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**Association between reproductive behaviour and
androgen metabolite excretion in free-ranging
male giraffes (*Giraffa camelopardalis*)**

by

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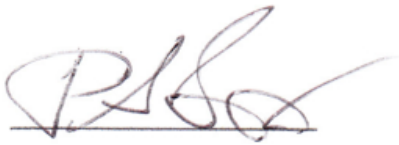
March 2012

Declaration

I declare that the dissertation

“Association between reproductive behaviour and androgen metabolite
excretion in free-ranging male giraffes (*Giraffa camelopardalis*)”

which I hereby submit for the degree MSc (Veterinary Science) at the
University of Pretoria and the work reported on is the result of my own
work and has not previously been submitted by me for a degree at this or
any other tertiary institution.



Peter Seeber, at *Krumpendorf* on this 27th day of March 2012

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Abbreviations

BSA	-	Bovine serum albumin
DMSO	-	Dimethyl sulfoxide
EA	-	Epiandrosterone
EGR	-	Entabeni Game Reserve
EIA	-	Enzyme ImmunoAssay
GC	-	Glucocorticoids
HNP	-	Hwange National Park
HPLC	-	High Performance Liquid Chromatography
m	-	meter
min	-	minute
RIA	-	Radio ImmunoAssay
s	-	second
SRE	-	Sexually Related Event
TMB	-	Tetramethylbenzidine

Abstract

Little is known on the giraffe's reproductive behaviour, particularly observations of male sexual behaviour are scarce. Most information results from studies on captive animals, and observations of free-ranging giraffes were mainly focused on social structure within herds and only yield limited data on the bulls' tendency of roaming between cows' herds. In addition, no data was available on endocrine mechanisms which modulate male reproductive behaviour. Male endocrinology in terms of androgens has only been studied on culled animals, hence no results correlating behaviour and individual androgen concentrations were available so far.

Simultaneous observation of behaviour and endocrine state has become an important tool to monitor reproduction of wildlife species and has brought up valuable information on the reproduction in different species. Non-invasive techniques to monitor endocrine functions facilitate studies of physiological factors, and play an increasingly important role because it is not necessary to interfere with the animal's natural behaviour. In this study, the reproductive behaviour of free-ranging giraffe bulls was monitored synchronously with changes in androgen levels using an Enzyme Immunoassay for non-invasive measurement of faecal androgen concentrations. Hwange National Park (HNP), Zimbabwe, was chosen as a main study site, as it is considered to represent near-natural conditions for the local abundant giraffe population. Behavioural observations were conducted on different classes of mature giraffe bulls in 2011. Additionally, faecal samples for hormone measurement were collected in Entabeni Game Reserve (EGR), South Africa, in order to compare between different populations and to increase sample size for assay validation. Sampling in Entabeni Game Reserve was executed twice, in spring and in autumn (2011) in order to investigate on differences in

androgen concentrations between seasons.

Behavioural data of a total of 77 individually recognizable male animals were recorded using a specifically compiled ethogram, which covered a range of presumably sexually orientated and not-sexually orientated behavioural patterns.

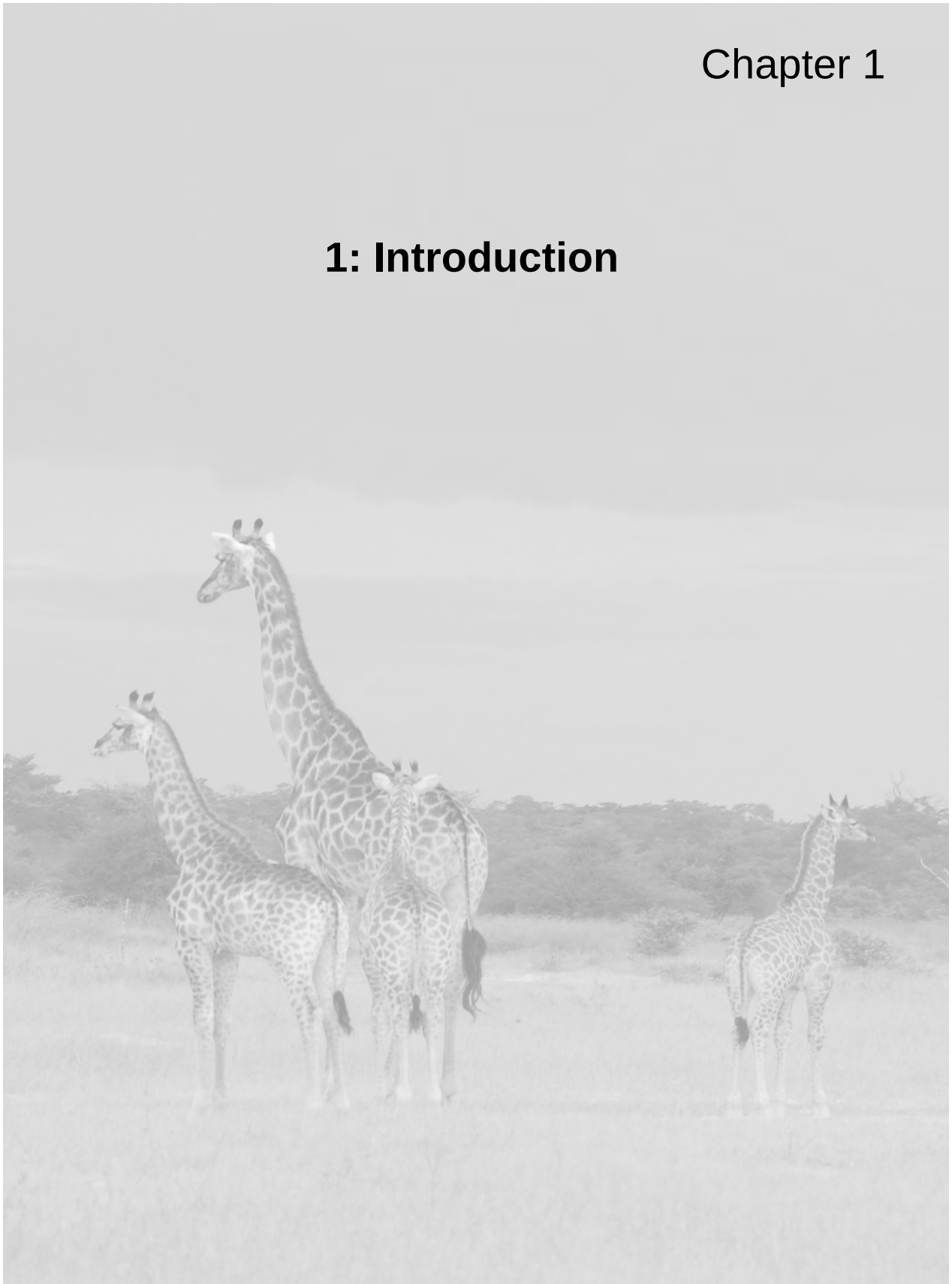
During behavioural observations faecal samples were collected in order to assign samples with individual mating and dominance behaviour. Samples analysed on testosterone (T) and epiandrosterone (EA) concentration at the laboratory facilities of the Endocrine Research Group, Faculty of Veterinary Science. Behavioural data in respect of obviously sexually orientated actions and dominance state were then compared to faecal androgen metabolites in order to investigate on a correlation between endocrine milieu and reproductive behaviour. By comparing EA levels from mature bulls, mature cows, and immature individuals collected in HNP and EGR, an EIA could be identified which reliably measures androgen concentrations in giraffe faeces. Regarding recorded sexual activity patterns of different age classes, the oldest bulls showed to engage significantly more often in sexual behaviour than younger age classes. Accordingly, faecal epiandrosterone concentrations differed significantly regarding age classes of mature bulls. Furthermore, differences between sample sets indicated EA variations between seasons.

This study was conducted in order to contribute to the understanding of the giraffe's mating system and male mating strategies in particular. The outcome advocates for future long-term studies in order to investigate on seasonal and ecological influences on the reproduction of giraffe.



Chapter 1

1: Introduction



The giraffe is the world's tallest land mammal and although numerous studies and reports on its general biology are available, surprisingly little is known about the giraffe's reproduction under natural conditions. Particularly male mating behaviour and its endocrine correlates have hardly been investigated on. Most studies on the reproductive system have been conducted with females, and with animals in captivity, but results on the male counterparts, particularly on animals in-situ, are very scarce. It must also be considered that findings on captive animals must not necessarily apply to their wild counterparts, neither in terms of behaviour nor regarding endocrinology (compare Veasey et al. 1995; Bashaw 2010; Wingfield et al. 1990).

According to Hall-Martin et al. (1978), there are significant individual and age-specific differences in androgen concentrations in giraffe bull testicular tissue. These differences, measured in culled material, remained unexplained, but could be interpreted towards the existence of more complex reproductive factors in giraffe. However, in the giraffe, testicular endocrine function has never been studied in-vivo or in-situ before, despite the fact that methods of non-invasive field endocrinology being a valuable tool to assess and correlate parameters of behaviour and reproductive physiology, as it has been done in a vast range of mammals (Schwarzenberger 2007), including African elephants (Rasmussen et al. 2008; Ganswindt et al. 2010), bison (Mooring et al. 2004), spotted hyaenas (Dloniak et al. 2006) or lynx spp. (Jewgenow et al. 2006). The tool of non-invasive hormone monitoring in a behavioural endocrinology context allows correlating behavioural observations with physiological parameters with no need to interfere with the target animal. This technique facilitates the observation of reproduction and have proven to be of broad use for conservation biology, since the obtained results can reveal crucial responses to stress, pollution and other ecological influences on the individual and therefor on the breeding population (Walker et al. 2005).

Regarding sexual behaviour of giraffe bulls, proximate mating behaviour has been described in captive and, to a much lesser extent, in free ranging animals, but still there is a fundamental lack of knowledge on male reproductive strategies and assumed alternative tactics (e.g. Dagg 1971; Bercovitch et al. 2006; Jolly 2003). Previous studies focusing on behavioural time budgets of giraffe showed that their general behavioural patterns are very adjustable to variations in ecological conditions (e.g. van der Jeugd & Prins 2000; Le Pendu et al. 2000; Ginnet & Demment 1999). This flexibility in behavioural patterns indicates that very specific results might not be relayed over a range of differing ecological conditions.

In order to understand the giraffe's reproductive system in terms of behaviour and physiology, results on animals under near-natural conditions are necessary. By using the technique of non-invasive endocrinology, a range of physiological parameters can be correlated with behavioural results, which facilitates to create a broader picture clarifying the giraffe's reproduction. The aim of this study was to contribute to the knowledge on male giraffe's reproductive behaviour and its endocrine correlates, and, in this course, to validate a tool for non-invasive measurement of faecal androgen metabolites in giraffes.

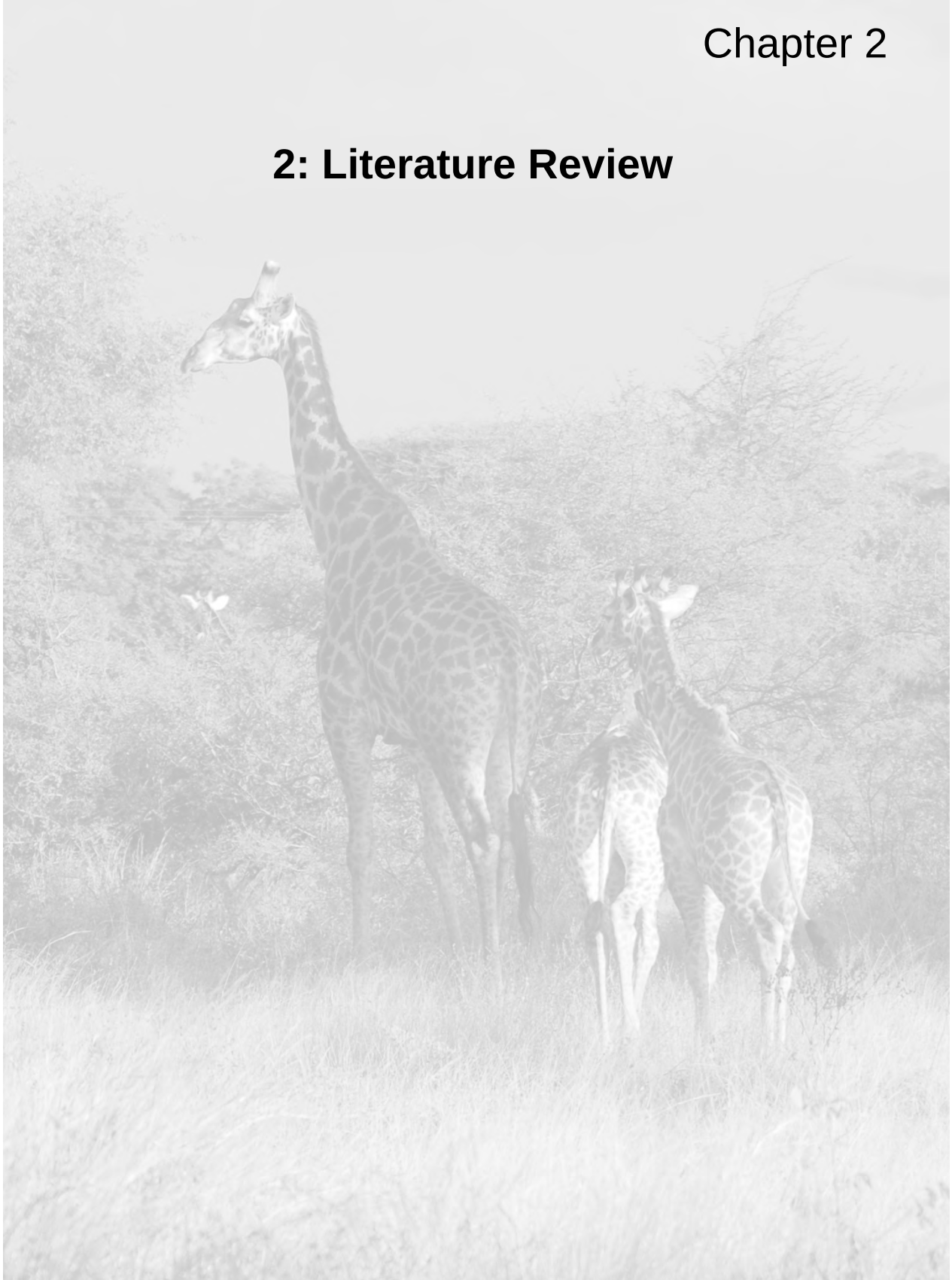
The specific objectives of this study were

- to investigate the reproductive behaviour and social preferences of adult giraffe bulls in different age classes
- to relate age class and dominance behaviour in giraffe bulls
- to establish and validate a method for non-invasive measurement of androgen metabolites in faeces of free-ranging giraffe bulls
- to correlate androgen levels, age class and reproductive / dominance behaviour in giraffe bulls
- to compare androgen levels of giraffe bulls living in different habitats.



Chapter 2

2: Literature Review



2.1 The Giraffe

2.1.1 Taxonomy

The giraffe (*Giraffa camelopardalis*; von Linné 1758) is the world's largest ruminant, belonging to the monospecific genus of *Giraffa* (order *Cetartiodactyla*, suborder *Ruminantia*, infraorder *Pecora*, family *Giraffidae*). The natural distribution of the giraffe is restricted to the African continent and so far, there is great uncertainty concerning number and taxonomy of giraffe subspecies (compare Dagg & Foster 1982; Kingdon 1997; Grupp 2005; Brown et al. 2007; Hassanin et al. 2007) but most commonly, nine subspecies are accepted (Ansell 1968; Table 2.1).

Several sources state that subspecies are distinguishable by their fur colour and spot patterns, which does not seem to be reliable though (Lydekker 1904; Dagg & Foster 1976). The geographic distributions do not overlap in most cases (Fig. 2.1), assumedly due to ecological barriers such as mountainous areas, constant water bodies, tropical rain forest or man built barriers, all of which are usually not trespassed by giraffe. According to recent studies, considerable genetic differences between the subspecies do not only support the division into subspecies but might even account for a division of the monospecific genus *Giraffa* into several species (Brown et al. 2008; Hassanin et al. 2007). Since interbreeding between most of the considered subspecies has been well documented, the apprehension of different giraffe species is, on the other hand, questionable (reviewed in Mitchell et al. 2009).

Subspecies	Common Name	Natural distribution	Census (wild)	Census (captivity)
<i>G.c. angolensis</i>	Angolan / Smoky Giraffe	Botswana, Zambia, Zimbabwe	< 20.000	21
<i>G.c. antiquorum</i>	Kordofan G.	Cameroon, Central African Republic, Chad	< 3.000	64
<i>G.c. camelopardalis</i>	Nubian G.	DR Congo, Ethiopia, Sudan, South Sudan	< 250	161
<i>G.c. giraffa</i>	South African / Cape G.	Botswana, Mozambique, South Africa, Zimbabwe	< 12.000	45
<i>G.c. peralta</i>	West African / Nigerian G.	Niger	< 220	50
<i>G.c. reticulata</i>	Reticulated / Somali G.	Ethiopia, Kenya, Somalia	< 5.000	477
<i>G.c. rothschildi</i>	Rothschild / Baringo G.	Kenya, South Sudan, Uganda	< 700	485
<i>G.c. thornicrofti</i>	Thornicroft / Rhodesian G.	Zambia	< 1.500	unknown
<i>G.c. tippelskirchi</i>	Maasai / Kilimanjaro G.	Kenya, Tanzania	< 40.000	96

Table 2.1. Subspecies of *Giraffa camelopardalis*, distribution and estimated census in the wild and in captivity.

Sources: Ansell 1968; ISIS 2011; IUCN 2010; Giraffe Conservation Foundation 2011; Nowak 1999.

The species' threat status is rated as *least concern* by the International Union for Conservation of Nature (IUCN) although two of the giraffe subspecies are rated as *endangered*, namely *G.c. rothschildi* and *G.c. peralta* (IUCN 2010). The recent distribution of giraffe has been reduced compared to the distribution observed in the late 18th century due to direct human impact such as poaching and habitat degradation as well as increasing aridity and periodic epidemics of rinderpest (Dagg 1971; Fennessy 2010). In the southern African subregion the giraffe has been reintroduced to many areas where the species has already been eliminated and today the giraffe is common inside and outside of sanctuaries in South Africa, Namibia, Botswana and Zimbabwe and to lesser extent in Zambia and Mozambique, without regard of subspecies (Fennessy 2010).

The most southern regions of Africa are inhabited by the South African and the Angolan subspecies (Fig. 2.1.). The present study has been conducted in western Zimbabwe and northern South Africa, hence the study animals presumably belonged to the Angolan and South African subspecies, respectively (Novak 1999; IUCN 2010), but genetic evidence for this assumption was not available.

The subspecies Angolan or *Smokey* giraffe *G.c. angolensis* was first described by Lydekker, 1903 (quoted in Dagg 1971). Its coat pattern shows characteristic large spots with some notches around the edges with spots extending down the entire lower leg. The colour of spots is quite variable and can range from a light reddish brown to a very dark chocolate brown. The Angolan giraffe is assumed to be extinct in Angola today but still thriving in western Zimbabwe, northern Botswana, parts of Namibia and in southern Zambia (Fennessy 2010). Genetic studies revealed that the southern group, consisting of *G.c. giraffa*, and *G.c. angolensis* is distinct from the eastern and

northern groups; taxonomic division on the level of species is supported by analysis of mitochondrial and nuclear markers (Brown et al. 2007; Brenneman et al. 2009).

The South African giraffe was first described as a subspecies by Schreber in 1784 (quoted in Dagg 1971). The South African giraffe has rounded, irregular or blotched spots, on a comparably light background, running down to the hooves. According to genetic studies on mitochondrial and nuclear markers the subspecies *G.c. angolensis* is the closest related group to the South African giraffe (Brown et al. 2007; Hassanin et al. 2007).

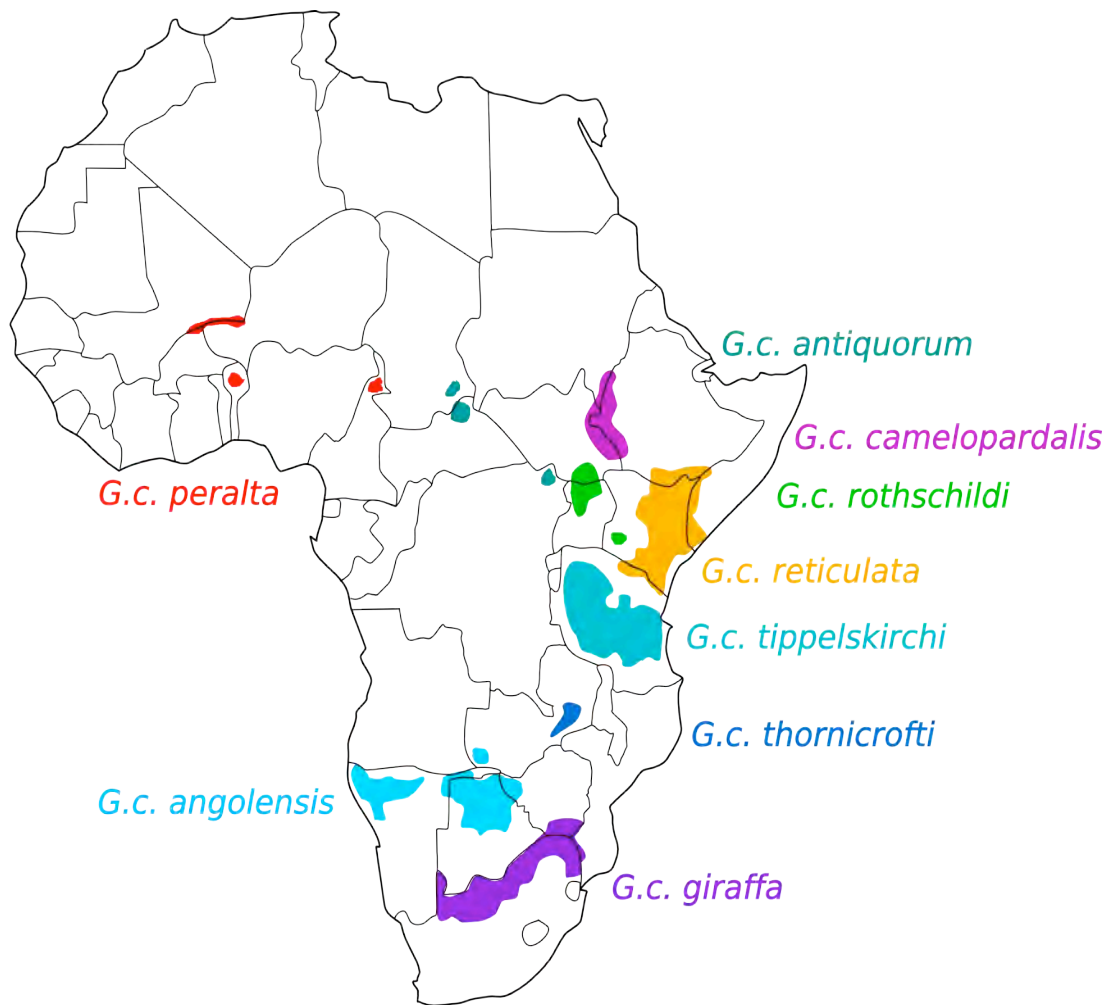


Fig. 2.1. Distribution of the nine *Giraffa camelopardalis* subspecies (modified after IUCN 2010; Nowak 1999; Brown et al. 2007).

2.1.2 Biology

The giraffe is a sexually dimorphic species with total height and body mass differing between adult bulls and cows. On average, cows measure 4.60 m and weigh 830 kg, while bulls measure 5.20 to 5.80 m and weigh about 1200 kg (Dagg 1971). Other secondary sexual characteristics are the bulls' bigger ossicones (which are sometimes referred to as *horns*, but are entirely built of bone mass) and additional bone structures on the forehead as well as the bulls' stouter musculature on the neck (Dagg 1971). The typical habitat of giraffe is an arid or semi-arid savannah, rarely a desert and never rain forest or mountainous area (Dagg 1971; Foster & Dagg 1972; Leuthold & Leuthold 1978; Fennessy 2009). The complex synusia of grasses, shrubs and trees of the savannah is essentially the habitat of choice for the giraffe, as it is commonly characterised as an exclusive browser, thus only foraging on trees and shrubs (Lamprey 1963; MacNaughton & Georgiadis 1986).

Studies on reproduction patterns agree that there is no strict seasonality in terms of an exclusive breeding season for the giraffe (Foster & Dagg 1972; Dagg 1971; Pratt & Anderson 1982; Bercovitch & Berry 2009), but annual precipitation patterns seem to greatly influence the calving rate; in case of the southern African giraffe populations, calving peaks were recorded during the wet season, while for populations closer to the equator, calving peaks were observed during the two dry seasons (Hall-Martin 1975; Dassman & Mossman 1962, respectively). In different habitats where a permanent source of groundwater ensures sufficient availability of browse all year long, there is no evidence of a correlation between birth rate and season (e.g. in the Luangwa Valley, Zambia; Bercovitch 2009). However, the giraffe's adaption of reproduction to ecological conditions has been speculated on by various authors.

Giraffe cows become sexually mature at the age of 4.5 years with average first parturitions at an age of 6.4 years (Bercovitch & Berry 2009). The gestation period varies between 394 and 488 days (Dagg 1971), with an effective calving interval of 550-650 days (Bercovitch et al. 2004; Hall-Martin & Skinner 1978). Lactation lasts 12-13 months and the ovarian cycling reappears shortly after a postnatal acyclic period (Hall-Martin & Skinner 1978; Bercovitch et al. 2006; Bercovitch et al. 2009), hence an adult cow is usually pregnant and/or lactating most of her mature life (del Castillo et al. 2005). Cycling has been observed to resume about 30 days postpartum. Mating activity appeared as early as about 60 days postpartum in captive animals, after passing two to five cycles, on average, without readiness to mate (Bercovitch et al. 2006). The average cycle length in giraffe cows was is 15 ± 3 days, ranging from 8-20 days, the luteal phase averages 6 ± 2 days, and the follicular phase 9 ± 2 days (Bercovitch et al. 2006).

In the giraffe bull, spermatogenesis is initiated at an age of three to four years on average, diagnosed by histological analysis of the seminiferous tubules (Hall-Martin et al. 1978). Bulls in captivity were observed to breed at an age of 32 months, but are not assumed to do so in the wild (Hall-Martin & Skinner 1978; Hall-Martin et al. 1978). Due to the bulls' behavioural immaturity and small body size at an early age, social maturity and actual ability to breed in the wild is likely to occur much later than mere physiological maturity (Hall-Martin 1975).

2.1.3 Social structure

The social system of the giraffe is mostly described as a loose association, referred to as *fission-fusion* system, suggesting that social relations are established and broken up randomly (Coe 1976; Dagg & Foster 1976). In this regard, individuals form or join groups and leave them again without any obvious social interaction. However, particular studies on free ranging

animals indicate evidence for more complex social systems, which in fact are influenced by individual preference and avoidance (Le Pendu et al. 2000; Pratt & Anderson 1985). Even within the stable social environment in a group of captive giraffes, social interactions have been shown to depend on a network of individual preferences (Bashaw 2007). The rather subtle social interactions which might often be oblique to the observer may have contributed to the erroneous conclusion that social affiliations are indiscriminate. According to observations by Pratt & Anderson (1982) young adult bulls associate significantly more with other giraffes and therefore are found in mixed or bachelor groups more often than older adult bulls.

2.1.4 Behaviour

Behaviour of wild and captive giraffes has been studied extensively regarding feeding behaviour. As a highly selective browser the giraffe was reported to feed on about 260 species with the most prominent genera in the giraffes' diet being *Acacia*, *Combretum*, *Croton*, *Lycium*, *Grewia*, *Diospyros*, *Spirostachys*, *Terminalia* and *Rhus* (e.g. Blomqvist & Renberg 2007; Ciofolo & Le Pendu 2002; Cooper et al. 2003; Hall-Matin 1974; Kok and Opperman 1980; Leuthold 1972; Parker 2004; Pellew 1983; 1984; Young & Isbell 1991). Being a browser, the giraffe can avoid competition with other ungulates by feeding from the canopy in a height of preferably 2-4 meters, which is particularly advantageous during the dry season when browse is scarce (Blomqvist & Renberg 2007; Pellew 1983). Despite numerous studies on giraffe feeding behaviour, there are still aspects of feeding behaviour which are only marginally explored, e.g. grazing, geophagy and osteophagy. The giraffe is sometimes referred to as an exclusive browser (Lamprey, 1963; MacNaughton & Georgiadis 1986) and numerous studies on its feeding ecology describe foraging on woody species only (e.g. Innis, 1958; Leuthold & Leuthold, 1972; Sauer, Theron & Skinner, 1977; Blomqvist & Renberg, 2007).

However, there are indications of an altered feeding behaviour as well, as Du Toit (2005) states that the giraffe only 'very occasionally' feeds on grass, and Ciofolo & Le Pendu (2002) report grass species as a negligible part of the giraffe's diet. Due to its physique, it is presumably a considerable effort for the giraffe to reach ground level with its mouth, since it has to adopt the typical 'drinking position', where the forelegs are splayed out laterally, and sometimes the carpal joints are also flexed. In this position the animal is constrained in its movements, and the risk of predation is increased (Périquet et al., 2010).

2.1.4.1 Reproductive Behaviour

In giraffe, social systems in respect of mating tactics have been studied on animals in captivity (e.g. Bercovitch et al. 2006) but little is known on male reproductive strategies in free ranging animals (compare van der Jeugd & Prins 2000). The considerably limited space of enclosures and a constant group structure actually represent an opposed system to the giraffe's natural surroundings, and even within considerably large enclosures, individuals eventually form a group of a constant social structure, which again appears to lead to a constant dominance hierarchy among males (Bercovitch & Berry 2009; Bercovitch et al. 2004; del Castillo et al. 2005; Dagg 1971).

In the wild, mature bulls tend to be solitary with movements of up to 20 km/day (Le Pendu 2000). According to Backhaus (1961) and Leuthold (1978), home ranges of bulls seem to overlap widely and there are no signs of an area exclusively used by one individual or other hints of territoriality. The tendency of solitary movement is positively correlated to the bull's age, i.e. young bulls at an estimated age of 5-7 years were observed to spend significantly less time alone than do bulls of older age classes, as distinguished by Pratt & Anderson (1982; 1985). Free-ranging giraffe bulls

exhibit variable patterns of behaviour regarding long distance movement, apparently depending on the ecology of their habitat (van der Jeugd & Prins 2000). In arid areas with little browse bulls roam within home ranges of up to 1950 km² (Fennessy 2009) whereas in habitats with sufficient browse home ranges were estimates as small as 5.2 km² per male, leading to a much higher density of animals (van der Jeugd & Prins 2000).

The social interactions of the giraffe are often rather subtle for the human eye. However, several patterns of intraspecific interaction have been observed and described regarding their presumed cause and context. Among bulls, social interactions with no obvious context of intrasexual competition are the most noticeable, in particular among younger bulls which seem to spend much more time with other males than older ones do (Pratt & Anderson 1985). One quite obvious example of these social interactions is the so-called *sparring* behaviour which is performed by bulls of all age classes with no regard of the hierarchic ranking but apparently is most common among adolescent ones (Innis 1958; Pratt & Anderson 1985). Sparring can be interrupted by long pauses and continue again after several minutes and it seems to be rather a social bonding ritual than having any relation to hierarchic orders (Innis, 1958 Pratt & Anderson 1985). In areas with a high density of giraffes, stable groups of males, females and immatures were observed without any agonistic encounters between the resident bulls, but only with new immigrant ones (van der Jeugd & Prins 2000; Coe 1967).

Free-ranging giraffe bulls that encounter a group of cows will usually engage in a sequence of investigation behaviours, presumably to assess the cyclic state of a potential mating partner: the bull nuzzles the cow's flanks and anogenital area, thereby stimulating her to urinate. When the cow urinates, the bull tests the urine by drawing some of it up into its mouth and performing a flehmen response (Dagg 1970; Dagg & Taub 1970), presumably using Jacobson's organ (Knappe 1964). Investigating is usually performed on most of

the cows encountered, but significantly more on cycling than on pregnant COWS (Bercovitch et al. 2006; Pratt & Anderson 1985). The frequencies of testing cows are strongly correlated with the bull's age as well (Pratt & Anderson 1985). As observed by Pratt & Anderson (1985) cows will allow young bulls significantly less often to test them, simply by not urinating or walking away. Once a receptive cow is located, the bull mate-guards her, i.e. he will steer the cow away from other giraffes and tries to restrict the approach of other bulls (Pratt & Anderson 1982). The bull keeps testing urine and nosing mane, neck and rump. Eventually if the cow allows it, the bull mounts her to mate. After mating, mate guarding will be continued if other bulls are in close proximity. When other bulls are absent, mate guarding will be interrupted and the courtship will be re-established some time later, as only observed in captive giraffe (Bercovitch 2006).

If other bulls are present, they keep watching but mostly get out of the way of a mate-guarding bull (Leuthold 1979). Rarely an established courtship will be disturbed by an intruder, who can either be attacked aggressively by the mate guarding bull or take over the courtship without obvious male-male interaction (Leuthold 1978; Pratt & Anderson 1985). If both bulls are equally motivated they will attack each other by vigorous bouts with head and neck. Fights are always started abruptly with no immediate sign of preceding dominance gestures or comment fighting, as the opponents approach lively and immediately begin to deliver powerful and quick blows against each other with head and neck, sometimes simultaneously (Pratt & Anderson 1985).

Bulls within a consistent social environment usually establish a stable dominance hierarchy and have a quite subtle way of communicating dominance, which must not always be obvious to an observer (van der Jeugd & Prins; Innis 1958; Foster & Dagg 1970). The descriptions of visual expression in terms of dominance and submission are divergent though, as different investigators report opposed observations: Pratt & Anderson (1982; 1985) and

also Leuthold (1978) have observed that a superior bull walks towards an inferior one with an erect posture and its head held high, presumably in order to appear as big as possible, while the inferior individual lowers its neck and head in order to look small and thereby to avoid an aggressive reaction. On the contrary, Dagg (1971) reports that a dominant bull lowers its head, perhaps assuming the typical fighting position, while the submissive bull carries his head high and pointing its nose upwards, resembling a feeding posture and thereby exposing its body to the dominant bull.

As one obvious expression of superiority agreed on, a dominant bull walks straight towards a subdominant one, while the latter, if inferior indeed, turns away and walks off, referred to as *displacement* (Pratt & Anderson 1985). Sometimes it is enough for the dominant individual to merely stare at a subdominant one for several minutes without showing any other visual signs in order to make the other one withdraw (Innis 1958). Usually larger individuals are dominant over smaller ones, so dominance is mostly expressed by pure physical appearance, which is therefore a rather obvious cue to the observer. Pratt and Anderson (1985) noted, that the expression of dominance is not directed randomly towards any other younger or smaller bull but only against selected individuals. Large vigorous bulls that join a group, only drive the formerly dominant bulls off and ignore lower ranked ones (Pratt & Anderson 1982; 1985). The same observations report that body size does not always predict the outcome of a dominance encounter, since small but more experienced bulls can drive bigger ones off, disregarding their physical inferiority.

2.2 Endocrinology of reproduction in mammals

Hormones are chemical structures released by glands or cells in order to influence cells, tissue or organs within the same organism (Nussey & Whitehead 2001). The different types of messenger molecules have a vast range of functions, with reproduction and correlated behaviour being one aspect of a multitude.

The hormonal system is affecting the individual at an early stage of embryonic development already - in the female embryo, the hormonal milieu is crucial for the Müllerian ducts to develop into ovary, oviduct and uterus, while in male embryo the gonads secrete hormones in order to inhibit the development of the Müllerian ducts but instead to form the Wolffian ducts which later develop into the male reproductive organs (Nussey & Whitehead 2001). In adult female mammals, the major hormones of the reproductive system are oestrogens and progestagens, and the female reproductive cycle as well as pregnancy depend heavily on secretion of oestrogens and progestagens (Poeggel 2009). In the adult male mammal, androgens are the most important hormones regarding reproduction and they are responsible for sexual behaviour, development of secondary sexual characteristics in puberty, and sperm production (Nussey & Whitehead 2001).

Most effective hormones of the reproductive system belong to the class of steroid hormones and are all derived from the parent structure cholesterol, which is synthesised in the mitochondria and metabolised into bioactive derivatives via a multitude of enzymatic processes (Kronenberg 2007).

2.2.1 Reproductive steroid hormones

Steroid hormones are low-molecular weight compounds and are responsible for various important physiological functions. The parental structure cholesterol consists of three hexagonal carbon rings and a pentagonal ring, which carries a side chain on the C₁₇ position of the whole polycyclic hydrocarbon system and two methyl groups attached on position C₁₈ and C₁₉ (Nussey & Whitehead 2001). The synthesis of active steroid hormones is executed by chemical substitution, and complete or partial removal of the side chain, with the first product of the enzyme cascade being a progestagen, which can further be transferred into either a glucocorticoid (cortisol, or corticosterone), a mineral corticoid, or an androgen (Kronenberg 2007).

Due to their lipophilic character steroid hormones are not soluble in blood plasma but are released into the blood circulation as a lipoprotein complex (Nussey & Whitehead 2001). When steroid hormones reach a target tissue, their hydrophobic character facilitates penetration through the cellular membranes (Kronenberg 2007). Within the cell, the hormone binds to a receptor, which is an intracellular protein, and the resulting hormone-protein-complex will subsequently penetrate through the nuclear membrane into the nucleus, where it affects DNA expression (Kronenberg 2007). Depending on the species of steroid hormone, specific genes are subsequently activated and the expression via protein biosynthesis is initiated (Kronenberg 2007).

Steroid hormones are synthesized continuously, hence inactivation is inevitable to ensure the homeostasis of a certain plasma hormone level (Nussey & Whitehead 2001). The major part of steroid hormone metabolism and degradation takes place in the liver and in the kidneys, hence steroid hormone metabolites are excreted in faeces, urine and saliva (Schwarzenberger 2007).

In a male mammal, the main source of androgens are the Leydig cells of the

testicles (Kronenberg 2007). During the embryonic development, the Sertoli cells, which nurture premature sperm cells during spermatogenesis in the mature individual, are developed first (Gilbert 2003). The Leydig cells develop in between the Sertoli cells and soon after differentiation, they begin to produce androgens, which trigger masculinization of the male foetus (Pineda & Dooley 2002). By influence of androgens, the Wolffian ducts develop into epididymis, vas deferens and seminal vesicles and meanwhile the Sertoli cells secrete an inhibitory hormone (Müllerian Inhibitory Hormone, MIH) which inhibits development of fallopian tubes and uterus from the Müllerian ducts (Nussey & Whitehead 2001). Besides masculinization of the foetus and maturation of sperm cells, androgens have a multitude of essential and characteristic effects on body and behaviour of male animals (Emerson 2000). In the mature male organism, the production and maturation of sperm cells depends on androgens, since differentiation of germ cells into sperms requires a high local concentration of testosterone (Pineda & Dooley 2002). Also, secondary sexual characteristics such as differences in body size, coat pattern or fur structure, increased musculature, horns, or transformation of the vocal cords, e.g. depend on the onset of androgen release during a certain phase of the individual's development, which is referred to as puberty (Emerson 2000; Pineda & Dooley 2002; respectively).

2.2.2 Androgens and intersexual reproductive behaviour

Display of mating behaviour is influenced by the endocrine milieu to a large part (Miles et al. 2007). Sexual behaviour depends on androgen levels in adult male mammals, as shown for several species, e.g. African elephant (Ganswindt et al. 2003), bison (Mooring et al. 2004), or white rhinoceros (Kretzschmar et al. 2004), and suppression of androgen release as by removal of the gonads results in significant decrease or nonappearance of mating behaviours (Payne & Bennett

1976). Vice versa, androgen peaks which can be triggered by ecological factors have been shown to be coincidental with significant increase in sexual behaviour (e.g. Ganswindt et al. 2005; Kretzschmar et al. 2004). On a smaller scale, androgen levels even differ between individuals that engage in alternative mating tactics (Rachlow et al. 1998).

Comparably to the giraffe's, also the African elephant's reproductive social system is classified as a mate-guarding polygyny (Poole 1989). For the elephant it has been shown that reproductive success depends on a male's strategy and behaviour, mostly due to female choice of dominant and vigorous mating partners being in a state of increased dominance and sexual behaviour, referred to as *musth* (Poole 1989). Regarding endocrinology, the state of musth is correlated with a significantly elevated level of androgens (Ganswindt et al. 2005; Ganswindt et al. 2010), hence reproductive success seems to be tightly correlated to variations in the endocrine milieu. Concerning the giraffe, an open social system and potential all-year mating activity imply an opportunistic sexual engagement of mature bulls, which is presumably also correlated to androgen levels.

As described in Wingfield (1990), androgen levels of non-breeding male vertebrates differ from androgen levels of breeding individuals due to environmental stimulation, e.g. breeding season or presence of receptive females.

In the bison it has been shown that during mating season males that mate-guarded cows had significantly higher androgen levels than males that did not guard (Mooring et al. 2004). As giraffe lack a mating season and reproduce throughout the year, bulls mate-guard whenever circumstances allow it, hence a general pattern of androgen secretion is hard to predict.

2.2.3 Androgens and intrasexual reproductive behaviour

Endocrine correlates of dominance and intrasexual agonism have been in focus of numerous studies on social animals, and usually testosterone was held responsible for most forms of aggression in various mammalian species (e.g. humans, flying foxes, red deer; Booth et al. 2006; Klose et al. 2009; Bartos et al. 2010; Mehta & Josephs 2010, respectively). However, the hypothesis of an exclusive relationship between aggressive dominance and testosterone levels has proven to be inaccurate (van der Meij et al. 2008; Mehta & Josephs 2010; Josephs et al. 2006). The recent approach to dominance mediated by endocrine correlates includes more complicated regulatory circuits, such as the influence of cortisol and its derivatives, which in fact antagonise the effect testosterone has on behaviour. Cortisol, as the most common stress hormone down-regulates androgen receptors and therefore mitigates the impact of testosterone on the target tissue (Burnstein et al. 1995).

In mammals, the plasma level of testosterone is apparently related to the individual's state of dominance but does not reflect a constant and proportional relationship, as shown in different studies on different species, e.g. African elephants (*Loxodonta africana*; Ganswindt et al. 2010), red deer (*Cervus elaphus*; Bartos et al. 2010), mice (*Mus musculus*; Machida 1981), men (Mazur 1998) and flying foxes (*Pteropus policephalus*; Klose et al. 2009). Sociosexual settings and other ecological cues also play a crucial role concerning individual androgen levels, e.g. in the white rhinoceros (*Ceratotherium simum*) the number of females one bull is housed with is positively correlated with the male's plasma testosterone concentration (Kretzschmar et al. 2004), and similar results were found in the male black rhinoceros (*Diceros bicornis*), whose testosterone levels also increase if other males are in close proximity (Christensen et al. 2009). Vice versa, a variation in faecal androgens has also been shown to be coincidental with behavioural

changes e.g. in African elephants (*Loxodonta africana*; Ganswindt 2008), white rhinos (*Ceratotherium simum*; Kretzschmar et al. 2004) or bison (*Bison bison*; Mooring et al. 2004). Hence the cause for the change in androgen and corticosteroid concentration can be found in a variation of (socio-) ecological conditions.

As described by Mazur (1998), competition between male mammals may sometimes involve fierce combat, but can also be mild and subtle, e.g. in a comment fight. In such case, the mere appearance of the superior animal may suffice to make the inferior one yield. A similarly subtle communication of dominance has been described for giraffe (Pratt & Anderson 1985; Innis 1958), but so far, no results on endocrine correlates of reproductive behaviour in giraffes were available. Release of androgens has been shown to be an effect of social cues, such as the presence of competing males, as described for male in birds (*Challenge hypothesis*, Wingfield et al. 1990).

However, results on androgens in giraffe bulls were so far only available from studies on formalin-fixed testicular tissue out of freshly culled animals, showing that testosterone concentration is significantly correlated with the individual's age (Hall-Martin et al. 1975; Hall-Martin et al. 1978); the same study reports a great variation in the testosterone amounts found, which remained unexplained.

2.2.4 Non-invasive hormone measurement

2.2.4.1 Principle and application

Endocrine assessments were originally performed by analysis of plasma, serum or tissue samples, which can be an accurate approach for studies on domestic or captive wild animals, but for research on free-ranging wildlife the

invasive approach is associated with a considerable logistic effort and substantial stress for the target animal as it has to be tranquilised and/or immobilised. Concerning the measurement of stress hormones, the invasive approach can produce misleading results due to acute handling stress prior to and during sample collection. Also regarding immobilisation, the risk of injury for animals and humans and interference with the animal's natural physiological functions and behaviour, a non-invasive approach is clearly favourable (Dumonceaux et al. 2006; Hodges et al. 2010). Non-invasive sampling requires less effort to obtain adequate samples and animal suffering and disturbance can be reduced to a minimum, for obvious reasons (Schwarzenberger 1996).

However, faeces are usually the easiest samples to collect from wildlife, because proximity to the animal is not necessary to obtain a useful specimen (Dumonceaux et al. 2006). Due to the physiological steroid hormone metabolism, derivatives of the active compounds are constantly eliminated and excreted from the blood stream into peripheral tissue, kidneys or liver (Kronenberg 2007). Thus, metabolites of the respective hormones can be found in the integument as well as in excretes such as saliva, urine, or faeces, which is the basis for the principle of non-invasive hormone measurement (Hodges et al. 2010).

Due to the duration of the gut passage faecal steroid metabolites represent a cumulative endocrine profile of the past hours (Schwarzenberger 2007). Peaks of steroid hormone concentrations in plasma samples often result from daily rhythm or acute stress; these influences are more dampened in faecal steroid profiles and therefore a more general result can be obtained (Schwarzenberger 2007). Thus, measurement of hormone metabolites in organic substrates such as faeces, hair or urine shows an average concentration of the relevant hormones, which is an advantage, in most cases. An averaged endocrine profile is particularly favourable because of its covering the entire period from food intake and digestion until defecation, while blood samples can be

influenced by circadian release rhythms, pulsatile hormone release or acute stress caused by taking of the sample and other short time factors (Schwarzenberger 2007). For assessment of an averaged hormonal profile faecal steroid hormone analysis can therefore give a more accurate picture.

Inactivated hormone metabolites are bound to a biological matrix for excretion, thus in order to measure the concentration of hormone metabolites it is necessary to dissolve the steroid metabolite from the matrix to which it is bound (Hodgson, 2010). Due to the lipophilic character of steroid derivatives, the hormone metabolites can be extracted by their solubility in ethanol. For quantitative measurement of hormone metabolites in the extract, a wide array of techniques has been in use (e.g. High Pressure Liquid Chromatography), but usually immunoreactive techniques are the method of choice. The most common approaches are radioimmunoassays (RIA) or enzyme-linked immunoassays (EIA). Both procedures are immunological methods to detect an antigen which can be a high or low molecular structure, and are based on the principle of an antibody's ability to specifically bind one antigen (Porstmann & Kiessig 1992). Subsequently, the bond complexes must be measurable in order to infer a relative concentration of antigen per volume of substrate, which is why the antibodies are labelled. In case of RIA, antigen is labelled with radioactive elements, which is favourable due to established exact and fast diagnostic methods and lower costs, and is therefore quite popular despite the hassle of radioactivity (Porstmann & Kiessig 1992). For EIA's, either antigen or antibodies are labelled with an enzyme, which, after addition of a substrate, signals the specific antigen-antibody bindings by a change in physical characteristics, such as light scattering or changes in the refractive index of the medium (Wild, 2008).

2.2.4.2 Assay validation

Regardless of the immunological method, RIA or EIA, a calibration is mandatory to evaluate effectiveness of the respective assay for the hormone metabolite, substrate and for the biological species in question, which is particularly crucial for quantitative assays (Wild, 2008). For qualitative and quantitative analysis of faecal hormones it must be considered that the parent hormones like oestrogen, testosterone or cortisol mostly do not appear in the faeces due to the metabolism in liver and kidneys, which is why only hormone metabolites can be traced (Schwarzenberger 2007). Thus, one considerable challenge of faecal steroid metabolite analysis is the vast number of different metabolites within closely related species (Möstl et al. 2002; Touma & Palme 2005). Oestrogen metabolites such as oestradiol or oestrone are present in faecal samples of female animals in much greater amounts, while testosterone and corticosterone are heavily metabolised and therefore are harder to identify in faecal samples (Schwarzenberger 2007). Buchanan and Goldsmith (2004) state the importance of validation of new measurement techniques for each taxonomic species and that it is essential to validate in order to verify the reliability of the results. Even steroid metabolites in closely related species can apparently differ tremendously (Touma & Palme 2005).

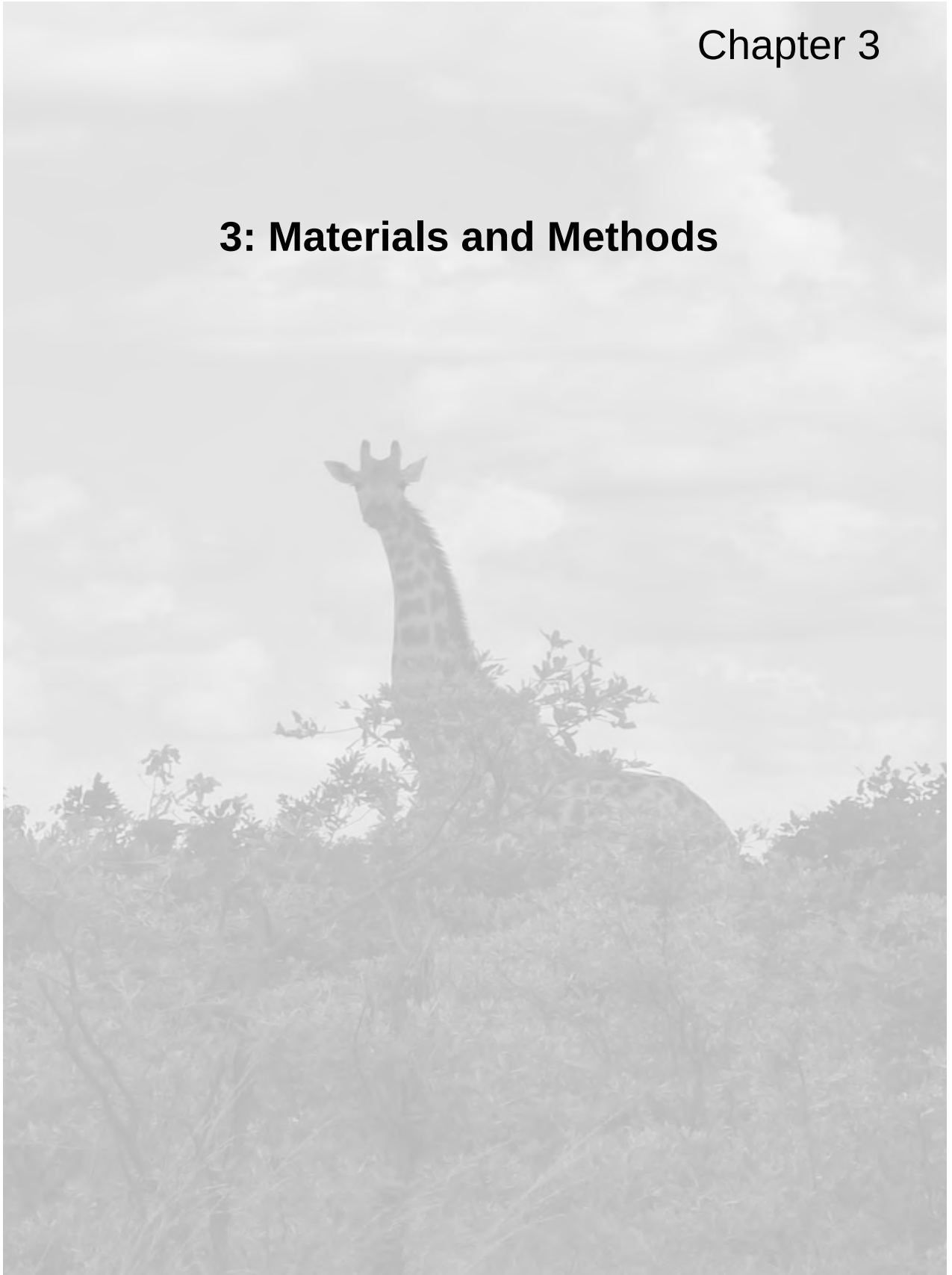
However, besides biological validation on species level, processing and storage of samples are also critical, which is why accuracy and transparency of the applied methods are crucial to ensure reproducibility and comparability of results (Hodgson 2010).

Chemical stability of steroid metabolites in a faecal sample is an error prone issue, because microbial activity can obscure hormonal profiles (Khan et al. 2002). Thus, in order to inhibit bacteria and bacterial enzymes, storage at temperature lower than -20°C is recommended for faecal samples (Pappano et al. 2010). Field work conditions might not always allow freezing of samples

immediately, but other options to ensure stability of endocrine concentrations are also in use, such as storage in ethanol, or in ethanol and sodium acid. In this chemical milieu faecal samples can be stored up to 3,5 years without significant changes in glucocorticoid concentrations, according to Curtis et al. (2000). Another approach of processing for storage is immediate drying of the samples in order to inhibit microbial metabolic activity by dehydration (Henderson et al. 1999). In this regard it is important to ensure a quick drying procedure, preferably under constant parameters for each set of samples. Also, the sample material should be homogenised to some extent to ensure a consistent dehydration.

Chapter 3

3: Materials and Methods



3.1 Study sites

The fieldwork of the present study was conducted at two different field sites, in Hwange National Park (HNP), Zimbabwe, and Entabeni Game Reserve (EGR), South Africa.

3.1.1 Hwange National Park

Hwange National Park is situated in the province of Matabeleland North in western Zimbabwe on the border to Botswana, about 200 km north-west from the city of Bulawayo (Fig. 3.1). Founded as a nature reserve in 1928 the area gained the status of a National Park in 1930 (World Conservation Monitoring Centre 2010), and today covers approximately 14.700 square kilometres of diverse habitat, including an ecotone between the dry western deserts of the Kalahari to rather moist savannah woodlands in the park's eastern regions, on an altitude ranging from 938 to 1152 masl (World Conservation Monitoring Centre 2010; Walter & Breckle 1999). The park expands approximately from 25°45' E to 27°30' E and from 18°27' E to 19°50' S. Several core filed sites within HNP were chosen, selected on basis of visibility of focus animals for behavioural observations; all core areas were within the north-eastern region of HNP, in a maximum distance of about 70 km from Main Camp (Table 2.1).

The climate is typically semi-arid with variable precipitation. The mean annual temperature is 20.3 °C, while temperatures vary in winter between -5 and 13°C, and in summer between 32° and 38°C (Nations Encyclopedia 2010). Rainfall is markedly seasonal, with a wet summer season between October and May, followed by a transitional season, during which both rainfall and

temperatures decrease; during the cool and dry season from mid-May to mid-August, frost may occur with ground temperatures of -5°C or even lower; subsequently, there is a warm and dry period, which again lasts until the onset of rains in October (World Conservation Monitoring Centre 2010). The annual precipitation is 606 mm on average with rainfall mainly from November to March (Chamaillé-Jammes et al. 2007). Most water bodies are only temporary during the humid period and dry out in winter, but approximately 60 waterholes have been drilled throughout the park, some of which are constantly pumped during dry season.

Hwange National Park covers a transition from sparse desert-like vegetation in the southern area to a dense tree population in the North-East, in analogy to precipitation (Childes & Walker 1987). According to the division of biosphere into ecosystems by Walter and Breckle (1999), the western part of Hwange National Park with its vast grasslands and scarce distribution of trees lies in Zonoecotone II/III, while the eastern area is part of Ecozone II. The dense wood vegetation of this part consists predominantly of the genera *Baikiaea*, *Pterocarpus*, *Guibourtia* and *Acacia*, the understorey is dominated by *Acacia* sp., *Combretum* sp. and *Terminalia* sp. (Childes & Walker 1987; Rogers 1994). Almost the whole grounding of HNP is considered a cambic arenosol covered by Kalahari sands, creating a ground profile characterised by its low water retention capacity (Oldeman & van Engelen 1993), hence the water supplies for green plants are considerably low during the dry season. Also, the vegetation in the HNP area thrives on soil of markedly low mineral concentration (Holdo et al. 2002; Holdo 2003) because this vegetation mostly grows on Kalahari sand, which is extremely poor in micronutrients and trace elements subsequently available to herbivores (Childes & Walker 1987).

Vast regions of HNP are either woodland dominated by *Baikiaea plurijuga* (*Rhodesian Teak*), or more open savannah and shrub land, dominated by *Terminalia sericea* and *Acacia* sp. (Rogers 1994), while only the latter areas are

assumed to be a suitable habitat for giraffe (Mitchell 2003).

The Park is not fenced at most of its boundaries, veterinary fences on the Botswana border to prevent foot and mouth disease built in the 1980's have been torn down to the largest part (Namibia Nature Foundation 2011).

Prior to the project, a preliminary evaluation of the study site has been conducted for four weeks. The behavioural observation and individual identification has in many cases been inhibited by the animals' movement into dense vegetation, therefore an array of adequate sites had been chosen, as indicated in the map in Fig. 3.2. The most suitable spots were in the area south of main camp, formed by open to half-open savannah vegetation which was dominated by *Terminalia*, *Acacia* and *Combretum sp.* The areas north-west of main camp had proven to be rather unsuitable for giraffe since the local vegetation is a dense forest, dominated by *Baikiaea plurijuga*, which is apparently not browsed by giraffe.



Fig. 3.1. Geographic location of Hwange National Park, Zimbabwe.

Site	Coordinates	masl	Site	Coordinates	masl
Airstrip	18°43'31"S; 26°57'13"E	1059	Livingi	18°42'11"S; 26°55'27"E	1059
Balla Balla	18°43'45"S; 26°55'17"E	1052	Longone	18°47'29"S; 26°48'00"E	1076
Caterpillar	18°47'59"S; 26°57'58"E	1073	Main Camp	18°43'37"S; 26°57'05" E	1060
Dopi	18°51'02"S; 26°55'40"E	1055	Makwa	18°47'24"S; 26°57'17"E	1049
Guvalala	18°49'50"S; 26°44'27"E	1072	Ngwashla	19°01'40"S; 27°06'12"E	1027
Kennedy I	18°52'32"S; 27°08'09"E	1015	Nyamandhlovu	18°46'32"S; 26°53'19"E	1061
Kennedy II	18°58'07"S; 27°05'13"E	1012	Sedina	18°43'14"S; 26°56'58"E	1058

Table 3.1. Main core field sites for behavioural observations in HNP.

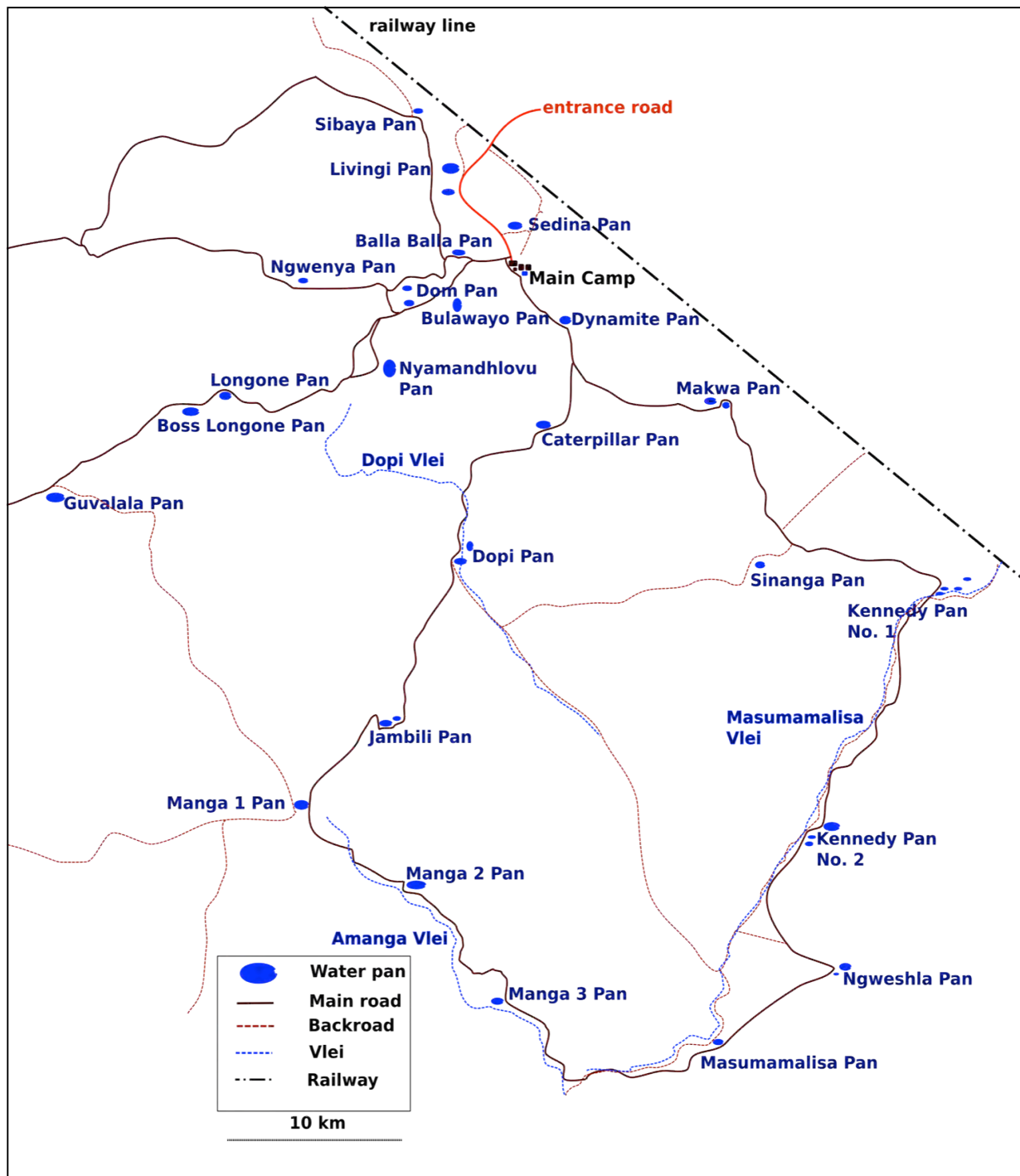


Fig. 3.2. Map of Hwange National Park, central region.

3.1.2 Entabeni Game Reserve

Entabeni Game Reserve is a private game reserve, situated in Limpopo Province, South Africa (Fig. 3.3.), and covers about 250 km². The reserve is entirely fenced and keeps a giraffe population of about 45 individuals. The giraffe's main predators such as lions and leopards are present.

The climate in this area is also semi-arid, but tempered by its height of about 1200 masl. Temperatures average 22°C in the humid summer, and 11°C in the dry winter season; average annual rainfall is 495 mm with December being the wettest month and July the driest (South African Weather Service 2010).

The area of EGR is divided into a lower and a higher escarpment, both of which are inhabited by giraffes. Due to a steep barrier in between them, the two subpopulations do not mingle, and only giraffes of the lower escarpment were used for this study.



Fig. 3.3. Geographic location of Entabeni Game Reserve, Limpopo, South Africa.

3.2 Study animals

Behavioural observations and faecal sample collection were conducted on adult giraffe bulls. For biological validation of the Enzyme Immunoassay, androgen metabolites were also measured in faecal samples of adult cows and immature animals of both sexes.

3.2.1 Hwange National Park study population

The giraffe population of Hwange National Park is assumed to belong to the Angolan subspecies, *Giraffa camelopardalis angolensis* (Novak 1999; IUCN 2010). In order to individual identify the study animals, all encountered bulls were recorded in an ID catalogue. On the first encounter, the animal was photographed (from both sides, ideally) and given a name consisting of three to four letters indicating the closest core site (water pan), and a continuous number.

The final study population of HNP consisted of 73 individually identified bulls, 17 of which were rated as age class A, 25 as age class B, and 31 as age class C. For 18 sightings the focus individual was classified into an age group, but could not be identified and/or recorded in the ID catalogue due to technical failure. Four of the unidentified individuals were classified as age class A, the remaining 14 as age class C.

3.2.2 Entabeni Game Reserve study population

In EGR, fourteen mature bulls were seen and recorded in the ID catalogue, according to the procedure described above. Seven bulls were considered class B, and seven class C. Furthermore, 32 mature adult female and

immature animals were identified.

3.2.3 Captive animals

For preliminary behavioural observations, animals in captivity were observed at the National Zoological Gardens (NZG), Pretoria, South Africa. The facility housed two adult giraffes during the conduction of this study (one bull and one cow). Behavioural observations were conducted on both animals.

3.3 Behavioural data collection

3.3.1 A giraffe ethogram

In order to assemble relevant behavioural patterns of adult giraffe bulls, a behavioural catalogue of the giraffe was compiled by extensive literature review and four weeks of preliminary observations in HNP. The complete catalogue was designed as an ethogram defining behavioural patterns in order to be used for future behavioural observations (see Chapter 4 Results, 4.1 'A compiled ethogram for the giraffe (*Giraffa camelopardalis*)').

3.3.2 Feeding behaviour

During the course of the studies at HNP, giraffe were occasionally observed feeding on grass. Although the observations did not indicate any link to reproduction, we investigated this non-core activity in a bit more detail and finally added it to the compiled catalogue of giraffe behaviours (see Chapter 4 Results, 4.1 'Grazing behaviour of the giraffe in Hwange National Park, Zimbabwe').

3.3.3 Identification, observation and recording techniques

Individuals were identified by the unique pelage pattern (Shorrocks & Croft 2009; Dagg 1970; Leuthold 1978), and additionally, by the shape of ossicones, skulls and exotoses, as well as body size, overall appearance and additional characteristics such as predation marks. Individual differences of coat patterns are exemplary demonstrated in figure 3.4.

According to previous studies on giraffe sociosexual behaviour and population structure, bulls were classified as adult if they had about the size of a mature cow (Pratt & Anderson 1982; 1985). Adult bulls were further categorised into three age classes A, B and C, based on physical appearance, i.e. body size, musculature of the neck, shape of skull and ossicones (Pratt & Anderson 1982; 1985); according the system applied, bulls are rated as class C and of an estimated age of five years once they attain the size of an adult female. Individuals mature from class C to class B by gaining body mass, a stouter neck and the size of their ossicones increases. Bulls of class A are larger than class B, but also have a considerably stout neck, massive ossicones and remarkable growth of additional bone mass on their foreheads. Exemplary pictures of four different individuals demonstrate the physical differences between the three age classes in figure 3.5.

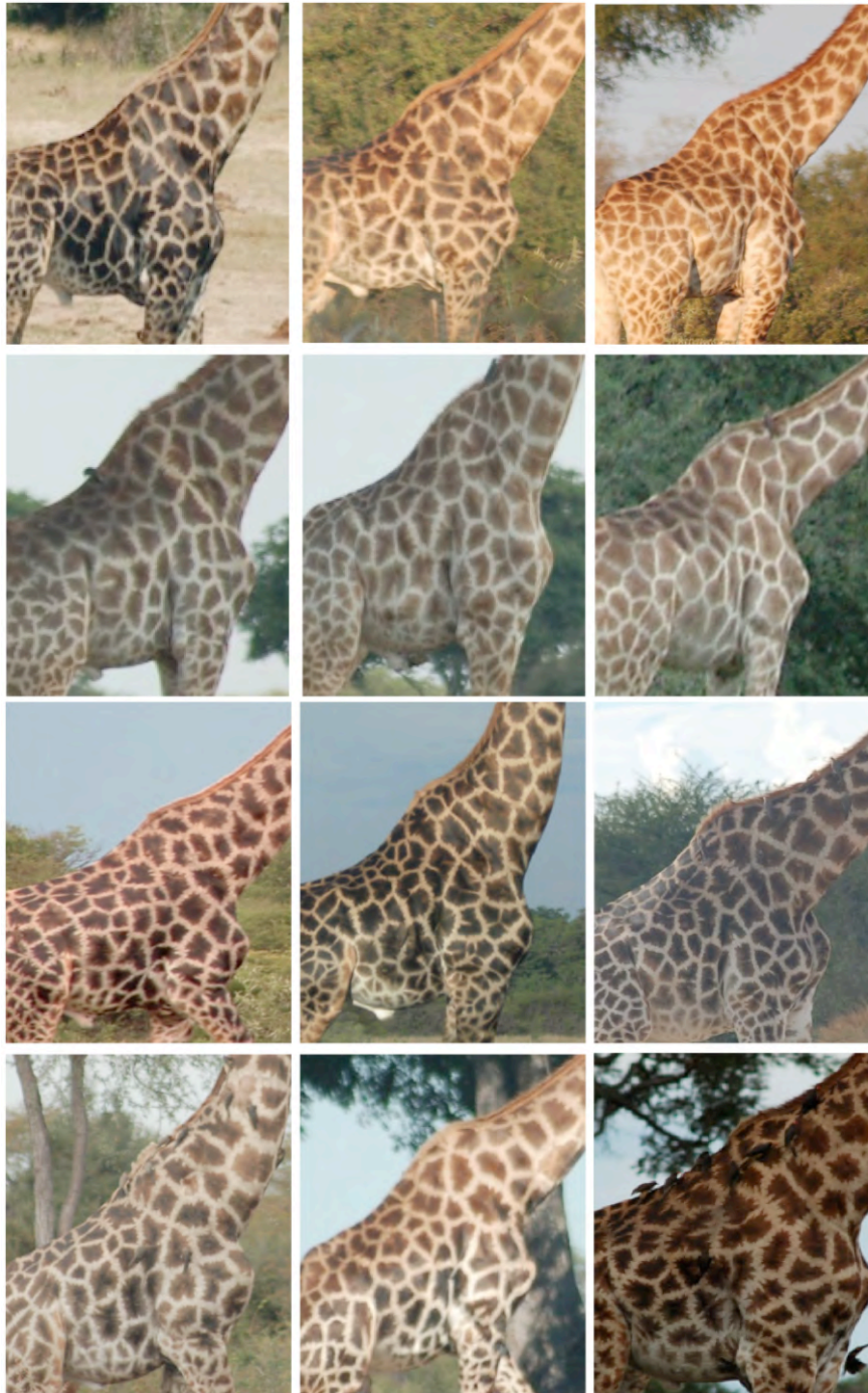


Fig. 3.4. Examples for individual differences in pelage patterns and colours in adult giraffe bulls.

Class C



Class B



Class A

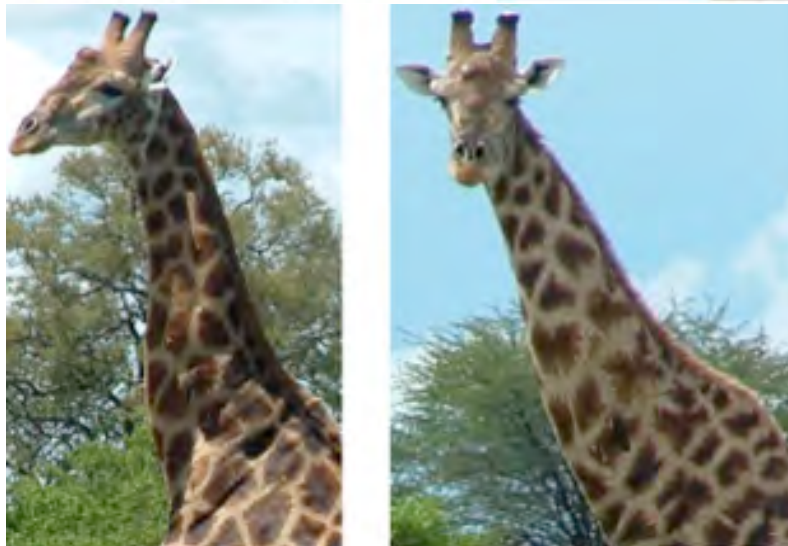


Fig. 3.5. Examples of the three distinguished age classes (note differences in size and structure of ossicones and forehead exostoses).

Behavioural observations of reproductive and dominance behaviour were exclusively conducted in HNP. Focus animals were chosen primarily in order to obtain a reasonable amount of data from all adult age classes, and secondarily with preference of bulls that had not been observed before. One to five focal animals were observed at a time. Animals were observed for the predefined behavioural patterns by the techniques of *focal animal sampling* and *ad libitum* sampling (Altmann 1974), for selected periods of time, depending on visibility of the animal. Behaviour was recorded by *all-occurrence sampling* (Martin & Bateson 2000).

Thirteen behavioural patterns, presumably related to reproductive activity or dominance hierarchy, were looked for. Due to the recording method, most behaviours were considered *events*, only one (mate guarding) was considered a *state* (table 3.2; Martin & Bateson 2000). The six intersexual behaviours are referred to as *sexually related events* (SRE's), consisting of *approach*, *follow*, *necking*, *investigate*, *urine test*, and *mating attempt*. The SRE's were further classified into *affiliation behaviours* and *courting behaviours*, according to a presumed sexual motivation (table 3.2). *Affiliation* behaviours seemed to be relevant, but less determined regarding the bull's intention. *Courting* behaviours were clearer, probably due to a stronger sexual motivation. Mate guarding was not included in these two groups as it was recorded as a state and not as an event.

Events were captured by the count of occurrences during the entire observation period, while a behavioural state was regarded as representative for the entire observation, thus based on sightings, individuals were categorised as *sexually active* if any one of the SRE's (as well as mate guarding) was seen during the observation period, and as *inactive* if a bull did not engage in any of the sexual behaviours in presence of cows during the observation period.

Category	Behaviour	recording method
Affiliation behaviours	<i>Approach</i>	<i>event</i>
	<i>Follow</i>	
	<i>Necking</i>	
Courting behaviours	<i>Investigate</i>	
	<i>Urine test</i>	
	<i>Mating attempt</i>	
	<i>Mate Guarding</i>	<i>state</i>

Table 3.2. Considered intersexual behavioural patterns.

3.3.4 Socioecological parameters

In addition to the individual's behaviour, the following social factors were recorded:

Group size

It must be assumed that the observer's sight distance is inferior to the giraffes' visual abilities (Du Toit 2005; Innis 1958), and therefore social interactions, visual communication and reactions probably take place over a considerable distance, hence two individuals might be in social contact with each other already before both of them are even in human eyesight (Pratt & Anderson 1982; 1985). Due to the mostly quite dense vegetation in between and around the open pans of HNP, the spatial observation range was in most cases restricted by visibility of an estimated 300-500 metres. The maximum sight distance was about one km, which was the case only at three of the seventeen core field sites (*Airstrip*, *Nyamandhlovu* and *Makwa*), and even at these larger sites it was never seen that two separate groups of giraffes appeared and remained separate. Thus, for the present study all giraffes in

sight were considered to belong to one social complex, and the parameter group size reflects the count of all mature and immature individuals seen during one observation session.

Group composition

In general, all sightings of giraffes were recorded by group size and group composition (count and sex of adult and subadult individuals). Concerning behavioural observations of adult bulls, only four categories of group composition were relevant, as defined in table 3.3.

Group composition	Definition
<i>solitary</i>	no other giraffe in sight
<i>bachelor group</i>	all individuals in sight were adult, or subadult males
<i>with cows</i>	the bull in focus was accompanied only by cows (and immatures)
<i>in mixed group</i>	the focus individual was in company of adult (and subadult) cows, and of one or more adult bull(s)

Table 3.3. Possible states of a bull's social association.

Male-male association

Intrasexual association was recorded by the presence of other adult bulls, and their respective age classes, within the social group of the focus animal. Associations in this regard were recorded from view of the respective focus animals, which could, but did not necessarily have to be, all adult bulls in sight.

Dominance state

Regarding intrasexual interactions that seem to be correlated to hierarchic behaviour, the frequency of dominance gestures was recorded, as well as a

state of dominance, which was representative for the respective observation session only.

A dominance gesture was defined as being exaggerated by the focus individual, it had to be obvious to the observer as such, and appeared to be an intended restriction of another male's access to females. In case other bulls responded with gestures of inferiority, the acting bull was regarded dominant. A dominance state was recorded when other bulls yielded and thereby avoided confrontation with an approaching bull, which itself did not necessarily have to engage in any agonistic behaviour. A bull that visibly restricted the approach of other bulls towards a cow, or whose approach made other bulls yield, was considered dominant. Yielding bulls therefore were recorded as subdominant, and in four cases individuals were obviously dominated by bigger bulls and simultaneously dominant over other ones, which was recorded as an intermediate hierarchy status.

3.4 Non-invasive hormone measurement

For non-invasive measurement of androgen metabolites, faecal samples were collected at both field sites. In HNP, collection of faecal samples was done subsequently to, or during behavioural observations when the focal or a different identified bull was seen defecating. In EGR, individual bulls were looked for, and attended until defecation.

3.4.1 Sample collection

Samples were collected within 20 minutes after defecation. About 5 to 8 faecal boli were collected into plastic bags and stored on ice during the

respective field trip in order to minimise microbial degradation of steroid metabolites (Khan et al. 2002). The further processing of the samples differed between the field sites due to different logistic assets.

Samples collected in HNP were dried in a low-heat oven at approximately 50°C for about 24 hours to achieve maximal dehydration. The dried material was stored in airtight vials and shipped to the laboratory facilities of the Endocrine Research Laboratory (ERL), University of Pretoria (UP).

Samples collected in EGR were stored deep frozen, and at the end of the respective field work period shipped to ERL, where the samples were lyophilised (Fieß et al. 1999; Ganswindt et al. 2002) in an Instruvac freeze-dryer (Air and Vacuum Technologies; Model VFDT 2.50), at approximately 50 mTorr and -40°C.

3.4.2 Steroid extraction

After the drying procedures, all sample sets were processed in the same manner according to well-established methods at the ERL (Ganswindt et al. 2010): the dried material was pulverised manually and sieved in order to remove undigested fibrous material, subsequently a weighed amount of 100 to 110 mg of each sample was suspended with 3 mL ethanol (EtOH 80%) and overhead-shaken for 15 minutes to extract steroid metabolites (Fieß et al. 1999; Ganswindt et al. 2002; Ganswindt et al. 2003). Extracts were then centrifuged for 15 minutes at 1500 RPM and aliquots to 1.5 mL of the supernatant were transferred into Eppendorf tubes for deep frozen storage until performance of enzyme immunoassays.

3.4.3 Enzyme Immunoassay

Microtiter plates (96 wells) were coated by application of 150 µl coating solution (chemical composition of all reagents see Appendix) and incubation at room temperature over night. Subsequently, the wells were saturated with a saturation solution. Antibodies raised in rabbits against 5α-androstane-3α-ol-17-one-HS and 5-α-androstane-3,17-dione-thioether, labelled with biotin (Palme & Möstl 1994), were applied before aliquots of each sample (50 µl), standards, and quality controls were pipetted in duplicate into each microtiter plate. Reactivity and cross reactivities of the used antibodies include EA (100%), 5α-androstane-3,17-dion (800%), 5α-androstane-3α-ol-17-on (40%), and 4-andro-stene-3,17-dion (15.6%; Palme & Möstl 1994).

The dilution of sample extract was adjusted to the linear range of the standard curve. Sample extracts of adult males were diluted with assay buffer in a ratio of 1:500, samples of females and juveniles in a ratio of 1:100. The plates were incubated over night at 4°C, then washed four times with a washing solution. A streptavidine-peroxidase solution was pipetted into each well (150 µl) before incubation at room temperature for 45 minutes on a shaker. The microtiter plates were washed again before adding substrate solution and incubation for about 40 minutes on a shaker. The enzymatic reaction was stopped by adding sulphuric acid (H₂SO₄, 2 mol/l), subsequently optical density was measured at 450 nm.

In order to monitor impurity of the sample material, assays were conducted in serial dilutions. The resulting displacement curves were checked for parallelism to the standard curve.

All hormone concentrations are expressed as µg/g dry weight (DW).

3.4.4 Validation of comparability of extraction methods

To ensure comparability of results obtained by different processing methods, the two procedures were validated. For this purpose a subset of samples collected in EGR (N=10) was divided into two aliquots each, one of which was lyophilised, the other was oven-dried according to the procedure applied in HNP (24 hours at 50°C). Subsequently to the differing drying procedures, all samples were processed and analysed as described above.

3.4.5 Biological validation of EIA suitability

For a biological validation, groups of animals which presumably varied in circulation hormone concentrations were compared. Faecal samples from adult bulls, adult cows, and from subadult individuals were measured to assess variations in androgen levels. In case of repeated sampling of one individual during one field work period, a median value was processed.

3.5 Data analysis

Statistical analyses were done with SPSS 19.0.0 and SPSS 20.0. software, performing standard procedures. Each variable was tested for normal distribution by a Shapiro-Wilk (ShW) test. Normally distributed data were further analysed by parametric tests, otherwise non-parametric statistics were employed.

The distribution of one variable within one group was tested by a standard χ^2 test for independent samples. In case of more than one data set per individual the arithmetic mean of all respective variables was used to avoid bias towards more frequently seen individuals.

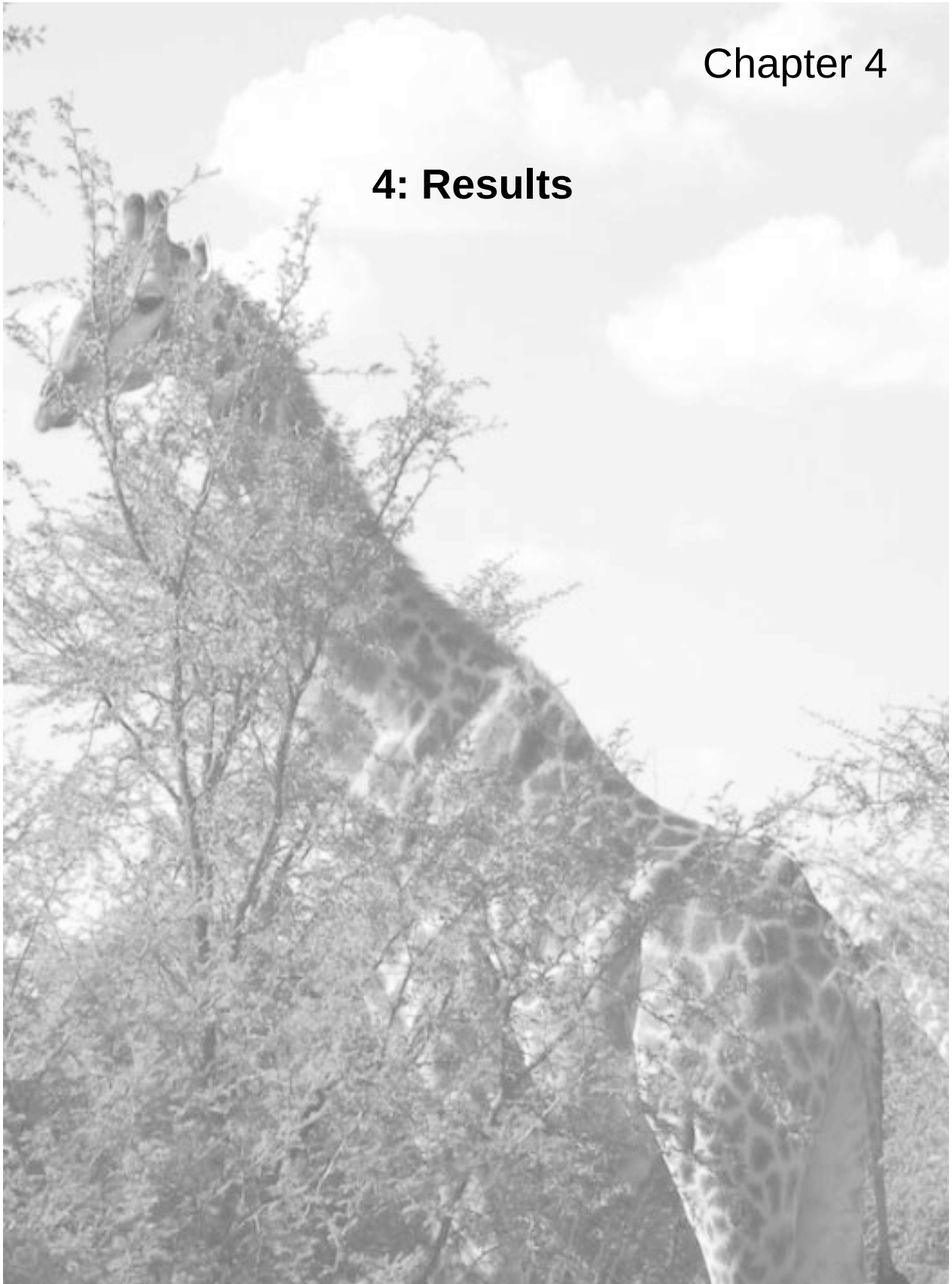
Regarding frequencies of reproductive behaviour, and sexual activity, the three age classes of mature bulls were compared. Measured EA concentrations were compared between the three age classes of mature bulls, and cows, and immature individuals.

For comparison between two groups, differences were analysed by a Mann-Whitney-U test (confidence level 95%). Three or more groups were compared by analysis of variances performing a Kruskal-Wallis-H test and in case of a significant result ($p < 0.05$), groups were *post hoc* analysed pairwise by a Mann-Whitney-U test, including a Bonferroni adjustment of the significance level, i.e. dividing the significance level by the number of executed post hoc tests (Hatzinger & Nagel 2009).

Correlations between EA concentration and reproductive behaviour were tested by a Spearman-Rho testing procedure. All data are presented as mean values $\pm$ standard deviation, unless otherwise stated.

Chapter 4

4: Results



4.1 A compiled ethogram for the giraffe (*Giraffa camelopardalis*)

PA Seeber, I Ciofolo & A Ganswindt; submitted for publication in *Journal of Ethology*.

4.1.1 Introduction

The giraffe (*Giraffa camelopardalis*) is the tallest land-living animal and the only extant species of its genus (Mitchell and Skinner 2003). Although there is still uncertainty about the exact number and distribution of subspecies within *Giraffa* (Dagg 1962), a division into nine subspecies are generally accepted (Dagg & Foster 1976). Two of these subspecies are currently listed as endangered by the International Union for Conservation of Nature and Natural Resources (IUCN 2010). The giraffe naturally inhabits a variety of habitats, from desserts to more heavily vegetated bush- and tree savannah (Fennessy 2009; Pratt & Anderson 1982), and there is evidence for habitat-related adjustments of occurring social structures and of particular behaviours (van der Jeugd & Prins 2000).

Over the past couple of decades, the number of giraffes has declined considerably across Africa, presumably due to direct and indirect anthropogenic impact, such as extensive poaching, habitat destruction, and induced epidemics of e.g. rinderpest (Ciofolo 1995; Dagg 1962; Dagg 1971). As a consequence, most of today's giraffe populations are isolated and live in detached habitat fragments or fenced reserves (Ciofolo 1985; Dagg 1962). By restricting the natural tendency of giraffes to roam vast areas in search for conspecifics, further implications in terms of behavioural alterations are easily conceivable (Brenneman et al. 2009; van der Jeugd & Prins 2000). These alterations might even have long-term effects on factors like intraspecific competition,

predator-prey relationships, or parasite transmission (Brown et al. 2007; Brenneman et al. 2009), and should therefore be considered in specially tailored conservation plans for such disturbed populations. However, for an integrated conservation approach, comprehensive and ideally *in-situ* collected ethological data should be available to effectively alter and implement adequate conservation strategies (Curio 1996).

Nowadays, the monitoring of behaviour is also a very valuable and frequently used tool to provide information about the health and welfare status of animals in captivity (Maple 2007; Clubb & Mason 2003). However, the interpretation of behavioural data is not absolutely robust though, and relies heavily on preliminary defined criteria (Castellote & Fossa 2006). In order to obtain reliable results, the respective behavioural patterns must be adequately defined (Veasey et al. 1995). To allow cross-institutional comparison of collected data and to contribute to a reliable base of information, behaviour must be measured in a distinct and standardised way. Thus, the use of an accurate established ethogram is highly recommendable (Lehner 1996), not least because it helps to prevent 'drift' during the course of observation (Martin and Bateson 2001).

In terms of available behavioural data for the giraffe, many of the contributing studies only cover specific behavioural classes and at times, these studies use inconsistent terminology or innovate purpose-built definitions for certain behaviours (e.g. Bashaw 2010; Bercovitch et al. 2006; Baxter and Plowman 2001; Pratt & Anderson 1982; Leuthold and Leuthold 1978; Nesbit Evans 1970; Innis 1958). Hence, sustainable data to build upon is rather limited. In this paper, we therefore aim to provide a full descriptive catalogue of the giraffe's behavioural repertoire for observations of wild and captive animals. The compiled ethogram is structured into several categories, which are, in the case of intraspecific interactions, subdivided by sex and age class of the acting individuals, and also into sex and age class of the animal the behaviour is presumably

directed to. The definitions and general remarks provided will hopefully be of practical value in terms of producing comparable and sustainable ethological data from future observations.

Study areas

Observations were conducted at three different study sites: Hwange National Park (HNP), Zimbabwe, Entabeni Game Reserve (EGR), South Africa, and at the National Zoological Gardens of South Africa (NZG) in Pretoria.

HNP covers 14.650 km² in western Zimbabwe and is entirely unfenced. A presumably stable population of roughly 3000 giraffes are estimated to live in HNP and adjacent areas (IUCN 1999). Lions as the giraffe's main predators are abundant (Loveridge et al. 2007), and also other large predators such as cheetahs and leopards are present. Observations were conducted at several water holes and open plains in the Main Camp area, ranging from Guvalala Pan to Ngweshla Pan.

EGR is a private game reserve, covering 250 km² and is entirely fenced. An otherwise unmanaged population of about 45 giraffes were kept in the reserve during the time of observation. Lions, cheetahs, and leopards are also present. During the time of observation in 2010, NZG housed two adult giraffes (1 male, 1 female).

4.1.2 Materials and methods

General method

In a similar approach to other studies (e.g. McDonnell and Poulin 2002; Lichtenberg and Hallager 2008), the behaviours reported in this paper were compiled from several sources. In order to cover as many of the behaviours shown by giraffes as possible, numerous publications, focussing on descriptions of giraffe behaviour or at least partly addressing the topic, were reviewed for

behavioural descriptions and definitions. In addition, observations were conducted in three different environments, in order to confirm, refine, and if necessary extend existing descriptions of giraffe behaviour.

All behaviours were described as brief and definite as possible, according to the descriptions by other authors and our own observations. In this regard, we tried to comment regarding the behaviours apparent social and/or ecological context, and assumed purpose. The resulting list of behaviours is divided into two main groups; *Activities* (characterised by the absence of any social context) and *Interactions* (characterised by the presence of some kind of interaction between animals).

Literature review

In total, 94 publications (92 scientific articles and 2 books) on giraffe ecology, behaviour and general biology were reviewed for descriptions of behavioural patterns in wild and captive giraffes. 59 published contributions contained descriptions, evidence or hypothetical explanations of behavioural patterns, and thus were used to compile the resulting ethogram (not all of them are mentioned as references).

Original observations

Giraffes were predominantly observed using *ad-libitum* and *all-occurrence* sampling (Altmann 1974). As an example of a near-natural environment, wild giraffes were observed in HNP for thirteen weeks, between November and December 2010, and from March to April in 2011. During a total observation time of 272 hours, 1264 sightings were recorded (345 sightings of mature males, 752 of mature females, 159 of juveniles / subadult individuals). As a top-up, about 40 giraffes were additionally observed on a daily basis in EGR for three weeks in September 2011. In order to collate behavioural

descriptions from animals in captivity, two adult giraffes (1 male, 1 female) housed at NZG, were also briefly observed for 7 hours in February 2011.

4.1.3 Results

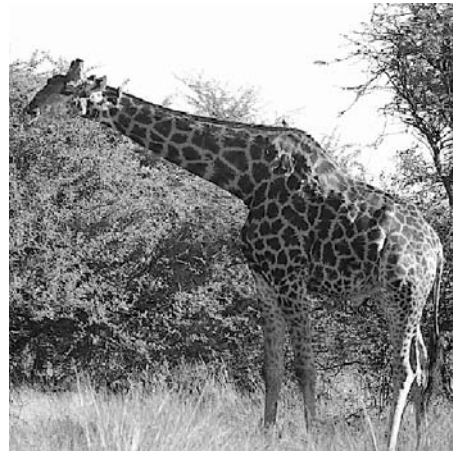
A total of 57 different behaviours could be identified. These behaviours were subdivided into 29 *Activities* and 28 *Interactions*. Activities were subdivided further into *General activities* (Table 4.1) and *abnormal repetitive behaviours* (Table 4.2). Interactions were structured by sex and age class of the acting animal, and of the animals the behaviour is presumably directed to. This resulted in *General interactions* (Table 4.3), *Bull-Cow behaviour* (Table 4.4), *Bull-bull behaviour* (Table 4.5), *Cow-Bull behaviour* (Table 4.6), and *Interactions by calves* (Table 4.7).

Activities

As mentioned above, behaviours allocated to the category *Activities* are not obviously related to any type of interactive behaviour and also not restricted to one sex or age class. Behaviours of the *Activities* category were further subdivided into *General Activities* (Table 1) and *abnormal repetitive behaviours* (Table 2).

browse

The giraffe uses tongue and/or lips to gather and ingest browse from trees or shrubs. Shoots, leaves, or leaflets are stripped off or picked from the plant. In free-ranging giraffe, a large part of the daily time budget is occupied by browsing (about 50 to 70%, Leuthold and Leuthold 1978, Pellew 1984, Blomqvist and Renberg 2007). The time spent chewing between bites is usually used to *scan*. Browsing is to distinguish from *feeding* and *grazing*.

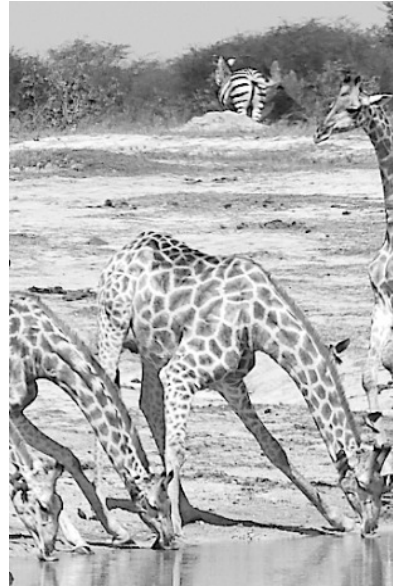


feed (in captive animals)

The giraffe ingests food other than browse, such as concentrates or hay. Captive animals are often fed on hay and concentrates from cribs or elevated feed buckets (Baxter and Plowman 2001). On the contrary to browsing, giraffe spend considerably less time feeding on concentrates due to a higher nutrient content and easier accessibility; while giraffe in the wild spend up to 16 hours browsing, captive individuals only feed as little as 4-6 hours (Veasey et al. 1996, Bashaw et al. 2001).

drink

In order to drink from a water source on or below ground level, the giraffe has to splay its forelegs out laterally, and additionally flex its carpal joints to reach the water surface. Water is swallowed in this position as well. One drinking bout, on average, lasts for about 17 seconds (own observation). Captive animals are sometimes provided with elevated troughs, obviously making the typical drinking position obsolete. In the wild, drinking is often preceded by long periods of scanning and pacing along the banks of the water body. Drinking is often interrupted to shake birds off or to merely scan, raising the head up but remaining with splayed out legs.



ruminate

Rumination is chewing a bolus of already ingested food after regurgitation. In the giraffe, regurgitation of a bolus is usually visible on the outside of the throat and therefore the subsequent chewing movement that is not proximately following prior food intake is considered rumination. The giraffe can ruminate while standing, lying, or even while walking (Du Toit and Yetman 2005, Blomqvist and Renberg 2007).

scan

The individual actively observes its surroundings. Vigilance is most obvious when the animal is standing still with an erect neck and appears to be actively watching, but scanning is apparently done synchronously with many other behaviours (e.g. while ruminating, walking, or between feeding bouts). The giraffe's visual abilities are assumed to be its most important feature of predator defence (Cameron and Du Toit 2005).



walk

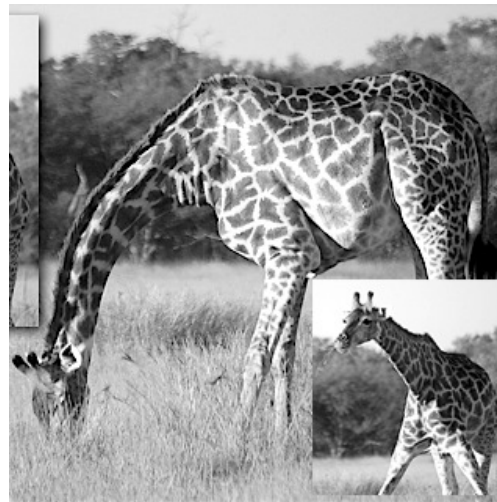
The animal moves in a four-beat locomotion which is distinct from the three-beat *cantering* also due to its lack of a phase of suspension. In walk, there is a tendency to pacing, as both legs on one side might swing forward almost contemporary. The giraffe spends a considerable proportion of the day walking (e.g. 20% according to Blomqvist and Renberg 2007) and travels roughly 3 to 5 km per day on average (Fennessy, 2009, Berry, 1978).

canter

The locomotion is a three-beat movement with a moment of suspension. The tail is usually curled up, sometimes over the back, while cantering. This swifter of the two gaits of the giraffe is only done on rather short distances and for short periods of time. In most cases, canter is an escape movement (e.g. from predators, vehicles or conspecifics), but can also be used as a targeted movement (e.g. towards a conspecific, apparently threatening). In canter, the giraffe can travel with 56 km per hour (Arbuthnot 1954 in Du Toit 2005). Small obstacles can be jumped over in canter or in a similar movement (Dagg and Foster 1976). The giraffe cannot maintain the canter movement for more than several minutes due to increasing blood pressure (Dagg 1971).

graze

The giraffe assumes a position as when *drinking* from a ground level water source (spreading legs, and often also bending carpal joints) to reach the ground. Literature states that the giraffe hardly ever makes use of feeding from the herbaceous layer (summarised by Du Toit 2005). In own observations though giraffes were seen to spend considerable time grazing (Seeber et al. 2012). During grazing, a giraffe can change the position of one of its forelegs to enhance its range. Grazing must not be confused with *geophagy*.



geophagy

The giraffe assumes the typical drinking position to reach down to ground-level where it licks soil or even bites pieces of it off to ingest it. Often followed by chewing and/or tongue movement / licking. Geophagy seems to be more common in regions of poor mineral supply (Langman 1978) and was seen frequently during own observations in Hwange National Park, where the sandy Kalahari soil is known to contain a very limited amount of micronutrients (Childes and Walker 1987).

***osteophagy***

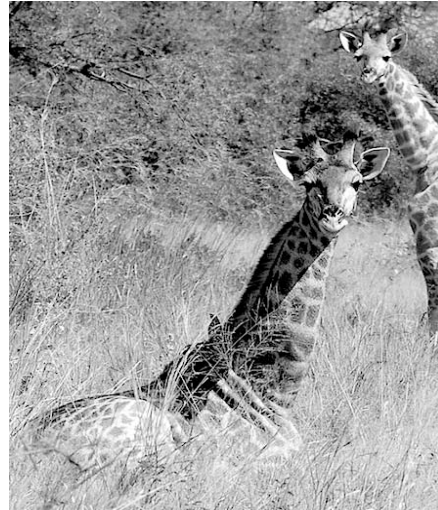
The animal bends down to the ground and picks up bone material to chew on it, eventually dropping the piece again (Ciofolo and Le Pendu 2002; Kok and Opperman 1980). Osteophagy has been reported in captive animals more often than in free-ranging ones (Du Toit 2005) and is assumed to be a result of poor supply of dietary minerals (Wyatt 1971).

scavenge

Parts of a carcass other than bones are chewed on, or ingested (Western 1971). Giraffe in captivity and in the wild were observed to scavenge on carcasses of different herbivore species, including giraffes (Western 1971, Ogrizek 1954 quoted in Du Toit 2005). Comparably to osteophagy and geophagy, scavenging in the giraffe is presumably an indicator of nutritional stress, as e.g. lacking minerals (Langman 1978). Giraffe cows have also been reported to eat the afterbirth (Kristal and Noonan 1979b).

rest (lying)

The trunk touches the ground with the giraffe lying in sternal position. The legs are tucked in or folded up under the body. The head is carried with an erect or slightly bent neck. When lying down, front legs are bent first, followed the hind legs. Mostly giraffe calves are seen to lie down, but animals of all age classes do so (Pratt and Anderson 1979). In adults it is only done for rather short periods of time, presumably to *sleep*, with the head resting on the body (Kristal and Noonan 1979a).



drowse

The animal stands with a rather relaxed neck and lowered head and seems not to *scan*. Rumination can occur while drowsing. Mostly, ears are not carried attentively but are relaxed as well. These resting phases are rather short and sometimes follow rumination; most apparent during the heat of the day (Innis 1958).

yawn

The animal opens its mouth and sometimes the tongue is protruded. The lower jaw might move sideways.

sleep

Lying on the ground in a sternal position (as when resting), neck curled, head resting on the animal's hip or thigh and eyes closed (Kristal and Noonan 1979a). The body position is quite obvious, but the physiological state of sleeping can only be assumed (comp. Boy and Duncan 1979). As in other ungulate species, juvenile giraffes seem to spend more time sleeping than adult ones (Kristal and Noonan 1979a).

rub (object)

The giraffe rubs its body / neck / head against an object. Rubbing on objects is presumably done to get rid of an itch, as the giraffe's rather unstable balance does not allow to scratch an itch with its own legs, as seen in other ungulates. Giraffes can also walk and stand over shrubs that are somewhat higher than animal's trunk to rub their bellies on it by moving back and forth.



lick object

The giraffe licks the surface of an object other than soil or other mineral donors, sometimes followed by nibbling on it. Licking an object seems to be more frequent in juveniles, than in adult giraffes. The tongue is presumably used to investigate taste and texture of an object. Not to confuse with stereotypic object licking in captive animals.

paw

The animal stomps the ground with one foreleg, once or repeatedly. Pawing is rather rare and seems to be a displacement activity caused by an external irritation which is considered as a possible threat by the giraffe (Innis, 1958). Pawing is sometimes followed by or precedent to *snorting*, which also seems to be a reaction to an irritation. It is mostly to be seen as an interruption of *scanning*. The function of pawing might be considered to be an alarm signal as well (comp. Hirth and McCullough 1977).

snort

The giraffe blows air out through widened nostrils by a short expulsive and clearly audible grunt. In most cases, the animal is standing and scanning one particular spot or direction. Snorting is interpreted in the giraffe as a reaction to a potentially threatening irritation, as it is observed in other ungulates as well (Dagg, 1971; comp. Hirth and McCullough 1977, respectively). It might function as a warning signal for conspecifics. In own observations, dominant giraffe bulls were seen and heard to snort immediately after the displacement of an inferior bull.

self-grooming

The animal licks or bites its own body or legs. Biting or licking the own body is assumed to provide a relief from an itch or another unpleasant local sensation. Only several parts of the body can be reached with the animal's own mouth, in other cases *rubbing* is presumably the method of choice.



buck

The animal jumps while cantering or even more or less on the spot, contemporary lowering its neck. Bucking in adult animals is not to confuse with playful jumping in calves, though it resembles the movement. It seems to be a defence reaction against birds such as oxpeckers and might in some cases be followed by *cantering* for a short distance. Bucking is often preceded by shaking of head and neck to get rid of birds (Dagg, 1971). Calves might buck for the same reason at times.

tail swishing

The giraffe uses its tail to brush off flies almost on its entire trunk (Innis, 1958). Tail swishing might also be a sign of irritation in alert situation.

lick urine

The giraffe licks another giraffe's urine from the ground. Licking urine from the ground is not to confuse with *urine testing*, which is performed by adult and sometimes by subadult bulls after stimulating a cow to urinate. Licking urine in bulls is sometimes followed by a *flehmen* response (Dagg 1970). Adult cows were also seen to lick urine of other giraffes from the ground.

predator defence

The giraffe lifts its front legs quickly (either successively or both at a time), kicking or stomping them hard on the ground or eventually on a specific target Dagg 1971; Dagg and Foster 1976); kicking is also done with the hind legs (Du Toit 2005). Giraffes have been observed to kill lions with these foreleg stomps (Dagg 1971, Dagg and Foster 1976). This defence behaviour is never directed towards conspecifics (Dagg, 1971). In young animals, a quite similar behaviour can be seen but is done in a playful context with other giraffe calves and is often combined with

running or even bucking.

Table 4.1. General activities.

Abnormal repetitive behaviours

As in other species, it is assumed that abnormal repetitive behaviours often develop in captive animals due to a time budget shift in the daily activity pattern (Mason 1991; Carlstead 1996). Giraffes in captivity spend considerably less time feeding compared to the amount of time giraffes *browse* in the wild (Baxter and Plowman 2001, Veasey et al. 1996).

tongue playing

A persistent movement of the tongue outside the mouth; not licking an object, and not during or shortly after *feeding*, *geophagy*, *browsing*, or *ruminating* (Bashaw et al. 2001; Fernandez et al. 2008).

object licking

The animal uses its tongue on an object that is neither food nor a mineral donator, repeatedly and persistently over a lengthy period of time (Bashaw et al. 2001; Veasey et al. 1996).

mane biting

Biting or chewing the mane of a conspecific for more than some seconds, repeatedly, and not in a grooming context (Sato and Takagaki 1991; Baxter and Plowman 2001).

vacuum chewing

The animal repeatedly performs a chewing motion without prior food intake, and when not ruminating (Baxter and Plowman 2001).

pacing

The animal walks a definite short path, repeatedly and without a discernible purpose (Veasey et al 1996). Pacing is a stereotypical behaviour which has been described in many different captive wildlife species, including the giraffe. However, in giraffe oral

abnormal repetitive behaviours seem to appear more often than locomotory ones.

Table 4.2. Abnormal repetitive behaviours

Interactions

As mentioned above, all behaviours which require any type of *Interactions* are allocated in this category. Behaviours of the *Interactions* category were further subdivided into *General Interactions* (Table 4.3), *Bull - Bull Behaviour* (Table 4.4), *Bull - Cow Behaviour* (Table 4.5), *Cow - Bull Behaviour* (Table 4.6), and *Behavioural Interactions by Calves* (Table 4.7).

approach

One animal moves towards a conspecific, obviously not in order to threaten, but seeking proximity. Approaching appears considerably frequent when bulls join groups and attempt to investigate each individual. These approaches might be followed by *investigation* or *nuzzling*.

nuzzling

A tactile encounter with conspecific by animal's nose or muzzle to conspecific's nose or any other area than flanks or anogenital area. This greeting ritual seems to be more frequent between cows and calves than among adult giraffes. Pratt and Anderson (1979) refer to it as *nasofrontal greeting*, Bashaw et al. (2007) as *muzzle/muzzle*. Nuzzling seems to strengthen the social bonds between animals, as it is seen very often in cows, directed to calves before suckling (Pratt and Anderson 1979).

follow

One animal stays in close proximity to another one and follows its movements in walk. Following is common in bulls, sometimes prior to the establishment of a mate guarding courtship, or when cows do not approve of the bull's affiliation. Also, calves have a tendency follow each other. Mature cows were described to follow the movement of their calves (Pratt and Anderson 1985).

yield

The giraffe gets out of the way of another giraffe or interrupts approach to change its

own way, avoiding the other individual. Yielding is often to be seen in subdominant bulls that get chased away by other bulls (see *displace*), but also cows sometimes yield from approaching bulls. Bashaw (2010) refers to yielding as 'avoiding'.

displace

One animal drives a conspecific off by an agonistic action, which can be an approach in walk or *canter*, or merely standing and staring. The dominance gestures in giraffe are rather subtle to the observer, but seem to be perceived by conspecifics over a considerable distance (Compare *dominance gesture*). Pratt and Anderson (1985) report a distance of 40-80 meters between two bulls showing obvious gestures of dominance and submission. In own observation, a distance of more than 100 meters was estimated in similar situations. Displacing is an enhanced form of the *dominance gesture*, aiming to drive the opponent off. In Bashaw (2010), dominance gestures are summarised as *non-contact aggression*.



necking / rubbing (social)

One giraffe rubs its head or neck against a conspecific's body, sometimes leading to an entwining of the necks. Rubbing (sometimes referred to as necking) seems to be more of a social purpose than to scratch an itch and is frequently seen in young bulls, sometimes leading to *sparring* (Bercovitch 2006; Coe 1967).



bump

One giraffe pushes another one with its chest. Bumping into another giraffe is common in calves, cows and most frequently seen in young bulls (Pratt and Anderson 1985; Bercovitch et al. 2006). In adult and subadult bulls it is not to confuse with a *mounting attempt*, where the bull investigates another giraffe first and then also pushes it with its chest. Bumping sometimes appears to be an invitation for further social behaviour, such as *sparring* or *necking*.

grooming

One animal grooms another one's body or crest by licking or biting. This is most common in mothers grooming their calves, and appears rather rarely in adult bulls (Horwich et al. 1983). Not to confuse with the licking of a cow's anogenital area or flanks by a bull, which is a part of *investigating*.

mount

One animal stands right behind or on the side of another one, lifting its front legs on to conspecific's body, attempting to mount it. In adult bulls, the mounting attempt is usually preceded by pushing the other animal with the chest and lower neck (Woods 1972). In calves, it is rather a sudden jump onto the other animal, usually in play with another calf. Mounting attempts seem to be very common among premature bulls. The mounted animal does sometimes not tolerate, but in other cases even ignores being mounted and continues feeding (Innis, 1958).

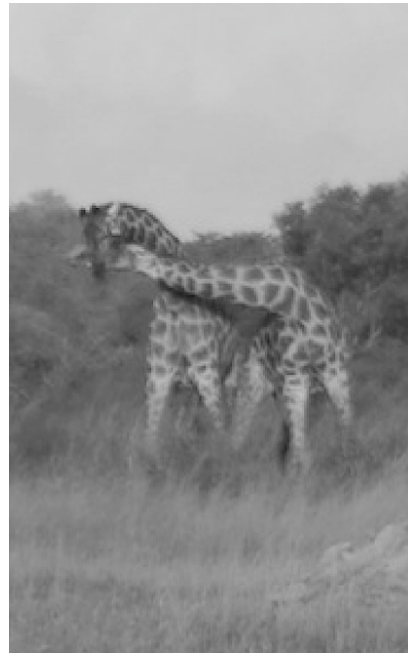


nursing attempt

One animal attempts to suckle on a cow's udders. The unsuccessful nursing attempts are mostly seen in calves, which approach a cow that is already nursing another calf (Pratt and Anderson, 1985). Sometimes subadult or adult animals also approach the nursing act, and try to suckle themselves. Cows are reported not to allow any other calf than their own to nurse (Pratt and Anderson 1985).

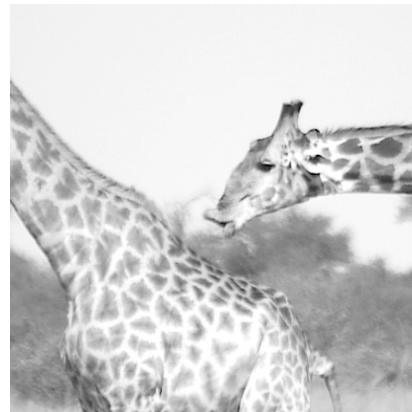
sparring

The giraffe swings its head against the body of the sparring partner with comparably soft blows. The two, or up to eight animals can stand parallel or antiparallel or in a different angle to each other (Dagg 1970). The motion of sparring resembles that of a fight, but sparring is considerably slower and less vigorous. Sparring seems to be a social behaviour, exaggerated by bulls, and, rarely, even by cows. Sparring develops slowly, sometimes initiated by *necking* and can continue over hours (Dagg 1970). It is usually interrupted oftentimes for several minutes to scan, or even to ruminate. In literature, only cows in captivity are mentioned to spar, usually against a bull (Dagg 1970). During our own observations in HNP, one young but mature cow was observed to join a sparring match between young bulls.



flehmen

The giraffe raises its head up, sometimes with the nose line tilted above the horizontal, and curls up the upper lip, inhaling deeply. The flehmen response is frequently performed by bulls after *urine testing* in the process of *investigating*, showing long saliva threads hanging from the mouth. By inhaling deeply, the giraffe presumably uses its Jacobson's organ to assess the cow's cyclic state (Knappe 1964; Dagg and Taub 1970).



Investigate

The animal licks or sniffs a conspecific's anogenital area or flanks. Investigating is most common in bulls directed towards adult and subadult cows. By investigating the bull intends to stimulate the cow to urinate, and subsequently might perform *urine testing*. The behaviour can be conducted by bulls or cows,



and in both cases directed to either sex.

vocalise

The giraffe produces infrasound vocalizations, sometimes by performing a 'head throw' behaviour (von Muggenthaler, Baes, Hill, Fulk, Lee, unpublished results). Infrasound in the giraffe is hypothesised to be a means of long- and short-distance communication. In captivity, infrasound vocalization has been reported in adult bulls and cows (von Muggenthaler, Baes, Hill, Fulk, Lee, unpublished results).

Table 4.3. General interactions.

dominance gesture

Any type of behaviour, often an erect posture while standing still or a tensed and arched neck when moving, by which a bull intends to express its social rank, or tries to challenge another individual. The visual signs of dominance differ remarkably and can only be interpreted by the social context (also see *displace* and *submission*; Pratt and Anderson 1982; Dagg 1971). A dominance gesture can only be assessed by its social context and, in some cases, might be somewhat arbitrary. In other cases dominance is made very clear to the counterpart animal and obvious to the observer (see Discussion).

submission

Any type of behaviour shown by a bull, most obvious is *yielding*, that subsequently results in an avoidance of or retraction from a social ranking dispute. Like *dominance*, submission is expressed in various ways and can only be interpreted from the social context.

fight

One bull attacks by swinging head and neck against his opponent's body and legs with very powerful and fast blows (Innis 1958; Pratt and Anderson 1982). To increase their stability, the fighting individuals spread their front and hind legs and lean against each other with their hindquarters touching (Innis 1958). Sometimes the attack with the head is preceded by a bump with the rear, as seeking to push against the opponent and lean against it at a time. It is also referred to as *contact aggression* (Bashaw 2010).

play fight

The movement resembles that of a *fight*. The blows with head and neck are delivered quite vigorous, but not as powerful and as fast as in a fight. As in a fight, the participants often lean against each other with their hindquarters to increase stability. A play fight is usually also interrupted at times for the participants to scan, as if taking a break and after several minutes go back to the play fight, which does not occur in a serious fight.



Table 4.4. Bull-bull behaviour.

mate guard

The bull directs one cow away from conspecifics, or intends to prohibit approach of other bulls. Bulls might keep proximity of less than one animal's body length, in other cases, the bull remains in a distance of up to 30 metres. The intensity of mate guarding and the proximity a bull keeps to the guarded cow seems to depend on presence of other bulls, and presumably on the cow's oestrus state.



urine testing

The bull tests cows urine (often when the cow urinates), usually leading to a *flehmen* response. Urine testing is a part of the investigation procedure. By nuzzling a cow's flanks the bull stimulates the cow to urinate. As she urinates, the bull catches some urine in its mouth. As suggested by Dagg (1958) the bull might be able to assess the cow's reproductive state by



urine testing.

laufsschlag

The bull stands close behind a cow or follows a moving cow and lifts one front leg (repeatedly) to touch the cow's hind leg or flank. It can be assumed that laufs Schlag is a preliminary investigation of the cow's readiness to mate. During our own observations this behaviour was only seen when the bull was *mate guarding*.

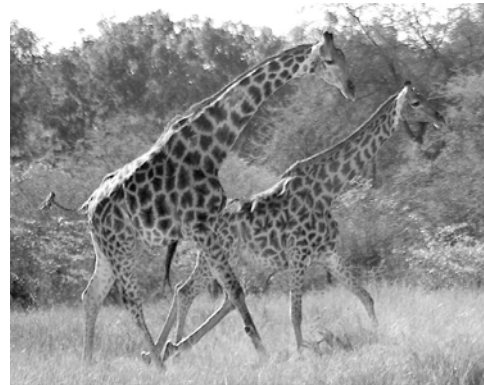
mating

The bull rocks its body weight on the hind quarters and lifts front legs to mount the cow. The bull's penis is erect, subsequent intromission and several vigorous pelvic thrusts (Innis 1958; Bercovitch 2006). The cow does not necessarily stand still during the copulation (Bercovitch et al. 2006). Ejaculation follows immediately to intromission, as within 2 s (Bercovitch et al. 2006). Since cows hardly stand still, mating is mostly preceded by several mating attempts (Bercovitch 2006, Pratt and Anderson 1985).

Table 4.5. Bull-Cow behaviour.

escape from bull

A cow escapes from a bull's affiliation attempt (*mate guard*, or *investigation*). Usually in canter, the cow intends to withdraw from a bull's affiliation. Persistent bulls keep up with the cow and restrict her from approaching conspecifics of both sexes. The cow's canter appears somewhat slower than when escaping from predators or vehicles.



tolerate affiliation

The cow tolerates the bull's investigation, or mounting attempts. The cow, presumably in oestrus, tolerates the bull's investigation and mounting (attempts) by standing still and not *walk* or *canter* away from the bull. The cow can even ignore the investigating bull and keep browsing (Innis 1958).



stimulated urination

The cow urinates subsequently due to stimulation by a bull's investigation. When bulls *investigate* cows, the cows often react by urinating, leading to *urine testing*. Sometimes cows are stimulated to urinate merely by a big bull walking past them (Pratt and Anderson 1985).

tolerate mating attempt

The cow tolerates bull's mounting attempt, eventually leading to copulation. Not necessarily standing still, but not obviously trying to escape from the bull's close-up. Cows which are just about to come into oestrus might tolerate the bull's investigation but not the mounting attempt. A cow might keep browsing while the bull mounts her, or she keeps walking, but not *escaping* from the bull.

Table 4.6. Cow-bull behaviour.

tail-chew

The calf chews on another individual's tail. In most cases, the calf chews its mother's tail, sometimes on the tail of another juvenile (Horwich et al. 1983).

nurse

Juvenile animal sucks milk from cow's udders. It was reported that the nursing relationship in giraffe is exclusively limited to one cow and her calf by Pratt and Anderson (1979). They also suggest that nursing serves as a strengthening of the mother-calf bond, and not only for nutrition.

play

A variety of behavioural patterns in the immature giraffe can be interpreted as play behaviour. This includes running, bucking, kicking with fore- or hind legs, and biting other individuals. Play behaviour appears most commonly among young calves, as they play with each other, perform play running and jumping solitarily, or try to play with adult giraffes (Pratt and Anderson 1982). This behaviour excludes *play fighting*.

Table 4.7. Interactions by calves.

4.1.4 Discussion

This ethogram, describing 57 different behaviours of giraffe, was compiled to serve as a basis for future studies designed to further examine the complex behavioural patterns of this remarkable species. Based on our own observations, several often older descriptions could be verified and even new insights added to what is stated in literature.

The classification of the described behaviours into *activities* and *interactions* might appear rather clear from a definition point of view, but should be used with precaution, because the complete intention and purpose of an observed behaviour always remains an interpretation based on a projection of the observer's conception. The animal's behaviour can not be reduced to the sum of different behavioural acts, which is why clear and precise definitions are essential to create a common language understandable among human observers and to contribute to the understanding of wildlife behaviour.

Interpretation and allocation of observed behaviours should be done on the largest possible temporal and spatial scale. An animal's original intention is in many cases difficult to evaluate and rather oblique, which applies particularly to large animals like the giraffe with its hardly assessable visual facilities (Du Toit 2005). Furthermore, olfactory cues and insufficient recognition regarding long distance communication via infrasound make it sometimes difficult to unequivocally relate a certain observed behavioural event to a specific category (von Muggenthaler, Baes, Hill, Fulk, Lee, unpublished results), therefore the division of interactions and activities not related to a social context remain somewhat arbitrary. It should be also taken into account, that several behaviours can be expressed in rather different ways (e.g. *dominance*). In this regard, it must be considered that individual variances of social behaviour might be clear to conspecifics but remain oblique to the observer.

Regarding social interactions not restricted to one sex or age class (*General Interactions*), it is worth noting that many of these behaviours were originally described as exclusively exaggerated by one sex, or by a specific age class. However, during our observations, we also register the performance of these behaviours by the respective opposite sex, or across age classes, respectively. For example, *sparring* in mature wild animals was assigned to bulls exclusively (Innis 1958, Pratt and Anderson 1982, 1985) but during observations in HNP, adult cows were seen to take part in sparring on two occasions.

It must be also mentioned, that for the visual communication of dominance, contradictory descriptions are given in literature. Pratt and Anderson (1982, 1985) report that a dominant bull will walk towards an opponent with its head held high, intending to look as big as possible. On the contrary, Dagg (1971) states that a dominant bull, threatening an opponent will carry his head deep with the neck parallel to the ground, as if assuming a fighting position. We suggest that both observations are adequate and that communication of dominance might vary with the distance between opponents. In this regard, the “head-high” posture could be assumed for a distance of more than two body lengths, while the “fight” posture would be assumed with the opponent in close proximity, as it has been seen during our own observations. The typical intention of a threatening giraffe bull is often expressed by an arched and tensed neck (see *Dominance gesture*), as it is also seen in other ungulates, e.g. horses (Ransom 2009) or reindeer (Lund-Larsen 1977). The visual communication of submission is contrary to that of *dominance* and thus is also described contradictory in literature. According to Pratt and Anderson (1982, 1985), the subdominant individual will carry its head low to look smaller than it is, in order to not provoke aggression. Dagg (1971) reports that inferior giraffe bulls stand with an erect neck and the nose pointed upwards, assuming a feeding position and thereby exposing the body to attacks. As

well as for *dominance*, a distance dependent expression for submission might be considered. In this regard, the plasticity of social behaviour and communication patterns should be borne in mind during conduction and interpretation of behavioural observations.

The section on play behaviour was kept rather short and comprehensive. For the sake of brevity, all behaviours of the same obvious (play) intention were summarised. Nevertheless, future studies might be able to reveal various forms of play behaviour in the giraffe, similar to that of other ungulates, although probably not as pronounced as e.g. in horses (McDonnell and Poulin 2002).

Several behaviours, although often only observed in form of an attempt (e.g. mounting, mating, nursing) are classified as separate behaviours in this ethogram, because these attempts often represent a considerable proportion of an animal's time budget. Therefore these behaviours might be considered as a separate sub-section in an ethogram used for observations.

A comprehensive and reliable tool to monitor giraffe behaviour in the wild as well as in captivity is a necessity to gain a better understanding of the giraffe's life-history requirements. Subsequently, gaining a better understanding of giraffe behaviour will help to develop more effective conservation strategies for improving giraffe management in the wild and in captivity to allow a long-term coexistence.

4.2 Grazing behaviour of the giraffe in Hwange National Park, Zimbabwe

PA Seeber, HT Ndlovu, P Duncan & A Ganswindt; published in *African Journal of Ecology*.

4.2.1 Introduction

The giraffe (*Giraffa camelopardalis*) is usually described as an exclusive browser, feeding only on shrubs and trees, preferably between 2 - 5 metres above ground (Ciofolo & Le Pendu, 2002; Lamprey, 1963; MacNaughton & Georgiadis, 1986). Although browsing seems to be an easier form of feeding for giraffes in terms of accessibility and vigilance (Young & Isbell, 1991), a few studies mention that the giraffe also 'very occasionally' feeds on grass (Du Toit, 2005; Pienaar, 1963). To be able to graze, a giraffe has to adopt the typical 'drinking position', where the forelegs are splayed out laterally, and sometimes the carpal joints are also flexed. In this position the animals are particularly vulnerable to predators (Périquet et al., 2010). In this Note, we show that grazing, although not a core activity, is a recurrent event in a nutrient-poor environment such as Hwange National Park, and suggest a possible function.

4.2.2 Materials and methods

Giraffes (*Giraffa c. angolensis*) were observed in Hwange National Park, Zimbabwe, from March to May 2011. The study area included several water holes and open grasslands in the Main Camp area (Guvalala to Ngweshla Pan). 188 hours of observations were conducted from about 50-100 m distance, and a total of 1107 giraffe sightings were made. Focal observations were made on individuals when they were seen to feed from the herb layer,

referred to as grazing. Grazing sites were examined after each observation and only herbaceous plants were found in each case. The local herb layer is dominated by grass, and visual evidence of grazing always accounted for grass, not forbs. We therefore consider that consumption of forbs was insignificant. In most cases of grazing (80%), the observation commenced an individual was opportunistically found grazing. On only three occasions individuals started to graze during an on-going observation.

Feeding related behaviours (Table 4.8) were recorded using *ad-libitum* and *all-occurrence* sampling (Altmann, 1974). *Grazing* was recorded only when other similar behaviours like *drinking* or *geophagy* could be excluded. All animals that were found grazing (Figure 1) were photographed for identification (Innis, 1958; Dagg, 1971), to allow a comparison of individual frequency and duration. A *grazing bout* was defined as starting when the giraffe assumed the bent-down position to feed on grass, and ended when it got up again (Figure 1A). The duration of grazing bouts as well as number of bouts per event were recorded. A *grazing event* started when a giraffe adopted the bent-down position for the first time during an observation and ended when grazing was terminated by shifting to a different behaviour or moving out of sight. For each event, the overall time an individual spent on grazing and directly associated behaviours, e.g. walking between grazing patches or scanning between bouts (*Inclusive grazing time*), was recorded. In addition, the total time an individual spent ingesting herbaceous plants in a bent-down position with head to the ground was calculated (*Exclusive grazing time*).

Behaviour	Description
<i>graze</i>	'drinking position' adopted by splaying its legs out laterally, in some cases also bending the carpal joints, and lowering the head to the ground, where the giraffe ingests herbaceous plants. Head and neck moves from one side to the other.
<i>drink</i>	'drinking position' adopted by splaying its legs out laterally and bending carpal joints, lowering the head to reach a ground-level water source. If the water source cannot be seen from a distance, drinking can be confirmed when the giraffe raises its head and water is dripping out of its mouth. Head and neck is kept in a steady position.
<i>geophagy</i>	'drinking position' adopted by splaying its legs out laterally, in some cases also bending the carpal joints, the head is lowered to ground level to ingest soil by licking the ground or biting the soil.
<i>browse</i>	the giraffe stands in an upright position and forages on trees or shrubs, the neck may be bent down, but legs are not spread apart or bent.

Table 4.8. Ethogram of feeding and associated behaviour.

4.2.3 Results & discussion

Grazing behaviour was observed on 31 occasions (27 times by adult/subadult cows, twice by adult males and juveniles, respectively), although typical woody food plants (e.g. species of *Acacia*, *Combretum*, *Terminalia*, *Dichrostachys*) were in leaf and browsed on during the study period. Grazing was observed for 174 min (*exclusive grazing time*), representing 1.5% of the total observation time, at seven different locations. For five of these locations, salt licks were found within 20 m of the grazing patch. A total of 21 grazing giraffes were identified, and three individuals have been repeatedly observed grazing on different days. Grazing individuals were almost exclusively found in groups, consisting of four to sixteen animals and up to four giraffes were seen grazing together. We only observed one case of a solitary individual grazing. In order to feed from the herb layer, the giraffe bends down adopting

the *drinking position* and grazes to the front and to the side as well (Figure 2). Grazing was followed by geophagy twice, within 15 min. To change grazing patches, giraffes usually got up. Only one giraffe was seen to switch the position of one of its forelegs while still bent down, thereby increasing its foraging range. Grazing was frequently interrupted by vigilance (Figure 2B), as seen during browsing and drinking (Cameron & Du Toit, 2005; Ginnett & Demment, 1997). Overall grazing times, number of bouts per grazing event, and duration of bouts are summarised in Table 4.9.

Our results support previous findings that giraffes do feed from the herb layer (Ciofolo & Le Pendu, 2002; Du Toit, 2005; Pienaar, 1963). However, our short-term study indicates that grazing seems not to be an exceptional event. During our observations the grass layer was already senescent, indicating very low digestible protein/energy content (Safari et al., 2011; Tefera et al., 2009). In contrast, some of the giraffes' preferred browse species reach their maximum protein content during this time (Sauer, 1983). We therefore assume that the function of feeding on herbs during the dry season is not principally to supplement protein/energy intake, but rather to access other nutritional factors not currently present in sufficient amounts in browse, since previous studies imply that the herb layer sometimes has more available micronutrients than browse (Bowell & Ansah, 1993; Milewski & Diamond, 2000). Giraffes are likely to require large amounts of minerals (Milewski & Diamond, 2000; Mitchell & Skinner, 2003), which is also assumed to be the driving force behind osteophagy (Wyatt, 1971; Kok & Opperman, 1980). The soil in Hwange consists principally of Kalahari sands, which are extremely poor in nutrients (Childes & Walker, 1987). Therefore further data on the concentration of micronutrients in the plants eaten would be of great interest to draw conclusions on the functions of grazing in giraffes in Hwange National Park.

	Time _{incl} ^a (min)	Time _{excl} ^b (min)	Count of bends	Duration of bout (s)
mean $\pm$ sd	20 $\pm$ 11	5 $\pm$ 4	13 $\pm$ 9	23 $\pm$ 10
min-max	4-42	1-18	2-36	4-49

Table 4.9. Distribution and length of grazing events.

^a Inclusive grazing time: amount of time spent on grazing and directly associated behaviour (walking between grazing patches and scanning between grazing bouts).

^b Exclusive grazing time: amount of time spent on ingesting grass in a bent-down position with head to the ground.

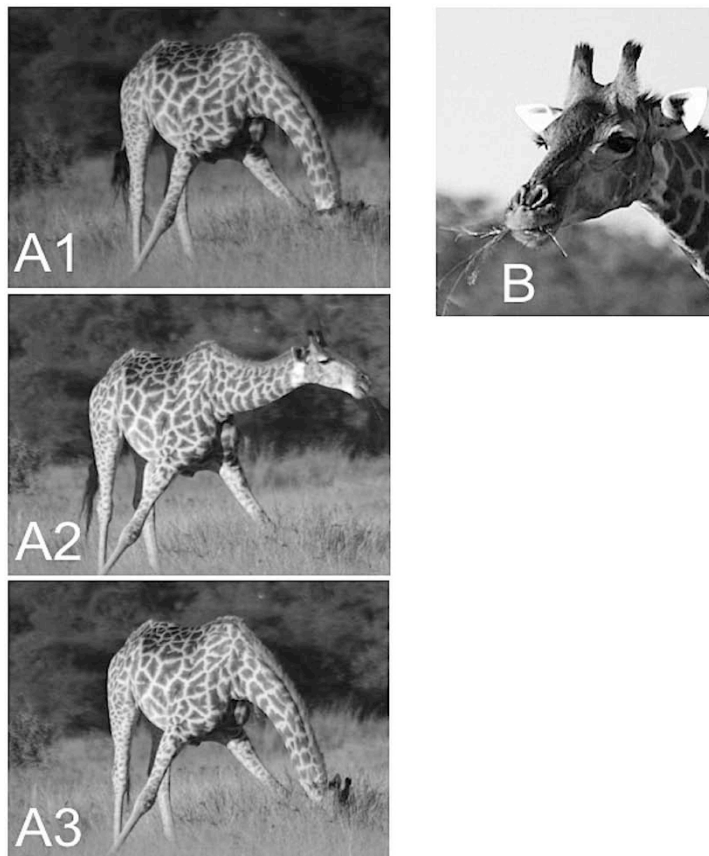


Figure 4.1. Grazing giraffe in typical position; legs splayed out laterally, but carpal joints not bent. A: Sequence of grazing (A1;3) interrupted by scanning (A2). B: close-up showing grass in the mouth.

4.3 Male reproductive and sex-specific behaviour

In preparation as

Seeber PA, Duncan P, Fritz H & Ganswindt A: Sexual and dominance behaviour in free-ranging giraffe bulls in Hwange National Park, Zimbabwe.

A total of 1107 giraffe sightings were recorded, consisting of 222 groups, including solitary individuals. A total of 94 mature bulls were captured in the ID catalogue. Adult bulls were seen 276 times, 31 times immature bulls, 12 times bulls of undetermined age, 616 times adult cows, 51 times immature cows, 131 times calves, and twice individuals were not allocated to a sex, or age class. A total of 264 individual behaviour observations were made - on 17 individuals allocated to age class A (81 observation sessions, in 49 hours; table 4.10), on 25 animals of age class B (91 observation sessions, total 59 hours) and on 31 bulls of age class C bulls (92 sessions, in total 75 hours).

Age class	A	B	C
No of Sightings	81 (30.7%)	91 (34.5%)	92 (34.8%)
Observation time (h)	49,2 (26.9%)	58,7 (32.2%)	74,7 (40.9%)

Table 4.10. Observation counts per age class and respective amount of observation time.

4.3.1 Social associations

Group size

Group size ranged from solitary individuals to one group of 22 giraffes (mean 5 ± 4). Excluding solitary giraffes, over 90% of all social groups were composed of 2 - 13 individuals.

Social associations and group sizes were analysed for an effect of hour of the day (Fig. 4.2 and Fig. 4.3). Mean group size recorded during the morning observations (between 6.30-10.30) as well as during the midday ones (between 10.30-14.30) was 4 ± 3 and was found to be significantly smaller than during afternoon observations (between 14.30-18.50) with a mean group size of 6 ± 4 (post hoc Mann-Whitney-U test morning-afternoon $p=0.016$; midday-afternoon $p=0.012$). Individuals were used as observational units.

During the morning all three group structures (solitary / mixed sex / unisex groups) were equally common ($\chi^2=0.118$; $df=2$; $p=0.943$; $\chi^2=9.588$; $df=5$; $p=0.088$, respectively), while during midday observations single animals were less common than mixed-sex, or all-cow groups ($\chi^2=10.825$; $df=2$; $p=0.004$, $\chi^2=19.450$; $df=5$; $p=0.002$; respectively). This trend was found for the afternoon observations as well, with an even higher frequency of one-bull-and-cows groups and bulls-and-cows groups ($\chi^2=28.110$; $df=2$; $p<0.001$, $\chi^2=37.110$; $df=5$; $p<0.001$, respectively). Mean group size only varied slightly between the age classes and was highest for C bulls (8 ± 5), and lowest for B bulls (6 ± 5), the mean group size A bulls were found with was 7 ± 5 . A statistically significant difference was in deed found between the age groups (Kruskal-Wallis-H test, $df=2$; $p=0.04$), the post hoc analysis with a Bonferroni adjusted confidence level ($p^*<0.017$) only found the age classes B and C to

differ from one another significantly ($p=0.013$), age classes A versus B, and A versus C did not produce a significant difference in group size.

Adult giraffe bulls were most often found to be associated with cows and other bulls, which is referred to as *mixed groups* (54%, $N=141$; table 4.11). Solitary individuals (11%, $N=30$) as well as bulls in bachelor groups (14%, $N=36$) each represent a rather slight proportion of the sample. Equal distribution of group composition over the sample population was found unlikely ($\chi^2=119.72$; $df=3$; $p<0.001$).

In general, solitary bulls were seen less frequently than expected, tested on the difference between solitary and 'in any social association' ($\chi^2=157.64$; $df=1$; $p<0.001$).

No statistically significant differences between the age classes were found regarding the four different categories of group composition ($df=2$; $p=0.274$), nor discriminating only between 'solitary' and 'in any social association' ($df=2$; $p=0.217$).

Age class animals sighted	A	B	C	Σ
<i>solitary</i> %	13 16.05	10 10.99	7 7.61	30 11.36
<i>in bachelor group</i> %	5 * 6.17	10 10.99	21 22.83	36 13.64
<i>with cows</i> %	28 34.57	19 20.88	10 10.87	57 * 21.59
<i>in mixed groups</i> %	35 43.21	52 57.14	54 58.70	141 53.41
Total	81	91	92	264

Table 4.11. Sightings of adult giraffe bulls associated with differently composed groups. Asterisked numbers indicate statistical significance.

Overall, adult giraffe bulls were associated with cows in 75% (N=198) of all sightings, which is significant at $p < 0.001$ ($\chi^2 = 66$; $df = 1$). A class bulls were associated with cows in 77% (N=63) of all sightings, B bulls in 78% (N=71), and C bulls in 70% (N=64), all of these produced the same statistical significance result as the distribution for all classes pooled ($p < 0.001$). There was no significant difference between the age classes regarding the frequency of association with cows ($df = 2$; $p = 0.330$).

Male-male association

Adult bulls were found to be associated with other bulls in 67% of all sightings (N=183). Regarding age classes, A-bulls were seen associated with other bulls to the least (53%, N=43), C-class bulls to the highest extent (83%, N=76), and B-class bulls represented an intermediate state (70%, N=64). Comparing age classes, an analysis of variances revealed a significant difference in the frequency of intrasexual associations ($df = 2$; $p < 0.001$). The

post-hoc procedure with Bonferroni-adjusted significance level produced the following results:

A-class bulls were found less often associated with other bulls than B-class ($p=0.013$), and C-class bulls ($p<0.001$). C- and B-class bulls did not differ significantly in observed frequencies of intrasexual associations ($p=0.037$).

Animals of class A were found with their own age class 20 times, and 45 times with younger ones. B-class bulls were seen 17 times with older bulls, and 33 times each with younger, and with their own age class. Bulls of age class C were found 65 times with older individuals, and 47 times with their own age class. Hence A bulls seem to only avoid their own age class, and B bulls seem to avoid older bulls, while C class bulls do not.

Dominance

In most cases (80%, $N=211$), a dominance rank for the focal animal could not be determined. However if rank was observed, frequencies of dominance / submission gestures were recorded to correlate them with potential determining factors such as presence of cows.

Overall, dominant bulls represented 10%, inferior bulls 9% and intermediate hierarchy states 1% of all observations. A-class bulls were most often seen to hold a superior hierarchy state, while an inferior state was seen most often in class C individuals (table 4.12). The frequency of submissive signals was, however, highest in age class B. Comparing the frequency of hierarchy observations, the age classes were found significantly different (Kruskal-Wallis test $p=0.002$), but only the dominance states of age class A and C differed significantly (Mann-Whitney-u-test $p=0.003$).

Age class	Signals / h observation		State		
	dominance	submission	Dominant	Inter-mediate	Inferior
A	1.05725 *	0.2643	17 *	1	4
B	0.18729	0.5448	6	2	9
C	0.02676	0.3345	3 *	1	10

Table 4.12. Frequency of dominance signals (events per hour of observation; mean) and hierarchy states per age class (total count). Asterisked numbers indicate statistical significance.

Regarding group composition, 96% (N=25) of all obvious dominant states were observed in the presence of cows, and 96% of all inferior states (N=22) were seen when cows were close. For frequencies of dominance gestures, bulls showed 0.432 gestures per hour, on average, when cows were in their group and to a 12-fold lesser extent when in all-male groups (0.036 times per hour).

4.3.2 Sexual activity

Behavioural observations were conducted in 96% of all sightings of mature bulls. In total, the highest frequency of sexual behaviour referred to as sexually related events (SRE's) was seen in bulls of age class A, higher than in class B and to the lowest frequency was recorded in age class C (table 4.13). Individuals were used as observational units.

Frequencies of affiliation and courting behaviours were analysed separately, using individuals as observational units. For affiliation, A bulls showed a significantly higher frequency than B bulls, differences between A and C, and

B and C class were not significant. For courting behaviours, A bulls had a significantly higher frequency than B class ($p=0.004$) and C class bulls ($p=0.001$). B- and C-class did not differ significantly.

Age class	Affiliation	Courting
A	2.603	4.005 *
B	1.311	1.396
C	0.682	0.776

Table 4.13. Frequencies of sexually related events in two categories, per age class (events per hour of observation). Asterisked number indicates statistical difference.

Mate-guarding was only witnessed thirteen times in total, eleven times of which were by A-class bulls and two by B-class bulls. Considering the state of mate-guarding to be representative for the entire respective observation session, A-class bulls were seen to mate-guard in 18.5% of the age class's total observation time, while in B-bulls, mate-guarding was seen in only 3.4% of the time.

Data on sexual behaviour were further analysed with respect to the presence of other males. A comparison of the association states for each age class showed a higher frequency of courting behaviours in A-class bulls when other bulls were present, than when they were the only male accompanying cows (Fig. 4.2). On the contrary, B-class bulls showed a frequency of courting behaviours reduced by 50% when other bulls were around and also C-class bulls had a frequency of courting behaviours reduced by two third when other males were present. The same tendency was found for affiliation behaviours. However, only in age class A the frequencies of both, affiliation and courting

behaviours differed significantly between presence and absence of other males (Mann-Whitney-U test $p=0.038$; $p=0.008$, respectively).

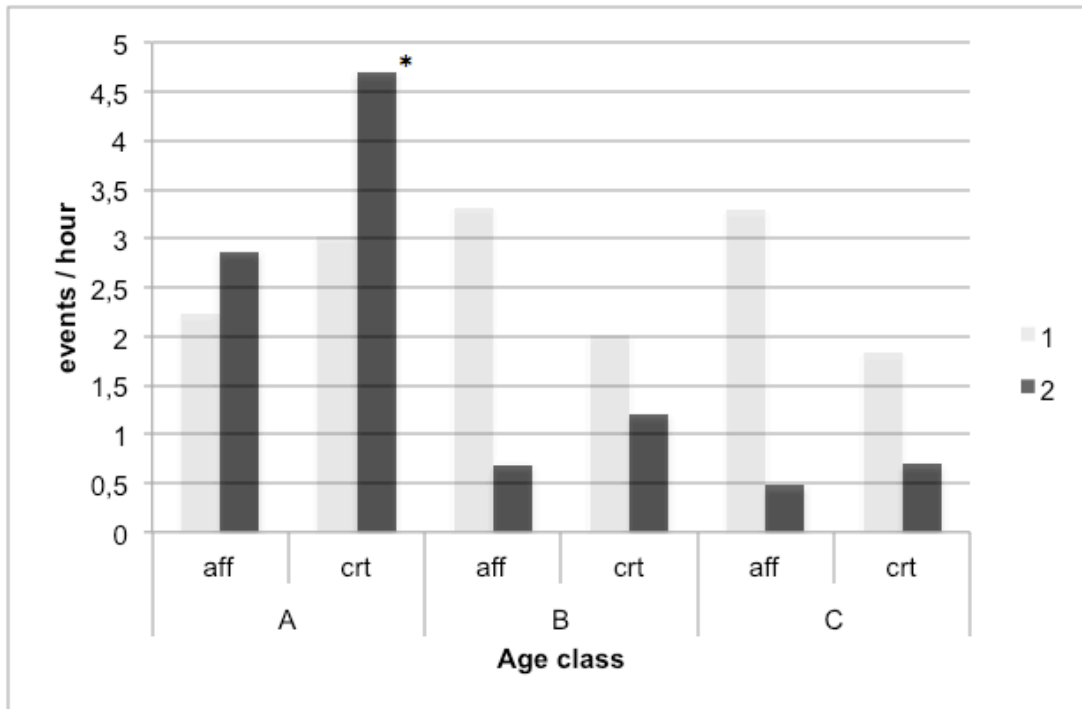


Figure 4.2. Frequencies of affiliation (*aff*) and courting (*crt*) behaviours per hour, in two states of the focus bull's social association, per age class (1=only bull; 2=other bulls present). Star indicates statistical significance.

Furthermore, the two dominance states were analysed for occurrence of SREs. Mate-guarding as such was considered a rather obvious sign of instantaneous dominance, which is why it was not included in this analysis. As a result, 36% of SREs had been performed by dominant bulls, 11% by inferior individuals and for the remaining 53% of all SREs a dominance state was not determined for the focal animal in the respective observation. Courting behaviours were seen significantly more frequently performed by dominant bulls than by inferior ones (Mann-Whitney-U test $p=0.001$), whereas differences between frequencies of affiliation behaviours were non significant.

Daytime

The results show change in frequency of reproductive behaviour with daytime. The frequency of SREs was highest in the afternoon, and both affiliation and courting behaviours seemed to increase during the day. For the morning observations, the average frequency of affiliation events was measured with 0.9 events per hour, 1.2 during midday, and 1.9 during afternoon observations. Courting behaviours rose from 1.3 in the morning to 1.9 during midday, and 2.1 events per hour during afternoon observations.

4.4 Androgen measurement

In preparation as

Seeber PA, Duncan P, Fritz H & Ganswindt A: Regulative endocrine mechanisms related to reproductive tactics in free-ranging male giraffes (*Giraffa camelopardalis*).

4.4.1 Validation of drying procedures

The sensitivity of the assay at 90% binding was 8 pg per well, the intra-assay coefficient of variation ranged between 3.51-7.72%, the inter-assay coefficient of variation between 7.73-11.58%. Serial dilutions of extracted fecal samples gave displacement curves that were parallel ($< 3\%$ variance in slope) to the respective standard curve of the assay.

Regarding the validation of the two different drying procedures, out of ten paired samples, nine pairs produced deviations between 2.8-10% (mean 9.66%; Fig. 4.3; appendix E). One outlier pair (B5) showed a deviation of 43.7%. Measured EA concentrations were distributed normally, a subsequent t-test for paired samples showed no significant difference between the sample sets ($p=0.305$; $df=9$), hence the approaches of low-heat drying and lyophilising were assumed to produce comparable results regarding androgen metabolite measurement.

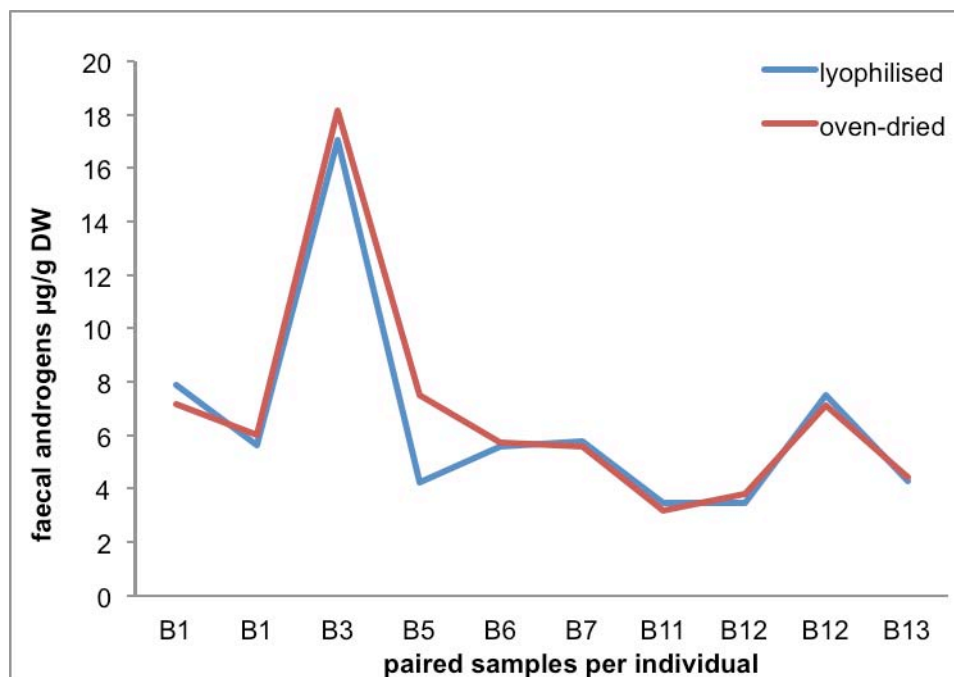


Figure 4.3. Epiandrosterone (EA) concentrations ($\mu\text{g/g DW}$) measured in faecal samples processed by different methods.

4.4.2 Biological validation of EIA suitability

For an overall biological validation, faecal EA values from class A, B, and C bulls as well as from females and juveniles monitored in autumn 2011 in HNP and EGR were compared. The differences in faecal EA levels between adult bulls, adult cows, and subadult individuals are shown in figure 4.4. Faecal EA levels of A class bulls were significantly higher compared to EA concentrations of females and subadult individuals (Kruskal Wallis ANOVA $p=0.004$, post hoc $p=0.005$ (females) and $p=0.007$ (subadults)). Faecal EA levels of B class and C class bulls did not differ compared to EA concentrations of females and subadults (B class: $p=0.242$ and 0.504 , C class: $p=0.117$ and $p=0.634$, respectively). In addition, no difference in faecal EA levels were found between females and subadults ($p=0.148$).

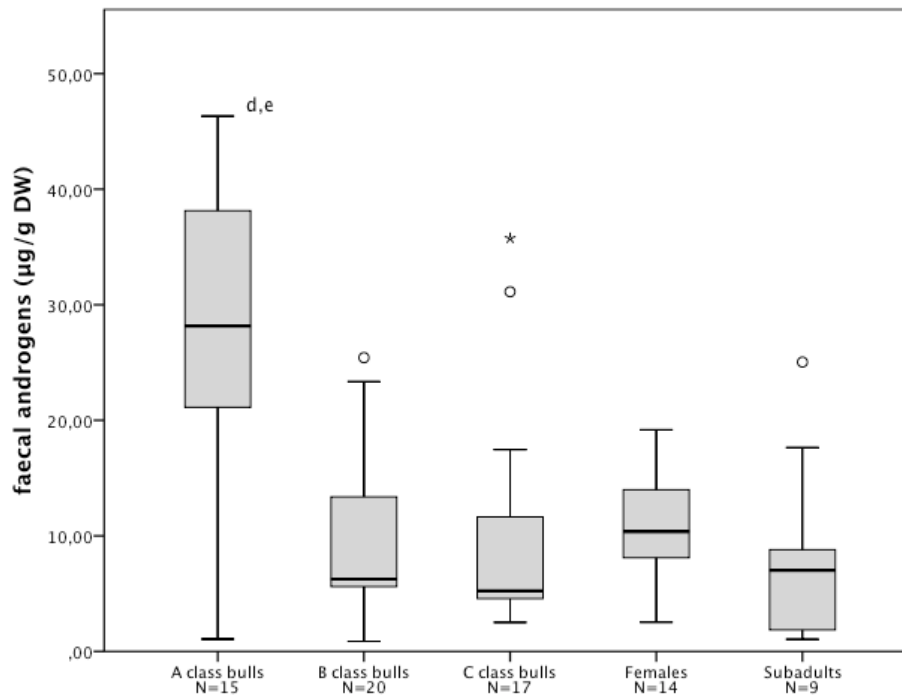


Figure 4.4. Boxplot of grouped median values of faecal EA concentrations (HNP and EGR samples pooled). Dots and stars indicate outlier values, superscripts indicate statistically significant differences between groups.

4.4.3 Differences in EA levels between age classes of adult bulls

Overall, faecal EA levels of A class bulls were significantly higher than faecal EA concentrations of B and C class bulls (Kruskal Wallis ANOVA $p=0.002$, post hoc $p=0.001$, and $p=0.004$, respectively). No difference in faecal EA levels were found between B and C class bulls ($p=0.243$).

For the HNP subset, faecal EA levels of A class bulls were significantly higher than faecal EA levels of B class bulls, but not of C class males (Kruskal Wallis ANOVA $p=0.014$, post hoc $p=0.004$, and $p=0.048$, respectively; figure 4.5). However, there is a trend of higher EA concentrations for A class bulls

compared to C class bulls ($p=0.048$ n.s. after Bonferroni-adjustment). EA levels between B and C class bulls did not differ statistically ($p=0.907$).

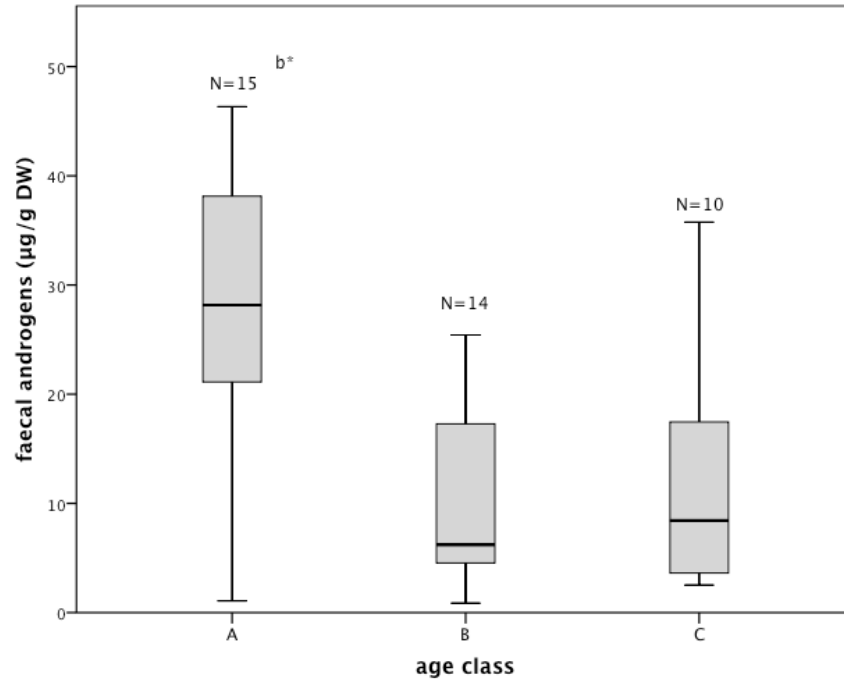


Figure 4.5. Boxplots of grouped concentrations of EA in faeces of class A, B, and C bulls of HNP. Superscript indicates significant difference.

A comparison between the two existing age classes (B and C) of bulls found in EGR showed a significant differences in faecal EA levels with higher hormone concentrations in class B bulls compared to class C bulls (MW U test $p=0.011$; figure 4.6).

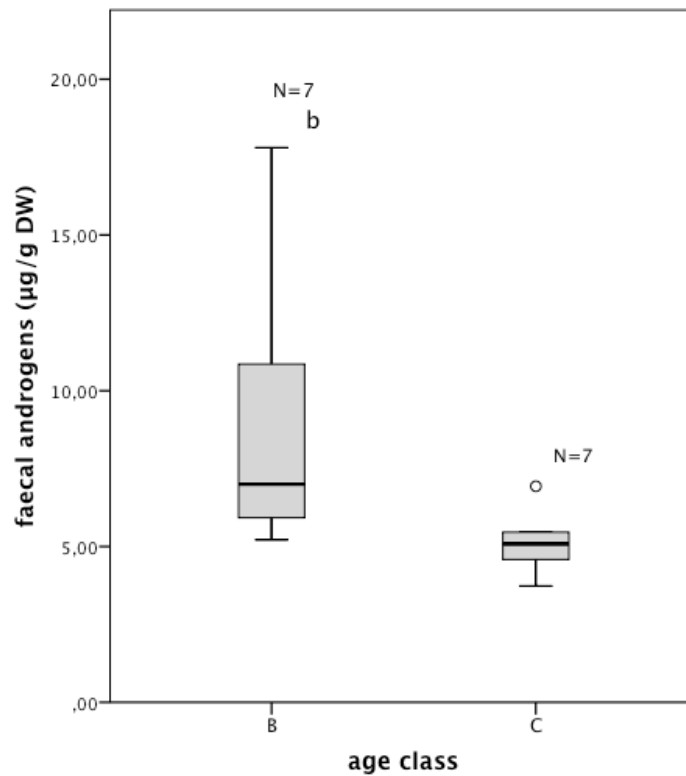


Figure 4.6. Androgen concentrations in class B, and C bulls in EGR. Dot represents outlier value. Superscript indicates statistically significant difference ($p=0.011$).

4.4.4 Differences in male giraffe EA levels across habitats

The difference in faecal EA concentrations of B class bulls monitored in HNP (mean: 6.21 $\mu\text{g/g DW}$) compared to B class bulls roaming in EGR (mean: 7.00 $\mu\text{g/g DW}$) was not significant (MW U-test $p=0.799$). Also no difference in faecal EA levels was found between C class bulls of HNP (mean: 8.44 $\mu\text{g/g DW}$) and in EGR (mean: 5.08, MW U-test $p=0.536$).

4.4.5 Seasonal differences in EA levels of giraffe bulls

Seasonal differences in faecal EA levels could only be compared for males monitored in EGR. Overall, faecal EA levels of samples collected in spring were higher than those collected in autumn (Wilcoxon test for paired samples $p=0.019$, Fig. 4.7) Regarding age classes, differences in faecal EA levels between seasons were not significant (Wilcoxon test $p=0.345$) in class B bulls, but in C class males with higher EA concentrations found in spring (Wilcoxon test $p=0.018$).

Furthermore, the significant difference in faecal EA levels between B and C class bulls mentioned under 2. ($p=0.011$) was only found when samples collected in autumn were compared. No differences in faecal EA levels between the age classes were found in the spring sample set ($p=0.628$).

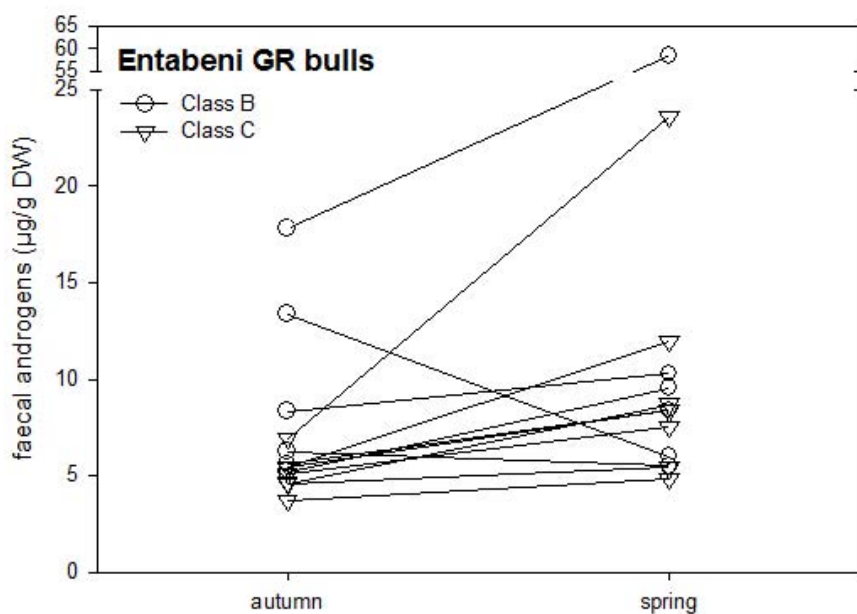


Figure 4.7. Individual variations in median EA values within two seasons.

4.4.6 Differences in EA levels of bulls in relation to sexual activity

Within the category of A class bulls, sexually active animals (determined as described in section 3.3.3) had significantly higher faecal EA levels, than inactive individuals (Student's t-test, $p=0.029$, Fig. 4.7). In class B animals no difference was found between faecal EA levels of the two states of observed sexual activity (Student's t-test, $p=0.438$, Fig. 4.8). In contrast, sexually active C class bulls had significantly higher faecal EA levels than inactive ones (Student's t-test $p<0.001$, Fig. 4).

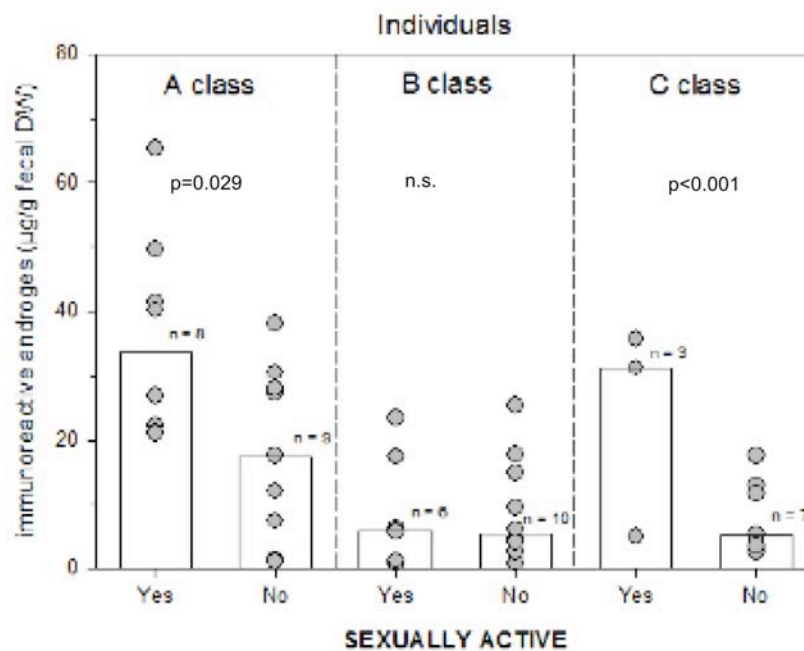


Fig. 4.8. Faecal EA levels of sexually active and inactive bulls divided by age class.

Faecal EA concentrations were significantly correlated with the frequency of sexually related events (SREs) observed (Spearman-Rho $\rho=0.628$, $p=0.007$).

By further dividing the SREs into either affiliation (approach, follow, necking) or courting behaviours (investigating, urine testing, mate guarding, and mating attempt), a similar trend was found for the relation of faecal EA concentrations and courting behaviours (Spearman-Rho $\rho=0.647$, $p=0.005$; Fig. 4.9a), but not for affiliation behaviours (Spearman-Rho $\rho=0.431$, $p=0.286$; Fig. 4.9b).

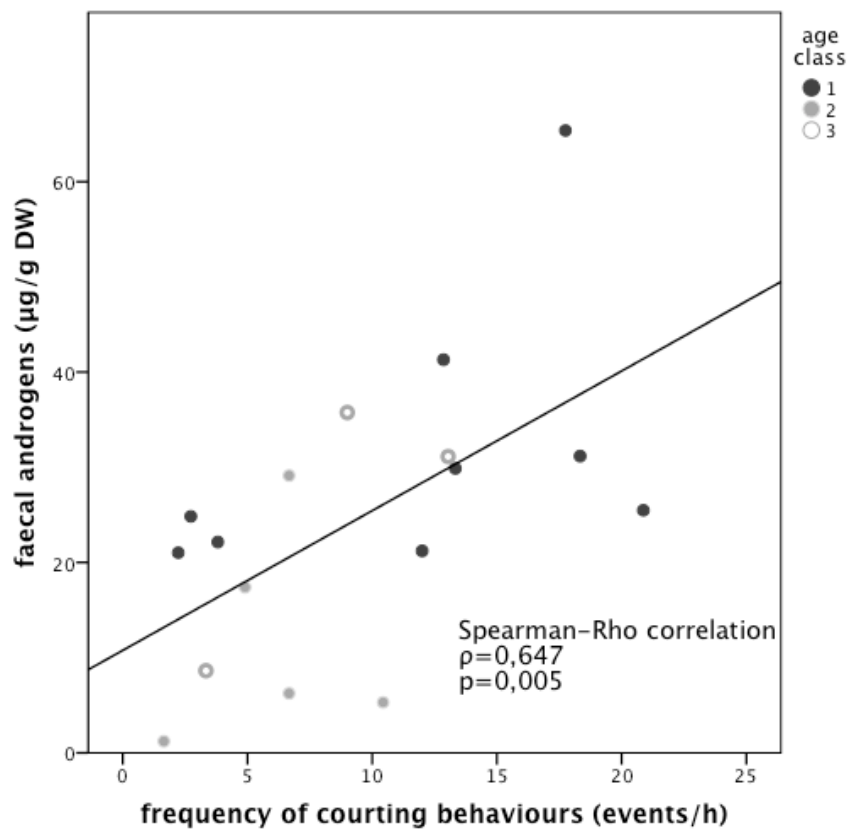


Fig. 4.9a. Correlation of faecal androgen metabolite concentration and frequencies of courting behaviours (events per hours), in the three age classes.

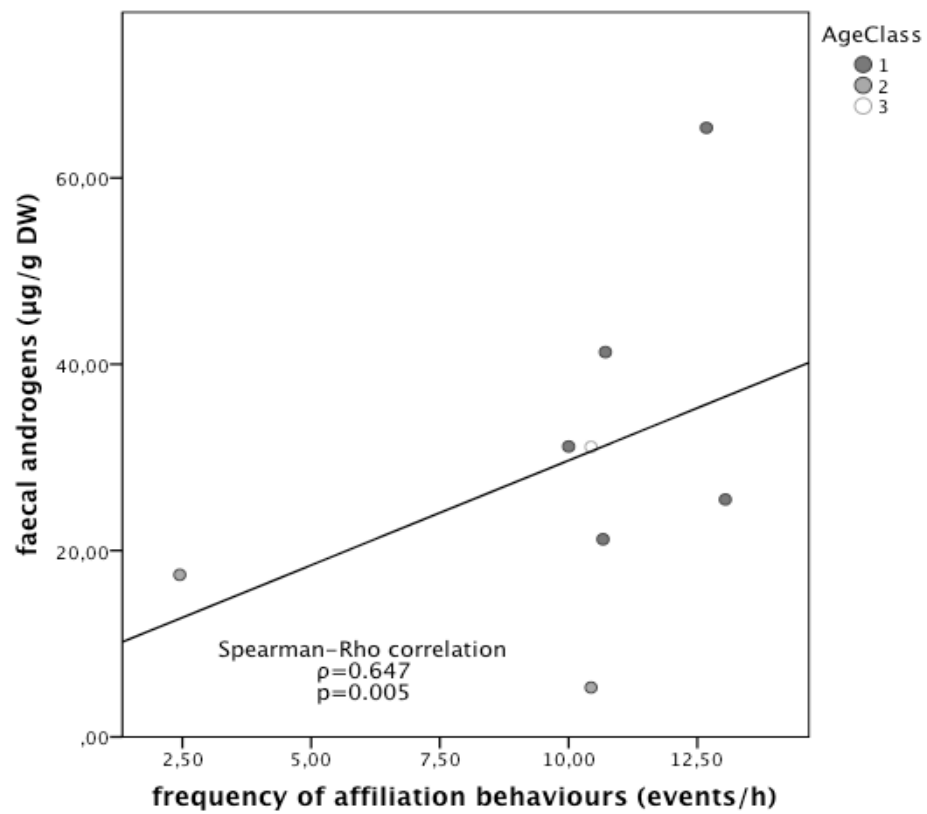
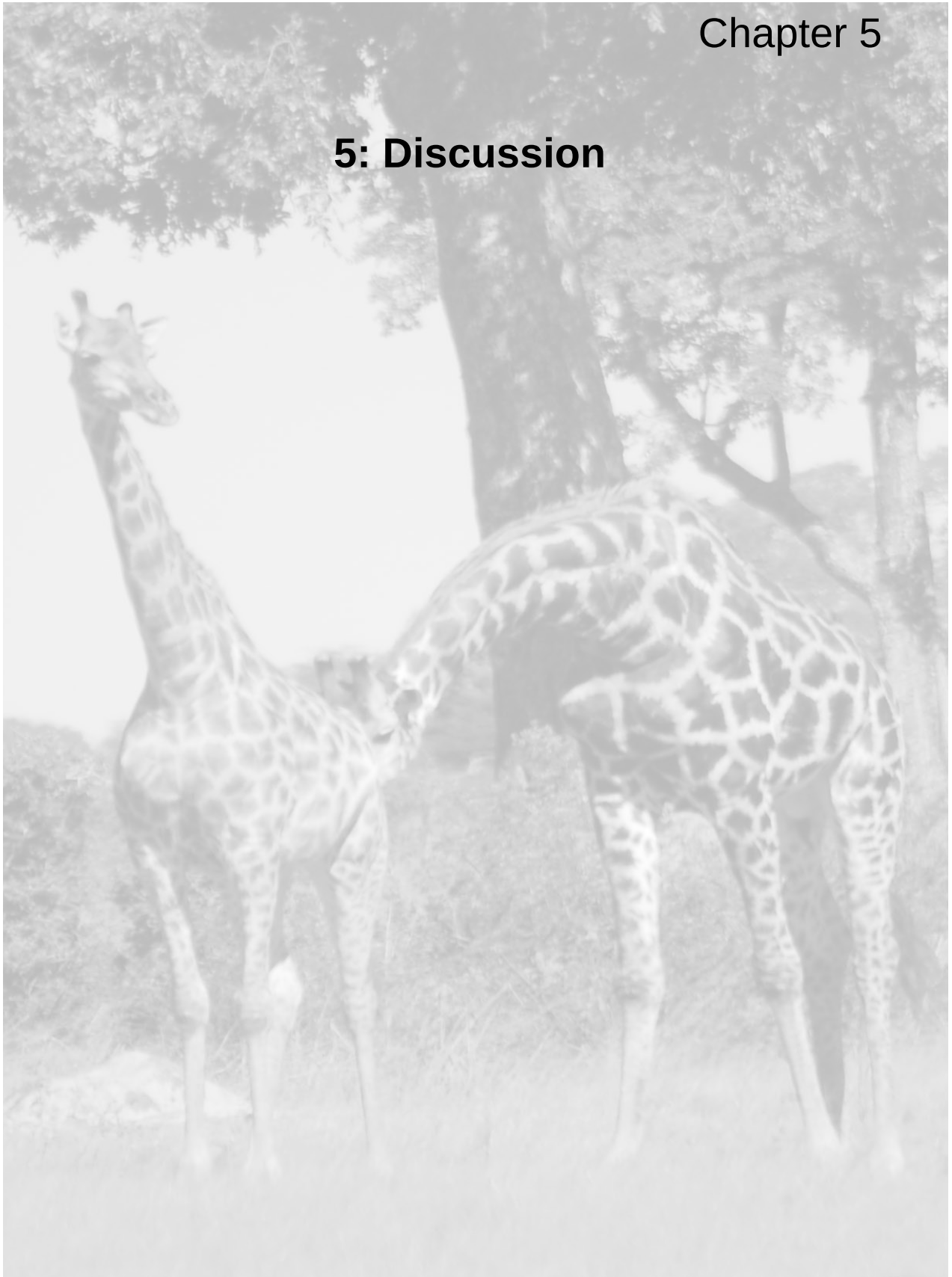


Fig. 4.9b. Correlation of faecal androgen metabolite concentration and frequencies of affiliation behaviours (events per hours), in the three age classes.



Chapter 5

5: Discussion



5.1 Male reproductive and sex-specific behaviour

A giraffe herd was roughly defined by Backhaus (1961) as a number of giraffes generally moving in the same direction and that are engaged in the same activity at any one time. For the present study, the behavioural pattern was not regarded crucial to determine social associations, since general activity varied notably between members of what was considered a social complex. Foster & Dagg (1972) defined a group as individuals less than 1 km apart and moving into the same general direction. Restricted by visibility, two different groups of giraffes were never seen within one observation session during our observations in HNP. It was seen though that a second group appeared during an on-going observation session and fused with the resident one. It sometimes seemed that individuals were separating from what was initially considered one group, but due to visibility restrictions, in most cases splitting-up of a group could only be assumed.

Sex ratios in groups of wild giraffes are known to be quite variable (Foster & Dagg, 1972). The present results show a sex ratio of 31% males, which is in fact within the range of previous observations (ranging from about 40% to 72% males, reviewed in Foster & Dagg, 1972). Sites similar to the selected study sites appear to be favoured by females due to local vegetation structure (Ginnett & Demment 1997, 1999; Bercovitch & Berry 2010), thus an actually higher ratio of males in HNP might be suspected.

Foster & Dagg (1972) state that there is no typical pattern of group composition in the giraffe. Neither fission of two groups or solitary animals joining a group, nor apparent splitting-up produced obvious social interactions, except for sexually associated behaviours by bulls.

Although two separate groups were never seen at a time, in some cases a

group appeared during an on-going observation and fused with the one being watched. Sometimes individuals also seemed to separate from what was considered their group, but due to visibility restrictions, it was not possible to see how far they went. Regarding the giraffe's daytime specific activity pattern (Leuthold & Leuthold 1978), a variation of group size depending on daytime was investigated on. Giraffe bulls spend a higher proportion of time feeding in the morning hours (Leuthold & Leuthold 1978), hence a gathering of giraffes at selected patches of high-quality food in the first hours of the day may be expected. In this study, group size varied through the day, with larger groups seen in the early morning, smaller groups towards the middle of the day, and often largest groups around dusk. This suggests a morning and an afternoon aggregation related to social associations and behaviour. However the causes of variation in group size merit a long-term study to investigate group dynamics. The typical food plants (species of the genera *Acacia*, *Combretum*, *Terminalia*, *Dichrostachys*) were still in leaf, available and browsed at the time of the observations.

The three age classes of males shared their time among the four types of possible association (*with cows*, *with bulls and cows*, *with bulls*, *solitary*) in ways which were not significantly different. Pratt & Anderson (1982) used the same categories of group composition, but obtained different results, as they reported significant preferences in association, by age class: in their study, A and B bulls were significantly more common with cows, but without other bulls (44%), and solitary (about 25%), but hardly with cows and other bulls (about 7%), while C bulls were seen associated with bulls and cows more often (26%), and hardly solitary (6%). The results of the present study show a different tendency, as the association with cows and bulls was by far most common in all three age classes (table 2).

It could be hypothesised that ecological conditions influence the choice of

social associations in the giraffe. The study by Pratt & Anderson (1982) was conducted in Arusha National Park, Tanzania, which covers a small area (119 km², about 1 % of HNP) and is also characterised by its paucity of large predators, as lions are absent. For relatively small and enclosed areas it has been reported that giraffes develop a stable social structure with a stable dominance hierarchy, presumably since all animals know each other (van der Jeugd & Prins 2000; Coe 1967). This could explain the very different social relationships between giraffes in Arusha NP and HNP. Furthermore, lions which are naturally the main predators of the giraffe (Du Toit 2005; Dagg 1970) are represented in HNP in considerable numbers (Loveridge et al. 2007), thus predation pressure might be considered as a factor influencing social decisions. In other ungulates predation pressure has been shown to influence social structures (e.g. impalas, Hunter & Skinner 1998). In case of the giraffe, predictions as to specific responses to predation pressure would need further investigation.

In this study, bachelor groups composed principally of age class C bulls, whereas solitary males in most cases belonged to class A. All three age classes occurred in mixed groups, also in contrast to the study of Pratt & Anderson (1982) who state that A and B bulls appear the least in mixed groups. The sex ratio observed by Pratt & Anderson (1982) is also different from the ratio of the present study (50% males in Arusha National Park, c. 30% males to females in HNP), which might account for the different social structure found by Pratt & Anderson (1982). In this study, males were found significantly more often in company of other males than on their own, which is different from other studies (see Van der Jeugd & Prins 2000). Once again difference between ecosystems may explain such differences: van der Jeugd & Prins (2000) observed giraffes in Lake Manyara National Park, Tanzania, which covers about 100 km², and also hosts a considerably smaller number

of giraffes than HNP does (estimated 85 individuals in Lake Manyara NP, and an estimated 3000 in HNP; IUCN 1998). Furthermore, the vegetation structure of tropical Tanzania is assumed to affect the social structure of giraffe groups compared to that in subtropical biomes, as hypothesised by van der Jeugd & Prins (2000). Regarding predation pressure, it might be assumed that the presence of large predators leads to a decreased percentage of solitary individuals, particularly regarding animals younger than class A, or B bulls. Also, increased vigilance behaviour and readiness for flight could reduce reproductive activity.

Van der Jeugd & Prins (2000) suggest that differences in mating strategies of male giraffes might exist over different environments. In this regard, intrasexual association might vary in relation to the same selective influences. As shown in Table 2., class A bulls were found less often in bachelor groups than solitary, C-class bulls represent the opposite, while B-class bulls show intermediate and equal percentages for these two states.

Concerning age classes of associated animals, it was found that associations with class C bulls are most common compared to associations with the two older classes. Pratt & Anderson (1982) also report that C bulls are more often associated with each other than with the two older classes, which led them to the conclusion that A and B bulls try to avoid other bulls of class A and B, in the presence of cows. From the data collected during our own observations, this conclusion could not be verified for the giraffes in HNP. In fact, a comparison of intrasexual associations concerning age classes showed no difference between the frequencies of intrasexual associations when the focus bulls were in company of cows.

Due to the observed social dynamics and fluent hierarchic structures, stable dominance hierarchies as found in small reserves (Bercovitch et al. 2009; van der

Jeugd & Prins 2000) are highly improbable in HNP. Leuthold (1979) observed giraffes for over three years in Tsavo East NP (11700 km²) and suggested that the bulls knew each other and thus established a stable hierarchy system; behavioural observations of this study were only conducted in between two rivers (Galana and Voi) which are constant water bodies and hence not crossed by giraffes, thus the population within the study area represents only a fraction of the park's population which is confined to a considerably smaller area (estimated 3000 km²).

The communication of dominance is not always obvious to the human observer, though the rare occurrence of aggressive encounters (Innis 1958; Pratt & Anderson 1985) advocates a hierarchy among giraffe bulls which is quite clear to the participants. Their physique seemed in most cases to be a sufficient indication of the actual dominance state, but particularly between large and slightly smaller bulls this conclusion showed not to be absolutely reliable as sometimes smaller bulls dominated larger ones, but class C bulls were never seen to dominate a class A bull. Several times dominance signals and displacement by big bulls were only directed towards bulls of roughly the same size, while bulls that were considerably smaller could approach the defended cows and perform investigation behaviour undisturbed.

The rather obvious visual communication of dominance was in most cases seen between bulls of roughly equal size. In several cases, the visual signs of dominance were quite clear to the observer, e.g. a large bull with an obviously tensed dorsal neck musculature and an arched neck, walking or cantering towards another bull, which yielded. Obvious tensing of the dorsal neck musculature seemed to be one of the most obvious dominance signs. In other ungulates tensing of the dorsal and lateral neck musculature is reported to be a clear sign of dominance or an agonistic intention (e.g. in the horse; Ransom 2009) and a rise of androgen levels during breeding season is reported

to stimulate hypertrophy of the neck musculature (e.g. in the reindeer; Lund-Larsen 1977), which thus might work as a subtle communication of dominance. Thus it can be assumed that in the giraffe the enhanced tensing of these muscles might also be in use to communicate a severe motivation of hierarchic superiority.

Concerning visual signs of submission, it was seen twice that a bull who had been displaced approached the dominant individual with a lowered neck, and opening and closing its mouth repeatedly, as if performing an exaggerated chewing motion. In equids, an exaggerated chewing motion with open mouth is a known sign of inferiority, referred to as *snapping* (Tyler 1969; Ransom & Cade 2009). During our observations, subdominant bulls were seen to perform a similar behaviour when dominant individuals approached them, thus we suggest that snapping as a communication of inferiority is applicable for the giraffe as well.

Dominance ranks were observed to vary between observations of the same pair of participants, within a few weeks. On one occasion, one bull who had been displaced by another bull two weeks before was seen mate guarding, and during the observation session encountered the other originally dominant bull. The bull guarding the female this time was able to displace the rival and to restrict his approach to the cow. The displaced bull, who had shown clear signals of dominance two weeks earlier, yielded readily. Variation in dominance over relatively short periods of time might be attributed to external cues, such as presence of competing males and their respective age classes or dominance states, or due to internal cues such as the endocrine state (Machida et al. 1991). The data set of the present study is insufficient to draw solid conclusions on variations in individual dominance of wild giraffe bulls, hence further observations on temporal and spatial social structures combined with endocrine investigation would be necessary.

The present results show that dominance in giraffe bulls is apparently displayed by a range of rather subtle signs, which might not always be obvious to the observer, in particular as communication of dominance might take place over considerable distance. In this regard it is worth noting that the means of intraspecific communication in the giraffe have been poorly investigated, and non-visual communication must also be considered. Infrasound vocalization has indeed been reported to occur in captive giraffes, but its actual communicative use is still unclear (von Muggenthaler, Baes, Hill, Fulk, Lee, unpublished data). However, due to the relatively low count of observed clear hierarchies the quantitative results on dominance and hierarchy structures among adult giraffe bulls must be considered preliminary.

The frequencies of both, *affiliations* and *courting* were highest in A class bulls, intermediate in B bulls and rarest in C bulls, which is in accordance with the results of other researchers (e.g. Pratt & Anderson 1985). Regarding each behavioural pattern separately, two behaviours of the affiliation category did not match this distribution, as *necking* was seen in C class bulls more often than in B and A bulls, and *approach* was seen in C bulls more frequently than in B bulls. In this regard it might be somewhat arbitrary to conclude a sexual motivation in these behaviours which confirms the chosen classification into *affiliation* and *courting* behaviours. In older bulls, necking with cows seemed to be associated with sexual behaviour, as it sometimes was done between two events of investigating, and sometimes preceded or followed urine testing. Necking among bulls in some cases induced sparring, which is considered a behaviour of social rather than of dominance context (Pratt & Anderson 1982; 1985). Regarding *urine testing*, it is apparently much less frequent than *investigating*, and was particularly rare in the youngest age class. In this regard, Pratt & Anderson (1985) report that giraffe cows

oftentimes ignore the investigative approaches of young bulls, but immediately respond to the mere appearance of bigger bulls by urinating. Thus *urine testing* is apparently limited by the cow's readiness to urinate as a reaction to the bull investigating her, rather than by the bull's intention to do so.

Mate guarding only occurred in age class A and B, and particularly in class A, corresponding to the frequencies of courting behaviours. Innis (1958; referring to it as an aspect of 'courting') reports that this behaviour is relatively rare, which can be confirmed by own observations. All events of mate guarding were accepted by other present bulls, an intrusion by another bull was never witnessed.

Bulls of the age classes B and C were apparently not influenced in their sexual behaviour by the presence of other bulls. Overall, observations C-class bulls generally produced rather variable results concerning sexual activity and social associations, which is why sexual behaviour of the youngest mature age class might not be considered comparable to reproductive behaviour of older bulls.

The considerable increase in sexual activity in A bulls when other males are present supports the idea of enhanced activity when in competition for mating partners. Increased time and effort spent investigating cows and eventually establishing a courtship may reduce a bull's fitness due to reduced foraging and rumination time, and it could be that older bulls can afford this loss of maintenance time but younger and growing bulls cannot. Also, younger bulls might not be able to sustain an established mate guarding relation once a bigger bull intrudes, which might be a reason why only A class bulls enhance their sexual behaviour in presence of other males. Considering the concept of female choice (Orians, 1969; Waltz, 1982), A bulls probably have the highest

chance of being accepted, whereas B and C class bulls might only be considered by a cow when no higher-ranking bull is available. This lack of acceptance of potential partners by females might influence the bulls' behaviour as well, since in younger bulls the arrival of higher ranking males reduced the chance of a successful mating, so the reduction of browsing and ruminating time for increased effort on sexual behaviour was not often compensated by successful mating.

As expected, the two categories of dominance states produced a significantly different result in terms of SRE frequency. Hence it seems that the communication of dominance, however obvious or hidden to a human observer, is an appropriate means for dominant bulls to restrict sexual activity in sub-dominant individuals. Comparing age classes though, the separation into dominance classes produced quite divergent results in respect of SRE frequencies. In oldest class bulls, the frequencies of sexually related events are higher in dominant than in subdominant ones, which is in accordance with the results of Pratt & Anderson (1982; 1985). In bulls of age class B though, there is no significant difference of SRE frequency between the dominance states, which forms an intermediate state to bulls of age class C, where subdominant individuals apparently show more sexually related behaviours than do dominant ones. The differences in C bulls are statistically non-significant, which may be due to the lack of power of statistical analysis due to small sample size, but it is in fact likely that SRE are not directly related to dominance for subordinate individuals.

The frequencies of sexually related activities did not vary according to the hour of the day, there tended to be an increase at the time of sunset. Lacking observations at night time, no further conclusions can be drawn on a day time pattern of reproductive behaviours. Leuthold & Leuthold (1978) also point out

the necessity of 24-hours observations in giraffes, since the species is nocturnal as well (van der Jeugd & Prins 2000; Baxter & Plowman 2001). Also, intrasexual hierarchies and communication of dominance seem to be insufficiently studied and would require further investigation. The present study revealed information on dominance and sexual behaviour of giraffes within a near-natural environment, and showed deviations from the results of previous studies conducted under different ecological circumstances. Although no quantitative data on reproductive behaviour was available, results imply the necessity of evaluating ecological factors for comparison of behaviour and social structures.

5.2 Androgen measurement

5.2.1 Validation

The validation of the drying methods applied advocates for a comparability of results obtained by the different processing methods. As shown in figure 4.1 the one outlier sample pair (B5) is not in accordance with the other 9 results and is therefore considered incidental. It must be considered that the faecal samples were not homogenised prior to separation into subsets and also a mix-up of another individual's dropping when collecting the respective sample could have led to this result.

No significant difference in androgen concentrations was found between the processing methods. Therefore, the approach of low-heat drying of faecal samples can be considered to be an adequate method where freezing and subsequent lyophilisation is not feasible as in remote study sites.

As shown for different other species like African elephants, the used EIA is considered to reliably measure androgen metabolites (Ganswindt et al. 2010). In terms of EIA reliability for species-specific measurement of androgen metabolites in giraffe faeces the comparison of age classes in mature bulls showed the expected pattern of significantly higher EA levels in age class A bulls, highest EA levels in older bulls compared to younger individuals and compared to cows/immature individuals; accordingly, class A bulls had higher EA concentrations in sexually active bulls than in inactive ones. The used assay therefore is considered to reliably measure androgen metabolites in giraffe faeces. As shown for other species, faecal androgen levels correspond with plasma androgen levels (e.g. Dloniak et al. 2004), which has not been proven for the giraffe yet. Also, concerning saliva as a substrate for non-

invasive measurement of androgens, a subsequent study would be necessary to ensure comparability with faecal hormone concentrations.

5.2.2 Androgen levels

Differences in EA levels between age classes of adult bulls

The results showed a considerable higher concentration of androgen metabolites in faeces of A-class bulls, compared to the two younger classes, Age class B and C in HNP had roughly equal EA levels. In EGR, where A class bulls are absent, a significant difference was found between the two resident age classes. It could be considered that the presence of A class bulls might influence the endocrine development of younger bulls, thus if younger bulls grow up without older conspecifics, a premature sexual development might be a result. The EGR population consisted of a small number of individuals kept within an enclosure of limited size, thus an established and rather constant dominance hierarchy is quite likely (van der Jeugd & Prins 2000; Innis 1958; Foster & Dagg 1970). In this regard, bulls of age class B represent the highest class in EGR, although they are considerably younger than the dominant class in HNP, hence social state and lack of older individuals probably influence the individual's endocrine development as well. However in order to draw further conclusions by comparing results from two different field sites, the mentioned factors would have to be investigated and standardised.

Differences in EA levels across habitats

For other large herbivores, differences in individual/population androgen levels, presumably as a result of ecological variability, were described (Kretzschmar et al. 2004; Christensen et al. 2009). In this study, no significant

differences in faecal EA concentrations were revealed between the two study populations. However the absence of A-class bulls in EGR is a socioecological factor which might influence the endocrine state of younger bulls, as comparably shown in other large herbivores as well, e.g. black rhinoceros, and white rhinoceros (Christensen et al. 2009).

Seasonal differences in EA levels

A variation in EA levels between autumn and spring was found in the EGR population. Since calving rates in free-ranging giraffes vary by season, an annual pattern in mating activity and endocrine correlates could be considered to depend on resource availability. During the field investigation period of the present study browse was sufficiently available (as described in section 4.2.). However, samples were only collected during one autumn and one spring season, thus for a conclusion on an annual endocrine pattern a further cycle of sampling would be needed. In this regard the correlation of season and sexual activity should also be investigated on. The tactics of male roaming and mate guarding are costly in terms of a loss of browsing time for large herbivores, and maybe also for the giraffe, which is why a tendency of seasonal reproduction might not only be influenced by varying female fertility, but also by differing investment in sexual activity by giraffe bulls.

Differences in EA levels in relation to sexual activity

Faecal androgen concentrations are positively correlated with aggressive and agonistic behaviour in other ungulate species (Fletcher 1978; Patton et al. 2001). Because social rank and fighting ability often increase with age, androgen levels have been found to be positively correlated with age in bovids and cervids (Ditchkoff et al., 2001; Pelletier et al., 2003). The results of this study are consistent with previous reports, as older giraffe bulls with higher androgen levels were more likely to engage in reproductive behaviour, determined by

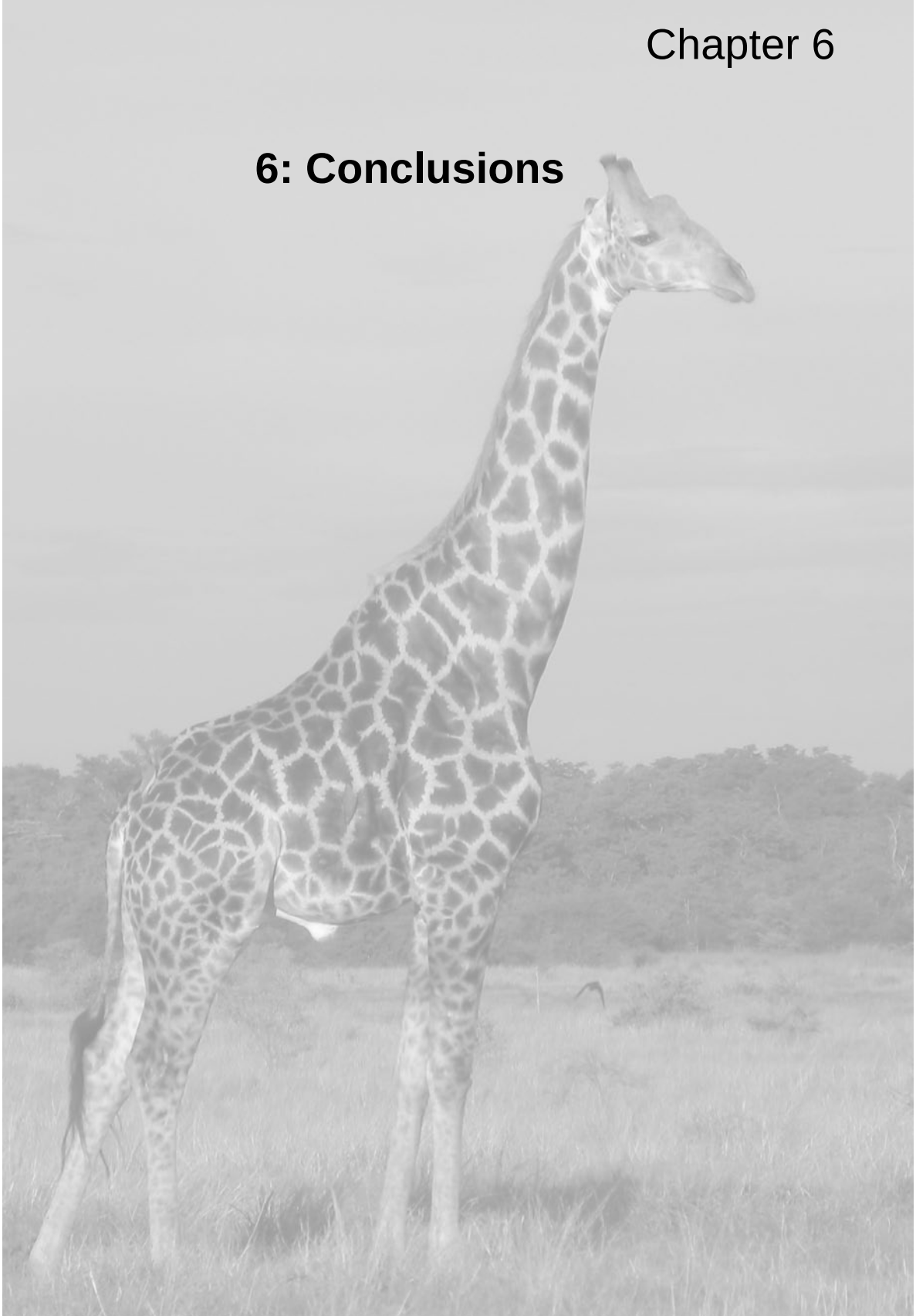
the frequency of SRE's, as well as courting behaviours. A correlation between affiliation behaviours and EA levels was not found, which is most probably due to the rather arbitrary and interpretative character of this behavioural category. However, the frequency of the more obvious courting behaviours was positively correlated with measured EA levels.

This study was designed to compare initial results of androgen levels in sexually active, and inactive individuals. Due to temporal limitations of behavioural observations, only the state '*sexually active*' could be determined with relative certainty, thus the results refer to challenge response levels of androgens for sexually active bulls (as described in Wingfield et al. 1990), and only indicate baseline levels of presumably sexually inactive ones.

In order to draw a broader picture on the correlation between androgen levels and reproductive behaviour in the different age classes, behavioural observation and sample collection on a larger scale would be necessary.

Chapter 6

6: Conclusions



This study is the first to investigate androgen excretion patterns in relation to observed reproductive behaviour in mature giraffe bulls in-vivo and in-situ. The biological validation of the used enzyme immunoassay confirms the reliability of the non-invasive approach used. For future studies, the identification of the measured androgen metabolite via HPLC could be considered in order to investigate on the species specific steroid metabolism of the giraffe. A subsequent study might also address a comparison of faecal androgen levels with plasma androgens.

Behavioural observations regarding male sexual activity corresponded with variations in faecal androgen concentrations regarding the expected pattern of increased EA concentrations in older bulls compared to younger ones. Also, higher EA levels in sexually active than in inactive bulls confirm a presumed correlation between the measured androgen metabolite and sexual maturity, and sexual behaviour, respectively. A comparison of the correlations between sexual activity and androgen excretion for each age class separately showed that in the middle age class both, active and inactive bulls had low and insignificantly different EA concentrations, whereas active males of age class A, and B had significantly higher EA levels. This gap of sexual activity and correlated androgen levels leaves room for further investigation, suggesting that a speculative suppression of sexual activity by dominant (A class) bulls only affects upcoming B class bulls, but not C class bulls due to directed dominance signals.

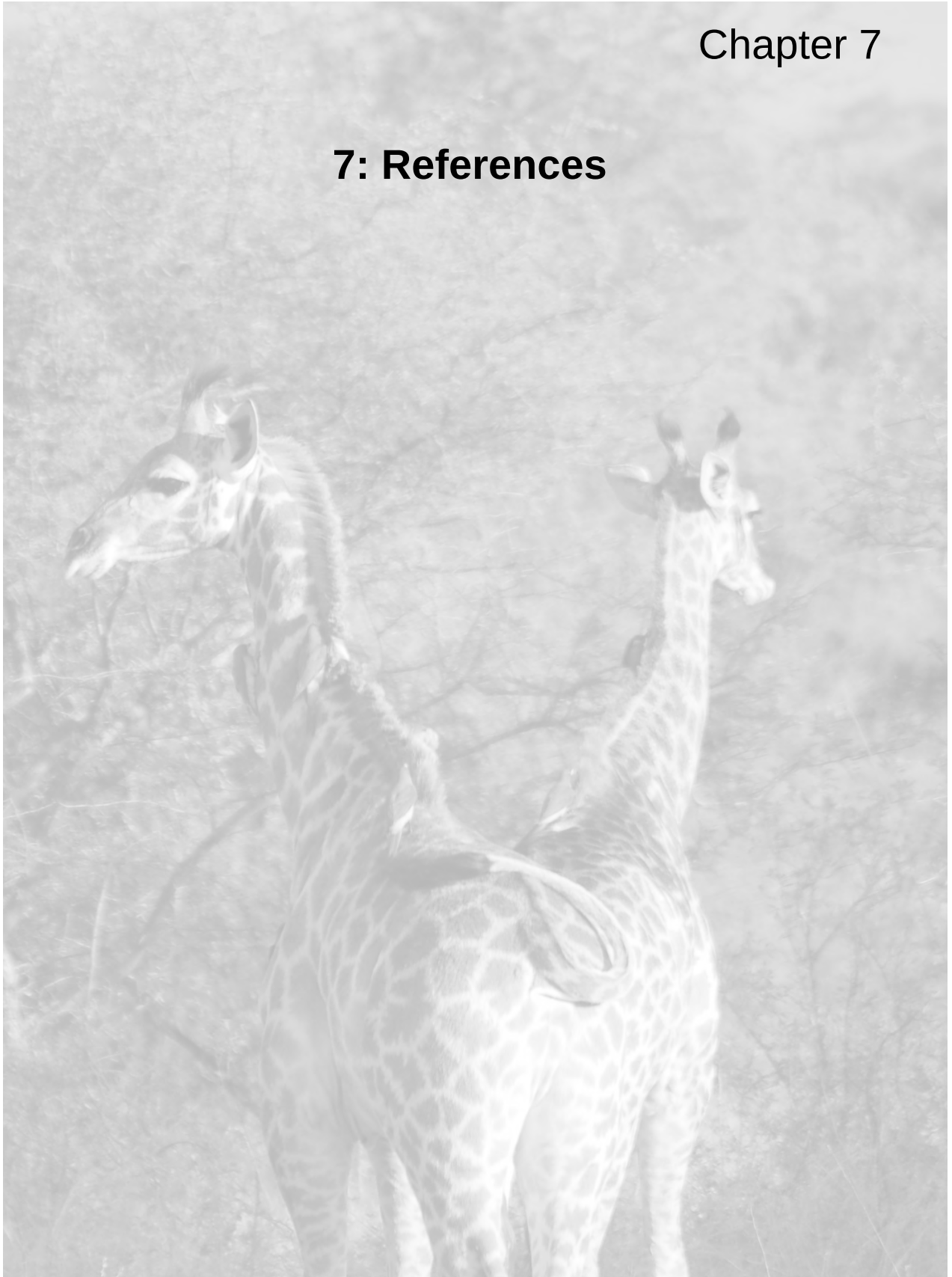
This study produced preliminary results showing need for further investigation in order to clarify the complex reproductive system of the giraffe. Due to tremendous fluctuations in individual EA concentrations, and statistically significant differences of individual EA levels between seasons, a rut-like behaviour in mature giraffe bulls might be considered for further investigation. Subsequently, the frequency of sexually active periods, as well as triggering factors for the suggested rutting behaviour should be analysed, such as

presence/absence of other potential rival males, receptivity stage of present cows, or other ecological factors such as seasonal resource availability. For future studies, long-term observation with simultaneous hormone monitoring would be useful in order to investigate on individual short-term variations as well as seasonal long-term variations of sexual behaviour and correlated androgen levels.

On a longer time scale, it might be considered to follow behavioural and endocrine patterns of individuals from prematurity to senescence. This would allow to ascertain age dependent variations in androgen excretion and correlated sexual behaviour. Regarding variations in androgen levels, ecological cues and physiological stress responses should be investigated as well. Social influences and ecological factors have been shown to influence sexual behaviour and correlated androgen levels in other large mammals (Ganswindt et al. 2010), which is why a synchronous monitoring of glucocorticoid metabolites might reveal further information on individual variations in the male reproductive system of the giraffe.

Chapter 7

7: References



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APPENDICES

Appendix A: Data capture sheet for behavioural observations

Date _____ Time ____ - ____ ID _____ Age class _____ Sheet No. _____			
GPS _____		Sample No. _____ probability _____ %	
Temperature _____ °C	weather _____		wind(Beaufort) _____
Vegetation _____			
Group size Σ _____		Other Individuals: _____ Cows _____ Calves _____ Bulls _____	

Intersexual behaviour		Intrasexual behaviour	
Approach		Approach	
Follow		Necking	
Necking		Sparring	
Guarding	Yes No	Follow	
Investigate		Dominance signal	
Urine test		SUBmission	
Mating attempt			
additional remarks:			

Appendix B: Chemical composition of reagents for EIA's

Coating Buffer (CBf)	1.59 g Na_2CO_3 +2.93 g NaHCO_3 added up to 1 l final volume with H_2O (RO), at pH=9.6
Coating Solution (CS)	50 μg protein A + 15 ml CBf
Saturation Solution (SS)	2.42 g $\text{C}_4\text{H}_{11}\text{NO}_3$ +17.923 g NaCl + 10 g bovine serum albumin (BSA) + 1 g NaN_3 added up to 1 l final volume with H_2O (RO), at pH=7.5
PBS Stock Solution (PBS-SS) for Washing Solution	0.399 g NaCl +5.752 g Na_2HPO_4 +1.005 g KCl +1.028 g KH_2PO_4 added up to 1 l final volume with H_2O (RO), at pH=7.2
Washing Solution (WS)	960 ml H_2O (RO) +40 ml PBS-SS +500 μl Polysorbate 20 (Tween 20 [®])
Assay Buffer (ABf)	8.5 g NaCl +5.96 g Na_2HPO_4 +1 g BSA added up to 1 l final volume with H_2O (RO), at pH=7.2
Using Solution (US) for Substrate Solution	4.75 g $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$ +3.9 g Na_2HPO_4 +0.25 g $\text{CH}_6\text{N}_2\text{O}_3$ added up to 500 ml final volume with H_2O (RO), at pH=3.8
TBM Solution (TMB-S)	125 mg TBM + 10 ml DMSO
Substrate Solution (SubS), per plate	250 μl TMB-S +17 ml US
Streptavidine Solution (StrS), per plate	16 ml ABf +2 μg Streptavidine (1 μg / 10 μl)

Appendix C: Index of study animals

Hwange, March - May 2011

ID	Age class	N faecal samples	cumulative observation time (hours)
AIR21	C	0	0.25
AIR23	C	1	7.32
AIR24	C	0	0.87
AIR25	A	4	9.37
AIR26	A	2	6.79
BOS1	A	0	0.73
DOM10	B	1	1.92
DOM11	B	0	0.48
DOM12	B	0	0.97
DOM13	C	1	1.26
DOM14	B	2	0.77
DOM15	A	0	0.43
DOM16	B	1	0.48
DOM17	A	0	0.75
DOM7	B	9	16.98
DOM9	B	1	0.73
FOR1	C	0	0.5
FOR2	C	0	0.5
FOR3	C	0	0.5
FOR4	C	0	5.33
GUV10	C	0	2
GUV11	C	0	2
GUV12	C	0	2
GUV13	C	0	2
GUV14	C	0	2
GUV15	C	0	2
GUV2	A	1	0.53
GUV3	B	0	0.67
GUV4	B	1	0.55
GUV5	C	1	2.85
GUV6	A	2	1.53
GUV7	C	0	1.18
GUV8	C	0	1.18
GUV9	B	0	1.18
KAT1	A	0	0.36

LIV3	C	0	1.32
LON1	C	0	0.45
MAI1	A	3	7.39
MAI2	B	3	3.02
MAI3	A	3	4.86
MAI4	A	2	4.23
MAI5	C	0	0.33
MAI7	B	1	1.85
MAK11	A	1	1.27
MAK37	C	2	1.90
MAK38	C	0	2.78
MAK39	B	3	8.16
MAK40	A	2	0.84
MAK42	B	1	4.52
MAK43	C	1	1.04
MAK44	C	1	2.10
MAK45	B	0	0.38
MAK46	C	0	2.88
MAK47	B	1	2.55
NYAM10	C	0	0.73
NYAM11	C	1	3.47
NYAM12	B	1	0.48
NYAM13	C	1	0.75
NYAM14	B	0	3.30
NYAM15	B	0	0.35
NYAM16	B	0	1.08
NYAM17	B	1	0.93
NYAM18	B	1	2.33
NYAM19	A	1	1.45
NYAM20	B	1	0.46
NYAM21	C	1	2.43
NYAM22	B	1	0.98
NYAM8	A	1	3.18
SED10	C	2	4.85
SED11	A	2	2.95
SED12	B	1	2.80
SED13	C	0	1.10
SED14	A	1	1.47

Entabeni, February - April 2011

ID	Age class	N faecal samples
B1	B	6
B2	B	2
B3	B	3
B4	C	4
B5	B	5
B6	B	4
B7	B	3
B8	C	5
B9	B	1
B10	C	1
B11	C	5
B12	C	5
B13	C	4
B14	C	3

Entabeni, September 2011

ID	Age class	N faecal samples
B1	B	5
B2	B	2
B3	B	2
B4	C	7
B5	B	2
B6	B	5
B7	B	5
B8	C	4
B9	B	0
B10	C	5
B11	C	4
B12	C	2
B13	C	3
B14	C	3

Appendix D: Validation of drying procedures

Epiandrosterone (EA) concentrations ($\mu\text{g/g DW}$) measured in faecal sample subsets dried by different procedures. Deviations in per cent of the respective higher value.

Animal ID	EA conc. ($\mu\text{g/g DW}$)		Deviation (%)
	<i>oven-dried</i>	<i>lyophilised</i>	
B3	17,08	18,19	6,10
B12	7,51	7,12	5,19
B5	4,22	7,49	43,66
B11	3,45	3,2	7,25
B13	4,28	4,45	3,82
B1	5,64	6,04	6,62
B7	5,79	5,59	3,45
B12	3,47	3,8	8,68
B6	5,56	5,72	2,80
B1	7,88	7,17	9,01