al. (2019).

In 2009, in a final attempt to save the subspecies, four of the world's seven remaining captive individuals (three females and one male) were translocated from Dvůr Králové Zoo in the Czech Republic to Ol Pejeta Conservancy in Kenya (Saragusty et al., 2016). It was hoped that returning them to their natural habitat would encourage breeding

(Saragusty et al., 2016). The females entered oestrus and were observed mating but no offspring were produced (Harley et al., 2016; Saragusty et al., 2016). Two of the translocated rhinos have since died, as have the other three in captivity, leaving two surviving individuals (Saragusty et al., 2016; Tunstall et al., 2018). The two survivors are female and so the species is now functionally extinct (Tunstall et al., 2018). Any chance of recovery relies on the application of assisted reproductive technologies, utilising *in vitro* fertilisation, surrogates and cryopreserved genetic material (Hildebrandt et al., 2018; Tunstall et al., 2018). A successful rescue effort will require advancements in the development of cloning by nuclear transfer and the artificial production of gametes via stem cell differentiation (Hildebrandt et al., 2018; Tunstall et al., 2018); it will also require habitat protection and restoration to occur in tandem (Crees et al., 2016). If these technological and logistical obstacles can be overcome, it will take at least 50 years for the subspecies to be abundant enough to move beyond its current Critically Endangered status (Saragusty et al., 2016).

Southern white rhinos inhabit a fraction of their former range in southern Africa (Rookmaaker and Antoine, 2012). Historically, they roamed between the Cape and the Zambezi River but were hunted extensively following European colonisation of the area (Rookmaaker and Antoine, 2012). By 1895 the global population had been reduced to a single population of 20 to 50 individuals restricted to the iMfolozi area in South Africa (Heller, 1914; Rookmaaker, 2000; Emslie and Brooks, 2002). To halt their decline to extinction, the area was declared a game reserve in 1897 and the hunting of rhinos declared illegal (Player, 1972; Rookmaaker, 2000). With hunting pressure removed, the population began to increase, and had grown to 437 individuals by 1953 (Player, 1972). In 1960, the world's entire population of southern white rhino were still restricted to just a single location (Knight et al., 2015). The high density of rhinos began to cause overgrazing and exceed the area's ecological carrying capacity (Player, 1972). The resultant deterioration in habitat conditions were worsened by increasing human pressures on the reserve, including illegal occupation and cattle grazing. This, along with poaching, placed the population at risk of decline and reduced its opportunities for further growth (Player, 1972). Settling

For these reasons, a programme of translocations to repopulate the southern white rhino over its historic range was begun in 1961 following the development of new capture techniques (Harthoorn, 1962; Player, 1972). Kruger National Park in South Africa was chosen as the destination for a significant number of early translocations due to its potential to support a high rhino population and its protected status (Pienaar, 1970). Translocations to Kruger began in 1961 and lasted for the next 12 years; between 1964 and 1968 nearly 100 rhinos were re-introduced to the park (Pienaar 1970; Player, 1972). Other populations were re-established across parts of their historic range and individuals were sent to foreign zoological gardens to create an international reserve population in case conditions in the wild deteriorated (Player, 1972). By 1968, the total rhino population in South Africa had increased to around 1,800 individuals distributed across several locations (Player, 1972). In 1980, a record drought in Hluhluwe-iMfolozi lead to the translocation of 446 white rhinos out of the park in just one year (Knight et al., 2015). By 1997 the southern white rhino had increased to 8,440 individuals in 248 discontinuous populations (Emslie and Brooks, 1999), peaking at 21,316 individuals in 2012 (Emslie et al., 2019). Following the effects of poaching and drought, numbers had decreased to 18,064 by 2017 (17,212 to 18,915 individuals at 90% CI; Emslie et al., 2019). Until this recent decline, population growth rates between 1992 and 2010 had averaged 7.1% per year (Emslie et al., 2019).

At the end of 2017, an estimated 15,625 white rhinos could be found in South Africa, representing 86.5% of Africa’s total white rhino population (Emslie et al., 2019). The single greatest population can be found in Kruger, estimated to number around 9,337 individuals in September 2015, representing around 49% of the continent’s white rhino population (Ferreira, et al., 2017). The species’ population recovery over the last century is largely attributed to good conservation practise and meta-population management in South Africa (Emslie and Brooks 1999; Knight 2017). It is now the most numerous of all rhino species and internationally recognised as one of the greatest conservation success stories of a large mammal (Emslie and Brooks, 1999; Sodhi et al., 2011; Knight, 2017). Aside from South Africa, populations within their native range can now be found in Botswana, Kenya, Mozambique, Namibia, eSwatini (previously known as Swaziland), Uganda and Zimbabwe, along with a 2018 reintroduction to DRC (Emslie et al., 2019).

Several individuals have also been introduced outside of their native range to Senegal and Côte d'Ivoire (Emslie et al., 2019).

1.3 Private management of the white rhino

White rhino population dynamics have been significantly influenced by the actions of the South African state and private sector (Taylor et al., 2014; Knight et al., 2015). In South Africa, private landowners may legally own the wildlife on their properties and utilise them for economic gain (Taylor et al., 2016; Rubino and Pienaar, 2017). In comparison, Kenya allows for wildlife ownership but banned consumptive use practises, including trophy hunting in 1977 (Baker, 1997; Emslie and Brooks, 1999; Taylor et al., 2016). Trophy hunting occurs in 23 African countries but is most common in southern Africa and Tanzania, in part due to differing levels of government support and varying regulatory regimes (Lindsey et al., 2007b). In these countries, ownership rights to wildlife and the right to derive income through consumption use (killing) were introduced in the 1960's and 1970's (Lindsey et al., 2007b). Existing under different legislature, much of the private wildlife industry in Southern Africa has developed under this consumptive use model compared to rhino range states such as Kenya and India where policy makers tend to take a more preservationist approach to conservation (Baker, 1997; Snijders, 2012; Taylor et al., 2016; Rubino and Pienaar, 2017). Preservationist methods aim to conserve wildlife in a pristine state and are based on views that consumptive use is unpalatable, unethical or ineffective (Baker, 1997; Lindsey et al., 2007b; Angula et al., 2018). Consumptive methods rely on sustainable use practises and take a more utilitarian approach to conservation (Lindsey et al., 2007b; Angula et al., 2018). The practise can have a positive economic and ecological impact when it relies on evidence-based population estimates, low off-takes, enforceable quotas and a fair distribution of proceeds (Baker, 1997; Lindsey et al., 2007b). However, in reality, many management systems may lack these properties (Baker, 1997). In South Africa, one of the earliest markets for translocated rhinos was in the game ranching industry (Emslie and Brooks, 1999). Demand from the private sector increased in 1968 when the South African government legalised regulated hunting, and ranches began to buy in rhinos to sell on as trophies to hunters (Player, 1972; Knight et al., 2015). In 1989, the Natal Parks Board lifted restrictions on the sale price of rhinos and began selling them at auction, with rhino sales allowed to reflect their full economic value, it became profitable for the private sector to buy in rhinos to breed rather than just trophy hunt (Taylor et al., 2014; Knight et al., 2015). These translocations to private land helped keep state owned rhino populations at sustainable levels and maximised birth and population growth rates by reducing density dependent effects (Taylor et al., 2014).

Private ownership increased the white rhino population (Knight et al., 2015) and expanded rhino range outside of formally protected areas (Taylor et al., 2014). By 1987, there were 791 rhinos across 80 private reserves; by 1997, 1,785 individuals existed across 163 private reserves (Emslie and Brooks, 1999); and by 2001, 2,534 individuals occurred across 242 properties (Castley and Hall-Martin, 2003). Survey data from 2001 showed almost all of these properties were previously used for cattle production and most of the landowners kept rhinos for commercial reasons, with 88.3% of the 223 properties citing ecotourism, hunting or breeding as their primary management objective (Castley and Hall-Martin, 2003). Thus, these commercial operations are often referred to as game ranches due to the breeding and management of wildlife rather than livestock (du P. Bothma and du Toit, 2010). By the end of 2015, approximately 6,140 white rhinos were conserved on private land (Emslie and Adcock 2016), the majority of which were privately owned (Knight 2017). This totalled 30.6% of Africa's white rhino population (Knight, 2017) and the reserves on which they were located had a combined area of around 1.7 million hectares, almost the size of Kruger National Park (Balfour et al., 2016). As of 2017, declines in state managed populations and continued increases in privately managed ones, mean that 49.3% (90% CI: of 46.9% to 51.6%) of white rhinos are now held under private ownership (Emslie et al., 2019). The single greatest privately owned white rhino population numbers 1,626 individuals and is owned by the rhino breeder John Hume in Northwest Province, South Africa (Save the Rhino, 2018). Furthermore, some of these breeders have invested in rhinos as a speculative investment, with an anticipation that future profits could be made if the international horn trade became legalised (Rubino and Pienaar, 2018). However, current trends indicate some private owners are beginning to disinvest in rhino; from 2012 to 2014 an estimated 63 to 70 South African reserves removed all rhinos from their properties (Balfour et al., 2016). This totals a loss of 200,000 hectares of land available for rhino conservation and equates to a loss of carrying capacity able to support 2,500 rhinos (CITES, 2016a). Which, given the increase in larger privately owned populations means there has been a trend towards consolidation (Emslie et al., 2019).

Rhino populations can be classified as wild, semi-wild, or captive (Leader-Williams et al., 1997). The African Rhino Specialist Group (AfRSG) use three key diagnostic features based on (1) the size of land the area they occupy, (2) whether breeding is natural or manipulated, and (3) the degree of compression (density of animals), food supplementation, husbandry, and veterinary care. They also classify locations into several categories based on their level of protection, these are: rhino conservation area, intensive rhino protection zone, rhino sanctuary, rhino conservancy, rhino ranch and outlying rhinos, although none of these have any legal status (Emslie and Brooks, 1999). The rhinos on many South African private reserves meet the criteria for wild free-ranging conditions, for example properties keeping rhino in 2008 had an average size of 5638 hectares (Hall-Martin et al., 2009), which is above the 1000 hectares required (Leader-Williams et al., 1997). The Red List of Mammals of South Africa, Swaziland and Lesotho considers all forms of private ownership to have a net positive effect on white rhino conservation regardless of the intensity of management (Emslie and Adcock, 2016). This includes operations that raise rhino in intensive semi-wild conditions with some supplementary feeding, which enables them to be stocked at higher densities than normal, and for anti-poaching effort to be concentrated. If their poaching rates fall below the national average then they may provide an insurance policy to wild populations, providing a source for future translocations that could supplement or found populations in other areas (Emslie and Adcock, 2016). However, such operations require careful management to prevent inbreeding or genetic erosion caused by small founder populations (Guerier, et al., 2012). Even so, rhinos kept within semi-wild conditions often have much higher rates of population growth than those reared within captive conditions (Swaisgood et al., 2006; Ververs et al., 2017).

1.4 Demand for horn

Asian rhino species have long been exploited for their use in animal-based traditional medicine, particularly in China. The earliest written record of this, the *Shen Nong Ben*

Cao Jing or Divine Peasants Herbal, dates horn's use to 2600 BCE (Nowel, 2012) and an extensive trade in horn products had developed as far back as the T'ang Dynasty (618-907 CE) (Schafer, 1963). In traditional Chinese medicine (TCM), horns are prescribed medicinally, in the belief that they treat ailments including fever, delirium, convulsions and headaches (Milliken et al., 2012; Cheung et al., 2018). Although several Chinese medical trials have reported horn to have pharmacological effects, the research has been of poor quality (Tang et al., 1999). A review by Nowell (2012) found limited evidence for the pharmacological effects of rhino horn apart from one randomised double-blind trial that reported mild antipyretic effects in humans (Tsai, 1995). Despite this, standard anti-inflammatory drugs perform better and horn substitutes from other species work at least as well (Nowell, 2012). Historically, most African rhino horn was exported to Arabia where it was used in ceremonial dagger handles, particularly in Yemen (Vigne et al., 2007). Much of this horn was sourced from East African rhino populations, and it is thought that the last free-ranging individuals of the northern white rhino were slaughtered for this market (Vigne et al., 2007). However, the demand in Yemen has largely ceased due to civil war and economic recession along with effective replacement of dagger handles with materials other than rhino horn (Milliken et al., 2012).

African horn is now largely sought for its use in both traditional and non-traditional medicine in Asian markets where it supplants the increasingly rare horn available from Asian rhino species (Vigne and Martin, 2018). China banned the use of rhino horn as a medical ingredient in 1993, however the country remains a major destination for horn despite its removal from modern TCM pharmacopeia (Emslie and Brooks, 1999; Emslie et al., 2019). Demand in other former major markets has radically decreased, including Japan, South Korea and Taiwan (Milliken et al., 2012). However, new markets have emerged, particularly in Vietnam, where data from questionnaires, CITES export data and illegal seizures indicate that horn consumption has intensely increased (Milliken et al., 2012; Truong et al., 2016; Emslie et al., 2019). This has been linked to the country's dramatic rise in economic prosperity along with non-traditional uses driving new customer demand (Milliken et al., 2012). Non-traditional uses include cures for hangovers and cancer, as a de-toxicant, and as a signifier of wealth (Truong et al., 2016).

It is hypothesised that the timing of a spike in poaching from 2008 onwards can be traced to press reports in which a Vietnamese Communist Party official attributed his recovery from terminal cancer to the use of rhino horn (Rademeyer, 2012).

The illegal trade is aided by widespread corruption in source, transit and consumer countries (Rademeyer, 2016a, 2016b). Horns are trafficked by transitional organised criminal syndicates with the involvement of corrupt or lawbreaking park rangers, professional hunters, soldiers, police officers, customs and conservation officials (Rademeyer, 2016a, 2016b). Data from the TRAFFIC Rhino Horn Seizures Database, shows an estimated 1,903 rhino horns or horn pieces (4,763 kg) were seized worldwide from illegal sources from 2009 to 2013 (Emslie et al., 2019). Seizures then increased to an estimated 2,955 horns or horn pieces (6,517 kg) from 2014 to September 2018. Over the 2009 to 2018 period, South Africa (2,994 kg), China and Hong Kong (1,715 kg), Vietnam (1,757 kg) and Mozambique (1,031 kg) were responsible for the greatest number of seizures (66% of all seizures by weight), with all other countries reporting total seizures of less than 500 kg. However, seizures do not represent the total flow of horn into illegal markets. From 2016 to 2017, an estimated 4,757 African rhino horns entered the illegal trade, 95% of which were sourced from poached rhinos (Emslie et al., 2019).

1.5 Current poaching epidemic

South Africa was spared the brunt of the rhino poaching onslaught that occurred across Africa during the 1970s and 1980s and accounted for much of the increase in the global population (Emslie and Brooks, 1999). Other range states experienced high losses of rhinos, and Mozambique and Angola saw the rhino become nationally extinct twice within the 20th century following, reintroductions (Emslie and Brooks, 1999). In South Africa, an average of just 15 African rhinos were poached per year between 1990 and 2007 (Di Minin et al., 2015). This changed in 2008, when a total of 83 rhinos were poached nationally, due to an increased demand for rhino horn in consumer countries and the high black-market price it commanded (Emslie et al., 2016; MacMillan et al., 2017). The year-on-year poaching rate then increased rapidly, with over 1000 rhinos poached per year from 2013 to 2017 (Figure 1.4; Emslie et al., 2016). In total, 7,130 rhinos were poached from 2008 to 2017, representing 86.3% of the continent's total poaching mortalities (Emslie et al., 2019). However, these numbers represent a likely under-estimate as poached carcasses can go undetected in large areas with low ranger densities such as Kruger, where poaching rates may be as much as 20% higher than those recorded (Emslie et al., 2019). In 2018, 769 rhinos were poached across South Africa, which while a significant decrease on previous years, still represents a rate of 2.1 rhinos poached per day (Mokonyane, 2019).

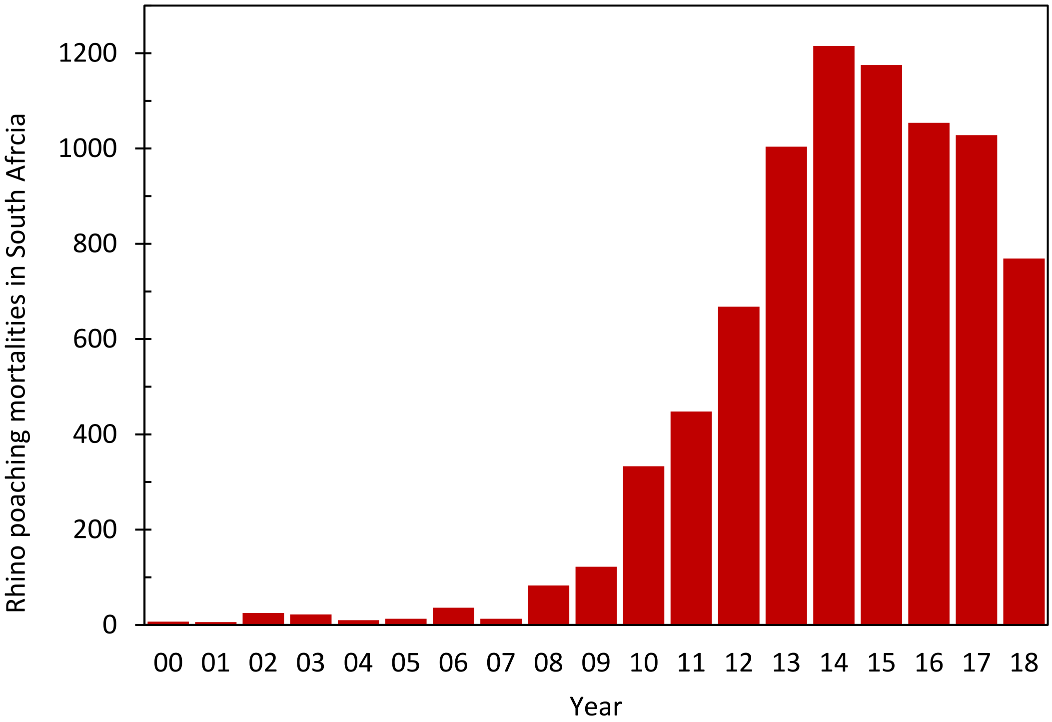

Figure 1.4. Reported white and black rhino poaching mortalities in South Africa between 2000 and 2018. These figures represent minimum numbers as some carcasses go undetected. Source: Rademeyer 2016a; Emslie et al., 2019, Mokonyane, 2019).

High poaching rates may erode the gains in rhino numbers made over the last few decades (Ferreira et al., 2018), with 4.6% of Africa's rhino population lost to poaching in 2017 (Emslie et al., 2019). Deaths from poaching mortalities are beginning to catch up with births and may overtake natural replenishment rates (Ferreira et al., 2018). From 2014 to 2015 the number of white rhinos born and surviving until the first year in South African National Parks (SanParks) were similar to the total number poached (Ferreira et al., 2017). While in the four years prior to 2016, 2,936 rhinoceros carcasses attributed to poaching were detected in Kruger (Ferreira et al., 2018) and a prediction that white rhinos would experience a detectable decline by 2016 (Ferreira and Okita-Ouma, 2012) was confirmed by a recent population estimate (Ferreira et al., 2018). Excluding reductions from translocations, white rhinos in Kruger National Park decreased by around 3,400 individuals from 2015 to 2017 (Emslie et al., 2019). However, this reduction may in part be due to drought which would have decreased reproduction and the survival of adults (Eberhardt, 2002; Ferreira et al., 2018). Individuals may also have moved out of Kruger into adjacent private reserves where higher densities of water resources can be found (Ferreira et al., 2018). Despite this, between 2014 and 2017 poaching removed as much as 10 to 12% of the white rhino population per year in Kruger, which being above the estimated maximum population growth rate of 9%, will have contributed towards the decline (Emslie et al., 2019). Throughout the same period, white rhinos within private reserves increased, likely because many properties supplementary-fed their populations during the drought (Emslie et al., 2019).

Di Minin et al. (2015) modelled changes in white rhino abundance from 2014 to 2023 under eight different policy scenarios; under existing management conditions, they predicted the species would go extinct in the wild within the next twenty years, with only medium and high increases in anti-poaching efforts resulting in continued population growth. Modelling by Emslie and Adcock (2016) also examined what would happen to the South African white rhino population under a range of possible poaching and growth scenarios. If the level of poaching in 2015 was further reduced or maintained, numbers were projected to grow over the next five years. If poaching levels were modelled on three- and five-year trends, a decline in numbers was projected over a five-year period. Under all modelled scenarios, population size after five years did not require up-listing from Near Threatened status; however, after ten years the species required up-listing to Vulnerable or Endangered status under the most extreme poaching scenarios (Emslie and Adcock, 2016). Recent data supports these predictions of decline; when including deaths from natural causes the continent-wide population reduced by 15.2% between 2012 and 2015 (Di Minin et al., 2015; Emslie and Adcock, 2016; Emslie et al., 2019).

1.6 Value of the white rhino

1.6.1 Economic value

In southern Africa, rhino owners can profit through tourism, live sales, and the limited trophy hunting of surplus bulls (Lindsey et al., 2007a; Cooney et al., 2017; Saayman and Saayman, 2017). In many cases, funds can then be fed back into conservation (Emslie et al., 2016). Rhino-related tourism is responsible for generating significant revenue for both private and state reserves (Saayman and Saayman, 2017). In Kruger, the economic value of rhinos per year was estimated to be $5.9-14.9 million (2017 USD) between 2011 and 2013, calculated by combining data on tourists' willingness-to-pay to see them and total visitor numbers. The same survey reported that the number of tourists who considered the rhino to be their most preferred species was increasing (Saayman and Saayman 2017). Other surveys of tourists at a Namibian communal conservancy indicated that 7 to 14% of total wildlife viewing value could be ascribed to rhinos (Spenceley and Barnes, 2005). Furthermore, revenue generated through live sales of white and black rhinos has also been high (Spenceley and Barnes, 2005). Hluhluwe-iMfolozi Park generated the equivalent of 60% of their conservation budget through live sales between 2000 and 2005, and Phinda, a private reserve in South Africa, made a profit of $221,041 (2014 USD) through sales of white rhino between 2002 and 2005 (Spenceley and Barnes, 2005).

In many private reserves revenue is also generated through trophy hunting, and while the practice remains controversial, its contribution to increasing the range of rhinos is well documented (Cooney et al., 2017; t Sas-Rolfes, 2017). Trophy hunting differs from subsistence hunting, in that it is carried out recreationally and targets specific animals for a pre-determined fee, trophy hunting also differs from poaching, which is illegal and unmanaged (Cooney et al., 2017). In the South African game ranching industry, several dozen white rhinos are hunted every year for an average fee of $85,000 (2012 USD) per rhino (n = 63 in 2012; Saayman and Saayman, 2017). Figures from 2005 show hunting offtake equated to 0.34% of South Africa and Namibia's total white rhino population (Emslie et al., 2016). However, between 2006 and 2011, demand for legal hunting was boosted by 'pseudo-hunts', in which horn was legally exported as a trophy but then illegally sold for consumption in Southeast Asia (Emslie et al., 2016). To tackle this, the South African government began a regulatory crackdown in 2012 whereby applicants without a trophy hunting track record were denied permits (Emslie et al., 2016). This predominately affected applicants from the Czech Republic, Ukraine, Vietnam, China, Bulgaria, Canada and Slovakia and largely brought pseudo-hunts under control (Emslie et al., 2016). From 2012 to 2017, an average of 83 white rhinos per year were hunted across Namibia and South Africa (Emslie et al., 2019). This offtake is equivalent to removing 0.5% of the population per year and is thus within sustainable limits (Emslie et al., 2019) as well as being significantly lower than the number of mortalities caused by poaching (Figure 1.4; Emslie et al., 2019). In comparison, black rhinos are rarely hunted due to their scarcity (Emslie et al., 2016), but Namibia's Ministry of Environment and Tourism sold an auctioned black rhino hunt for $350,000 (2014 USD) in 2014, with the entire fee placed in a trust fund to support rhino conservation efforts (Lee and Du Preez, 2016). Hunting revenue can contribute a high proportion of a game ranch’s income, for example a private reserve managing 195 rhinos in South Africa paid for 63% of its operating costs through trophy hunting over an eight-year period (Cooney et al., 2017). All funds made from rhino hunting were then spent on rhino protection and conservation management.

The current poaching epidemic is starting to erode the value of keeping rhinos as the price of protecting them continues to rise (Rubino and Pienaar, 2018). The revenue generated from rhino tourism, trophy hunting and breeding is now in many cases inadequate to meet the security costs in protecting them (Emslie et al., 2019). As costs begin to exceed revenues, owners may view rhinos as a financial liability and are beginning to remove them from their properties (Balfour et al., 2016). The cost of recruiting and retaining skilled staff, deploying them across often vast areas of terrain, and equipping them with sophisticated anti-poaching technologies are high (Collins et al., 2013). Interviews of rhino owners conducted in 2015-2016 estimated security costs to be between $1500–3700 (2017 USD) per month, with one large rhino breeder spending $227,000 per month (Rubino and Pienaar, 2018). Reserve managers were not willing to share exact amounts but the research indicated the security costs accounted for 30 to 60% of reserve budgets (Rubino and Pienaar, 2018). More recently, internal survey data from the Private Rhinos Associated stated owners were paying on average $7,900 (2019 USD) per rhino per year (Jones, 2019). While in 2018, several news outlets reported that John Hume's ranch, which represents the largest privately owned population of rhino in the world, was at risk of financial collapse due to unsustainable security costs (Save the Rhino, 2018). Cost increases have affected government actors too, leading to a reallocation of existing conservation resources towards rhino protection (Biggs et al., 2013). Governments of range states including South Africa, Namibia and Zimbabwe, have all increased their funding of anti-poaching and rhino protection services since the onset of the poaching crisis in 2008 (Ferreira and Okita-Ouma, 2012). For example, between 2008 and 2012 South Africa upped state funding from $20,000,000 per year to $57,000,000 (2012 USD) per year (Ferreira and Okita-Ouma, 2012). Despite this, between 2012 and 2017, ten populations of white rhino and three populations of black rhino were lost from government-run reserves in South Africa after all remaining individuals were either poached or the survivors were translocated to safer locations (Emslie et al., 2019). Voluntary donations fund an increasing amount of anti-poaching efforts, with monetary donations increasing from ZAR110 million during 2010/2011 ($13,613,600 in 2011 USD) to around ZAR1 billion during 2013 ($95,147,500 in 2013 USD; Saayman and Saayman, 2017). Between January 2010 and June 2016, over $1.3 billion (2016 USD) of donations was committed by NGOs, states and multilateral partners to combat all forms of the illegal wildlife trade in Africa and Asia (The Global Environmental Facility, 2016). This total excluded a further US $70 million of committed funding, including a $23.7 million (in 2014 USD) donation from the Howard G. Buffett Foundation to combat poaching in Kruger National Park (The Howard G. Buffet Foundation, 2016; The Global Environmental Facility, 2016). However, Di Minin et al. (2015) predicted that to prevent future population decline in South Africa current anti-poaching efforts were not enough and investment must increase to $147,000,000 (2014 USD) per year to maintain or increase rhino numbers.

This increase in costs coupled with an increase in incidents that threaten human life and the perception that there are fewer incentives to conserve them have reduced live sale prices of white rhinos and increased the number of owners seeking to disinvest (Taylor et al., 2014; Balfour et al., 2016; CITES, 2016a; Rubino and Pienaar, 2017, 2018). Furthermore, as the live sale prices of rhinos are market-driven, the value of animals may have begun to decrease if changes in demand have led to an oversupply in live rhinos (van der Merwe et al., 2004). The trend in rhino removals threatens to decrease population growth rates, reverse the expansion of the species' range and impact the management of meta-populations (Emslie and Adcock, 2016; Rubino and Pienaar, 2017). From 2007 to 2018, the live sale price of white rhinos in South Africa has reduced by 58% in the South African Rand and 67% in the United States Dollar (Emslie et al., 2019). In 2018, the average live sale price of a white rhino at auction (n = 12) was approximately $29,000 (2019 USD; Wildlife Auctions, 2019) valuing it substantially lower than the black-market price of a dead rhino where horn can cost as much as $65,000 (2014 USD) per kilogram (Lunstrum, 2014). Decreases in live sale prices significantly reduce conservation budgets (Emslie and Adcock, 2016). For example, SANParks and Ezemvelo-KZN Wildlife experienced a decline in turnover of around $3,800,000 (2014 USD) in the sales of live rhinos from 2008 to 2012 because of the drop in price but also because they had fewer surplus animals to sell due to poaching (Emslie and Knight, 2014).

1.6.2 Ecosystem value

The white rhino is the world's second largest terrestrial mammal (after the elephant), with males weighing between 2000 and 2300 kg, and females between 1400 and 1700 kg (Shorrocks and Bates, 2015). White rhinos are selective grazers with a preference for short grasses such as *Cynodon*, *Digitaria*, *Heteropogon*, *Enneapogo*n and *Chloris* species (Jordaan et al., 2015; Shorrocks and Bates, 2015). During the dry season they will also feed on taller, less nutritious grasses when short grass is unavailable (Owen-Smith, 1997) or on new growth in previously burnt *Themeda* grasslands (Shrader et al., 2006). However, seasonal declines in forage quality do not necessarily lead to increased levels of food intake, indicating that during dry periods, rhinos may balance energy expenditure through the mobilisation of fat reserves rather than compensate behaviourally (Shrader et al., 2006).

White rhinos consume the equivalent of around 2% of their body mass per day (Shrader et al., 2006), and their regular grazing can help transform the structure and composition of grasslands into a lawn-like state (Hempson et al., 2015). Frequent cropping aids lawn persistence through the removal of tall-grass invaders and stimulates the foliar regrowth of high-quality short grasses (Anderson et al., 2013; Hempson et al. 2015). Following rhino removals in Hluhluwe-iMfolozi Park, Waldram et al. (2008) found the grass height of lawns increased in mesic (750 mm pa) but not semi-arid savannahs (600 mm pa). Thus, in wetter areas rhinos act as facilitators for other short grass grazers, such as wildebeest (*Connochaetes* spp.) and warthogs (*Phacochoerus africanus*; Hempson et al., 2015). However, in drier areas, these smaller grazers are able to maintain short grass communities (Waldram et al., 2008). The same study also found rhino removals resulted in larger, less patchy fires, indicating rhinos affect the fire regimes of grassland savannahs. This is because the creation and maintenance of grazing lawns act as grazer-maintained firebreaks due to their reduced fuel load (Waldram et al., 2008). Cromsigt and te Beest (2014) monitored the effects of rhino recolonization on vegetation in Kruger and found short grasses covered a greater proportion of high rhino impact areas (17.5%) than low rhino impact areas (10.7%). They also reported around 20 times more grazing lawns in the high rhino impact landscape. The high impact areas had a greater density of rhinos than the low impact areas (high area: 8.6 observations per km^2; low: 2.0 per km^2) and also had a longer history of use. Thus, white rhinos can be considered 'ecosystem engineers' because they drive spatial and structural heterogeneity at a landscape level.

Rhinos also modify predator-triggered trophic cascades due to their status as apex consumers and megaherbivores (>1,000 kg; le Roux et al. 2018). Rhinos distribute their dung more evenly over a landscape than mid-sized herbivores. This is because their lower susceptibility to predation reduces the need to defecate in the high-visibility areas, which enable predator detection, favoured by species that are more fearful. Without this exchange, safer open areas would disproportionally accumulate more nutrients than areas of dense thicket. This occurs despite white rhinos' tendency to defecate in communal latrines (known as middens), which concentrate nutrients locally (Veldhuis et al., 2018), as midden position remains unrelated to the landscape of fear (Laundré et al., 2010; le Roux et al., 2018). In this context, the landscape of fear represents the relative levels of predation risk that reflect the level of fear of predation that prey species experience across an area (Laundré et al., 2010). Thus, given their place as the largest terrestrial grazer, rhinos are responsible for a wide range of ecological interactions and ecosystem services and their removal could lead to a range of trophic cascades (Ripple et al., 2015; Everatt et al., 2016).

1.7 Anti-poaching strategies

Anti-poaching strategies can be split into reactive policies that respond to current threats, such as through increases in security and law enforcement (Haas and Ferreira, 2017), and proactive polices, that look to prevent the threats occurring, such as through consumer demand reduction schemes or a sustainable legal supply (Taylor et al., 2017; Olmedo et al., 2018). Much of conservation spending only tackles the symptoms of poaching and does nothing to satiate demand (Ferreira et al., 2014). Thus, without disruptions in consumer markets or reductions in poverty and inequality in range states, poaching may overtake future enforcement efforts (Challender and MacMillan, 2014). However, these polices take time to implement and in the meantime, measures that increase rhino security are becoming ever more important. Field responses range from strategic translocations of rhinos from areas of high risk to areas of low risk (Ferreira et al., 2015; Hayward et al., 2018), increases in anti-poaching security (Collins et al., 2013), and the dehorning of rhinos to deter poaching (du Toit and Anderson, 2013). Many of these conservation actions are complementary and require an integrative approach if poaching is to be reduced (Heitmann, 2014).

1.7.1 Dehorning

Dehorning, also known as horn trimming, refers to the controlled removal of a portion of a rhino’s horn by veterinary procedure (Badenhorst et al., 2016). It may be utilised as a wildlife management technique for several non-exclusive reasons: the first is to prevent a rhino injuring itself or another individual (Trendler, 2014; Patton et al., 2018a), the second is to reduce the risk of poaching to more manageable levels (du Toit and Anderson, 2013), and the third is harvesting or stockpiling horn for financial gain (Taylor et al., 2014). On rare occasions, rhino horns may also break off naturally or during translocations (Taylor et al., 2014). This can occur during fights if the horn is exposed to heavy force (Lindeque, 1990) or subject to unnatural leverage during transport (Rees, 2019).

Horn structure and growth

Rhino horns are made entirely of keratin and are not attached to the skull; this makes them different to the keratin-and-bone horns found in the Bovidae or the solid bone horns of the Cervidae (Hieronymus et al., 2006). It is thus neither enervated nor vascularised, and similar in composition to the hooves of ungulates and the beaks of birds (Ryder, 1962; Hieronymus et al., 2006). Horn's keratin based structure has in turn led to comparisons being made with human hair or fingernails in several consumer demand reduction campaigns (Sato and Hough, 2016; Smith, 2018). Average adult horn masses totalled 6.24 kg in males and 5.10 kg in females for a population of white rhinos (Kock and Atkinson, 1993), and 2.65 kg in a population of black rhinos which show no intersexual variation (Pienaar et al., 1991). Measurements by Pieenar et al. (1991) reported a mean anterior horn mass of 5.16 kg ± 2.01 SD (n = 163) and mean posterior horn mass of 1.86 kg ± 0.98 SD (n = 153) in adult white rhinos, for a mean total of 7.02 kg per individual. Horn grows continuously throughout a rhino's life, increasing in both diameter and length (Pieenar et al., 1991). The horns of male white rhinos grow faster than females (Rachlow and Berger, 1997), with male horns increasing in mass 35.6% faster than females per year (Ververs, 2018). In white rhinos, the front, or anterior horn is larger and averages 90 cm long in adults but can reach 150 cm in the oldest individuals (Lydekker, 1908; Estes, 1991). Horn has also been shown to regenerate faster in the first year after dehorning (Pieenar et al., 1991). For white rhinos this equates to a height increase of around 7 cm per year for the anterior horn and 3 cm per year for the posterior horn (Kock and Atkinson, 1993; Rachlow and Berger, 1997). This equates to a mean increase of 0.861 kg per year in male white rhino horns and 0.635 kg per year in females (Ververs, 2018). The rate of growth slows as rhinos age, eventually reaching an asymptote in the oldest individuals (Pienaar et al., 1991; Rachlow and Berger, 1997; Ververs, 2018). For individuals older than a year in age, horn rubbing behaviour causes the observed growth rate to be lower than the intrinsic growth rate due to wear on the tip (Pienaar et al., 1991).

Dehorning procedure

Dehorning involves removing most of a rhino's horn mass without inflicting injury on the animal (Kock and Atkinson, 1993). Care is taken not to damage the growth plate, allowing healthy regrowth to occur (Badenhorst et al., 2016). The procedure requires animals to be immobilised. Darting is usually done from a helicopter to increase precision when firing, but also to increase the ease and speed of locating subjects and aid the direction of ground crews (Morkel and Kennedy-Benson, 2007). Following anaesthesia, the anterior and posterior horns are trimmed with chainsaws. Practitioners face a compromise between the risk of damage to the horn base and the desire to remove as much horn mass as possible. The trimming procedure does not affect any afferent nerve endings; thus the rhino will not experience pain (Badenhorst et al., 2016). If horn is cut too closely to the germinal layer it can predispose the animal to infection and deform regrowth (Kock and Atkinson, 1993). Following the conventional method, a stump of horn around 9 to 11 cm above the growth plate is left (Ververs, 2018). Under the Kock and Morkel method, more horn is removed by rounding the remnant stump with an angle grinder (Ververs, 2018). The precise amount of horn removed varies, but initial dehorning removes around 90% of a white rhino's horn mass (Figure 1.5; Kock and Atkinson, 1993). The frequency of repeat dehorning for anti-poaching purposes depends on the management criteria, poaching threat and the costs involved, but usually a time of 12-36 months is advocated (Figure 1.6; Rachlow and Berger, 1997; Lindsey and Taylor, 2011). To minimise poaching risk, it is recommended that the procedure is carried at a similar time on as many individuals in a population as possible (Milner-Gulland, 1999; Lindsey and Taylor, 2011) but it can also be performed opportunistically (du Toit, 2011; du Toit and Anderson, 2013).

Immobilisation during dehorning allows for routine veterinary checks to be performed and enables the notching or tagging of ears for monitoring purposes (Morkel and Kennedy-Benson, 2007). Dehorning also provides an opportunity to collect DNA samples for South Africa's Rhino DNA Index System (RhODIS). This makes individual DNA profiles available to law enforcement agencies enabling them to match horns to carcases and wildlife crime scenes through molecular analysis (Harper, 2011). In South Africa, dehorning is regulated by the government agency Nature Conservation, who mandate that owners or custodians must mark, microchip and register all horn cut-offs (Taylor et al., 2014). The costs of dehorning vary depending on terrain, vegetation type, population size and density (Kock and Atkinson 1993; Rachlow and Berger 1997), but in South Africa tend to average around $1000 (2011 USD) per rhino (Lindsey and Taylor, 2011).

Figure 1.5. Typical differences in white rhino horn size and shape, between (A) a horned adult female, and (B) a dehorned adult female with 513 days of horn growth after the procedure. Neither of the calves have been dehorned.

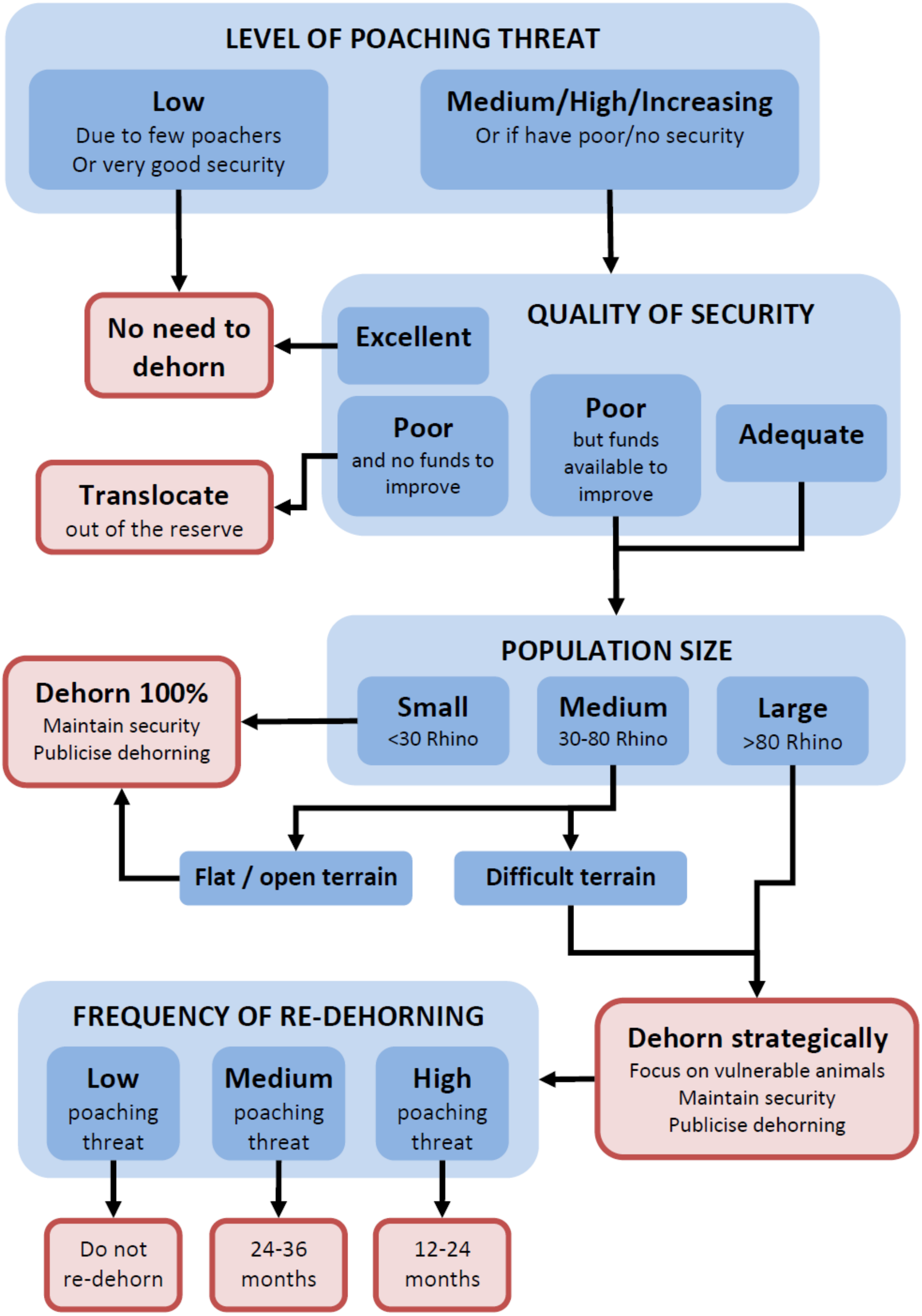

Figure 1.6. Conditions under which dehorning can help reduce poaching threat. Reproduced from Lindsey and Taylor (2011).

Historic and current use of dehorning

The procedure was pioneered in Namibia in 1989 when a wild population of twelve black rhinos were dehorned in Damaraland in response to a significant increase in poaching risk (Martin, 1994). Several more Damaraland rhinos were dehorned in 1991 and as no veterinary or poaching mortalities occurred, the operation was considered a success. Dehorning was then expanded to other areas in Namibia, including the Waterberg Plateau and Etosha National Park, and continued to be practised until 1995 (Lindsey and Taylor, 2011). Throughout the 1990's no dehorned rhinos were poached in Namibia, however, some of this success may be attributed to concurrent improvements in anti-poaching strategies, including the toughened criminal sentences introduced in 1990 (Martin, 1994) and increased security enforcement throughout the period (Lindsey and Taylor, 2011).

The first documented dehorning of wild white rhinos occurred in Zimbabwe in 1991 following high levels of poaching incursions in Hwange National Park (Kock and Atkinson, 1993). It was then expanded to rhino populations throughout Zimbabwe and by 1993 over 158 black and 112 white rhinos were dehorned (Kock and Atkinson, 1993). By 1994 approximately 81% of Zimbabwe's total population had been dehorned (Kock and Atkinson, 1994). Poaching of dehorned rhinos was initially lower than horned individuals (Kock and Atkinson, 1993). However, in 1993 around 94 rhinos were poached from Hwange, the majority of which had been dehorned (Kock and Atkinson, 1996). Conservationists concluded this was due to a complete vacuum in security that occurred following their dehorning, coupled with at least 18 months of horn regrowth (Kock and Atkinson, 1996). Despite this initial setback, dehorning continues in Zimbabwe to the present day, and dehorned rhinos are considered to have lower poaching rates than horned rhinos, especially when levels of security are high (Lindsey and Taylor, 2011; du Toit and Anderson, 2013; Kagande and Musarurwa, 2014).

Dehorning in South Africa only became common as an anti-poaching tool from 2008, after poaching rates dramatically increased (Lindsey and Taylor, 2011). Until recently, dehorning in the country was predominately performed on smaller populations in private reserves rather than provincial and federal run reserves (Taylor et al., 2014).

However, the current poaching epidemic has seen dehorning become an increasingly common conservation intervention in areas that suffer high poaching rates as the cost of effective security rises (Lindsey and Taylor, 2011; Taylor et al., 2014). Survey data published in 2014 indicated that 37% of 52 private owners dehorned their rhinos. Extrapolated out to approximately 400 South African private rhino owners, this equates to 148 reserves that may have dehorned their rhinos within the last few years (Taylor et al., 2014). Several larger national parks have now committed to dehorning at least some of their rhinos, with Kruger National Park beginning the selective dehorning of females in 2019 (SANParks, 2019).

Dehorning as an anti-poaching tactic

Dehorning acts to reduce the monetary reward available to poachers, which once offset against the associated risks of poaching, decreases their incentive to poach (du Toit and Anderson, 2013; Kagande and Musarurwa, 2014). It is considered effective when the costs of adequate security cannot be met and the risk of rhino poaching mortalities outweighs any possible adverse veterinary, behavioural or ecological costs (Lindsey and Taylor, 2011; du Toit and Anderson, 2013; Derkley, et al., 2019). However, the high price of horn can make even a smaller remnant stub attractive to poachers, and without adequate security enforcement dehorned rhinos may still be poached (Kock and Atkinson, 1996). Thus, its success depends on the degree of concomitant security practises relative to poaching pressure (Kock and Atkinson, 1996; Lindsey and Taylor, 2011). Du Toit and Anderson (2013) suggested poaching pressure could be described by the following function:

$$Poaching\ Pressure = \frac{Reward\ to\ poacher\ from\ the\ illegal\ sale\ of\ horn}{Risk\ to\ poacher\ of\ being\ arrested\ \times\ Effort\ required\ to\ poach}$$

A 2011 survey of 67 African rhino stakeholders, made up of government workers, private rhino owners, non-governmental organisation staff, veterinarians, tourism operators, and biologists, reported 63% of interviewees considered dehorning was effective at reducing poaching risk under at least some circumstances, while 72% considered it to deter poachers from targeting a particular reserve (Lindsey and Taylor, 2011). Dehorning is more effective if the procedure is publicised (Martin, 1994) since poachers may target a population if they are unaware of the animal's horn status (Kock and Atkinson, 1993). Approximately 53% of respondents in the same 2011 survey of rhino stakeholders (n = 67) thought that once a poacher had entered a reserve and encountered a rhino they were as likely to shoot a dehorned rhino as a horned one (Lindsey and Taylor, 2011). Further criticisms include that dehorning displaces poaching risk from a dehorned population to a horned one and thus does not tackle the wider causes of the problem (Daly et al., 2011). Furthermore, as authorities must be notified in advance, there have been cases where rhinos have been poached before dehorning has occurred, due to the leakage of information to a wider audience (Taylor et al., 2014). Dehorning can also transfer risk from the rhinos onto rhino owners leading them to be targeted by criminal gangs for the horn that they hold (Rubino and Pienaar, 2018).

Dehorning to reduce injury and horn loss

Dehorning may also be used to prevent rhinos injuring themselves during translocation or in some captive situations (Trendler, 2014), and to reduce injury infliction on other individuals (Patton et al., 2018a). Rhinos may injure themselves in translocation if they knock or lean their horns against a hard surface such as a metal crate (Taylor et al., 2014; Rees, 2019). Knocks typically occur when an animal is irritated and result in shards of horn breaking off at the tip (Taylor et al., 2014). While entire horn loss can occur if an animal is over-sedated and places heavy pressure on the horn base plate by leaning its body weight against its horn (Morkel and Nel, 2019). The frequency that complete breakoffs occur is dependent on differences in rhino body condition and veterinary care, but during translocation it probably ranges from 0.25% to 3% (Taylor et al., 2014). Thus, to prevent damage to the horn or growth plate rhinos may be dehorned prior to transportation (Taylor et al., 2014; Trendler, 2014). Dehorning may also be utilised to minimise the frequency of fighting or reduce the risk of serious injuries from fights (Patton et al., 2018a). Populations dehorned for security reasons can also experience the benefits of injury reduction. Fighting mortality monitored over a 6-year period in the Bubye Valley Conservancy in Zimbabwe showed the annual rate in dehorned black rhinos to be 11% lower than horned rhinos in the same area (du Toit and Anderson, 2013). While data for the entire Zimbabwean Lowveld indicated the yearly fighting mortality rate of dehorned black rhinos to be around 36% lower than in horned rhinos (du Toit, 2011).

Dehorning for financial gain

Dehorning provides a non-lethal and sustainable mechanism for the repeat harvesting of horn (Taylor et al., 2014). However, the exploitation of horn for financial gain is complicated by variations in domestic and international law around its trade (Derkley et al., 2019). The primary conservation motive behind such a trade is that it could provide funds to pay for rhino protection and increase the economic incentives for the keeping and breeding of rhinos (Taylor et al., 2014; Rubino and Pienaar, 2017). While other proponents for a legal trade theorise that flooding the market with horn will reduce its sales price and consequently decrease incentives to poach (Crookes, 2017). The merits of such a trade are controversial (Derkley et al., 2019), and generally there has been a trend towards increasingly restrictive legislation that aims to kerb the sale of rhino horn and protect rhino species from further decline (Table 1.1). In 1977, high rates of poaching lead to all rhino species being placed on Appendix I of the newly established Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which prohibited the international trade in rhino and rhino products for all signatories (Emslie and Brooks, 1999). By the mid-1990s the majority of Asian consumer countries, including China (together with Hong Kong), Japan, South Korea and Taiwan had followed with domestic bans on trade (Milliken et al., 2012). However, in many jurisdictions entrenched corruption meant enforcement was poor (Emslie and Brooks, 1999; Rademeyer, 2012). In 1994, South Africa's white rhino population was down-listed to Appendix II after a recovery in numbers, ending prohibition but heavily regulating any further trade (Emslie and Brooks, 1999). This was followed by the transfer of the white rhino population of eSwatini to Appendix II in 2004 (CITES, 2016a). These populations are still subject to the ban in trading horn, but not to the export of hunting trophies or limited sale of live individuals (Taylor et al., 2014).

In South Africa, where the domestic trade in horn remained legal, the increasing exportation of horn to Asia through loopholes such as pseudo-hunts, led to a moratorium being placed on all horn trade in 2009 (Taylor et al., 2014). Following the domestic ban, the practice of dehorning rhinos to stockpile or store horn for financial reasons appeared to decline, but increased for anti-poaching purposes (Lindsey and Taylor, 2011). In 2016, eSwatini lodged a bid for a limited lifting of the international trade ban at the 17th Conference of Parties to CITES but the majority of CITES members voted against it and the proposition failed (CITES, 2016a, 2016b). However, all white rhino range states, aside from Botswana and Kenya, voted for it (CITES, 2016b). Following the domestic ban, several legal challenges were made against the government to reverse its decision (Toyana, 2015; Molewa, 2016; Molewa 2017a). The court challenges, brought by rhino owners Johan Kruger and John Hume, and supported by Wildlife Ranching South Africa and the Private Rhino Owners Association of South Africa, eventually led to the moratorium on domestic trade being revoked in 2017 (Molewa 2017A). While legal, the sale of rhino horn in South Africa remains strictly regulated (Molewa, 2017b); horn must be registered on the national database, with a DNA certificate and documentary proof of its legality, while the buyer must have no criminal record (Vigne and Martin, 2018). However, without access to international markets, it is unclear how a domestic trade in rhino horn can supply consumer demand (Jakins, 2018).

The recent changes in domestic legislation in South Africa and ongoing discussion for a re-establishment of the international trade may lead to increased levels of dehorning if rhino owners seek to capitalise on the value of their herds (Taylor et al., 2014). A governmental report published in 2014 found that 51% of a representative sample of private rhino owners would consider dehorning their rhino if the domestic trade was legalised (Taylor et al., 2014). While 65% of the same sample would consider dehorning their herds if the international moratorium were lifted (Taylor et al., 2014). In 2019, eSwatini submitted a new proposal to allow for a limited export of horn to East Asia while Namibia proposed moving their white rhino to Appendix II but both motions failed to pass (CITES, 2019).

Table 1.1. Major changes in policy affecting the domestic and international trade in rhino horn. CITES: Convention on International Trade in Endangered Species of Wild Fauna and Flora. CoP: Conference of Parties.

Date	**Description of legal change or policy**
04/02/1977	Implementation of an international ban in the trade of rhino and rhino parts following the listing of all Rhinocerotidae spp. under CITES Appendix I. Source: Emslie and Brooks (1999).
1993	China bans the sale, importation, exportation and possession of horn. Rhino horn medicines are removed from the official pharmacopoeia of China and a ban is placed on the manufacturing of rhino horn medicines. Source: Emslie and Brooks (1999).
17/11/1994	Transfer of South Africa's white rhino population to CITES Appendix II, adopted at CoP8 (Prop 17) with the following annotation: “For the exclusive purpose of allowing international trade in live animals to appropriate and acceptable destinations and hunting trophies. All other specimens shall be deemed to be specimens of species included in Appendix I and the trade in them shall be regulated accordingly”. Source: Emslie and Brooks (1999).
1997	Domestic bans have come into force in Japan, South Korea and Taiwan. Source: Milliken et al. (2012).
02/01/2004	Transfer of eSwatini’s white rhino population to CITES Appendix II, adopted at CoP13 (Prop 9) with the same annotation as South Africa. Source: CITES, 2016a.
2006	Exploitation of South Africa's trophy hunting policy emerges as a serious issue. Termed 'pseudo-hunts', rhinos horns are legally exported as trophies and then sold on the black market. Source: Emslie et al. (2016).
13/02/2009	South Africa places a moratorium on the domestic trade in rhino horn (Gazette No. 31889, Government Notice 148). Source: Taylor et al. (2014).
2012	South Africa takes measures against pseudo-hunters, denying permits to those without a trophy hunting track record. Source: Emslie et al. (2016).
01/06/2013	South Africa announces its plans to propose a regulated international trade in rhino horn at CoP17. Source: Molewa (2013).
26/11/2015	South Africa's high court overturns the 2009 domestic ban on horn trade with immediate and retrospective effect after two of South Africa's biggest rhino owners, John Hume and Johan Kruger win on a technicality. Source: Toyana (2015).
21/04/2016	South Africa announces that it will not propose legalising the international trade in rhino horn at CoP17. Source: CITES (2016a).
23/05/2016	South Africa’s Supreme Court dismisses the governments appeal to keep the domestic moratorium in place. The Department of Environmental Affairs appeals to the Constitutional Court. Source: Molewa (2016).
03/10/2016	eSwatini’s proposal to export rhino horn is rejected at Cop17. "Prop 7: To alter the existing annotation on the Appendix II listing of Swaziland’s white rhino, adopted at the 13th Conference of Parties in 2004, so as to permit a limited and regulated trade in white rhino horn which has been collected in the past from natural deaths, or recovered from poached Swazi rhino, as well as horn to be harvested in a non-lethal way from a limited number of white rhino in the future in Swaziland". Source: CITES (2016a, 2016b).

Date	Description of legal change or policy (cont.)
05/04/2017	The domestic trade is legalised after South Africa's Constitutional Court rejects the government's appeal with no further chance of appeal. Source: Molewa (2017b).
30/10/2018	China states its intention to authorise parts of captive rhinos for scientific, medical and cultural use. Two weeks later the State Council Executive Deputy Secretary-General Ding Xuedong announced that the regulations had been postponed after study and that the ban would continue. Source: Ding (2019).
28/08/2019	eSwatini proposal to remove the existing annotation listed in Appendix II (Prop. 8) is rejected at CoP18. "With the intention of allowing limited and regulated trade in stockpiles of *C. s. simum* horn which has been legally collected in the past or recovered from poached eSwatini rhino (totalling 330 kg), as well as horn to be harvested annually in a non-lethal way in the future (amounting to up to 20 kg per year).
28/08/2019	Namibia's proposal to transfer their white rhino population to CITES Appendix II with the same annotation as South Africa (Prop. 9) is rejected at CoP18 Source: CITES (2019).

Biological effects of dehorning

The procedure carries the risk of health complications, but may also exert negative behavioural or physiological changes on individuals and populations (Lindsey and Taylor 2011; Derkley et al., 2019). Risks during anaesthesia and immobilisation include muscle damage (Morkel and Kennedy-Benson, 2007), hypertension (Hattingh and Knox, 1994), and death from hyperthermia or respiratory depression (Bush et al., 2004). Additionally, while improvements in veterinary science mean immobilisation-induced rhino mortalities are far lower than they were in the past (Emslie et al., 2009), the need for regular immobilisations during repeat dehorning procedures increases the overall level of risk (Fahlman, 2008).

Evaluating an animal's welfare requires knowledge of its health, comfort and mental state (Mellor, 2016). Derkley et al. (2019) considered how a rhino's welfare could be influenced by several domains of suffering: nutritional impacts (water deprivation, food deprivation, and/or malnutrition), behavioural effects, environmental challenges, health effects (disease, injury, and/or functional impairment) and mental effects (anxiety, fear, pain, distress, thirst, hunger, boredom). Dehorning has the potential to affect a variety of behaviours (Lindsey and Taylor 2011), as rhinos use their horns during social interactions (Estes, 1991; Owen-Smith, 1973), when defending against predators

(Goddard, 1967) and when accessing resources (Van Strien, 1985; Owen-Smith and Danckwerts, 1997). For example, in black rhinos horn asymmetries within a population may affect dominance (Berger and Cunningham, 1998) and reductions in horn size appear to affect agonistic social (Patton et al., 2018a) and defensive behaviours (Berger et al., 1993; Berger and Cunningham, 1996). Additionally, research on white rhinos in an intensively reared population showed increases in stress immediately after a dehorning procedure (Badenhorst et al., 2016). The relative paucity of research around dehorning, particularly with white rhinos, has led to a number of calls for further study, including on potential social and behavioural impacts (Lindsey and Taylor, 2011; du Toit and Anderson, 2013; Trendler, 2014; Badenhorst et al., 2016; Patton et al., 2018a) and l stress-related physiological parameters (Ververs, 2018).

1.7.2 Reducing consumer demand

Behavioural change interventions aim to cut or reduce demand for rhino horn by modifying consumer behaviour, thereby reducing poaching rates (Rubino and Pienaar, 2017). Awareness and education campaigns work on two main premises: exposing the risks to the rhino populations and the barbarity that is inflicted on them, and exposing horn’s lack of medicinal value (Olmedo et al., 2018). Other policies seek to change behaviour through the implementation of stricter laws against consumption (MacMillan et al., 2017). In Vietnam, many demand reduction polices lack clear measurable objectives and it is unclear whether they are having an effect (Truong et al., 2016; Olmedo et al., 2018; Veríssimo and Wan, 2019). Surveys of Vietnamese horn consumers by Dang and Nielsen (2018) found that half of users explicitly expressed no interest or concern in the conservation plight of rhinos (46%, n = 16) and that the majority were not deterred by existing law enforcement efforts (69%, n= 13). Other surveys of horn consumers have suggested that awareness campaigns would have more of a discouraging effect if they were used alongside stronger penalties such as increased fines or imprisonment (MacMillan, et al., 2017; Hanley et al., 2018). China and Vietnam are yet to eliminate demand (MacMillan, et al., 2017), but significant reductions have occurred in other markets (e.g. in Japan, South Korea, Taiwan and Yemen) indicating that consumer behaviour can change despite the existence of deeply entrenched cultural beliefs (Milliken et al., 2012).

Infusing rhino horns with toxic chemical compounds and discolouring them with dye is another anti-poaching tactic that aims to reduce the desirability of horn to end-users (Rhino Rescue Project 2019). However, if poachers ignore, or are unaware of the difference between infused and untreated horn it will have no impact on poaching rates (Ferreira, et al., 2014). Additionally, it relies on the conception that rhino horn is made up of hollow tubules and inter-tubular hollow spaces (Van Orden and Daniel, 1992; Hieronymus and Witmer, 2004; Hieronymus et al., 2006), which would allow for internal fluid distribution (Rhino Rescue Project, 2019). More recent analysis, using light, digital and stereomicroscopy, indicated that horns were more solid in structure and that previous work was erroneous (Boy et al., 2015). There are also ethical ramifications if poisoned end-users suffer harm as well potential legal consequences for the actors responsible if consumers become plaintiffs (Ferreira et al., 2014). Thus, the efficacy of the infusing horn is likely to be poor and ineffective as a conservation tool (Rubino and Pienaar, 2017).

1.7.3 Reducing illegal supply

Although some countries have shown significant reductions in demand, the current CITES trade ban has not stopped the trading of horn or lead to a decrease in poaching (Rubino and Pienaar, 2017). If the international ban was lifted, income from a sustainable legal trade could be used to fund the increasing costs of rhino protection (Biggs et al., 2013; Di Minin et al., 2015; Taylor et al., 2017). Furthermore, if increased supply led to a drop in prices it could decrease incentives to poach and reduce profits for criminal syndicates (Rubino and Pienaar, 2017; Taylor et al., 2017). However, the issue is controversial and has divided rhino stakeholders, scientists, and policy-makers worldwide (Collins et al., 2015; CITES, 2016a, 2016b; Crookes, 2017; Taylor et al., 2017). Advocates state that a sustainable horn supply, sourced from natural mortalities, dehorning, trophy hunting and stockpiled horn, could produce between 5,319 and 13,356 kg of horn per year (Taylor et al., 2017). Although long-term sale prices are difficult to estimate, socioeconomic modelling by Di Minin et al. (2015) predicted a sustainable legal horn trade could generate profits of $717,000,000 (2014 USD) / year. Furthermore, Martin et al., (2016) suggested that a sustainable trade in rhino horn offered the highest-valued land use for ranchers of semi-arid savannahs.

Biggs et al. (2013) suggested that the high price in horn is driven by the restrictions placed on its supply coupled with stable or growing demand. Thus, for the market price to decrease supply must keep up with or exceed demand and consumers must switch from an illegal supply to a legal supply or be dissuaded from buying horn (Taylor et al., 2017). For example, the illegal trade in Southeast Asian porcupines (*Hystrix brachyuran)* persists despite the existence of commercial farming operations because wild adult porcupines retail for half the price of farm-bred adults and thus are laundered into the system (Brooks et al., 2010). Additionally, some Chinese consumers continue to prefer wild sourced bear bile than farmed stock despite its more expensive price (Dutton et al., 2011). Similar preferences appear to exist for animal-based traditional medicine users in Vietnam, where users showed a greater willingness to pay for horn from wild-sourced animals than farmed animals (MacMillan et al., 2017). Similar findings were reported by Dang Vu and Nielsen (2018), with horn users preferring sources from wild rhino over farmed rhinos due to a perceived higher potency. Thus, if the primary source of legal horn is from intensively managed or captive rhinos, consumers may choose to buy from the illegal market instead (Dang Vu and Nielsen, 2018). This also undermines suggestions of satisfying the market with fake or imitation horn (Pembient, 2019), which is already mis-sold on Vietnamese markets (Milliken et al., 2012). Despite this, users also showed a preference for non-lethally harvested horn over lethal methods (MacMillan et al., 2017), suggesting horn harvested from dehorned free-ranging rhinos could provide a suitable sustainable source. The survey by MacMillan et al. (2017) also found consumers were less willing to pay high prices if legalisation occurred, potentially because of an associated loss of prestige and exclusivity (MacMillan et al., 2017), one of the current drivers of demand (Truong et al., 2016). Lastly, the study by MacMillan et al., (2017) anticipated that legalisation would cause a slight increase in the number of horn users, which in turn could increase in demand (MacMillan et al., 2017). Furthermore, findings from Dang Vu and Nielsen (2018) indicated that the majority of surveyed existing users would increase horn consumption following legalisation.

Modelling by Crooks (2017) indicated that for rhino poaching to be reduced, increased costs for poachers must occur alongside a lowering of the price of horn. Thus, for a sustainable trade to have a positive conservation impact, profits would have to be re-invested in rhino security (Di Minin et al., 2015). The main concerns among detractors are that if demand outstrips supply, poaching could be increased, and that the resultant illegal supplies of horn could be laundered onto the legal market depleting more fragile populations, such as those in Asia (Collins et al., 2015; Crooks, 2017; Ellis, 2017; Derkley et al., 2019).

1.7.4 Modifying poaching risk

Foot and vehicle patrols are the primary method of apprehending poachers and reducing incursions (Hart et al., 2015; Barichievy, et al., 2017; Haas and Ferreira, 2017). These increasingly incorporate technological solutions into their tactics, including acoustic monitoring (Wrege et al., 2017), aerial surveillance (Mulero-Pázmány et al., 2014; Shaffer and Bishop, 2016), animal tracking tags (O'Donoghue and Rutz, 2016), camera trapping (Hossain et al., 2016), and thermal imaging (Hart et al., 2015). In addition to this, rhinos are increasingly translocated from areas subject to high poaching rates to areas of relative safety due to the rising costs of effective security (Lindsey and Taylor 2011; Ferreira et al., 2015; Hayward et al., 2018; Rees, 2019). As well as establishing a new breeding nucleus, these populations may act as a reserve from which individuals for future re-colonisations can be drawn (Emslie and Adcock, 2016). For example, the organisation 'Rhinos Without Borders' aims to move at least 100 white rhinos threatened by poaching in South Africa to the Okavango Delta in Botswana (Joubert and Kent, 2016). While in the 1990s, black rhinos in Zimbabwe were moved into 'Intensive Protection Zones' where security efforts were more concentrated (Lindsey and Taylor 2011). Despite this, the success rate of translocations is extremely variable, due to the effects of injuries, failed acclimatisation, or introductions into unsuitable environments (Dickens et al., 2010; Miller et al., 2016; Morkel and Nel, 2019). This can lead to poor reproductive outcomes or even death of some or all of the translocated individuals (Emslie et al., 2009; Linklater et al., 2010; Miller et al., 2016; Rees, 2019). For example, in previous translocation events of white rhinos in Botswana 5 of 27 individuals died and in Zimbabwe 4 of 12 individuals died (Rees, 2019). Even for translocated populations, poaching risk is not homogenous and rhinos may require *in situ* movement away from poaching hotspots such as internal perimeter zones (Park et al., 2015; Haas and Ferreira, 2017; Rees, 2019). Thus, in these conditions, movement of at-risk rhinos away from areas of danger at the local scale maybe a more appropriate anti-poaching tactic (Rees, 2019).

1.8 Research aims and thesis outline

Against a backdrop of rampant poaching, population decline and changing legislation, white rhino populations are increasingly subject to a range of conservation interventions. Despite the wide-scale practise of dehorning as an anti-poaching tactic, significant gaps exist on whether it impacts the behaviour and physiology of the white rhinoceros. Additionally, the need to mitigate poaching risk in game reserves coupled with a lack of *in situ* techniques to do so, could be aided by the design of novel methods to manage rhino movement. The research that forms the basis of this thesis employs a combination of physiological analyses, field-based monitoring, and behavioural experiments to examine some of these gaps and inform future conservation strategies for the protection of the white rhino.

The remainder of this thesis is divided into six chapters. Chapter two details the general methods used throughout data collection. Chapters three to six present the results of the research whilst chapter seven summarises the findings, their conservation implications and potential directions for further work.

Specifically the aims of this thesis were:

1) To determine whether dehorning affects aggressive social behaviours and dominance in white rhinos (Chapter 3). Chapter Three employed focal behavioural studies to observe changes in the type and rate of agonistic social interactions and explore differences in social position following dehorning.

2) To determine whether dehorning causes any short or long-term physiological effects in white rhinos (Chapter 4). Chapter Four employs endocrine analyses to monitor changes in stress in dehorned and horned populations of rhinos. The research relied on changes in adrenal and gonadal steroid hormones after dehorning procedures with rhinos sampled non-invasively through faecal collection.

3) To determine whether dehorning affects wallowing, digging and rubbing behaviours in white rhinos (Chapter 5). Chapter Five combines camera trapping techniques with field observations to generate data on infrequent horn-use behaviours at rubbing posts, mud wallows and salt licks.

4) To design and evaluate novel deterrent-based techniques that can be utilised as an anti-poaching tactic for white rhino conservation (Chapter 6). Chapter Six exposed rhinos to acoustic, olfactory and drone-based stimuli and monitored their responses. The deterrents were considered effective if they repeatedly elicited avoidance behaviour.

Chapter 2 – General methodology

2.1 Introduction

This chapter provides details of the study sites and general methods used during this research. Methods and statistical analyses that relate to specific results presented in this thesis are outlined in subsequent chapters.

2.1.1 Study sites

Given the current rhino poaching situation in South Africa (Emslie et al., 2019), the names of field site locations have been removed for security reasons, following African Rhino Specialist Group Protocol. The data used in this study were primarily collected from two sites in Northwest Province, South Africa (Figure 2.1) and are referred to as Site A and Site B throughout the thesis. Data were collected between October 2015 and November 2017 over five separate field seasons of between 2 weeks and 6 months (53 weeks total). Field sites were selected for their rhino management policy, proximity to one another, and accessibility. Permission to conduct research was received from the land owners and management staff. The field sites were separated by a straight line distance of 3.5 km with the intervening landscape consisting of human settlement and low-intensity pastoral land (Yarnell et al., 2015). The sites' close proximity helped standardise environmental conditions such as climate and habitat type thus minimising the potential for confounding experimental effects. Site A covers an area of 4,932 ha, is privately owned, occupies the safety buffer around an explosives depot and has been managed for conservation and ecotourism since 1982 (Yarnell et al., 2015). Site A's revenue is generated through ecotourism, education, commercial hunting and game breeding (James, 2014). Site B covers an area of 48,029 ha, is state owned, and managed by North West Parks Board (NWPB), with an additional 2,000 ha of private land incorporated into the north-western boundary. The park was proclaimed in 1979 but not fully established until 1984 (NWPB, 2015). Site B's operating objectives are 'to maintain the system's biodiversity in all forms and to minimise any visual impairment of the natural and cultural landscape'. Site B's revenue is generated from domestic and international tourism (NWPB, 2015).

Topographically, Site A is relatively flat, ranging from 1050 m to 1170 m above sea level. In contrast, Site B is almost entirely located in the eroded base of a volcanic intrusion producing rings of concentric hills and valleys that range from 1050 to 1675 m above sea level (Carruthers, 2011). Both sites occur within the savannah biome (Shorrocks and Bates, 2015) but more specifically the Central Bushveld Bioregion (Mucina et al., 2006). The vegetation consists of broad-leaved deciduous bushveld, with a mosaic of pediment grasslands, thickets and woodland (Mucina et al., 2006; Yarnell et al., 2007; NWPB, 2015). In both sites the vegetation is within the Central Bushveld Bioregion (Table 2.1; Mucina et al., 2006).

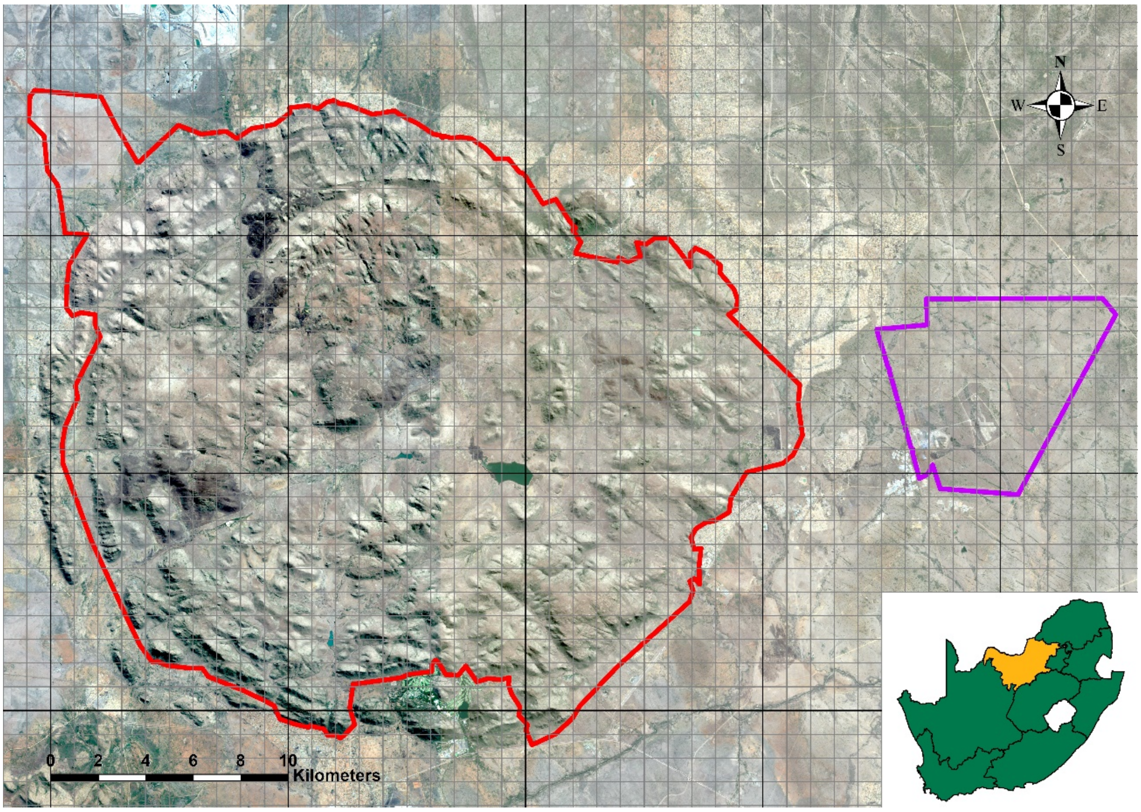

Figure 2.1. Boundaries of Site A [purple] and Site B [red], in Northwest Province [yellow inset] within South Africa [green inset].

Table 2.1. Floristically important taxa of the Central Bushveld Bioregion. Central Sandy Bushveld occurs at Site A; Pilanesberg Mountain Bushveld at Site B. Important taxa include species (and lower taxa) that are high in abundance or prominent at the landscape scale. Source Mucina et al., 2006.

Vegetation Unit	**Growth Form**	**Floristically Important Taxa**
Central Sandy Bushveld	Tall trees	*Acacia burkei*, *A. robusta*, *Sclerocarya birrea* subsp. *caffra*
	Small trees	*Burkea africana*, *Combretum apiculatum*, *C. zeyheri*, *Terminalia sericea*, *Ochna pulchra*, *Peltophorum africanum*, *Rhus leptodictya*
	Tall shrubs	*Combretum hereroense*, *Grewia bicolor*, *G. monticola*, *Strychnos pungens*
	Low shrubs	*Agathisanthemum bojeri*, *Indigofera filipes*, *Felicia fascicularis*, *Gnidia sericocephala*. Geoxylic Suffrutex: *Dichapetalum cymosum*
	Woody climber	*Asparagus buchananii*
	Graminoids	*Brachiaria nigropedata*, *Eragrostis pallens*, *E. rigidior*, *Hyperthelia dissoluta*, *Panicum maximum*, *Perotis patens*, *Anthephora pubescens*, *Aristida scabrivalvis* subsp. *scabrivalvis*, *Brachiaria serrata*, *Elionurus muticus*, *Eragrostis nindensis*, *Loudetia simplex*, *Schmidtia pappophoroides*, *Themeda triandra*, *Trachypogon spicatus*
	Geophytic herbs	*Hypoxis hemerocallidea*
	Succulent herbs	*Aloe greatheadii* var. *davyana*
Pilanesberg Mountain Bushveld	Small trees	*Combretum apiculatum*, *C. molle*, *C. zeyheri*, *Strychnos cocculoides*, *Croton gratissimus*, *Englerophytum magalismontanum*, *Rhus leptodictya*, *Vangueria parvifolia*
	Tall shrubs	*Diplorhynchus condylocarpon*, *Elephantorrhiza burkei*, *Grewia flava*, *Hibiscus calyphyllus*, *Mundulea sericea*, *Steganotaenia araliacea*, *Vitex rehmannii*
	Graminoids	*Chrysopogon serrulatus*, *Elionurus muticus*, *Panicum maximum*, *Themeda triandra*, *Enneapogon scoparius*, *Hyperthelia dissoluta*, *Panicum deustum*
	Herbs	*Abutilon pycnodon*, *Chamaesyce inaequilatera*, *Hermannia depressa*, *Nidorella resedifolia*, *Xerophyta retinervis*
	Succulent herbs	*Crassula lanceolata* subsp. *transvaalensis*

Table 2.2. Presence and absence of medium to large bodied (> 10 kg) grazers and browsers at Site A and Site B. + indicates species presence and – indicates species absence. Source: NWPB (2015); pers. comm. D. MacTavish (2017).

Order	**Species**	**Vernacular**	**Site A**	**Site B**
Artiodactyla	*Aepyceros melampus*	Impala	+	+
	Alcelapus buselaphus	Red hartebeest	+	+
	Antidorcas marsupialis	Springbok	-	+
	Connochaetes taurinus	Blue wildebeest	+	+
	Damaliscus lunatus	Common tsessebe	+	+
	Damaliscus pygargus	Blesbok	+	+
	Giraffa camelopardalis	Giraffe	+	+
	Hippopotamus amphibius	Hippopotamus	-	+
	Hippotragus niger	Sable	+	-
	Kobus ellipsiprymnus	Waterbuck	+	+
	Oreotragus oreotragus	Klipspringer	-	+
	Oryx gazella	Gemsbok	+	+
	Phacochoerus aethopicus	Warthog	+	+
	Raphicerus campestris	Steenbok	+	+
	Redunca arundinum	Common reedbuck	+	+
	Redunca fulvorufula	Mountain reedbuck	+	+
	Sylviacapra grimmia	Common duiker	+	+
	Syncerus caffer	African buffalo	+	+
	Taurotragus oryx	Common eland	+	+
	Tragelaphus strepsiceros	Greater kudu	+	+
	Tragelaphus sylvaticus	Cape bushbuck	+	+
Perissodactyla	*Ceratotherium simum*	White rhinoceros	+	+
	Diceros bicornis	Black rhinoceros	-	+
	Equus burchelli	Burchell’s zebra	+	+
Proboscidea	*Loxodonta africana*	African bush elephant	-	+
Struthioniformes*	*Struthio camelus*	Common ostrich	+	+

*__Struthioniformes__ are members of Class Aves, all other families are within Mammalia

The sites are surrounded by an electrified wire fence to restrict animal movement of large bodied species (Yarnell et al., 2015; Pekor et al., 2019). The majority of native medium to large bodied (>10 kg) browsers and grazers are present at both sites following reintroductions of extirpated species or pre-existing occurrence (; Slotow and van Dyk, 2001; NWPB, 2015; Stuart and Stuart, 2015; pers. comm. D. MacTavish, 2017). Large carnivores, including lion (*Panthera leo*), cheetah (*Acinonyx jubatus*) and Nile crocodile (*Crocodylus niloticus*) are present at Site B but not Site A, with both sites containing leopard (*Panthera pardus*). Being fenced, the sites are managed to maintain the ecological carrying capacity of the habitat and controlled for fluctuations in animal species and vegetation resources (du P. Bothma and du Toit, 2010; Deere, 2011; NWPB, 2015). Wildlife management practises include the maintenance of year-round supplies of water but do not extend to supplementary feeding. Burn regimes are managed to maintain grassland heterogeneity and reduce bush encroachment through a mixture of controlled and natural burns (Yarnell et al. 2007; van Oudtshoorn, 2014; NWPB, 2015). Both sites are traversable by vehicle due to a network of surfaced and unsurfaced roads.

The field sites have a sub-arid climate, with rainfall in the summer and dry winters. The year can be roughly split into three seasons: a cool dry season from May to mid-August, a hot dry season from mid-August to October, and a hot wet season from November to April (Mucina et al., 2006). Mean monthly maximum and minimum temperatures measure c. 5 °C to 30 °C (Figure 2.2a; Mucina et al., 2006) but daily temperature extremes can reach lows of -6°C and highs of 40°C (Yarnell et al., 2007). Mean annual precipitation for the area is reported as c. 650 mm (Mucina et al., 2006; Yarnell et al. 2007). Rainfall has also been measured daily at six different weather stations distributed evenly over Site A. The daily average of the stations was then used to calculate monthly rainfall for the whole site. Mean annual precipitation rates at Site A from 2010 to 2017 were lower (mean plus standard deviation: 539 mm ± 185 mm; n = 8 years) than those reported by Yarnell et al. (2007) due to a province wide drought in 2015/2016 (Figure 2.2c).

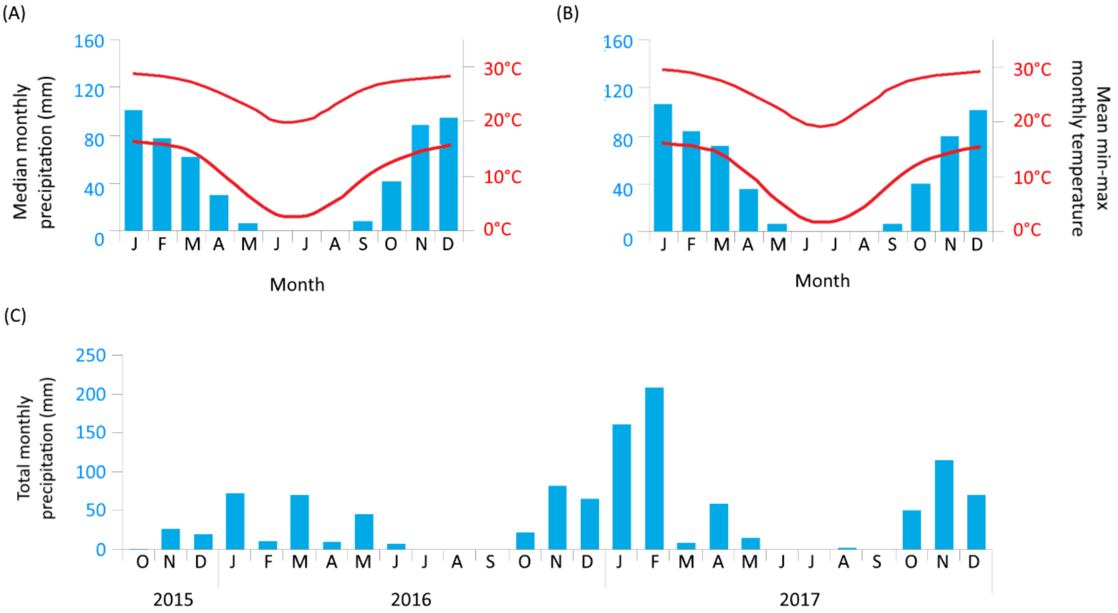

Figure 2.2. Climate data for (A) Pilanesberg Mountain Bushveld vegetation region, representative of Site B; (B) Central Sandy Bushveld vegetation region, representative of Site A; and (C) Rainfall data measured over the study period for Site A. Bars indicate monthly precipitation. Upper and lower lines indicate mean daily maximum and minimum temperature respectively. Source A-B: Mucina et al., 2006.

2.2 Study populations

Both white rhino populations originate from re-introductions, with the area falling within white rhino historic range (Rookmaaker and Antoine, 2012). At both sites, the white rhinos (from here on referred to as just ‘rhinos’) live off natural food sources throughout the year but have access to several artificial mineral licks and water sources. The rhinos receive limited husbandry and veterinary care, have a natural breeding strategy and undergo biological management (see Table 2.3). Since the initial introduction, the demographics of the two populations have changed due to births, natural mortalities, translocations, and poaching losses (Slotow and van Dyk, 2001; pers. comm. NWPB, 2016; pers. comm. D. MacTavish, 2016). Additionally, both locations are patrolled by anti-poaching units. Despite being fenced, the populations in both locations meet the African Rhino Specialist Group (AfRSG) criterion for a wild population (see Table 2.3; Leader-Williams et al., 1997; Emslie and Brooks, 1999). These conditions mean the populations and field sites are representative of those found elsewhere in Southern Africa, where rhino populations are usually fenced with some degree of management interventions (Thompson et al., 2016).

Table 2.3. Terms and definitions as defined by the African Rhino Specialist Group (AfRSG). Source: Leader-Williams et al. (1997); Emslie and Brooks (1999).

Term	Definition
Biological management	Management of rhino populations (primarily through adjusting rhino stocking densities, but also managing the densities of other browsers and habitat management) to maintain rapid, healthy population growth, to minimise inbreeding and loss of genetic diversity. Rhino removal and introduction decisions are based on a population's breeding performance, social behaviour, genetic relationships, the rhino density relative to an area's habitat carrying capacity, vegetation conditions etc.
Natural breeding strategy	Situations where mating opportunities are not controlled. This definition excludes the removal of individuals to minimise inbreeding between closely related individuals in wild free-ranging populations, and the introduction of additional rhinos to wild free-ranging populations to enhance genetic diversity and population viability.
Wild population	Free-ranging wild rhinos can be defined as those that live in large to medium (>10 km2) areas, generally in the historical range of the taxon, at natural densities and spacing, without routine food supplementation, and with only very occasional husbandry and veterinary intervention. They have a natural breeding system.

2.2.1 Rhino classification

Rhinos observed in this study were classified into three age classes: adults, subadults and calves (Shrader and Owen-Smith, 2002). The calf class extends from birth until dispersal away from the mother (Figure 2.3a-d), which occurs around two to three years old, when the calf is chased away following the mother's next parturition. The subadult period extends from maternal independence until a rhino reaches socio-sexual maturity (Figure 2.3d-f). For males, the adult class begins when they become solitary and/or territorial at ten to twelve years old (Figure 2.3f), and for females on the birth of their first calf at around seven years old (Figure 2.3e-f). In Chapter 4, a pregnant subadult female was classified as an adult to reflect the resultant physiological changes that occur during pregnancy (MacDonald et al., 2008); this was calculated by back-counting from parturition the average white rhino gestation time of 495 days (Linklater, 2007). The ages of rhinos in Site A were known precisely through a long-term monitoring effort which recorded generational lineages. In Site B, ages were classified through known age or a combination of rhino body size, horn size, horn wear (Pieenar et al., 1991; Hillman-Smith et al., 1987; Figure 2.3) and social grouping (Shrader and Owen-Smith, 2002).

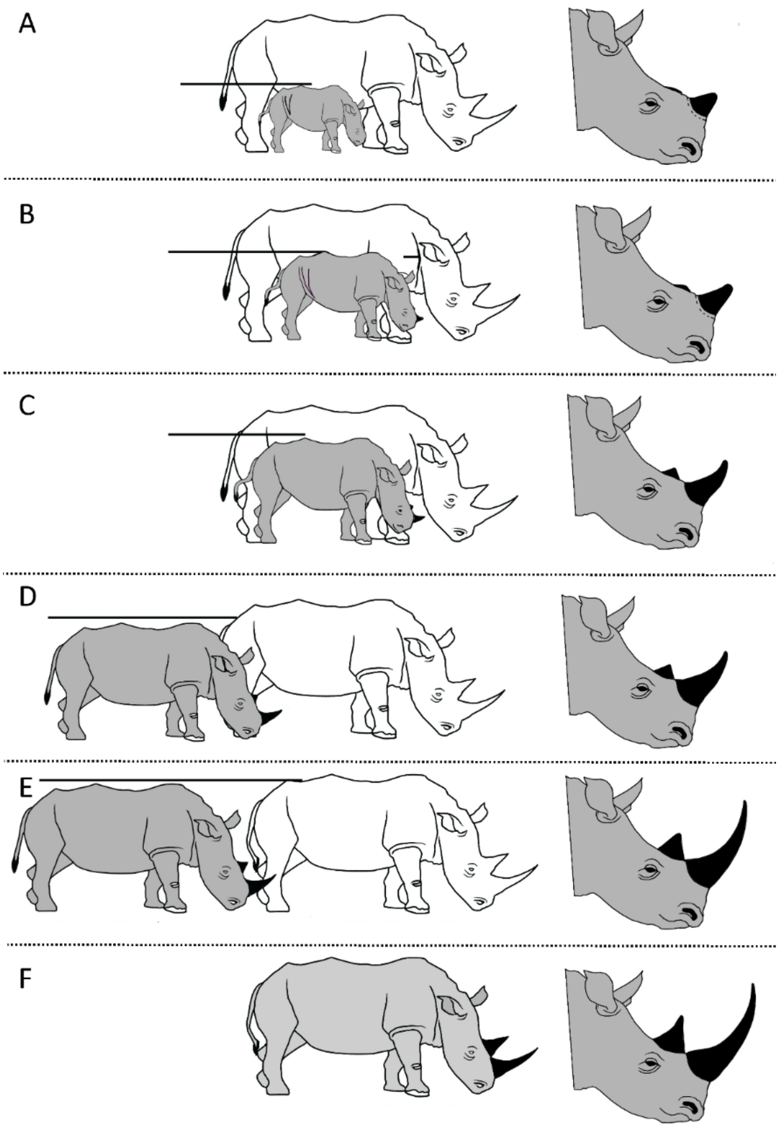

Figure 2.3. Approximate body size and horn appearance of white rhinos across different age brackets. Horizontal lines mark approximate height against an adult (unshaded rhino). Sizes representative of (A) 0 to 3 months old; horn near 6 months. (B) 3 months to 1 year; horn near 1 year. (C) 1 to 2 years; horn near 2 years. (D) 2 to 3.5 years; horn near 3 years. (E) 3.5 to 7 years; horn near 5 years. (F) 7 years and over; horn over 7 years. Adapted from Adcock and Emslie (2003).

All adult rhinos at Site A and the majority of adult rhinos at Site B are marked by a unique ear notch number that enables their identification and long-term monitoring (Figure 2.4). Notching involves clipping small sections (usually triangular or square in shape) from a rhino's ear (Emslie and Brooks, 1999). Rhinos with easily identifiable naturally occuring tears were left unnotched at both sites. This, along with characteristics such as body size, body condition, sex, horn appearance and social grouping (e.g. unnotched calves with notched mothers) allowed individual identities to be established to a high degree of certainty.

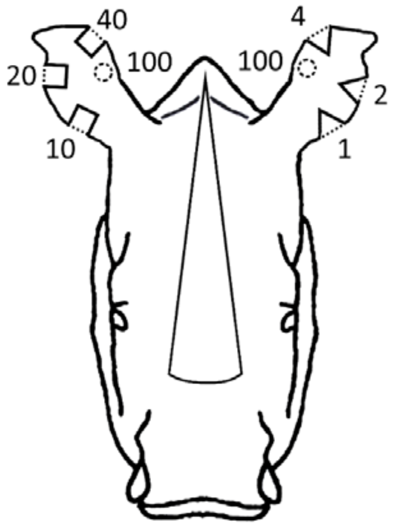

Figure 2.4. Ear notch patterns enable the individual identification of a rhino. Each position corresponds to a number, which summed together provide an exclusive identifier. Duplicate 4 and 40 notches can be cut at the top of the ears to increase available combinations, and outer notches cut as either squares or triangles in shape. Adapted from Adcock and Emslie (2003).

2.2.2 Site A population

Rhinos were introduced to Site A in 1992. During the course of the study period the rhino population at Site A increased from fourteen individuals to seventeen due to the birth of three calves (Table 2.4). At the end of the study period the population had a density of 290 hectares / rhino. The rhinos at Site A are regularly dehorned (Table 2.4), with one calf, all subadult and all adult rhinos having been dehorned once each prior to the start of the study, with the first dehorning occurring after a poaching incident in 2014. Throughout the study period, rhinos were dehorned a further zero to two times each. Thus, the population at Site A had been subject to between zero and one dehorning procedures each at the start of the monitored period, and between zero and three times each by the end of the monitored period. The shortest gap between re-dehorning procedures was 369 days and the longest period was 961 days.

Table 2.4. Population characteristics of rhinos at Site A. Age classes are given for the start (24/10/15) and end (05/11/17) of data collection. Age classes: A (adult), SA (subadult), C (calf), U (unborn).

Name	**ID**	**Arrival**	**Age class**		**Sex**	**Dehorning procedures**		
			Start	**End**		**1**	**2**	**3**
Nkombi	AF1	20/12/94*	A	A	F	20/10/14†	20/04/17	
Kelly	AF2	01/12/97	A	A	F	20/10/14†	02/06/16	
Mish	AF3	07/04/05	A	A	F	20/10/14†	20/04/17	
Jodi	AF4	15/11/08	A	A	M	20/10/14†	20/04/17	
Brutus	AM1	11/09/15*	A	A	M	13/09/15†	06/06/17	
Luke	AM2	06/02/06	A	A	M	20/10/14†	02/06/16	21/07/17
Reine	SAF1	22/03/10	SA	A	F	20/10/14†	02/06/16	
Dougie	SAM1	06/12/08	SA	A	M	20/10/14†	02/06/16	06/06/17
Chilli	SAM2	10/04/11	SA	SA	M	27/10/14†	02/06/16	06/06/17
Courtney	SAM3	18/05/12	C	SA	M	20/10/14†	02/06/16	06/06/17
Logan	SAM4	28/05/12	SA	SA	M	27/10/14†	02/06/16	06/06/17
Willis	SAM5	13/02/13	SA	SA	M	20/10/14†	02/06/16	06/06/17
Phoenix	CM1	15/05/15	C	C	M	20/04/17		
Mac	CM2	21/06/15	C	C	M	20/04/17		
Jimbo	CM3	25/01/16	U	C	M	20/04/17		
Madolo	CM4	02/04/16	U	C	M	20/04/17		
Storm	CM5	10/04/17	U	C	M			

* indicates a rhino arrived at the reserve by translocation rather than through birth.
† indicates a dehorning took place prior to the start of data collection.

2.2.3 Site B population

The first rhinos were introduced to Site B from Hluhuwe-Umflozi Park between 1979 and 1982 (Slotow and van Dyk, 2001). During the study period, there were over 300 white rhinos in Site B (pers. comm. NWPB, 2016) giving a minimum density of approximately 167 hectares per rhino. However, as 14.7% of the park consists of slopes with a gradient greater than 60% (Fajji et al., 2018), the effective density of rhinos is likely to be closer to 142 hectares per rhinos due to their avoidance of steep terrain (Park et al., 2015; Sheil and Kirkby, 2018). The rhinos at Site B were not dehorned but undergo periodic immobilisations for ear notching, DNA sampling, veterinary procedures and microchipping. This helps maintain a register of known individuals and estimate population growth rates (NWPB, 2015).

2.2.4 Dehorning procedure

Prior to each dehorning, the necessary permits were obtained from North West Department of Economic Development, Environment, Conservation and Tourism (DEDECT) by the reserve manager (information available on request). Representatives of Nature Conservation were present at each dehorning to confirm that all official guidelines were followed throughout the procedure. Dehorning personnel consisted of the primary researcher (SGP), several reserve management staff and at least two veterinarians. Individual rhinos were immobilised independently, except for mother-calf groupings which were immobilised together to avoid distress. Rhinos were immobilised by shooting a dart into the rump or the upper part of the hind leg from a helicopter (Figure 2.5). The immobilisation cocktail consisted of intramuscular etorphine hydrochloride (M99), azaperone (Stresnil) and hyaluronidase with the dose varied according to animal body size (Morkel and Nel, 2019). Following immobilisation, ear and eye covers were placed on the rhino to avoid sensory damage or distress. The anterior and posterior horns were then trimmed with chainsaws and the edges smoothed with a disk sander. Care was taken to cut above the skin-horn interface (9–11 cm) to prevent damage to the growth plate and sinuses. Between 0.2 kg and 2.6 kg of horn were removed per rhino for each dehorning. Throughout the procedure, rhino respiratory rate was constantly monitored to assess any signs of stress related to immobilisation. During the trimming procedure measurements and photographs were made of the horn and the rhinos were inspected for wounds or illness. Rhinos were also ear-notched if required. Rectal faecal samples were taken by the primary researcher (SGP; see Chapter four). Finally, to reverse the effects of the opioids, the rhinos were injected intravenously with butorphanol and naltrexone. The complete dehorning procedure, from initial darting to full recovery took around 15 to 20 minutes per rhino.

Figure 2.5. The dehorning procedure: (A) Rhinos are immmobilised by a dart fired from from a helicopter, (B) their eyes and ears are covered to reduce exposure to particles and noise (C) both psterior and anterior horns are cut with a chainsaw; (D) the remnant stub is sanded to further reduce horn mass and file sharp edges. Images taken at Site A during the study and courtesy of L MacTavish.

2.3 Data collection and analysis

2.3.1 Behavioural observations

Data were collected by behavioural observations in Chapters 3, 5 and 6. Observations of rhino social behaviour took place at Site A and Site B between 27/01/2016 and 27/10/2016 for Chapter 3. Observations of rhino horn use behaviour took place at Site A and Site B between 24/10/2015 and 15/11/2017 for Chapter 5. For Chapter 6, observations of rhino behavioural responses to acoustic, olfactory and drone-based stimuli were made between 01/10/2016 and 05/11/2017 at Site A only, where the need for repeat experiments with the same individuals was aided by the reporting of rhino locations and identities by the reserve management staff. In comparison, the larger area and population of Site B made finding the same rhinos several times logistically more challenging.

Behaviours were scored directly in the field or from recordings taken by handheld cameras (Chapters 3 and 6), from recordings taken by camera traps (Chapters 5 and 6) and from recordings taken by a drone (Chapter 6). All field observations and video footage were conducted and reviewed by the primary researcher (SGP) unless otherwise stated. Prior to the start of all observations, rhinos were identified and assigned a unique identification code (age class: sex: notch number) to keep track of repeat sampling events. If their identities could not be established, rhinos were categorised by their horn size/shape, age class and sex.

Observations were conducted through either instantaneous scan sampling (Chapter 3) or all occurrences sampling (Chapters 3, 5 and 6). For all occurrences sampling, the incidence of a specific behaviour is recorded in totality across the entire observation period (Altmann, 1974). This makes it appropriate for recording rare or infrequent interactions such as social behaviours (Lehner, 1992). For instantaneous scan sampling, the behaviour of a subject is only recorded at set intervals throughout an observation period (Altmann, 1974). This makes it suitable for determining activity budgets of common behaviours over a period of time (Lehner, 1992). These two sampling techniques have benefits over behaviour collected *ad libitum* (non-systematically) which will only provide anecdotal descriptions of data, which are less suitable for experimental analyses. As well as techniques such as one-zero sampling that be used to record the presence or absence of behaviours but provide only basic estimations of behavioural frequencies and durations (Simpson and Simpson, 1977). Behaviours of interest were defined by ethograms adapted from past studies of rhino behaviour and are listed in the appropriate chapters. Prior to the start of all observations, except those taken during camera trapping, rhino locations were calculated with Equation 2.1. This uses the geographic co-ordinate of the observer's position, taken by handheld GPS (Garmin GPSMAP 64), the distance of the rhino, taken by a rangefinder (Leica Rangemaster CRF 1600-R) and angle of the rhino taken by a compass.

Equation 2.1. Projection of a geographic co-ordinate by a known bearing and distance to a new latitude (x_2) and longitude (y_2). Adapted from Zwillinger (2002).

$$x_2 = \sin^{-1}\left(\frac{\pi}{180} \times x_1\right) \times \cos^{-1}\left(\frac{D}{6371000}\right) + \cos^{-1}\left(\frac{\pi}{180} \times x_1\right) \times \sin\frac{D}{6371000} \times \cos\left(\frac{\pi}{180} \times B\right) \times \frac{180}{\pi}$$

$$y_2 = \frac{180}{\pi} \times \left(\left(\frac{\pi}{180} \times y_1\right) + \tan^{-1} 2\left(\cos\frac{D}{6371000} - \sin\left(\frac{\pi}{180} \times x_1\right) \times \sin\left(\frac{\pi}{180} \times x_2\right), \sin\left(\frac{\pi}{180} \times B\right) \times \sin\frac{D}{6371000} \times \cos\left(\frac{\pi}{180} \times x_1\right)\right)\right)$$

Where,

X2 = rhino latitude (decimal degrees)

Y2 = rhino longitude (decimal degrees)

X1 = observer latitude (decimal degrees)

Y1 = observer longitude (decimal degrees)

D = distance of rhino from the observer position (metres)

B = bearing of rhino from the observer position (degrees)

Camera trapping has been demonstrated as a successful method to monitor both black and white rhinos (Stein et al., 2010). All camera trap data were collected within Site A only. The camera traps used throughout the project were either Bushnell Trophy Cam (Essential and Aggressor) or Ltl Acorn (5610WA) models. Video recordings were triggered by passive infrared (PIR) sensors which detect sudden changes in thermal radiation within a detection zone (Welbourne et al. 2016). Thus, they began when an animal entered the frame with a sufficiently different surface temperature (either hotter or colder) to that of the background environment. Camera traps were set to operate over the entire 24-hour period over several days (see Chapters 5 and 6). Behaviours could be observed throughout the day and night as the cameras emitted ‘low glow’ (850 nm) or ‘no glow’ (940 nm) infrared light in low light conditions. To avoid the risk of disturbing the animals, only cameras that emit the 940 nm wavelength were used in the deterrent trials (Chapter 6). Cameras were positioned 1 to 1.5 m above ground height and placed between 5 and 10 m from the focal point. Recording times were set at 30 second durations with intervals of 2 (Chapter 6) or 10 seconds (Chapter 5) between triggers to enable near continuous observations of behaviour.

2.3.2 Faecal sample collection

Fresh faecal samples were collected from rhinos for endocrine analysis from both Site A and Site B between 24/05/16 and 22/10/17. Samples were collected from dung or from the rectum during immobilisations, but only if rhino identity could be ascertained. Samples were assayed for adrenal and gonadal steroid concentrations using the methods described in Chapter Four.

2.3.3 Data analysis

Details of the relevant statistical analyses are listed in the relevant chapters. All statistical analyses were performed in R (version 3.5.1; R Core Team, 2018). Data were plotted in R using the package 'ggplot2' or in Microsoft Excel, except for social network analyses which were plotted in Gephi (version 0.9.3; Bastian et al., 2009). For the results of statistical analyses to be considered accurate, the data must meet a variety of assumptions specific to the test (Kabacoff, 2015). All analyses that utilised a continuous outcome variable were checked for normality following Kabacoff (2015). For dependent tests of difference with two groups, normality tests were performed on the differences between those groups. For independent tests of difference with two groups, the distribution of the data in each group was checked for normality. For all tests of difference with a continuous predictor variable or a predictor with greater than two groups, the standardised residuals were tested for normality. Normality tests were performed by consulting P-P plot and histograms of standardised residuals, or through testing the data or residuals using the base R function 'shapiro.test'. Tests of other assumptions are described in the methods section of subsequent chapters.

Usage of generalised linear mixed models

In Chapters 3 and 4 generalised linear mixed models (GLMMs) were run with the 'glmer' function from the 'lme4' package (version 1.1-17) using methods outlined by Bolker (2018). GLMMs allow for the modelling of a wide range of response distributions, including of non-normal data, and for the inclusion of multiple sources of random variation (known as random effects), including when repeat measurements are taken from the same individual (Bolker et al., 2009; Johnson et al., 2015). This makes them suited for the analysis of ecological data and overcomes problems with classical ANOVA tests where non-normal datasets require transformation, and of pseudo-replication, which can occur if random effects are excluded (Bolker et al., 2009). The GLMMs used here fit fixed-effects parameters and random effects by maximum likelihood (ML) utilising Laplace approximation. Laplace approximation was selected over penalised quasi-likelihood methods because it reduces bias and approximates the true GLMM likelihood rather than a quasilikelihood (Bolker et al., 2009). Continuous predictor variables were centred and scaled using the base R function 'scale'. Random terms were retained in the model unless their variance components were found to equal zero. Models were selected by comparing their Akaike Information Criterion (AIC) and the associated Chi-square test statistic from the deviance output of the standard R 'anova' function (Bolker, 2018). Models with lower AICs ($\Delta < 2$) were selected as these will generally have a better statistical fit and utilise fewer parameters than those with higher AICs (Mazerolle, 2006; Symonds and Moussalli, 2011).Type 3 Wald χ^2 tests were used to extract the significance of fixed effects following checks of over-dispersion (Bolker et al., 2009; Kabacoff, 2015; Bolker, 2018). The Wald χ^2 tests were performed with the 'Anova' function from the 'Car' package (version 2.1-6) which extracts the test statistics without refitting the model (Bolker et al., 2009; Bolker, 2018).

2.3.4 Ethics and permissions

Ethical approval was granted by the Animal Welfare and Ethics Review Board of the University of Brighton (Ref: 2018-1127). Project methods were reviewed and approved by the game reserve managers and abide to ethical guidelines set by the American Society of Mammalogists (Sikes et al., 2016). Permission was granted from North West Parks & Tourism Board (Permit No. 58956) to conduct research inside Site B and by D. MacTavish to conduct research at Site A.

Chapter 3 – The social effects of dehorning

3.1 Introduction

White rhinos utilise their horns in a wide variety of social behaviours (Owen-Smith, 1973). This includes as an armament during intra-sexual competition between territorial males (Berger et al., 1998), but also as a defensive or attacking weapon during agonistic encounters among other age-sex classes (Owen-Smith, 1973). In black rhinos horn size can be a predictor of dominance during agonistic confrontations (Berger and Cunningham, 1998), but in white rhinos the role of horn size is not as well understood (Du Toit and Anderson, 2013). Abnormal rates of aggression have been widely posited as a theory for poor reproductive success in captive white rhinos (Swasigood et al., 2006; Metrione and Harder, 2011; Cinková and Bičík 2013), while changes in the frequency of agonistic behaviours have been linked to increases in physiological stress (Schmidt and Sachser, 1997). As such, there has been a call for more intensive studies to evaluate whether dehorning, which significantly shortens a rhino's horn, disrupts or modifies the social behaviour of free-ranging white rhinos (Lindsey and Taylor, 2011; Du Toit and Anderson, 2013; Trendler, 2014; Badenhorst et al., 2016; Patton et al., 2018a).

3.1.1 Social organisation

White rhinos can live for over forty years and undergo several changes in social organisation across their lifespan (Hillman-Smith, 1986; Patton et al., 2016). These changes affect their engagement with and exposure to a range of agonistic and cohesive behaviours (Rachlow et al., 1999; Cinková et al., 2016). Female white rhinos give birth to a single calf after a gestation period of around 495 days (Linklater, 2007). Calves remain with their mothers until they are chased away shortly before or after the birth of a sibling; this occurs at around 2 to 3 years old and marks the stage they reach sub-adulthood (Owen-Smith, 2013; Patton et al., 2016). Subadults rarely remain solitary, forming persistent associations with other subadults or non-maternal adult female-calf pairs (Shrader and Owen-Smith, 2002). Rhinos in these associations will orientate their movements towards one another and stay within close proximity (often less than 25 m) for continuous periods of time (Shrader and Owen-Smith, 2002; Cinková et al., 2016).

Thus, individuals can be found in stable social aggregations of around two to six individuals, which can persist from several days to several years (Shrader and Owen-Smith, 2002; Cinková et al., 2016). It is likely that the subadult members of these groups benefit from a reduction in the costs associated with dispersal through a decreased risk of inter- and intra-specific attack and an increased knowledge of local resources through accompaniment of environmentally familiarised individuals (Shrader and Owen-Smith, 2002; Shrader et al., 2013). Persistent associations end when subadults reach reproductive age, or if the female they are associated with gives birth to a new calf (Shrader and Owen-Smith, 2002). Additionally, groups of all age-sex classes will form larger temporary social aggregations (for up to several hours) when resting, around wallows or water holes (Owen-Smith, 2013) and following disturbance from a threat such as a carnivore or human (Owen-Smith, 1988). In congregations triggered by disturbance, rhinos stand rump to rump and face outwards towards the threat in a defensive social behaviour referred to as group guarding (Owen-Smith, 1973).

Male white rhinos reach sexual maturity at 10 to 12 years old, at which point they become predominantly solitary (Owen-Smith, 1975). Around a third of adult males hold territories (non-overlapping home ranges) which provide them with spatially-exclusive reproductive access to concurrent females (White et al., 2007). In contrast, adult females, subordinate adult males, and subadult groupings hold overlapping home ranges that may encompass the territories of several territorial males (Rachlow et al., 1999; Shrader and Owen-Smith, 2002). Dominant males will accompany cows in oestrus until they are sexually receptive and remain with them until several days after copulation (Owen-Smith, 1973, 1975). Territories are patrolled and demarcated through urine spraying and the kicking and scraping of dung (Marneweck et al., 2018). Females and non-territorial subordinate males do not kick their dung and urinate in a single stream (Rachlow et al., 1998). Rhinos can detect one another’s sex, age, territorial and reproductive condition from the odour of their dung, making it an effective communication tool (Marneweck et al., 2017).

Females reach sexual maturity at 6 to 7 years old (Shrader and Owen-Smith, 2002). Adult females appear to show no direct mate choice and will breed with any territorial male with which their home range overlaps (White et al., 2007). Females spend the greatest proportion of time in territories with the most grassland, thus males can maximise reproductive success by defending these areas (White et al., 2007). Home range and territory size vary, depending on an area's productivity, water source distribution and conspecific population density (Pienaar et al., 1993; Thompson et al., 2016). Females typically have larger home ranges than males (Conway and Goodman, 1989; Pienaar et al., 1993; White et al., 2007), but this is not always the case (Thompson et al., 2016). Range sizes measure between 4 – 59 km^2 in adult females and territories between 0.8 – 50 km^2 in dominant males (Rachlow et al., 1999; Owen-Smith, 2013). Subadults either adopt the home range of the female that they are accompanying or establish temporary ranges of 2 to 7 km^2 before moving elsewhere (Shrader and Owen-Smith, 2002). Once reaching sexual maturity males may challenge a dominant male to take over a territory or become subordinate to one (Rachlow et al., 1999).

3.1.2 Agonistic social behaviour

Social behaviours associated with conflict, threat or aggression are common in white rhinos with their form, frequency and function dependent on individual social position, age-sex class and motivational state (Owen-Smith, 1975; Meister, 1998). Territorially dominant males confront other adult males encountered in or on the boundary of their territories by exhibiting aggressive behaviour such as charging (Owen-Smith, 1975). If the encounter is between dominant males, the interloper will meet the charge but then progressively retreat until reaching his own territory (Owen-Smith, 1973). Sub-dominant satellite males will also meet a charge but roar and show gestures suggestive of fear or submission, such as a tendency to step backwards, a curling of the tail, and a rise in pitch of the roar to a shriek (Owen-Smith, 1975). If these submissive behaviours occur, the challenge will then cease and the sub-dominant male is allowed to remain on the territory. If the encountered male does not retreat, or is encountered far from its own territory, the challenge may break into a fight which can result in territorial displacement if the interloper wins (Owen-Smith, 1975). If the interloper loses it will retreat from the territory it has entered (Owen-Smith, 1975). In fights, rhinos repetitively thrust their horns and forcefully press their bodies against one another which can result in minor to serious injury or even death (Owen-Smith, 1973; Patton et al., 2018a). On occasion, dominant males will also aggressively challenge the subadults of both sexes (Rachlow, 1997; Owen-Smith, 1975). However, these encounters are rare and those that do occur are usually brief (Shrader and Owen-Smith, 2002).

In free-ranging white rhino populations, dominance is only known to occur within adult males that compete for territories that give reproductive access to females (Cinková et al., 2016). In this context, changes to their horns, which are used in fighting, may affect their capacity to defeat other individuals and thus their resource holding potential (Rachlow et al., 1998; Lindsey and Taylor, 2011). Only a few studies have documented the effect that horn size asymmetries may have on dominance in African rhinos (Kock and Atkinson 1993; Berger and Cunningham, 1998; Du Toit and Anderson, 2013).

A free-ranging population of black rhinos in the Zimbabwean Lowveld were monitored for changes in location after dehorning (Du Toit and Anderson, 2013). As the mean distance of sightings of dehorned bulls from dehorning sites (3.3 km, n = 30) was no greater than the distances observed between repeat sightings of horned bulls in the same population (n = 30) the authors concluded that there was no evidence of displacement. However, no comment was provided on the timings between sightings or whether the bulls were territorially dominant. Research on black rhinos indicates that horn mass appears to affect intrasexual dominance in males but not in females (Berger and Cunningham, 1998). In male-female interactions, both parous and nonparous females were most often observed to be dominant over males independent of the size of their horns (81% of the time when horns were larger and 74% when smaller, n = 16, 23 events respectively). In male-male interactions, rhinos with anterior horns longer than their opponents were more likely to dominate their dyadic interactions (65% of the time, n = 128 events). The effect was most prominent for individuals that had anterior horn size differences greater than 10 cm in length. Furthermore, the relationship between horn mass and dominance remained when age was controlled for. In female-female interactions, horn size did not predict dominance (only 20% of interactions were won by the female with the larger horn, n = 46 events). However, Lindeque (1990) reported the case of a black rhino bull in Namibia remaining dominant after its horn broke off naturally.

Evidence of the effects of horn size on dominance in white rhinos are also unclear. Kock and Atkinson (1993) made several observations of free-ranging white rhinos, where dehorning had no apparent effect on fighting ability. This included the ability of four dehorned but large-bodied males in Hwange National Park, Zimbabwe, to maintain their territories despite several horned males being present in the area. Additionally, they witnessed a dehorned territorial male dominate a horned intruder. However, Daly et al. (2011), reports that in confined areas, social interactions are more intense and that on occasions dehorned bulls have been killed by 'lesser' bulls and cows. Research by Rachlow et al. (1998) found that age and body size were correlates of male territoriality in free-ranging white rhinos. While horn size was not monitored, as it continues to grow as rhinos age before reaching an asymptote (Pienaar et al., 1991; Rachlow and Berger, 1997; Ververs, 2018), it may be a further predictor of dominance. The study found that older adults were more likely to hold territories than younger adults, with older rhinos being similar in body length to, but wider in girth (measured by neck and chest circumference) than younger individuals. Thus, the heavier mass but also greater social experience of territory holders increased their chance of winning, and indicates that their behavioural interactions can in part be explained by their resource-holding potential (Maynard Smith and Parker, 1976). Research by Patton et al. (2018a) reports how when three adult male rhinos were dehorned on a small reserve in Uganda, the number of fights they engaged in reduced by a mean 73% in the two years after their dehorning compared to the two years before. Additionally, the number of fights they engaged in with three subadult horned males decreased by 57% over the same period. Thus, despite some ambiguities, prior-attributes of individuals clearly contribute to fighting ability and influence dominance relationships in both species of African rhino. Rhinos are more closely matched in physical attributes following dehorning due to the age-dependent differences in horn growth (Rachlow and Berger, 1997), so if individuals usually pursue a strategy of avoidance or appeasement with superior competitors (Owen-Smith, 1973), then the procedure may increase the frequency of aggressive confrontations. This is visible in some species where closely matched individuals are more likely to fight (Setchell and Wickings, 2005).

Outside of their function in territorial dominance, agonistic behaviours in white rhinos can also have space-maintaining functions to prevent other individuals from approaching too close (Owen-Smith, 1973; Rachlow 1997) and in competition for resources such as food, shade and space (Metrione et al., 2007). In a study of free-ranging white rhinos resident to Hluhluwe-iMfolozi National Park, South Africa, females were rarely observed to engage antagonistically towards one another despite sharing overlapping home ranges (Owen-Smith, 1975). For subadults, threatening behaviours, such as horn clashes, were also only infrequently observed between them. In comparison, subadults associating with adult females were often observed as the recipients of aggressive behaviour (Owen-Smith, 1975). Free-ranging rhinos resident to smaller reserves or held in captivity show similar ranges of agonistic behaviours to those found in larger populations (Cinková and Bičík, 2013; Cinková et al., 2016). Research by Cinková et al. (2016) indicates how different management conditions can affect agonistic behaviours in free-ranging white rhinos and that comparisons between different populations can provide useful insights into rhino behavioural ecology. In their study, a population with supplementary feeding was found to have higher rates of agonistic interactions than a population that was not supplementary fed, suggesting aggression was related to resource competition.

3.1.3 Non-territorial dominance

Dominance can be defined as a structural attribute of the pattern of recurring, dyadic agonistic interactions between two individuals, where the resultant outcome consistently favours one dyad member over another without the need for escalation (Drews, 1993). Thus, dominance hierarchies exist when the individuals within a group show asymmetries in aggressive and submissive behaviours, with some individuals giving way to others (Hinde, 1978; Alcock and Rubenstein, 2019). Such dominance hierarchies are common in situations where clumped resources can be appropriated or controlled by single individuals (Archie et al., 2006). In free-ranging white rhino populations, dominance hierarchies are only known among territorial males (Owen-Smith, 1975). Dominance hierarchies have also been observed amongst all age-sex classes in captive-held white rhinos (e.g. Mikulica, 1991; Meister, 1998, Kuneš and Bičík, 2002, Metrione et al. 2007, 2011, Cinková and Bičík, 2013). Although, in captive conditions, a rhino’s position in a social hierarchy does not necessarily lead to an advantage in their access of resources (Kuneš and Bičík, 2002; Cinková and Bičík, 2013).

Metrione et al. (2007) monitored aggressive confrontations within a group of seven adult females housed together in a single enclosure and found specific individuals were more likely to win their dyadic interactions than others. As the agonistic interactions between pairings reversed in outcome at least some of the time and several intransitive relationships were present, their social ranking was not strictly linear. For a hierarchy to be completely linear, all dyads must have a ‘transitive’ dominant-subordinate relationship, where if individual ‘A’ dominates ‘B’, and ‘B’ dominates ‘C’, then ‘A’ must also dominate ‘C’ (Shizuka and McDonald, 2012). Asymmetries in aggression of captive held populations of adult females were also reported by Swaisgood et al. (2006), who found that wild born individuals elicited greater levels of threatening behaviours towards captive bred females than the other way around. However, as submissive behaviours were not observed and the rates of most aggressive behaviours were rare, there was only weak evidence for the presence of a dominance hierarchy. Rees (2019) found that a group of six wild caught subadult rhinos formed a stable dominance hierarchy when placed in a boma together during translocation. They concluded that the hierarchy may have been induced by the environmental stressors of the boma and intragroup competition for resources.

Enclosures for captive rhinos are far from representative of natural conditions, being smaller in size than typical game reserves and providing punctuated access to food resources (Hutchins and Kreger, 2006). Furthermore, social groupings can be at higher densities than that found in free-ranging rhino populations, or involve the housing of adult males with females on a permanent basis (Swaisgood et al., 2006). Thus, the dominance relationships observed may only occur in rhinos kept at high population densities with abnormal social structures (Mikulica, 1991; Cinková and Bičík 2013). Furthermore, in non-supplementary fed, free-ranging white rhino populations food resources are dispersed and abundant and so cannot be monopolised (Cinková et al., 2016). In these conditions individuals are more likely to show poorly differentiated dominance rank relationships or non-hierarchal egalitarian social structures (Isbell, 1991; Meister et al. 1998; Archie et al. 2006; Cinková et al., 2016). This may in part explain the apparent absence of social dominance found in free-ranging white rhinos outside of reproductive competition (Owen-Smith, 1973; Cinková et al., 2016). Research on free-ranging white rhinos in two small private reserves indicates that subadults and calves are more submissive to adults at feeding places and suggests that a dominance hierarchy may be present in some contexts (Cinková et al., 2016). Thus, more intensive studies may establish whether the lack of a hierarchy is an artefact of inadequate sampling or a true absence (Clutton-Brock and Harvey, 1976; Cinková et al., 2016).

3.1.4 Aims

This study sought to determine whether dehorning affects the social behaviour of free-ranging white rhinos. Rhinos use their horns during agonistic social interactions and so dehorning has the potential to alter the frequency and form of these behaviours. The findings have the potential to aid management decisions on the optimum frequency of dehorning procedures. The primary research questions were (1) whether dehorning caused a change in the rate of agonistic social interactions, (2) whether dehorned rhinos displayed the same range of agonistic behaviours as horned rhinos and (3) whether dehorning induced changes in social dominance.

Objective 1 was to determine whether the range and rate of aggressive social behaviours in a population of dehorned rhinos differed to those in a horned population. The two populations were geographically close (3.5 km apart at their nearest points) but separated by fencing (See Chapter 2 for further details). Dehorning was predicted to reduce the range of horn-reliant behaviours exhibited during aggressive confrontations compared with horned rhinos. Additionally, rhinos that had been dehorned once were predicted to engage in a higher number of agonistic behaviours than rhinos that had never been dehorned before, if horn size affected their probability of dominating an interaction or influenced their tolerance of conspecifics. Adult bulls were expected to show greater rates of aggressive behaviours than other age-sex classes given their function in maintaining territorial dominance (Owen-Smith, 1988).

Objective 2 was to determine whether the rate of agonistic social interactions changed following a re-dehorning procedure (the period before and after a subject’s second dehorning). Rhinos were predicted to engage in an increased number of aggressive behaviours following the procedure (both with the individuals inside of and outside of any long-term social groupings).

Objective 3 was to determine whether rhinos within a stable long-term social grouping had asymmetric outcomes in their aggressive interactions and if so, whether the social positions occupied before dehorning differed to the positions occupied after the procedure. A levelling of horn size was predicted to disrupt existing social rankings as physical attributes are known to affect the outcomes of dyadic encounters in white rhinos (Rachlow et al., 1998).

3.2 Methods

3.2.1 Experimental design

Behavioural observations of white rhinos took place between 27/01/2016 and 19/10/2016. Observations were split between two sites, Site A and Site B (site parameters are listed in Chapter 2). The population at Site A consisted of 15 rhinos at the start of the behavioural study, but increased to 16 after the birth of a calf (see Chapter 2). Twelve of the rhinos had been dehorned once prior to the start of the study, eight of which were dehorned again during the behavioural observation period. Each rhino had been dehorned between 0 and 2 times by the end of the data collection period (see Chapter 2). The population at Site B consisted of between 300 and 350 individuals that had never undergone dehorning. A greater sampling effort was concentrated at Site A than Site B to enable repeat observations of the same individuals. A full listing of dehorning dates, subject parameters and a methodology of the dehorning procedure are provided in Chapter 2.

3.2.2 Behavioural monitoring

Behavioural observations typically took place in the morning (sunrise to 11 am) and afternoon (3 pm to sunset) to coincide with peaks in rhino behavioural activity (Patton et al., 2018b). To locate the animals, haphazard routes were driven or walked until a rhino or group of rhinos were encountered. Haphazard sampling, also known as convenience sampling, is used in field studies to simulate random sampling (Eikan et al., 2016). This relies on the assumption that the spontaneously followed search technique selected individuals from a homogeneous target population and did not give subjects an unequal chance of selection (Eikan et al., 2016). Furthermore, as observations were collected from the entire population in Site A, the technique did not lead to any individuals being excluded from the dataset. To limit observer-triggered disturbance, rhinos were followed from a distance and observed using binoculars. Observations took place both on foot and from a stationary vehicle. Rhinos were identified via their unique ear notch pattern or in the case of calves, by that of their mother (See Chapter 2). This avoided inducing pseudo-replication in analyses of repeat observations. The rhinos were categorised into three age classes: calves, subadults and adults (see Chapter 2). Behavioural observations of all rhinos in the group were selected as focal animals, unless it was a temporary aggregation that would go onto split spatially (e.g. two cows with two calves). In the latter case, one independent grouping was selected *ad libitum* as the focus when the geographic split occurred. Behavioural observations ended when rhinos lay down to rest (for greater than 60 minutes), were lost from sight (for greater than 15 minutes), or it was too dark to identify them. Each of these monitored periods were classified as a separate observation session, with multiple observation sessions conducted for some individuals. On occasion, pre-existing knowledge of concurrent complex or rare social behaviours (e.g. fights between individuals) led to specific individuals being sought out for observation. Due to the inherent observer bias in these sessions they were coded separately as ‘opportunistic’ observations. Additionally, 11 observation sessions conducted by a research assistant, M. Withey, for which the primary researcher, SGP, was not present were coded as opportunistic observations. All other observation sessions were conducted by SGP.

Rhino activity was recorded by scan sampling every 2 or 5 minutes (see Table 3.1). For each rhino, counts of inactivity, observer-biased activity, or rhino absence were summed, multiplied by their time interval and subtracted from the total time of the observation session to calculate the effective observation time. Observer-biased activity was defined as disturbance by observer presence, indicated by vigilance towards the observer, or running from the observer, until the rhinos settled back to their prior undisturbed behaviour (Rachlow et al., 1998; Shrader and Owen-Smith, 2002). If a focal individual interacted with any other rhino, the focal rhino’s social behaviours were recorded as well as the social behaviours directed towards it by the other individuals. Social behaviours were recorded by all occurrences sampling with an ethogram (Table 3.2) adapted from other studies of white rhino social behaviour (Owen-Smith, 1973; Cinková et al., 2016).

Table 3.1. The behavioural states of the focal subjects were classified into one of several exclusive categories. Data was recorded at two or five-minute intervals. The definition of vigilance was adapted from Owen-Smith (1973) and Shrader and Owen-Smith (2002).

Data	State	Description
Included	Active	All behavioural states other than those listed below (e.g. foraging, walking, drinking).
Excluded	Resting	Lying or sitting on the ground in rest.
	Running from observer	Rapid movement (run or gallop) faster than a walk away from observer(s).
	Vigilant to observer	Alert to observer(s), or to an animal spooked by observer(s). Rhino is standing stationary with its head held above the ground and both ears focused towards the disturbance.
	Absent	Rhino was not visible or not under observation.

Table 3.2. Social behaviours recorded by all occurrences sampling. Adapted from Owen-Smith (1973) and Cinková et al. (2016). Behaviours were categorised as either agonistic (A): associated with conflict, threat or aggression; cohesive (C): socio-positive, or other (O): unclear or ambiguous.

Behavioural unit	**Type**	**Description**
Approach with threat	A	Directed movement (walk) towards a rhino leading to agonistic behaviour. Head up and ears forward, or head lowered and ears back if close.
Approach without threat (direct)	C	Directed movement (walk or run) towards a rhino leading to no agonistic behaviour. Rhinos remain in proximity to one another.
Approach without threat (indirect)	O	Indirect movement (walk) towards a rhino (e.g. during foraging). Only recorded if an agonistic or cohesive behaviour followed.
Move-away	A	Directed movement (walk) away from rhino following agonistic behaviour.
Charge	A	Rapid movement (run) towards a rhino for a distance of at least several meters, including feinted attacks.
Chase	A	Rapid movement (run) towards a fleeing rhino.

Behavioural unit (cont.)	**Type**	**Description**
Flee	A	Rapid movement (run) away from a rhino following agonistic behaviour. Is not necessarily being chased.
Turn	A	Fast turning and raising of head and/or turning of body towards a disturbing rhino. Often includes a few quick steps.
Shoulder	A	Forcefully pressing against recipient with body/neck/head.
Nudge	O	Accidental physical contact. E.g. brushing/touching while walking/foraging.
Non-aggressive contact	C	Deliberate non-aggressive physical contact. Includes head/neck/body rubbing or mouth/lips contact against a recipient.
Naso-naso contact	C	Standing, head up, face to face, sometimes allowing noses to meet. Often the first interaction when rhinos from different groups meet.
Stare	A	Standing, horn to horn, staring at opponent. If head down and ears back, indicates submission.
Group guard	A	Two or more rhinos standing side-side or rump-rump with their heads facing outwards towards a threat. Defensive formation.
Courtship	C	Proceptive or receptive sexual behaviours including mounting and mating.
Horn thrust	A	Forceful thrusting of horn against the body of a recipient.
Horn to horn blow	A	Forceful thrusting of horn against the horn of a recipient.
Horn fencing	A	Forceful and repetitive thrusting of horn against the body/horn of a recipient. Recipient usually responds with the same. Main element of territorial fights*.
Horn wrestling	C	Less-aggressive repetitive thrusting of horns against the body/horn of a recipient. Recipient usually responds with the same. Likely develops fighting skills and acts as a form of play. Instigator usually a subadult or calf.
Horn contact	C	Gentle contact of horn against the body of a recipient, includes rubbing and leaning.
Aggressive vocalisation	A	Bellow or loud roar directed at opponent or threat.
Submissive vocalisation	A	A singular/series of trumpeting shrieks. An intensive shrill sound, reminiscent of the trumpeting of an elephant; made by subordinate bulls or by territorial bulls out of their home territories.
Panting contact call	C	A series of inhalations and exhalations when a rhino is isolated from its group and when approaching or staring at other animals.
Squeak	C	A singular/series of high-pitched squeaks made by calves in proximity to their mothers. Suckling usually follows.
Other vocalisation	CAO	A singular/series of snorts, puffs, squeals or grunts, often without clear function. Part of defensive, submissive, alarm or contact call.

*Fights were distinguished from other forms of agonistic interactions by the presence of at least one territorial bull and an engagement in horn fencing from both individuals.

Sequential social behaviours (Table 3.2) between the same individuals (that occurred within one minute of the preceding social behaviour) were classed as a single independent social 'event'. If there was a change in participants or a gap between social behaviours greater than one minute then a new social event was coded. For short social behavioural events (less than 60 seconds in duration) social behaviours were scored in the field, for social behavioural events longer than this, video recordings were made to aid in their breakdown. Behavioural studies by Metrione et al. (2007) and Cinková et al. (2016) included a snort vocalisation, defined as a threat with a nasal exhalation or inhalation, as an agonistic interaction in their ethograms. However, in this study snort vocalisations that were not part of a behavioural sequence or occurred without reciprocation were not scored as agonistic events due to the difficulty in identifying a vocaliser and the recipient, as well as their sometimes ambiguous function. For each event, the identity of the instigator and receiver were recorded. In some instances, the identity of a participant was unknown; e.g. if multiple rhinos were in a group and their notches were not visible. Each event was classified as either agonistic (associated with conflict, threat or aggression), cohesive (socio-positive) or other (unclear or ambiguous), similar to existing studies of white rhino behaviour (Cinková and Bičík, 2013; Cinková et al., 2016) and based on previous functional inference (Owen-Smith 1973). For each participant, the outcome of an agonistic event was classified as either a win or a loss. Rhinos won an interaction if they instigated threatening behaviour and/or if the recipient retreated. Rhinos lost an interaction if they displayed submissive behaviour or showed a tendency to step back or retreated first from the interaction (Owen-Smith, 1973). If the outcome was obscured or ambiguous, neither a win nor loss was designated.

Rhinos were classed as group members if they were consistently seen together for over three days. If social events occurred between members of the same group, they were classed as intra-group interactions; if they occurred between members of shorter-term or temporary social groupings they were classed as inter-group interactions (*sensu* Cinková et al., 2016). An exception was made for social events involving adult males and females, all of which were classed as inter-group interactions, even during periods of persistent accompaniment. This is because such associations are usually limited to courtship and occur if a female is in oestrus (Owen-Smith, 1975; Rachlow et al., 1998) and are different to more stable cohesive associations (Cinková et al., 2016). The largest stable grouping was observed at Site A, where six subadult individuals remained together for over six months; the group formed two months prior to dehorning (on 05/04/2016) and persisted until after the end of behavioural observations (19/10/16).

3.2.3 Data analyses

Tests investigated whether dehorning influenced the rate of agonistic social interactions. All analyses were two-tailed, and all alpha levels were set at 0.05, except for analyses of the intra-group interaction rate (analyses 2-3) which were interpreted against a Bonferroni-adjusted p-value of 0.025 to control for an increased risk of type-1 errors during repeat analysis of the dataset.

Analysis 1-3. Agonistic social interaction rate

Agonistic interaction rates were calculated by dividing the total number of agonistic events by each rhino's effective observation time. Behavioural differences between rhinos lead to variation in their effective observation times per session, while variable rhino detection rates resulted in an unequal number of repeat observations per individual. Observations of non-focal rhinos were also removed, as these were only recorded during encounters with focal individuals and thus have inflated counts of social interaction relative to their observation time. For the same reason, opportunistically sampled sessions were also excluded. Finally, all observations of calves were removed as only one calf was dehorned throughout behavioural observations.

Analysis 1. Difference in agonistic social interaction rate between horned and dehorned rhinos

The rate of agonistic social interactions were contrasted between horned and dehorned rhinos considering differences in age-sex class. Behavioural observations from rhinos that had been subject to one dehorning only (from Site A) or no dehorning procedures (from Site B) were selected for analysis. To standardise effective observation lengths per session, sessions that totalled fewer than 60 minutes were excluded from the dataset, while those greater than this were truncated at 120 minutes. The resultant dataset contained 168 observation sessions from 46 rhinos (Table 3.3). Replicate observation sessions were largely confined to the dehorned rhinos from Site A, where 12 rhinos accounted for 131 of the observations, with the 34 horned rhinos from Site B accounting for just 37 of the observation sessions. For the dehorned rhinos, a mean 491 days had elapsed between the dehorning procedure and date of observations, ranging from 138 to 692 days for the 12 individuals. The dataset had a mean effective observation length of 99 minutes, ranging from 62 to 120 minutes in length.

Table 3.3. Age-sex class of subjects in the dehorned population (Site A) and horned population (Site B).

Age-sex class	**Dehorned**	**Horned**	**Total**
All	12	34	46
Adult female	4	9	12
Adult male	2	9	11
Subadult female	1	5	6
Subadult male	5	11	16

A generalised linear mixed model (GLMM) was performed to test whether the rate of agonistic social interactions (a continuous positive response variable) was influenced by two fixed effects (‘Age-sex class’, ‘Horn status’ and their interaction term), when considering one random effect (Individual identity). Counts of agonistic social interactions were input as the response variable but output as an interaction rate through the fitting of an offset. The offset related each datum to the length of its observation session, thereby controlling for the variation within sampling length. However, the model failed to converge due to the inclusion of a singular subject at the dehorned subadult female level. To address this, age-sex class was excluded and the model was rerun with just one fixed effect (‘Horn status’). The data were non-parametric counts so a Poisson distribution with a log-link function was fitted. A check for over-dispersion was performed by dividing the model’s residual deviance by its residual degrees of freedom.

A subset of the data was selected to test whether the rate of agonistic social interactions (the continuous positive response variable) was influenced by ‘Age-sex class’ and ‘Horn status’ (and their interaction term) through the use of a generalised linear model (GLM).

This utilised the glm() function from the base R package. One session from each rhino was selected for inclusion in the model. Selection of the data was made via a random number generator, using the sample() function included in the base R package. The resultant dataset had a mean effective observation length of 93 minutes, ranging from 70 to 120 minutes in length. As with the first model, counts of agonistic social interactions were input as the response variable but output as an interaction rate through the fitting of an offset. Again, the data were non-parametric counts so a Poisson distribution with a log-link function was fitted. Following a check for over-dispersion (again, by dividing the model's residual deviance by its residual degrees of freedom), the resultant ratio was larger than 1, indicating that the observed variance was larger than that predicted by the model and that over-dispersion was present (Kabacoff, 2015). This was confirmed through the qcc.overdispersion.test() function accessed through the qcc R package. To control for over-dispersion, the model was refit with a quasi-Poisson distribution, which left the model's parameter estimates unchanged but increased the standard error (Kabacoff, 2015).

Analysis 2. Difference in agonistic social interaction rate following a re-dehorning procedure

The rate of agonistic social interactions were contrasted before and after a dehorning procedure. Knowledge of long-term group composition allowed for separate investigation of the intra- and inter-group agonistic interaction rate. Eight dehorned rhinos at Site A were monitored before and after they experienced a second dehorning. Two of the eight rhinos did not form stable groupings with any other adult or subadult throughout the monitored period, and thus were excluded from the intra-group rate analysis. Dehorning removed a minimum of between 0.5 to 2.6 kg of horn per rhino. Thus, individuals showed greater variation in horn size prior to their dehorning than they did after despite the procedure being a re-dehorning. This is due to the differential rates in horn growth observed between individuals of different ages and sexes (Pienaar et al., 1991; Kock and Atkinson, 1993; Rachlow and Berger, 1997; Ververs, 2018).

The effective observation time of these sessions were summed for each individual prior to and after their second dehorning. The resultant observation lengths totalled between

1080 and 2601 minutes prior to their second dehorning procedure, and 1083 to 2250 minutes after it. To standardise the cumulative observation length for each rhino, the higher of the two totals was rounded down the lower of the two totals (Table 3.4). To select which observation sessions to exclude, each observation session was assigned a random number. The sessions were summed following the randomised order until the specified observation length was reached. If the final session increased the cumulative observation total beyond the specified length, the remainder was excluded. The resultant 16 periods were divided by their concomitant number of inter and intra-agonistic social interactions to calculate the hourly rate per rhino. For the observations taken prior to their second dehorning procedure, rhinos had between 458 days and 548 days of horn regrowth since their most recent dehorning (Table 3.4). For the observations taken after the second procedure, rhinos had between 1 day and 135 days of horn regrowth since their dehorning.

To test whether the inter-group rate of agonistic social interactions were affected by dehorning, a Wilcoxon-signed rank test was run using the function wilcox.test() from the base R package, as the difference in the groups were non-normally distributed (See Chapter 2). To test whether the intra-group rate of agonistic social interactions were affected by dehorning, a paired-t test was run using the function t.test() from the base R package, as the data were normally distributed.

Analysis 3. Difference in agonistic social interaction rate within a stable social grouping following a re-dehorning procedure

A separate analysis was performed to ascertain whether any differences in the rate of intra-agonistic interactions pre- and post- dehorning extend towards rhinos found within a stable social grouping. The selected social grouping consisted of five subadult males and one subadult female. The resultant combined observation lengths totalled between 890 and 1015 minutes prior to dehorning and 1605 and 1878 minutes post dehorning. In line with the previous analysis, the lower of the two totals per rhino (either pre- or post-dehorning) the higher of the two totals was rounded down the lower of the two totals was selected for analysis (Table 3.4). A randomised sequence was used to select the sessions necessary to meet this minimum (see above). The resultant 12 periods were divided by their concomitant number of intra-agonistic social interactions to calculate the hourly rate per rhino. To test whether the intra-group rate of agonistic interactions from within a stable social grouping were affected by dehorning, a paired-t test was run using the function t.test() from the base R package, as the differences between the two groups were normally distributed (See Chapter 2).

Table 3.4. Total effective observation length of subjects pre- and post- a dehorning procedure under any group composition and as a stable sub-grouping of six individuals. Days since 'last dehorning' shows the minimum and maximum number of days elapsed between behavioural observations and the most recent procedure. Rhinos are ordered by age-sex class then age within each class. AF = Adult female, AM = adult male, SAF = subadult female, SAM = subadult male.

ID	**Group composition**	**Observations (mins)**		**Last dehorning (days)**	
		Pre-	**Post-**	**Pre-**	**Post-**
AF2 (Kelly)	Any	1083	1083	466-543	7-102
	Six-member	-	-	-	-
AM2 (Luke)	Any	1252	1252	468-540	1-101
	Six-member	-	-	-	-
SAF1 (Reine)	Any	2086	2086	465-548	2-135
	Six-member	890	890	533-548	38-110
SAM1 (Dougie)	Any	2224	2224	465-548	2-110
	Six-member	1015	1015	533-548	12-107
SAM2 (Chilli)	Any	2250	2250	458-541	1-135
	Six-member	890	890	526-541	15-135
SAM3 (Courtney)	Any	1080	1080	532-548	6-135
	Six-member	980	980	533-548	6-135
SAM4 (Logan)	Any	1923	1923	458-541	1-135
	Six-member	975	975	526-541	15-110
SAM5 (Willis)	Any	1941	1941	472-548	2-135
	Six-member	965	965	533-548	6-110

Analysis 4. Changes in social dominance hierarchy within a stable social grouping following a re-dehorning procedure

The social position of each rhino within the stable six-member grouping relative to its conspecifics were contrasted pre- and post-dehorning. All rhinos were subadults but differed in age (and correspondingly in size): SAM1 (Dougie) 89 months, SAF1 (Reine) 74 months, SAM2 (Chilli) 61 months, SAM3 (Courtney) 47 months, SAM4 (Logan) 47 months and SAM5 (Willis) 38 months. SAF1 was the only female. The six subadult individuals were ranked in order of body size during the dehorning procedure (02/06/2016) using a tape measure (body length). Body size rankings corresponded with age and sex, with SAM1 (Dougie) the largest in size, followed by AF2 (Reine) and SAM2 (Chilli) which were similar in size, and then SAM3 (Courtney), SAM4 (Logan) and SAM5 (Willis) which were the smallest in size.

All dyadic encounters for which the identities of both actors were known were included if the win-loss score outcome was clear. Dyadic interactions were taken from all observation sessions in which the rhinos were together, with no constraints on length, whether the observations were opportunistic or scheduled, or whether the individuals were focal or non-focal subjects. Interactions were split between those that occurred before dehorning (05/04/2016 to 01/06/2016) to those that occurred after the procedure (02/06/2016 to 19/10/2016). Rhinos had a combined effective observation time of 15,453 minutes [257.55 hours] prior to dehorning (mean: 42.92 hours per rhino) and 14,781 minutes [246.35 hours] after the procedure (41.06 hours per rhino; Table 3.5).

The selection of a suitable dominance index requires knowledge of the factors that will affect social status (in this case win-loss ratios) and consideration of its underlying algorithmic properties and assumptions (de Vries and Appleby, 2000; Bayly et al., 2006). The Batchelder–Bershad–Simpson (BBS) index as published by Jameson et al. (1999) was selected as the most appropriate method to calculate rank positions. The derived scores can be used to rank the animals in terms of predicted dominance outcomes. Through an iterative procedure, the BBS index determines a scale-score for each individual from (1) the proportion of wins in its encounters with others, (2) its proportion of losses, and (3) the scale scores of the others that it has met in agonistic encounters. The latter of which allows the ranking of scale scores from a relatively small number of pairwise encounters as well as the formulation of winning probabilities for pairs of individuals that are not observed to interact. This makes it well suited for use with ecological studies that may have incomplete or relatively sparse datasets introduced through the sampling constraints of behavioural fieldwork with wild study subjects (Jameson et al. 1999; Croft et al., 2008). The BBS procedure also relies on the assumption that the outcomes of dyadic encounters are independent of all previous encounters (Jameson et al. 1999) making it more suited for the formation of hierarchies based on physical attributes rather than winner-loser effects. This makes it similar to the scale developed by Clutton-Brock et al. (1979) for the evaluation of dominance in red deer stags (*Cervus elaphus*). The BBS scale predicts the closeness of ranks by calculating how much one animal dominates another in terms of probability (Jameson et al. 1999). This makes it superior to cardinal dominance scales, where the distance between adjacent rankings are equal (Drews, 1993) allowing for the detection of more realistic dominance distances that can separate certain animals.

Table 3.5. Total observation lengths for all observation sessions in which six stable rhinos were together as a stable long-term grouping. No constraints were placed on observation session length, sampling type (opportunistic or scheduled), or whether the subjects were focal or non-focal individuals. Age given for the start of group observations (05/04/2016).

ID	**Age-sex class**	**Age (months)**	**Observation length (mins)**	
			Pre-dehorning	**Post-dehorning**
SAF1 (Reine)	Subadult female	74	2511	2487
SAM1 (Dougie)	Subadult male	89	2622	2515
SAM2 (Chilli)	Subadult male	61	2529	2699
SAM3 (Courtney)	Subadult male	47	2567	2380
SAM4 (Logan)	Subadult male	47	2582	2312
SAM5 (Willis)	Subadult male	38	2642	2388

The BBS index relies on the assumption that the underlying distribution of dominance is continuous and normal. First, each rhino was assigned an initial scale score as a function of the proportion of agonistic encounters it won through Equation 3.1. Using equation 3.2, the scale scores were recursively rescaled until they converged for each individual.

Equation 3.1:

$$s(A_i) = \frac{\alpha(2W_i - N_i)}{2N_i}$$

Equation 3.2:

$$s(A_i) = \frac{2(W_i - L_i)}{N_i} + Q_i$$

Whereby,

$\alpha = \sqrt{2}\pi = 2.50663$, taken from the Taylor expansion of the normal distribution

A_i = animal identity. For a pair of rhinos A_1 and A_2 that have the final scale scores $s(A_1)$ and $s(A_2)$, A_1 is judged to be dominant to A_2 if $s(A_1)$ is greater than $s(A_2)$

W_i = the number of encounters in which animal A_i was observed to have won.

N_i = the number of encounters in which A_i was involved

L_i = the number of encounters in which A_i lost

Q_i = the mean initial scale score of those animals that A_i met in agonistic encounters

3.3 Results

3.3.1 Agonistic social interaction rate

Analysis 1. Difference in agonistic social interaction rate between horned and dehorned rhinos

A total of 115 agonisitc behavioural events were observed in 22 out of the 46 rhinos during 278.4 hours of observations. Statistical analysis, showed that the agonistic social interaction rate of dehorned rhinos (mean of individual means ± SEM: 0.423 ± 0.057 / hour; n = 12) was not significantly different to the rate found within horned rhinos (0.301 ± 0.089 / hour; n. = 34; GLMM: p = 0.516; Table 3.6). There were two predominant forms of agonistic interaction, in the first form, one rhino would stray too close to another in an indirect approach and be met with a turn or charge, often accompanied by a vocalisation (aggressive or snort), followed by one, both or neither rhino moving away. In the second form, an individual would directly approach another individual and charge or chase them. Rhinos were involved in at least one charge or chase during 26.1% of the social events (30 out of 115 events); this amounted to 27.3% of the events (27 out of 99 events) from the dehorned population and 18.8% of those (3 out of 16 events) from the horned population. Agonistic physical contact was rare; rhinos either delivered or received at least one horn thrust in 7.0% of the social events (8 out of 99 events); this amounted to 8.1% of the events (7 out of 86 events) from the dehorned population and 6.3% of those (1 out of 16 events) from the horned population. Rhinos engaged in horn-to-horn blows or horn fencing in 4.4% of social events (5 out of 115 events); this amounted to 3.0% of the events (3 out of 99 events) from the dehorned population and 12.5% of those (2 out of 16 events) from the horned population.

Table 3.6. Statistical output from GLMM analysis indicating the effect of horn status on the rate of agonistic social interactions (n = 45, obs = 168). Type 3 Wald χ^2 tests were used to extract the significance of fixed effects. df = degrees of freedom.

Fixed effects	Estimate	Standard error	χ^2	df	P
Intercept	-2.328	0.250	-	-	-
Dehorning : Class (overall)	-0.244	0.378	0.419	1	0.517

In the behavioural subset, 19 agonistic behavioural events were observed in 14 out of the 46 rhinos during 70.78 hours of observations. As with the main dataset, statistical analysis, showed that the agonistic social interaction rate of dehorned rhinos (mean ± SEM: 0.237 ± 0.149 / hour; n = 12, obs. = 12) was not significantly different to the rate found within horned rhinos (0.302 ± 0.089 / hour; p = 0.831; n = 34, obs. = 34; Table 3.7). Furthermore, differences in age-sex class (mean ± SEM; adult female: 0.103 ± 0.070 / hour, n = 13; adult male: 0.334 ± 0.151 / hour, n = 11; subadult female: 0 / hour, n = 16; subadult male: 0.507 ± 0.170 / hour, n = 6) were also non-significant (p = 0.444), as was the interaction between horn status and age-sex class (p = 0.652; Table 3.7; Figure 3.1).

Table 3.7. Statistical output from GLM analysis indicating the effect of sex-age class and horn status on the rate of agonistic social interactions (n = 45, obs. = 45). AF = adult female; AM = adult male; SAF = subadult female; SAM = subadult male. Type 3 Wald χ2 tests were used to extract the significance of fixed effects. df = degrees of freedom.

Fixed effects	Estimate	Std. error	χ^2	df	P
Intercept	-3.142	1.075	-	-	-
Horn status	-0.361	1.520	0.056	1	0.813
Class AF vs AM	-16.828	4229.402	-	-	-
Class AF vs SAF	-17.160	6146.681	-	-	-
Class AF vs SAM	0.877	1.241	-	-	-
Class (overall)	-	-	2.678	3	0.444
Dehorning : Class AM	18.252	4229.4020	-	-	-
Dehorning : Class SAF	0.628	6726.625	-	-	-
Dehorning : Class SAM	0.881	1.681	-	-	-
Dehorning : Class (overall)	-	-	1.633	3	0.652

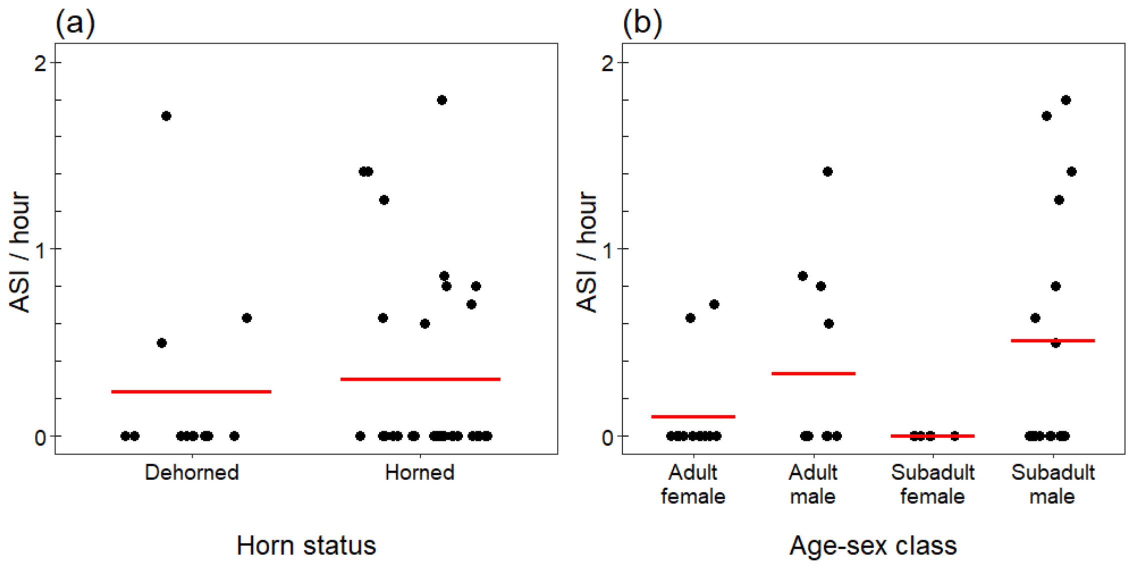

Figure 3.1. The rate of agonistic social interactions between rhinos in (a) a dehorned and horned population and (b) between different age-sex classes. Horizontal bars represent group means. Points have been horizontally jittered. N = 46.

Analysis 2. Difference in agonistic social interaction rate following a re-dehorning procedure

For eight rhinos that were monitored before and after dehorning, a total 248 agonistic behavioural events were recorded across 462 hours of effective observation time; 86 events occurred prior to dehorning and 162 events after the procedure (231 hours of observations per period). Prior to dehorning, most agonistic events occurred during encounters between individuals within long-term groupings (intra-group: 67.5% of interactions) rather than in encounters between rhinos in different groups (inter-group: 32.5%) (n = 86 events). After dehorning, the overall proportion of agonistic encounters in long-term groupings increased further (intra-group: 85.2% of interactions) relative to the number of between group interactions (inter-group: 14.8%) (n = 162 events).

The eight rhinos were involved in a total of 52 inter-group agonistic behavioural events, 28 occurred prior to dehorning and 24 after the procedure. Full individual breakdowns are listed in Appendix 2 (Table A2.1-A2.2). The median rate of inter-group agonistic interactions was lower after a dehorning (0.059 / hour) than before (0.093 / hour). However, this was not statistically significant (Wilcoxon-signed rank: $V = 17$, $p = 0.673$, n = 8). Two adult rhinos experienced an increase in inter-group agonistic interactions after their dehorning with six subadults experiencing no change or a slight decrease in their interaction rate (Figure 3.2).

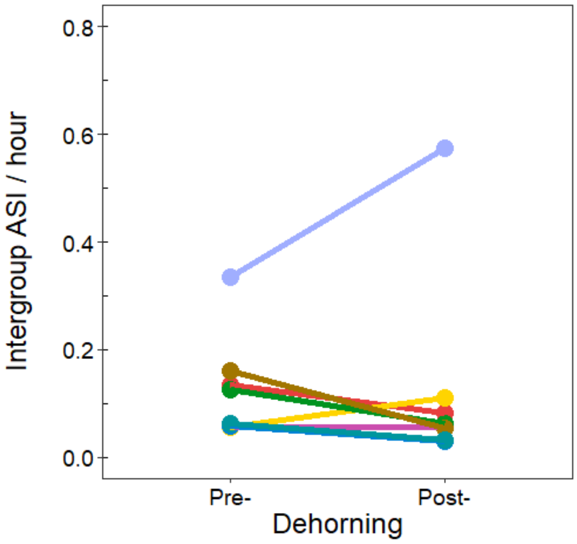

Figure 3.2. The rate of inter-group agonistic social interactions monitored in eight rhinos before and after their dehorning (n = 8). Individuals colours represent AM2 (purple), AF2 (yellow), SAM1 (red), SAF1 (blue), SAM2 (gold), SAM3 (pink), SAM4 (green), SAM5 (turquoise).

Eight rhinos were involved in a total of 196 intra-group agonistic behavioural events, 58 occurred prior to dehorning and 138 after the procedure. Two of the six rhinos did not form long-term associations with other individuals throughout the study period and so were not included in the analysis. The rate of intra-group agonistic interactions in six rhinos was higher after a dehorning (mean ± SEM: 0. 293 ± 0.075 / hour) than before (0.752 ± 0.008 / hour). This difference (mean change: 0.456 / hour) was significant ($t_{(5)}$= -3.624, p = 0.016, n = 6; Figure 3.3a).

Typical dyadic interactions involved the same behavioural activities outlined in Analysis 1, including an approach (direct or indirect), a turn, charge or chase with or without a vocalisation, followed by one, both or neither rhino moving away. Rhinos were involved in at least one charge or chase during 26.2% of the social events (65 out of 248 events); these behaviours occurred in 14.0% of events prior to dehorning (12 out of 86 events) and 32.7% of the events after dehorning (53 out of 162 events). Several of the agonistic social events also involved physical contact; rhinos either delivered or received at least one horn thrust in 10.9% of the social events (27 out of 248 events). With horn thrusts occurring in 6.9% of pre-dehorning events (6 out of 86 events) and 12.9% of post dehorning events (21 out of 162 events). Rhinos engaged in horn-to-horn blows or horn fencing in 7.3% of social events (18 out of 248 events); which included 8.1% of social events occurred prior to dehorning (7 out of 86), and 6.8% of the events after dehorning (11 out of 162 events).

Analysis 3. Difference in agonistic social interaction rate within a stable social grouping following a re-dehorning procedure

When only those interactions that occurred within a stable six member grouping were considered, a total of 126 intra-group agonistic behavioural events were observed during 191 hours of observations; 24 events occurred prior to dehorning and 107 occurred after (during 95 hours each). The intra-group agonistic interaction rate showed a greater increase (mean change: 0.844 / hour) following dehorning (Pre: 0.241 ± 0.043 / hour; post 1.09 ± 0.183 / hour; Figure 3.3b) than in Analysis 2 where group composition was not held constant. The difference was significant ($t_{(5)}$ = -3.973, p = 0.011, n = 6) when considered against a Bonferroni-adjusted alpha level of 0.025. Full individual breakdowns are listed in Appendix Two.

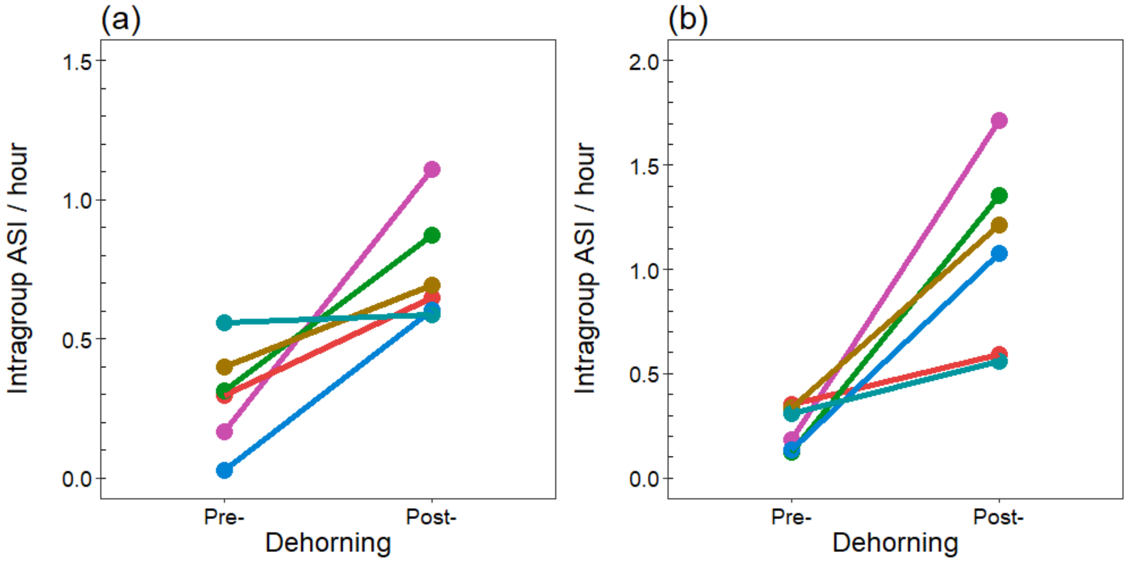

Figure 3.3. The rate of intra-group agonistic social interactions monitored in six rhinos before and after their dehorning within (a) any group composition and (b) under a stable six-member group composition (n = 6). SAM1 (red), SAF1 (blue), SAM2 (gold), SAM3 (pink), SAM4 (green), SAM5 ‘(turquoise).

3.3.2 Social dominance hierarchy

Analysis 4. Changes in social dominance hierarchy within a stable social grouping following a re-dehorning procedure

Rhinos within the stable six-member social grouping participated in a total of 30 intra-group dyadic encounters prior to dehorning (during a combined effective observation length of 257.55 hours) and 80 dyadic encounters after dehorning (observation length of 246.35 hours) for which a clear winner and loser could be identified (Table 3.8). The resultant win-loss ratios ranged in proportion from 0.10 to 1.00 of all agonistic interactions won prior to dehorning and 0.33 to 1.00 interactions won after dehorning (Table 3.8). Dominance rank relationships were highly asymmetrical within dyads and near-transitive within the group. Win-loss matrices for all agonistic dyadic encounters are listed in Appendix 2 (Table A3-A4). Following recursive scaling, BBS scores converged on the 78th iteration prior to dehorning and 46th iteration after dehorning (Appendix 2: Table A5-A6). Prior to dehorning, these scores placed SAF1 (Reine), the only female in the group, in the top ranked social position, with SAM1 (Dougie), the oldest of the males, in second place. Third place was held by SAM4 (Logan), fourth place by SAM2 (Chilli), fifth place by SAM5 (Willis) and sixth place by SAM3 (Courtney). Dominance positions changed in four out of the six individuals following the dehorning procedure (Figure 3.4). The bottom two ranked individuals, SAM5 and SAM3, climbed to the fourth and third ranked places respectively, with SAM4 and SAM2 dropping two places each accordingly. The two top ranked social positions did not change, nor did their win-loss ratios.

Table 3.8. Social breakdown of dyadic agonistic interactions between a stable group of six subadult rhinos prior to and after their dehorning. Encounters were observed between 05/04/2016 and 21/05/2016 pre-dehorning, and post-dehorning between 04/06/2016 and 12/10/2016. Table shows the Batchelder-Bershad-Simpson (BBS) index as outlined by Jameson et al. (1999) as a measure of social rank. a_i = animal identity; w_i = the number of encounters in which animal a_i was observed to have won; N_i = the number of encounters in which a_i was involved; L_i = the number of encounters in which a_i lost. Age given for the start of observations (05/04/2016).

Ai	**SAF1**	**SAM1**	**SAM4**	**SAM2**	**SAM5**	**SAM3**	**Total**
	Reine	Dougie	Logan	Chilli	Willis	Courtney	
Pre-dehorning							
W_i	6	11	5	4	3	1	30
L_i	0	4	4	3	10	9	30
N_i	6	15	9	7	13	10	60
BBS index	1.578	0.505	0.393	0.041	-0.706	-1.152	-
BBS rank	1	2	3	4	5	6	-
Win-loss ratio	1.00	0.73	0.56	0.57	0.23	0.10	-
Age (months)	74	89	47	61	38	47	-
Post-dehorning							
W_i	23	17	10	10	7	13	80
L_i	0	7	20	18	11	24	80
N_i	23	24	30	28	18	37	160
BBS index	1.639	0.576	-0.446	-0.589	-0.406	-0.388	-
BBS rank	1	2	5	6	4	3	-
Win-loss ratio	1.00	0.71	0.33	0.36	0.39	0.35	-
BBS rank change	0	0	-2	-2	1	3	-

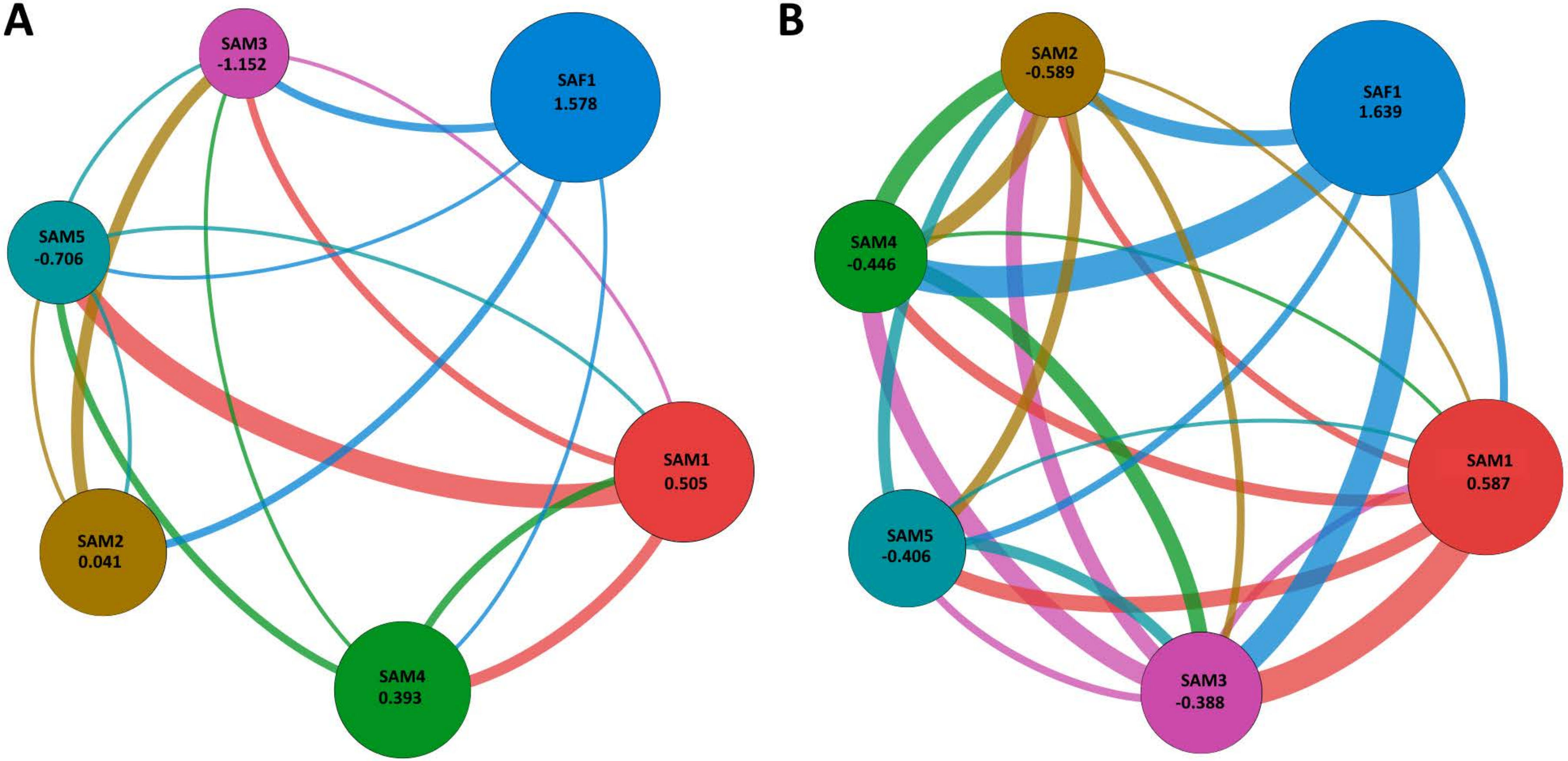

Figure 3.4. Social network of agonistic interactions between six subadult rhinos before (A) and after (B) a dehorning procedure. Node size indicates the difference in social rank by BBS scale scores (increasing in size anti-clockwise). Edges (lines) that match their node colour represent dyadic interactions that resulted in a win. Edge width indicates the number of interactions a rhino won, varying from one (thinnest) to eight (thickest). Ages: SAM5 (Willis) 38 months, SAM4 (Logan) and SAM3 (Courtney) 47 months, SAM2 (Chilli) 61 months, SAF1 (Reine) 74 months, SAM1 (Dougie) 89 months.

3.4 Discussion

The results provide evidence of limited dehorning-induced social changes in free-ranging white rhinos. The range and frequency of agonistic confrontations of dehorned rhinos were similar to those observed within horned rhinos, indicating that a reduction in horn size does not functionally constrain social behaviour or lead to increased rates of aggression. Although re-dehorned rhinos underwent a significant increase in their intra-group agonistic interaction rate, this may only represent a temporary response. Additionally, asymmetries in their aggressive behaviours provide some evidence that dehorning can be socially disruptive to dominance relationships.

3.4.1 Agonistic social interaction rate

Individuals from the two populations showed considerable overlap in their rates of antagonistic activity, suggesting dehorning had a minimal effect on social behaviour. The aggressive behaviours observed had functions in space-maintenance, courtship, resource competition and territorial dominance (Owen-Smith 1973; Rachlow et al., 1998; Metrione et al., 2007; Patton et al. (2018a). However, the difference in density between the two sites may have occluded the detection of a significant effect. The estimated density of white rhinos within the horned population was greater (approx. 167 hectares per rhino) than that within the dehorned population (308 hectares per rhino). Research on captive white rhinos suggests that increases in density can affect the rate of agonistic social behaviours (Metrione et al., 2007). A study by Metrione et al. (2007) found that females housed in small enclosures (0.033 km^2) with four calves exhibited more space maintenance vocalisations (mean 6.19 / hour) than females housed in larger enclosures (0.06 km^2) with one calf (0.182 / hour) or enclosures (mean 0.65 km^2) with no calves (mean 0.086 / hour). Although there is likely to be a threshold where the effect is seen, if similar density-dependent effects hold for free-ranging populations, then a higher agonistic rate induced by dehorning may have been missed when compared against the higher density of the comparative population. However, if density-dependent effects were present they would have led to an overestimation in aggression attributable to dehorning rather than an underestimation. Furthermore, if the observed rates of aggression in the horned population are representative of previous years, then it is not to the detriment of reproductive success, as Site B is classified as a 'key one' population by the AfRSG due to its large population size and historically positive growth rate (Emslie and Brooks, 1999; NWPB, 2015).

For the within-population analysis of rhinos before and after their second dehorning, the non-significant difference in the inter-group agonistic interaction rate further supports the case that the procedure had a minimal impact on white rhino social behaviour. This was largely driven by the near static change in the rate of inter-group aggressive encounters within subadult individuals. Conversely, the increase in aggressive behaviours observed for the dominant adult male [AM2 Luke] and adult female [AF2 Kelly] (Figure 3.2) indicates rhinos of different age-sex classes or social statuses may experience the effects of dehorning unevenly. Even so, the heightened levels of inter-group agonistic encounters observed for the adults still falls within the range of agonistic rates observed within the horned population. Additionally, the increased rate of aggression in the male does not appear to relate entirely to territorial challenges, with a greater number of interactions occurring with adult females and subadults but fewer number of interactions occurring with the other adult male after dehorning (Appendix 1: Table A2). The increase in inter-group aggressive behaviours of the adult female [AF2] may have in part been driven by reproductive changes. AF2 gave birth to a new calf on 15/03/2018. The average gestation period in white rhinos is 495 days (Linklater, 2007), meaning conception occurred approximately 54 days after behavioural observations had ended. White rhinos have both 30 day and 70 day oestrous cycles (Patton et al., 1999; Roth et al. 2018) so it is likely that AF2 was cycling during at least the second of the two observation periods. Given that Metrione et al. (2007) reported higher rates of aggressive space maintaining vocalisations in females once they had entered oestrus, this may explain the increase in aggression observed. In comparison, oestrus cannot be applied to the pattern of behaviour from the subadult female [SAF1] (an increase in intra but not inter-group aggression) as she was pregnant before the study commenced until almost six months after it ended, as verified by her later parturition.

In comparison, there was an increase in intra-group aggression in subadults, including after the effects of group composition had been controlled for. As horn size differences decreased, but body size differences remained unchanged, the hypothesis that dehorning contributed to social change is supported. Individuals with similar physical attributes may show higher levels of aggression than individuals that are less closely matched, as the results of such interactions cannot easily be predetermined (Setchell and Wickings, 2006). Additionally, agonistic interactions may increase in frequency following the formation of a new group as a result of unstable dominance relationships (Clutton-Brock and Harvey, 1976). Such a response was observed by Rees (2019) in a grouping of translocated subadult white rhinos, where agonistic interactions decreased after 35 days of capture as dominance rankings stabilised. However, as higher levels of agonistic interactions were observed within the stable social grouping of subadult rhinos after dehorning rather than prior to it, the results further support the direction of change was dehorning induced. For the dehorned rhinos in the between-populations analysis, between 138 and 692 days had elapsed since their first dehorning procedure. In the within-population analysis, dehorned rhinos with 458-548 days of horn growth (prior to their second dehorning procedure) were compared against rhinos with 1-135 days of horn growth (after the second dehorning procedure). Thus, the lack of a significant effect in the between-populations analysis, but presence of one in the within-population analysis may indicate that increased levels of aggression occur only in the days immediately after a dehorning procedure or when horns are most similar in size. Thus, the social effects of dehorning may only be temporary if the observed social changes relate to the invasive procedure itself, or if the adaptive benefits of increased aggression reduce as horns regenerate. This would be expected as time progresses and horn mass approaches its pre-dehorned state. However, it cannot be ruled out that the two populations differed in their rates of aggression due to demography and density.

Despite the observed increases in aggression, the six-member grouping of subadults did not break-up during the months after the dehorning procedure. Social groupings of subadults are not obligatory and in other populations can show a comparatively high turn-over rate (Owen-Smith, 1973), for example the majority of subadult associations in Hluhluwe-iMfolozi National Park were observed to last less than a month (93%, n = 180 associations; Shader and Owen-Smith, 2002). Thus, in this case it seems that the benefits of group membership likely outweighed any costs associated with an increased rate of aggression. Either the costs of dispersal were high (Shader and Owen-Smith, 2002) or the effects of any agonistic-related changes, such as increased energetic expenditure (Riechert, 1988), a higher risk of injury (Patton et al., 2018a) or elevated levels of physiological stress (Schmidt and Sachser, 1997), were minor or absent (see Chapter 4).

Research by Metrione et al. (2007) shows that the majority of agonistic interactions in captive rhinos relate to competition for food and shade. While Meister (1998) and Cinková et al. (2016) reported higher levels of space maintaining aggression when food resources are clumped rather than dispersed. For non-supplementary fed free-ranging populations grass cannot be monopolised, so the observed increase in the rate of agonistic interactions are unlikely to be induced by seasonal fluctuations in food resources. However, given that behavioural observations took place throughout the dry season, rhinos will have had access to a decreasing number of wallows and drinking pools that also became smaller in size. The presence of these usurpable resources could create competition which along with an expected reduction in individual proximities could have partly explained the observed rise in aggression. Similar behaviour is observed in African bush elephants (*Loxodonta africana*), which rely on abundant and widely distributed food resources, but must still compete for access to other rarer but important resources such as water, minerals, rubbing posts and some foods (Archie et al., 2006).

Following sexual maturity, male rhinos become solitary, while females cease membership of existing social groups (Shrader and Owen-Smith, 2002). Therefore, the changes in aggression and dominance, which were primarily observed among subadult groupings are unlikely to continue into adulthood. Thus, the observed effects are unlikely to impact on future mating opportunities or have negative short- or long-term implications for reproductive success, such as a loss in genetic diversity or an increase in inbreeding (Guerier, et al., 2012). Such effects could occur in a closed population if dehorning caused the reproductive dominance of bulls to become skewed towards a fewer number of individuals (Purisotayo et al., 2019). Future studies on the impacts of dehorning would benefit from considering genetic parentage as well as behaviour, as although territory-holding is usually considered necessary for male reproductive success

(Rachlow et al., 1998; White et al., 2007), behaviourally-subdominant bulls have been documented to sire calves (Guerier, et al., 2012).

Even so, dehorning could still influence reproductive success if it affected the ability of territorial bulls to maintain territories and win fights (Rachlow et al., 1998; 1999). The number of fights observed in the study were too low to compare statistically (n = 3). Although this matches the low rates of fights observed in other free-ranging horned populations (Owen-Smith, 1973), the lack of data means it was not possible to evaluate whether the fighting rate was affected by the procedureor confirm observations made by Patton et al. (2018a) who reported a reduction in serious fights following dehorning. However, fighting mortality in a population of black rhinos was found to be similar before and after a dehorning procedure (du Toit and Anderson, 2013), indicating injuries rates may not necessarily decrease. The fighting behaviours observed in this study were similar in both horned and dehorned populations, indicating there was no reduction in horn-reliant behaviours or apparent functional constraints. While it was not possible to disentangle the effects of horn size, body size and social experience in the fights observed, it is likely that all of these attributes contribute towards territorial dominance (Rachlow et al., 1998). Further data on territoriality in dehorned white rhino populations with horn and body size disparities would be able to confirm that this is the case.

3.4.2 Social dominance hierarchy

This study is the first to report strong evidence for the presence of a non-territorial dominance hierarchy in free-ranging white rhinos. The observed hierarchy arose through differential success in the outcomes of dyadic encounters among the individuals (Hinde, 1978). Previous studies of free-ranging individuals have lacked the intensive observations of dyadic agonistic interaction rates that have taken place in captivity (Mikulica, 1991; Meister, 1998; Swaisgood et al., 2006; Cinková and Bičík 2013; Rees, 2019). Thus, asymmetries in aggressive behaviours between companions may have been missed in other studies due to their rare frequency rather than being unique to the study population (Owen-Smith, 1973; Swaisgood et al. 2006; Metrione et al., 2007; Cinková et al. 2016).

The majority of confrontations appeared to have had space maintaining functions and comparatively few instances of physical contact. Unlike with foraging resources (grass), preferential within-group access to wallows, shade, rubbing posts and salt licks may still be obtained in free-ranging populations. The formation of stable social hierarchies can reduce intra-group aggression, particularly if submissive signals towards aggressive displays prevent more serious episodes of combat (Maynard Smith and Parker, 1976; de Vries et al., 2003). Well-defined dominance relationships are common in species for which the potential for injury during altercations are high even when competition for resources is low (Crowley, 2001). However, the benefits and costs of such a hierarchy will not necessarily be uniform for individuals across different social rankings (de Vries et al., 2003). Although dominant individuals may benefit from preferential access to resources, high ranking animals within unstable hierarchies may engage in more frequent aggressive challenges to reinforce their social status (Fairbanks, 1994), this in turn can lead to higher levels of social stress (Creel et al., 1996). For resources that are not finite the payoff asymmetries will be relatively small (Hammerstein, 1981). Thus, there are likely to be few benefits for escalating conflicts beyond the minor aggressive behaviours observed (Crowley, 2001). Given that the benefits of occupying a higher social ranking (such as preferential access to resources) are likely to exceed the costs of aggression (time and energy), and may even minimise the potential for injury, the presence of a social hierarchy is likely to be adaptive.

The upper positions of the hierarchy were occupied by the two oldest individuals prior to and after dehorning. These individuals consistently dominated smaller, younger members of the group. This corroborates research by Rachlow et al. (1998) who found that prior attributes in white rhinos, namely age and its correlate body size, can explain the outcomes of dyadic encounters during territorial confrontations. Metrione et al. (2007) found a similar relationship for rhinos in captivity, whereby in female pairings, the eldest rhino was usually dominant over its companion. The need to resolve dominance relationships among physically similar individuals may also explain the observed increase in the number of agonistic interactions following dehorning (Clutton-Brock and Harvey, 1976).

Prior to dehorning, when horn size differences were greatest, the rankings did not correlate precisely with either horn size or age, indicating that physical attributes are not the sole causal factor behind social position. Traits such as sex and behavioural history may provide alternative explanations for the hierarchy (Chase et al., 2002; Cinková and Bičík 2013). The top-ranked social position was held by the only female within the group [SAF1] and results of several other studies indicate that captive male white rhinos occupy lower social rank than females, receiving and retreating from the most challenges within a group (Mikulica, 1991; Meister, 1998; Cinková and Bičík 2013). However, Metrione et al. 2011 reported that females placed in enclosures with the same male since birth or early adolescence were subordinate to it. Research by Rees (2019) found that six white rhinos kept together in a boma developed a strong linear hierarchy. The two oldest females won more agonistic interactions than two similarly aged males (5.5 to 6 years old) and these four older individuals were in turn ranked higher than the two youngest individuals (a 5 year old female and four year old male). Furthermore, the oldest and highest ranked female had suffered a horn breakage during translocation, suggesting that body size or past experience were more important factors than larger horn size. Such a hierarchy based on body size could be beneficial if it indicates the likelihood of achieving a win or a loss, and thus whether to pursue a strategy of appeasement or escalation (Archie et al., 2006). Research on captive northern white rhinos showed a change in dominance following an enforced change in group membership (Cinková and Bičík, 2013). In this case, the number of altercations increased following the removal of the oldest female from a group of six individuals and two initially low-ranked females increased their social position. Thus, dynamic winner-loser feedback effects may provide a parallel regulatory mechanism for establishing social positon in white rhinos alongside physical attributes. These are present in many social species, where winning (or losing) a previous agonistic encounter can influence the probability of winning (or losing) the next one (Chase et al., 1994; Chase et al. 2002).

Despite this, the shift in social positions among the bottom four ranked individual indicates that a sudden change in horn size can be socially disruptive for within-group dominance hierarchies. The reduction in dominance distances as shown by the reduced range of the rhinos’ BBS scores indicate that the new relationships were almost intransitive and lacked a strong linear structure, thus the resource holding potential of each individual were somewhat equalised. Most notably, the third oldest individual [SAM2] became the lowest ranked individual despite being the third largest in body size. It thus seems that a levelling in horn size can cause a concomitant levelling in agonistic win-loss ratios.

3.4.3 Conclusion

An increase in aggression and disruption in dominance was observed in rhinos following a re-dehorning procedure; however, as this increase was predominantly among subadult individuals and their levels of aggression were similar to rates observed from a horned population, dehorning is likely to have a negligible effect on long-term white rhino social dynamics and fitness. Dehorned rhinos demonstrated similar agonistic and cohesive behaviours to those observed in horned rhinos and other free-ranging populations described in the literature (Appendix 1; Owen-Smith, 1973; Rachlow et al., 1998; Cinková et al., 2016). Aggressive behaviours had functions in space-maintenance, resource competition and territorial dominance but most interactions resulted in avoidance or appeasement and thus carried little risk of physical injury.

Current guidelines for dehorning as an anti-poaching tactic based on threat level and cost recommend the procedure be conducted every 12 to 36 months (Lindsey and Taylor, 2011). When considering impacts on social behaviour, the results of this study indicate that recommendations on the frequency and need of dehorning procedures are unlikely to be in need of major revisions. However, asymmetries in horn size should be avoided where possible as these may confer some social disadvantages if the effects extend to dominant bulls. Even so, the effects may be only temporary and given the potential for a reduction in more serious fights, a serious cause of injury (Patton et al., 2018a), they are unlikely to pose risks to reproductive success. Still, more research is required to understand whether dehorning has other impacts on white rhino fitness, such as through changes in territoriality or breeding success.

Chapter 4 – Physiological effects of dehorning

4.1 Introduction

Exposure towards anthropogenic stressors can lead to a maladaptive endocrine stress response in vertebrates (Dickens et al., 2010; Dickens and Romero, 2013). Consequently, physiological stress can correlate with detrimental animal welfare conditions and indicate an adverse response to anthropogenic disturbance (Romero, 2004). The hormonal mediators of the stress response can provide a valid measure of overall animal health (Dickens et al., 2010; Dickens and Romero, 2013; McCormick and Romero, 2017). It is essential to evaluate whether dehorning acts as a long-term stressor in rhinos and consequent reductions in fitness (reproductive success) (Lindsey and Taylor, 2011; Taylor et. al., 2014; Ververs, 2018). A prior study reported that intensively reared female white rhinos experienced a spike in physiological stress in the first four days after dehorning (Badenhorst et al., 2016). However, it is unknown whether the physiological effects extend beyond this period, or transfer to free-ranging or wild populations (Ververs, 2018). Information on the longer-term physiological effects of dehorning will aid the design of effective rhino conservation policies and aid management decisions (Badenhorst et al., 2016; Ververs, 2018). For example, if regular dehorning results in chronic stress, then decisions on the frequency of the procedure can act to minimise future health effects. Alternatively, if no negative long-term response occurs, then wildlife managers may wish to increase dehorning rates to reduce the risks of poaching.

4.1.1 Function and mechanism of stress

A stressor is a noxious or unpredictable stimulus that leads to a range of adaptive physiological or behavioural changes, known as a ‘stress response’ (Romero, 2004; Hill et al., 2018). In vertebrates, early physiological effects of the stress response include heightened cardiovascular tone (e.g. via an increase in heart or breathing rate), increased energy mobilisation (e.g. the transfer of energy from stores to exercise muscle), and a decline in proceptive and receptive sexual behaviours (e.g. erection loss) (Sapolsky et al., 2000; Dickens et al., 2010). In the short-term, an acute stress response acts to increase an animal’s chances of surviving hostile or challenging conditions (Hill et al., 2018). However in the long-term, if the perturbation is chronic or a consecutive series of acute stressors, then the stress response can become maladaptive and deleterious to an animal's health or reproductive fitness (Dickens et al., 2010; McCormick and Romero, 2017; Hill et al., 2018). In the long-term, pathologies may arise through processes such as cardiovascular change (e.g. hypertension or myocardial infarction; Rupp, 1999), immunosuppression (e.g. reduced cell-mediated immune responses; Dhabhar and McEwen, 1997) and reproductive suppression (e.g. reduced conception and increased abortion rates; Young et al., 2006).

The hypothalamic–pituitary–adrenal axis is the fundamental neuroendocrine system involved in the mammalian stress response (Hill et al., 2018). The perception of a stressor triggers a hormonal cascade, whereby the hypothalamus releases adrenocorticotropic hormone (ACTH) which stimulates the adrenal cortex to synthesise glucocorticoids (cortisone, cortisol and corticosterone; Sapolsky et al., 2000). The pituitary gland secretes these glucocorticoids into the bloodstream, which along with catecholamines (epinephrine and norepinephrine) trigger an adaptive physiological response (McCormick & Romero 2017). If the stress response is acute, glucocorticoids inhibit their own production and return to normal levels (Sapolsky et al., 2000). However, chronic stressors can disrupt the negative feedback of glucocorticoids, resulting in their prolonged elevation (Romero, 2004). Fluctuations in glucocorticoids have been used to monitor rhino responses to environmental or human-induced changes, including the effects of seasons and translocations (white rhino [Schmidt and Sachser, 1997, Meister, 1998; Metrione and Harder, 2011; Badenhorst et al., 2016; Yang et al., 2019]; white and black rhino [Brown et al., 2001; Turner et al., 2002]; black rhino [Freeman et al., 2014; Göttert et al., 2015]; Indian rhino [Capiro et al., 2014]. Thus, glucocorticoids function as a mediator of physiological stress make them an ideal marker for how white rhinos respond to a stressor (Linklater et al., 2010; Palme, 2018).

Glucocorticoid levels may eventually return to pre-stressed levels in chronically stressed white rhinos (Linklater et al., 2010). Thus, during longer-term monitoring it can be useful to observe additional indicators of health, such as markers of fertility. In rhinos, potential markers include androgens and progestogens (Linklater et al., 2010), which have functions in male and female rhino reproduction respectively (Kretzschmar et al., 2004; Roth et al., 2018). Progestogens have functions in the oestrous cycle, gestation, and embryogenesis (Brown, 2018). While androgens have functions in spermatogenesis (Preston et al., 2012) and are correlated with reproductive success in black (Edwards et al., 2015) and white rhinos (Kretzschmar et al., 2004). In vertebrates, progestogens, such as progesterone, are primarily secreted by the corpus luteum (Hill et al. 2018) while androgens, such as testosterone, are primarily secreted by the Leydig cells of the testes (Moyes and Schulte, 2016). Fluctuations in the levels of gonadal steroids are reliable indicators of reproductive health in rhino species (Schwarzenberger et al., 1998; Kretzschmar et al., 2004; MacDonald et al., 2008; Hermes et al., 2012; Van der Goot 2013; Freeman et al., 2014; Edwards et al. 2014, 2015; Roth et al., 2018). This makes gonadal steroirds suitable for monitoring long-term responses to stressors alongside changes in glucocorticoids (Linklater et al., 2010).

4.1.2 Factors influencing stress

Immobilisations and translocations are both documented sources of anthropogenic stress in rhinos (Kock et al., 1990; Linklater et al., 2010; Capiro et al., 2014; Göttert et al., 2015; Yang et al., 2019). The potential effects of these management interventions include reductions in fertility (Linklater et al., 2010) and changes to the birth sex ratio (Linklater, 2007). Only a single study has assessed whether white rhinos show a physiological stress response to dehorning (Badenhorst et al., 2016). The study monitored the faecal corticosterone metabolites (FCM) levels of 15 adult (>72 months) and seven juvenile (<72 months) females from two days before their dehorning until four days after (Badenhorst et al., 2016). All of the adults had been dehorned prior to the study, as had two of the seven juveniles. Compared to the pre-dehorning baseline, median FCM concentrations were highest on the second day after dehorning, with a 32% (range: -7% to 293%) increase in adults and 33% (range: 14% to 174%) increase in juveniles. On the fourth day, FCM levels for adults and juveniles were 16% (range: -24% to 42%) and 11% (range: -34% to 25%) higher than the baseline respectively. It is likely that the observed stress response was at least partially due to chemical immobilisation, which is a documented source of physiological stress for many species of wild animal, including in black rhinos (Kock et al., 1990, Dickens et al., 2010). Although immobilisation is inseparable from the horn trimming procedure, horn removal itself does not punctuate afferent nerve endings thus the increase in stress is unlikely to be triggered by pain (Badenhorst et al., 2016). Monitoring only took place until four days after the dehorning procedure, so the welfare implications beyond this date are unknown.

Additionally, the rhinos lived in limited free-ranging conditions, were supplementary-fed, and had an average stocking density of 9 hectares per rhino (Badenhorst et al., 2016). Reports from 2018, indicate the 8,000 hectare ranch held around 1,626 rhinos, had a gender skew towards females and maintained a proactively managed studbook (Save The Rhino, 2018). It is also defined as a Captive Breeding Operation (CBO) under South African Threatened Or Protected Species (TOPS) and CITES regulations (Save The Rhino, 2018). Thus, the conditions reported by Badenhorst et al., (2016) were not representative of those of other freer-ranging populations, occurring at lower-densities, or kept under different management conditions.

When evaluating the success of a veterinary or management procedure, it is important to monitor whether an animal experiences any delayed or latent physiological effects beyond immediately apparent injuries and mortalities (Brivio et al., 2015). For example, a study of free-ranging pampas deer (*Ozotoceros bezoarticus*) found individuals subject to capture and radio collaring were significantly more fearful, less sociable and defensive post-capture than controls, and additionally experienced a spike in FCMs (Munerato et al. 2015). Dehorning has the potential to cause stress beyond the initial immobilisation and trimming procedure if the resultant smaller horns disrupt behaviours such as reproduction, resource access, or sociality (Schmidt and Sachser, 1997; Meister, 1998; Lindsey and Taylor, 2011; see Chapter 2 for a description of the procedure). Chronic stress can occur via exposure to a single persistent stressor, or to several sequential, or multiple simultaneous acute stress responses (Dickens et al. 2010). Psychosocial factors are one potential mechanism that can influence concentrations of FCMs. A review of the endocrine corticoid profiles reported from different animal species, including studies of both wild caught and freely living individuals, found those individuals exposed to chronic social stress (defined by *a priori* knowledge of how species' FCM levels related to social position or social instability) generally underwent increases in baseline FCMs for prolonged periods (Dickens and Romero, 2013). A study of captive female white rhinos found stress levels were associated with social position, whereby females that retreated from the most agonistic interactions had higher levels of FCMs (Meister, 1998).

Immobilisations of wildlife can also decrease their subsequent reproductive success (e.g. through abortion in mountain goats (*Oreamnos americanus*) (Côté et al., 1998) or post-natal calf loss in moose (*Alces alces*) (Solberg et al., 2003). Immobilisation-induced declines in rhino productivity are thus of concern when dehorning (Lindsey and Taylor, 2011). In a study of inter-calving intervals (ICIs), free-ranging black rhinos that underwent the most frequent immobilisations (for either radio collaring, dehorning, ear notching, or translocation) had the longest inter-calving intervals. However, Atkinson et al. (2002) contested this conclusion due to flaws in the study’s statistical analysis and design. This included pseudo-replication and that a rhino’s immobilisation history may have in part been a function of its health and age. Furthermore, a more recent study of black rhinos found no link between immobilisations and ICIs (Du Toit and Anderson, 2013). Whereby the ICIs of a dehorned population in Zimbabwe were no longer than the southern African regional average (Du Toit and Anderson, 2013).

Translocations are also a source of physiological stress in rhinos. Rhinos may be subject to stress during the initial capture process (Kock et al. 1990), transport (Yang et al., 2019) and during confinement, which can restrict their movement (Linklater et al., 2010), reduce their retreat space (Carlstead and Brown, 2005), and create abnormal social configurations (Metrione and Harder, 2011). Stress may also continue beyond the confinement period, with captive Indian rhinos experiencing elevated FCMs for up to 9 weeks after their movement (Capiro et al., 2014). Linklater et al. (2010) measured stress levels in wild white and black rhinos following their capture and placement in enclosures (bomas) over an 11-week period. The study analysed fluctuations in FCMs, faecal progestogen metabolites (FPMs) and faecal androgen metabolites (FAMs). White rhinos of both sexes experienced a sustained rise in FCM levels of two to five times that of pre-capture levels for up to the first 17 days of their captivity. In male white rhinos, FCM profiles then declined, ultimately dropping below pre-capture levels. In comparison, their FAM levels continued to decrease throughout the remaining 8 weeks of captivity. Thus, in male white rhinos a return of FCMs does not necessarily indicate the absence of chronic stress (Linklater et al., 2010). The FCMs of female white rhinos remained high throughout captivity, while levels of FPMs decreased. In captive black rhinos, translocation-induced physiological stress peaked within 3 to 4 days, indicating that for some individuals the procedure can be minimally invasive (Göttert et al., 2015). Furthermore, a review by Dickens (et al., 2010) found that strategies that minimised chronic stress during the translocation of wild animals had the greatest chances of increasing future reproductive success.

Stress from translocation can also affect the birth sex ratios of white, black and Indian rhinos (Linklater, 2007). An analysis of 104 rhino births reported male bias (86% male births) if translocation occurred during early gestation (0 to 0.19 gestation equivalent period), but female bias (38% male births) if it occurred during mid-gestation (0.2 to 0.79 gestation equivalent period), and further male bias (67% male births) if conception occurred after a rhino arrived in captivity. The effect that sex-differential glucose metabolism has on embryo mortality rates is likely responsible for this skew, whereby female embryos are most vulnerable pre-implantation and male embryos most vulnerable after implantation has occurred (Linklater, 2007). In comparison, dehorning appears to have no effect on sex ratios (Ververs, 2018) indicating it may not be a significant cause of stress. An analysis of an intensively-reared game-ranched population of white rhinos that underwent frequent dehorning procedures found no significant bias in the birth sex ratio of calves (52.74% male births, n = 562, Ververs, 2018), similar to the birth sex ratio seen of wild white rhinos (approximately 50% male births, Owen-Smith, 1973).

4.1.3 Validating rhino endocrine responses

Although endocrine pathways are highly conserved across animal groups, hormonal analyses must be validated to be considered biologically and physiologically meaningful (Wheeler et al., 2013; Roth et al., 2018; Palme, 2018; Taff et al., 2018). It is possible to detect the analytes of hormones from a range of bodily fluids and excretions including blood and plasma, saliva and exhalations, urine and faeces, in addition to feathers, hair and scales (McCormic et al., 2017). However, the suitability of a biological matrix for endocrine analysis will depend on species-specific mechanisms of steroid metabolism and excretion, as well as the practicality of sample collection (McCormic et al., 2017;

Palme, 2018). Faeces have been validated as a suitable medium to monitor adrenal and gonadal changes in white rhino steroid metabolites by several studies (e.g. Hindle and Hodges, 1990; Brown et al., 2001; Kretzschmar et al., 2004; Riato, 2007). Furthermore, faecal samples can be collected non-invasively without disturbing the animal (Kretzschmar et al., 2004). Blood samples have also been used (Brown et al., 2001) but require capture, and provide a near-instantaneous measure of stress (Whitten et al., 1998). This makes faeces more appropriate for studies of large free ranging mammals than blood sampling if pre-capture responses are desired (Fusani, 2017).

To determine whether there is a causal relationship between the physiological response of a rhino and a potential stressor, steroidogenic activity must be linked to the timings of external events (Brown et al., 2001). Riato (2007) calculated the metabolic lag between adrenal activity and the excretion of glucocorticoids in captive white rhinoceros by injecting them with ACTH and monitoring the resultant changes in FCM concentrations. Faeces collected within 2 days of the ACTH challenge showed a several-fold increase in corticoid levels compared to baseline levels, with peak clearance occurring 24 to 48 hours after stimulation and returning to baseline levels four days after. This lag is at least partially dependent on the duration it takes digestive matter to pass through the intestinal tract and is reflective of the mean retention times of consumed particles (43 ± 5 hours) and fluids (28 ± 4 hours) in white rhinos (Steuer et al., 2010). Thus, FCM concentrations represent a pooled value of steroidogenic activity rather than an instantaneous reading (Whitten et al., 1998). This misses the more acute events found in measures of circulating hormones but diminishes circadian, ultradian, and pulsatile flux (Metrione and Harder; 2011; Wheeler et al., 2013; Palme, 2018).

Kretzschmar et al. (2004) determined the lag between white rhino testosterone secretion and the appearance of its metabolites in faeces. Synthetic gonadotropin-releasing hormone was injected int a free-ranging adult male to stimulate testosterone production. Faecal samples collected over 5 days showed peak clearance of FAMs 24 hours after the stimulation of testicular activity and remained elevated for the monitored period. Additionally, Edwards et al. (2014) validated the field extraction and quantification of native testosterone in black rhino faeces. Hindle and Hodges (1990) determined the lag between circulating progesterone and its appearance in faeces. An adult female white rhino was injected with radioactively-labelled progesterone and monitored for a four-day period. The labelled compound was recovered in both urine and faeces, with the highest levels occurring on the second day after the injection.

4.1.4 Aims

This study aimed to determine whether dehorning causes any physiological effects in free-ranging white rhinos. Changs in hormon levels were monitored over time to elucidate whether the horn removal process was the primary stressor, or whether the potential impacts of dehorning extended beyond this. Information on the physiological effects of dehorning will aid management decisions on the frequency of dehorning operations. Current recommendations are based on poaching threat (in part predicted by horn regrowth rate) and economic cost rather than animal welfare (Milner-Gulland et al., 1992; Lindsey and Taylor, 2011; Du toit and Anderson, 2013). The primary research questions were (1) whether dehorning caused a detectable stress-induced change in adrenal and gonadal steroids; (2) whether the number of times a rhino was dehorned influenced the rate of this change; and (3) at what timescales these changes occurred.

Objective 1 was to determine whether age-sex class influenced an animal’s FCM or FAM baseline. This would resolve whether subjects from different classes could be pooled for further analysis. Age was predicted to influence FCM concentrations in rhinos, in line with the findings of other studies (Brown et al., 2001; Badenhorst et al., 2016). Age was also predicted to influence FAM concentrations of male rhinos, as it can increase with sexual maturity (Kretzschmar et al., 2004; Verver, 2018). All female rhinos were in the same age grouping (adult) so no comparisons of FPMs were made.

Objective 2 was to determine whether the stress levels of a population of once-dehorned rhinos differed to those of nearby horned populations. Only FCM concentrations were monitored, as singular measures of gonadal hormones do not provide biologically meaningful data on stress (Linklater et al., 2010). Once-dehorned rhinos from the focal population were predicted to exhibit higher FCM concentrations than neighbouring populations of horned rhinos.

Objective 3 was to investigate whether dehorning induces a detectable change in stress within the first week of the procedure (i.e. whether there was an acute physiological response) and understand whether any subsequent longer-term physiological trends began from a relatively low or high baseline. FCM concentrations were predicted to be higher in the week after dehorning than the week before.

Objective 4 was to determine whether dehorning acts as a stressor beyond the initial horn removal procedure (i.e. whether there was a chronic physiological response). The FCM profiles of dehorned rhinos were predicted to differ to rhinos that did not undergo the procedure. If dehorning did not act as a stressor then no changes in FCM levels were expected.

Objective 5 was to determine whether the number of times a rhino is dehorned could predict changes in physiological stress. The number of dehorning procedures a rhino was subject to was predicted to supress FCM profiles if the procedure acts as a cumulative stressor.

Objectives 6-7 were to determine whether the number of times rhinos are dehorned can predict changes in gonadal activity. Dehorning was predicted to supress FPM and FAM concentrations in female (Objective 6) and male (Objective 7) rhinos respectively if the procedures act as a cumulative stressor.

4.2 Methods

4.2.1 Experimental design

Faecal samples were collected from 25 rhinos from 24/05/2016 until 22/10/2017. Samples were collected from rhinos located geographically close to one another and resident to one of three different reserves (Site A, Site B, Site C. For details on Site A and Site B see Chapter 2, Site C is a fenced reserve (450 hectares) contiguous with Site B that holds a population 5 free-ranging rhinos. The rhinos sampled at Site A were dehorned; the rhinos sampled from Site B and Site C were not dehorned (referred to as horned rhinos here on) and were included in the study for comparison. The majority of sample collection was focused on Site A (165 samples from 16 rhinos), with several samples also collected from Site B (7 samples from 7 rhinos) and Site C (2 samples from 2 rhinos). All three reserves were within 30 km of each other. The population at Site A had been subject to between 0 and 1 dehorning procedures each at the start of the monitored period, and between 0 and 3 times each by the end of the monitored period. See Chapter 2 for a full listing of dehorning dates, subject parameters and a methodology of the dehorning procedures. In addition to this, a single sample from a recently deceased female that had succumbed to poaching related injuries was collected from Site B on 17/02/2016. This was to biologically validate the FCM assay via the detection of a known stressor. Samples were only collected if they could be matched to a specific individual (see Chapter 2). Rhinos were observed until they defecated. To prevent behavioural disturbance, faeces were not collected until rhinos had moved away from the site of defecation, with a maximum of one hour waited. Faeces were collected from the centre of a dung bolus or scraping to avoid contaminating the sample with soil microbes. Thirteen samples were also collected rectally during chemical immobilisation. Faecal samples were not collected if the peak excretory period (24 to 48 hours after hormone release) (Riato, 2007) overlapped with experimental disturbance (see Chapter 6).

Faecal metabolite concentrations must reflect *in vivo* conditions if analyses are to be representative of physiological change. To minimise the effects of microbial breakdown and environmental degradation on metabolite concentrations, faeces should be collected soon after defecation (Shutt et al., 2012). In white rhinos, faecal metabolites can remain robust for at least 12 hours after defecation, varying less than 5% over this period (Turner et al., 2002). To determine the length for which faecal metabolites remained stable and identify an appropriate timeframe for sample collection, the relationship between defecation time and hormone concentration was established. FCM values were sampled from a single pile of unshaded dung 3, 6, 14, 25, and 48-hours after defecation and compared against the concentrations detected in fresh dung (< 1 hour old). Samples were sealed in airtight screwcap bijou bottles within one hour of defecation and frozen within four hours of collection. The only exception to this was for the deceased rhino, for which dung was collected between 36 and 48 hours after defecation.

4.2.2 Metabolite extraction and detection

To halt storage effects, faecal metabolites can be frozen between collection and analysis (Ziegler and Wittwer, 2005). Alternatively, if the infrastructure to store and transport them frozen is not available, metabolites can be extracted and stored in buffer (Ziegler and Wittwer, 2005). To extract the metabolites, faeces are dissolved in an aqueous solution of water and ethanol then filtered or centrifuged (Santymire and Armstrong, 2010). Faecal metabolites were extracted with a modified protocol based on that of Wheeler et al. (2013). Faecal samples were defrosted within 3 months of freezing and extracted on site. An aliquot (0.150 ± 0.005 g) of each sample was weighed on a balance (VWR LPC-213; accurate to 0.001 g) and then added to a solution of 1-part ethanol (750 µl, 90%) to 1-part distilled water (750 µl). Next, the mixture was agitated in a vortex shaker for 5 minutes (Fisherbrand Mini Vortex Mixer; 2.8K rpm) to create a faecal slurry. The slurry was centrifuged for 15 minutes (SciSpin Mini Microfuge; 7,000 rpm) and then 500 µl of the resulting supernatant was pipetted into a screw-cap tube (Eppendorf; 2 ml). To prevent evaporation of the sample extract, the tubes were sealed with Parafilm and stored at 4°C. Extracted metabolites are stable for up to a year when stored as a liquid (Kalbitzer and Heistermann, 2013). To establish the dry weight of each sample, a second aliquot of defrosted faecal matter was air-dried and weighed until it was found to have stabilised.

Endocrine analyses of faecal metabolites predominantly utilise enzyme-linked immunosorbent assays (ELISAs). These have largely supplanted radioimmunoassays due to their safety and ease of use (Melmed et al., 2016) and remain cheaper than chromatographic methods (e.g. liquid chromatography) with mass spectrometry (Palme, 2018). Endocrine analyses were conducted in the UK at the University of Brighton by the primary researcher (SGP) with permission to import rhino faecal extract from South Africa granted from DEFRA (Authorisation no: ITIMP16/1052). To determine the concentration of FCMs (both sexes), and either FPMs (females) or FAMs (males), samples were analysed with competitive immunosorbent assays. In these, the steroids from the sample compete against labelled steroids for binding sites on the antibodies (Palme, 2018). Commercial enzyme immunoassay kits for corticosterone (4-pregnene-11ß,21-diol-3,20-dione), progesterone (Pregn-4-ene-3,20-dione) and testosterone (Androst-4-en-17β-ol-3-one) were purchased from Enzo Life Sciences (lot numbers: ADI-900-097, ADI-901-097, ADI-900-011, ADI-900-065). Corticosterone, testosterone and progesterone are heavily metabolised before excretion, thus the assays rely on the cross-reactivity of their metabolites (Palme, 2018). This makes it more accurate to refer to the measured concentrations from each assay as faecal corticosterone metabolites (FCMs), faecal androgen metabolites (FAMs) and faecal progestogen metabolites (FPMs) rather than as native hormones. In total, 172 of the samples were analysed for FCMs, 50 for FPMs, and 114 for FAMs.

Samples were vortexed before analysis and then diluted into sample assay buffer to eliminate matrix interference. To determine optimal detection rates, serial dilutions of samples were made for each hormone. Dilutions were selected that fell within the most linear part of the standard curve and did not generate absorbance values beyond the highest standards (corticosterone: 20,000 pg/mL; testosterone: 2000 pg/mL; progesterone: 500 pg/mL). Samples were run in duplicate in 96-well microtiter plates in parallel with a series of known concentrations of target analyte. Following the manufacturer's instructions (Enzo Life Sciences; Table 4.1), the samples were added to each well and target hormones were then immobilised by the addition of an antibody (sheep polyclonal antibody to corticosterone, or mouse monoclonal antibodies to testosterone and progesterone). A conjugate (alkaline phosphatase conjugated to either corticosterone, testosterone or progesterone) and a substrate (p-nitrophenyl phosphate in buffer) was then added to each well. Following a further incubation period, the enzyme reaction was halted by the addition of a stop solution (trisodium phosphate). The resultant colour intensities were read on a spectrophotometer (EZ Read 2000 Microplate reader) at a 405 nm wavelength.

A sample's optical density is inversely proportional to its concentration of hormone (Gaw et al., 2013). This relationship was plotted with a four-parameter logistic curve detailed in Equation 4.1. The optical densities of unknown concentrations could then be interpolated from the curve (see Figure 4.1). Once determined, initial concentrations were adjusted by the water content of their initial weight, to equal mass hormone per mass faecal dry weight (*sensu* Ezenwa et al., 2012).

Table 4.1. Assay protocol flow chart for endocrine analyses. Steps taken (unshaded cells) and their prerequisites (shaded cells) for the detection of hormones by ELISA from diluted faecal extract. Reagents were added to the wells of micro-titre plates coated with donkey anti-Sheep immunoglobulin G (for corticosterone) or goat anti-Mouse immunoglobulin G for progesterone and testosterone.

Corticosterone ELISA	**Progesterone ELISA**	**Testosterone ELISA**
Add samples	Add samples	Add samples
100 µl	100 µl	100 µl
Add conjugate	Add conjugate	-
50 µl	50 µl	
Add polyclonal antibody	Add polyclonal antibody	Add monoclonal antibody
50 µl	50 µl	50 µl
Incubate plate	Incubate plate	Incubate plate
2 hr, 21°C, 500 rpm	2 hr, 21°C, 500 rpm	1 hr, 21°C, 500 rpm
Add wash solution	Add wash solution	-
3 x 400 µl	3 x 400 µl	-
-	-	Add conjugate
		50 µl
-	-	Incubate
		1 hr, RT, 500 rpm
-	-	Add wash solution
		3 x 400 µl
Add substrate	Add substrate	Add substrate
200 µl	200 µl	200 µl
Incubate plate	Incubate plate	Incubate plate
1 hr, 21°C	0.75 hr, 21°C	1 hr, 37°C
Add stop solution	Add stop solution	Add stop solution
50 µl	50 µl	50 µl
Read plate	Read plate	Read plate
405 nm	405 nm	405 nm

Equation 4.1. Four-parameter logistic curve.

$$y = d + \frac{a - d}{1 + \left(\frac{x}{c}\right)^{b}}$$

Where,

$y =$ unknown concentration of target hormone

$x = percent\ bound = \frac{net\ optical\ density}{net\ maximum\ binding\ well\ optical\ density} \times 100$

a = the minimum value that can be obtained

$d =$ the maximum value that can be obtained

$c =$ the point of inflection (i.e. the point on the S shaped curve halfway between a and d)

$b =$ Hill's slope of the curve (i.e. this is related to the steepness of the curve at point c)

Several immunological criteria were monitored to ensure that rhino faecal metabolites could be reliably detected by assay (Palme, 2018). To calculate the percentage recovery of each hormone, undiluted faecal extracts were spiked with known concentrations of each hormone, these were then diluted in buffer and assayed alongside un-spiked samples. Additionally, the manufacturer (Enzo Life Sciences) had previously tested the corticosterone assay against rhinoceros faeces and reported a sample recovery of 93.9%. The dilutions that resulted in the greatest recovery of hormone were selected for further use. Serial dilutions of faecal extracts were checked for parallelism between their binding inhibition curves and the hormone standards. The degree of cross-reactivity between each target metabolite and potential cross-reactants were conducted by the assay manufacturer (Enzo Life Sciences) and are listed in

Table 4.2. High cross-reactivities will also extend to other closely related metabolites not tested for in commercial assays (Palme, 2018).Assay sensitivity, which equates to the lowest detectable concentration of hormone (Kretzschmar et al., 2004), was calculated as two standard deviations from the zero (no antigen present) along the standard curve. Average intra-assay variabilities were calculated from the coefficients of variation of each sample, all of which were run in duplicate. Inter-assay variabilities were calculated from repeats run across multiple assays.

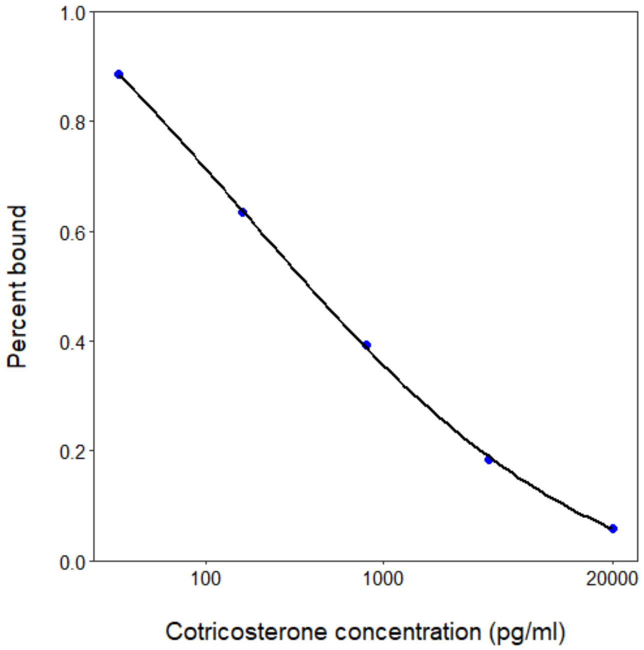

Figure 4.1. Example of a standard curve plotted via a four-parameter logistic function. X-axis plotted as a log-scale. Percentage bound equals the observed optical density divided by the maximum optical density obtainable in the assay.

Table 4.2. Cross-reactivates listed as a percentage of the actual concentration of cross-reactant in the sample against the level detected in the assay. Cross-reactants were dissolved in assay buffer and compared with the binding rate of the ELISA target hormones. Source: Enzo Life Sciences, (2015, 2016a, 2016b).

Progesterone cross-reactivity	**Corticosterone cross-reactivity**	**Testosterone cross-reactivity**
Progesterone [100%]	Corticosterone [100%]	Testosterone [100%]
5α-Pregnane-3,20-dione [100%]	Deoxycorticosterone [28.6%]	4-androsten-17ß, 19-diol-3-one [14.64%]
17-OH-Progesterone [3.46%]	Progesterone [1.7%]	4-androsten-3,17-dione [7.2%]
5-Pregnen-3ß-o1-20-one [3.46%]	Testosterone [0.13%]	5-androsten-3ß-ol-17-one [0.72%]
Corticosterone [0.77%]	Tetrahydrocorticosterone [0.28%]	Estradiol [0.4%]
4-Androstene-3,17-dione [0.28%]	Aldosterone [0.18%]	5α-androsten-17ß-ol-3-one [<0.001%]
Deoxycorticosterone [0.06%]	Cortisol [0.05%]	Estriol [<0.001%]
DHEA [0.01%]	Pregnenolone [<0.03%]	Aldosterone [<0.001%]
17ß-Estradiol [<0.001%]	ß-Estradiol [<0.03%]	Corticosterone [<0.001%]
Estrone [<0.001%]	Cortisone [<0.03%]	Cortisol [<0.001%]
Estriol [<0.001%]	11-dehydrocorticosterone acetate [<0.03%]	Cortisone [<0.001%]
Testosterone [<0.001%]		Estrone [<0.001%]
Hydrocortisone [<0.001%]		Progesterone [<0.001%]
5α-Pregnane-3α,20α-diol [<0.001%]		5-pregen-3ß-ol-20-one [<0.001%]
Danazol [<0.001%]		

4.2.3 Data analyses

All analyses were two-tailed, and all alpha levels were set at 0.05. Seven sets of analyses were undertaken to address the seven objectives of the study (Figure 4.2). Analysis 1 tested for baseline physiological differences of age-sex class in FCMs (Analysis 1a) and FAMs (Analysis 1b); Analysis 2 for differences in FCMs between a dehorned and a horned rhinos; Analysis 3 for the presence of an acute (or short-term) stress response to dehorning; and Analysis 4 for a chronic (or long-term) response to dehorning. Analysis 5 tested whether FCMs were influenced by the number of dehorning procedures. Analysis 6a tested whether FPMs were influenced by pregnancy, Analysis 6b whether FPMs were correlated with FCMs, and Analysis 6c whether FPMs were influenced by the number of dehorning procedures. Analysis 7a tested whether FAMs were correlated with FCMs and Analysis 7b whether FAMs were influenced by the number of dehorning procedures. Full methods are outlined in the appropriate sections below, with statistical variables listed in

Table 4.3.

Analysis 1. Physiological differences in age and sex

FCM concentrations were contrasted between age-sex classes (Analysis 1a). FAM concentrations of males were contrasted between Age Class only (Analysis 1b). Faecal samples were collected between 24/05/2016 and 22/10/2017 from Site A. For FCMs, 162 samples were collected from 17 rhinos. For FAMs, 115 samples were collected from 12 rhinos. A GLMM was run to test whether FCM concentration (a continuous positive response variable) was influenced by age-sex class, when considering one random effect (‘rhino Identity’). Another GLMM was run to test whether FAM concentration (a continuous positive response variable) was influenced by age class, when considering one random effect (‘rhino Identity’). For both models, the error was non-normally distributed (See Chapter 2), and the variance was non-constant, so a gamma distribution was selected, along with a log link function.

Analysis 2. Physiological differences between populations

The FCM concentrations of horned rhinos were compared against rhinos that had been dehorned once. Samples were collected from 24 rhinos between 24/05/2016 and 04/10/2017 at the three field sites. If repeat measures were available for the same individual then the median value was taken. The FCM levels of 15 dehorned rhinos (from 45 samples) were compared against the FCM levels of nine horned rhinos (from 9 samples). The data were non-normally distributed (See Chapter 2), so an independent Wilcoxon rank sum test was run with the base R function 'wilcox.test' to analyse whether rhinos of different 'Horn Status' varied in their FCM concentrations (

Table 4.3).

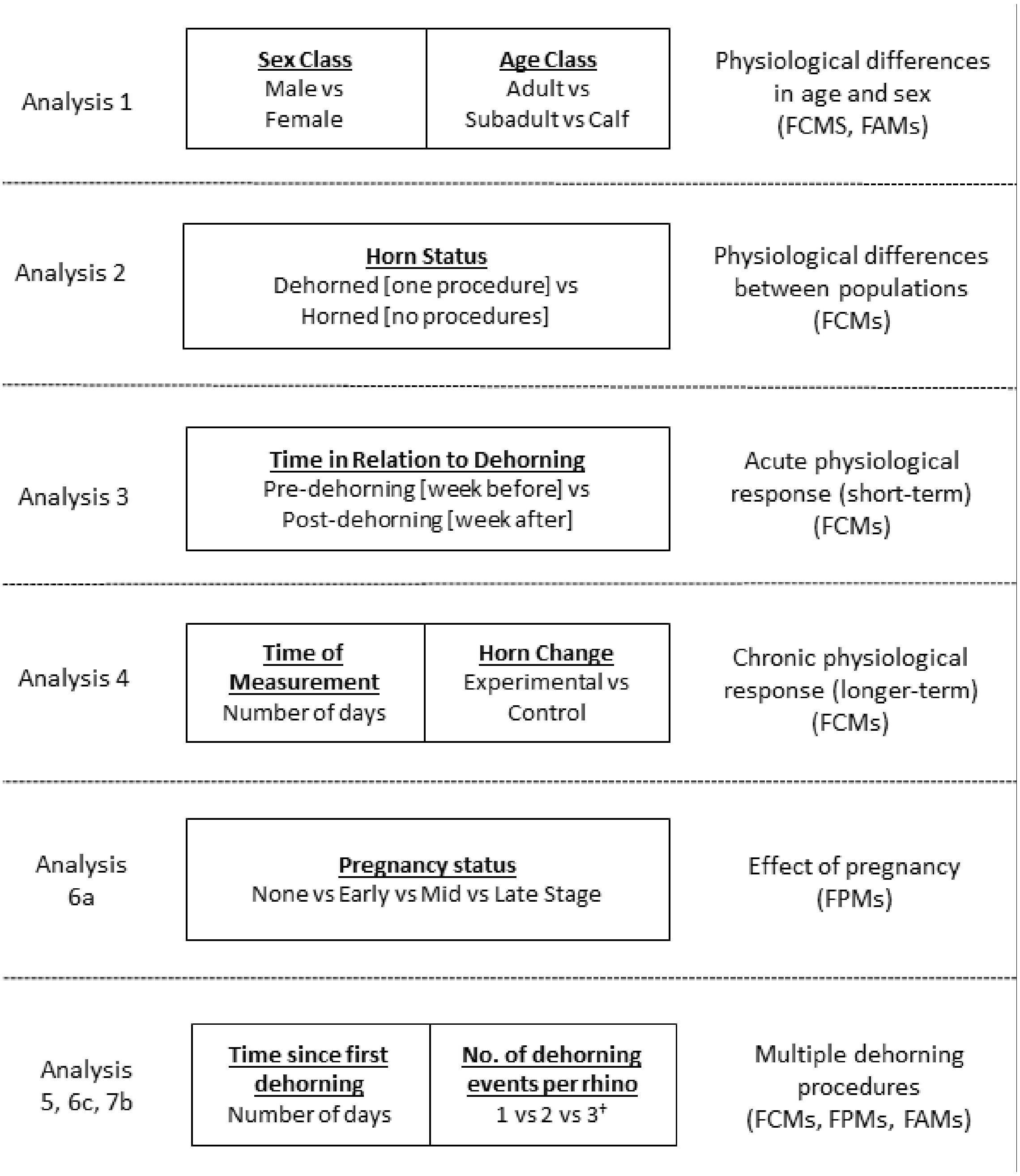

Figure 4.2. Schematic of the physiological analyses used in the study. Boxes show major independent variables (underlined) and the levels beneath them. A description of each analysis is listed to the right of the box with dependent variables of interest listed in parentheses beneath. Analyses of correlations between FCMs and FPMs (Analysis 6b) and correlations between FCMs and FAMs (Analysis 7a) are not shown. All samples aside from those collected in Analysis 2 were collected in Site A only. † No analysis of FPMs occurred.

Table 4.3. Variables included in the seven sets of phsyiological analysis. Response variables are marked with an asterisk.

Analysis	Variable	Levels	Description
1, 2, 3, 4, 5, 6b, 7a	FCMs*	Scalar	Faecal corticosterone metabolites (µg /g)
1, 7a, 7b	FAMs*	Scalar	Faecal androgen metabolites (µg /g)
6	FPMs*	Scalar	Faecal progestogen metabolites (µg /g)
1	Age Class	3	Age class of each rhino [Calf, Subadult, Adult]
1	Sex Class	2	Sex class of each rhino [Male, Female]
2	Horn Status	2	Whether the rhinos were dehorned once [dehorned] (collected within Site A) or never dehorned (collected within Site B and Site C) [horned]
3	Time in Relation to Dehorning	2	Whether FCM concentration was sampled in the week prior to [Pre] or after dehorning [Post]
4	Time of Measurement	Scalar	Timing of FCM measurement within the monitored period [day 1-142]
4	Horn Change	2	Whether or not a rhino was dehorned immediately prior to the monitored period [experimental] or if no change occurred [control]
5	Time Since First Dehorning	Scalar	Time elapsed between the date of first dehorning and sample collection for FCM analysis [no. of days 111-1097]
6c			Time elapsed between the date of first dehorning and sample collection for FPM analysis [no. of days 581-1062]
7b			Time elapsed between the date of first dehorning and sample collection for FAM analysis [no. of days 258-1097]
6a	Pregnancy Status	3	Whether a rhino was within the first third [early], second third [mid] or final third [late] stages of pregnancy or was not pregnant [none].
5, 6c, 7b	No. of Dehorning Procedures	3	The number of instances a rhino had been dehorned on the hormone sampling date [1, 2, 3]
5, 6c, 7b	Rainfall	2	Amount of precipitation during the hormone sampling period [Dry, Wet]
All	Rhino Identity	Up to 24	Each faecal sample was matched to a specific rhino [Rhino identity number]

Analysis 3. Acute physiological response

FCM concentrations measured up to one week before a dehorning procedure were compared with those collected up to one week after. Samples were collected from eight individuals dehorned on the same day (02/06/16) at Site A. All eight individuals had been dehorned once prior to this (584-591 days earlier). One sample was collected from each rhino 0 – 117 hours before their second dehorning and 24 – 166 hours after the procedure, allowing subjects to act as their own control. The paired differences were normally distributed (See Chapter 2) and the variance constant, so a dependent t-test with the base R function ‘t.test’ was run on collection ‘Time in Relation to Dehorning’ (

Table 4.3).

Analysis 4. Chronic physiological response

FCM profiles of rhinos that underwent a dehorning procedure were contrasted against those that did not. The profiles of sixteen rhinos were monitored over a contiguous 142-day period from 03/06/16 to 22/10/16 at Site A. Eight of the rhinos were dehorned immediately prior to the monitored period (Experimental group) and eight were not (Control group). At least 24 hours was left between the time of dehorning and the first collected sample to account for the lag time between stressor and response (Riato, 2007). A total of 89 samples met this criteria and were included in the analysis. The length of the monitored period was limited to 142 days as additional dehorning procedures occurred after this date. The analysis investigates the effect of a repeat dehorning event, as this was the second time that the rhinos in the experimental group had undergone the procedure. A period of 584-591 days had elapsed between the first dehorning and the second dehorning. The control group contained four rhinos that had undergone an earlier dehorning procedure and four that had not; the dehorned rhinos had between 269 - 604 days of horn regrowth at the date of the first collected sample. All rhinos experienced similar environmental conditions and were exposed to comparable levels of forage availability, disturbance (e.g. from anti-poaching units, vehicles, and people) and climatic variation. The inclusion of the control group enables FCM profiles to be decoupled from other potentially confounding sources of stress.

A GLMM was run to test whether FCM concentration (a continuous positive response variable) was influenced by two fixed effects and their interaction term (‘Time of Measurement’ and ‘Horn Change’), when considering one random effect (‘Rhino Identity’). The interaction indicated whether the effect of horn type had a differential impact over time of measurement. The error (standardised residuals) was non-normally distributed (See Chapter 2), and the variance was non-constant, so a gamma distribution was selected, along with a log link function. To account for the pooling of horned and once-dehorned rhinos in the control group, each subject was given the freedom to vary by the inclusion of a random intercept and random slope respectively. This allows for variations in the initial concentration of FCMs and individual-specific rates of change. The model then measures how each rhino’s slope differed from the population average slope. If the variance of these measures equalled zero it would indicate that the slope of time on FCM concentration was the same for all rhinos in a group. Without this freedom to vary, variation within individuals may have masked any between-group effects (Taff et al., 2018).

Analysis 5. Multiple dehorning procedures – FCM profiles

Trends in FCM profiles were analysed in relation to the number of dehorning procedures rhinos were subject to. The profiles of fifteen rhinos were monitored for up to 516 days from 24/05/2016 to 22/10/2016 at Site A. Data from rhinos that had never been dehorned were excluded from the model. Throughout this period, the rhinos were subject to 22 dehorning procedures in total, and had experienced an additional 12 procedures prior to the start of data collection. Each of the rhinos had been dehorned between 1 and 3 times by the end of the data collection period (see Chapter 2). No data was available for the first 110 days following any rhino’s first dehorning. Rainfall was included in the model as a random effect to account for potential season-dependent changes in FCMs over the study period. This can be brought on by rain-dependent changes in resource availability and body condition, such as those reported by Pokharel et al. (2017) in Asian elephants (*Elephas maximus*). Data were classified by whether sampling occurred during a high or low rainfall period. This was defined by the onset of the 2016/17 rainy season which marked the end of a period of drought. Data collected in the year before 18/10/2016 had a year rainfall of 262 ml, while that collected over the subsequent year had a year total of 659 ml (See Chapter 2).

A GLMM was run to understand whether FCM concentration (a continuous positive response variable) were influenced by two fixed effects (‘Time Since First Dehorning’ and ‘Number of Dehorning Procedures’) and their interaction term, accounting for two random effects (‘Rhino Identity’ and ‘Rainfall’). For Rhino identity both a random intercept and random slope were included, while for Rainfall only a random intercept was included to reduce the complexity of the model. A gamma distribution with log link function was selected as the best fitting model. Rainfall was excluded from the final model as the random effect variance was estimated to be zero; indicating that either the slope of FCM concentration on time (‘Time Since First Dehorning’) was the same for both levels, or that the model was over fit due to the low number of levels in the variable (Gelman, 2006).

Analysis 6a. Multiple dehorning procedures - effect of pregnancy on FPMs

Four rhinos gave birth within or soon after the monitored period. To calculate periods of pregnancy, conception times were estimated by back counting the average white rhino gestation length of 495 days from parturition (Linklater, 2007). The effects of pregnancy on FPM concentration were established prior to those of dehorning. Between 24/05/17 and 15/10/17, 50 samples were collected from five females at Site A. Values were then characterised by whether they occurred out of pregnancy or within early, mid or late gestation. Gestation was classified into three 165 day periods to cover the entire approximated gestation period and account for the comparatively lower levels of progesterone expected in the first months after conception (MacDonald et al., 2008). FPM concentrations that exceeded the maximum detectable range of the assay were included in the model (8 out of 50 samples). Thus, an analysis of clipped FPM concentration was conducted rather than one of true levels. A GLMM was run to investigate how clipped FPM concentrations (a continuous positive response variable) were influenced by one fixed effect (‘Pregnancy Status’) when considering one random factor (‘Rhino Identity’). The error (standardised residuals) was non-normally distributed (See Chapter 2), and the variance was non-constant, so a gamma distribution was selected, along with a log link function. To account for variations in the initial concentration of FPMs, each subject was given the freedom to vary by the inclusion of a random intercept.

Analysis 6b. Multiple dehorning procedures – relationship between FPMs and FCMs

To determine the relationship between FCMs and FPMs measured from the same sample, a Pearson's correlation test was performed with the base R function 'cor.test'. Pregnant females were excluded from the analysis following the results of analysis 6a, as were males. No FPM concentration exceeded the maximum detectable range of the assay. Thus, an analysis of true levels was conducted rather than one of clipped FPM values.

Analysis 6c. Multiple dehorning procedures – FPM profiles

Trends in FPM profiles were analysed in relation to the number of dehorning procedures female rhinos were exposed to. The profiles of five rhinos were monitored for up to 482 days from 24/05/2016 to 17/09/2017. Values recorded during pregnancy were excluded from the analysis. Throughout this period, the rhinos were subject to five dehorning procedures in total, and had experienced an additional five procedures prior to the start of data collection. Each of the rhinos had been dehorned 2 times by the end of the data collection period (see Chapter 2). No data were available for the first 580 days following any rhino's first dehorning. Rainfall patterns were also monitored, as fluctuations in rainfall can influence the timings of births in white rhinos (Owen-Smith, 1973; Penny, 1987).

A GLMM was run to understand whether FPM concentration (a continuous positive response variable) were influenced by two fixed predictor variables and their interaction term ('Time Since First Dehorning' and 'No. of Dehorning Procedures') when considering two random effects ('Rhino Identity' and 'Rainfall'). The error was non-normally distributed (See Chapter 2), and the variance was non-constant, so a gamma distribution was selected, along with a log link function. For Rhino identity both a random intercept and random slope were included in the model, while for Rainfall only a random intercept was included to reduce the complexity of the model. Rainfall was excluded from the final model as its inclusion resulted in a singular fit. This indicates that the random effects structure was too complex to be supported by the dataset and that the model was overfit, (Long, 2018) likely due to the small sample size of just 35 observations.

Analysis 7a. Multiple dehorning procedures – relationship between FAMs and FCMs

To determine the relationship between FCM and FAM levels measured from the same sample, a Pearson’s correlation test was performed with the base R function ‘cor.test’. Females and calves were excluded from the analysis.

Analysis 7b. Multiple dehorning procedures – FAM profiles

Trends in FAM profiles were analysed in relation to the number of dehorning procedures rhinos were exposed to. The profiles of seven male rhinos were monitored for up to 516 days from 28/05/2016 to 22/10/2017 at Site A. Rhinos that had never been dehorned and all calves were excluded from the analysis. Throughout this period, the rhinos were subject to 13 dehorning procedures in total, and had experienced an additional seven procedures prior to the start of data collection. Each of the rhinos had been dehorned between 2 and 3 times by the end of the data collection period (see Chapter 2). No data was available for the first 258 days following any rhinos first dehorning. Rainfall patterns were also monitored as fluctuations in rainfall can influence FAM levels in white rhinos (Kretzschmar et al., 2004).

A GLMM was run to understand whether FAM concentration (a continuous positive response variable) were influenced by two fixed predictor variables and their interaction term (‘Time Since First Dehorning’ and ‘No. of Dehorning Procedures’) when considering two random effects (‘Rhino Identity’ and ‘Rainfall’). The error was non-normally distributed (See Chapter 2), and the variance was non-constant, so a gamma distribution was selected, along with a log link function. For Rhino identity both a random intercept and random slope were included in the model, while for Rainfall only a random intercept was included to reduce the complexity of the model. Rainfall was excluded from the final model as the random effect variance was estimated to be zero. The random slope for Rhino identity was also excluded from the final model as a ‘singular fit’ warning indicating the model had been overfit.

4.3 Results

4.3.1 Assay validity

The minimum detectable concentration of corticosterone was 7.90 pg / ml (n = 7 replicates), 2.41 pg / ml for testosterone (n = 4 replicates) and 10.79 pg / ml for progesterone (n = 2 replicates). Average intra- and inter-assay coefficients of variability were <16% for FCMs, and <10% for FAMs and FPMs. Serial dilutions of faecal extracts yielded parallelism between binding inhibition curves and the hormone standards. Significant recovery of exogenous hormone was demonstrated for corticosterone (91%), testosterone (75%), and progesterone (78%).

Faecal samples collected within the first few hours of defecation (at 3, 6 and 14 hours) had comparable FCM concentrations to those collected immediately after defecation (< 1 hour; Figure 4.3); samples collected at 0, 3, 6 and 14 hours had a coefficient of variation of just 4.07%. Samples had weak comparability after this period, with those collected 25 and 48 hours showing a coefficient of variation of 27.24% and 63.18% respectively when grouped with fresh dung. The recently deceased female rhino showed the highest recorded value of the study at 60.905 µg /g (approximately 10 times the population mean), thus biologically validating the FCM assay. However, this result should be interpreted with caution as its collection occurred outside the window of metabolite stability.

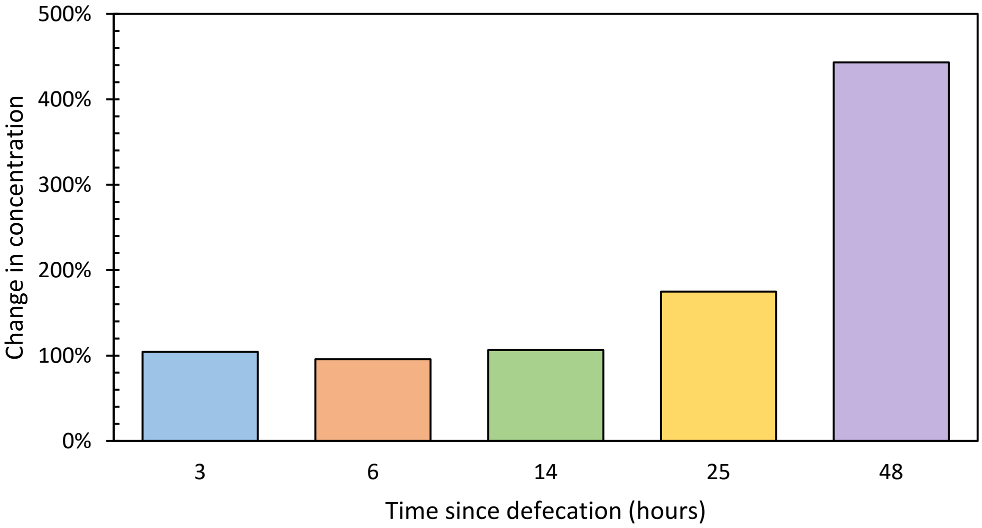

Figure 4.3. Change in FCM concentration between samples collected at 3, 6, 14, 25 and 48 hours after defecation when compared with a sample collected immediately after defecation (<1 hour). N = 5.

4.3.2 Analysis 1. Physiological differences in age and sex

FCMs did not differ significantly between rhino age-sex class (p = 0.469; Table 4.4) illustrated in Figure 4.8Figure 4.4. Adult females had the highest FCM concentrations (mean ± sd: 0.720 ± 0.501 µg /g, obs. = 51, n = 5), followed by male calves (0.701 ± 0.536 µg /g, obs. = 25, n = 5); subadult males (0.598 ± 0.391 µg /g, n = 5, obs. = 69) and adult males (0.498 ± 0.346 µg /g , n = 2, obs. = 17).

Table 4.4 Statistical output from a GLMM analysis indicating whether rhinos of different age-sex classes show differences in FCM concentration. Type 3 Wald χ^2 tests were used to extract the significance of the fixed effect. df = degrees of freedom.

Fixed effects	**Estimate**	**Standard error**	**χ^2**	**df**	**P-value**
Analysis 1a – 162 observations					
Adult female vs adult male	-0.328	0.254	-	-	-
Adult female vs subadult male	0.026	0.206	-	-	-
Adult female vs male calf	-0.164	-0.187	-	-	-
Age sex-class (overall)	-	-	2.534	3	0.469

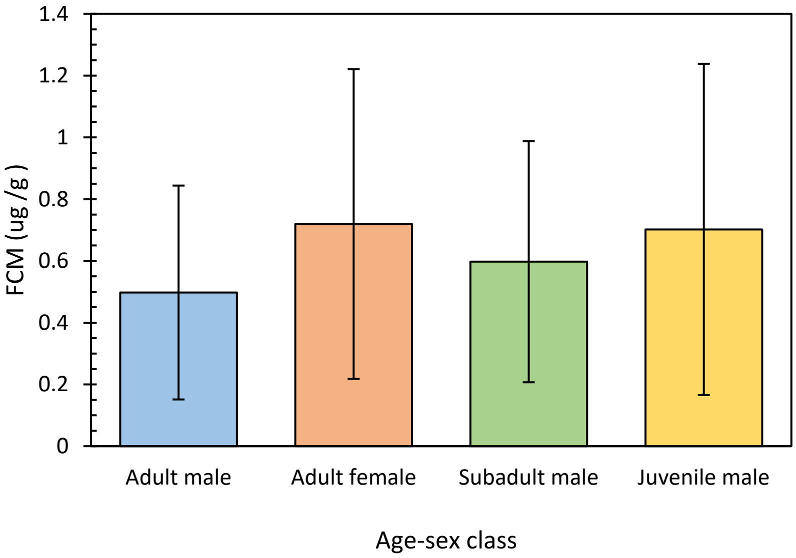

Figure 4.4. Mean FCM concentrations within age-sex classes. Error bars specify one standard deviation from the mean; observations = 162, n = 17.

FAMs did not differ significantly between rhino age-sex class (p = 0.450; Table 4.5) illustrated in Figure 4.5Figure 4.8. Higher mean FAM concentrations were detected from adult males (mean ± sd: 0.355 ± 0.168 µg /g, obs. = 17, n = 2), than from subadult males (0.273 ± 0.105 µg /g, obs. = 72, n = 5) or male calves (0.288 ± 0.142 µg /g, obs. = 26, n = 5; Figure 4.5). Furthermore, a breeding bull had the maximum-recorded value (0.387 ± µg /g) and a calf the minimum value (0.218 µg /g).

Table 4.5. Statistical output from a GLMM analysis indicating whether rhinos of different age classes show differences in FAM concentrations. Type 3 Wald χ2 tests were used to extract the significance of the fixed effect. df = degrees of freedom.

Fixed effects	**Estimate**	**Standard error**	**χ^2**	**df**	**P-value**
Analysis 1b – 115 observations					
Adult male vs subadult male	-0.201	0.158	-	-	-
Adult male vs male calf	-0.124	0.179	-	-	-
Age class (overall)	-	-	1.786	2	0.450

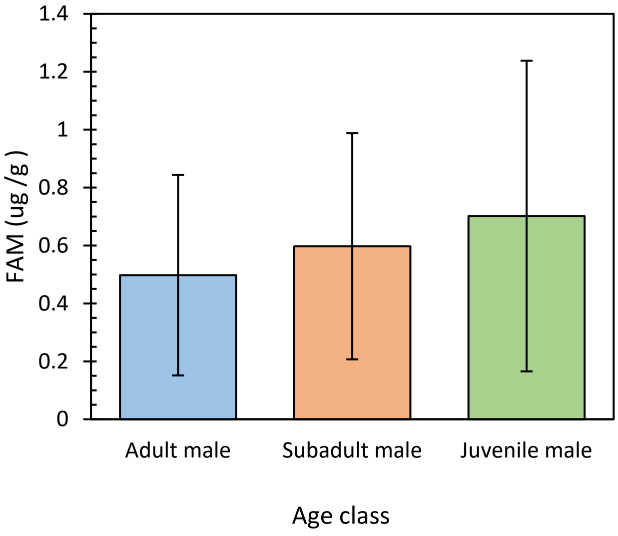

Figure 4.5. Mean FAM concentrations within age classes. Error bars specify one standard deviation from the mean; observations = 115, n = 17

4.3.3 Analysis 2. Physiological differences between populations

The FCM concentration of rhinos that had been dehorned once did not differ significantly to those measured in rhinos that had never been dehorned before (Wilcoxon rank sum test: *W* = 59, df = 1, *p* = 0.640, *n* = 24). The FCM levels of the dehorned rhinos had a median concentration of 0.575 µg /g (*n* = 15), while those from the horned group had a median concentration of 0.583 µg /g (*n* = 9; Figure 4.6).

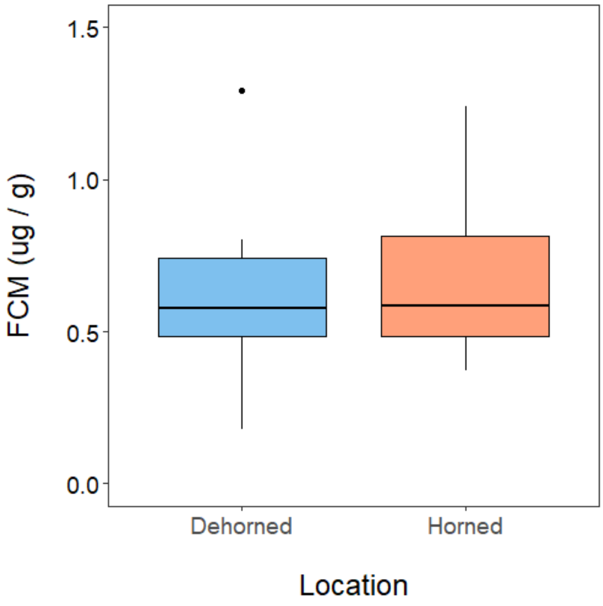

Figure 4.6. Median FCM concentrations of once dehorned and horned rhinos. Whiskers specify the lowest datum still within 1.5 IQR of the lower quartile, and the highest datum still within 1.5 IQR of the upper quartile; outliers mark data beyond this; n = 24.

4.3.4 Analysis 3. Acute physiological response

FCM concentrations of samples collected in the week prior to a dehorning procedure did not differ significantly to samples collected over the following week (Paired t-test: t = -1.674, df = 7, p-value = 0.138, n = 8). The mean difference in FCM levels of rhinos pre- and post-dehorning was -0.180 ± 0.304 µg /g (n = 8), with the values after dehorning being lower on average (0.435 ± 0.083 µg /g, n = 8) than those collected before dehorning (0.615 ± 0.309 µg /g, n = 8; Figure 4.7).

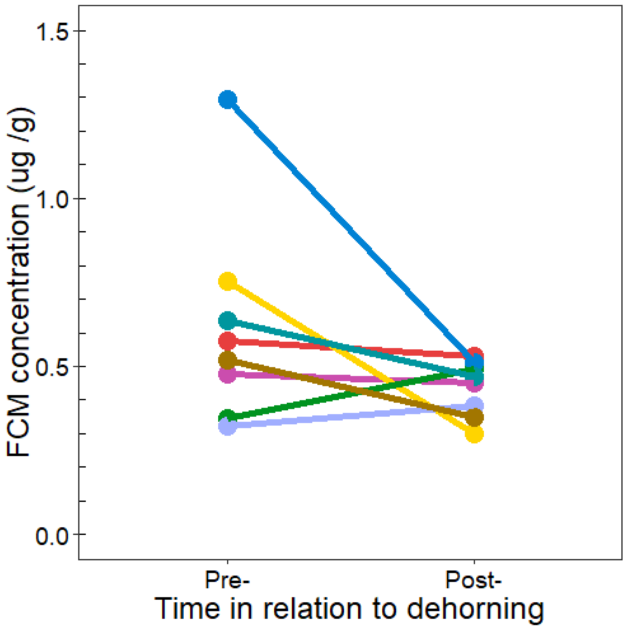

Figure 4.7. FCM concentrations sampled up to one week before and one week after a dehorning procedure (n = 8).

4.3.5 Analysis 4. Chronic physiological response

Trends in FCMs monitored over a 141-day period did not differ significantly between the recently-dehorned group of rhinos and the horned control group, as shown by the non-significant interaction term (p = 0.547; Table 4.6) illustrated in Figure 4.8. Furthermore, when considering the terms of the individual fixed effects, FCM concentration was not influenced by time (p = 0.973; Table 4.6) nor was it influenced by which group a rhino was in (p = 0.934; Table 4.6; Figure 4.9).

Table 4.6. Statistical output from a GLMM analysis indicating whether rhinos experience a chronic physiological response to dehorning. Type 3 Wald χ^2 tests were used to extract the significance of fixed effects. df = degrees of freedom.

Fixed effects	**Estimate**	**Standard error**	**χ^2**	**df**	**P-value**
Analysis 4 – 89 observations					
Time of measurement	-0.011	0.330	0.001	1	0.973
Horn change	0.021	0.254	0.007	1	0.934
Interaction (overall)	-0.194	-0.194	0.363	1	0.547

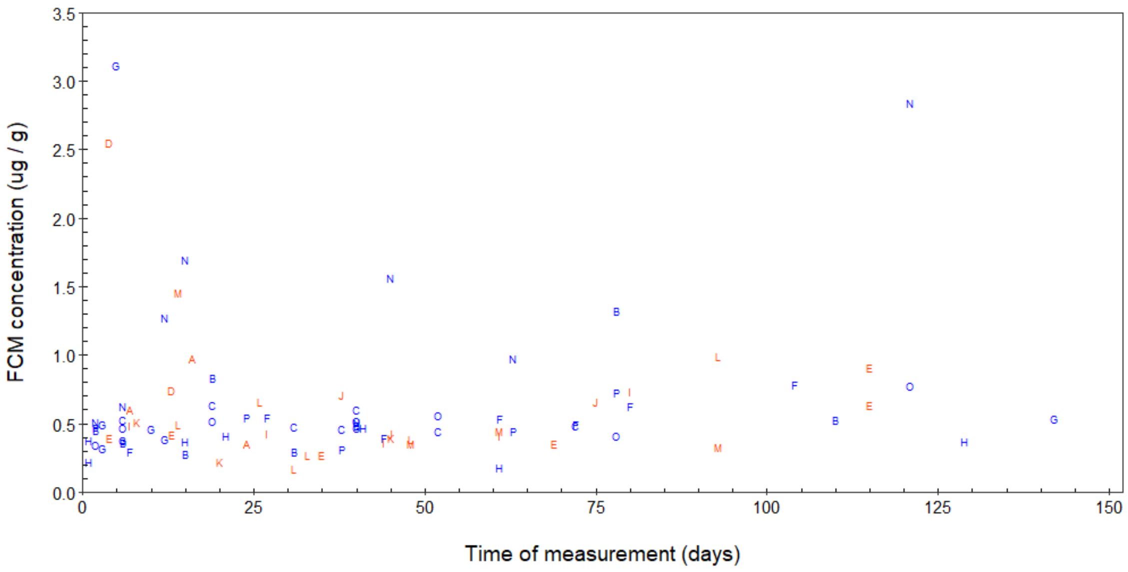

Figure 4.8 Trends in FCMs of rhinos monitored over a 141-day period. Showing rhinos that underwent a change in horn status (orange letters) and those that did not (blue letters). Letters A-M indicate individual rhino identities. Observations = 89; N = 16).

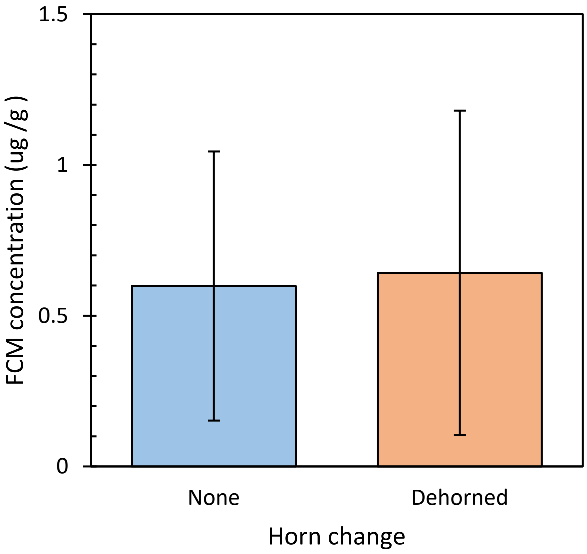

Figure 4.9. Comparison of mean FCM concentrations within the recently dehorned (experimental) group and no change (control) group pooled over a period of 142 days. Error bars specify one standard deviation from the mean; observations = 89; n = 16).

4.3.6 Analysis 5. Multiple dehorning procedures – FCMs

The number of times a rhino was dehorned did not influence the rate of change in FCM concentration, as shown by the non-significant interaction term (p = 0.229; Table 4.7; Figure 4.10). When considering the terms of the individual fixed effects, FCM levels were neither significantly influenced by time since first dehorning (p = 0.110; Table 4.7) nor by the number of dehorning procedures (p = 0.097; Table 4.7; Figure 4.11).

Table 4.7. Statistical output from GLMM analysis indicating whether FCMs are influenced by multiple dehorning procedures. Type 3 Wald χ^2 tests were used to extract the significance of fixed effects. df = degrees of freedom.

Fixed effects	**Estimate**	**St. error**	**χ^2**	**df**	**P-value**
Analysis 5 – 143 observations					
Time since first dehorning	0.615	0.104	2.546	1	0.110
No. of dehorning procedures (1 vs 2)	0.267	0.136	-	-	-
No. of dehorning procedures (2 vs 3)	-0.698	0.786	-	-	
No. of dehorning procedures (overall)	-	-	4.666	2	0.097
Interaction (1 vs 2)	-0.274	0.160	-	-	-
Interaction (2 vs 3)	0.095	0.558	-	-	-
Interaction (overall)	-	-	2.949	2	0.229

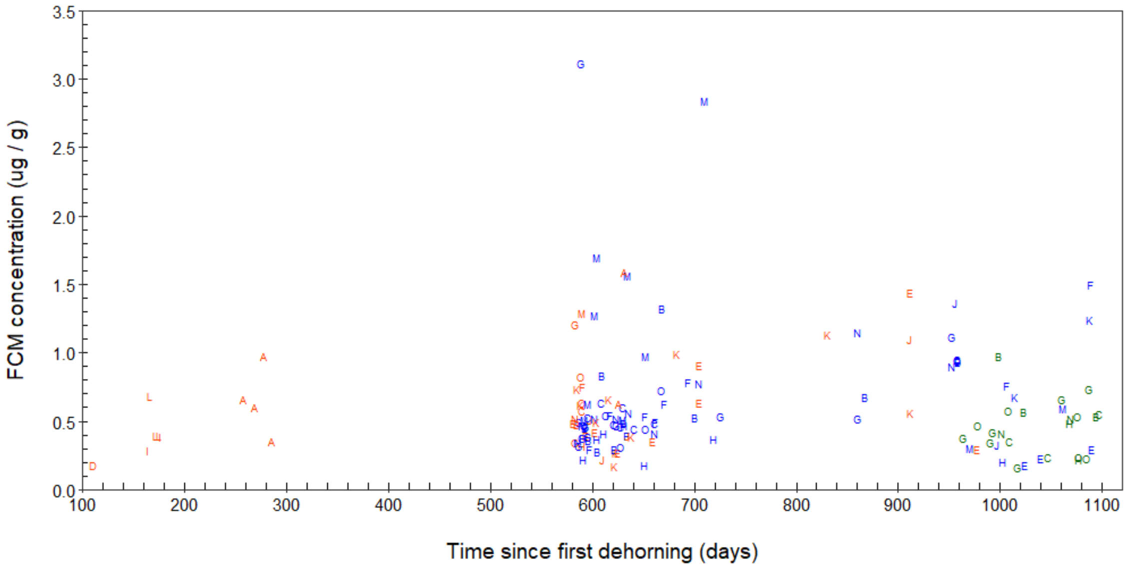

Figure 4.10. Trends in FCMs following multiple dehorning procedures. Colours indicate rhinos after one (orange), two (blue) and three (green) dehorning procedures. Letters A-O indicate identities of individual rhinos. Observations = 143; n = 15.

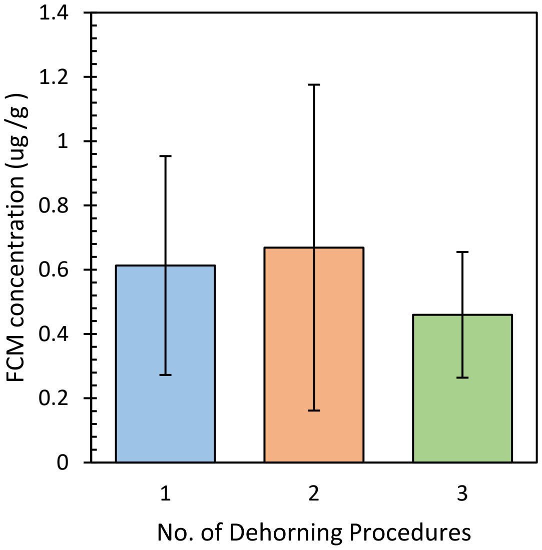

Figure 4.11. Mean FCM concentrations of rhinos after their first, second and third dehorning procedures. Error bars specify one standard deviation from the mean; observations = 143; n = 15.

4.3.7 Analysis 6. Multiple dehorning procedures – FPMs

The clipped FPM concentration of rhinos was significantly greater during pregnancy ($p < 0.001$, Table 4.8; Figure 4.12). Clipped FPM concentrations sequentially increased throughout pregnancy, values recorded during early pregnancy showed a mean concentration of 1.609 µg /g (n = 2 observations), in mid-pregnancy 3.601 µg /g (n = 12 observations) and in late pregnancy 8.499 µg /g (n = 2 observations). In comparison mean clipped FPM concentrations recorded out of pregnancy measured 1.106 ± 0.393 µg /g (n = 34 observations).

Table 4.8. Statistical output from GLMM analysis indicating whether FPMs are influenced by pregnancy status (analysis 6b) or multiple dehorning procedures, but in non-pregnant female rhinos only (analysis 6c). Type 3 Wald χ^2 tests were used to extract the significance of fixed effects. df = degrees of freedom. * = statistically signifcant.

Fixed effects	**Estimate**	**St. error**	**χ^2 value**	**df**	**P-value**
Analysis 6b – 50 observations					
Pregnancy (non vs 1)	0.421	0.281	-	-	-
Pregnancy (non vs 2)	1.158	0.145	-	-	-
Pregnancy (non vs 3)	2.081	0.281	-	-	-
Pregnancy (overall)	-	-	111.7703	3	<0.001*
Analysis 6c – 35 observations					
Time since first dehorning	0.102	0.090	1.291	1	0.256
No. of dehorning procedures	0.005	0.199	0.001	1	0.979
Interaction (overall)	0.110	0.135	0.659	1	0.417

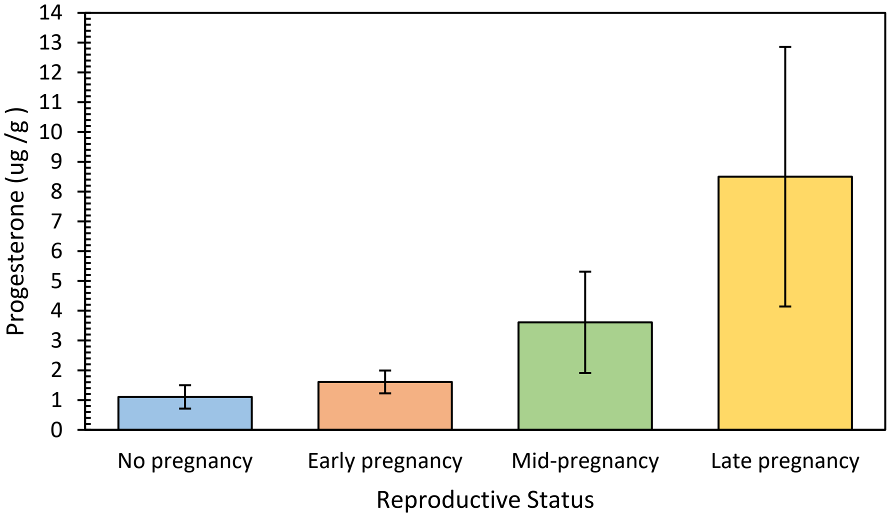

Figure 4.12. Clipped FPM concentrations of non-pregnant rhinos and of rhinos monitored during early (first third), mid- (second third) and late (final third) pregnancy. Error bars specify one standard deviation from the mean; observations = 50, n = 5.

The levels of FPMs detected in the dung of dehorned non-pregnant females did not correlate with their FCM levels (Pearson's product-moment correlation: t = -0.057, df = 33, p = 0.955, r = 0.094, n = 35). The number of times a non-pregnant rhino was dehorned did not influence the rate of change in FPM concentration, as shown by the non-significant interaction term (*p* = 0.417; Table 4.8; Figure 4.13). Considered in isolation, the individual fixed effects were also non-significant (time since first dehorning: *p* = 0.256; number of dehorning procedures *p* = 0.979; Table 4.8; Figure 4.14).

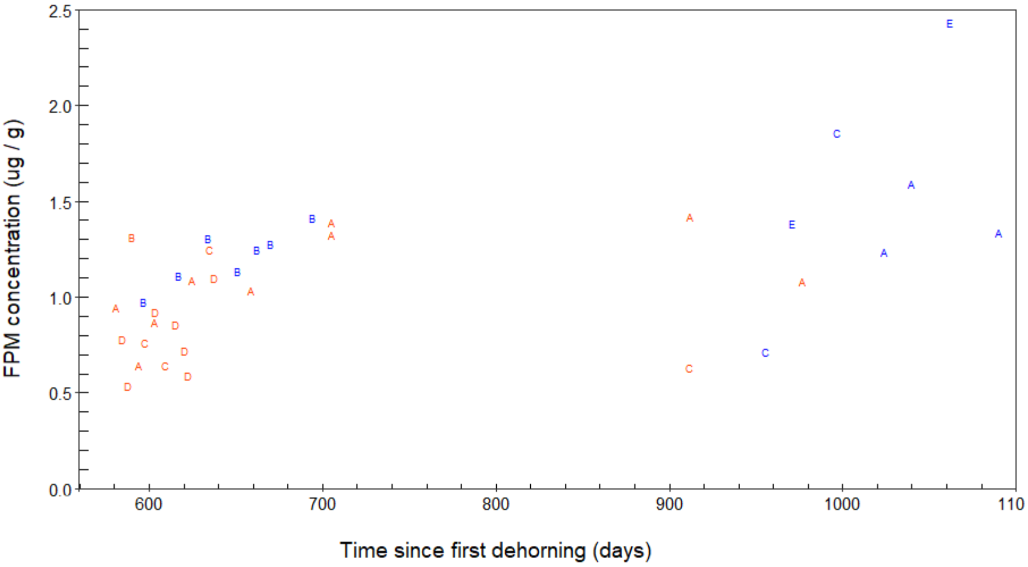

Figure 4.13. Trends in FPMs of non-pregnant female rhinos following multiple dehorning procedures. Colours indicate rhinos after one (orange) and two (blue) dehorning procedures. Letters A-E indicate identities of individual rhinos. Observations = 35, n = 5.

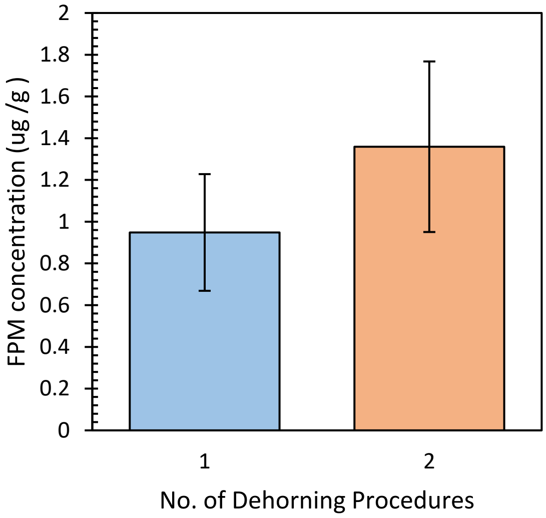

Figure 4.14. Mean FPM concentrations of non-pregnant female rhinos after their first and second dehorning procedures. Error bars specify one standard deviation from the mean; observations = 35, n = 5.

4.3.8 Analysis 7. Multiple dehorning procedures – FAMs

The levels of FAMs detected in the dung of dehorned male adults and subadults did not correlate with their FCM levels (Pearson's product-moment correlation: t = 0.867, df = 84, p-value = 0.389, r = 0.094, n = 86). The number of times a rhino was dehorned influenced the rate of change in FAM levels over time, as shown by the significant interaction term (*p* = 0.002; Table 4.9; Figure 4.15). When considering the terms of the individual fixed effects, FAM concentrations were significantly influenced by time since first dehorning (*p* < 0.001; Table 4.9) but not by number of dehorning procedures (*p* = 0.135; Table 4.9; Figure 4.16).

Table 4.9. Statistical output from GLMM analysis indicating whether FAMs are influenced by multiple dehorning procedures in subadult and adult male rhinos. Type 3 Wald χ^2 tests were used to extract the significance of fixed effects. df = degrees of freedom. * = statistically significant.

Fixed effects	**Estimate**	**St. error**	**χ^2 value**	**df**	**P-value**
Analysis 7 – 86 observations					
Time since first dehorning	-1.329	0.072	15.495	1	<0.001*
No. of dehornings (1 vs 2)	-0.250	0.162	-	-	-
No. of dehornings (2 vs 3)	-0.602	0.462	-	-	
No. of dehornings (overall)	-	-	4.003	2	0.135
Interaction (1 vs 2)	-0.549	0.171	-	-	-
Interaction (2 vs 3)	0.372	0.40	-	-	-
Interaction (overall)	-	-	12.517	2	0.002*

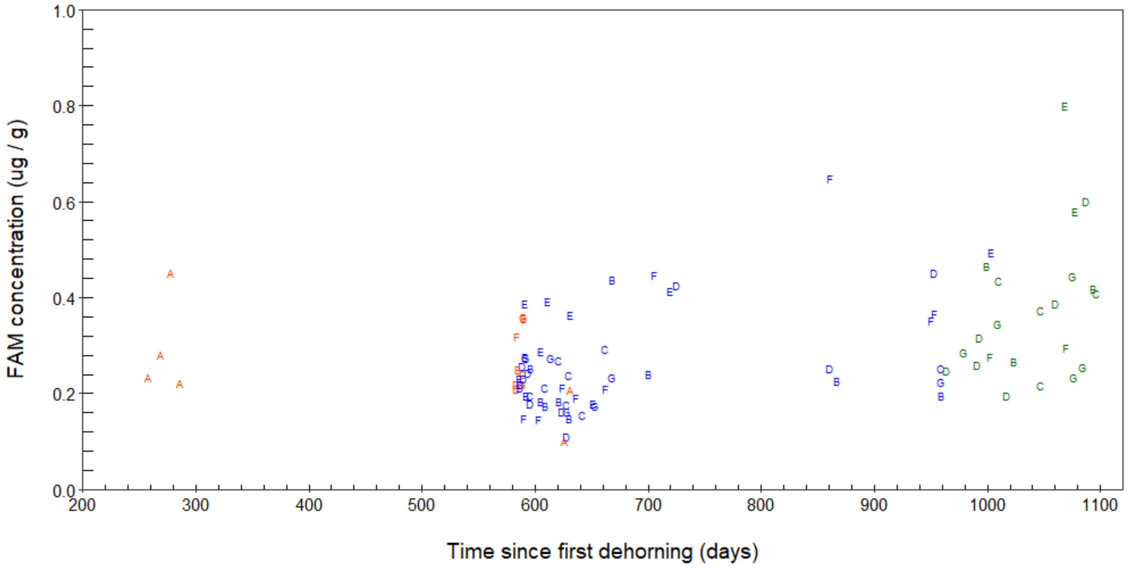

Figure 4.15. Trends in FAMs of adult and subadult male rhinos following multiple dehorning procedures. Colours indicate rhinos after one (orange), two (blue) and three (green) dehorning procedures. Letters A-G indicate identities of individual rhinos. Observations = 86, n = 7.

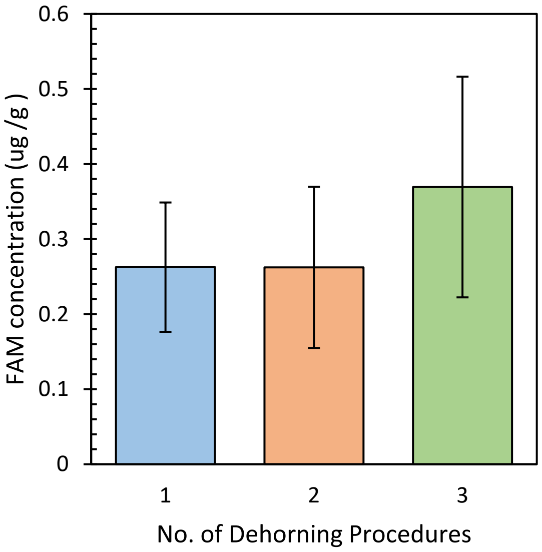

Figure 4.16. Mean FAM concentrations of rhinos after their first, second and third dehorning procedures. Error bars specify one standard deviation from the mean; observations = 86, n = 7.

4.4 Discussion

This study is the first to explore whether dehorning influenced endocrine activity in a non-intensively managed limited free-ranging population of rhinos, and the first to test for an effect beyond a short-term stress response and in response to repeat procedures.

The results indicate dehorning does not appear to influence physiological stress in white rhinos, and that the procedure does not appear detrimental to their welfare or health. The link between gonadal steroid change and dehorning was less clear due to limitations within the study design.

4.4.1 Physiological differences in age and sex

Rhino age and sex did not significantly influence FCM levels, allowing subjects to be pooled for analysis. FAM levels did not significantly differ between age groups; this was counter to the findings of Ververs (2018), who reported age-related FAM differences in a population of game ranched white rhinos. However, the highest overall value, recorded from a breeding bull (a confirmed sire of calves), was in accordance with other research (Kretzschmar et al., 2004; Ververs, 2018). It is likely that fluctuations in FAMs induced by season or social structure (Kretzschmar et al., 2004) may in part have concealed a relationship with age. Only adults and subadults were included in further analyses of FAMs, as calves do not engage in breeding or sexual behaviours (Owen-Smith, 1975), and thus levels of gonadal activity were not of biological interest.

4.4.2 Physiological differences between populations

The dehorned population of rhinos were no more stressed than the neighbouring horned populations according to FCM analysis. This corroborates comparisons made by Badenhorst et al. (2016) who found no significant difference between a range-limited dehorned population and a free-roaming horned population. It also extends their findings beyond a range-limited and heavily managed dehorned population to the more freely ranging dehorned population monitored in this study. Importantly, the stocking density of the rhino population in this study (294 hectare per rhino) are more representative of the conditions of rhinos conserved on private land in South Africa (277 hectares per rhino) than those of the game ranched population reported (9 hectare per rhino) (Sims-Castley et al., 2005; Badenhorst et al., 2016; Thompson et al., 2016). High population densities lead to increased glucocorticoid concentrations in a range of species (Creel et al., 2013). When white rhinos are kept at higher densities they can show greater levels of aggressive social behaviours (Metrione et al., 2007) and atypical social structures (Cinková and Bičík, 2013) which in turn can also influence their physiological stress levels (Meister, 1998).

4.4.3 Acute physiological response

FCM values sampled up to one week after dehorning were no higher than the pre-dehorning baseline. The lack of a short-term acute response runs counter to the trend observed by Badenhorst et al. (2016) whereby rhinos underwent a spike in FCM levels immediately after their dehorning. The only key methodological difference in the dehorning procedure between this study and that of Badenhorst et al. (2016) was that the latter did not utilise a helicopter for darting because of the limited-ranging ability of the rhinos (Koro, 2018). Use of a helicopter when darting, sometimes necessitating pursuit, is standard practise in other dehorning operations (Lindsey and Taylor, 2011), including this one. However, as helicopter pursuit can increase stress in black rhinos (Kock, 1990) the absence of a detectable response here is even more surprising.

The differences between the two studies may be explained by variation in the timings of sample collection. Badenhorst et al. (2016) reported a peak in FCMs 24 - 48 hours after dehorning, which rose to a median 32% (range: −7.0-293%) above the baseline, whereas by 72 to 86 hours after the event FCM had decreased to a median of just 16% (range: −24 to 42%) above the pre-dehorning baseline. In comparison, the samples in this study were collected a mean 80 hours (range: 24 to 166) after dehorning and had declined a mean 11% (range: -0.58-0.15%) below the pre-dehorning level. Thus, the sampling times of the current study will have missed the peak period of FCM excretion if it occurred soon after the dehorning procedure.

Additionally, it remains possible that the rhinos in this study did not undergo an increase in FCMs following dehorning. Not all capture methods are stressful or result in an endocrine response. Chemically immobilised Alpine ibex (*Capra ibex*) showed no significant hormonal change in either FCMs or FAMs up to 100 hours after capture; even when considering individual trends (Brivio et al., 2015). Furthermore, the adrenocortical activity of animals kept under different management conditions can vary substantially, for example FCMs of captive bushbabies *Galago moholi* were significantly different to those of free-ranging individuals (Scheun et al. 2017). Whether a stress-induced FCM peak was truly absent, or occurred prior to sample collection, the physiological effects of dehorning did not extend to the week after the procedure.

4.4.4 Chronic physiological response

Stress profiles of the recently-dehorned group were no different to those animals that did not undergo the procedure. Together with the above analysis, this provides evidence that the dehorning-induced stress response reported by Badenhorst et al. (2016) was triggered by immobilisation and the horn removal procedure rather than any resultant behavioural changes caused by a reduction in horn mass. It thus seems likely that the spike observed by Badenhorst et al. (2016) was an adaptive acute stress response, with no perceivable long-term effects.

The chemical immobilisation and collaring of red colobus monkeys (*Procolobus rufomitratus*) appears to show a similar response (Wasserman et al., 2013). Three days after collaring, the FCM levels of two monkeys showed a two-fold increase above their baseline levels. However, in data collected over a 14 month period, beginning 11 months after the collaring, no differences in FCMs were apparent between 10 collared individuals (68 ± 28 ng/g) and 14 un-collared individuals (71 ± 30 ng/g) living in the same social group. However, no control was made for pseudo-replication and so results may have been affected by intra-individual variation (Cockrem, 2013). Even so, this indicates how invasive procedures may cause an acute stress response but not a chronic one. Similarly, non-significant differences in FCM profiles between collared and non-collared African wild dogs (*Lycaon pictus*) reported over a 500 day period, indicated no chronic stress (Creel et al., 1997).

In comparison, other research has shown even minor variations in management conditions may lead to changes in physiological stress. Schmidt and Sachser (1997) monitored FCM levels in a herd of captive white rhinos in response to different foraging conditions. Levels of FCMs were higher in conditions where foraging resources were clumped (one heap per group) than in conditions where feed was dispersed (one heap per individual). The clumped foraging conditions coincided with increased frequencies of agonistic interactions, which were 2-3 times higher than those that occurred in dispersed foraging conditions. However, as the effects of higher FCMs extended to a separated solo male the precise relationship was unclear. The absence of a detectable long-term stress response following dehorning means the technique compares favourably to other forms of rhino management, including translocation, a commonly used conservation intervention that can elevate physiological stress for several weeks (Linklater et al., 2010; Yang et al., 2019).

4.4.5 Multiple dehorning procedures – FCMs

The number of dehorning procedures did not influence rhino FCM profiles. It appears that repeat immobilisations along with the punctuated reductions in horn mass (around 1 kg / dehorning) do not act as a long-term stressor. This corroborates the results of the above FCM analyses and extends the findings to repeat dehorning procedures. If the effects of the GLMM are considered in isolation, 'Time Since First Dehorning' showed a significant positive association with FCM concentration. Thus, rhinos appeared to experience an incremental increase in physiological stress that was unaffected by the number of dehorning procedures. However, the results of the prior analyses do not support dehorning to be the causative factor. The GLMM design means the observed trend is correlative and so environmental, physiological or psychosocial stressors experienced over the course of the study may provide an alternative explanation for the rise in FCMs (Creel, 2001; Kretzschmar et al., 2004; Palme, 2018). For example, reduced availability of foraging resources (Parry-Jones et al., 2016), differences in reproductive state (Edwards and Boonstra, 2018), and changes in social interactions or dominance ranks (Creel et al., 2013) are all factors known to affect animal glucocorticoid responses.

4.4.6 Multiple dehorning procedures – FPMs

FPM concentrations were significantly higher during pregnancy. The increase in FPMs during pregnancy match the findings of MacDonald et al. (2008) who reported variation by as much as two orders of magnitude throughout gestation. To increase the detectability of progestogen suppression, values recorded during pregnancy were excluded from the dehorning analysis. Concentrations of FPMs in non-pregnant rhinos were independent to concentrations of FCMs, qualifying FAMs for use as an additional indicator of stress. Although the observed pregnancy-dependent increases provide no insight into dehorning, they biologically validate the hormone extraction procedure and assays by matching the literature. The non-significant relationship between the number of dehorning procedures and rhino FPM profiles provides evidence that the absence of a stress-induced FCM response was real rather than undetected (Linklater et al., 2010).

Aside from changes in gonadal hormones, differences in fecundity can also aid the evaluation of rhino reproductive health (Rachlow and Berger, 1998). The ICIs from rhinos at Site A (calculated from all historic live births; Appendix 3A) were lower prior to dehorning than after (mean ± sd: pre 35.7 ± 4.6 months, n = 4, births = 20; post: 37.3 ± 6.1 months; n = 4, births = 7; Appendix 3B). However, if only those rhinos for which both pre- and post-dehorning ICIs exist are considered, the pattern reverses (mean ± sd: pre 37.7 ± 9.1 months, n = 2, births = 9; post: 33.0 ± 5.9 months; n = 2, births = 4). Consequently, there was no reduction in fecundity following dehorning and the ICIs corroborate the non-significant influence of dehorning on FPM profiles. Additionally, these ICIs fall within the ranges reported from white rhinos in a reserve in Zimbabwe (mean 34.8 - 39.6 months; Rachlow and Berger, 1998). However, they are higher than those reported in a dehorned game-ranched population (median: 29.2 months; Ververs et al., 2017) and horned captive rhinos (mean: 34 months; Skinner et al. 2006). Although in these cases rhinos are heavily managed to maximise reproductive success and are not kept under natural conditions (Skinner et al. 2006; Ververs et al., 2017). Thus, dehorning does not appear to influence the reproductive health of female white rhinos.

4.4.7 Multiple dehorning procedures – FAMs

Concentrations of FCMs were independent to concentrations of FAMs. This qualified FAMs for use as an additional indicator of stress and matched the findings reported by Brown et al. (2001) on captive white rhinos. The number of dehorning procedures had a significant effect on the rate of change in FAM concentration. Interestingly, FAM levels increased after the third dehorning, rather than an expected suppression if rhinos were experiencing chronic stress. This may indicate a release from chronic stress if rhinos acclimatised to repeat procedures. There is no consensus for what constitutes a stereotypical hormone change in chronically stressed animals (Dickens and Romero, 2013) however, an increase in FCMs is usually anticipated (Palme, 2018). As this was not detected, the observed trends in FAM profiles are unlikely representative of chronic stress, unless FCMs peaked prior to the monitored period and then declined to pre-stressed levels (Linklater et al., 2010). Either way, the potential negative effects on rhinoceros reproductive health appear to be short-term, with FAM levels rising towards the end of the monitored period.

Alternatively, if dehorning disrupted breeding behaviour or dominance, it could influence their reproductive health through a mechanism other than chronic stress (Rachlow and Berger, 1998; Kretzschmar et al., 2004; Ververs et al., 2018). In other species, dominance related changes in hormone level may not just be due to psychosocial factors (Creel, 2013), for example, differing concentrations of FCMs and FAMs in chimpanzees (*Pan troglodytes*) are due to the differing metabolic demands between dominant and sub-dominant individuals (Muller and Wrangham, 2004a, 2004b).

4.4.8 Limitations of the study and future work

It was not possible to test for the presence of sex or age related differences in response to dehorning. However, the overall trends across subjects provide robust group-specific averages as the parameters of the GLMMs controlled for individual variation. The inclusion of a larger number of fixed effects within the GLMMs would have been desirable but the sample size and correlations between variables of interest (e.g. ‘Time Since First Dehorning’ and rhino ‘Age Class’) limited the complexity of the models (Bolker, 2018). While the lack of age related differences justified the pooling of subjects in this study, Badenhorst et al. (2016) excluded juveniles from their analysis after finding them to have lower baseline FCM levels than adults. Studies of other species, including rhino, have also reported no age related effects in FCMs (Brown et al., 2001; Viljoen et al. 2008; Goncalves et al., 2016) or even FAMs (Brvio et al., 2015). Whereas a study of African buffalo (*Syncerus caffer*) found adults but not subadults experienced a spike in in FCMs after chemical immobilisation (Spaan et al. 2017). Thus, the pattern of age related effects is far from clear and acts to underline the difficultly in comparing studies of endocrine profiles between and even within species (Dickens and Romero, 2013; Palme, 2018).

The lack of suitable control conditions for the multiple procedure GLMM analyses means that the apparent link between FAM profiles and dehorning should be interpreted with caution. A major limitation with this study is that the timings of repeat dehorning procedures were inseparable to the increasing age of the study subjects. Throughout the study, several subadults began to exhibit sexual behaviours, such as urine spraying and foot scraping (Owen-Smith, 1975). Sexual behaviours are a predictor of FAM levels in subadult rhinos (Ververs, 2018), although this wasn’t detected in this study. Thus, the observed relationship may reflect developmental changes brought on by the onset of puberty and sexual maturity rather than those caused by dehorning. While cause and effect remain unclear, it appears that repeat procedures do not supress FAM levels over the long-term and are not an ongoing cause of stress. These problems extend to the population-level analysis (analysis 2), for which conditions were not held constant across groups. Rhinos from the horned populations had access to a wider range of habitats NWPB, 2015) than those of the dehorned population. This may in turn have influenced their access to resources (Shrader et al., 2006; Jordaan et al., 2015) which could have affected their endocrine profiles to an extent that would disguise any dehorning-related differences. Even so, the results still indicate that the conditions of the dehorned population were at least no more stressful than those of the horned population.

The precision of the analyses were limited by the inherent variation found within the datasets (Taff et al., 2018). Intra- and inter-assay variation may have occluded the detection of more minor changes in hormone concentration (changes below <16% for FCMs and <10% for FAMs and FPMs). However, as FCMs of white rhinos may increase by two to five times in response to a stressor (Linklater et al. 2010) and up to ten times in the case of the deceased rhino, assay precision is unlikely to have affected detection of a significant trend. Additionally, the choice of antibodies may have been sub-optimal as native hormones are heavily metabolised before excretion (Möstl and Palme, 2002). The previous study on dehorning by Badenhorst et al. (2016) utilised a group specific (5α-pregnane-3β,11β,21-triol-20-one) assay which can detect a wider range of FCMs than a standard corticosterone (4-pregnene-11ß,21-diol-3,20-dione) assay, potentially boosting the signal (Palme, 2018). However, as past studies of white rhino have successfully detected FCM peaks via corticosterone assays in response to a stressor, the approach used here remains valid (Brown et al. 2001; Linklater et al. 2010). Furthermore, the concentrations quantified from a corticosterone assay are likely to include multiple faecal corticoid metabolites due to high cross-reactivates between similar substances (Palme, 2018).

It would be beneficial if future longitudinal studies on rhino stress physiology controlled for life histories, resource availability, habitat type and anthropogenic disturbance, as all of these can influence the endocrine profiles of wildlife species (Creel et al., 2002; Brown and Fuller, 2006; Goymann, 2012; Pokharel et al. 2017; Webster et al., 2018). This could best be achieved through studies of captive or range-limited populations for which the logistics of sample collection and the standardisation of conditions are less challenging (Palme, 2018), as a previous short-term study of rhinos demonstrated (Badenhorst et al., 2016). Future work may also benefit from reductions in the price of genetic analyses that allow for the linking of faecal samples to an individual without requiring direct observation (Coppes et al., 2018).

4.4.9 Conclusion

This research shows that dehorning causes no major alterations in hormone levels, and consequently that the procedure is unlikely to be a cause of chronic stress. Compared to results in the literature, dehorning compares favourably to other large mammal veterinary and management interventions. Dehorning rarely occurs as a one-off procedure (Lindsey and Taylor, 2011), and so the apparent absence of a long-term physiological response to dehorning should give wildlife managers confidence that its practise is not to the detriment of rhino welfare. The frequency of the procedures need not take consideration of induced levels of chronic stress when all other procedural risks are minimised. However, as physiological stress is not the only consideration in animal welfare, further behavioural research is required to understand whether dehorning causes behavioural changes to reproduction, resource access, and sociality. Thus, dehorning should only be used as conservation method when other less invasive anti-poaching techniques are not available or feasible (such as an increased security budget), and the frequency of dehorning should be set to the level that most effectively reduces poaching risk.

Chapter 5 – Horn use in a non-social context

5.1 Introduction

Rhino species use their horns in intra-specific social interactions during courtship, combat and play (Estes, 1991) but also for accessing resources (Richie, 1963; Van Strien, 1985; Owen-Smith and Danckwerts, 1997) and in interspecific defence (Goddard, 1967; Sillero-Zukiri and Gottelli, 1991). Rhinos also regularly rub their horns against objects such as rocks and trees (Pienaar et al. 1991; Rachlow, 2001). While several studies have investigated how differences in horn size relate to the ability to defend against predators (Berger et al., 1993; Berger et al., 1996; du Toit and Anderson, 2013) few have considered how it may affect other horn-based behaviours (Lindsey and Taylor, 2011; Trendler, 2011).

5.1.1 Defensive behaviours

African rhino species use their horns to defend against attacks from predators (Goddard, 1967; Owen-Smith, 1973; Sillero-Zukiri and Gottelli, 1991). However, such attacks are rare, with the calves and subadults of black rhino (*Diceros bicornis*) experiencing infrequent predation from lions (*Panthera leo*) (Western, 1982; Brain et al, 1999; Patton, 2009) and occasional predation attempts from spotted hyenas (*Crocuta crocuta)* (Sillero-Zukiri and Gottelli, 1991). Despite this, research by Berger et al. (1993) showed that for black rhinos sympatric with spotted hyenas, a dehorned population had lower rates of calf survivorship than a horned population. Though given the absence of observed predator attacks, high levels of resource competition with livestock and the effects of an extended drought may have provided an alternative explanation to predation (Loutit and Montgomery, 1994; Berger and Cunningham, 1996; Lindsey and Taylor 2011). In contrast, a study of black rhinos in the Zimbawean Lowveldt found no difference in black rhino calf mortality rates before and after a dehorning procedure (du Toit and Anderson, 2013). As is the case with black rhinos, white rhinos rarely experience non-human predation (Owen-Smith, 2013), with only isolated reports of crocodile- (*Crocodylus niloticus*) and lion-induced mortality in Hluhluwe-Umfolozi National Park, South Africa (Hitchins and Anderson, 1983) and apparent interspecific damage to ears and tails in

Hwange National Park, Zimbabwe (Kock and Atkinson, 1993). Thus, dehorning is not considered to have a major impact on calf survival for either species (Lindsey and Taylor, 2011).

5.1.2 Resource access

Asian and African rhino species use their horns to access resources (Richie, 1963; Van Strien, 1985; Owen-Smith and Danckwerts, 1997). Both Sumatran (*Dicerohinus sumatrensis*) and black rhinos utilise their horns to pull down branches (Van Strien, 1985; Awaliah et al., 2017) and break stems to bring them within reach of feeding (Owen-Smith and Danckwerts, 1997). Similar foraging behaviours are observed in other ungulates, for example eland (*Taurotragus oryx*) and kudu (*Tragelaphus strepsiceros*) will use their horns to break branches and knock down small trees (Owen-Smith 1993; Nyengera and Sebata, 2010). In comparison, there are no reports of white rhinos using their horns as a foraging tool. This is because they are predominantly short grass grazers (Shrader et al., 2006; Jordaan et al., 2015) with forbs consisting of no more than 1% of their diet (Owen-Smith, 2013).

There are no accounts of rhinos using their horns to dig for water, although black rhinos have been observed using their forelimbs to dig holes during dry environmental conditions (Richie, 1963). However, both Sumatran and black rhinos have been observed using their horns to break up earth and soft rocks at salt licks (Richie, 1963; Borner, 1979). The soil or rock is then ingested, in a behaviour known as geophagy (Abrahams, 2013). In large mammals, soil intake is generally selective (Klaus et al., 1998) and can act to supplement minerals otherwise missing from a diet (Brightsmith and Muñoz-Najar, 2004), to assist in digestion (Wings and Sander, 2006) and to aid the absorption of tannins, alkaloids, bacteria or toxins (Gilardi et al., 1999; Brightsmith et al., 2008). Other horned geophagus species such as the bongo (*Tragelaphus eurycerus*) and African buffalo (*Syncerus caffer*) are also known to use their horns to dig up mud (Hillman, 1986). In contrast, Indian rhinos (*Rhinoceros unicornis*) will use their large incisors to dig into earth instead (Hazarika and Saikia, 2010). A black rhino was also observed digging with its horn prior to placing its mouth in mud in an apparent attempt to relieve buccal irritation following consumption of a toxic plant (Wood et al, 1997). Given that white rhinos also deliberately ingest soil and are known to dig with their horns (Owen-Smith, 1973), dehorning could affect their access to some minerals.

Rhinos also use their horns during wallowing behaviours (Owen-Smith, 1973; Van Strien, 1985) where they lie and roll in shallow pools of muddy water (Estes 1991). Wallowing acts to protect the skin from parasites and aid in thermoregulation on hot days (Balfour and Balfour 1991; Metrione and Eyres, 2014). However, unlike the horns of several tropical bovid species (Picard et al., 1999) rhino horns have no thermoregulatory function. This is because they lack a vascularized bony core that in other species can be structured to facilitate heat loss (Picard et al., 1999; Boy et al., 2015). Prior to wallowing, Sumatran rhinos dig soil from the banks of pits with their horns and feet, a practise that likely acts to thicken the consistency of the mud (Van Strien, 1985). White rhinos have also been observed digging their horns into mud before wallowing (Owen-Smith, 1973). Such behaviour may allow them to test the consistency of the mud, with individuals observed to move elsewhere when the mud is too thick (Owen Smith, 1973). If dehorning reduces or prevents digging in white rhinos it may affect their ability to wallow.

5.1.3 Rubbing behaviour

Rhinos often rub their bodies against objects such as dead wood, rocks and trees to aid in the removal of parasites and dead skin, particularly after wallowing (Balfour and Balfour 1991; Pienaar, 1994; Metrione and Eyres, 2014). All rhino species also regularly rub their horns against objects (Laurie 1978; Van Strien, 1985; Pienaar et al., 1991; Hariyadi et al., 2010) with the resultant lateral and anterior wear contributing towards its tapered shape (Hieronymus et al., 2006). This is obvious in captivity when rhinos that have had access to abrasive materials such as concrete and steel wire show excessive amounts of horn wear (Hutchins and Kreger, 2006; Metrione and Eyres, 2014). Without wear or keratin degradation, horns would form a gently curving cylinder rather than a cone (Hieronymus et al., 2006). The functional significance of horn rubbing may extend beyond the maintenance of horn shape (Rachlow, 2001). In a study of free ranging white rhinos in Zimbabwe, adult males were observed to rub their horns on paperbark commiphora trees (*Commiphora marlothii*) for longer periods than other tree species

(Rachlow, 2001). Additionally, an estimated 30% of commiphora within the reserve showed signs of debarking that were likely caused by horn or body rubbing behaviour. In this case, rhinos may have been selecting trees to ingest resin to satisfy a nutritional or medicinal need, or to convey information through scent deposition. In contrast, when Sumatran rhinos purposefully twist saplings with their horns and head it may act as a visual signal alongside olfactory marking behaviours as the plants show no evidence of browsing (Borner, 1979; Van Strien, 1985).

The frequencies of horn rubbing behaviours can vary between individuals but are assumed to remain near constant throughout an adult rhino's life (Pienaar et al. 1991). For adult white rhinos, the rate of horn growth decreases with age (Rachlow and Berger, 1997), and thus the horns of some of the oldest individuals may decrease in size if the rate of rubbing exceeds the horn's intrinsic growth rate (Pienaar et al. 1991). Reports suggest that male white rhinos rub their horns more frequently than females (Pienaar et al. 1991; Pienaar and Hall-Martin, 1991). Observations of immobilised rhinos in Kruger National Park indicated that the horn bases of adult males were often smoother than the horn bases of females, which were more frayed and fibrous (Pienaar et al., 1991). In subadults (<8 years old) the difference in smoothness was not so defined, indicating higher rates of rubbing in adult male rhinos over females. Additionally, Pienaar and Hall-Martin (1991) observed differential rates of wear in horns implanted with transmitters, further suggesting that males rub their horns more frequently than females. Additionally, observations of free-ranging Indian rhinos indicate that males show faster rates of horn wear than females (Laurie 1978).

Pienaar et al. (1991) noted that the horns of both black (Bigalke, 1946) and white rhinos (Klös, 1969), appear to grow back faster in the first year after horn loss. Faster rates of growth were also observed in dehorned populations than horned populations (Rachlow and Berger, 1997), with the observed growth rate of dehorned individuals almost as high as the intrinsic growth rate of horned individuals (Pienaar et al., 1991; Lindsey and Taylor, 2011). While Rachlow and Berger (1997) reported lower rates of wear on the dorsal surfaces of regrown horn stubs than in horned rhinos. Recent work by Verver (2018) failed to conclude whether the number of dehorning events or age of a rhino at first dehorning influenced horn regrowth rates because older animals, which experience lower growth rates than younger individuals, had experienced more dehorning events, (Lindsey and Taylor, 2011). Thus, it is possible that decreased horn rubbing rates may in part be responsible for the apparent stimulation of horn growth following dehorning.

5.1.4 Aims

Very few studies have reported non-social horn use behaviours within either horned or dehorned free-ranging white rhinos. This study sought to document and describe these horn use behaviours and to determine whether dehorning impacts on their frequency or function. The primary research questions were: (1) does dehorning influence resource access or wallowing behaviours and (2) does dehorning cause a reduction in horn rubbing behaviours? The secondary research question was does the frequency of horn rubbing differ between the two sexes. Objective one was to investigate whether horn digging behaviours occurred during geophagy or wallowing in both dehorned and horned rhinos. Dehorned rhinos were predicted to show fewer horn digging behaviours than horned rhinos. Objectives two and three were to investigate whether the frequency of horn rubbing behaviours changed relative to levels of horn growth or sex. Horn rubbing was predicted to be lower after dehorning when horn mass was at its lowest and males were predicted to rub their horns more than females.

5.2 Methods

5.2.1 Experimental design

Behavioural observations of white rhinos took place between 24/10/2015 and 15/11/2017. Observations were split between Site A (dehorned population) and Site B (horned population) (site parameters are listed in Chapter 2). Behaviours of interest (Table 5.1) were scored directly in the field and through camera trapping. Rhinos were classified by their identifiable features (See Chapter 2). Field observations occurred in both Site A and Site B but all camera trapping occurred within Site A only, due to the difficulty of searching for and revisiting locations on foot in Site B. Behaviours recorded during camera trapping were scored through all occurrence sampling (See Chapter 2). Camera traps were placed at salt licks, mud wallows and rubbing posts that showed recent signs of rhino activity (Table 5.2). Cameras were attached to trees around 1.5 metres high and 5-10 metres away from the focal point. Videos recorded for 30 seconds and were separated by intervals of 10 seconds (See Chapter 2). The operating period of each camera was calculated by subtracting the time of the last recorded video from the time of camera setup. Rhinos were located for field observations by following haphazard search routes across both reserves. Behaviours observed in the field were recorded through all occurrences sampling. Field observations continued ended when rhinos lay down to rest or it was too dark to identify them.

Table 5.1. Behaviours of interest that were recorded by all occurrence sampling during field observations and from camera trap footage.

Behaviour	**Definition**
Geophagy	Active ingestion of earth or rock
Wallowing	Rolling or lying in mud
Limb digging	Repetitive movement of earth by the feet or limbs
Horn digging	Repetitive movement of earth by the horn
Body rubbing	Repetitive movement of the body against an object
Horn rubbing	Repetitive movement of the horn against an object

5.2.2 Digging

All acts of geophagy and wallowing were monitored for limb and horn digging behaviours. In geophagy, soil intake is usually selective (Abrahams, 2012) but no examples of natural salt licks could be located. Thus, cameras were placed at two artificial salt blocks on six occasions to establish their usage by rhinos (Figure 5.1). Cameras were also placed at 10 mud-filled depressions on 30 occasions (Figure 5.1). Cameras at the salt blocks operated for 567:33 hours (Table 5.2) and had an average operational period of 81:04 ± 42.32 hours (mean + SD) (n = 6). Cameras at the mud wallows operated for 3306:35 hours (Table 5.2) and had an average operational period of 110:13 ± 49.55 hours (mean + SD) (n = 30).

5.2.3 Rubbing

Rubbing posts were identified as tree stumps, trunks and branches with evidence of wear between 0.5 m and 1 m high along trails frequented by rhinos. Cameras were placed at 9 rubbing posts on 36 occasions (Figure 5.1). Cameras operated for 4687:43

hours (Table 5.2) and had an average operational period of 130:13 ± 4.01 hours (mean + SD) (n = 36).

Table 5.2. Number of locations, sessions and lengths of operating periods for camera traps placed at rubbing posts, mud wallows and salt licks. Location numbers correspond with those listed in Figure 5.1. Camera traps that failed to record were excluded from the totals, as were periods for which the feature became obscured (e.g. due to animal interference).

Feature	Location ID	No of Sessions	Total period (H:M)	Average period (H:M)
Rubbing Post	1	4	520:38	130:09
	2	2	600:44	300:22
	3	10	1177:05	117:42
	4	5	926:26	185:17
	5	1	70:28	70:28
	6	1	118:36	118:36
	7	3	311:09	103:43
	8	5	476:48	95:22
	9	5	485:49	97:10
	Total	36	4687:43	130:13
Salt Lick	10	4	346:56	86:44
	11	2	178:05	89:02
	Total	6	567:33	81:04
Mud Wallow	12	1	128:38	128:38
	13	2	469:06	234:33
	14	2	213:05	106:32
	15	7	674:07	96:18
	16	2	260:27	130:13
	17	4	500:50	125:12
	18	1	62:12	62:12
	19	2	144:04	72:02
	20	7	602:02	86:00
	21	2	252:04	126:02
	Total	30	3306:35	110:13

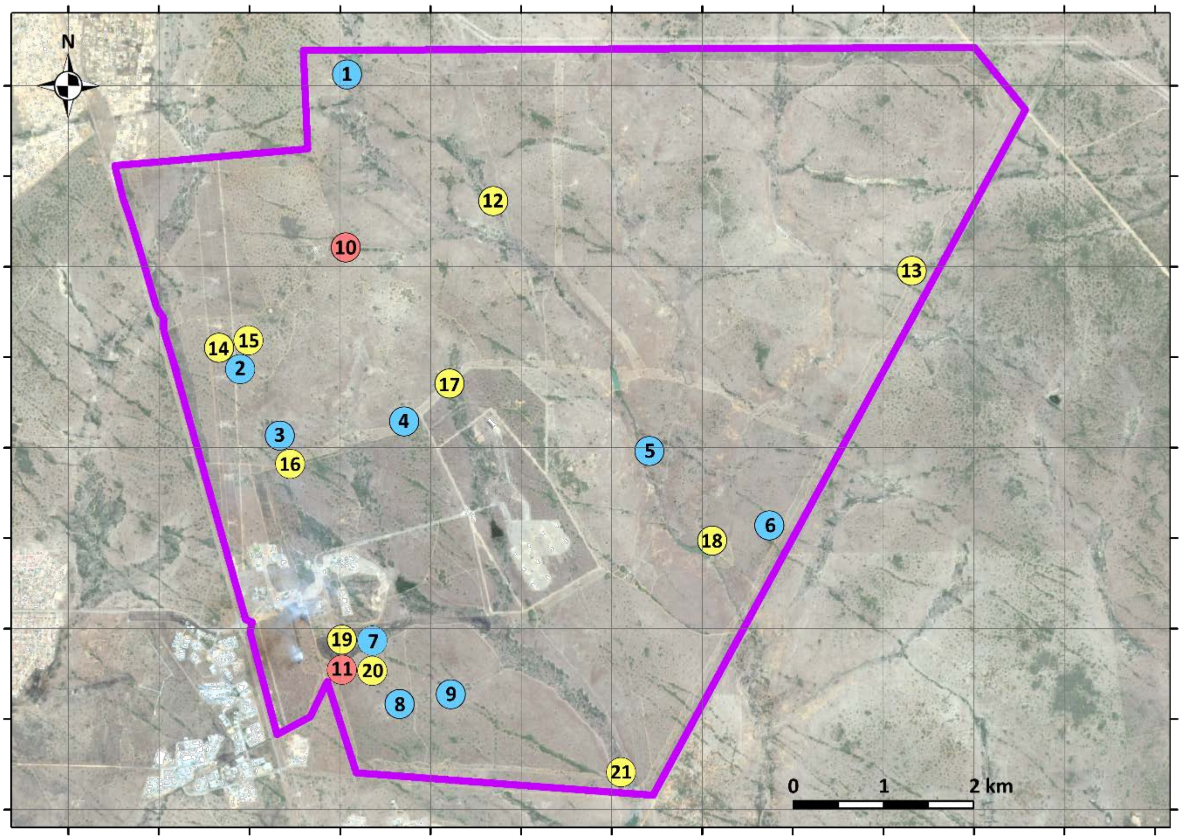

Figure 5.1 Camera trap locations of rubbing posts (1-9 blue), salt licks (10-11 red) and mud wallows (12-21 yellow) within Site A. Location numbers correspond to those listed in Table 5.1.

5.2.4 Data Analysis

For horn digging, no statistical analyses were undertaken due to its rarity of observation. Instead, these data are presented descriptively. For horn rubbing, statistical analysis was conducted on data collected during camera trapping but not on data collected during field observations, again due to the latter's rarity of observation. Data were only included in analyses if the rhino could be identified. All analyses were two-tailed with alpha levels set at 0.05. The multitude of potential rubbing posts across the reserve prevented their monitoring in totality. Thus, it was not possible to determine how true rates of horn rubbing were affected by dehorning, as monitored changes in rubbing rates over time may have represented changes in the frequencies of post visitation. Instead, the first analysis investigated whether the duration of horn rubbing behaviours (the dependent variable) was influenced by the amount of horn growth a rhino had at the time of rubbing (the independent variable). Discontinuous horn rubbing behaviours were summed to create a single total if there was a pause of less than 15 minutes between them. The amount of horn growth at the time of rubbing was categorised into one of two groups: greater than 12 months of growth or less than 12 months of growth (measured from the time since a rhino's last dehorning or the time since birth, whichever was most recent). The data in each group was non-normally distributed (See Chapter 2) and so a Mann-Whitney U test was performed.

The second set of analyses looked at changes in the likelihood of horn rubbing when a rhino visited a post. A visit was defined as engagement in either body rubbing and/or horn rubbing by a rhino at a post. Discontinuous horn and/or body rubbing behaviours separated by a pause of less than 15 minutes were considered part of the same visit. Likewise, discontinuous rubbing behaviour separated by a pause greater than 15 minutes were counted as separate visits. Thus, the dataset consisted of counts of the presence or absence of horn rubbing behaviour at posts during visits. A chi-squared test was used to establish the degree of independence between engagement in horn rubbing while at a post (the dependent variable) and horn growth (the independent variable). As with the first analysis, horn growth was categorised as having either greater than or less than 12 months of uninterrupted growth. A second chi-squared test was used to establish the degree of independence between engagement in horn rubbing while at a post (the dependent variable) and sex (the independent variable).

5.3 Results

5.3.1 Horn digging

No instances of horn digging were observed during geophagy in either horned or dehorned rhinos. However, two rhinos exhibited limb digging during the four instances of geophagy recorded from horned rhinos. In the first observation, two horned adult males ingested dirt from a low earthen bank (Male 1 for 470 seconds; Male 2 for 330 seconds). To loosen the earth, the males dug with their feet but neither used their horns (the first male dug for 6 to 13 seconds on seven occasions; the second male for 10 seconds on one occasion). During digging, the animals lowered their heads and touched their anterior horns to the ground but their horns did not break the soil (**Error! Reference source not found.**a). In the second observation of geophagy, a horned adult female and her calf ingested dirt from an area of flattish ground without demonstrating limb- or horn-based digging behaviours. During the six instances of geophagy recorded from dehorned rhinos (5 during a field observation and one during camera trapping) neither horn nor limb digging was observed. In the one field observation, a group of five dehorned subadult rhinos ingested dirt for up to 20 minutes each. The five rhinos directed aggressive behaviours towards one another throughout consumption but no horn or limb-based digging occurred. One observation of geophagy was observed during the 567 hours of camera trapping at salt licks. In this case, a dehorned adult female ingested minerals from an artificial block without digging. In addition to this, two dehorned rhinos were recorded approaching and then sniffing a mineral block but did not engage in geophagy.

One observation of horn digging was recorded from a horned rhino and seven from dehorned rhinos during wallowing behaviour (six during field observations and one during camera trapping). The horned rhino was an adult male that dug the frontal base of its anterior horn into the ground (for 61 seconds; **Error! Reference source not found.**b) and a forelimb (for eight seconds) before wallowing in the freshly churned mud (for 533 seconds). After wallowing, the rhino rubbed its anterior horn on a nearby branch until all large clods of mud had been dislodged. In the observations of dehorned rhinos, a group of five subadult rhinos dug their anterior horns into a mud-filled depression (for up to ten seconds each) with one rhino also digging with a forelimb (**Error! Reference source not found.**c). The rhinos had 541 to 548 days of horn growth at the time of the observation. Immediately after digging, two of the rhinos wallowed (for 70 seconds) but all five rhinos then left to follow a sixth rhino that had not stopped at the depression. In the second observation, a dehorned adult male dug its anterior horn into mud (for 5 seconds) during 15 minutes of wallowing. The rhino had been dehorned recently and had just 10 days of horn growth. During the 3,306 hours of camera trapping at mud wallows, rhinos were observed wallowing on 20 occasions, however only one of these included horn digging behaviour. In this case, a 12-month-old calf stood during wallowing to dig its anterior horn into the mud (for 10 seconds) then lay down again. The individual had been dehorned 42 days prior to the observation.

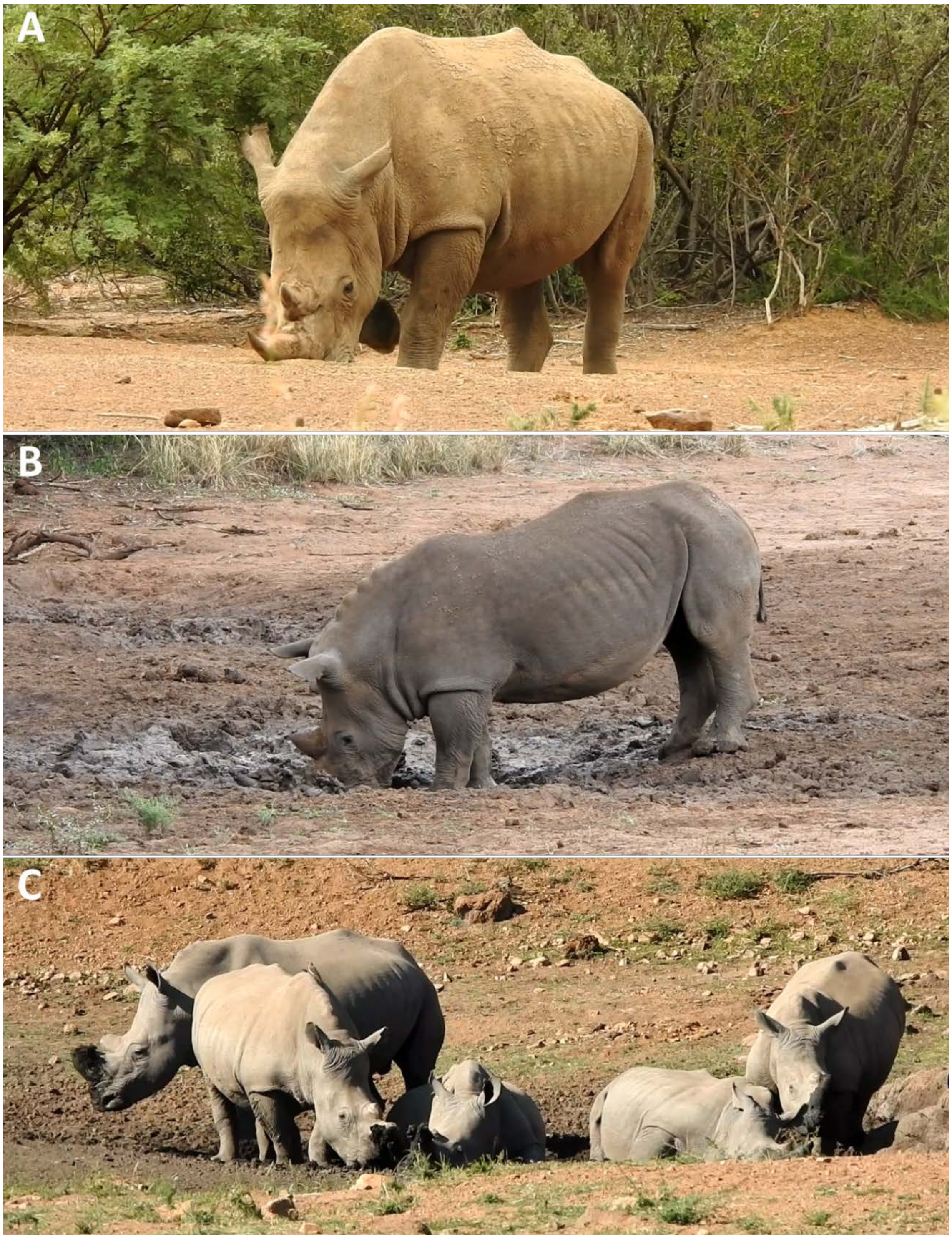

Figure 5.2. Examples of digging behaviour in white rhinos recorded during the study: A) A horned white rhino digs with a forelimb while pressing its horn to the ground to loosen earth before ingestion; B) a horned rhino digs with its horn before wallowing in mud; C) five dehorned rhinos with fresh earth on their horns after digging in a muddy depression.

5.3.2 Horn rubbing

Two instances of horn rubbing were detected from horned rhinos and 29 from dehorned rhinos (2 during field observations and 27 during camera trapping). In all cases, rhinos rubbed their horns against wooden branches, trunks and stumps (Figure 5.3A-B). For the observations of horned rhinos, an adult male rubbed its horns (for 41 seconds) and body against a stump after wallowing; and a horned adult male rubbed its horns against a branch (for 110 seconds) after wallowing but did not exhibit body rubbing. For the two field observations of dehorned rhinos, a subadult male rubbed its horns against a trunk (for 20 seconds) and a subadult female rubbed its horns against a branch (for 40 seconds) after wallowing.

During the 3,306 hours of camera trapping, rhinos visited all nine of the monitored rubbing posts. Rhinos visited the posts on 87 occassions (where either body or horn rubbing occurred; calculated from 307 records of rhinos in 267 camera trap videos) but rhino identity could only be established in 80 of these. The most common time for using rubbing posts was between 14:00 and 16:00 (34.9% of 307 records) and the least common time was between 04:00 and 6:00 (0.3% of of 307 records; Figure 5.4). Rhinos were observed horn rubbing during 27 of the 87 visits but individual identities could only be established for 25 of these (for 15 different individuals). The first horn rubbing was observed 2 days after dehorning and the last instance 702 days after the procedure.

Horn rubbing duration for rhinos with less than 12 months of horn growth (median: 20.5 sec, n = 16) did not differ significantly to rhinos with greater than 12 months of horn growth (20 sec, n = 9) (Mann-Whitney: W = 70, p = 0.932, n = 25; Figure 5.5). The frequency of horn rubbing during post visits for rhinos that had less than one year’s uninterrupted horn growth (30.2% of 53 visits) was similar for those with greater than one year’s growth (33.3% of 27 visits) as shown by the lack of a significant association ($\chi2$ (1) = 0.001, p = 0.975, n = 80; Figure 5.6). Additionally, there was no significant association between the frequency of horn rubbing during post visits and a rhino’s sex (male: 27.5% of 58 visits; female: 40.9% of 22 visits; $\chi2$ (1) = 0.771, p = 0.380, n = 80; Figure 5.7).

Figure 5.3. Horn rubbing and licking behaviours at rubbing posts. A) A horned rhino rubs it anterior horn against a stump B) a dehorned rhino rubs its anterior horn against a tree trunk; C) a horned calf nuzzles a rubbing post before horn rubbing. The individuals also rubbed their heads and bodies against the rubbing posts.

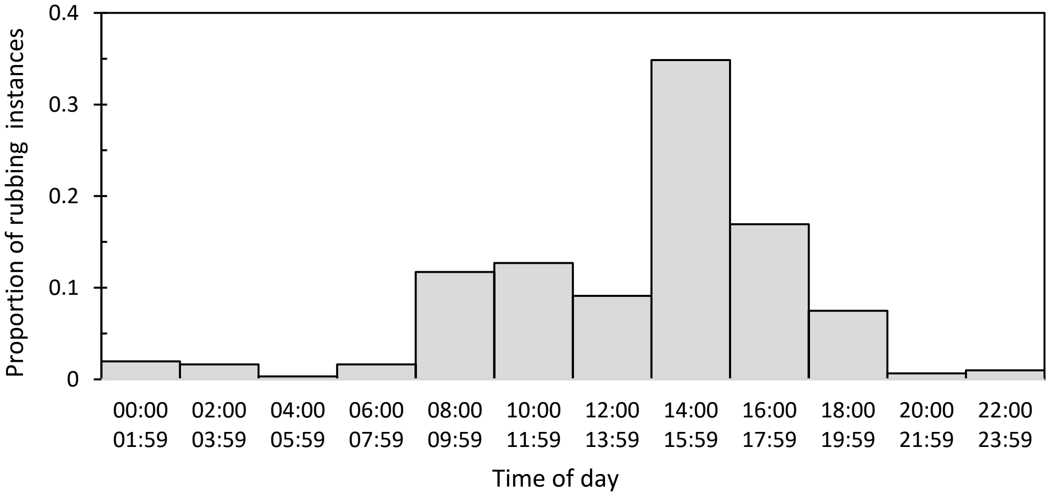

Figure 5.4. Breakdown of time spent horn or body rubbing at rubbing posts from 307 records during camera trapping. Totals represent the proportion of the 307 records that occurred within each two-hour period.

Rhinos were also observed rubbing their mouths against, licking and apparently smelling the surfaces of posts. For example, a dehorned adult male was recorded intermittently licking the sap from the tilted trunk of a Sweet thorn (*Vachellia karroo*) tree and rubbing its body against it. While a horned calf was detected licking a polished wooden stump for over a minute that had no sap residue (**Error! Reference source not found.**C). During and after nuzzling the stump, the calf lifted its head and exhibited the flehmen response, before proceeding to rub its body and horn. Both the licked branch and polished stump showed evidence of regular wear and were observed being rubbed on by other rhinos on several other occasions.

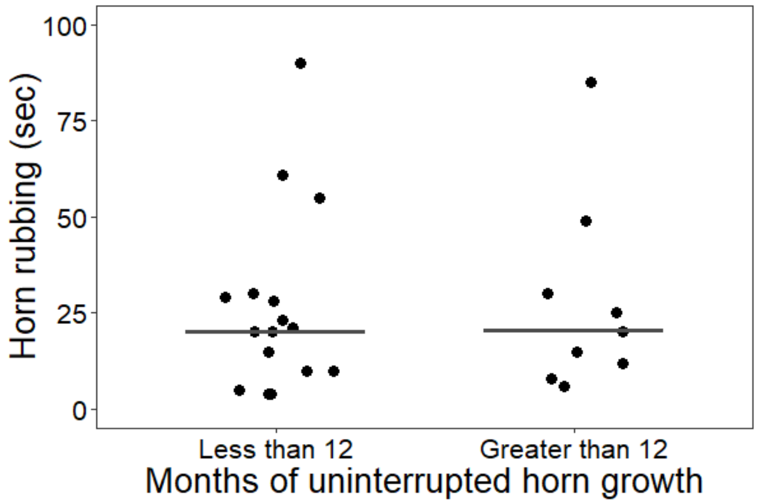

Figure 5.5. Duration of horn rubbing behaviours recorded for rhinos with less than 12 months of uninterrupted horn growth (n = 16) and rhinos with greater than 12 months of uninterrupted horn growth (n = 9). Horizontal bars show medians; data are horizontally jittered.

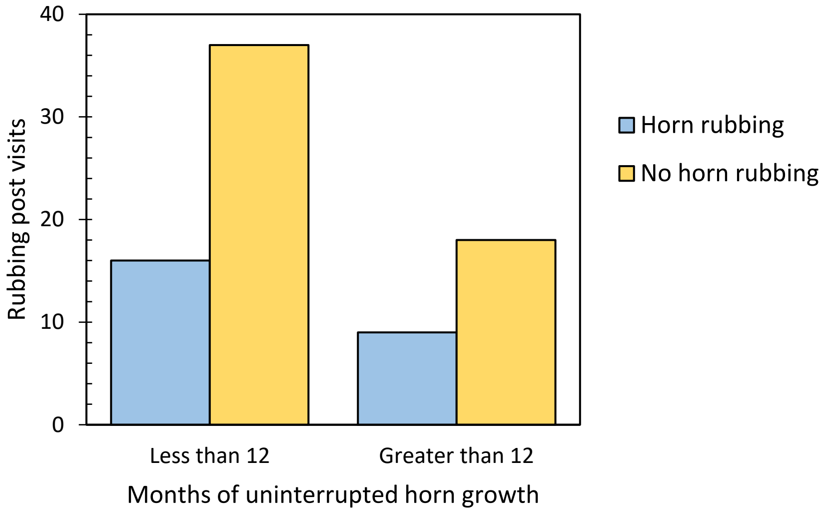

Figure 5.6. Frequency of horn rubbing during post visits for rhinos with less than and greater than 12 months of horn growth.

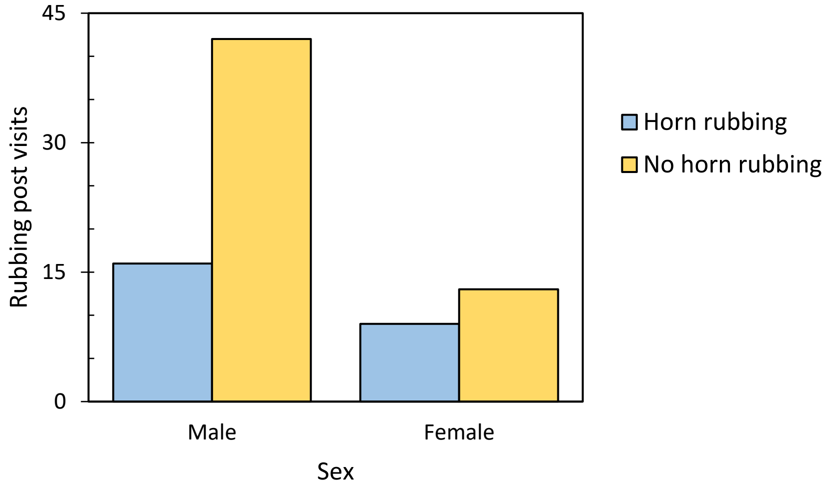

Figure 5.7. Frequency of horn rubbing during post visits for male and female rhinos.

5.4 Discussion

Dehorning did not have a noticeable impact on digging behaviours during geophagy and wallowing, or influence the frequency of horn rubbing behaviours. Rhinos were observed to dig with their feet during geophagy rather than their horns, indicating that dehorning is unlikely to affect a rhino's ability to access minerals. The observed usage of artificial mineral licks suggests the animals were ingesting salts to satisfy a mineral deficiency, rather than consuming grit to aid digestion (Wings and Sander, 2006) or to neutralise toxins from clays (Brightsmith et al, 2008). There are few instances of free-ranging white rhinos engaging in geophagy described in the literature, but the records reported here differ to those described by Owen-Smith (1973), where calves in Hluhluwe–Imfolozi Park were occasionally seen nibbling at and ingesting soil around termite mounds, but adult rhinos were not observed engaging in geophagy at all. Differences between populations may relate to how underlying soil geomorphologies contribute towards plant mineral concentrations (McNaughton, 1988), with rhinos in some habitats apparently able to satisfy their dietary requirements from food or water without the need to ingest soil (Owen-Smith, 1973). This can be seen in areas of the Serengeti, where high densities of grazers survive without the need of salts licks because the mineral rich grasses are nutritionally sufficient (McNaughton, 1988).

Both horned and dehorned rhinos utilised their horns when digging at wallows, with individuals also using their feet. It was not possible to calculate horn digging frequencies at wallows as the behaviours were observed only infrequently. Digging at wallows may have been more common than observed (at just 5% of camera trapped wallowing events), as the large size of some wallows and the placement of rhinos relative to the camera meant that clear observations were not always possible. Additionally, cameras were not out permanently and many locations were not monitored. Given that rhinos dug for periods after wallowing had already commenced, it seems likely that the behaviour acts to improve the consistency of the mud, as suggested by Van Strien (1985) rather than simply as a test of whether or not the mud is suitable for wallowing as suggested by Owen-Smith (1973).

The amount of horn growth had no detectable effect on horn rubbing frequency or duration and horn rubbing was observed just two days after a dehorning procedure. The hypothesis that increased horn growth rates after dehorning could be explained by a decrease in horn rubbing frequency was therefore not supported. There was also no evidence that male rhinos rubbed their horns more frequently than females as suggested elsewhere (Pienaar and Hall-Martin, 1991). Investigative sniffing and mouthing behaviours indicated rhinos were aware of previous users and that rubbing posts play some role in olfactory communication as suggested by Rachlow (2002). Additionally, apparent ingestion of tree sap shows there may be some nutritional basis for the selection of certain rubbing locations. Future research could identify whether rhinos rub on a preferred species of tree.

5.4.1 Conclusion

Despite suggestions that dehorning may impact on non-social horn-based behaviours in rhinos (Trendler, 2011) no evidence was found that dehorned white rhinos were functionally constrained when engaging in digging or rubbing behaviours. The dehorned rhinos engaged in similar behaviours to those of horned rhinos reported elsewhere in the literature (Owen-Smith, 1973; Hutchins and Kreger, 2006). The infrequent observance of horn use in both horned and dehorned populations show that the social function of horns during agonistic encounters, as documented in Chapter 3, are likely to be a more important consideration when planning management strategies for their conservation.

Chapter 6 – Eliciting rhinoceros avoidance behaviour using novel deterrents

The research from this chapter appears in the following publication:

Penny SG, White RL, Scott, DM, MacTavish L, Pernetta AP. 2019 Using drones and sirens to elicit avoidance behaviour in white rhinoceros as an anti-poaching tactic. Proc. R. Soc. B 20191135. http://dx.doi.org/10.1098/rspb.2019.1135

6.1 Introduction

Poaching risk for rhino populations is not homogenous across their distribution and may vary from the national to the protected area scale (Park et al., 2015; Haas and Ferreira, 2017; Emslie et al., 2019). To reduce poaching risk, rhinos are increasingly translocated between reserves and even countries (Ferreira et al., 2015; Joubert and Kent, 2016; Hayward et al., 2018; Rees, 2019). Deterrents, which act to establish avoidance behaviours in animals (Götz and Janik, 2010), have the potential to move at-risk rhinos away from areas of danger at a more localised scale. This study focuses on the design and evaluation of deterrent-based techniques for use as an anti-poaching tactic. Such conservation strategies could help wildlife managers respond to spatially and temporally dynamic variations in poaching risk.

6.1.1 Poaching risk

Rhino poaching risk is influenced by biophysical (Ferreira et al., 2015), geopolitical (Büscher and Ramutsindela, 2015) and socioeconomic factors (Challender and MacMillan, 2014). Limited conservation resources are therefore focused on those areas exposed to the greatest levels of poaching risk (Haas and Ferreira, 2017). Several factors are predictive of the probability of rhino poachers operating in a given area, including the distance from the nearest water sources, buildings, vegetation, and roads, the number of rhinos in the area and the topography (Park et al., 2015). A spatiotemporal analysis of poaching patterns in African bush elephants found similar results, with the density of conspecifics, roads and rivers, condition of the vegetation, and distance from anti-poaching bases all acting as indicators of poaching risk (Maingi et al., 2012). Other studies have confirmed poaching risk to be a function of an area's proximity to boundaries, rivers and roads or centred on geographic features such as waterholes or specific land cover types (Kyale et al., 2011; Ouko, 2013; Shaffer and Bishop, 2016; Sibanda et al., 2016). Rhino poaching risk is also dependent on the time of day, and phase and position of the moon (Koen et al., 2017). Twilight and night are the preferred time of poaching, particularly when they coincide with increased levels of lunar illumination (Koen et al., 2017) which will aid hunting but also poacher interdiction by rangers (Haas and Ferreira, 2017). Poachers will also take advantage of bad weather conditions, which may limit the scope of patrols and increase their probability of escape (Koen et al., 2017).

In South Africa, Kruger National Park has experienced the greatest losses to poaching, but saw a 24% reduction in poaching between 2016 and 2017, linked to improvements in anti-poaching security (Molewa, 2018). Over the same period, poaching increased in the provinces of KwaZulu-Natal, Northern Cape, Mpumalanga, Free State and North West (Molewa, 2018), with a particularly large increase in Hluhluwe-Imfolozi National Park (Ezemvelo KZN Wildlife, 2018). Poaching mortalities can be reduced by permanently translocating rhinos out of areas with high poaching pressure into areas where they can be better protected (Ferreira et al., 2015; Joubert and Kent, 2016; Hayward et al., 2018). However, if translocations are not possible or desirable, and poaching hotspots still occur, rhinos can be moved *in situ* instead (Rees, 2019).

Physical barriers, such as secondary interior fences are one method of controlling animal movement, however, barriers may reduce an area's carrying capacity and involve considerable expense to construct and maintain (Hayward and Kerley, 2009). Other strategies rely on 'pull' factors (attractants) to encourage an animal into an area by modifying the distribution and availability of desirable resources such as forage, waterholes or saltlicks (Smit et al., 2007; Jackson et al., 2008). For example, white rhino populations readily consume the new growth of burnt *Themeda* grasslands (Shrader et al., 2006). Thus, if grasslands were actively burned in areas with low poaching risk it could help attract rhinos to safer zones. Non-physical structures such as sounds and smells can also act as 'metaphorical fences' (Hayward and Kerley, 2009). Known as deterrents, these 'push' factors work actively, to drive an animal from an area it has already entered, or passively, by preventing or dissuading an animal from entering an area in the first place (Osborn and Parker, 2003). Used in this context, deterrents could inhibit rhino encroachments into specific areas and represent a prospective low-cost and *in situ* method to reduce poaching risk.

6.1.2 Mechanism of deterrents

Deterrents evoke avoidance behaviours in animals by exploiting defensive or anti-predator behavioural responses (Götz and Janik, 2010). For example, aversive or threatening stimuli can elicit fear or anxiety in a target subject, increasing real or perceived risk to a point where the costs of utilising a resource or area exceed its benefits (Schakner and Blumstein, 2013). Fear results in a fight or flight response, while anxiety increases risk assessment (such as vigilance) or results in the avoidance of the anxiety-associated stimulus or area (Stankowich and Blumstein, 2005; Grillon, 2008; Schakner and Blumstein, 2013). The strength of a behavioural response depends on the level of perceived risk (Frid and Dill, 2002), which in turn depends on an animal's life history and motivational state (Staddon et al., 1993; Epple et al., 1995, Cunningham and Berger, 1997; Gilsdorf et al., 2002; Götz and Janik, 2010). Given the analogies with predation risk, deterrents can be categorised as: stimuli reliant on pain (e.g. exposure to electric shocks in coyotes *Canis latrans* [Andelt et al., 1999]); stimuli predictive of threat (e.g. broadcast of alarm foot stomps to western grey kangaroos *Macropus fulignosus* [Biedenweg et al., 2011], or stimuli aversive through disruption (e.g. light from reflective prisms in Chacma baboons *Papio ursinus* [Kaplan and O'Riain, 2015]).

However, repeated stimulation can diminish a behavioural response through habituation, sensory fatigue or motor fatigue (Rankin et al., 2009), and thus limit the long-term effectiveness of a deterrent (Osborn and Parker, 2003). Habituation occurs when a response decreases in frequency and/or magnitude until it reaches an asymptotic level (Rankin et al., 2009. It can be distinguished from sensory fatigue and motor fatigue by the process of dishabituation, in which a diminished response will at least partially recover on presentation of a different stimulus (Rankin et al., 2009). Habituation will not always occur (Biedenweg et al., 2011), and can be delayed if an animal curtails its exposure to the stimulus through avoidance behaviour (Schakner and Blumstein, 2013).

6.1.3 Usage of deterrents

Deterrents are widely used as a means to reduce or mitigate human-wildlife conflict, acting to disrupt an animal's behaviour that would otherwise negatively influence a human goal. Deterrents have been used to reduce crop raiding (Wallace and Hill, 2016), decrease depredation in commercial fisheries or livestock holdings (Morton and Symonds, 2002), and to prevent of injury or loss of life (Smith et al., 2008). For a strategy to be successful it must be tailored towards a species' susceptibility to a stimulus (Gilsdorf et al., 2002; May et al., 2015). Consequently, methods to curtail or modify animal movement vary widely, but frequently employ olfactory (e.g. Seamans et al., 2002; Chelliah et al., 2010), visual (e.g. Musiani et al., 2003, Hsiao et al., 2013) and auditory stimuli (e.g. Morton and Symonds, 2002; King et al., 2007), or even rely on novel technologies such as drones (e.g. Hahn et al., 2017). To date, only two studies have reported the use of deterrents in rhino management, in which electric fences were found to be effective at reducing crop raiding in Indian rhinos *Rhinoceros unicornis* in Nepal (Bailey, 2011) and foot and vehicle patrols were used to herd white rhinos away from unsafe locations in Botswana (Rees, 2019). White rhinos will usually show vigilance towards a disturbance, only responding with flight if the threat is deemed significant (Owen-Smith, 1973). For example, white rhinos will respond to a moving person at upwards of 80 m, but flight only consistently occurs at a range of less than 50 m (Owen-Smith, 1973). Acoustic deterrents

White rhinos have a large acoustic repertoire and are strongly reliant on sound to detect changes in their environment (Owen-Smith, 1973; Policht et al., 2008; Cinková and Policht, 2014; Linn et al., 2018). This predisposes them towards acoustic disturbances (Owen-Smith, 1973) and may make them susceptible to broadcasts of sound stimuli. Acoustic deterrents can elicit a generalised threat response through loud or novel noises, such as with bangs in rabbits *Sylvilagus* and *Lepus* spp. (Gilsdorf et al., 2002) or repel animals through pain and discomfort, such as with artificial tones in phocid seals *Halichoerus grypus* and *Phoca vitulina* (Götz and Janik, 2010). Alternative strategies exploit biological cues, such as the warnings of hetero- or con-specifics, as with the broadcast of bird alarm calls (Bomford and O'Brien, 1990), or mimic direct risks, such as the playback of felid growls and human shouts to Asian elephants *Elephas maximus* (Thuppil and Coss, 2016). Deterrents that utilise bioacoustics require high fidelity copies of the original signal or risk failure (Gilsdorf et al., 2002). For example, the ineffectiveness of foot-thump broadcasts at deterring red-necked wallabies (*Macropus rufogriseus banksianus*) may have been due to the absence of an infrasound component (Ramp et al., 2011). Other acoustic deterrents rely on conditioned responses towards unpleasant experiences (King et al., 2007). For example, African bush elephants exposed to the noise of African honeybees *Apis mellifera scutellata* will flee from the sound source (King et al., 2007). Subsequent studies have confirmed that elephant's will avoid the noise of bees, likely because animals have experienced past exposure to stings, or witnessed the stinging of conspecifics (King et al., 2011; 2017). White rhinos regularly disturb vegetation when rubbing against branches and moving through scrub (Rachlow, 2001), behaviour which may provoke defensive swarms of African honeybees (Gould and Gould, 1988), and make them susceptible its use as a deterrent.

6.1.4 Aerial deterrents

Many ungulate species, including white rhinos, will flee from the pursuit of a helicopter; this behaviour is widely utilised during wildlife monitoring, game captures and veterinary procedures to herd animals into desirable locations (Kock et al., 1990; Morkel and Kennedy-Benson, 2007; du P. Bothma and du Toit, 2010). The disturbance effects of drones have also been reported across a range of taxa (Ditmer et al., 2015; Pomeroy et al., 2015; Brunton et al., 2019), with low altitude flights eliciting flight behaviour in many ungulate species, including giraffe *Giraffa camelopardalis*, wildebeest *Connochaetes taurinus* and zebra *Equus quagga* (Bennitt et al., 2019). Although smaller and quieter than helicopters, drones have been successfully demonstrated as a tool to herd and control the movement of African bush elephants as a means to reduce crop raiding (Hahn et al., 2017).

Given their apparent cross-species effects, drones have the potential to work as a deterrent for white rhinos, aided by the species' preference for relatively open savannah grasslands (Shrader et al., 2006) which are conducive to aerial pursuit. The magnitude of a behavioural response is likely to depend on the model of the drone, propulsion system and the flight trajectory taken (Mulero-Pázmany et al., 2017). For example waterfowl will respond differently if approached by a fixed-wing or rotary-bladed airframe (McEvoy et al., 2016). Drones are frequently used for monitoring wildlife (Krause et al., 2017; Barnas et al., 2018; Su et al., 2018) but their employment as an anti-poaching tool is limited to the detection and interdiction of poachers (Shaffer and Bishop, 2016; Jiménez López and Mulero-Pázmány, 2019. Hambrecht et al., 2019). Observations of white rhinos made during aerial reconnaissance with a fixed wing-drone at altitudes of 100 to 180 m found no evidence of alarm or flight behaviour (Mulero-Pázmany et al., 2014). However, it is possible that rhinos will react to flights performed at altitudes lower than this.

6.1.5 Olfactory deterrents

Many ungulate species rely on olfactory signals for communication (Eisenberg and Kleiman, 1972), and possess well-developed olfactory systems (Brown, 1979). Unlike acoustic signals, odours persist in the environment after their initial formation, which can be advantageous when signal persistence or repeat exposure are desirable (Eisenberg and Kleiman, 1972). Deterrents reliant on olfactory cues can succeed in driving animals from undesirable areas by exposing individuals to unfamiliar scents (Schulte, 2016). Such strategies rely on neophobia, such as with the use of synthetic odours to deter mountain beavers (*Aplodontia Rufa*) but may suffer from habituation after repeat exposure (Epple et al., 1995). Other approaches co-adopt the signalling of threats, such as through the placement of coyote hair to repel White-tailed deer *Odocoileus virginianus* (Seamans et al., 2002), or alternatively act to incite pain or discomfort in a target subject, such as with the use of chilli-based aerosols against bears *Ursus* spp. (Smith et al., 2008). Chilli's aversive properties, low economic cost (as little as £1 per kg) and minimal technical requirements have seen it widely trialled as a deterrent (Wallace and Hill, 2016). The substance can produce a burning sensation when inhaled or ingested as capsaicin, the active ingredient, stimulates sensory afferent neurons causing irritation (Miller, 2001; Osborn, 2002). Strategies of deployment vary but include as a noxious smoke (Graham and Ochieng, 2008), as an aerosol (Osborn and

Rasmussen, 1995) and as a linear barrier (Chelliah et al., 2010). For example, a rope barrier coated in a paste of chilli powder, tobacco and oil was successful at reducing breaches from female-led herds of African bush elephants, and halved the number of breaches from solitary males (Chelliah et al., 2010). In this case, it is likely that the females were more risk averse than the solitary males due to their calves greater susceptibility to predation (Chelliah, 2010). Similarly, Sitati and Walpole (2006) reported how elephants would follow a chilli-soaked rope to its end-point before crossing, although the parallel use of sirens may have also influenced their behaviour (Sitati and Walpole, 2006). Rope barriers coated in a chilli-water mix have also been successful in repelling blue monkeys *Cercopithecus mitis* (Wallace and Hill 2016) but chilli-grease-covered fences did not deter Sumatran elephants *E. maximus sumatrensis* from raiding crops (Hedges and Gunaryadi, 2010).

Chemical communication has an important function in organising the social structure and spatial distribution of white rhinos (Marneweck et al., 2017). Adult bulls demarcate their territories with dung and urine and all individuals often defecate in communal dung piles, from which individuals are able to discriminate the familiarity and sex of conspecifics (Cinková and Policht, 2015; Marneweck et al., 2018). White rhinos also tend to walk with their head held low and their nostrils close to the ground (Owen-Smith, 2013). Whilst the large size of their nasal sinuses, which take up the same amount of space in the skull as the brain, is further evidence of a highly developed sense of smell (Owen-Smith, 2013). Thus, their acute sense of smell may predispose them towards olfactory-based deterrents.

6.1.6 Aims of the study

The application of deterrents to move animals away from areas of danger, remain unstudied in anti-poaching contexts beyond the simple herding of animals by vehicle and on foot. This study aimed to design and evaluate novel deterrent-based techniques that could be utilised in anti-poaching management approaches for white rhino conservation. This included the investigation of stimuli with different sensory modalities and mechanisms, and the degree to which this made them effective as a deterrent.

Successful deterrents could be utilised to move rhinos from areas of high poaching risk to areas of refuge providing a useful conservation tool for wildlife managers.

The primary research questions were whether stimuli could be utilised to elicit avoidance behaviours in rhinos, and to understand if these responses would diminish after repeat exposure. It was predicted that exposure to some stimuli would induce fear or anxiety in rhinos, inciting avoidance of the stimulus via a flight response. Mother-calf pairings were predicted to be more responsive to deterrents than either subadults or territorial bulls. For the acoustic deterrent, rhinos were exposed to three stimuli: the noise of disturbed African honey bees (from here on shortened to 'bee'), to the territorial calls of Cape turtle dove (*Streptopelia capicola*) (from here on shortened to 'dove') and the noise of an oscillating siren. It was predicted that the noise of bees would elicit a flight response (locomotion away from the deterrent), the noise of an oscillating siren would elicit an alert response (vigilance towards the deterrent), and the noise of the dove would elicit no response. Responses to bee noise were expected to diminish more slowly than those to the siren if rhinos had undergone past aversive conditioning to stings. For the drone deterrent, rhinos were exposed to the approach of a drone flying at three different altitudes (≤20, 60, 100 m). Animals were predicted to flee further from the lower altitude trajectories (≤20 and 60 metres), than from the high-altitude trajectory (100 metre), where noise could be expected to be minimal and non-intrusive. Habituation towards the drone was also expected to occur, with reduced avoidance expected after repeat exposure. For the olfactory deterrent, rhinos were exposed to the scent of chilli oil than to the scent of sunflower. It was predicted that rhinos would demonstrate greater avoidance behaviour and reduced investigative behaviour to the scent of chilli oil than to the scent of sunflower oil, due to the potential irritating properties of chilli.

6.2 Methods

6.2.1 Experimental design

Rhino behavioural responses were recorded following exposure to acoustic, olfactory and drone-based stimuli (Table 6.1). Experiments were conducted from October 2016 to November 2017 on a population of dehorned white rhinos on a private reserve (see

Chapter 2). Experiments occurred after social behavioural studies were completed (see Chapter 3). All experiments took place within bushveld savannah (Shorrocks and Bates, 2015) where grasses made up between 50 and 100% of the groundcover. Habitat type was standardised to avoid it influencing an animal's perception and response to risk (Frid and Dill, 2002 Prior to the start of acoustic and drone experiments, rhinos were identified via their unique ear notch patterns to prevent pseudo-replication (see Chapter 2). Following identification, rhinos were left for a five minute acclimatisation period. If disturbance (vigilance towards the experimenter) occurred before the experiment began, then the experiment was delayed until the rhinos settled back to their prior undisturbed behaviour. In mother-calf pairings, only mother behaviour was recorded as calf behaviour could not be considered independent.

The deterrents were selected for their potential to exploit fear and anxiety in rhinos and thus had the potential to effect animal welfare and cause stress (Mellor, 2016; Hernández et al., 2018; Derkley et al., 2019). Throughout the study, several steps were taken to ensure exposure to the deterrents did not exceed what was necessary to elicit and observe avoidance behaviour (locomotion away from the deterrent). Broadcasts of the acoustic stimuli and pursuit by drone were not continued beyond a one minute period, which provided adequate information to assess their efficacy as a deterrent. Furthermore, the stimuli were directed from the fence line towards the rhinos, so that the animals always had a means of escape. In addition to this, experiments were not performed on mothers with young calves (less than three months old) and repeat experiments on the same individuals were only conducted if a period of at least 24 hours had elapsed since the last exposure. In comparison, the olfactory experiments were passively deployed, meaning rhinos could choose the proximity to which they approached a scent."

Table 6.1. Experimental design for the testing of how white rhinos respond to acoustic, olfactory- and drone-based stimuli, detailing deterrent type, treatment within deterrent type and number of replicates per rhino.

Deterrent	Mechanism	Treatments	Replicates per rhino
Acoustic	Active	Disturbed African honey bee	4
		Oscillating siren	4
		Cape turtle dove territorial calls	4
drone	Active	Low-altitude trajectory (≤20 m)	3
		Mid-altitude trajectory (60 m)	3
		High-altitude trajectory (100 m)	3
Olfactory	Passive	Chilli infused sunflower oil	-
		Sunflower oil	-

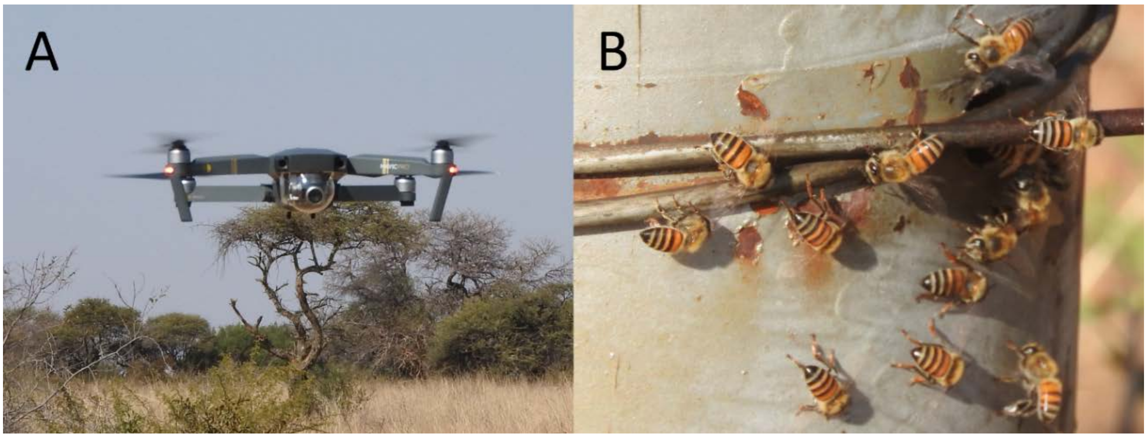

Figure 6.1. Images of (A) the quadcopter drone (a DJI Mavic Pro) that flew trajectories towards the rhinos and (B) the hive of African honey bees (*Apis mellifera scutellata*) that were recorded for the acoustic deterrent.

Behavioural responses were video recorded following exposure to each stimulus (acoustic: Canon VIXIA HF R400; drone: DJI Mavic Pro (Figure 6.1a); olfactory: Bushnell Trophy Cam). These were coded by the primary researcher (SGP), and in the case of olfactory deterrents coded without prior knowledge of the treatment type to avoid bias. The absence or presence of a behavioural change was recorded, and if a behavioural change occurred, then the type of behavioural response was noted (Table 6.2).

Table 6.2. Behavioural classifications and definitions used to measure rhino responsiveness towards a deterrent. Letters denote the trials for which behaviours are of relevance: acoustic (A); drone (D) and olfactory (O) deterrent. All behaviours marked by an asterisk were summed as a measure of awareness.

Behaviour	**Deterrent**	**Definition**
Investigative*	A, D, O	Locomotion (directed walking or running) towards the deterrent.
	O	The sniffing or chewing of the deterrent.
Alert*	A, D, O	Vigilance towards the deterrent (standing stationary with the head held above the ground).
Flight*	A, D, O	Locomotion away from the deterrent (directed walking or running). Head held high, tail often curled.
Crossing	O	Incidents of stepping over and past the rope.
Ignore	A, D, O	All other behaviours were classified as unresponsive e.g. social and foraging behaviours. Alert behaviours were coded as unresponsive if they occurred before exposure, or if vigilance was towards another stimulus e.g. towards hetero- or con-specifics. Locomotive behaviour was not considered flight if it was undisturbed or not directed from the stimulus e.g. walking during territorial marking and during foraging.

6.2.2 Acoustic deterrents

For the acoustic deterrent experiments, twelve rhinos were exposed to each of the three treatments up to four times each (Table 6.2). The order of exposure to the bee and dove treatments were randomised, with the oscillating siren treatment introduced after. The broadcast of bee noise has the potential to incite a flight response in rhinos, if as occurs in African bush elephants *Loxodonta africana* (King et al., 2007), individuals have experienced past aversive conditioning to stings. The siren was selected to test whether a novel noise could incite a flight response. The siren had a broad bandwidth to ensure a relatively high loudness, a spectral frequency within the range that rhinos vocalise (Cinková and Policht, 2014; Linn et al., 2018), and a fast frequency modulation to maximise roughness (Götz and Janik, 2010). The calls of the dove were selected as a control for the other two treatments due its ubiquitous occurrence and apparent neutral presence in the local soundscape.

Recordings of bee noise and dove calls were made on-site (Figure 6.1) with an ME 66 Sennheiser microphone; frequency response: 40 – 20,000 Hz, ± 2.5db) equipped with windshield. The microphone was connected to a Roland R-05 recorder (44.1 Hz sampling rate, 16 Bit, uncompressed wave format). Audio sequences (Figure 6.2) were edited in Audacity (version 2.1.1) and clipped to 60 seconds in length. To attenuate extraneous abiotic noise the bee recording was low-pass filtered at 4500 Hz with a six dB per octave roll-off. The siren consisted of a repeated ascending tone this consisted of a sine waveform rising in spectral frequency from 500 Hz to 5000 Hz looped to a 2 Hz cycle. Sounds were broadcast louder than the recorded volume to compensate for speaker distance. The amplitude of the three sequences at 50 to 150 m measured 76 to 66 dBc for the bee, 86 to 75 dBc for the dove and 79 to 74 dBc for the siren in field conditions. This was similar to the level broadcast by King et al. (2007) during bee playback experiments on elephants (66.1 dB at 10 m). For reference, the background sound levels were measured at 52 dBc.

Sounds were broadcast through a Roland R-05 sound recorder connected to two 30-watt horn speakers (frequency range: 250 Hz - 10 kHz; TOA Corporation) via an amplifier (Sony Stereo Power Amplifier Serial 6508687), powered by an external 12 V battery. Speakers were placed on the roof of a vehicle (2 m high), facing towards the rhinos. Playbacks were started when rhinos were downwind, and between 50 and 150 m of the speakers. Rhino behavioural responses were measured for the one-minute duration of the playback experiments (Table 6.2). Observations were truncated at one minute to ensure that rhinos remained visible throughout the experiment and to aid their comparability with data taken from the drone. The duration of investigative, alert and flight behaviours was a measure of ‘awareness’ of the stimulus. The ‘distance travelled’ in response to the stimulus was a measure of flight response. The length of shorter distances (<10 seconds of movement) were estimated from rhino body length (approx. 3 m) relative to features in the video, for longer distances changes in rhino location were calculated via a rangefinder (Leica Rangemaster CRF 1600-R).

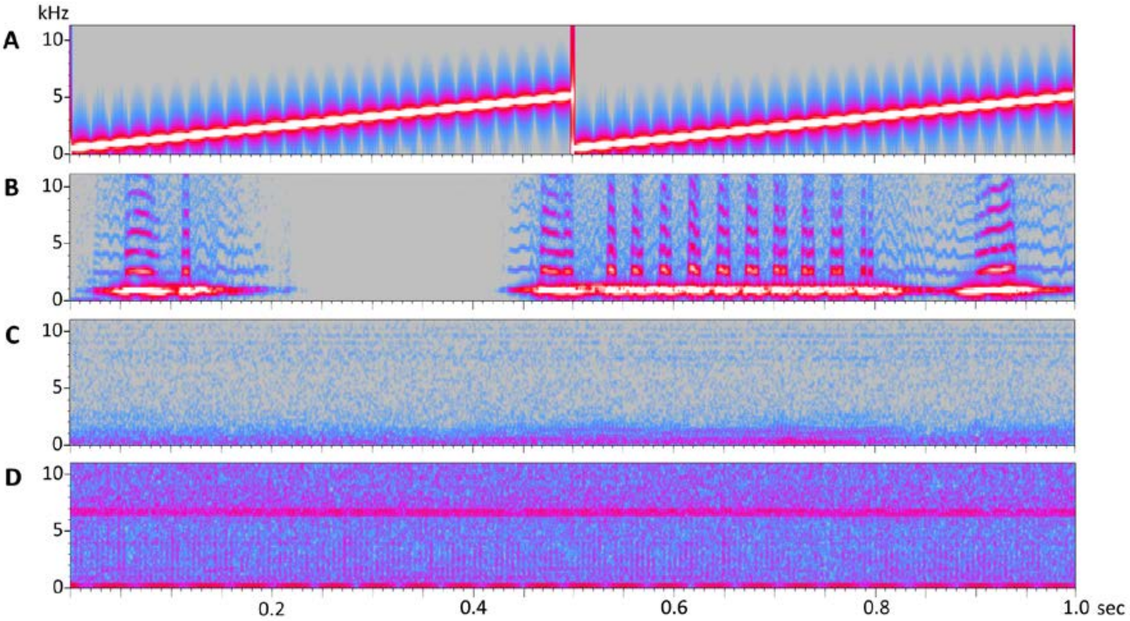

Figure 6.2. Spectrograms of a representative one-second section of the acoustic and drone treatments: (A) oscillating siren, (B) dove territorial calls, (C) disturbed bee, and (D) drone [DJI Mavic Pro]. FFT length 512, frame size 100%, overlap 87.5%, Hamming window.

6.2.3 Drone deterrent

For the drone experiments, rhinos were exposed to flights at three different altitudes three times each. To avoid bias from condition order, the initial drone altitude was randomised with each subsequent exposure a different altitude to the preceding one. All drone experiments were performed with a multi-rotor DJI Mavic Pro (Shenzhen, China). The drone was selected for its manoeuvrability, portability, availability as an off-the-shelf model, and its similarity to the drone models used by Hahn et al. (2017) to scare elephants. The drone flights were initiated at least 150 m from the rhinos to avoid prior/post experimental exposure. Following launch, the drone ascended to one of the three selected altitudes and flew in a straight, steady, level trajectory towards the epicentre of each rhino or rhino grouping. If the drone reached this overhead point, it hovered above the rhino for up to five minutes. If the rhinos moved, the drone pursued them for up to one-minute. The drone was then flown back to the launch site. The speed of the drone was kept to approximately 10 m / sec during the approach, pursuit and return stages of the experiment. To achieve a desirable conservation outcome, drone trajectories were flown from the boundary fence lines of the reserve towards the rhinos to move them towards more central areas.

To standardise experimental conditions, experiments were only carried out when wind speed was perceived to be minimal (Beaufort scale 0-2). The amplitude of the drone was measured from 1.5 metres above the ground at three altitudes (76 dBc at 20 m; 67dBc at 60 m, 61 dBc at 100 m) along with background sound levels (48 dBc with the drone shutdown). Peak spectral frequency was measured at 6,494 Hz (Figure 6.2d), which is within white rhino hearing range (Cinková and Policht, 2014).

Rhino 'awareness' was recorded for a one-minute period following the first observed investigative, alert, or flight behaviour towards the drone (Table 6.2), if these behaviours were not observed a value of zero was recorded. Rhino 'reaction distance' was recorded as the distance between the rhino and drone on the first observation of awareness, if no response occurred the closest distance reached between the rhino and drone (the drone altitude when hovering overhead) was recorded. To calculate this, a rhino's spatial location, recorded before launch (see Chapter 2), was compared to the drone's location recorded every tenth of a second by an on-board GPS. If rhinos had moved significantly (> 20 m) between a five-minute acclimatisation period and the drone launch, the start location was updated.

Rhino flight response was quantified as the 'distance travelled' during a one-minute period following their first locomotive response to the drone (Table 6.2). This was calculated by comparing the difference between the rhinos start-, mid- (taken if the rhino stopped or changed direction) and end-positional co-ordinates following exposure to the drone (Equation 6.1). Mid- and end-positional co-ordinates were calculated from the position of the rhino in relation to the drone's location using the drone video output, internal compass, video timings and satellite imagery (Sentinel 2, European Space Agency).

Equation 6.1. Finding the distance between two points on the earth. Adapted from Zwillinger (2002).

$$D = \cos^{-1}\left(\cos\left(\left(\frac{\pi}{180}\right)\times(90-x_1)\right)\times\cos\left(\left(\frac{\pi}{180}\right)\times(90-x_2)\right)+\sin\left(\left(\frac{\pi}{180}\right)\times(90-x_1)\right)\times\sin\left(\left(\frac{\pi}{180}\right)\times(90-x_2)\right)\times\cos\left(\left(\frac{\pi}{180}\right)\times(y_1-y_2)\right)\right)\times 6371000$$

Where,

D = distance between two co-ordinates (metres)

X_1 = rhino latitude (decimal degrees)

Y_1 = rhino longitude (decimal degrees)

X_2 = drone latitude (decimal degrees)

Y_2 = drone longitude (decimal degrees)

6.2.4 Olfactory deterrent

For the olfactory experiments, rhinos were exposed to ropes infused with chilli (*Capsicum frutescens*) powder or sunflower (*Helianthus annuus*) oil. Accurate individual identification was not always possible and so responses were taken from a pool of 17 individuals with each exposure event treated as an independent data point. Thus, no tests of habituation were conducted. Chilli was selected as a potential olfactory stimulus due to capsaicin's irritating properties for some mammals (Miller, 2001). Chilli powder (specifically *skopdonner*, a local cultivar of South African bird's eye chilli, which scores around 50,000–175,000 Scoville Heat Units) was mixed with sunflower oil (1:10 ratio) to enable easy application. A pure sunflower oil treatment was selected as a control to identify whether rhino responses were associated with the medium of scent deployment or the scent itself. Lengths of 5 m natural fibre sisal rope were infused with scent by soaking them in one of the two treatment types for 24 hours. After five days, the scents were reapplied by re-submerging the ropes in the appropriate treatment type.

The lengths of scent-infused rope were laid across well-trodden animal trails that led to water bodies and showed recent signs of rhino activity (fresh spoor). Pairs of each treatment type were placed at up to four different locations across the study site at any time. The two treatment types were placed within 150 m of each other to increase the chances of rhinos experiencing an even encounter rate for each treatment. Rhino exposures were monitored by camera traps (see Chapter 2 for details) attached around 1.5 m above ground height and approximately 10 m away from the rope. Videos were recorded for 30 seconds, with 2 second intervals. Batteries and SD cards were checked every 5 days. Responses were recorded for the period that the rhinos stayed within 5 metres proximity of the rope (Table 6.2). It was not possible to record distances moved due to the narrow field of view provided by the camera traps (10-15 m). Avoidance of the stimulus was also determined by noting from recordings whether or not rhinos stepped over the scent stimulus.

6.2.5 Data analysis

For the acoustic and drone deterrents, the first set of analyses tested for differences in behavioural response between treatment types following a rhino's initial exposure to each stimulus. The second set of analyses, tested for differences in behavioural response between replicates of each treatment type, as an indicator of habituation. Friedman's tests were used to account for the non-parametric distribution of the data and the one-way repeated measures designs, whereby each subject appeared in greater than one treatment and/or replicate. Dunn's tests with Bonferroni corrections were performed on significant results to establish any directions in trend and account for the family-wise error rate. For the olfactory deterrents, the absence of subject IDs precluded the use of a repeated measures design. Consequently, a Mann-Whitney U was used to test for differences in the duration of behaviours towards each treatment type due to the non-parametric distribution of the data in each group (see Chapter 2) and a chi-squared test was used to establish the degree of independence between treatment type and behavioural counts. All analyses were two-tailed, and all alpha levels were set at 0.05.

6.3 Results

6.3.1 Acoustic deterrent

The rhinos in this study exhibited significantly longer durations of awareness in response to the siren (median = 57.5 seconds) than to either the bee (median = 8.5 seconds) or dove (median = 0 seconds) treatments (Friedman χ^2 (2) = 15.591, p < 0.001, n = 12, obs. = 36; Figure 6.3;Table 6.3). The distances rhinos' travelled also showed significant variation between acoustic treatments (Friedman χ^2 (2) = 15.250, p < 0.001, n = 12, obs. = 36; Table 6.3); with rhinos moving further in response to the siren (median = 46 m) than to either the bee (median = 0 m) or dove treatments (median = 0 m). When responding to the siren, subadults fled further (median = 55m, n = 4) than both mother-calf pairs (median = 37 m, n = 5) and adult bulls (median = 3 m, n = 3).

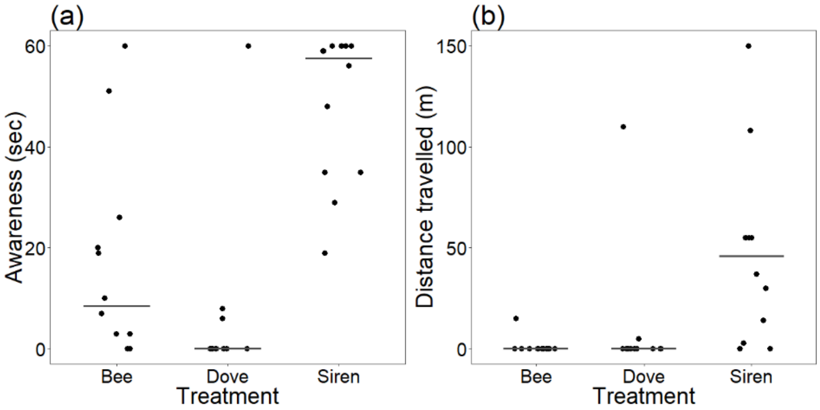

Figure 6.3. Rhino behavioural responses following initial exposure to each of the acoustic treatments for (a) awareness (n = 12, obs. = 36) and (b) distance travelled (n = 12, obs. = 36). Horizontal bars show medians; data are horizontally jittered.

Replicates of the siren resulted in no detectable change in awareness levels (Friedman χ^2 (3) = 0.857, p = 0.835, n = 6, obs. = 24) or distance travelled (Friedman χ^2 (3) = 4.932, p = 0.177, n = 6, obs. = 24) between experiments. Similarly, no changes were observed for either the dove (Awareness - Friedman χ^2 (3) = 0.875, p = 0.832, n = 8, obs. = 32; Distance travelled - Friedman χ^2 (3) = 3, p = 0.392, n = 8, obs. = 32) or bee (Awareness - Friedman χ2 (3) = 7.393, p = 0.060, n = 8, obs. = 32; Distance travelled - Friedman χ^2 (3) = 5.857, p = 0.112, n = 8, obs. = 32) treatments.

Table 6.3. Pairwise comparisons of rhino awareness duration and distance travelled in response to acoustic deterrents. Analyses performed on responses with significant effects via Dunn's tests with Bonferroni corrections (Per pair: n = 12, obs. = 24).

		Pairwise comparisons		
Parameter	**χ^2**	**Siren x Dove**	**Siren x Bee**	**Dove x Bee**
Awareness	18.427	< 0.001	0.001	0.173
Distance travelled	17.042	< 0.001	< 0.001	0.954

6.3.2 Drone deterrent

Rhinos could perceive the drone up to at least 100 metres in altitude (Figure 6.4) and showed a near full minute of awareness to the initial drone experiments (Figure 6.5). Rhino reaction distance and awareness to the initial drone experiments did not differ significantly between the three treatments (Reaction distance - Friedman χ^2 (2) = 3.455, p = 0.178, n = 11, obs. = 33; awareness - Friedman χ^2 (2) = 0, p = 1, n = 11, obs. = 33). However, the distances rhinos travelled in response to the initial drone experiments differed significantly between the three treatments (Distance travelled - Friedman χ^2 (2) = 6.681, p = 0.035, n = 12, obs. = 36). Rhinos moved significantly further in response to the drone flying at the low-altitude treatment (median = 61 m, n = 12) than they did to the high-altitude treatment (median = 10 m, n = 12), with the distance travelled in response to the mid-altitude treatment falling in between the two (median = 20 m, n = 12;Figure 6.5; Table 6.4). Distance travelled was consistently high in mother-calf groupings (median low = 65 m, mid = 40 m, high = 45 m, n = 5), with greater levels of variation between treatments seen in subadult groupings (median low = 49 m, mid = 12.5 m, high = 0 m, n = 4), and adult males (median low = 67 m, mid = 20 m, high = 0 m, n = 3).

Several behaviour responses diminished following replicates of the drone stimuli (Figure 6.6. Rhino reaction distance varied significantly following repeat exposure to the low-altitude treatment (Friedman χ^2 (2) = 11.561, p = 0.003, n = 11, obs. = 33) and mid-altitude treatment (Friedman χ^2 (2) = 9.657, p = 0.008, n = 10, obs. = 30), but not the high-altitude treatment (Friedman χ^2 (2) = 4.667, p = 0.097, n = 11, obs. = 33), with reaction distance declining over time (Table 6.4; Figure 6.6).

Figure 6.4. Some of the recorded responses to the drone and scent deterrent experiments. (a) Mother and calf alert to the drone hovering at the high-altitude treatment; (b) mother and calf running from the drone flown towards them at the low-altitude treatment; (c) subadult male chewing a sunflower oil-treated rope; (d) Adult bull crossing over and past the chilli-treated rope.

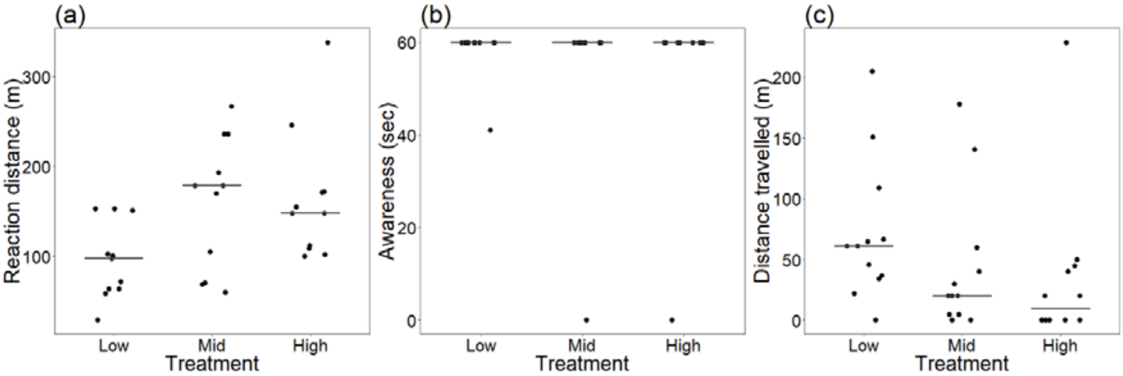

Figure 6.5. Rhino behavioural responses following initial exposure to each of the drone altitudes for (a) reaction distance (n = 11, obs. = 33), (b) awareness (n = 11, obs. = 33) and (c) distance travelled (n = 12, obs. = 36). Horizontal bars show medians; data are horizontally jittered.

Table 6.4. Pairwise comparisons of rhino reaction distance, awareness duration and distance travelled in response to drone flights at three altitudes. Analyses were performed on responses with significant effects after first exposure (between treatments) and repeat exposure (within treatments) via Dunn's tests with Bonferroni corrections. Sample sizes and observation numbers are listed per pair in subscript.

Parameter		**χ2**	**Pairwise comparisons**		
First exposure			**Low x Mid**	**Low x High**	**Mid x High**
	Distance travelled $_{(12;24)}$	7.269	0.013	0.101	0.634
Repeat exposure			**1st x 2nd**	**2nd x 3rd**	**1st x 3rd**
Low	Reaction distance $_{(11,22)}$	7.950	0.066	0.903	0.017
Mid	Reaction distance $_{(10,20)}$	8.175	0.025	1.000	0.016
Mid	Awareness $_{(10,20)}$	11.204	0.141	0.141	0.001
High	Awareness $_{(11,22)}$	9.310	0.075	0.442	0.004
High	Distance travelled $_{(12,24)}$	5.314	0.044	0.995	0.122

Awareness towards the stimuli did not vary significantly in response to replicates of the low-altitude treatment (Friedman χ^2 (2) = 2.10, p = 0.350, n = 11, obs. = 33). However, significant changes in awareness were detected after replicates to the mid-altitude (Friedman χ^2 (2) = 11.438, p = 0.003, n = 10, obs. = 30) and high-altitude treatments (Friedman χ^2 (2) = 9.920, p = 0.007, n = 11, obs. = 33); decreased levels of awareness were apparent for the later replicates (Table 6.4). Despite these drops in awareness and reaction distance, rhinos travelled a similar distance across replicates of the low-altitude (Friedman χ^2 (2) = 5.070, p = 0.079, n =11, obs. = 33) and mid-altitude treatment (Friedman χ^2 (2) = 3.706, p = 0.157, n = 12, obs. = 36). However, distances travelled in response to the high-altitude treatment did show significant variation between replicates (Friedman χ^2 (2) = 11.20, p = 0.004, n = 12, obs. = 36); with the greatest difference between the first and second replicates (Table 6.4; Figure 6.6).

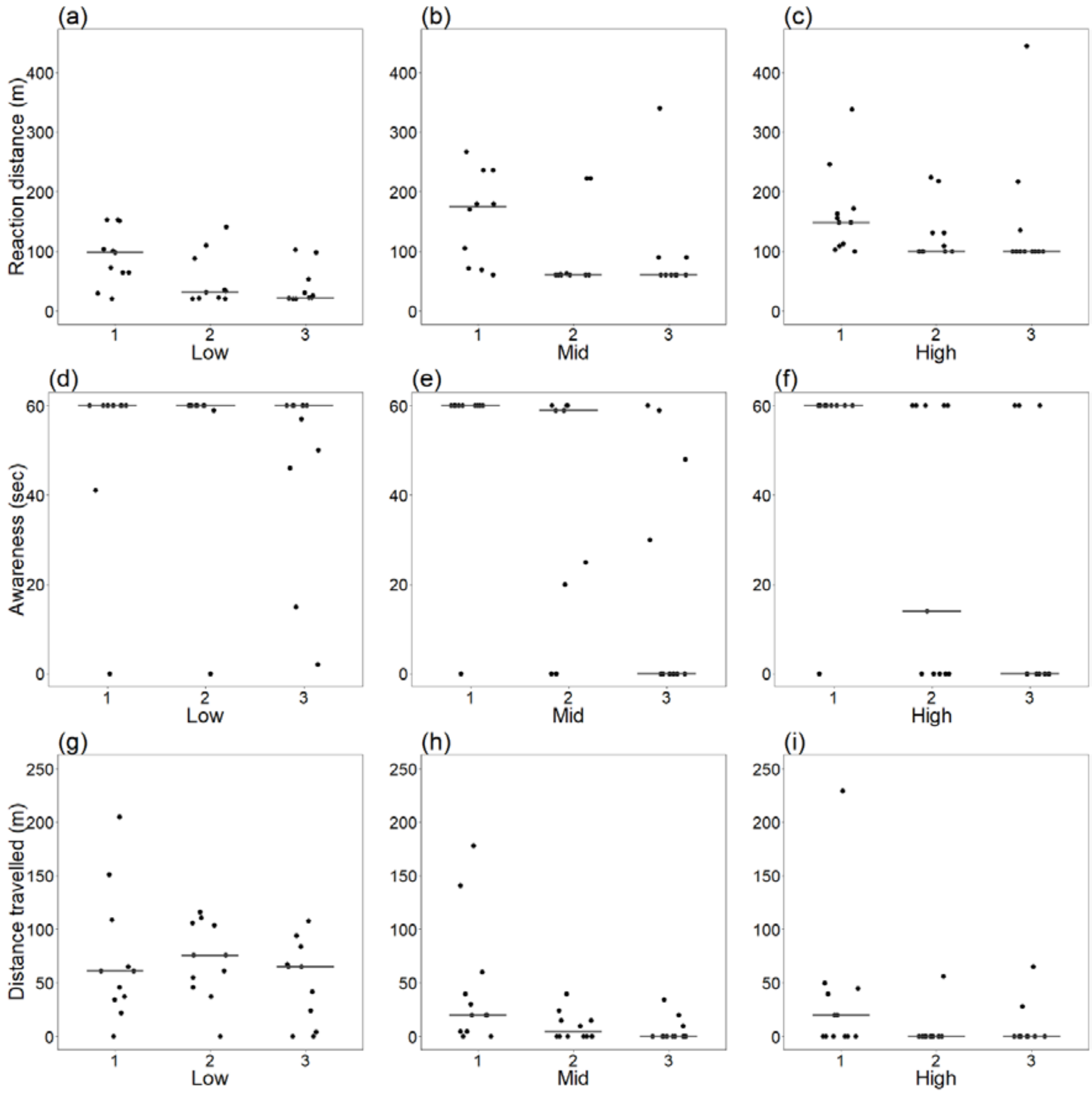

Figure 6.6. Rhino behavioural responses to the drone deterrent following three replicates per rhino at each altitude: (a-c) reaction distance, (d-f) awareness, and (g-h) distance travelled. Horizontal bars show medians; data are horizontally jittered (a, c, d, f: n = 11, obs. = 33; b, e: n = 10, obs. = 30; g, h, i: n = 12, obs. = 36).

6.3.3 Olfactory deterrent

Awareness towards the olfactory deterrent did not differ significantly between the chilli and sunflower oil treatments (W = 873, p = 0.255, n = 78), with sniffing, chewing and alert behaviours observed towards both treatment types (Figure 6.4, Figure 6.7). No association was found between the tendency of a rhino to cross over a rope following their approach of it and treatments type ($\chi2$ (1) = 0.915, p = 0.339, n = 78). Thus, following approach of the rope, most rhinos continued to travel along the game trail, crossing over the olfactory deterrents (Figure 6.4).

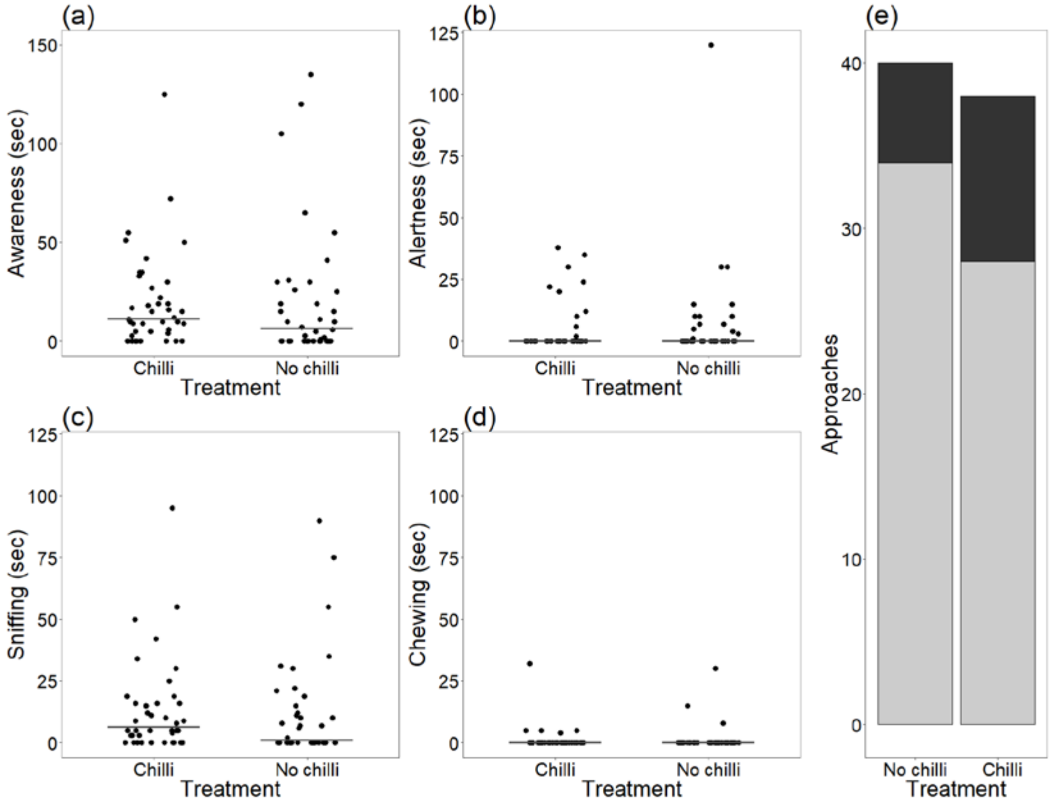

Figure 6.7. Rhino behavioural responses following exposure to the olfactory deterrents, showing: (a) awareness, and behavioural subsets of awareness: (b) alertness, (c) sniffing, and (d) chewing, whereby horizontal bars show medians and data are horizontally jittered; (e) tendency to cross the rope, whereby light and dark shading indicate a crossing and no crossing, respectively (n = 78).

6.4 Discussion

Exposure to both the siren and low-altitude drone treatment repeatedly elicited a flight response from the studied rhino, enabling the movement of animals away from undesirable areas with higher poaching risks. Although the distances travelled were short, in many cases the rhinos continued to flee after observations had ended; on two occasions rhinos ran over 500 metres in response to the drone, even without pursuit, and on four occasions over 250 metres from the siren. Whilst previous studies with elephants have shown the olfactory stimulus of chilli powder (Chelliah et al., 2010) and auditory stimulus of bees (King et al., 2007) to be effective deterrents in conflict scenarios the results of this study show neither to be effective for rhinos.

Rhinos may soon return to an area if the costs of avoiding the stimulus are outweighed by the benefits of staying put and foraging (Lima and Dill, 1990; Gill et al., 2001). Thus, the chances of return could be lowered if higher quality areas of habitat are maintained in other more suitable areas of a reserve. Deterrents may be less effective in periods of reduced resource availability, such as drought, when rhinos have a more limited choice of grazing areas or may not flee as far due to low energy reserves (Shrader et al., 2006). Dominant males will also be more inclined to return to an area than other social classes, given their need to regularly patrol and demarcate their territories (Owen-Smith, 1973; Marneweck et al., 2017).

The sample sizes (≤17 rhinos per analysis) are comparable to existing studies of deterrents [Epple et al., 1995; Ohrens et al., 2019) and reflect the difficulty of exposing free-ranging mammals to experimental stimuli and the need to minimise undue stress (Sikes, 2016). Furthermore, unlike several previous studies [Hahn et al., 2017; Chelliah et al., 2010, Thuppil and Coss, 2016), the repeated measures design of the acoustic and drone analyses provide a robust control for individual variation.

6.4.1 Effectiveness of the acoustic deterrent

Whilst bee noise resulted in significantly raised awareness levels when compared with dove calls, our study shows this to be an ineffective acoustic deterrent for rhinos. Despite the sympatric distribution of African honey bees with rhinos, their aggressive behaviour and their readiness to form swarms (King, 2010), the rhinos in this study did not appear to perceive the risk from the bees as great enough to initiate flight behaviour. This contrasts with the growing body of evidence for their use with African bush elephants (Vollrath and Douglas-Hamilton, 2002; King et al., 2011, 2017) perhaps because the thick skin of rhinos, adapted to shield against attacks from conspecifics, provides sufficient protection against aggressive swarms of bees (Shadwick et al., 1992).While elephants are also thick skinned, they are likely sensitive to stings around the eyes, behind the ears and inside the trunk (Vollrath and Douglas-Hamilton, 2002). Alternatively, if stinging incidents were rare the rhinos may not have been conditioned to flee; this was seen in elephants by King et al. (2007) who reported no bee-induced flight behaviour in a group of the youngest, most inexperienced individuals. Or, if the sound broadcast was an inaccurate reproduction of a bee swarm it may not have induced conditioned behaviour (Ramp et al., 2011). In this case it would indicate that acoustic neophobia alone was not enough to induce a flight response in rhino, and that other characteristics were required. In comparison, the repeated avoidance of the siren suggests rhinos responded to its roughness, with the fast frequency modulation inducing a psychophysiological unpleasantness (Gotz and Janik, 2010) which resulted in the observed flight response.

Repeat exposure to the siren did not lead to any significant behavioural changes, despite the lack of inherent fitness costs in staying put. Habituation to such signals can be minimal (Biedenweg et al., 2011) as animals are more likely to overestimate than underestimate risk, particularly if they have incomplete information, which can lead to imperfect decision-making (Frid and Dill, 2002). Lost foraging opportunities from rhinos avoiding the siren will have a lower fitness cost than underestimating a potential lethal risk (Bouskila and Blumstein, 1992; Frid and Dill, 2002). However, as only four replicates were analysed for each treatment type, the study can only conclude that responses do not significantly diminish over the three month long test period. Further monitoring of the acoustic deterrents at the study site are required to determine how long-term usage may affect rhino responses.

6.4.2 Effectiveness of the drone deterrent

The drone elicited a strong flight response in rhinos when flown at the low-altitude treatment, indicating the stimulus was effective at manipulating rhino habitat usage and suitable for use as a rhino deterrent. The differences in flight response exhibited towards the three drone altitudes confirm rhinos were reacting towards the drone rather than potential disturbance from human experimenters. The levels of acoustic and visual disturbance caused by the drone are a function of its proximity to the rhinos, with the drone becoming louder and more intrusive as it approaches. In most instances, rhinos perceived the drone at distances near to or greater than 100 metres; for there to be a detectable difference between treatments, the rhinos’ threshold of response would need to occur closer than 100 metres (the maximum possible proximity of the drone at the high-altitude treatment) at the low- or mid-altitude treatments, as up to this point the trajectory of all drone flights are subject to the same positional distances during approach. Additionally, as rhinos reacted to the drone when facing away from the drone's angle of approach, acoustic output alone can be enough to initiate a response. The wide variation in reaction distance found within each altitude treatment may in part be explained by variation in terrain or vegetation across experiments. These features may restrict visual and acoustic detection distances if they protrude above the drone-rhino plane, masking the line-of-sight from the rhino to the drone, or impeding sound transcendence through attenuation and/or reflectance. The acute angles inherent to the lowest flight treatment may explain the lower, but non-significantly so, values of reaction distances at this altitude. Despite care taken to conduct experiments under standardised wind conditions, speeds of wind at higher altitudes were difficult to assess, and may have affected sound transmission, introducing variation in perceived drone amplitude.

Mother-calf pairings fled from all three altitudes of the drone, suggesting they may perceive risk differently to other social groupings and be more susceptible to the deterrent than solitary males or subadult groupings. Sex differences in flight response have also been observed in free-ranging black rhinos following human disturbance (Cunningham and Berger, 1997). In habitats with more cover, females ran on average 4 km before resting compared to the 1 km of males, even without pursuit. In habitats with less cover, females ran more than 6 km before resting and males more than 3.5 km. Individual variation in traits such as sex or body size can influence trade-offs between the avoidance of perceived risk and fitness-enhancing activities (Epple et al., 1995; Frid and Dill, 2002). For example, males of both Asian elephants and mountain beavers (*Aplodontia rufa*) are less susceptible following exposure to aversive stimuli than females (Epple et al., 1995; Chelliah et al., 2010). In these cases, the males appear to be less risk-averse due to their comparatively greater body size and/or greater energy requirements (Epple et al., 1995; Chelliah et al., 2010), with females acting more cautiously due to their accompaniment of juveniles (Sukumar and Gadgil, 1988; Chelliah et al., 2010). Such behaviour is also visible in Mexican Spotted Owls (*Strix occidentalis lucida*) following disturbance by helicopters, which remain in their nests when accompanied by chicks but flee once the chicks are fledged (Delaney et al., 1999). White rhino mother-calf pairings may thus show differing perceptions of risk and a stronger response to deterrents than either adults or subadults due to their calves' greater vulnerability to predation (Owen-Smith, 1973).

Rhinos became somewhat habituated to the drone following repeat exposure over a short period. A greater level of exposure was necessary to induce a behavioural response at low- and mid-altitude treatments, as shown by decreases in reaction distance in the later replicates. The lack of decline seen for the high-altitude replicates appears to be an artefact of recording the minimum reaction distance for the drone's altitude when no response occurred. Diminishing rates of awareness in the mid- and high-altitude replicates indicate rhinos perceived the drone to decline in threat. Although there was no change in awareness in response to the low-altitude treatment, the concurrent decrease in reaction distance meant that greater levels of acoustic or visual exposure to the drone were necessary to maintain a similar degree of responsiveness. Rhinos continued to travel the same distance across replicates of the low- and mid-altitude treatments but reduced to near zero at the high-altitude treatment indicating rhino habituate quickly to nominal drone exposure but continue to flee from more intense levels of exposure. A similar effect occurred with the heart rates of captive black bears (*Ursus americanus*) which were elevated in response to initial drone exposure, but returned to baselines levels after repeated flybys (Ditmer et al., 2019). In comparison, habituation was not reported from experiments with African bush elephants, which continued to flee from drones over two seasons of exposure (Hahn et al., 2017). It remains possible that the distance travelled from the drone would diminish across all altitudes following further replicates, however, as rhinos fled from the drone after a cumulative nine replicates, the deterrent can work without a significant reduction in affect at least at this level of exposure.

As well as the total exposure amount, the frequency of exposure can influence habituation rates. Diminished responses have the potential to recover fully if the stimulus is withheld over time, in what is known as spontaneous recovery (Rankin et al., 2009), a less frequent exposure rate than that used in the study (9 times over 90 days) may see responses maintained over a longer period. The continued monitoring of drone usage at the study site will determine whether this is the case, and what the long-term effects of drone deterrents are on rhino behaviour. Minor changes to a signal may be enough to restore the original behavioural response (Talling et al., 1998), with exposure to a single strong or different stimulus leading to dishabituation (Rankin et al., 2009). Talling et al. (1998) demonstrated this in pigs, showing that their response to a uniform acoustic broadcast diminished over time, but was re-established if the sound was altered. To prolong the use of the deterrent, exposure to the drone can be intensified by piloting at lower altitudes than that tested in the study, or by piloting drones at faster speeds to increase the level of noise output and reduce time for decision making. The model of drone will also have an impact on the type of response (Mulero-Pázmany et al., 2017) and the DJI Mavic used in the study could be substituted for one with a louder acoustic output. Factors that influence the sound output of a drone include its propulsion system, electric motors, frame, weight and speed (Christie et al., 2016; McEvoy et al., 2016; Brunton et al., 2019). Numerous commercially available drone models allow for interchangeable or modular attachments, which would allow for speaker attachment, so sound output need not be restricted to the operating noise, with some speakers marketed explicitly for drone attachment (e.g. the Sky Speaker-I Megaphone; Shenzhen Yangda Security). This would allow for the broadcast of the siren directly from the drone and remove the need for a static terrestrial speaker, as was trialled in this study. A similar technique is already used in the aerial herding of wildlife by helicopter during game capture, where pilots broadcast loud noises towards animals (du P. Bothma and du Toit, 2010).

6.4.3 Effectiveness of the olfactory deterrent

Neither the chilli nor sunflower oil treatments were successful in affecting rhino movement, suggesting their ineffectiveness for use as a rhino deterrent. Rhinos could perceive the stimuli as shown by investigative sniffing and chewing behaviours. However, rhinos chewed both rope types and crossed over them a similar number of times, thus neither substance is aversive, nor appears to be causative of pain or irritation. Those instances where rhino did not cross were likely due to neophobia, either towards the scent or towards the experimental setup itself. As rhinos paused to investigate the treatments, the stimuli encouraged rhino to stay within their vicinity for longer, with both treatments acting as an attractant in these situations rather than as a repellent. Changes in behavioural responses over time were not monitored; however, incidents where rhino crossed the rope appeared evenly distributed throughout the data collection period. Thus, the variation appears to relate to differences in individual rhino motivational state and risk-benefit analysis rather than the effects of habituation.

Hedges and Gunaryadi (2010) failed to elicit an aversive response to a chilli rope deterrent in Asian elephants and argued that the reported successes of several similar chilli-based deterrent studies may have been due to their parallel usage of other deterrents such as increased levels of farmer vigilance (Osborn and Parker, 2002; Sitati and Walpole, 2006; Graham and Ochieng, 2008). In studies of human-wildlife conflict mitigation robust factorial designs are not always possible, as the failure of non-effective controls can have a direct impact on people’s livelihoods (Thuppil and Coss, 2016). It remains possible that rhinos could show a response to the aerosol deployment of chilli, but as the application of sprays has a greater potential to cause undesirable symptoms such as apnoea and temporary blindness (Miller, 2001; Smith et al., 2008), they are less suitable for exploratory use.

6.4.4 Conservation implications

While care was taken to reduce exposure to the stimuli during the experimentation stage, during periods of high poaching risk it may be necessary to expose rhinos to acoustic broadcasts and drone flights for longer than the one minute trialled in the study to encourage animals to move further. Although conservation techniques should be adapted to minimise undue stress (see Chapter 4), the welfare of a manoeuvred rhino will always be substantially higher than that of a poached rhino, which usually ends in death (Derkley et al., 2019). Additionally, if the stimuli do act as a source of stress but elicit anxiety, it may lead to future avoidance of the anxiety-associated stimulus or area reducing the need for increased exposure (Schakner and Blumstein, 2013). However, further study will be needed to establish this. Furthermore, rhinos are already herded directly by humans as an anti-poaching tactic (Rees, 2019), the use of the deterrents, which involve no direct human exposure, reduces the risk of rhinos habituating to people (Muntifering et al., 2018), which in turn could make them less susceptible to poaching.

The distances rhinos travelled were constrained by the one-minute period of measurement rather than a termination of behavioural response. The drone is most suited to increasing these distances, as it can remain within a rhino's flight zone as the animal tries to move away in contrast to the diminishing strength of the sound broadcasts. Pursuit by the drone is only limited by transmission range and battery life, which are much greater than the short periods tested in the experiment (>25 minutes and >2 km for the DJI Mavic Pro). For example, elephants were moved distances of over one kilometre following continued pursuit by a similar model of drone (Hahn et al., 2017). The same study also showed that animals could be moved at night despite the absence of a drone-mounted thermal camera by relying on artificial lights. Drone deterrents would be most applicable to small private reserves, where rhinos have access to perimeter zones or exposed areas, particularly during heightened periods of risk (e.g. around the full moon (Koen et al., 2014) or when poaching syndicates are known to be operating in the area (Mulero-Pázmany et al., 2014). They are less suited for use in larger state or national parks with semi-porous borders and near-constant poaching activity (Rademeyer, 2016a).

Aside from its capability of pursuit, the drone is superior at manipulating rhino movement than the siren for several other reasons. First, the drone's manoeuvrability allows for the more precise control of rhino movement as it can be flown into positions in response to changes in direction. In comparison, animals frequently ran parallel or diagonal to the sound source following the broadcast of the siren, resulting in unpredictable directionalities of response. Additionally, the drone is less limited by terrain and vegetation type than the siren, as it can be flown into position without any ground-based infrastructure. Furthermore, the drones' additional surveillance functions could incentivise poachers to avoid areas where they operate (Mulero-Pázmany et al., 2014). In contrast, the use of the acoustic deterrents risks advertising the prior position of rhinos to poachers.

The flight responses elicited by drones may show application in circumstances outside of anti-poaching security. A strong reaction to the drone was witnessed across several sympatric ungulates, with flight responses in wildebeest, giraffe, warthog *Phacochoerus africanus*, red hartebeest *Alcelaphus buselaphus*, zebra, kudu *Tragelaphus strepsiceros* and impala *Aepyceros melampus* observed. The aerial herding of these species for game counts, veterinary procedures and game captures, is currently fulfilled by helicopters (du P. Bothma and du Toit, 2010), but it is likely that a drone can be used to perform at least some of these functions at a reduced financial cost. Drones could also aid dehorning procedures of rhinos, by searching for and identifying rhinos and herding them towards a position that a ground team can work from, however a helicopter would still be required for the aerial darting stage.

6.4.5 Conclusion

In addition to identifying abiotic auditory stimuli as effective deterrents, this research is the first to identify the potential of drones and sirens as a management tool for the active movement of rhinos in protected areas. In contrast, the scent stimuli were ineffective at inciting avoidance behaviour. By reducing the altitude of flights this research demonstrates a technique that reserve managers can use drones to readily respond to reports of at-risk animals. For anti-poaching units that already use drones for surveillance purposes there is no additional outlay in equipment costs and their use as a deterrent can add an additional function to reconnaissance (Hambrecht et al., 2019).

Chapter 7 – General discussion

7.1 Introduction

The removal of white rhinos from savannah ecosystems could have wide ranging effects on grassland communities, impacting on vegetation, nutrient cycling and fire regimes (Waldram et al., 2008; Cromsigt and te Beest, 2014; Ripple et al., 2015; Veldhuis et al., 2018). Such a loss could also result in a significant decline in revenue for the wildlife and conservation industries, given white rhinos' high cultural and economic value (Spenceley and Barnes, 2005; Boeyens and Van der Ryst, 2014; Saayman and Saayman, 2017). Without past and continuing conservation efforts, the population size of white rhinos and many other threatened species would be substantially lower than they are today (Hoffmann et al. 2010, 2015). Yet conservation resources are limited, so it is increasingly important to incorporate evidence-based research into all conservation management decisions (Sutherland et al. 2004; Adams and Sandbrook, 2013; Mupepele et al., 2016). This thesis investigated whether dehorning had any behavioural or physiological effects on white rhinos and evaluated the effectiveness of novel deterrents. By applying a range of methodological approaches, the resultant research provides important new information on understudied aspects of white rhino biology. The following chapter summarises these research findings and highlights how the work contributes towards rhino conservation techniques. The chapter also discusses the management implications of the findings and outlines the direction that future studies could take.

7.2 General discussion

Many past studies of free-ranging white rhinos have focused on territoriality and home range size (e.g. Conway and Goodman, 1989; Pienaar et al., 1993; Rachlow et al., 1999; White et al., 2007; Thompson et al., 2016) or on wider ecological interactions (e.g. Waldram et al., 2008; Cromsigt and te Beest, 2014; Jordaan et al., 2015). In contrast, studies of white rhino social behaviour have primarily focused on captive rhinos (e.g. Meister, 1998; Kuneš and Bičík, 2002; Swaisgood et al., 2006; Metrione et al., 2007, Metrione and Harder, 2011; Cinková and Bičík, 2013) or have been largely observational in nature (e.g. Owen-Smith 1973, 1975; Patton, et al., 2012, 2016; 2018b). Only a minority of studies have applied some form of quantitative analysis in understanding the social behaviour of free-ranging white rhinos (Rachlow et al., 1998; Shrader and Owen-Smith, 2002; Cinková et al. 2016, Rees, 2019). The current work demonstrates that with intensive study, behavioural changes that are rare or difficult-to-observe can be quantified in wild populations and therefore also contribute towards evidence-based conservation policies.

The findings from this thesis suggest that dehorning has a negligible impact on white rhino behaviour and physiology, in terms of agonistic social interactions, stress response and resource access. Specifically, the findings from Chapter 3 indicate that dehorned and horned individuals exhibit similar types of agonistic and cohesive social behaviours. Although some changes in aggression were detected following dehorning, these were primarily observed among subadults and thus likely to have a minimal impact on reproductive success and individual fitness. However, the effects of dehorning on long-term male reproductive success were outside the scope of the study due to the rare occurrence of territorial challenges (Owen-Smith, 1973) and the relatively short time scale of the field work. Despite these constraints, fights were observed to be similar in length to those of horned rhinos reported in the literature (Owen-Smith, 1973) and their infrequent incidence coupled with the lack of serious injuries supports observations of adult and subadult dehorned rhinos made by Patton et al. (2018a). Additionally, by considering historic trends in inter-calving intervals of females (Appendix 1), it was found that the rhinos at Site A did not undergo a reduction in fecundity following dehorning. This supports work by Ververs et al. (2017) who reported healthy population growth rates from a population of dehorned but game ranched white rhinos which had shorter inter-calving intervals than those reported from a free-ranging population (Rachlow and Berger, 1998).

The apparent minor effects of the dehorning were further validated by the endocrine analyses in Chapter 4. No major trends in gonadal or adrenal hormone levels were detected in response to dehorning; consequently rhinos that underwent the procedure did not exhibit any endocrinological signs of chronic stress. The lack of a detectable long-term physiological response suggests that the acute stress response detected following dehorning in game ranched rhinos by a previous study (Badenhorst et al., 2016) relates to the effects of immobilisation and the trimming procedure rather than psychosocial or behavioural changes resulting from the smaller size of horns. Although the potential link between social behaviour and stress was not modelled in this study, the results suggests that the higher levels of aggression observed in the recently-dehorned group of rhinos did not result in a spike in FCMs. This is contrary to a study of captive rhinos by Schmidt and Sachser (1997) who detected higher levels of FCMs following an increased frequency of agonistic interactions. In Chapter 5, a combined camera trapping and focal behavioural study found no evidence that dehorning affected behaviours relating to resource access, digging or rubbing. Thus, the concerns that dehorning may result in behavioural consequences beyond social interactions and predator defence (Berger and Cunningham, 1996; Lindsey and Taylor, 2011; Trendler, 2011) were not supported. The existence of such apparent behavioural plasticity in response to artificially shortened horns may be an adaptation to the natural changes in horn size that occur throughout a white rhino's lifespan due to growth and wear (Pienaar et al., 1991).

Lindeque and Erb (1995) hypothesised that if the shape and length of horns were important for calf protection in black rhinos, then horns would have evolved towards an optimum size, and that the high levels of variation found within populations indicated that such characteristics could be non-adaptive. A similar argument could be made for white rhinos, given that they also exhibit high levels of individual variation in horn parameters (Pienaar et al., 1991; Kock and Atkinson, 1993; Rachlow and Berger, 1997). However, such a hypothesis does not consider the existence of a threshold effect or how progressive changes in physical traits throughout an individual's life can strongly affect fitness, for example the relationship observed between fecundity and female body size in many egg laying species (Honěk, 1993) or the phenotypic optimality point of sex changes in hermaphroditic species (Munday et al., 2006). It is possible that significant changes would only be observed after the complete removal of horn where no stub remains. However, this cannot be employed as an anti-poaching tactic, as it would involve a medically invasive procedure that could severely compromise the welfare of individual rhinos (Derkley et al., 2019). Despite this, the lack of a significant behavioural or physiological changes observed in this research may mean that such a non-adaptive explanation can be applied, but only in narrow terms of resource access and non-territorial forms of social interaction within the range of horn sizes observed.

The rhinos at Site A were dehorned prior to the start of the research as a crisis response to a previous poaching incident. This prevented observations being made prior to and after the first dehorning, where individuals could have acted as their own control. This would have made for a more desirable experimental design than the comparison between populations, where populations varied in both size and density. However, the sites' geographic proximity reduced the potential of other confounding effects, such as climate and habitat variability. While the differences in populations limited the strength of the conclusions, the paucity of data on the subject make the findings reported here valuable and unique in the field of rhino conservation. Furthermore, observations made both prior to and after repeated dehorning events within the Site A population allowed for a robust consideration of longer-term effects resulting from multiple procedures. Such insights are important, as it is now common practise for southern African reserves to re-dehorn their entire rhino populations every 18 months (Emslie et al., 2019). The asymmetries in horn size within the Site A population resulted from differences in age and sex related growth rates and the variable timings of dehorning events (Pienaar et al., 1991; Rachlow and Berger, 1997; Ververs, 2018). This is representative of the conditions found within many private reserves, where it is best practise to dehorn as many individuals in a population as possible to minimise poaching risk (Milner-Gulland, 1999; Lindsey and Taylor, 2011). However, no comparisons were made between adult horned and dehorned rhinos within the same population and so the effects of larger horn size asymmetries on rhino behaviour were not evaluated.

In addition to providing insights into rhino responses to dehorning, this research is the first to demonstrate the existence of dominance hierarchies among a stable grouping of free-ranging rhinos outside of territoriality. This is contrary to previous research that has considered rhinos to only form non-territorial hierarchies under captive conditions (Owen-Smith, 1973; Mikulica, 1991; Cinková and Bičík 2013; Rees, 2019). Thus, the lack of a hierarchy reported in other studies is likely an artefact of inadequate sampling size due to the difficultly in observing repeat social interactions between the same individuals in free-ranging populations (Clutton-Brock and Harvey, 1976; Cinková et al., 2016).

African rhino populations continue to face high levels of poaching pressure and require continued protection even after dehorning (Lindsey and Taylor, 2011; Emslie et al., 2019; Rubino and Pienaar, 2018). The work in Chapter 6 demonstrates how novel deterrents could represent a method to move at-risk rhinos away from areas of high poaching threat to areas of relative safety. The research established how exposure to abiotic auditory and visual stimuli can incite repeat avoidance behaviour. Exposure to both the siren and low-altitude drone treatment repeatedly elicited a flight response from rhinos. In comparison exposure to the scent stimuli was ineffective at manipulating movement despite rhinos' strong reliance on olfaction (Owen-Smith, 2013). Furthermore, rhinos often continued to flee after exposure to the stimulus had ended indicating that animals can be moved at significantly longer distances than those reported in the study.

7.3 Conservation implications

The lack of detectable physiological effects and limited behavioural effects observed during this study, support that dehorning should be pursued as a conservation tactic in situations where it can reduce the probability of poaching below that of immobilisation-induced mortalities. Dehorning is increasingly employed as a conservation strategy throughout private and state reserves in southern Africa (Taylor et al., 2017; Emslie et al., 2019; SANParks, 2019). For example, Kruger National Park, which holds the single largest global population of white rhinos, only began selectively dehorning animals in May 2019 (Emslie et al., 2019; SANParks, 2019). Recent analysis by Derkley et al. (2019) suggests that the fitness costs of physiological and psychological distress experienced during dehorning by the immobilisation process, such as hypertension, hyperthermia and acidosis, will always be substantially lower than that experienced by a poached animal (which usually ends in death). However, few studies have sought to understand the potential for more wider-scale or longer-term behavioural implications of dehorning until now. This research should aid conservation managers in understanding the biological implications of dehorning and ensure future decisions consider rhino welfare when evaluating conservation outcomes.

The absence of a detectable short or long-term physiological response suggests that dehorning compares favourably to other conservation techniques, such as translocation, which can increase stress levels and supress reproductive hormones over several weeks (Linklater et al., 2010; Capiro et al., 2014; Göttert et al., 2015; Yang et al., 2019). Dehorning also preserves habitat availability for the global population, which decreases when all individuals are translocated from an area (Emslie et al., 2019). Thus, when considering the data from this study, the current guidelines on the frequency of dehorning, which is set to the level that most effectively reduces poaching risk, do not require revision (Lindsey and Taylor, 2011).

Dehorning can reduce poaching pressure when the monetary reward available to the poacher decreases relative to the effort required to poach (Du Toit and Anderson, 2013). However, the procedure can also displace poaching to neighbouring horned populations as long as the demand for horn remains (Daly et al. 2011). Thus, dehorning can increase the chances that rhinos will persist in an area where they would otherwise not survive but not decrease poaching risk across the entirety of the species' range (Du Toit and Anderson, 2013). Dehorning can can be used to focus protection efforts on specific groups of animals (SANParks, 2019), such as genetically distinct individuals. For example the last two northern white rhinos have been dehorned in Kenya (Save The Rhino, 2015) despite (or perhaps because of) the procedure's limited practise in the country. However, if rhinos were dehorned across the entirety of their range but remained the only source of horn available to poachers, there would be little protective benefit from the procedure. Although in this situation, advocates for the wide scale practise of dehorning suggest that income from the legal sale of horn could be used to fund rhino protection (Taylor et al., 2017).

It was not possible to analyse the degree to which physical differences between combatants affected the frequencies of fights or the turnover rate of territories because of the rarity of these behaviours. Given these interactions between males are likely to have the greatest effects on individual fitness (Rachlow et al., 1998; White et al., 2007;

Guerier, et al., 2012), it remains possible that there are longer-term reproductive implications for dehorned populations that were not detected by this study. Thus, where funds are available for other less invasive anti-poaching techniques, such as increased patrols or the full-time guarding of rhinos (Patton et al., 2011; Haas and Ferreira, 2017), it would be precautionary to pursue these instead. If dehorning is to be carried out, the framework outlined by Milner-Gullard (1999) which suggests the optimal anti-poaching strategy is to dehorn as many rhinos in a small population as possible, should still be adhered too. In contrast, fitness effects are likely to be less important in more intensively managed populations, where reproductive outcomes are heavily influenced by translocations, skewed towards female sex ratios, or split into individual breeding camps (Uys, 2017; Ververs, 2018). In these conditions, natural social behaviours, such as territoriality, will have less of an influence on individual reproductive success and so dehorning can be pursued without disrupting this.

Rhinos are kept under an extensive range of management conditions, and vary in population size, demographic structure and density (Owen-Smith, 1973; Hutchins and Kreger, 2006; Uys, 2017; Emslie et al., 2019). Individuals will also differ in health status, internal physiology, social experience and social status (Rachlow et al., 1998; Shrader and Owen-Smith, 2002; Kretzschmar et al., 2004; Shrader et al., 2006; Patton et al., 2018a). Thus, given that responses to dehorning may vary under these different contexts it is essential to take all possible measures that reduce or eliminate the potential for stress during and after the procedure. Standard welfare practises performed during dehorning include cutting the horn above the germinal layer to avoid abnormal regrowth or infection (Kock and Atkinson, 1993), minimising the durations of procedures to reduce health problems such as hyperthermia and hypertension (Morkel and Nel, 2019), and the covering of an animal’s eyes and ears to prevent injury and reduce their perception of the procedure (Lindsey and Taylor, 2011). Given the potential for the separation of companions after the immobilisation procedure it may also be advantageous to dart stable groups of rhinos together where resources allow for it and, as has been stated by previous authors, attempting to minimise asymmetries in horn size within a population where possible (Lindsey and Taylor, 2011).

The existence of a non-territorial dominance hierarchy is of relevance for rhino management policies, as it indicates that similar behaviours observed within captivity do not necessarily result from unnatural conditions (Mikulica, 1991; Kuneš & Bičík, 2002; Metrione et al., 2007; Cinková & Bičík, 2013). This is important, as unusual social conditions have been hypothesised to contribute towards the lower reproductive success observed in some captive populations of white rhinos (Swaisgood et al., 2006). Additionally, the effects of the dehorning procedure on captive populations may be of increasing relevance in the future if poaching spreads to populations held outside of Africa. For example in 2017, Dvůr Králové Zoo in the Czech Republic dehorned all 21 of their rhinos, one of the largest captive populations in Europe, following the poaching of a white rhino in Thoriy Zoo, France (The Guardian, 2017). Observations of rhino social bonds prior to capture and translocation may also aid in the selection of suitable companions for the release site. For example, the social position of white rhinos kept in a boma was found to be a predictor of post-release group structure, with animals adjacent in dominance ranking forming pairs (Rees, 2019).

This research also identified new methods that could be used alongside existing anti-poaching tactics. The rising costs of effective anti-poaching security are putting significant financial pressure on both national parks and private reserves (Rubino and Pienaar, 2018), where the apprehension of poachers and reduction of incursions are primarily achieved through foot and vehicle patrols (Barichievy, et al., 2017; Haas and Ferreira, 2017). Given the need for continued anti-poaching protection whether or not a population has been dehorned (Lindsey and Taylor, 2011), there is a clear requirement for effective, low-cost and readily applicable techniques to aid on-the-ground conservation efforts. Deterrents could act as a useful anti-poaching tactic if they allow for the movement of rhinos away from poaching hotspots. Poaching risk varies both temporally and spatially due to changes in lunar illumination, accessibility, weather, patrol intensity, and poacher presence (Mulero-Pázmány et al., 2014; Park et al., 2015; Barichievy, et al., 2017; Koen et al., 2017). Thus, the flexibility in choosing where and when to deploy deterrents could aid the efficiency of existing patrols. Such a strategy would be most suited for use in private reserves, which are usually fenced and smaller than state or national parks (Sims-Castley et al., 2005; Thompson, et al., 2016) but hold approximately 50% of Africa's white rhino population and are typically subject to more intensive management than national park populations (Emslie et al., 2019).

Poaching tactics can vary depending on whether they are carried out by an individual or orchestrated by an organised criminal syndicate (Rademeyer, 2016a). While there are reports of increasingly sophisticated rhino poaching gangs (e.g. instances with helicopters), the technologies poachers' use are generally restricted to firearms, GPS and mobile phones (Duffy, 2014) with poachers typically operating on foot (Haas and Ferriera, 2018). Thus, reserves' are likely to continue to experience differential levels of risk in space and time (leading to zones of relative refuge and danger) regardless of any changes in poaching tactics.

The majority of faecal samples were collected prior to the start of the deterrent experiments, with no samples collected if the peak excretory period of FCMs overlapped with disturbance, so it was not possible to establish whether rhinos responded to the deterrent stimuli with an acute physiological response. To ensure that the welfare of a manoeuvred rhino outweighs any costs of exposure to the deterrent (e.g. lost foraging opportunities and increased anxiety), the deterrents should only be utilised when an animal's risk of being poached is high. Additionally, it will be important to determine the minimum amount of exposure needed to trigger avoidance behaviour and if a single exposure event can lead to continued avoidance of an anxiety-associated area (Schakner and Blumstein, 2013).

The existing usage of drones as an anti-poaching tool is largely limited to the detection and interception of poachers (Mulero-Pázmany et al., 2014; Shaffer and Bishop, 2016; López and Mulero-Pázmány, 2019. Hambrecht et al., 2019). However, as prices fall and technology improves, their usage in conservation is likely to become more widespread (Koh and Wich, 2012; Chabot et al., 2015). To minimise disturbance during reconnaissance and monitoring, flights should be restricted to altitudes of 100 m or greater during reconnaissance or monitoring, or models should be selected with a lower acoustic output. This would also reduce the chances of rhinos habituating to their usage as a herding tool. Additionally, the drone induced flight behaviours across several ungulate species and its capability of pursuit and ability to be deployed beyond the location of an operator may make such effects useful to management practises beyond anti-poaching. For example, the aerial pursuit of wildlife is often a necessary part of game capture operations, veterinary procedures and population monitoring (du P. Bothma and du Toit, 2010; Lindsey and Taylor et al., 2011) and in some instances drones could fulfil this function at a significantly reduced financial cost while also being used for reconnaissance.

The drone used in this study (a DJI Mavic Pro) retailed for around £1000 (2016 GBP). The costs of using the drone must also take into account the employment of an operator. In South Africa, drones can be used for an individual's personal and private purposes where there is no commercial outcome, interest or gain (The South African Civil Aviation Authority, 2019). This requires operating the drone within a restricted visual line of sight (up to 500 m from the operator) and flying drones with a mass less than 7 kg. Both of these criteria can be met when operating the drone as a deterrent, with the drone used in the study weighing less than 1 kg in mass. To fly at distances greater than 500 m, the South African Civil Aviation Authority (SACAA) requires the operator to obtain a 'Remote Pilots Licence'. Certification can be obtained from a range of commercial companies, with one operator registered with the SACAA charging 25,500 rand (ZAR 2019) for theory and practical training (UAV Industries, 2019). These costs are cheaper than other conservation techniques used to reduce poaching risk, such as translocation, which includes capture, quarantine, transport, release and monitoring (Rees, 2018). For example, the organisation Rhinos Without Borders estimated it would cost around $45,000 (USD 2014) per rhino when translocating ten animals from South Africa to Botswana (Rhinos Without Borders, 2019).

7.4 Opportunities for future research

By considering several unstudied aspects of white rhino behavioural ecology in relation to dehorning, this study provides a deeper understanding into how rhinos respond to the procedure. However, there remain several behavioural aspects in which further study would be of benefit. Principally, detailed data on individual changes in territoriality in dehorned white rhino populations could answer whether dehorning has any influence on male long-term reproductive success. Analyses of changes in genetic parentage would reduce the need for intensive behavioural observations to be conducted over long periods (Guerier, et al., 2012), with immobilisations providing opportunities to collect genetic samples from dehorned individuals (Purisotayo et al., 2019). For example, rates of territorial turnover within a population could be estimated by tracking changes in reproductive dominance before and after dehorning. Furthermore, although the rhinos in this study showed horn asymmetries through different timed procedures and varying rates of regrowth, the differences were not as significant as those found in populations where only a percentage of individuals are dehorned. This occurs in some larger national parks, where it is unfeasible to dehorn all individuals due to the prohibitive costs and logistical difficulties of working in remote areas (Lindsey and Taylor, 2011). In situations such as these, dehorning may be performed opportunistically, resulting in high asymmetries in horn size within the population (SANParks, 2019). A recent dehorning operation in Kruger National Park, dehorned only adult female rhinos because of their high contribution to population growth but also because of the lower perceived chance of social disruption (SANParks, 2019). Further study is vital to explore whether male rhinos with high horn and body size disparities experience changes in territoriality or whether there is a horn size threshold at which such effects occur. Such a threshold apparently exists naturally in male black rhinos, where individuals with horn sizes at least 10 cm greater than their opponents were more likely to dominate the interaction (Berger and Cunningham, 1998). Observations of hornless rhinos, which have survived following poaching incidents after intensive medical interventions could also provide valid insights into horn function. In one instance, a male rhino that survived having both its horns hacked off went on to sire a calf after being placed in captivity with a horned female although no other males were present (Saving the Survivors, 2019).

Investigations into whether dehorned black rhinos exhibit a short or long-term physiological response would also be of interest. The stress monitoring methods used in the study should be applicable to the species, especially given that many of the endocrine validations needed to establish a link between physiological response and stressor have already been performed (Brown et al., 2001; Turner et al., 2002; Steuer et al., 2010). As outlined in Chapter 4, future work may benefit from studying captive or range-limited populations, where the presence of fewer confounding conditions may increase the chances of establishing a causal link. Although the lower proportion of black rhinos held on privately managed land than white may limit the transferability of such conclusions to larger less intensively managed populations (Emslie et al., 2019). The potential effects of dehorning on Asian species of rhino are unknown, as the practise remains rare outside of southern African countries (Lindsey and Taylor, 2011).

Further work is needed to establish whether the effects of the deterrents persist for a time frame beyond that demonstrated in the study and whether the distances that the rhinos move can be reliably increased. It should also be established whether rhinos will habituate to the stimuli following increased rates of exposure, as has occurred in other studies that rely on deterrents (Osborn and Parker, 2003). It will also be important to calculate the lag between a rhino's removal from a site and its return. Additionally, this study provides a foundation for future investigations wishing to develop deterrents for use with white rhinos, providing methods for the evaluation or exploratory use of other stimuli. Potentially favourable avenues of research may include conspecific scents or the scents of predators, for example, white rhinos can identify the sex, age, territorial and oestrous state of conspecifics from their dung (Marneweck et al., 2017). Furthermore, speaker-mounted drones would allow for a combination of aerial pursuit and the continued modification of acoustic stimuli to reduce the chances of habituation (Talling et al., 1998).

7.5 Final statement

The southern white rhinoceros has been actively conserved for over 120 years (Rookmaaker, 2000) resulting in a dramatic increase in population size (Emslie and Brooks, 1999; Knight, 2017). However, recent losses threaten to push the species back on the path towards extinction (Di Minin et al., 2015; Ferreira et al., 2018; Emslie et al. 2019). Ultimately, the current poaching crisis cannot be solved by the *in situ* management of poaching risk alone. The long-term safety of the species can only occur following the implementation of strategies that tackle the causes of poaching, such as ceasing demand from consumer states, reducing poverty and inequality in range states, or even the establishment of a legal sustainable trade.

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Appendix 1 – Other social behaviours

A) Territorial fights observed within dehorned and horned populations of white rhinos

Fights were observed between rhinos within both the dehorned and horned populations (two fights in Site A and one within Site B). Fights were distinguished from other forms of agonistic interactions by the presence of at least one territorial bull and an engagement in horn fencing from both individuals. Fights were only observed during opportunistic observations and so were excluded from analyses in Chapter 3. During fights similar ranges of social behaviours were exhibited within both the dehorned population and horned population. Additionally, two dehorned adult males that were dominant at the start of behavioural observations retained their territories throughout the monitored period. In Site B, a fight was observed between two adult bulls of a similar body and horn size. The rhinos stared at one another nose-to-nose and engaged in horn fencing. One of the two rhinos repeatedly emitted submissive vocalisations, had its tail curled, and ears back. Its opponent had its tail straight and ears forward, identifying it as dominant (Figure A1.1). The fight was already underway on arrival and observations were halted after just 4 minutes when the subjects moved into scrub.

In Site A, a fight was observed between a subadult rhino [SAM1 'Dougie'] and a territorial bull [AM1 'Brutus']. The adult (12 years 194 days old) was over five years older than the subadult (7 years 188 days) but was slightly smaller in body size (confirmed by a veterinarian during darting). Additionally, the adult had a bigger horn than the subadult, having been dehorned 273 days prior to the fight, compared with eight days prior. The dehorned rhinos exchanged horn-to-horn blows, several horn thrusts, and engaged in shouldering, horn-to-horn staring and horn fencing. The fight was underway on arrival and persisted for a further 70 minutes (Figure A1.1). The adult rhino was judged to be dominant throughout the fight as it repeatedly gained ground on its opponent. The subadult attempted to walk away and flee during a break in contact 22 minutes and 40 minutes into the fight respectively but in both cases was pursued by the adult and fighting resumed. Both rhinos suffered superficial injuries, including small open sores on their heads and cracked skin around their horns, but otherwise appeared unharmed.

The subadult was observed with its five long-term group members a minimum of 368 minutes prior to the fight, and re-joined them within 104 minutes after the fight had ended.

A second fight in Site A was between an adult [AM2 'Luke'] and the same subadult [SAM1 'Dougie']. Both rhinos were dehorned 34 days prior to the fight. The fight was underway on arrival and persisted for a further 75 minutes, with a similar breakdown of horn-to-horn blows, horn thrusts, shouldering and horn fencing. Towards the end of the fight the adult rhino pushed the subadult into a dry riverbed. On reaching the riverbed, the subadult roared and then squealed intensely, signalling submission, at which point the fight ended. Throughout the fight, the five members of the subadult's long-term group were intermittently foraging and alert approximately 400 metres away and the subadult re-joined them within 10 minutes of the fight ending. The adult (10 years 153 days old) was nearly three years older than the subadult (7 years 214 days) but similar in body size.

B) Cohesive social behavioural observed within dehorned and horned populations of white rhinos

Rhinos within the dehorned and horned populations were observed engaging in similar sequences of cohesive behaviour. Cows frequently approached one another if foraging in close proximity, crossing paths, or utilising resources such as salt licks, waterholes and wallows. In a typical interaction the adult females approached until they stood head to head, sometimes making physical naso-naso contact but usually stopping just short of one another. This behaviour lasted for a few seconds to several minutes, after which one or both rhinos would move away. Calves often took this opportunity to horn-wrestle or chase members of other groups (Figure A1.2), particularly other calves or sub-adults, but on occasion with permitting adults too. Horn wrestling also occurred between subadults within social groupings in the dehorned and horned populations. The shortest observed horn wrestling events lasted less than a minute, but in two cases horn wrestling was observed for greater than an hour (both at Site B). Outside of mother-calf interactions and horn wrestling behaviour, cohesive physical contact was predominately observed during apparently accidental physical nudges and during defensive group guarding behaviours and resting, where rhinos touched flanks. Lip nuzzling from one rhino towards another's back was also observed within stable sub-adult groupings but only on two occasions.

Rhinos in long-term social groupings actively maintained their group membership by pursuing one another when separated. Contact calls, consisting of a panting vocalisation, were frequently made by group members that became separated from one another (usually after a distance greater than 50 metres) or during the active approach of a non-group member. Contact calls were not observed in adult females or calves, which stayed in close proximity together and did not call after other individuals associating with them. Six subadult rhinos were within a stable long-term grouping prior to the dehorning procedure but became separated during darting. Within six hours of the event, four of the six rhinos were back grazing together, but the fifth and sixth rhinos remained independent of the main group for up to 50 and 80 hours after the dehorning respectively. On another occasion four individuals that became separated from their two other group members searched for their missing companions for over 100 minutes without stopping to forage. Contact calls were emitted throughout the entire search, but not always by all four rhinos simultaneously, as they intermittently ran and walked across the reserve.

Figure A1.1. Fighting behaviours in horned and dehorned rhinos. Upper panel: the left rhino's forward facing ears indicate dominance over its opponent; conversely the right rhino's backwards facing ears and curled tail indicate submission, as did its concurrent vocalisations. Middle panel: An adult rhino [AM1] and subadult [SAM1] staring horn to horn between horn fencing, horn-to-horn collisions and shouldering during a fight. Lower panel: An adult rhino [AM1] shouldering the subadult [SAM1] during the same fight. The subadult was violently pushed back a distance of approximately 40 metres.

Figure A1.2. Horn wrestling behaviour between a dehorned subadult [SAM1] and horned calf [CM1] of 292 days old. The rhinos horn wrestled for 3 minutes 28 seconds, separated for five minutes then wrestled for a further 35 seconds.

Appendix 2 – Social behaviour data

Table A2.1. Individual breakdown of inter- and intra-group agonistic social interactions among eight rhinos before and after a dehorning event. Subsets represent rates sampled from a stable group of six individuals. Encounters were observed prior to dehorning on 02/06/2016 between 28/01/2016 and 20/04/2016, and post-dehorning between 02/06/2016 and 15/10/2016.

ID	Obs. length (min)	Intergroup agonistic interactions				Intra-group agonistic interactions			
		Pre-dehorning		Post dehorning		Pre-dehorning		Post dehorning	
		Count	Rate	Count	Rate	Count	Rate	Count	Rate
AF2	1083	1	0.06	2	0.11	-	-	-	-
AM2	1252	7	0.34	12	0.57	-	-	-	-
SAM1	2224	5	0.14	3	0.08	11	0.30	24	0.65
Subset	1015	-	-	-	-	6	0.35	10	0.59
SAF1	2086	2	0.06	1	0.03	1	0.03	21	0.60
Subset	890	-	-	-	-	2	0.13	16	1.08
SAM2	2250	6	0.17	2	0.06	15	0.40	26	0.69
Subset	890	-	-	-	-	5	0.34	18	1.21
SAM3	1080	1	0.06	1	0.06	3	0.17	20	1.11
Subset	980	-	-	-	-	3	0.18	28	1.7
SAM4	1923	4	0.25	2	0.06	10	0.31	28	0.87
Subset	975	-	-	-	-	2	0.12	22	1.35
SAM5	1941	2	0.06	1	0.03	18	0.56	19	0.59
Subset	965	-	-	-	-	5	0.31	9	0.62

Table A2.2. Individual breakdown of inter-group agonistic encounters between eight rhinos and four age-sex classes. Counts of interactions are shown pre- and post- a dehorning procedure.

ID	**Adult male**		**Adult female**		**Subadult**		**Calf**	
Class	**Pre-**	**Post-**	**Pre-**	**Post-**	**Pre-**	**Post-**	**Pre-**	**Post-**
AF2 (Kelly) Adult female	1	2	0	0	0	0	0	0
AM2 (Luke) Adult male	3	0	2	8	1	4	1	0
SAM1 (Dougie) Subadult male	3	2	2	1	0	0	0	0
SAF1 (Reine) Subadult female	2	0	0	1	0	0	0	0
SAM2 (Chilli) Subadult male	4	1	2	1	0	0	0	0
SAM3 (Courtney) Subadult male	0	0	1	1	0	0	0	0
SAM4 (Logan) Subadult male	3	0	1	2	0	0	0	0
SAM5 (Willis) Subadult male	1	0	1	1	0	0	0	0

Table A2.3. Association matrix of agonistic dyadic encounters between six individuals. Totals indicate the number of agonistic dyadic encounters between rhino pairings and the sum of all encounters they engaged in (*Ni*). Data is shown for encounters for which both a winner and loser could be ascertained (See Chapter 3). Encounters were observed prior to dehorning on 02/06/2016 between 05/04/2016 and 21/05/2016, and post-dehorning between 04/06/2016 and 12/10/2016. The matrices are required in the calculation of the mean scale score (*Qi*) of animals that a_i met in agonistic encounters for the Batchelder-Bershad-Simpson index (BBS) as outlined by Jameson et al. (1999).

a_i	SAF1	SAM1	SAM2	SAM3	SAM4	SAM5	N_i
Pre-dehorning							
SAF1	-	0	2	2	1	1	6
SAM1	0	-	0	3	5	7	15
SAM2	2	0	-	3	0	2	7
SAM3	2	3	3	-	1	1	10
SAM4	1	5	0	1	-	2	9
SAM5	1	7	2	1	2	-	13
Post-dehorning							
SAF1	-	2	4	7	8	2	23
SAM1	2	-	3	10	4	5	24
SAM2	4	3	-	6	9	6	28
SAM3	7	10	6	-	9	5	37
SAM4	8	4	9	9	-	0	30
SAM5	2	5	6	5	0	-	18

Table A2.4. Win-loss matrix for individuals involved in agonistic social interactions prior to and after a dehorning procedure. The number of encounters in which animal a_i was observed to have won (W_i) and the number of encounters in which a_i lost (L_i) are required in the calculation of the BBS index.

		Loser						
	a_i	SAF1	SAM1	SAM2	SAM3	SAM4	SAM5	**W_i**
	Pre-dehorning							
Winner	SAF1	-	0	2	2	1	1	6
	SAM1	0	-	0	2	3	6	11
	SAM2	0	0	-	3	0	1	4
	SAM3	0	1	0	-	0	0	1
	SAM4	0	2	0	1	-	2	5
	SAM5	0	1	1	1	0	-	3
	L_i	0	4	3	9	4	10	-
	Post-dehorning							
Winner	SAF1	-	2	4	7	8	2	23
	SAM1	0	-	2	8	3	4	17
	SAM2	0	1	-	2	4	3	10
	SAM3	0	2	4	-	5	2	13
	SAM4	0	1	5	4	-	0	10
	SAM5	0	1	3	3	0	-	7
	L_i	0	7	18	24	20	11	-

Table A2.5. Calculation of BBS ratings (s(ai)) from the dyadic encounters of six rhinos prior to their dehorning. The ratings had begun to converge after the first ten iterations but did not become invariant until the 78th step (intervening iterations not shown), which represents the final BBS values. a_i = animal identity; w_i = the number of encounters in which animal a_i was observed to have won; N_i = the number of encounters in which a_i was involved; L_i = the number of encounters in which a_i lost.

	a_i	**SAF1** (Reine)	**SAM1** (Dougie)	**SAM2** (Chilli)	**SAM3** (Courtney)	**SAM4** (Logan)	**SAM5** (Willis)
	W_i	6	11	4	1	5	3
	L_i	0	4	3	9	4	10
	N_i	6	15	7	10	9	13
Iteration steps	**0**	1.25331	0.58488	0.17904	-1.00265	0.13926	-0.67486
	1	1.70558	0.46429	0.02128	-1.17372	0.42504	-0.69374
	2	1.52344	0.51652	0.07179	-1.14008	0.38509	-0.71735
	3	1.59518	0.49892	0.02742	-1.15204	0.39237	-0.69902
	4	1.5728	0.50751	0.04803	-1.15373	0.3933	-0.7096
	5	1.57856	0.50254	0.03789	-1.15041	0.39304	-0.70352
	6	1.57746	0.50596	0.04269	-1.15321	0.39265	-0.70709
	7	1.57749	0.5036	0.04016	-1.15136	0.39332	-0.70487
	8	1.57756	0.50523	0.04159	-1.15253	0.39271	-0.70629
	9	1.57753	0.50413	0.04071	-1.1518	0.39318	-0.70537
	10	1.57753	0.50486	0.04127	-1.15226	0.39285	-0.70597
	~	…	…	…	…	…	…
	77	1.57753	0.50457	0.04105	-1.15208	0.39298	-0.70573

Table A2.6. Calculation of BBS ratings ($s(a_i)$) for six rhinos from dyadic encounters after their dehorning. The ratings had begun to converge after the first ten iterations but did not become invariant until the 46th step (intervening iterations not shown), which represents the final BBS values. a_i = animal identity; w_i = the number of encounters in which animal a_i was observed to have won; N_i = the number of encounters in which a_i was involved; L_i = the number of encounters in which a_i lost.

	a_i	**SAF1** (Reine)	**SAM1** (Dougie)	**SAM2** (Chilli)	**SAM3** (Courtney)	**SAM4** (Logan)	**SAM5** (Willis)
	W_i	23	17	10	13	10	7
	L_i	0	7	18	24	20	11
	N_i	23	24	28	37	30	18
Iteration steps	**0**	1.25331	0.52221	-0.35809	-0.37261	-0.41777	-0.27851
	1	1.7002	0.61011	-0.61024	-0.41367	-0.48203	-0.38299
	2	1.62006	0.56625	-0.58883	-0.376	-0.4391	-0.40438
	3	1.6445	0.58064	-0.58769	-0.392	-0.4486	-0.40787
	4	1.63748	0.57384	-0.58988	-0.38608	-0.44462	-0.40522
	5	1.63992	0.57666	-0.5885	-0.38828	-0.44628	-0.40697
	6	1.63901	0.57548	-0.58923	-0.38747	-0.4455	-0.40607
	7	1.63938	0.57597	-0.58886	-0.38776	-0.44587	-0.40651
	8	1.63922	0.57577	-0.58904	-0.38766	-0.44569	-0.4063
	9	1.63929	0.57585	-0.58896	-0.3877	-0.44578	-0.4064
	10	1.63926	0.57582	-0.589	-0.38768	-0.44574	-0.40635
	~	…	…	…	…	…	…
	46	1.63927	0.57583	-0.58898	-0.38768	-0.44575	-0.40637

Appendix 3 – Inter-calf Intervals in Site A

Table A3.1. Adult females present at Site A between 25/11/1992 and 13/01/2019. Emboldened dates indicate a mother was dehorned prior to calf birth. Asterisk indicates a stillbirth. Jubalani was sold from the reserve prior to the dehorning, while Cheeky Cow was killed in a poaching attack.

Calf birth	Female ID - Cheeky Cow	 AF1 Nkombi	 - Jubulani	 AF2 Kelly	 AF3 Mish	 AF4 Jodi	 SAF1 Reine
1	01/12/1997	01/12/1997	Unknown	06/02/2006	18/05/2012	**25/01/2016**	**10/04/2017**
2	15/01/2001	20/07/2001	23/01/2002	15/11/2008	**02/04/2016**	**13/01/2019**	
3	04/04/2004*	07/04/2005	24/05/2004	28/05/2012			
4	01/01/2006	29/03/2008	20/04/2006	**21/06/2015**			
5	15/04/2008	22/03/2010	06/12/2008	**15/03/2018**			
6	10/04/2011	13/02/2013					
7	17/03/2014	**15/05/2015**					
8		**04/03/2018**					
Age at First Calf (months)	Unknown	Unknown	Unknown	99.6	86.6	87.6	85.9

Table A3.2. Historic inter-calf interval (ICI) data for rhinos that gave birth to more than one calf at Site A between 01/12/1997 and 14/01/2019. Data shows the number of births, number of ICIs, mean length of ICIs in months, standard deviation of ICI lengths and number of independent subjects per ICI calculation. Pre-dehorning ICIs are calculated from all births that occurred prior to a rhino's first dehorning procedure. Post-dehorning ICIs are calculated from all births for which conception (estimated as 495 days prior to birth) occurred after a rhino's first dehorning procedure. All known pregnancies resulted in live births except for one stillbirth (SB). For Cheeky Cow, calculations are shown both with and without the stillbirth, as the stage of pregnancy at birth was not recorded. Total means are calculated from the independent ICI lengths per rhino rather than all ICI data. Means are also shown for Kelly (AF2) and Nkombi (AF1), the only two females for which ICI data were available both prior to and after their dehorning.

Female ID	**Total**					**Pre-dehorning**					**Post-dehorning**				
	Births	**ICI no.**	**ICI**	**SD**	**n**	**Births**	**ICI no.**	**ICI**	**SD**	**n**	**Births**	**ICI no.**	**ICI**	**SD**	**n**
Cheeky Cow +SB	7	6	33.1	6.42	1	7	6	33.1	6.42	1					
Cheeky Cow -SB	6	5	39.7	10.95	1	6	5	39.7	10.95	1					
Jubulani	4	3	27.9	3.63	1	4	3	27.9	3.63	1					
Nkombi	8	7	35.2	7.26	1	6	5	37.0	7.62	1	2	2	30.8	3.38	1
Kelly	5	4	36.8	3.88	1	3	2	38.4	4.62	1	2	2	35.3	2.02	1
Mish	2	1	47.2	-	1	1	-	-	-	-	1	1	47.2	-	1
Jodi	2	1	36.1	-	1						2	1	36.1	-	1
All +SB	28	22	36.0	5.78	6	21	16	34.1	4.08	4	7	6	37.3	6.03	4
All –SB	27	21	37.2	5.74	6	20	15	35.7	4.63	4	7	6	37.3	6.03	4
Kelly & Nkombi	13	11	36.0	8.35	2	7	7	37.7	9.14	2	4	4	33.0	5.94	2