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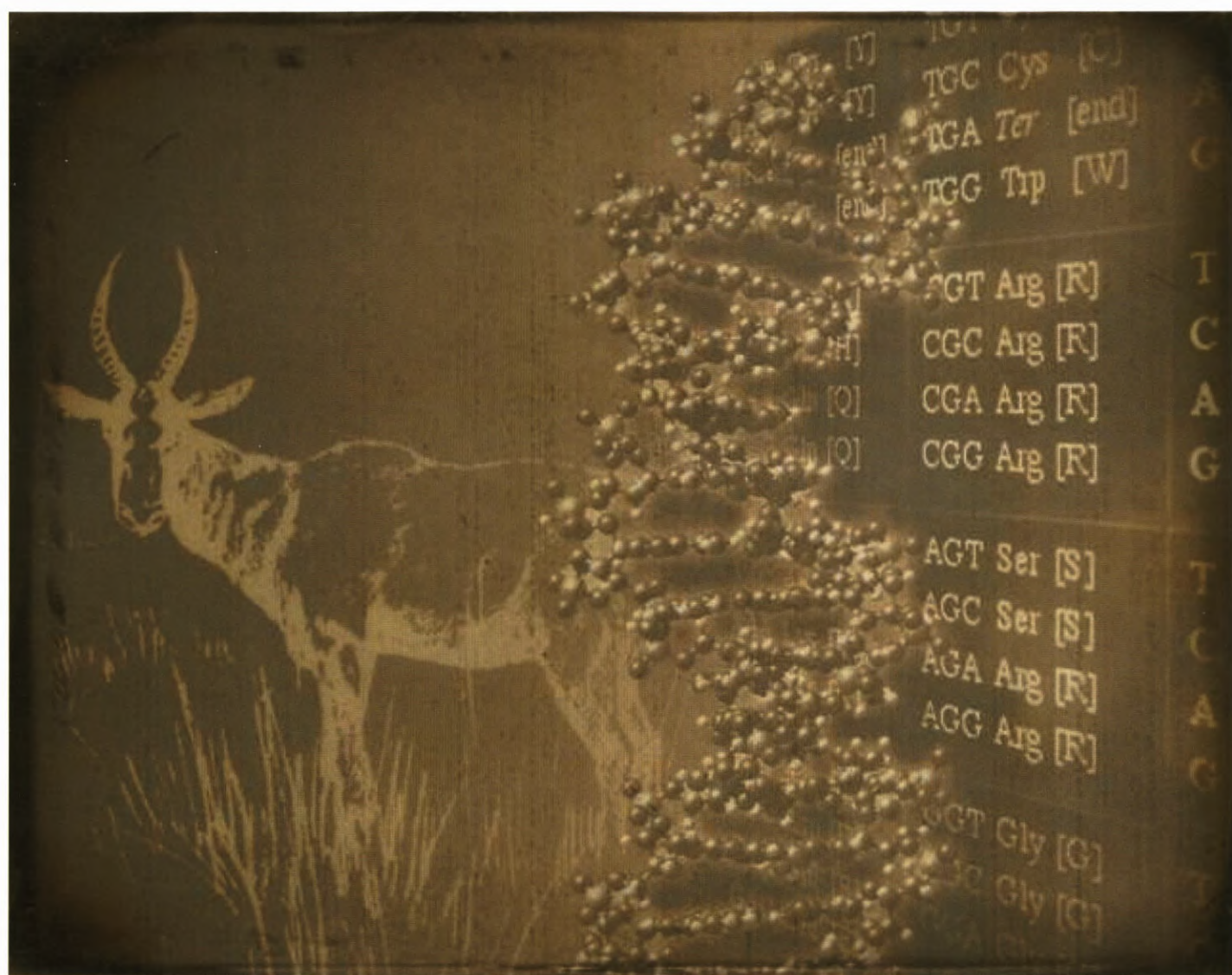
**USING ACTIVITY PATTERNS AND STR MARKERS TO  
DISTINGUISH BETWEEN BLESBOK (*Damaliscus  
pygargus phillipsi*) AND BONTEBOK (*Damaliscus  
pygargus pygargus*) IN THE FREE STATE**

BY  
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BLOEMFONTEIN 2011





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Chapter 1:  
Introduction





# 1 INTRODUCTION



## 1.1 WILDLIFE INDUSTRY

Legal authority granted to landowners by the South African government, allowing them to manage wildlife on their private rural land, has brought about a well-developed wildlife ranching industry. The term “wildlife ranching” refers to the management system where game animals kept on private properties either wander freely or are enclosed in large grazing camps and serve to provide the owner with a source of earnings (Benson, 1991).

This division is conjoined with other components of the economy, from one point of view with the agricultural sector and the other with tourism and conservation. The profitability of wildlife ranching depends on the income generated by several facets. These include ecotourism, sale of live game at auctions, harvesting (commercial hunting and venison trade) as well as trophy and sport hunting and each of these income sources have different financial implications.

Hunting is the most absolute recreational activity of wildlife ranching in South Africa, therefore hunting and hunting-related services will be accentuated. This sector has expanded considerably over the past few decades and it has been estimated that South Africa has the largest hunting industry in sub-Saharan Africa, boasting a multitude of game species including more than 70 antelope species, available for hunting (East, 1999; Damm, 2005; Lindsey *et al.*, 2006). According to Patterson & Khosa (2005), an estimated 7 000 hunters visited the country during the reporting period in 2003/04 and an added 200 000 resident hunters that pursue recreational hunting should also be taken into account. Hence, Damm (2005) estimated the total revenue directly generated by hunting for South Africa to be more than R4.5 billion. Of the total gross revenue generated, foreign trophy hunters contributed

approximately 18% of this income against the 53% contributed by resident hunters, the balance being made up by taxidermy, venison sales, trade of live game (private or at auctions) and game farming (Patterson & Khosa, 2005). Therefore, interest in wildlife ranching, partially as a result of the basal demand for both game-viewing tourism and hunting, has resulted in an extensive shift in private landowners moving away from conventional farming towards managing wildlife and recreation as a business on their lands. Other contributing factors include surplus animals from reserves that became available to private owners, relatively recent decreases in the productivity of cattle farming, increased incidents of stock being stolen as well as the re-acceptance of South Africa into the world community (Cousins *et al.*, 2008).

In South Africa there are an estimated 9 000 farms that are used for wildlife production, covering roughly 20.5 million hectares (16.8% of the total land) with a further 15 000 farms that are involved in both mixed stock and wildlife production (Patterson & Khosa, 2005; National Agricultural Marketing Council, 2006). More or less 300 of these game farms are found in the Free State Province and following Van Ee (1962), the majority of ungulates kept on these game farms and reserves are non-indigenous species. It should however be noted that numerous parties associated with this industry have been much acclaimed for their contributions towards national conservation of wildlife. Hunters, game farmers and game capturers have all played a central role in saving many species by restocking populations in areas where natural populations have suffered local extinctions (Flack, 2002). Blesbok and bontebok are two of the popular game varieties kept on various Free State wildlife farms and are the focus of this study.

## 1.2 TRANSLOCATION OF WILDLIFE IN SOUTH AFRICA

Following Matson *et al.*, (2003) translocation is viewed as the intentional release of animals in an effort to establish, re-establish or augment a population. In South Africa, translocations of game animals between ranches and regions were taking place at an accelerated pace over several years. Relocation of animals within their range, or to areas of their known historical range, can be viewed as a practical approach to conserve biodiversity and has been a key factor in ensuring the continuing survival of many species. Given that numerous small populations are reproductively isolated within

fenced areas, translocation practices at a local scale, allows for gene flow between different populations which helps to alleviate risks of inbreeding depression and subsequent reduction of genetic variation which may be manifested in reduced growth rates, lower fecundity and increased juvenile mortality. The success of relocations, however, is fairly unpredictable. According to Bothma *et al.* (2002), the present and historical distribution ranges should be considered when identifying suitable species for reintroduction. Bothma *et al.* (2002) stressed that this should be applied in association with sufficient knowledge of the ecological and wildlife ranching regions. Furthermore, thorough understanding of habitat requirements, feeding behaviour, water use and population structure is vital when making the final choice of whether or not to manage a specific species on a ranch (Bothma *et al.*, 2002).

Certain translocations can ultimately have unexpected and disastrous consequences as has been the case with several species throughout southern Africa. Existing dangers include destruction or degradation of habitat, competition, spread of foreign pathogens and parasites as well as aberrant defects. For instance, gemsbok (*Oryx gazelle*) have been reintroduced in conservation areas in the succulent Karoo. Even though they have been translocated to areas that are at the outskirts of their former range, they have never occurred there in such high numbers. Concerns have been raised on what impact their feeding habits (digging out succulents during dry periods) could have on the susceptible renosterveld habitat (Hamman *et al.*, 2005). Giraffe (*Giraffa camelopardalis*) have been translocated to unsuitable areas in the Eastern Cape. Given that their ecological requirements were not thoroughly researched prior to the translocation, their feeding habits have severely altered the habitat (Parker, 2004). Another example involves the nyala (*Tragelaphus angasi*) and bushbuck (*Tragelaphus scriptus*). Where nyala have been introduced into the Western Cape Province, they are out-competing and displacing the indigenous bushbuck (Department of Environmental Affairs and Tourism, 2005). Springbok (*Antidorcas marsupialis*) were resettled outside their natural distribution areas at the southern parts of the West coast and often contract foot-rot disease as they are not adapted to the humid winter conditions (Hamman *et al.*, 2003). The endangered Cape mountain zebra (*Equus zebra zebra*) in the West coast dune lands exhibit abnormal hoof growth, because the sandy substratum is inadequate to wear down their fast-growing hooves (Hamman *et al.*, 2005).



Apart from unanticipated outcomes of translocations, careless introductions of closely allied taxa into the natural distribution ranges of one another have brought about various afflictions affiliated with the hunting industry. Many landowners do follow good management practices with their land and wildlife populations, but for some, investment in game conservation is not the foremost concern and their focus is rather on generating maximum profits (Patterson & Khosa, 2005). These landowners neglect to take into account ecological and social requirements of wildlife populations. Numerous wildlife species are fenced off within the same area seeing that preferences of local hunters, foreign hunters and game-viewing tourists are a major driving factor (Cousins *et al.*, 2008). The division of large areas into smaller enclosures and overstocking these enclosures has led to several managerial complications.

### 1.3 NICHE MARKETS

Given the rapid growth of the industry, competition amongst wildlife farmers has, moreover, led to the advance of certain niche markets. Attention should be directed to these practices that have been created within the game ranching industry as they ultimately confute biodiversity conservation principles and the economic feasibility of this industry.

There is a large and growing market to supply highly profitable animals to overseas trophy hunters. As a means to increase the diversity of available trophies, a variety of exotic species such as common reindeer (*Rangifer tarandus*), Himalayan tahr (*Hemitragus jemlahicus*) and water buffalo (*Bubalus bubalis*) have been introduced. Some game farmers also practise selective breeding of animals specifically for trophy hunting. Demand for colour variants amongst such farmers and trophy hunters have led to the emergence of yet another niche market. Artificial selection for recessive colour phenotypes has led to an increasing number of colour variants being sold at game auctions or offered as trophy animals (Hamman *et al.*, 2003). Two colour variants of blesbok, white and yellow, have been selectively bred for such purposes. In view of live sale price differences, common blesbok attained average auction sale prices of R1 067 and R1 328 during 2008 and 2009, respectively (Cloete & Taljaard, 2009). White blesbok were sold on average for R1 558 (2008) - R1 785 (2009), while yellow blesbok proved to be the most popular and average sale prices varied between R4 100

(2008) and R3 418 (2009) (Cloete & Taljaard, 2009). According to Damm (2005), none of the abovementioned practices contribute in any way towards South Africa's international and national obligations on biodiversity conservation; as a matter of fact they transgress the South African Biodiversity Act. These practices could result in significant lessening of genetic variation and diversity of small populations and could undermine the sustainability of these populations.

Another distressing development involves the deliberate cross-breeding of subspecies or species which is one of the greatest concerns to conservation authorities (Hamman *et al.*, 2005). From a biological perspective, hybridisation may cause the loss of not only genetic, but also behavioural, morphological and ecological characteristics that have evolved in local populations over time (Latch *et al.*, 2006). This practise has become financially viable since hybrids are often bigger in size and are then sold as prized trophy animals to hunters. Deliberate interbreeding between two of the three acknowledged springbok subspecies is a familiar example. Some breeders purchase larger springbok rams at game auctions in the northern provinces as to breed bigger trophy animals from the smaller southern subspecies (Hamman *et al.*, 2003).

Reports of interspecific hybridisation have mainly been limited to animals maintained under captive conditions where males of the one species and females of the other species are forced together. Some published as well as unverified reports of hybrids resulting from various cross-breeding cases have been made. Lichtenstein's hartebeest (*Alcelaphus lichtensteini*) is not recognised as naturally occurring in South Africa and possibly hybridises with the endemic red hartebeest (*A. buselaphus*). Impala (*Aepyceros melampus melampus*) introductions have also led to hybridisation with black-faced impala (*A. m. petersi*). Black wildebeest (*Connochaetes gnou*) and blue wildebeest (*C. taurinus*) are not normally found sympatrically due to their different habitat preferences. Even though a great deal of the re-introductions of black wildebeest have been to areas which formerly fell within their natural distribution range, hybridisation with blue wildebeest has been identified as a main conservation threat and has led to several populations that had to be destroyed (Fabricius *et al.*, 1988). A further case involves blesbok and red hartebeest (Robinson *et al.*, 1991). Under natural conditions, blesbok and red hartebeest are disinclined to cross-breed due to behavioural differences, though hybridisation between the respective species has been documented

when kept under artificial conditions or in cases where the owner failed to ensure sustained existence of both sexes in each population.

Of greatest concern is when these cross-breeding incidents yield fertile offspring as has been the case for the two wildebeest species as well as blesbok/bontebok hybrids. Hybridisation between blesbok and bontebok populations in the Free State threatens the sustainability and conservation of both subspecies and thus warrants imperative action.

#### 1.4 EVOLUTIONARY HISTORY & NATURAL DISTRIBUTIONS OF *D. pygargus*

Essop *et al.* (1991) suggested that the distance in time divergence from a shared ancestor is a key factor in establishing a management policy to address hybridisation issues. Blesbok and bontebok fall under the genus *Damaliscus* of the family Bovidae (Mammalia, Artiodactyla, Ruminantia), subfamily Alcelaphinae. Following the Miocene origin (23 million years ago) of Bovidae, their evolutionary history was shaped by factors such as global immigrations, adaptive radiations and mass extinctions producing the existing diversity of 49 genera consisting of more than 140 species (Kingdon, 1989; Matthee & Davis, 2001). Around 5 - 6 million years ago the African Alcelaphinae underwent rapid radiation, while the genus *Damaliscus* evolved relatively recently (less than 1 million years ago) (Vrba, 1979; Capellini, 2006). The genus subsequently diverged into two species, *Damaliscus lunatus* (tsessebe) and *Damaliscus pygargus* (Capellini, 2006). At present, the bontebok is classified as *D. p. pygargus* (Pallas, 1767) and the blesbok as *D. p. phillipsi* (Harper, 1939). The previous classification as *D. dorcas* became redundant when the type locality was amended, restricting this locality to the Swart River (Grubb, 1993).

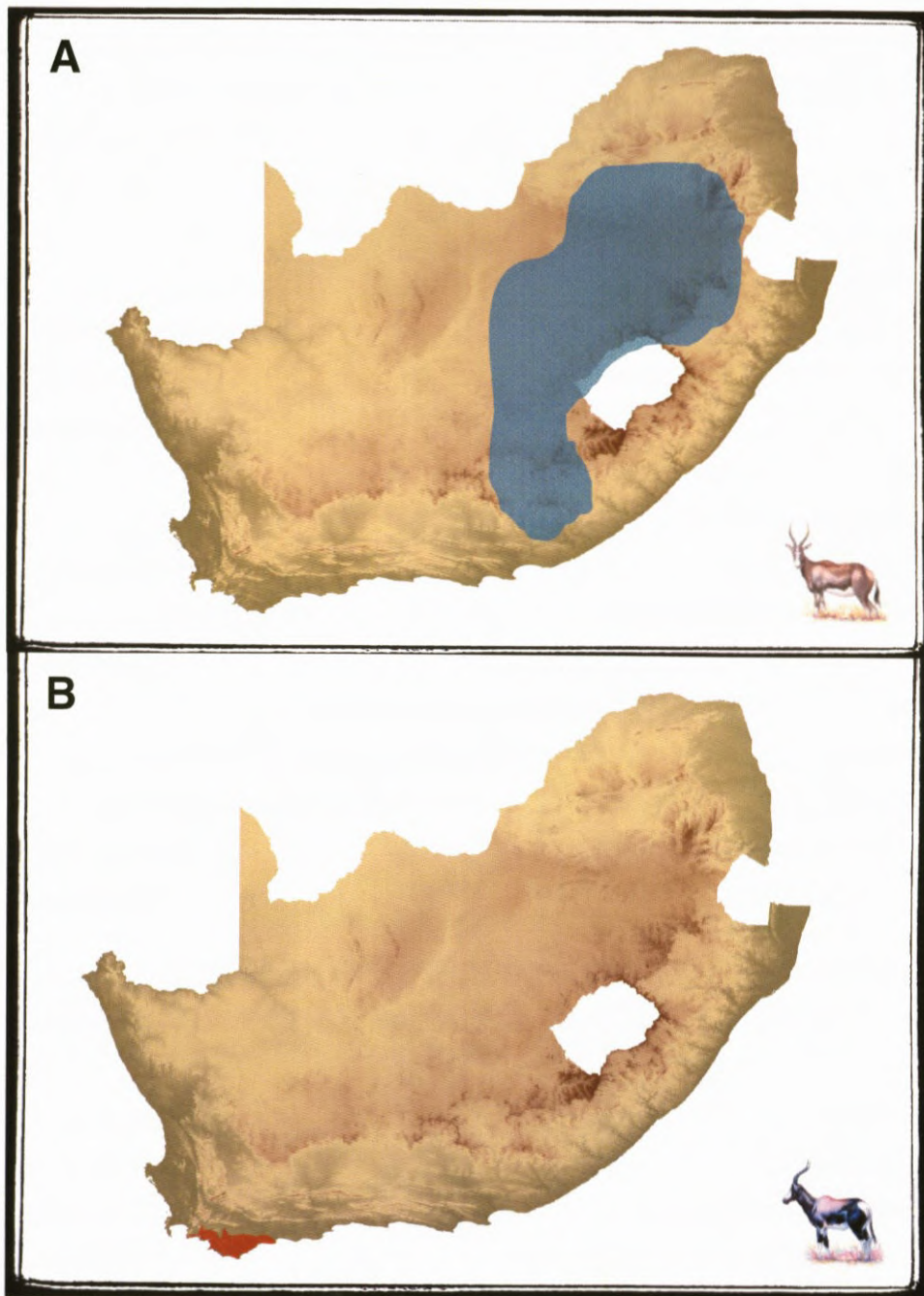
It has been a matter of controversy whether the blesbok justifies a separate specific or subspecific status relative to bontebok. Confusion arose due to disagreements about the relationship between the two as well as historical inconsistencies (Rookmaker, 1989). They were once considered separate species, but presently they are regarded as conspecifics.

Vrba (1979) suggested that the ancestral lineage of *D. pygargus* must have had an extensive and continual distribution in the subcontinent, covering an area from the south-western Cape to the

southern border of Zimbabwe. A sudden unfavourable climate change coupled with habitat changes, split the species into separate interior and coastal populations that evolved as unique, geographically isolated subspecies (Skinner & Chimimba, 2005). According to Lloyd (2001), the separation probably occurred after the last glacial period, towards the end of the Late Pleistocene, when the sea-level rose and the presently submerged continental shelf were no longer accessible as a grazing plain to their common ancestor. This caused the coastal plains habitat to be diminished to the constricted strip that is present today and during this time Fynbos presumably replaced grassland habitat in the southern Cape region (Vilakazi, 2009). Allopatric speciation was set in motion when a barrier was formed after the last glacial period and by the time of the early settlement it was noted that the two subspecies were separated by a 320 km stretch of arid Karoo veld, isolating the interior population from the coastal population (Skinner & Chimimba, 2005).

Given that the subspecies divergence is estimated to have taken place around 250 000 years ago, they are able to hybridise as reproductive isolating mechanisms that would prohibit gene flow has not yet evolved. However, this segregation allowed minor yet significant phenotypical differences such as pelage pattern and colour to arise in each group. There are also noticeable differences in terms of behaviour and social structure that support the present reclassification as subspecies (Bigalke, 1955). To determine whether behavioural differences will prohibit cross-breeding in areas where blesbok and bontebok occur sympatrically, it is imperative to investigate behavioural aspects of bontebok kept in the Free State, thus allowing comparison with known blesbok literature (Van Aswegen, 1994).

The present conservation status and distribution of *D. pygargus* is more apprehensible through the lens of history. In the days of the early settlers, antelopes were present in vast hordes, but historical records signified a shocking decline in both blesbok and bontebok numbers over the years following Dutch settlement (Ward, 1899; Fitzsimmons, 1920; Ellerman *et al.*, 1953; Skead, 1980). Blesbok are one of the four grazing herbivores that historically inhabited the open plateau of the South African Highveld (Estes, 1992) with populations that numbered hundreds of thousands. The Limpopo River formed the northern border of their historical range (Fitzsimmons, 1920), while south of this border they were present in highveld regions of the northern Karoo in the Eastern and Northern Cape, Gauteng and Mpumalanga (former Transvaal), the Free State (former Orange Free State) as well as certain areas of western and north-western Kwazulu-Natal (Potgieter, 1971;



**Figure 1.1** Native distributions of *Damaliscus pygargus* in South Africa.  
**A**, natural distribution of blesbok; **B**, natural distribution of bontebok.

Lloyd & David, 2008) (Figure 1.1a). Beyond the borders of South Africa, their historical range also included western Lesotho, but these populations went extinct before 1900 (Lynch, 1994). Blesbok were also abundant in the western highveld regions of Swaziland, but suffered the same fate as the Lesotho population due to overexploitation (East, 1999).

Hunting pressure extirpated the subspecies from much of its original range and their numbers were reduced to roughly 2 000 individuals by the late nineteenth century (Bryden, 1893). The relentless slaughter of populations persisted until 1899 when the Boer War ended. Thereafter, the greatest parts of the grassland areas were taken over by settlers forcing the surviving populations to survive only on fenced farms (Skinner & Chimimba, 2005). Nevertheless, blesbok developed within a larger diversity of habitats compared to the isolated bontebok that was restricted to a mosaic of fragmented habitats that totaled more or less 250 000 ha (2 500 km<sup>2</sup>) in size (Furstenburg, 2006). Consequently, blesbok developed the advantage of being better adaptable to changing environments (Furstenburg, 2006).

According to Furstenburg (2006), bontebok are considered the more specialised of the two subspecies. Bontebok are endemic to the coastal plain of the Western Cape Province (Fig. 1.1b) and have become adapted to endure the conditions of a restricted habitat. Bontebok were described around 100 years prior to blesbok seeing that blesbok were only discovered after expeditions extended to inland regions. It is likely that some of the early European settlers may have confused the two and incorrectly documented blesbok as bontebok. Burchell (1823), for example, referred to the location of a campsite on the map of his expedition throughout southern Africa as 'Bontebok Station'. This campsite was, however, beyond the range of bontebok and situated within the precedent range of blesbok. Furthermore, various geographical names in the Eastern Cape, such as Bontebokvlakte near Cathcart, might also actually refer to sightings of blesbok herds (Furstenburg, 2006). Misleading records consequently led to the assumption of a broader distribution range for bontebok than what was in reality the case.

Historically, their distribution was limited to a small area between the Bot River and the Heidelberg/Riversdale region and inland to the Sonderend and Langeberg mountain ranges (Bigalke, 1955; Skead, 1980; Rookmaker, 1989). As a true grazer it mostly utilised the lowland fynbos



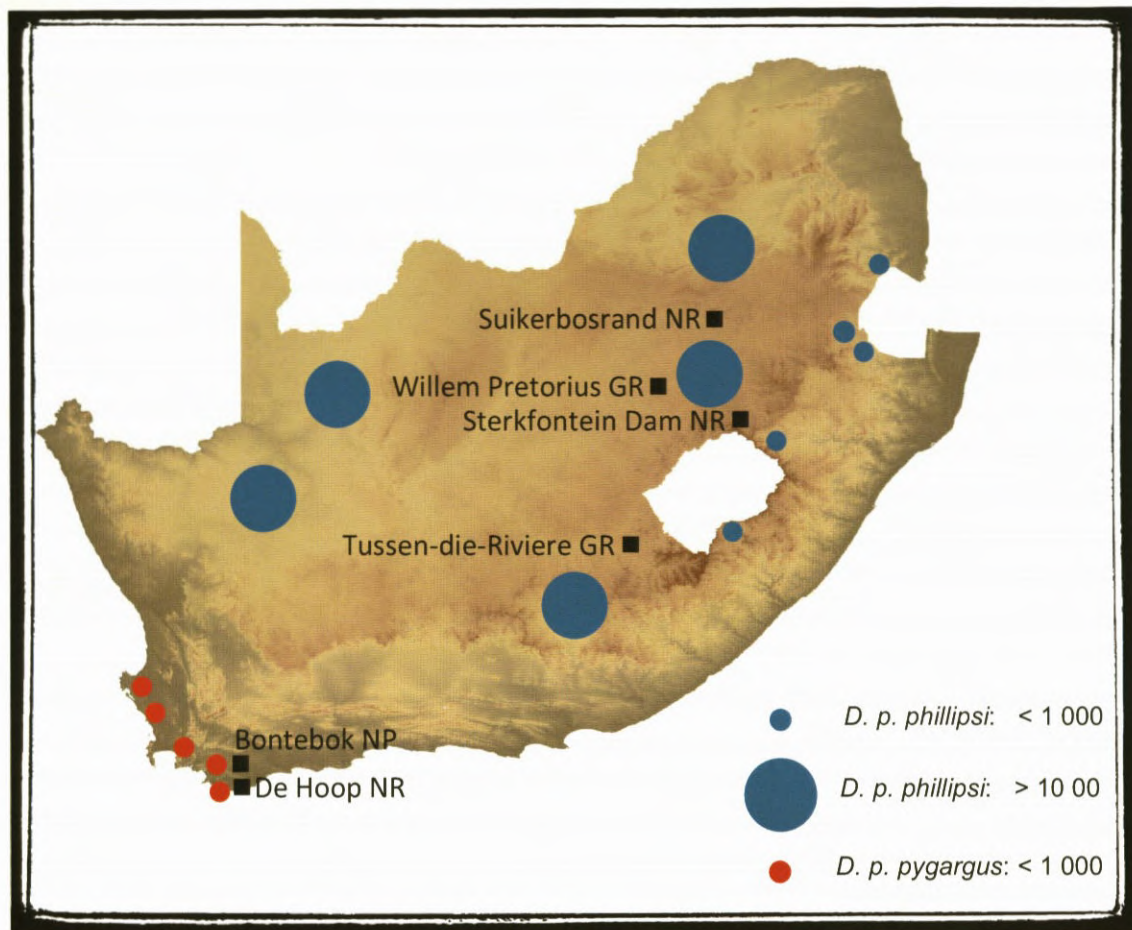
areas richest in short grass, namely the so-called Renosterveld (Lloyd, 2001). By the mid-nineteenth century, after extensive hunting by settlers and habitat loss, this once locally abundant antelope were on the verge of disappearance (Fitzsimmons, 1920). From all remaining larger mammal species of southern Africa, the bontebok was the closest to becoming extinct (Furstenburg, 2006). Potgieter (1971) gives the following summary of records indicating the drastic decline: "In 1689, Isaq Schriver recorded seeing "more than one thousand 'bonte harteboeken' running". Less than a century later, during 1777, William Paterson stated that the country between Botrivier and Caledon "abounds with game, in particular bontebok". A mere 21 years thereafter (1798), Barrow recorded that the previously numerous bontebok were "rarely seen in troops exceeding a dozen" in the Swellendam area. Further estimates of declines from a variety of sources have been documented by Van Rensburg ranging from a total of 300 in 1898 until only 12 individuals of these stately antelope were recorded in 1912." Fifteen years later during 1927, the number of bontebok surviving on farms in the Bredasdorp district and Swellendam area were recorded to be a total of 120 individuals (Bigalke, 1955). Thus, within a very short period of time the number of surviving bontebok experienced a further decline of more than 50%. Fortunately, several farmers within the Bredasdorp vicinity took it upon themselves to conserve the remaining bontebok by setting aside part of their farms as a reserve. Were it not for their efforts, this subspecies would most likely have suffered the same fate as the now extinct blue antelope (*Hippotragus leucophaeus*). Conservation efforts of the Myburgh, Van Breda and Van der Byl families helped to protect a relic population of 27 animals on their farmlands where they struggled to survive, but nevertheless this added to their slow recovery (Van der Walt *et al.*, 2001).

Two conservation approaches were used to protect them from further hunting pressure. During the early 1900's the government prohibited hunting and a fine of R75 was payable by any person that did not abide by this law. The second preventative measure involved the proclamation of the first Bontebok National Park in 1931. This conservation area was situated near Bredasdorp and was stocked with less than 20 individuals (Apps, 2000). Here their numbers steadily recovered, but the park was unsuccessful due the small size (722 ha) of the conservation area as well as inadequate grazing conditions. Furthermore, half of the herd died from various worm infestations as well as copper deficiency and related syndromes (Van der Walt *et al.*, 2001). Thus, in 1961, a second

Bontebok National Park was proclaimed in the vicinity of Swellendam and 84 bontebok were translocated to this area (Skinner & Chimimba, 2005). Translocation to this park proved to be more successful and the population have subsequently thrived there. Twenty years following translocation to the new park it was estimated that their numbers had increased to a viable population of 320 individuals and their numbers have been maintained at more or less 250 individuals.

### 1.5 PRESENT DISTRIBUTION RANGES OF *D. pygargus*

As is the case with various antelope species, there are at present no wild populations of either blesbok or bontebok. Even though blesbok have been reduced to a small fraction of their original numbers, they have remarkably recovered and recent assessments put their numbers between 235 000 - 240 000 (stable or increasing) (Sidney, 1965; Hey 1966; East, 1999). Their recovery has been achieved by the protection given to them by many reserves in the Free State and by the demand for venison which has encouraged farmers to introduce and preserve them on their farms. They are viewed as one of the most popular and commercially important game species in South Africa, facilitating reintroduction into large areas of their former range. Nearly all (97%) blesbok are kept on private land with the largest populations present in the Free State, Gauteng and Northern Cape provinces (East, 1999). The remaining 3% are found in more than 30 provincial reserves and three national parks, with the largest populations at Tussen-die-Riviere Game Reserve, Willem Pretorius Game Reserve and Sterkfontein Dam Nature Reserves in the Free State as well as Suikerbosrand Nature Reserve in Gauteng (East, 1999) (Fig. 1.2). They have also been introduced to areas beyond the borders of South Africa such as Botswana, Namibia, Swaziland and Zimbabwe. Where translocations have been successful, their natural population growth is fairly high. As many as 100% of adult ewes can give birth in one season, translating roughly to a 40% increase per annum (Bothma *et al.*, 2002). Hence, herds are thinned out on a seasonal basis and the carcasses generate a feasible income. According to Lynch (1983), blesbok are the most abundant game species in the Free State distributed throughout the province, with the largest concentrations occurring in the northern, north-eastern and south-western parts. Results obtained from a survey performed by Terblanche & Kok (1995) estimated blesbok occurring in the Free State to be in the range of 70 000 individuals.



**Figure 1.2** Estimated relative abundance of *D. pygargus* populations in South Africa (adapted from East, 1999) and locations of relevant National Parks (NP), Nature Reserves (NR) and Game Reserves (GR) with the largest populations.

The successful increase in bontebok numbers from Bontebok National Park meant that surplus animals could be removed and relocated to form reintroduced populations in other reserves and protected areas. According to Lloyd & David (2008), extralimital populations have been successfully translocated to the West Coast National Park as well as two local reserves. Relocation of surplus animals is imperative for the survival of any species, since single populations within a restricted area are more vulnerable to catastrophic events. In addition, introduced populations have been established in the Eastern Cape and Free State provinces (East, 1999). Recent estimates put their numbers in South Africa at roughly 2 300 - 3 500 animals (increasing), half of which occur on private land (East, 1999; Skinner & Chimimba, 2005; David & Lloyd, in press in: Lloyd & David, 2008). These translocations to privately-owned game farms were motivated by both conservation and commercial interests. Many of these populations have been established for sustainable use by trophy hunters and bontebok are continued to be sold at game auctions as they have a relatively high financial value. The record auction price for a bontebok during 2009 was R15 500, while the highest price paid for a blesbok was R3 100 (Cloete & Taljaard, 2009). A survey conducted in 2001 revealed that of the national estimated population, only about 1 500 bontebok actually occur within their historical distribution range with the largest populations (700) at De Hoop Nature Reserve (Fig. 1.2) and adjacent Overberg Test Range (David & Lloyd, in press in: Lloyd & David, 2008).

## 1.6 THE HYBRIDISATION THREAT

Lloyd & David (2008) stated that as a species, the continuing survival of *D. pygargus* is under no threat, but at a subordinate taxonomic level interbreeding between these phylogenetically closely related taxa is viewed as a great risk affecting both blesbok and bontebok conservation. At the outset, translocations of surplus bontebok aided in saving them from extinction, but prior introductions into the Free State were carried out without taking ecological requirements of bontebok and the genetic integrity of *D. pygargus* into consideration.

Bontebok/blesbok hybrids are not a novel anomaly, having been observed as early as 1920 when Fitzsimmons affirmed that *D. pygargus* interbreed freely and produce fertile progeny. By 1955, Bigalke reported that hybrids have been widely dispersed throughout southern Africa. Following the

introduction of bontebok during the 1960's, there has been extensive hybridisation among these closely related native and non-native subspecies, consequently having produced numerous hybrids on private land in the Free State. Anthropogenically-driven hybridisation is directly working against the natural processes of evolution and speciation.

The problem at hand is threefold. The species *D. pygargus* is categorised as least concern according to the IUCN Red List of Threatened Species, however categorisation below the species level assigns each subspecies to a separate category. Bontebok are viewed as the rarest antelope in South Africa and their numbers are still relatively low, hence the status of bontebok are presently listed as near threatened (Lloyd & David, 2008). This implies that there is an eventuality to move into the vulnerable or even endangered category if the serious threats are not adequately addressed. Given catastrophic historical population depletion, their ultimate security is not yet assured. Human-mediated hybridisation could devastate their genetic purity and eventually lead to their disappearance, which would result in a loss of endemic biodiversity.

Secondly, the hybridisation problem also translates into severe economic implications affecting both the wildlife ranching and hunting industries. Hybrids are possibly sold as pure bontebok at game auctions to unknowing buyers. This is of concern when considering the major price variation - bontebok are sold for approximately R5 500 and blesbok for R1 328 according to the game auction prices for 2009 (Cloete & Taljaard, 2009). These hybrids are then moved into areas where pure-bred populations of either parental subspecies are kept. In addition, international organisations responsible for the registration of hunting trophies, for example Safari Club International (SCI) and Rowland Ward, have recently claimed that many of the registered bontebok trophies are perhaps hybrid animals. Blesbok are sometimes cross-bred with a bontebok ram and offered to trophy hunters as pure bontebok. Yet again the great price difference is very concerning. According to cost estimates for 2006, a trophy hunter pays R5 000 - R7 000 to hunt bontebok and in the region of R1 500 to hunt blesbok.

Thirdly, hybridisation also affects the conservation of pure blesbok. They are listed as lower risk (conservation dependant) according to the IUCN categories (East, 1999) and it is feared that they

could vanish entirely from the Free State in the near future. The focus has always been on bontebok due to their vulnerable status, but in the Free State the focus has shifted to the endemic blesbok seeing that pure blesbok populations are favoured in terms of conservation priority. Despite their higher numbers and greater distribution, the genetic integrity of Free State populations is also at risk. At present, it is uncertain to what extent pure blesbok populations have been genetically contaminated and conservation authorities believe that if hybridisation were allowed to continue they would certainly go extinct. This would not only also result in the loss of endemic biodiversity, but would also have a tremendous impact on the local hunting industry.

The question arises as to what could be possible solutions to the present problem. In order to remedy this situation fairly stringent controls over the translocation of these animals are of utmost importance. Past policies did not provide any regulations on the housing of the subspecies' on the same property. However, it is evident that conservation authorities are taking this threat very seriously and are attempting to implement strict regulations. Movement of bontebok into or outside of the province have already been prohibited and translocations within the Free State are only permitted for legal existing populations. The hybridisation risk is, however, continued to be driven by existing practices that disregard these translocation restrictions and persist in moving bontebok beyond its historical range.

## **1.7 REGULATIONS**

In view of the National Environmental Management: Biodiversity Act (Act No. 10 of 2004) the possible classification of bontebok as "alien" is of significance to conservation managers given that the brief of these managers is often interpreted as being the conservation of indigenous biodiversity. This Act defines an alien species as "a species that is not indigenous; or an indigenous species translocated or intended to be translocated to a place outside its natural distribution range in nature, but not an indigenous species that has extended its natural distribution range by natural means of migration or dispersal without human intervention". If one considers bontebok as an "alien" species in the Free State a person may not import, possess, breed, translocate or trade bontebok without applying for the compulsory permits. Managers are thus faced with difficult and controversial



decisions such as the unviable choice of either removing bontebok from game farms and private land, or accepting that this non-indigenous subspecies, with significant impact on the endemic blesbok, are to remain in the province. If the legalisation that all bontebok were to be removed were to come into effect, it is certain that tensions would arise. This would necessitate removal of around 400 existing legal animals kept on 20 different game farms as well as the removal of illegal populations within the natural range of the blesbok. Some of these bontebok stocks were legally established by means of permits prior to the establishment of the "Translocation Policy", whereas others have been brought in without the co-operation of Nature Conservation authorities.

Recent regulations have been stipulated to assist with the coordination of uniform legislation on a provincial level and to direct the wildlife ranching industry in a more conservationist direction (Cousins *et al.*, 2010). The new Threatened or Protected Species (TOPS) regulations aim to provide a countrywide managerial standard for listed threatened or protected species. Various species that are of significant conservation or national importance, such as bontebok, will have standardised conservation standings across South Africa (Cousins *et al.*, 2010). The Biodiversity Act as well as the TOPS regulations in terms of the Biodiversity Act provides very important principles, but they will be challenging to put into practice. Nevertheless, successful implementation of these principles can be achieved if conservationist and all facets of the wildlife ranching industry work in partnership to ensure the sustainable use of wildlife.

The key challenge to overcome in order to guarantee the long-term survival of blesbok and bontebok is to prevent further hybridisation, for this reason all parties affiliated with the game ranching industry have important roles to play. The new TOPS regulations should lower the hybridisation threat as these regulations stipulate that bontebok (TOPS species) cannot be relocated to a farm where blesbok are also being kept. A further incentive involves income prospects. Despite the much higher market prices fetched by bontebok, blesbok hunting offers greater revenue opportunities. Figures obtained from Free State Nature Conservation indicated that over a period of six years (2000 - 2006) only 96 bontebok were hunted in the province compared to 3 008 blesbok. According to these figures, R403 2000 added profit was obtained from blesbok hunting and this must be seen as encouragement to farmers to rather keep populations of the endemic subspecies. Besides,

in the opinion of Cornus (2005), blesbok are also a far more challenging game animal to hunt. He also stated that from a sportsman's point of view, bontebok are collected rather than hunted as they are less astute.

## 1.8 HYBRID IDENTIFICATION

Apart from strict translocation controls, hybrid blesbok/bontebok stocks have to be identified and eliminated as live game. Unlike first generation black and blue wildebeest hybrids that are easily identifiable (Fabricius *et al.*, 1988), accurate visual identification of blesbok/bontebok hybrids is an extremely difficult task. These hybrid animals express a mosaic of parental phenotypes making it next to impossible to distinguish them merely by sight. To protect hunters, game auctioneers and owners of pure bontebok against hybrid deception, a photographically-based statistical technique was developed by Fabricius *et al.* (1989) that allows distinction of pure herds from genetically contaminated herds on the basis of coat pattern. Nature Conservation authorities are presently applying this method to deal with the hybridisation threat by means of objectively certifying pure bontebok and issuing certificates to owners of pure populations. Although it has proven to be effective, this method is not consistently accurate and in cases where doubtful results are obtained an alternative method based on genetic analysis of these populations could prove to be extremely useful.

## 1.9 GENETIC MARKERS

Fairly recent advances in biotechnology have made available a great number of genetic markers that can be applied to the study of animal populations (Anderson, 2008). Sunnocks (2000) describes genetic markers as simple heritable characters with multiple states at each character. In a diploid organism each individual can have one or two different states (alleles) per character (locus). Genetic markers reflect differences in DNA sequences and have been applied to various studies involving the Alcelaphinae (Allard *et al.*, 1992; Grobler & Van der Bank, 1995; Matthee & Davis, 2001). In relation to *D. pygargus*, research carried out by Osterhoff *et al.* (1972) represented the first effort to research blesbok populations by using genetic markers, but this study was restricted to five blood proteins.

Essop *et al.* (1991) were first to genetically assess both subspecies. This involved comparing restriction maps of mtDNA to determine the genetic distance between blesbok and bontebok. The obtained results were supportive of the present subspecies classification, though the study was conducted from very small sample sizes. Samples were obtained from only 17 blesbok individuals and one bontebok.

Allendorf *et al.* (2001) commented that the use of molecular genetic markers have also aided to abridge the often difficult task of identifying hybridised populations. This procedure was instigated with the development of protein electrophoresis (allozymes) in the mid-1960's (Ayala & Powell, 1972). This technique as well as examination of variation in the nuclear genome by utilising DNA fingerprinting probes have been performed on black and blue wildebeest, but were unsuccessful in revealing a diagnostic test for hybrids (Corbet, 1991). G- and C-banding techniques have also been applied to give an indication of whether stable meiosis was possible in cases of hybridisation between the two wildebeest species. Both species have the same diploid chromosome number ( $2n = 58$ ), indicating that meiosis would be stable and not impair fertility (Corbet, 1991). As a result, distinctions were not possible by using staining techniques.

With reference to the present study, assessment of *D. pygargus* chromosomes by Kumamoto *et al.* (1996) indicated that the chromosomal compliments of  $2n = 38$  in both blesbok and bontebok were consistent with previous studies (Wurster & Benirschke, 1967; Robinson *et al.*, 1991; Gallagher & Womack, 1992; Claro *et al.*, 1995). This method would therefore not be applicable to the identification of blesbok/bontebok hybrids as was the case for the two wildebeest species. Several other studies have also been carried out with the aim of yielding a diagnostic test for blesbok/bontebok hybrid identification. Characterisation of major histocompatibility complex DRB diversity concerning *D. pygargus* was assessed by Van der Walt *et al.* (2001) and these authors suggested that DRB screening could be used to examine the genetic purity of both blesbok and bontebok herds. Control region variation of the mitochondrial genome has also been investigated via single-strand conformation polymorphism (SSCP) as a means to screen for pure or hybrid populations of *D. pygargus* populations (Van der Walt, 2002). Results obtained from control region variation analysis found that only female introgression could be identified using mitochondrial haplotypes that

were unique to blesbok or bontebok (Van der Walt, 2002). This technique would therefore not allow detection of any male introgression since the mtDNA inheritance is characterised as maternal.

Although all of these genetic markers were demonstrated to be useful in the abovementioned studies, there are a number of drawbacks to consider. Despite the fact that allozymes provide a large amount of data and mtDNA provides data to a lesser but still significant extent (Wright & Bentzen, 1994), one needs to bear in mind that they are mainly invariant in various species, particularly in large mammals and a number of endangered species (Hedrick, 1999). Data is of limited comparability among studies and allele frequencies are rarely available (Hedrick, 1999). Paetkau & Strobeck (1998) also put forward that studies using restriction digests of mtDNA or allozymes yielded limited results concerning genetic variation and proved unsuccessful in identifying noteworthy genetic differences between populations.

Consequently, the selection of suitable genetic markers and techniques are vital to the successful application of molecular genetics in population biology. In particular, there is still a vast need for genetic markers showing substantial variation in order to resolve differences between individuals and populations. More recently new DNA-based methods have become available offering greater potential than the above mentioned earlier marker systems. Most importantly, recent progressions in molecular techniques have tremendously increased the number of loci that can be used to identify hybridisation. In the last few years, microsatellites also known as STR (short tandem repeat) or SSR (simple sequence repeat) markers have developed into one of the most popular molecular markers. Microsatellites are nuclear markers that consist of short tandem repeats of nucleotide motifs between two and six base pairs long (Goldstein *et al.*, 1995; Paetkau & Strobeck, 1998). Luikart & England (1999) considers microsatellites to be amongst the most practical and powerful markers due to their numerous useful attributes. Microsatellites have been widely employed in many fields, including phylogeny (Ritz *et al.*, 2000), evolutionary studies (Forbes *et al.*, 1995), population genetics (O'Ryan *et al.*, 1998; Simonsen *et al.*, 1998; Zschokke *et al.*, 2003; Alpers *et al.*, 2004; Harley *et al.*, 2005; Heubinger *et al.*, 2006), parentage and kinship (Queller *et al.*, 1993), conservation and management of biological resources (Garcia-Moreno *et al.*, 1996; Maudet *et al.*, 2004), wildlife forensics (Singh *et al.*, 2004) as well as DNA barcoding for taxonomy (Hebert & Gregory, 2005). Most

importantly, STR markers have proven to be successful in a wide range of hybridisation studies. These include hybridisation between blue and black wildebeest (Grobler *et al.*, 2005), common and black-faced impala (Lorenzen & Siegismund, 2004), wolf-like canids (Gotelli *et al.*, 1994; Roy *et al.*, 1994), domestic cats (*Felis domesticus*) and wildcats (*F. silvestris*) (Beaumont *et al.*, 2001; Pierpaoli *et al.*, 2003) as well as barred (*Strix varia*) and spotted owls (*S. occidentalis*) (Haig *et al.*, 2004; Funk *et al.*, 2006). Given their useful application pertaining to hybridisation studies, microsatellites were thus selected as the genetic marker of choice for this study.

### 1.10 STUDY OBJECTIVES

The present study aims to assess activity budgets of bontebok as these behavioural aspects are not well described by other authors. David (1973) comprehensively studied territorial behaviour of bontebok in the Western Cape Province, making only a few casual observations with regards to diurnal activity patterns. A further objective of this study is therefore to investigate behaviour of bontebok outside of its natural range, in the Free State, by quantifying activity budgets and patterns; assess variations in activity regarding seasonal changes, thus allowing comparison of known literature on diurnal and seasonal variation with blesbok in the Free State. A better understanding of their ecological needs and general behaviour outside of their natural range seems necessary to aid in addressing the hybridisation issue.

A further objective is to determine whether certain bovine, camelides, caprine, equine, ovine and porcine microsatellite primers can successfully amplify in blesbok and bontebok and if so, whether these markers have the potential to be used as a diagnostic marker for blesbok/bontebok hybrids. This could provide an alternative method for the identification of pure blesbok and bontebok populations as well as hybrid stocks, ultimately aiding the conservation of both subspecies.



Chapter 2:  
Study Areas



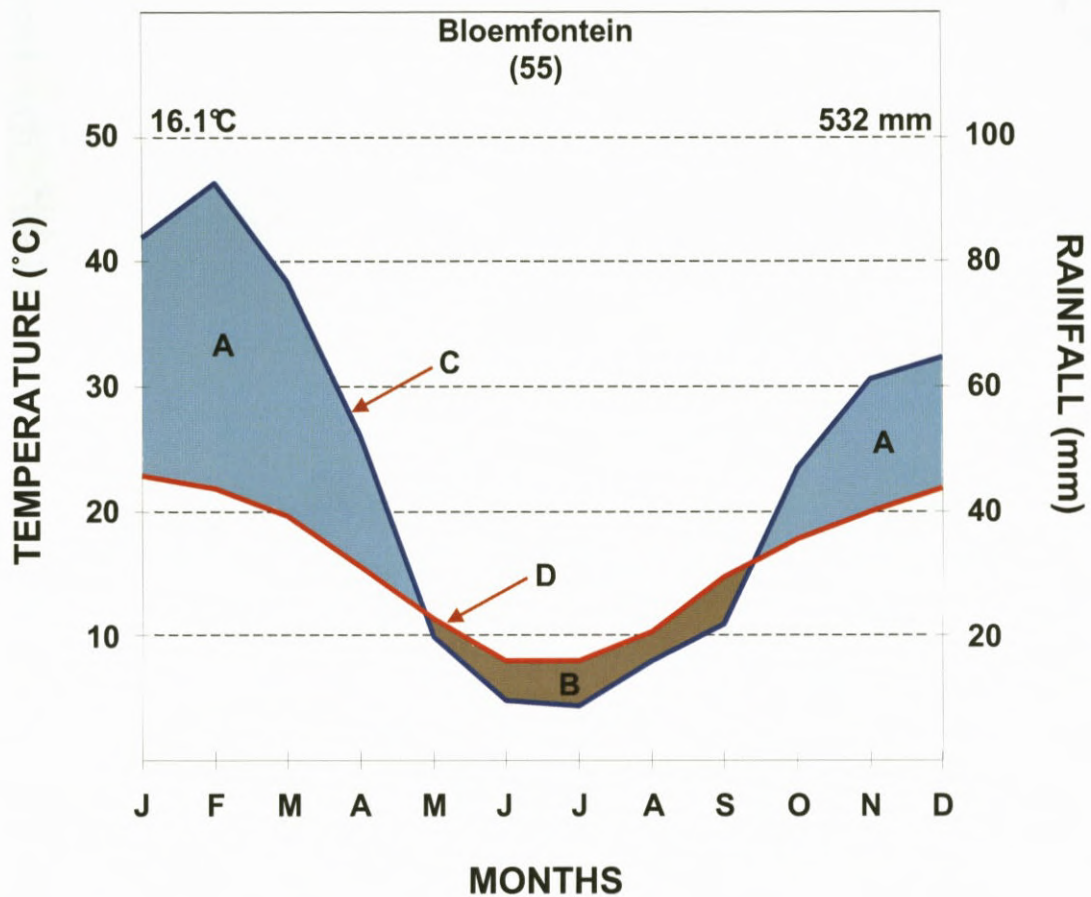
## 2 STUDY AREAS



The study was conducted in the Free State Province ( $26^{\circ}30' - 30^{\circ}45' \text{ S}$ ;  $24^{\circ}15' - 29^{\circ}45' \text{ E}$ ) situated on the central plateau of South Africa. The province borders six of the other provinces, the exceptions being the Northern Province and Western Cape Province. This rural region with farmland, mountains, goldfields and widely dispersed urban settlements lies between the Vaal River in the north and the Orange River in the south. It is considered the third-largest province in the country, encompassing a total surface area of approximately  $129\,464 \text{ km}^2$  (Lynch, 1983). The greatest area of the Free State consists of flat rolling grasslands that cover the central-eastern part of the highveld. The northern region is considered an important agricultural production area, while the southern parts of the province constitute dry and open plains with extensive farming of mainly sheep. The western area is predominantly known for the Free State Goldfields and is also regarded as an important agricultural area, whereas the eastern region that borders Lesotho is hilly to mountainous with scattered hills.

The Free State endures hot, arid conditions that vary rather drastically from season to season. The climate is typical of the interior plateau and this means that almost all precipitation falls in the summer months, with aridity increasing towards the west. A climatogram of the Free State (Fig. 2.1) was compiled from information supplied by the Bloemfontein Weather Service using the method described by Walter (1964). As shown in the figure, the wet season (September - April) is represented by the period when the rainfall curve exceeds the temperature curve. The province receives more than 500 mm rainfall during this period, thus almost three-quarters of the annual precipitation. The remainder of the year is considered the dry season (May - August) and during this period the rainfall curve falls drastically below the temperature curve (Fig. 2.1). The province experiences warm to hot summers and extremely cold winters frequented by severe frost, especially towards the eastern mountainous regions where temperatures can drop as low as  $-9.5^{\circ}\text{C}$ . Mean monthly temperatures





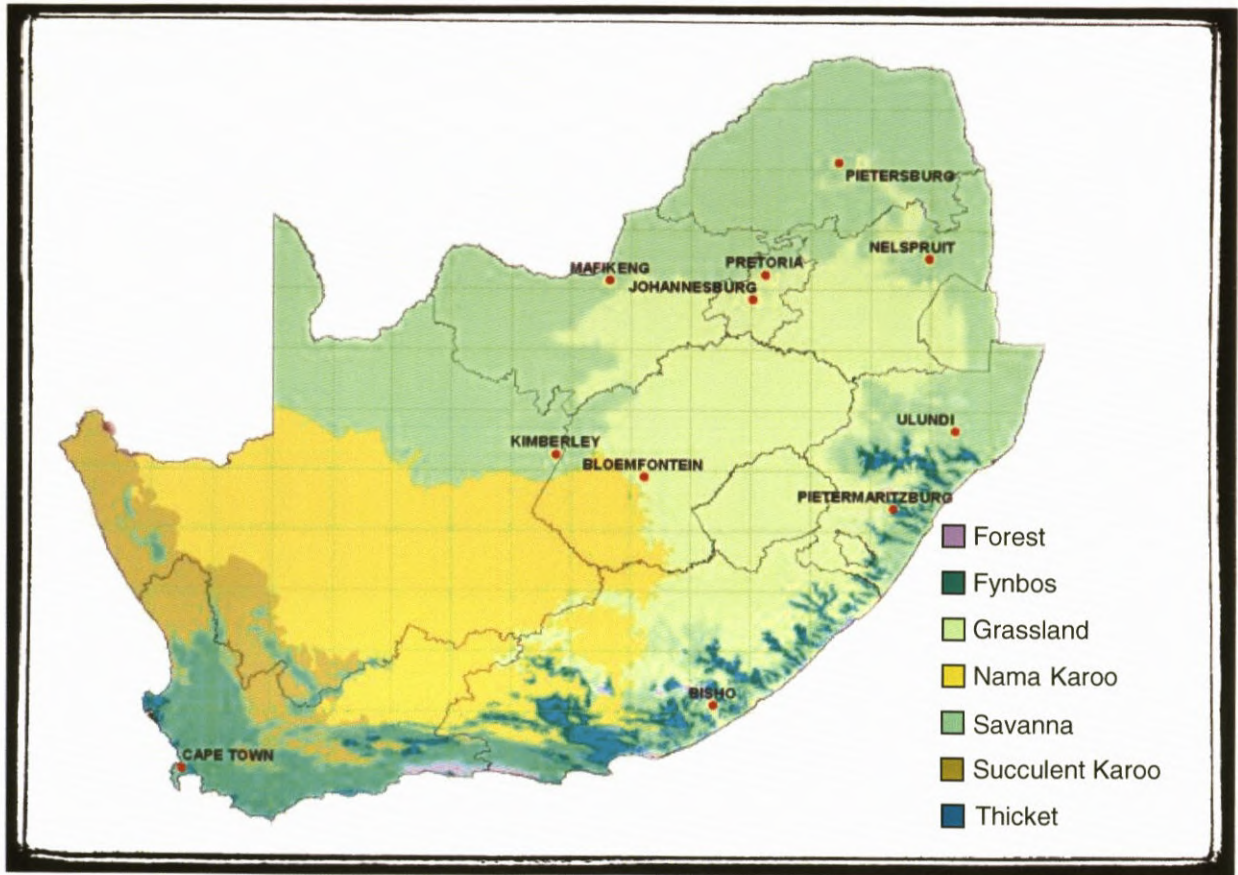
**Figure 2.1** Climatogram of Bloemfontein according to the method of Walter (1964). Figure between brackets indicates the number of years of observation. Mean annual temperature and rainfall are indicated in the top left and right corner, respectively. A, wet season; B, dry season; C, average monthly rainfall; D, average monthly temperature.



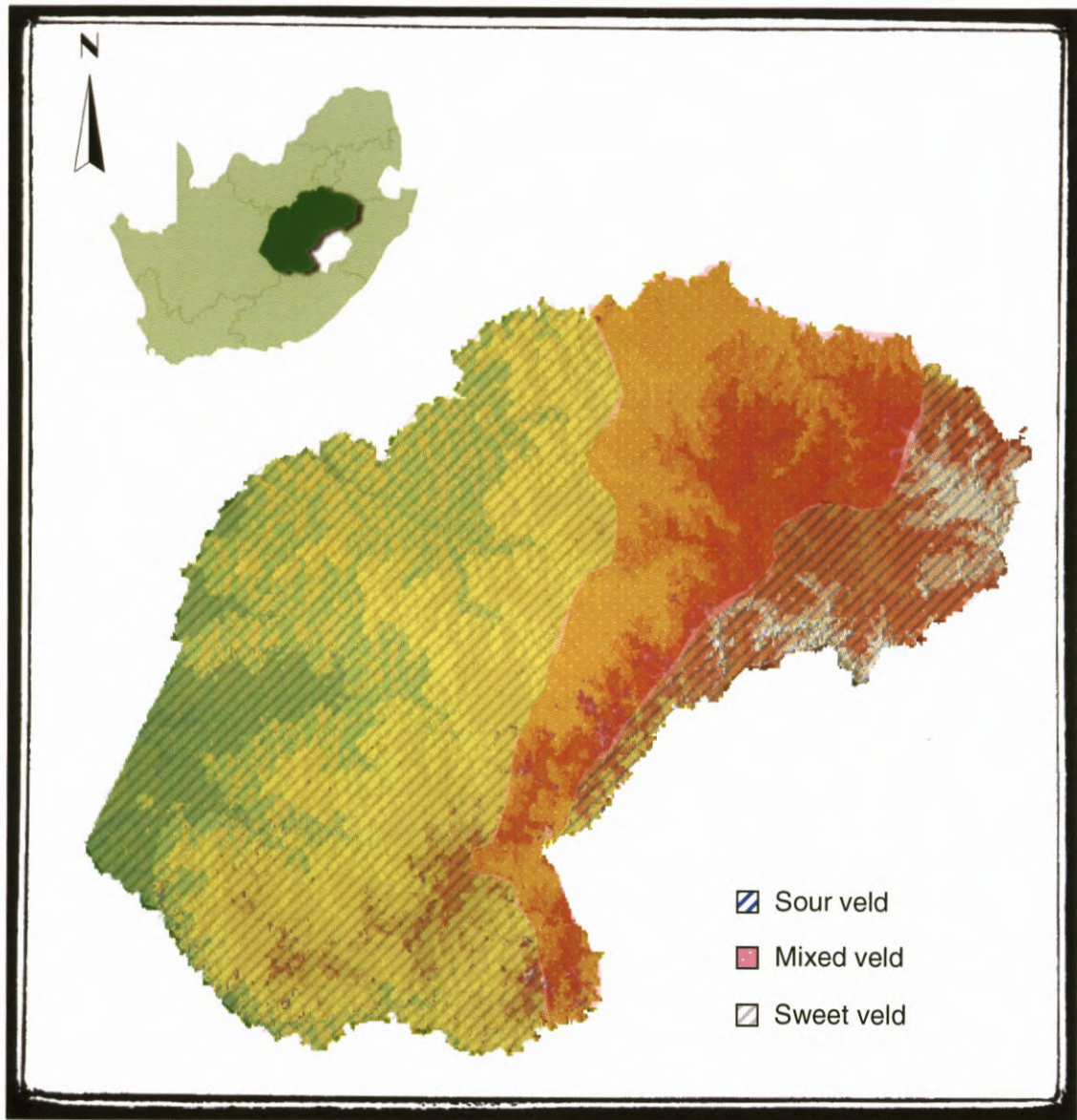
deviate from the eastern to the western regions, but in general average daily maximum and minimum temperatures for the hottest and coldest months reach 30.5°C during January and - 0.6°C in July, whilst absolute temperatures range from 34.1°C and - 4.9°C. Frost, ranging from severe to light, occurs throughout the province during winter and 120 - 170 frost days per year can be expected.

According to Lynch (1983) the Free State is typical Highveld in topography with altitudes ranging from 1 200 - 1 500 m. The region is almost treeless, consisting mainly of widespread grasslands with some Karoo vegetation in the south. However, due to regional differences in temperature, altitude, rainfall and soil several vegetation types occur in the province. Accordingly, Low & Rebelo (1996) distinguishes four biomes and 14 vegetation types in the Free State. Figure 2.2 shows that the Grassland Biome covers the greatest area of the province, hence it is considered the principal biome and includes a diverse range of Highland Grassland (no. 34 - 37, 39 - 41) as well as Mountain Grassland types (no. 43, 46). The Nama Karoo Biome includes the Upper Nama Karoo (no. 50), Orange River (no. 51) and Eastern Mixed (no. 52) vegetation types and covers the second largest area of the province (Fig. 2.2). Kimberley Thorn Bushveld (no. 32) represents the smaller Savanna Biome (Fig. 2.2), whereas Afromontane Forest vegetation (no. 2) of the Forest Biome comprises a mere 0.05% of the Free State. In general, more than 70% of the Free States' vast vegetation variety is dominated by the Moist Cool Highveld Grassland (no. 39), the Dry Sandy Highveld Grassland (no. 37) and the Eastern Mixed Nama Karoo (no. 52) vegetation types (Low & Rebelo, 1996). The majority of the central Highveld grass species are perennial, though as a result of disturbance, development and overutilization there are few remaining grassland areas that do not exhibit some degree of retrogression (Van Rooyen, 2002a). Red grass (*Themeda triandra*) constitutes most of the landscape and the presence of this grass species is considered indicative of stable grassland in the climax stages (Dews, 1918). Grazing is of importance in these areas, although overgrazing can cause *Eragrostis* spp. to replace red grass. Overgrazing can also lead to invasions of karroid shrubs such as bitter karoo (*Pentzia globosa*) and certain pioneer grasses, including *Aristida* spp. and *Tragus* spp. (Low & Rebelo, 1996).

From a grazing perspective, the habitat varieties in the grasslands and savannas can generally be classified as sweet veld, mixed veld or sour veld (Van Rooyen, 2002b) and Figure 2.3 shows the



**Figure 2.2** Biomes of South Africa. Biomes of vegetation units adapted from Low & Rebelo (1996).



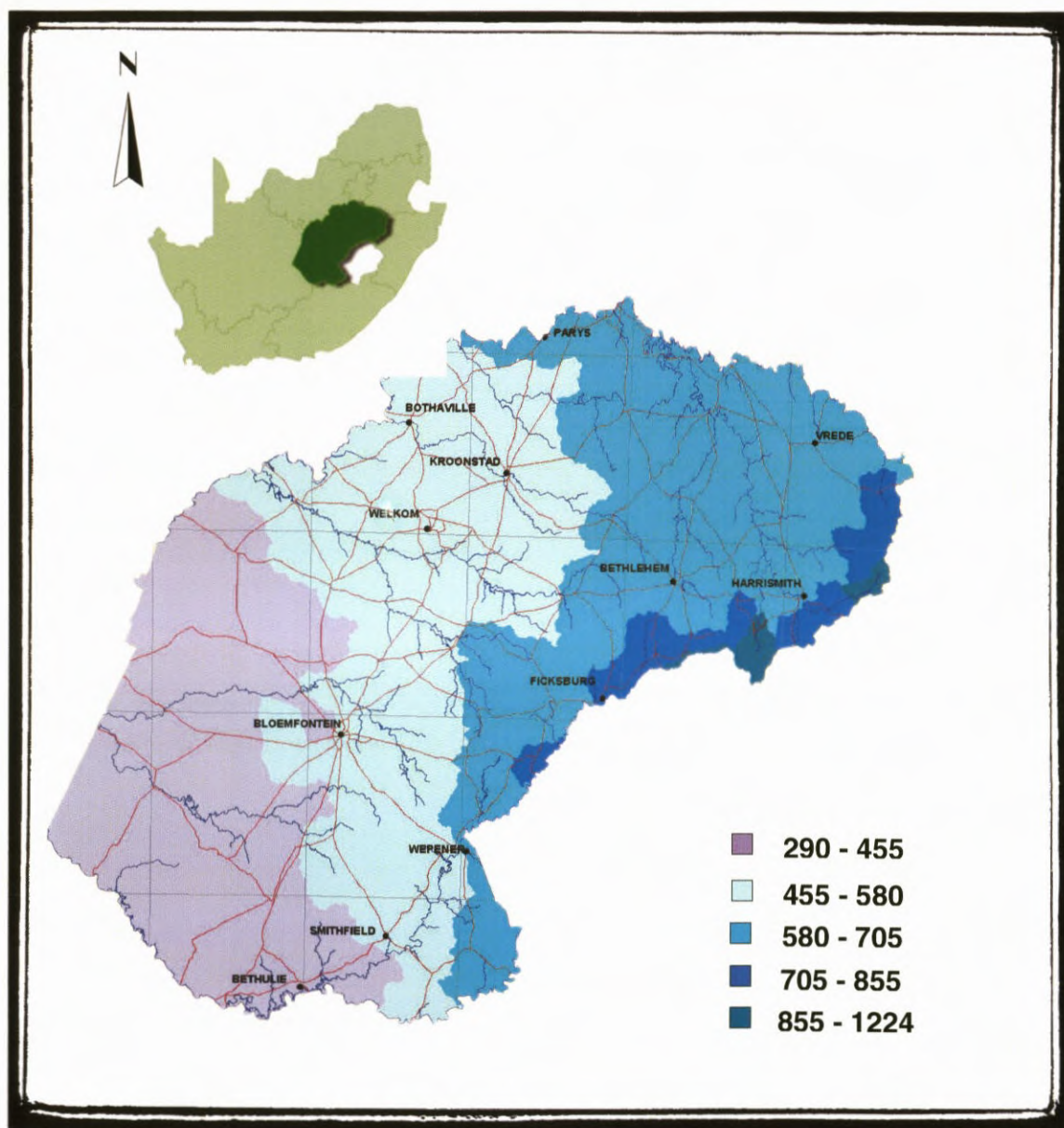
**Figure 2.3** Distribution of sour, mixed and sweet veld in the Free State Province.

distribution of these respective veld types across the province. In relation, the Free State is characterised by somewhat low and irregular rainfall and there is an increasing gradient of rainfall from east to west. Sour veld is found in the high-lying regions where the rainfall is 580 mm per year or higher, while sweet veld occurs in the lower-lying, semi-arid savannas that receive from 200 - 500 mm rainfall annually (Low & Rebelo, 1996) (Fig. 2.4).

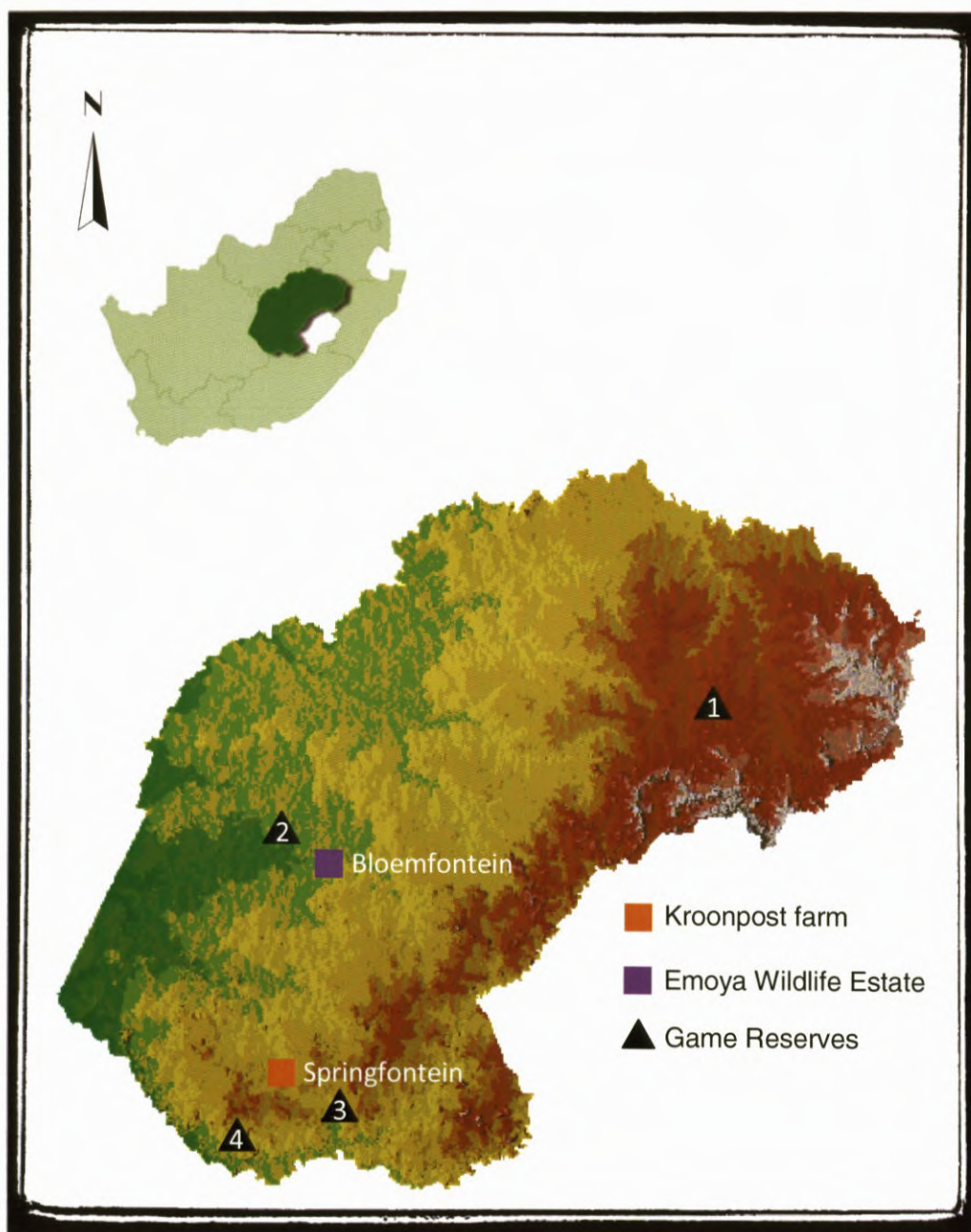
Morphometric data of blesbok body measurements were obtained from game reserves across the province, while field observations pertaining to aspects of bontebok behaviour were carried out at two separate locations in the central and southern Free State (Fig. 2.5). The first study area was at a mixed wildlife and livestock farm, namely Kroonpost (30°15'27.2" S; 25°57'55.3" E) in the southern Free State. The study area is situated approximately 32 km southeast of Springfontein at an altitude of 1 363 m above sea-level. The enclosure covers a small surface area of 160 ha, physiologically characterised by relatively level and open grasslands, a low-lying pan and two rocky outcrops (Fig. 2.6). Very few scattered patches of trees, including blue bush (*Diospyros lycioides lycioides*), broom karee (*Searsia erosa*) and karee (*S. lancea*) are found in the vicinity of the larger rocky outcrop as well as surrounding area of the low-lying pan.

Owing to a lack of available climatological data for the Springfontein area, weather data was obtained from the closest weather station located at Fauresmith about 76 km southeast of Springfontein. As shown in the climatogram compiled for the study site three seasons namely, early summer, late summer and winter can be distinguished (Fig. 2.7). Early summer (September - December) and late summer (January - April) are represented by the two periods when the rainfall curve exceeds the temperature curve. Of the annual precipitation (470 mm), approximately 60% is received during the latter season. January represents the onset of the late summer season and is considered the hottest month of the year when average daily and absolute maximum temperatures of 31.2°C and 34.4°C are reached. The winter season is over a period of four months (May - August), while the harshest weather conditions regarding average daily (- 2.5°C) and absolute minimum (- 4.7°C) temperatures are experienced during July. Frost can be expected in the region of 47 days for the duration of this dry and cold period.





**Figure 2.4** Mean annual precipitation (mm per quaternary catchment) in the Free State Province.

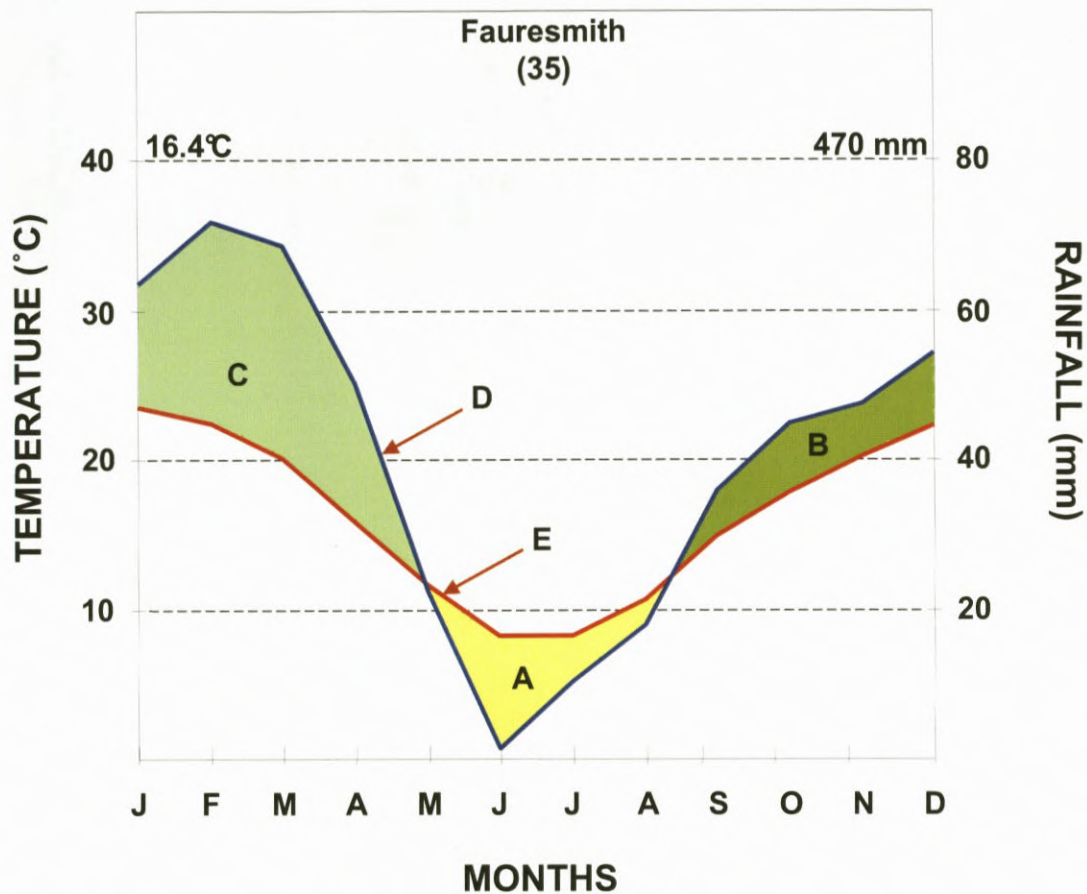


**Figure 2.5** Locations of the four game reserves (1, Wolhutterskop; 2, Soetdoring; 3, Tussen-Die-Riviere; 4, Gariep Dam) and the two main study areas in the Free State Province.





**Figure 2.6** Satellite image of the study site at Kroonpost situated in the Springfontein area, Free State, showing the location of the bontebok enclosure and surface area. Modified from Google Earth (2010).



**Figure 2.7** Climatogram of Fauresmith according to the method of Walter (1964). Figure between brackets indicates the number of years of observation. Mean annual temperature and rainfall are indicated in the top left and top right corner, respectively. A, winter; B, early summer; C, late summer; D, average monthly rainfall; E, average monthly temperature.



The study site near Springfontein falls under the Eastern Mixed Nama Karoo vegetation type (no. 52) as specified by Low & Rebelo (1996). The area is synonym of the False Upper Karoo (no. 36) and False Karroid Broken Veld classification (no. 37) of Acocks (1988). Within this region plant life consists of a mix of grass- and shrub-dominated vegetation types, while species composition changes in accordance with seasonal rainfall events. Grasses such as *Aristida* spp., *Eragrostis* spp. and red grass may dominate the landscape following sufficient summer rains. Common shrubs include bitter karoo, kapokbos (*Eriocelaphus ericoides*), thornkapok (*E. spinescens*) as well as doll's roses (*Hermannia* spp.).

The second site was located at Emoya Wildlife Estate (29°04'03, 76" S; 26°10'18, 47" E) in the central region of the Free State (*vide* Fig 2.5). The study area is situated approximately 6 km north of Bloemfontein at an altitude of 1 432 m above sea-level, encompassing a moderate surface area of 400 ha (Fig. 2.8). Physiological features of this site were comparatively similar to the Kroonpost study area and were also characterised by fairly level, open grasslands and three low-lying pans, but no rocky outcrops. Dense patches of trees (broom karee and karee) surround the two eastern pans, while trees were absent in the surrounding area of the central pan. Given that the climatogram (*vide* Fig. 2.1) for the Free State was based on data from the Bloemfontein weather station, the weather data is also applicable to the second study area.

Low and Rebelo (1996) classified the Bloemfontein area as part of the Dry Sandy Highveld Grassland vegetation category (no. 37), while Mucina *et al.* (2005) categorised Bloemfontein in the Dry Highveld Grassland Bioregion. The latter encompasses three vegetation types, namely Bloemfontein Dry Grassland, Winburg Grassy Shrubland and Bloemfontein Karroid Shrubland. Features of the Bloemfontein Dry Grassland vegetation type are applicable to the study area at Emoya Wildlife Estate. This grassland region is dominated by several lovegrass types (*Eragrostis* spp), small buffalo grass (*Panicum coloratum*) and large bushmansgrass (*Stipagrostis uniplumis*), while red grass, bitter karoo and sweet thorn (*Acacia karroo*) trees (found along water courses) are also considered to be important vegetation species that are representative of this particular bioregion (Low & Rebelo, 1996).



**Figure 2.8** Satellite image of the study site at Emoya Wildlife Estate situated in the Bloemfontein area, Free State, showing the surface area. Modified from Google Earth (2010).



Chapter 3:  
Methods & Material



### 3 METHODS AND MATERIAL



#### 3.1 MORPHOMETRICS

##### 3.1.1 Body measurements

A variety of morphological characteristics were collected from fresh blesbok and bontebok carcasses at several locations across the Free State Province (*vide* Fig. 2.5). Linear body measurements were obtained with a flexible tape measure and determined to the nearest 0.5 cm. The following morphometric data were collected:

- (i) Live body weight; body weight of a freshly killed specimen measured to the nearest 0.5 kg using a nylon sling attached to a spring scale (Fig. 3.1).
- (ii) Dead carcass weight; weight of an individual from which the head, feet and intestines have been removed. Weight was recorded to the nearest 0.5 kg.
- (iii) Total body length; measure from the tip of the muzzle to the last sacral vertebrae of a fully extended specimen.
- (iv) Chest girth; circumferential measure around the chest at the level of the third to fourth cervical vertebrae.
- (v) Shoulder height; vertical distance from the ground to the shoulder.
- (vi) Head length; measure from the tip of the muzzle to the first sacral vertebra.
- (vii) Ear length; measure from the bottom of the ear notch to the most distant tip of the ear.
- (viii) Hindfoot length; distance from the tip of the hoof to the proximal end of the calcaneus.
- (ix) Tail length; measured from the tail base to the most distal point of the straightened tail.





**Figure 3.1** Determining live weight from a dead bontebok individual by using a nylon sling attached to a spring scale.

- (x) Horn length; measurement along the front curve from the lowest edge of the base to the tip of the horn (Fig. 3.2).
- (xi) Distance between horn tips; distance from the tip of the left horn to the tip of the right horn (Fig. 3.3).
- (xii) Horn spread; distance between the left and right horn measured from the greatest outside curve of the horn (Fig. 3.4).
- (xiii) Horn circumference; circumference of the horn above the swelling of the prong (Fig. 3.4).

## **3.2 ACTIVITY PATTERNS**

### **3.2.1 Behavioural aspects**

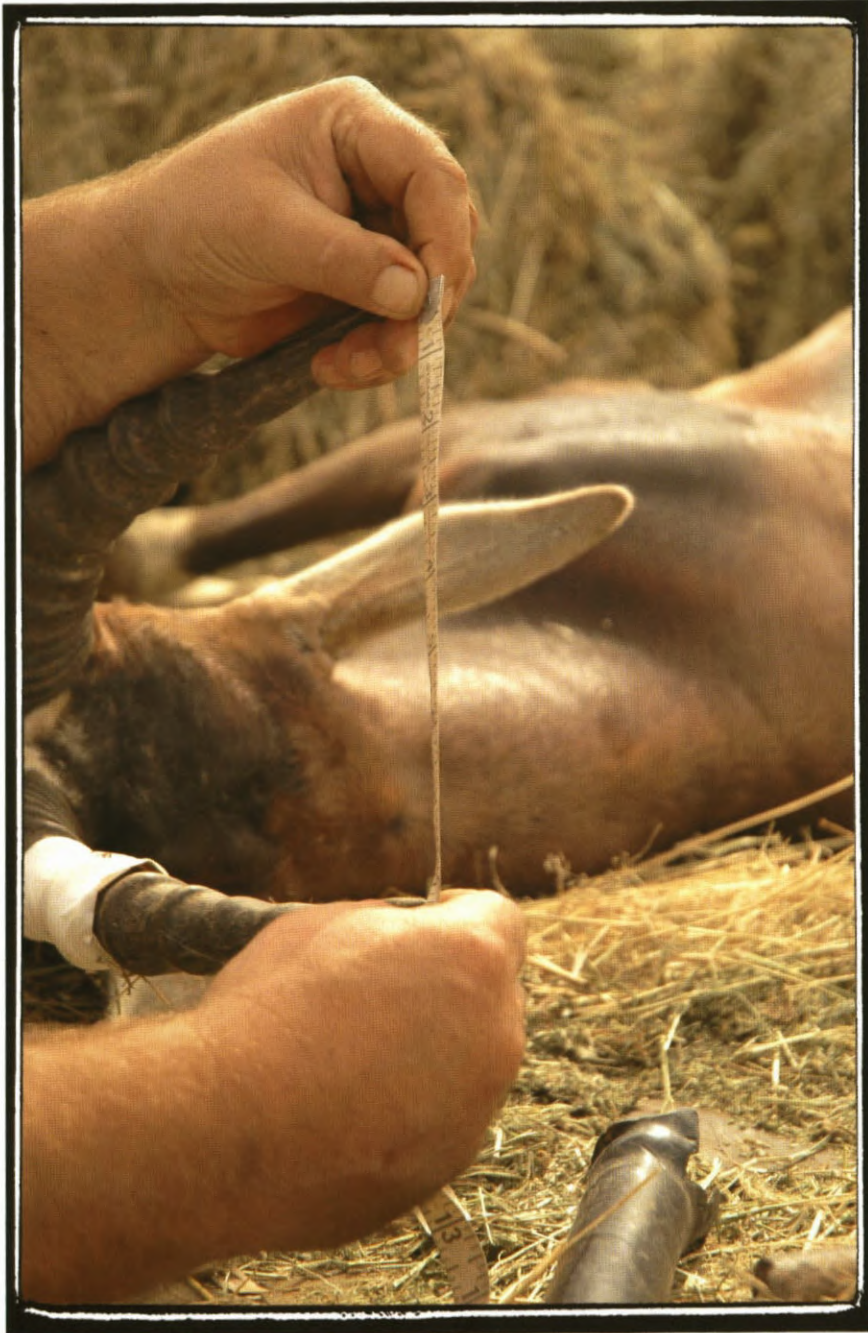
Activity data on bontebok totalling 1 098 hours were collected during August 2006 - April 2007 (Kroonpost farm) and February - July 2009 (Emoya Wildlife Estate). Measurements involved two types of observations, namely instantaneous scan sampling and focal animal sampling (Altmann, 1974).

At the Kroonpost study site, observational distances were too large to discern between the sexes, hence a scan sampling method with instantaneous sampling for the recording rule was used to quantify behaviour of the herd ( $n = 14 - 18$ ). Bontebok have a fairly stable social structure on a year-round basis and the herd consisted of one territorial ram, eleven ewes and the number of juveniles (younger than 12 months) varied between two and six. Direct field observations were accomplished using Pentax 10 x 25 binoculars from a vehicle, while following the animals as closely as was viable. Observations were carried out seasonally for three consecutive days from sunrise to sunset. Depending on the season these observations could last for a continuous period of up to 14 hours. Behavioural aspects of each visible bontebok in the population at a given moment were recorded, regardless of what the individual was doing beforehand. The population was always scanned from left to right at periodic observational intervals of 10 minutes during which all individuals were classified according to the activity in which they were engaged.



**Figure 3.2** Horn measurements taken from a dead specimen. Horn length is measured from the base of the skull to the tip of the horn along the front curve.





**Figure 3.3** Measurement of the distance between the horn tips of a dead bontebok adult male.





**Figure 3.4** **A**, horn spread determines the distance between the left and right horn measured from the greatest outside curve of the horn; **B**, horn circumference measures the circumference of the horn above the swelling of the prong.

Instantaneous scan sampling of the herd ( $n = 13$ ) at the second study site was carried out for two consecutive days on a weekly basis. The same methodology, as described above, was applied to determine herd activity. In contrast to the first site, the second study area allowed for closer observational distances allowing distinction between the territorial ram, ewes ( $n = 6$ ), yearlings ( $n = 3$ ) and juveniles ( $n = 3$ ). Juveniles were individuals younger than 12 months, while yearlings represented bontebok aged 12 - 24 months. In addition to the instantaneous scan method, a focal sampling procedure for the territorial ram was applied. All occurrences of specified actions of this particular individual were recorded, for as long as he was in view, during a predetermined sample period (sunrise to sunset).

To determine the veld condition of the study sites plant surveys were conducted in accordance with the wheel-point method as described by Tidmarsh & Havenga (1955). In total, 1000 observations of the nearest plant species were recorded along transects covering the entire area of the Kroonpost site as well as the specific area utilised by the herd at Emoya.

### **3.2.2 Body orientation**

Body orientation of bontebok in relation to sun angle and wind direction was also monitored. In total, 108 hourly observations were obtained during winter (5 - 7 August 2006), early summer (3 - 5 November 2006) and late summer (20 - 22 April 2007). The following were recorded on an hourly basis from sunrise to sunset:

- i) Angle between orientation of the sun and the long body axis.
- ii) Wind speed and direction were determined by using an Extech mini thermo-anemometer and expressed as  $\text{ms}^{-1}$ .
- iii) Screened ambient temperature (not exposed to rays of the sun) was measured with an Extech mini thermo-anemometer and expressed in  $^{\circ}\text{C}$ .
- iv) Weather conditions; animals in direct sunlight or sun obscured by cloudy conditions.
- v) Bontebok moving or stationary.

### 3.2.3 Statistical analysis

A statistics and analytics software package, Statistica (vers. 10), was applied to determine statistical significance of data for chapters 4 and 5. The following tests were performed: t-test for unrelated independent groups assuming homogeneous variances in combination with Cohen's effect size ( $d$ ) to confirm the meaningful significance of the statistical significance, Chi-square test supplemented with the Cramér's  $V$  effect size statistic to adjust chi-square significance to factor out sample size, Spearman's rank order correlation coefficient and the Z-test.

## 3.3 GENETIC ANALYSIS

### 3.3.1 DNA sampling

Hair samples are recognised as an increasingly important DNA source for wildlife research (Goossens *et al.*, 1998; Roon *et al.*, 2003) and have been used in the study of several mammal species (Morin *et al.*, 1994; Woods *et al.*, 1999; Frantz *et al.*, 2004; Anderson *et al.*, 2006). Non-invasive techniques involve not only using freshly plucked or shed hairs as a DNA source, but also faecal materials. The latter has, however, been found to be less consistent in genetic analysis (Anderson *et al.*, 2006). Hence, hair plucked from blesbok ( $n = 12$ ) and bontebok ( $n = 28$ ) individuals provided a practical alternative for genetic sampling as it required less skill, time and money than invasive techniques such as collecting blood or biopsy samples. Samples were collected from live specimens (Fig. 3.5) as well as fresh carcasses killed for venison or leisure hunting. At least 15 rooted hairs were plucked from each individual and stored in paper envelopes at room temperature. All of the bontebok sampled in the Free State were certified and registered as pure bontebok with Free State Nature Conservation. At present, no legislation exists to compel game farmers to certify blesbok and therefore no certified samples could be obtained. Sampling localities were thus restricted to farms with no history of bontebok kept on the same property as blesbok herds.





**Figure 3.5** Collecting hair from the tail of a live bontebok specimen at a game auction in the central Free State.

### 3.3.2 DNA extraction

Cells at the bases of fresh hairs provide high-quality DNA for amplification (Valderrama *et al.*, 1999). Accordingly there was no need to extract DNA from the hair samples before the polymerase chain reaction. An associated advantage was that even semi-degraded DNA could potentially hold sufficient undamaged copies of a short microsatellite target sequence to permit amplification. Two hair roots from each specimen were cut into a 1.5 ml eppendorf and the work surface and scissors used were wiped clean with bleach after each specimen to avoid cross-contamination.

### 3.3.3 PCR method

Genetic analysis basically consisted of two steps. The first step was to test cross-species amplification success in *D. pygargus* in order to further investigate potential subspecies-specific microsatellite loci for blesbok and bontebok. The first step entailed screening a preliminary panel of 72 ungulate primers (Table 3.1) in a subset of two individuals from each subspecies ( $n = 4$ ). PCR conditions were kept constant, except for annealing temperatures kept at the optimal temperature for the primer and the species for which it was designed. A total of 60 primers were excluded from the original panel based upon failure to amplify, yielding non-specific amplification products or deficient polymorphism in the subspecies. The remaining microsatellite loci yielded consistent amplification results and included MAF46, TGLA53, OarFC304, CSSM18, OarCP26, BMS1237, BMS2113, SPS115, TGLA126, ETH225, BMC3224 and MCM527. Funding constraints did not allow for further analysis of all 12 microsatellite loci, hence the concluding panel was narrowed down to five microsatellite loci (Table 3.2) that, based on consistent amplification, showed the most potential as possible diagnostic loci. The final panel consisted of two loci (CSSM18, TGLA53) originally isolated for use in domestic cattle (*Bos taurus*) and three loci (MAF46, OarCP26, OarFC304) specifically designed for sheep (*Ovis aries*).

Reactions were multiplexed, with reaction mixtures consisting of 2 U AmpliTaq Gold, 1 x PCR buffer (with 1.5 mM MgCl<sub>2</sub>), an additional 1.5 mM MgCl<sub>2</sub>, 0.1 - 0.5  $\mu$ M of each primer and 250  $\mu$ M per

**Table 3.1** Microsatellite loci tested on *D. pygargus* ( $n = 4$ ) hair samples.

Bovine	Camelides	Caprine	Equine	Ovine	Porcine
BM1824	LCA63	BM1329	AHT4	AGLA293	SO006
BMC3224	LCA66	BM1818	AHT5	BMS1237	SO035
BMS2113	VOLP03	ILSTS19	ASB2	CMC527	SW035
CSSM18	VOLP10	ILSTS87	HMS2	CSSM36	SW1041
ETH10	VOLP32	INRA005	HMS3	HSC	SW21
ETH225	VOLP67	INRA006	HMS6	ILSTS002	SW2402
ETH3	YWLL08	INRA63	HMS7	INRA23	SW749
INRA23	YWLL38	INRA172	HTG4	MAF46	
SPS115	YWLL144	INRA231	HTG6	MCM185	
TGLA53		MAF65	HTG7	MCM527	
TGLA122		OarRFCB11	HTG10	MGTG4B	
TGLA126		RM4	VHL20	OarCP26	
TGLA227		SRCPRSP5		OarFCB20	
		SRCRSP8		OarFCB304	
		SRCRSP23		TGLA57	
		SRCRSP24			

**Table 3.2** Characteristics of the five loci tested on *D. pygargus* ( $n = 40$ ), showing reported size ranges and primer sequences.

Locus	Size range (bp)	Dye label	Primer sequence (5' → 3')	Reference
CSSM18	116 - 134	JOE	Forward: TGTGCATAATTTGTGTCCGTCCGGA Reverse: AGGAATTCCCTCTAGAAAAGCAGGG	Moore <i>et al.</i> , 1994
MAF46	94 - 110	FAM	Forward: AAATACCCTATAAGGCACAGTACCAC Reverse: CACCATGGCCACCTGGAATCAGG	Swarbrick <i>et al.</i> , 1992
OarCP26	120 - 170	TAMRA	Forward: GGCCTAACAGAATTCAGATGATGTTGC Reverse: GTCACCATACTGACGGCTGGTTCC	Ede <i>et al.</i> , 1995
OarFCB304	150 - 188	JOE	Forward: CCCTAGGAGCTTTCAATAAAGAATCGG Reverse: CGCTGCTGTCAACTGGGTCAGGG	Buchanan & Crawford, 1993
TGLA53	151 - 187	FAM	Forward: GCTTTCAGAAATAGTTTGCATTCA Reverse: ATCTTCACATGATATTACAGCAGA	Georges & Massay, 1992

dNTP with deionised water added to 7.5  $\mu$ l. A forward primer for each locus was labelled at the 5' end with a fluorescent tag of either 5-FAM (blue), JOE (green) or TAMRA (yellow).

The Perkin Elmer GeneAmp PCR System 9700 was used to amplify the DNA. PCR conditions comprised of an initial denaturing step at 94°C for 10 minutes followed by 30 cycles of 30 seconds at 95°C and between 50°C - 60°C (depending on the primer used), and 60 seconds at 72°C. Following completion of the 30 cycles, the samples were left for 60 minutes at 72°C. The PCR product was thereafter stored at 4°C.

#### **3.3.4 Genescan Electrophoresis and analysis software**

The second step involved thorough assessment of the final panel to establish the number of alleles, allele length variation, allele size range and polymorphism by testing it on a larger sample size of blesbok ( $n = 12$ ) and bontebok ( $n = 28$ ) individuals. Analysis of microsatellite loci was performed on an ABI 377 automated sequencer and analysed with GeneScan software in order to automatically size and quantify DNA fragments. The GeneScan system allows for labelling DNA fragments with different colour fluorescent dyes and the results can be displayed as electropherograms, as tabular data or alternatively as both. Electropherograms specify fluorescence intensity as a function of fragment size and each electropherogram is representative of a single gel lane, while tabular data presents precise sizing and quantitative data. Genotyper software in turn analyse the data obtained from the GeneScan software. As a result fragments are displayed as labelled peaks in plot displays and individual genotypes can be determined.





Chapter 4:

# Morphometrics



## 4 MORPHOMETRICS



The subfamily Alcelaphinae originated in Africa and was distributed over numerous areas of the continent. Two million years ago, alcelaphines consisted of more than eight genera with at least 15 different species. At present only seven species from four genera remain. Nevertheless, this group is still one of the most numerous and widely distributed tribes in Africa (Estes, 1992).

In southern Africa these large to medium-sized antelopes are represented by three genera, namely *Alcelaphus*, *Connochaetes* and *Damaliscus*. These genera are morphologically characterised by forequarters that are higher than the hindquarters, resulting in a sloping profile of the back, long narrow heads with narrow slit-shaped nostrils along the edge of the semicircular rhinarium, the presence of horns in both sexes (subequally developed in females) and the presence of one pair of inguinal mammae in females (Bigalke, 1955; Estes, 1992).

The genus *Damaliscus* is represented by *D. lunatus* (topi & tsessebe) and *D. pygargus*, the latter endemic to South Africa. General morphological attributes of this genus include an elongated and slender muzzle, lyrate or complexly recurved horns which are ringed, a short and glossy pelage that varies from plain tan to chestnut colour with dark and white markings and a lighter coloured rump. The hock-length tail ends in a coarse tuft of hair (Estes, 1992). By comparison, *D. pygargus* is considered to be the smallest and most striking members of this group.

### 4.1 DISCRIMINANT IDENTIFICATION

Even though there are some characteristic differences between bontebok and blesbok regarding size and general physical appearance, it is fairly difficult to distinguish them by sight. Bigalke (1955)

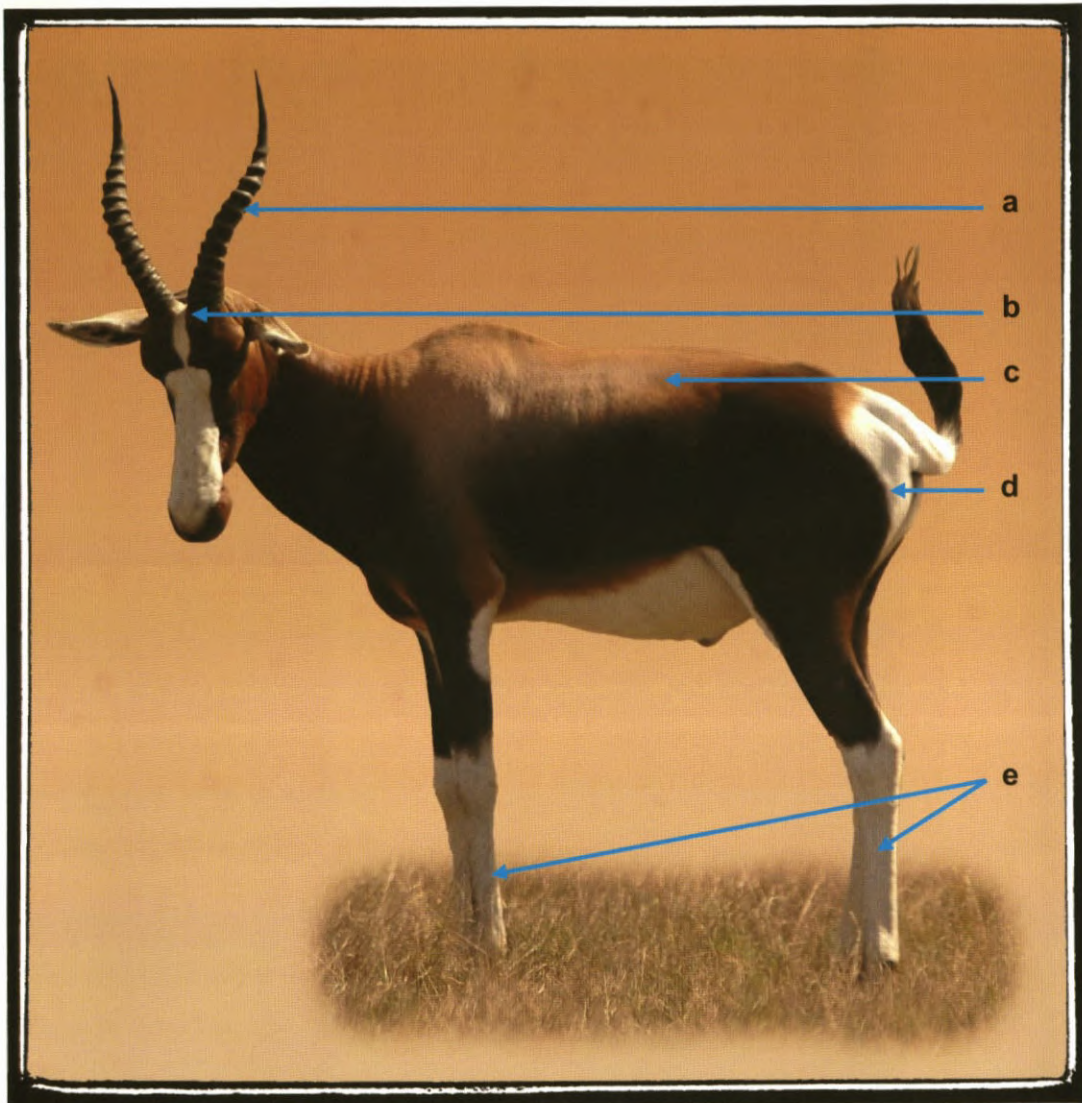
qualitatively summarised the morphological separation of the subspecies in terms of body colour, coat pattern and horn shape, specifically emphasising differences with regards to the white buttocks, upper legs and belly. In particular the bontebok has a fairly conspicuous appearance in terms of its striking patterns and rich colours (Fig. 4.1). The overall colour is dark brown with a gleaming purple sheen, fading to a silvery fawn on the shoulders and back (Fig. 4.2). The rump, lower limbs, base of the tail and the abdominal area are pure white. The white blaze that reaches from the base of the horns to the nostrils narrows sharply between the eyes and is usually not broken. David (1973), however, observed that 19% of individuals at Bontebok National Park exhibited a divided blaze. In comparison blesbok are less striking in appearance (Fig. 4.3). The general colour of the pelage varies between light brown, reddish brown or a darker brown and lacks the purple sheen that distinguishes bontebok. The saddle is a dull fawn-like colour, distinctive snow-white areas are absent and appear more beige in colour and the rump, which does not extend around the base of the tail, is also pale. Other noticeable differences are the lower legs which are mainly dark, while the white blaze is divided by a narrow brown bar just above the eyes. The name blesbok actually refers to the white blaze on the brow and muzzle. Although the horns of both subspecies are very alike in appearance, the rings of blesbok horns are closer together, do not encircle the horns and are a pale brown colour (Cornus, 2005), while bontebok horns are uniformly black in colour. Furthermore, blesbok horns are larger and lyre-shaped nor as heavily ringed. Males and females of both subspecies have similar horns, although those of females tend to be smaller and thinner at the base of the horns.

Owing to the usefulness in distinguishing the subspecies, Fabricius *et al.* (1989) chose the features of the white buttocks, upper hindleg and abdomen as criteria to develop a discriminant function that differentiates between pure bontebok and bontebok/blesbok hybrid populations. This method is based on body measurements determined from colour photographs taken of a representative fraction of the herd from which exact dimensions of the abovementioned criteria are determined. It is well known that alarmed bontebok individuals tend to turn away from a disturbance at a 90° angle (Fabricius *et al.*, 1989) and this position allows for usable photographs. It is important that the root of the tail be clearly visible and the animal needs to cover at least 20% of the photographic frame. These photographs are projected onto a white paper sheet mounted on a vertical board, ensuring that the height of the animal is between 80 - 120 mm after which the profile is traced onto paper. The borders

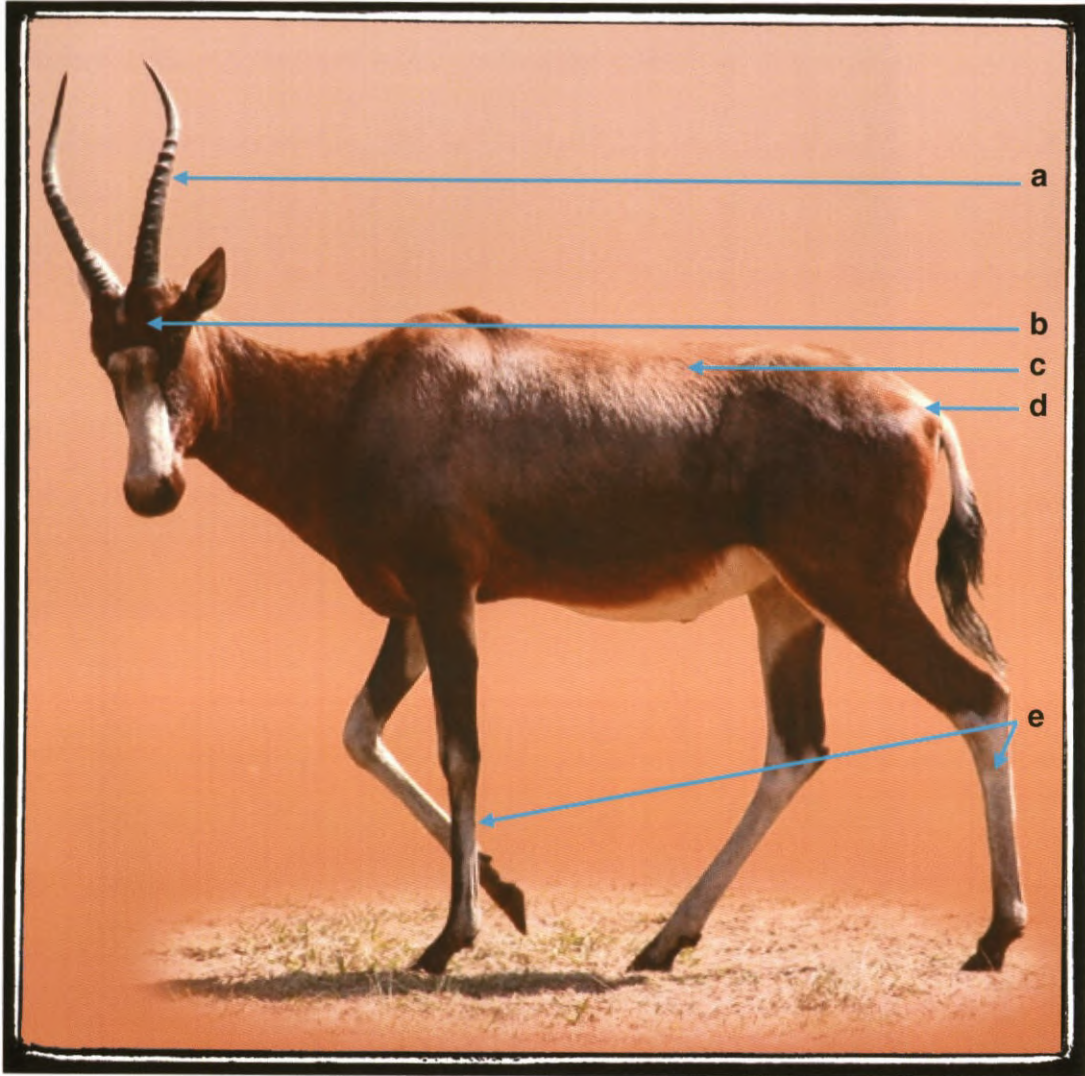


**Figure 4.1** Posterior view of an adult bontebok male shows the distinctive pied coat pattern with a strongly contrasting white rump and black lower tail.





**Figure 4.2** An adult bontebok. Morphological differences of importance are in terms of a) horns; b) white face blaze; c) body colour; d) rump and e) fore- and hind limbs.



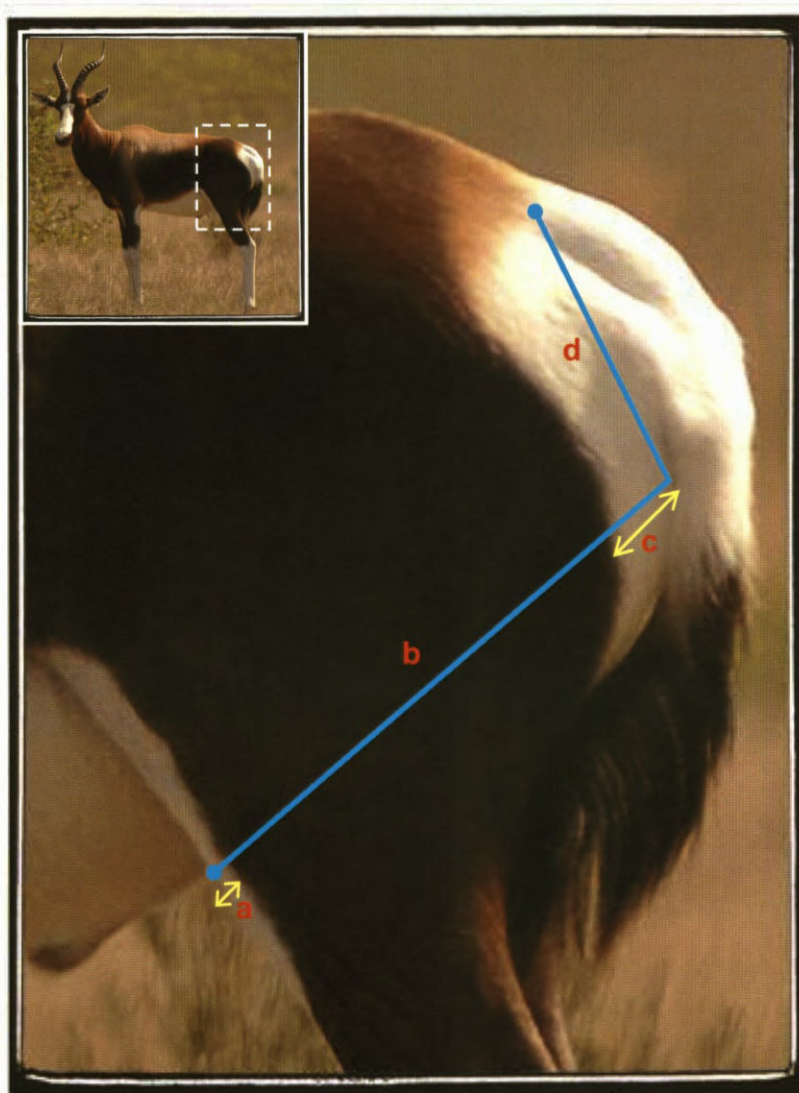
**Figure 4.3** An adult blesbok. Morphological differences of importance are in terms of a) horns; b) white face blaze; c) body colour; d) rump and e) fore- and hind limbs.

between the brown and white parts on the buttocks as well as the border of the narrow white patch on the anterior part of the upper hindleg are drawn onto the profile allowing for measurements a, b, c and d (Fig. 4.4) of the rump patch. All measurements are taken with callipers and rounded to the nearest 0.1 mm. These measurements are analysed by means of a computer programme according to the *C* test that was developed and determines whether populations suspected of genetic contamination differ markedly from pure bontebok and Figure 4.5 shows that the discriminant scores for bontebok/blesbok hybrids are intermediate between the pure populations.

At present, this relatively simple method is effectively carried out by Free State Nature Conservation staff on a routine basis. It has the advantage of offering a non-invasive method as no animals need to be killed or captured in the process. In addition, the history of the herd is also taken into consideration before proof of purity is issued. Certificates are not issued if the history traces back to a population that was formerly classified as impure. Certificates are also denied if bontebok and blesbok are kept on the same property or if blesbok previously occurred on the farm.

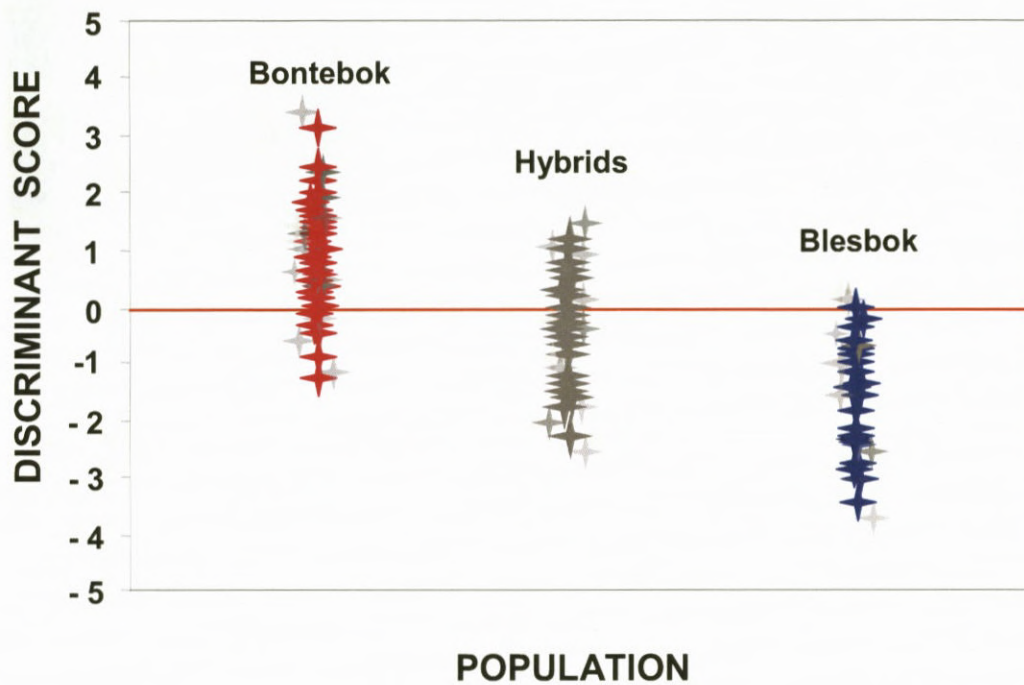
Since results of this method are subjected to sample size, it is vital that test populations should be above a certain minimum. In view of the rather small population sizes of bontebok kept in the Free State (Fig. 4.6), it is doubtful that many farmers own populations large enough for this test to be applied. Nevertheless, in numerous cases where the test was performed on small populations in the province that were suspected of impurity, they were in fact rejected. There is also the likelihood that some populations will not have been contaminated to a great extent and under these circumstances it can be expected that not all hybrid populations will be rejected. However, if a small sample is taken and the population is rejected, then it is considered to be a relatively sound indication that the population is indeed contaminated. Furthermore, difference from the 'type' population that is considered acceptable is not consistent and subject to human error, since it is impossible to determine precise guidelines for the amount of difference that should be considered as acceptable as there is some uncertainty on where the actual mean of bontebok lies. Therefore, even though this method has proven to be very useful it is not consistently accurate and it is recommended that this method should not be used as the only accepted criterion for sample sizes below the minimum set by the person performing the test. In these cases genetic analysis of the population in question could



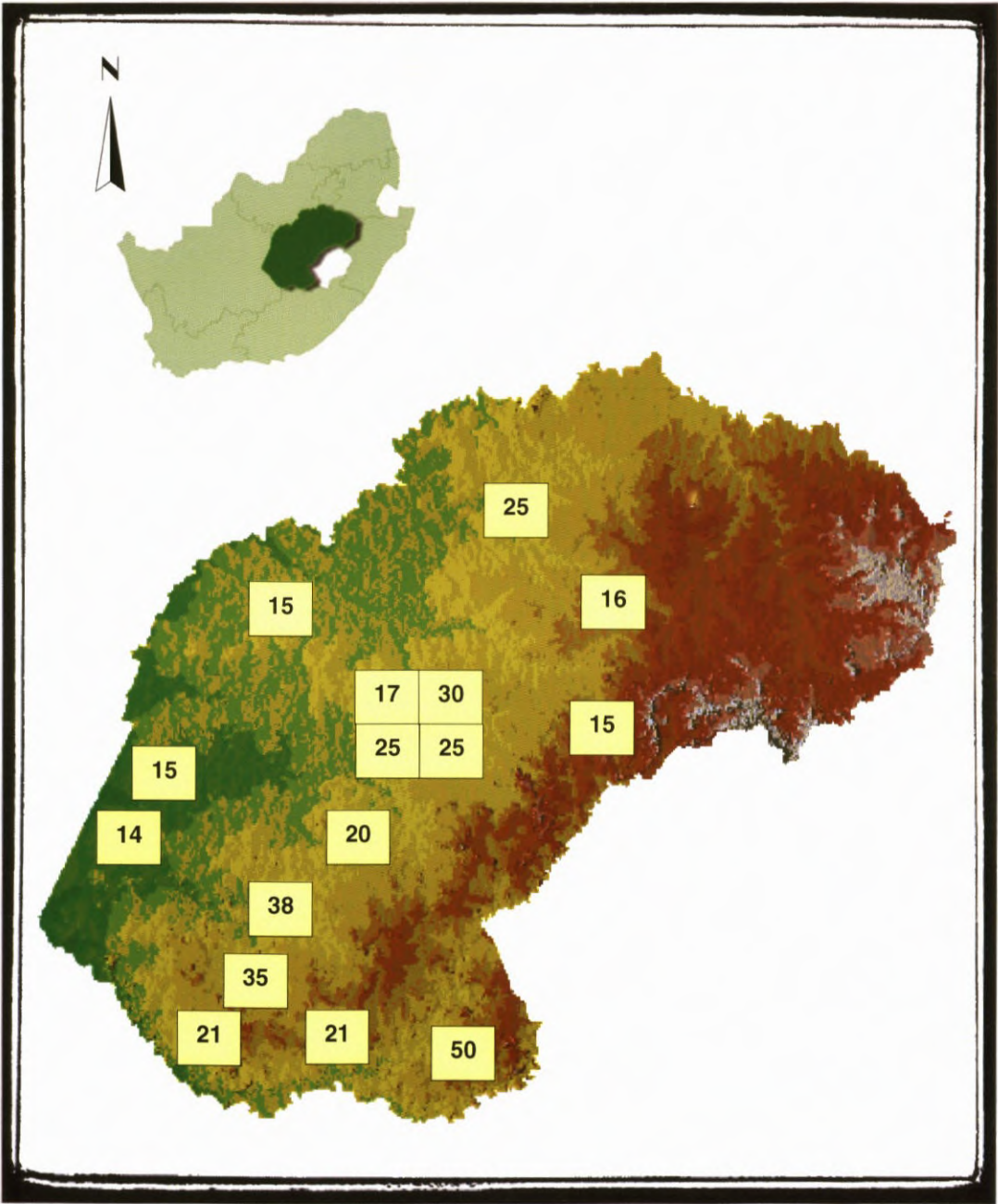


**Figure 4.4** Enlarged hindquarters of a bontebok individual. Measurements are taken along the straight blue lines, allowing measurements a, b, c and d.





**Figure 4.5** Hypothetical distributions on the discriminant function (red line) of bontebok, blesbok and hybrid populations. Adapted from Fabricius *et al.* (1989).



**Figure 4.6** Distribution and population sizes of registered pure bontebok herds in the Free State Province.

provide a practical alternative to distinguish between pure bontebok, pure blesbok and hybrid populations.

## 4.2 BODY MEASUREMENTS

In addition to the use of body measurements as a means to identify inter-specific hybrids, morphometric data has proven to be of use in a range of studies including sex-specific characteristics, inter-specific comparisons and intra-specific population comparisons (Anderson & Koen, 1993). Several researchers noticed the value of developing equations predicting body weight from linear measurements and success has been obtained in studies involving Alpine ibex (*Capra ibex*) (Bassano *et al.*, 2003), Dall's sheep (*Ovis dalli dalli*) and mountain goats (*Oreamnos americanus*) (Bunnell, 1980) as well as some east African mammals (Talbot & McCulloch, 1965). Furthermore, body measurements of Arabian horses (*Equus caballus*) proved to be practical in determining body conformation and, in turn, allowing evaluation and comparison of breeds (Sadek *et al.*, 2006). Boniface (2002) suggested that further aspects of applicability be investigated and established that an index derived from measures of variation in body weight among large grazing mammals appeared to be advantageous in determining species diversity. In addition, measurements of body mass of individuals are also considered to be a good indication of potential reproductive success (Bourgarel *et al.*, 2002).

At present, published data on the morphometrics of blesbok and bontebok focused primarily on body weight, shoulder height and horn length (Estes, 1992; Kingdon, 1997; Apps, 2000; Stuart & Stuart, 2000; Cillié, 2004; Skinner & Chimimba, 2005). Sample sizes and locality were seldom referred to and when indicated, body measurement means were calculated from less than 30 individuals. It also appears that these estimates were limited to adult specimens, since no mention was made of data regarding sub-adults. On average, male blesbok (70.0 kg) are heavier than females (61.0 kg) (Apps, 2000; Stuart & Stuart, 2000; Cillié, 2004) and body weight ranges between 65.0 - 80.0 kg and 55.0 - 70.0 kg for males and females, respectively (Kingdon, 1997). According to Bothma *et al.* (2002), there is little noticeable difference in shoulder height of males (95.0 cm) and females (93.0 cm), while average horn length measurements (38.0 cm) are only specified for rams (Apps, 2000;

Stuart & Stuart, 2000). Significantly less data is presented for bontebok, in particular regarding females. Skinner & Chimimba (2005) stated that males weigh between 59.6 - 63.6 kg and that the mean weight (61.0 kg) is 9.0 kg less compared to blesbok. Bontebok are thus 15 - 20% smaller in terms of built. According to Estes (1992) a female bontebok has a mean weight of 56.0 kg, while other literature simply stated that females are smaller and lighter compared to males. Once more, shoulder height was found to be similar for both sexes (males, 90.0 cm; females, 87.0 cm) (Bothma *et al.*, 2002) and estimates of mean horn length (36.0 - 38.0 cm) were only available for males (Roberts, 1951; Stuart & Stuart, 2000). Consequently, it is obvious that additional standard morphometric measurements are lacking for both subspecies.

Annual hunting and culling provided ample opportunities for collection of body measurements from a great number of blesbok throughout the Free State. Game reserves often have to crop high density populations to prevent or alleviate perceived habitat degradation, whereas surplus blesbok at game reserves were also utilised by the public for sport hunting. On the contrary, body measurements obtained for bontebok in the Free State were restricted to a small sample size ( $n = 3$ ) of only adult males. Owing to their fragile nature and the high market value of these animals, obtaining measurements from a live bontebok is next to impossible and data could only be obtained from dead specimens. Taking measurements during capture operations for translocation purposes were often abandoned after a short while, since the risk of individuals dying from stress related causes are too high. In cases of successful capture, physical handling of animals were kept to a minimum, allowing just enough time to administer a tranquiliser before loading them onto a transport vehicle. Despite these precautions, during the actual transportation to a new location, the risk of stressed individuals dying is still relatively high. The extent of their fragility was observed first hand at a game auction in the central Free State. An adult male died within a couple of minutes whilst being handled following its arrival. Accumulated stress of the capture, transport and physical handling caused it to die almost instantly.

Data of body measurements collected over a four-year period in the Free State are shown in Table 4.1. On average adult males were roughly 7.0 kg heavier compared to females (73.8 kg vs. 67.2 kg). Live weight (LW) of both sexes also showed great variation, ranging from 53.0 - 93.0 kg and

**Table 4.1** Body measurements of blesbok in the Free State Province. Weight (kg); other measurements (cm); standard deviation ( $\delta$ ).

MEASUREMENTS	MALES					FEMALES				
	Adults				Sub-adults	Adults				Sub-adults
	<i>n</i>	$\bar{x}$	Range	$\delta$	( <i>n</i> = 9)	<i>n</i>	$\bar{x}$	Range	$\delta$	( <i>n</i> = 10)
Weight/length ratio	176	0.4		0.1		129	0.4		0.0	
Live weight	336	73.8	(53 - 93)	7.2	58.1	195	67.2	(52 - 81)	5.3	55.1
Total body length	176	175.1	(104 - 200)	17	173.7	129	173.4	(136 - 193)	14.3	166.4
Head length	175	43.3	(26 - 57)	3.3	41.0	129	41.1	(33 - 48)	2.6	38.6
Chest girth	175	101.9	(76 - 120)	4.4	92.7	134	100.4	(89 - 123)	4.9	94.3
Hindfoot	176	43.5	(31 - 47)	1.6	42.9	129	43.1	(40 - 46)	1.3	41.6
Tail length	176	33.8	(22 - 45)	3.8	33.8	129	32.1	(21 - 43)	3.4	31.6
Shoulder height	174	104.4	(89 - 125)	4.0	99.7	132	101.0	(88 - 109)	3.4	97.6
Horn length	182	38.7	(30 - 46)	2.9	33.5	155	35.1	(24 - 42)	2.9	31.1
Horn circumference	175	17.6	(16 - 27)	1.3	16.5	130	13.7	(12 - 21)	1.1	12.3
Horn spread	155	23.7	(17 - 35)	2.4	22.8	126	21.4	(15 - 28)	2.1	17.7
Distance horn tips	172	17.0	(11 - 31)	3.2	13.9	129	15.9	(9 - 24)	2.4	13.0
Ear length	176	15.5	(14 - 18)	0.8	15.7	130	15.4	(14 - 18)	0.8	13.0

52.0 - 81.0 kg for rams and ewes, respectively. Variation of body weight between sexes and individuals are in all probability influenced by nutrition (Van Rooyen, 1993) as seasonal changes determine vegetation availability. Variation is also influenced by sexual cycles as the sexes have different lifetime strategies (Van Rooyen, 1993; Capellini, 2006). In relation, some authors have established that male impala are more susceptible to changes in food resource abundance and quality than ewes and they suffered a seasonal decrease in body mass during the rut (Van Rooyen, 1993; Bourgarel *et al.*, 2002). This trend was also observed for male nyala (Van Rooyen, 1993). Therefore, it is probable that territorial adult blesbok rams would also lose weight during the rutting season (March - May) due to a combination of reduced food intake and high energy expenditure. On the other hand, various studies have confirmed that female ungulates are seemingly less affected by seasonal variation of available resources (Post *et al.*, 1999; Le-Blanc *et al.*, 2001), though their weight ought to vary in accordance with reproductive status (pregnancy and lactating). One of the widespread features of the majority of species is that sexual activity takes place during a particular time of the year to ensure that birthing takes place at the optimal time of year (Santiago-Moreno *et al.*, 2006). With reference to blesbok, the peak birthing period is from November - December, continuing into January (Cillié, 2004). This guarantees that calf growth takes place under suitable environmental conditions. Females reach sexual maturity at the age of 2 - 3 years and following a gestation period of around eight months, a single lamb is born. Therefore, it is reasonable to assume that variation in female body weight can be expected during these months associated with high energy requirements for gestation and lactation.

An additional factor to consider is that hunting is authorised from the beginning of May to the end of August and as a result, the majority of measurements were taken at the onset of the hunting season (May/June). According to Bothma *et al.* (2002) blesbok have already achieved their highest weight gain during February, followed by a loss of 12% of their body weight during the rutting season and winter months (March - August). Even though the mean live weights (for both sexes) are greater compared to available literature, it is probable that the measurements would have been even higher if obtained three months earlier.

Carcass weight (CW) refers to the weight of a carcass which has been flayed, thus the head, feet and contents of the thoracic cavity has been removed. Carcass weight of rams ( $n = 5$ ) ranged between 48.0 - 54.0 kg with a mean of 43.4 kg, while that of females ( $n = 4$ ) were found to measure around 10 kg (33.0 kg) less and fell within a range of 37.0 - 45.0 kg. Reliable data of carcass weight is relatively scarce, but Bothma *et al.* (2002) estimated that the carcass weight of wild ungulates generally varies from 50 - 58% of the live weight of the animal. The obtained measurements concurred with these estimates, seeing that carcass weight represented 55% and 49% of LW of males and females, respectively. One can assume that carcass production and individual growth rates are similar for bontebok.

Minimal differences between the sexes were noticeable concerning mean measurements of total body length (< 2.0 cm), head length (< 2.5 cm), chest girth (1.5 cm), hindfoot (< 0.5 cm), ear length (0.1 cm) and tail length (< 2.0 cm) measurements (Table 4.1). Pertaining to the latter, it is a general rule that the tuft of hair on the tail is longer and has more white hair for rams than ewes (Du Plessis, 1968).

Shoulder height as well as the various horn measurements showed greater differences. In general, horns of males are longer and thicker than those of females and in both sexes the horns are strongly ridged for 75% of the horn length, except at the tip. Differences concerning horn length (HL) and horn circumference (HC) of the left and right horn were not noteworthy (< 0.4 cm), therefore left and right horn measurements were added together and a mean was calculated. Mean HL of adult rams (38.7 cm) measured 3.6 cm longer than the mean determined for adult ewes (35.1 cm). The ridges of the horns were also found to be more prominent in the rams. Maximum horn lengths of 46.0 cm and 42.0 cm were obtained for males and females, respectively. Horn spread and the distance between horn tips were larger in adult rams than in adult females and differed 2.3 cm and 1.1 cm, respectively, while HC of ewes (13.7 cm) measured 3.9 cm less than that of the males (17.6 cm). This difference is clearly visible when viewing an animal from the front or from the side. In males, it is generally accepted that horns function as weaponry for intra-specific combat (Clutton-Brock, 1982; Packer, 1983) and horn size is expected to be larger relative to body weight in polygynous bovid species as conflict associated with male mating success increases and male-male



competition becomes more intense (Clutton-Brock & Harvey, 1980). By comparison, no general consensus regarding functional explanations for horns on female ungulates exists. Conflicting explanations have taken account of defence against predators (Packer, 1983), intra-specific competition for resources among conspecifics (Geist, 1977; Clutton-Brock, 1982; Roberts, 1996) as well as heterosexual mimicry to protect male offspring against aggression of dominant males (Estes, 1991).

All of the morphometric results obtained indicated that males and females exhibit similar built, since outstanding differences were limited to body weight, several horn measurements and to a lesser extent shoulder height. These findings possibly explain why available data focussed in particular on these measurements.

In comparing the means of the genders, the *t*-test for independent groups assuming homogeneous variances was used when the *F*-test variances were not significant, whereas the *t*-test assuming heterogeneous variance was used when the *F*-test for homogeneity was significant. Complimentary to the *t*-test, effect size was calculated according to Cohen's *d*, to estimate the strength of statistically significant results. This is particularly important when analysing such large sample sizes seeing that effect size indices are independent of sample size. As illustrated in Table 4.2, highly significant gender differences between adult blesbok were marked for nearly all body measurements, total body length and ear length being the only exceptions.

From Table 4.1 mean measurements of adults and sub-adults can be compared for both sexes. According to Bothma *et al.* (2002) male lambs have a linear growth rate from about 6.4 kg at birth and attain a weight in the region of 50.0 kg within a year, while female lambs reach 41.0 kg within a period of 9 months. During the following three years the growth rate steadily decreases until rams reach a body weight ranging from 75.0 - 80.0 kg and around 70.0 kg in ewes. Mean LW of sub-adult males (*n* = 9) measured 58.1 kg, while sub-adult females (*n* = 10) measured 55.1 kg. Compared to adult estimates, differences of 15.7 kg and 12.1 kg were evident for males and females, respectively.

**Table 4.2** *T*-test statistics, degrees of freedom, *p*-values and effect size for gender body measurement differences of blesbok in the Free State Province.

MEASUREMENTS	t-value	df	p	Cohen's <i>d</i>	Significance
Weight/length ratio	4.429	302	0.000	0.5	$p < 0.001$
Live weight	11.990	501	0.000	1.0	$p < 0.001$
Total body length	0.912	297	0.363	0.0	
Head length	6.665	302	0.000	0.7	$p < 0.001$
Chest girth	2.830	307	0.005	0.3	$p < 0.01$
Hindfoot	2.611	300	0.001	0.3	$p < 0.01$
Tail length	3.782	303	0.000	0.4	$p < 0.001$
Shoulder height	7.763	304	0.000	0.9	$p < 0.001$
Horn length	11.111	335	0.000	1.2	$p < 0.001$
Horn circumference	27.915	303	0.000	3.2	$p < 0.001$
Horn spread	8.723	279	0.000	1.1	$p < 0.001$
Distance horn tips	3.674	299	0.000	0.4	$p < 0.001$
Ear length	0.601	304	0.549	0.1	

Total body length (TBL) did not differ much between male age classes (< 1.5 cm). Sub-adult females, however, measured 7.0 cm less than adult females. Measurements pertaining to head length, hindfoot length, tail length and ear length did not differ much between the age groups of both sexes. Chest girth (CG), shoulder height (SH) and the respective horn measurements showed more noticeable differences. In relation to CG, lesser values of 9.2 cm and 6.1 cm were obtained for sub-adult males (92.7 cm) and sub-adult females (94.3 cm), while SH measurements of adults and sub-adults of both sexes differed less than 5.0 cm. When comparing horn measurements of adult and sub-adult males, the latter measured 5.2 cm less for HL, 1.1 cm for HC, only 0.9 cm less for horn spread and 3.1 cm less in terms of distance between horn tips. Sub-adult females showed similar values of 4.0 cm for HL, 1.4 cm for HC, 3.7 cm for horn spread and 2.9 cm in terms of distance between horn tips.

Various authors have commented on the possible influences of season, rainfall, habitat quality and geographic location regarding morphometric variations (Talbot & McCulloch, 1965; Kutilek, 1979; Bourgarel *et al.*, 2002; Garroway & Broders, 2005; Capellini, 2006). With regards to the abovementioned influences, Table 4.3 shows the morphological measurements determined for blesbok from three different geographical regions, allowing comparisons within the Free State. Morphometric data was obtained from four different game reserves located in the eastern, central and southern regions of the province (*vide* Fig. 2.5). There are thus noticeable regional divisions and the two main factors concerning differentiation include the distribution of rainfall and changes in altitude (Smith, 2007).

In general, all measurements obtained from specimens in the central region of the Free State coincide with the mean ranges determined for the entire province (*vide* Table 4.1). Noticeable differences are evident when comparing live weight and total body length of the eastern and southern regions. Both sexes representative of the eastern region yielded higher weight measurements, seeing that males weighed nearly 13.0 kg more (84.6 kg vs. 72.1 kg), while a difference of 14.5 kg was noted pertaining to females. Given that the nutritional value of the veld varies during different seasons of the year and this factor being a major determinant of herbivore body condition (Van Rooyen, 2002b), the measurement dissimilarities could possibly be linked to

**Table 4.3** Sample size, mean body measurements and standard deviation of adult blesbok from different geographical areas in the Free State Province. Weight (kg); other measurements (cm).

MEASUREMENTS	MALES									FEMALES								
	Eastern			Central			Southern			Eastern			Central			Southern		
	n	$\bar{x}$ -bar		n	$\bar{x}$ -bar	$\delta$	n	$\bar{x}$ -bar	$\delta$	n	$\bar{x}$ -bar		n	$\bar{x}$ -bar	$\delta$	n	$\bar{x}$ -bar	$\delta$
Weight/length ratio				19	0.5	0.1	147	0.4	0.1				16	0.5	0.1	97	0.4	0.1
Live weight	8	84.6		19	74.7	9.0	159	72.1	6.7	1	81		18	68.2	7.3	98	66.5	5.0
Total length	8	146.4		19	146.9	15	147	180.2	12.0	1	142		16	147.8	12.0	97	177.1	11.0
Head length	8	45.3		19	42.7	3.1	146	43.3	3.4	1	46		16	41.5	2.9	97	41.1	2.4
Chest girth	8	99.5		19	100.7	4.1	146	102.2	4.5	1	105		16	97.8	16.0	98	100.2	5.0
Hindfoot	8	44.1		19	44.2	1.2	147	43.4	1.7	1	44		16	42.9	1.5	97	43.1	1.4
Tail length	8	34.9		19	34.0	6.0	147	33.5	4.0	1	32		15	30.2	2.5	98	32.2	3.6
Shoulder height	8	107.1		19	105.0	5.6	145	104.2	3.6	1	109		16	102.2	1.7	98	101	3.5
Horn length	8	38.6		19	37.7	2.2	116	38.9	3.0	1	36		18	35.7	1.8	87	35.6	2.5
Horn circumference	8	17.8		19	18.6	0.9	110	17.5	1.2	1	14		16	14.1	0.8	87	13.6	1.2
Horn spread	8	25.4		19	22.9	1.7	126	23.8	2.4	1	26		17	21.1	2.9	93	21.5	2.0
Distance horn tips	8	19.1		19	17.5	3.8	143	16.9	3.1	1	19		16	16.9	2.0	91	15.6	2.5
Ear length	8	16.2		19	16.0	0.9	147	15.4	0.8	1	17		16	16.5	2.5	98	15.3	0.8

differences in rainfall across the province, which in turn determines the quantity and quality of available grass. The Free State is characterised by somewhat low and irregular rainfall and there is an increasing gradient of rainfall from east to west (*vide* Fig. 2.4). In relation, sampling localities from the eastern and southern regions fall under sour veld and sweet veld habitats (*vide* Fig. 2.3). Sour veld is found in the high-lying regions where the rainfall is 580 mm per year or higher, while sweet veld occurs in the lower-lying, semi-arid savannas that receive from 200 - 500 mm rainfall annually (Low & Rebelo, 1996).

The most important grazing plants of the sour veld region tend to lose their palatability and dietary value subsequent to the growing season, hence animals are capable of maintaining good condition for about six to eight months of the year, while the primary grazing plants of sweet veld can be utilised throughout the year as palatability and nutritional value is retained for the complete life cycle (Van Rooyen, 2002b). Moreover, protein, calcium and phosphate scarcity are often associated with the grasses of sour veld. Although food quantity increases as the season progresses, its quality decreases towards the end of the growing season. Only half of the crude protein content of grasses available at the beginning of the growing season is still available at the end of it (Van Rooyen, 2002b). Nutrient deficiencies are not as pronounced in sweet veld areas compared to sour veld habitats, though given the high palatability of sweet veld grasses it is easily overgrazed (Van Oudtshoorn, 1999). On the other hand, the environment of high rainfall regions is considered fairly invariable, while the dynamics of semi-arid grazing systems are heavily subjected to rainfall. Despite the lower nutritional values of food resources associated with sour veld, food is more readily available in the eastern region, while the availability and species composition of forage is strongly limited by the lower and irregular rainfall in the southern areas of the province. Another factor to consider is that sweet veld are generally characteristic of areas with mild winters and nearly no frost (Van Oudtshoorn, 1999). The southern region of the Free State, however, tends to experience harsh winters with frequent frost and this should also influence the availability of food resources. Taking all of these factors into consideration, the observed weight variations could possibly be a result of the greater rainfall in the eastern area that provides more plentiful food leading to improved condition of animals. In relation, Bourgarel *et al.* (2002) found that impala populations inhabiting areas subjected to high annual rainfall were noticeably heavier compared to other populations from areas of lower

rainfall. However, the small sample sizes of the current study could also be a factor contributing to the differences between body weights among regions.

Pertaining to total body length, a contrasting trend was evident in both sexes (*vide* Table 4.3). Individuals from the southern region measured appreciably more in terms of TBL, regardless of measuring the lowest mean LW for all three regions. Toïgo *et al.* (2006) established that along a scale from favourable to unfavourable conditions, body weight is expected to show a decrease. Body size should remain rather stable, so that body condition should thus decrease. Therefore, the effects of available food resources influenced by climatic factors in the southern region were possibly responsible for a decrease in weight, but did not affect other aspects of body size. Even so, these TBL measurements are considered average compared to the results for the entire province, since TBL of males showed great variation ranging from 104.0 - 200.0 cm (*vide* Table 4.1).

Due to limited sample sizes for blesbok from the eastern region, this category was excluded from statistical analysis, hence only blesbok from the central and southern regions are presented in Table 4.4. Genders from each region were combined to determine if there are any statistically significant regional differences irrespective of gender. Differences in measures of live weight were small between the two regions, however greater variation was again evident when comparing total body length. Accordingly the latter proved to be of statistical significance at the 0.1% confidence level. Regional LW differences were not significant, hence the ratio between LW and TBL were calculated as it appears to be a good indication of body condition and an important variable to take into account. Table 4.4 shows that the relation between these two variables were highly significant ( $p < 0.001$ ). HL and EL measurements differed by less than 1.0 cm, but were nevertheless found to be statistically significant at the 5% and 1% confidence levels, respectively.

In addition, regional differences between males from the central and southern regions and between female populations representative of these two areas were analysed (Table 4.5). By comparison, regional differences of TBL and weight/length ratio were significant ( $p < 0.001$ ) for both genders. Measurement differences for HC and EL were also highly significant ( $p < 0.001$ ) pertaining to males, whereas TL and SH measurements of females were found to be statistically significant at the



**Table 4.4** Descriptive statistics of the sample size, mean and standard deviation for blesbok from the central and southern regions of the Free State Province. *T*-test statistics, degrees of freedom, *p* values and effect sizes are also indicated.

MEASUREMENTS	CENTRAL			SOUTHERN			t-value	df	p	Cohen's <i>d</i>	Significance
	<i>n</i>	$\bar{x}$ -bar	$\delta$	<i>n</i>	$\bar{x}$ -bar	$\delta$					
Weight/length ratio	35	0.5	0.1	244	0.4	0.1	- 8.573	38	0.000	2.11	$p < 0.001$
Live weight	37	71.5	8.8	257	70.0	6.6	- 1.063	42	0.294	0.23	
Total length	35	147.3	13.6	244	179.0	11.5	14.908	277	0.000	2.70	$p < 0.001$
Head length	35	42.2	3.0	243	42.4	3.2	0.447	276	0.655	0.08	
Chest girth	35	99.4	11.0	244	101.4	4.8	1.077	36	0.289	0.37	
Hindfoot	35	43.6	1.4	244	43.3	1.6	- 1.127	277	0.261	0.20	
Tail length	35	32.3	5.1	245	33.0	3.9	0.740	38	0.464	0.17	
Shoulder height	34	103.7	4.4	243	102.9	3.9	- 1.148	276	0.252	0.21	
Horn length	35	36.7	2.2	251	37.6	3.3	2.062	62	0.043	0.27	$p < 0.05$
Horn circumference	37	16.6	2.4	244	15.9	2.3	- 1.583	277	0.115	0.29	
Horn spread	35	22.0	2.5	219	22.8	2.5	1.719	253	0.087	0.31	
Distance horn tips	36	17.2	3.1	234	16.4	2.9	- 1.519	267	0.130	0.28	
Ear length	35	16.3	1.8	245	15.4	0.8	- 2.965	36	0.005	1.03	$p < 0.01$

**Table 4.5** *T*-test statistics, degrees of freedom, *p* values and effect sizes for male and female blesbok from the central and southern regions of the Free State.

MEASUREMENTS	MALES					FEMALES				
	<i>t</i> -value	df	<i>p</i>	Cohen's <i>d</i>	Significance	<i>t</i> -value	df	<i>p</i>	Cohen's <i>d</i>	Significance
Weight/length ratio	- 7.256	20	0.000	2.34	<i>p</i> < 0.001	- 5.755	17	0.000	2.15	<i>p</i> < 0.001
Live weight	- 1.585	176	0.115	0.39		- 0.926	20	0.366	0.31	
Total length	11.185	164	0.000	2.74	<i>p</i> < 0.001	9.951	111	0.000	2.69	<i>p</i> < 0.001
Head length	0.667	163	0.506	0.16		- 0.533	111	0.595	0.14	
Chest girth	1.411	163	0.160	0.34		0.601	16	0.557	0.37	
Hindfoot	- 1.904	164	0.059	0.47		0.476	111	0.635	0.13	
Tail length	- 0.365	20	0.719	0.12		2.116	111	0.037	0.59	<i>p</i> < 0.05
Shoulder height	- 0.639	20	0.530	0.22		- 2.159	40	0.037	0.37	<i>p</i> < 0.05
Horn length	1.616	170	0.108	0.39		- 0.170	114	0.865	0.04	
Horn circumference	- 3.942	163	0.000	0.96	<i>p</i> < 0.001	1.703	112	0.091	0.46	
Horn spread	1.555	143	0.122	0.38		0.555	19	0.586	0.19	
Distance horn tips	- 0.800	160	0.425	0.20		- 1.872	105	0.064	0.51	
Ear length	- 3.588	164	0.000	0.88	<i>p</i> < 0.001	- 1.912	15	0.075	1.20	

5% confidence level. On the whole, differences between populations representative of the various climatic regions appear to be somewhat trivial and perhaps easily accounted for by small-sample variation and/or regional nutritional differences.

There are currently three measuring systems in use to measure a trophy for its recognition as a record and these include the Rowland Ward system, Safari Club International (SCI) and the South African systems. According to Bothma *et al.* (2002), available data suggests that a limited 5 - 10% of all the animals in the majority of herbivore populations develop into adults that qualify as trophy quality. As a general rule trophy hunting is restricted to males and Bothma *et al.* (2002) commented that the optimal age for harvesting a male trophy is around 5 years, seeing that from then on the use of its horns in territorial disputes as well as day to day wear can have an undesirable effect on the quality and size of the trophy.

Irrespective of the system used, a general measuring method is applied. The length of each horn is measured on its front surface whilst carefully following the direction of the grain for the full length of the measurement, while circumference is measured at the base or as close to the base or hairline as possible. The distance between the horn tips is measured in a straight line from the tip of the one horn to the tip of the other horn. The Rowland Ward system originated in 1892 and trophies from any part of the world can be registered for publication in the record book if the specimen surpasses the minimum requirements. This system necessitates that measurements are taken by an official measurer using the imperial scale and fractions are approximated to the nearest  $1/8^{\text{th}}$  of an inch. All of the abovementioned horn parameters are taken, but it is the horn length measurement that determines the position obtained on the trophy list (Van Rooyen & Van Rooyen, 2002). The SCI system was published for the first time in 1977 and it is the only system that provides utmost recognition regarding horn growth, since horn length and horn circumference of both horns are taken into consideration. Though, only official measurers appointed by SCI are allowed to evaluate trophies. The scores of all four measurements are totalled and the individual is ranked accordingly, a minimum total score (in inches) of 39 is permitted for blesbok and a score of 36 is the allowed minimum regarding bontebok (Cornus, 2005). The Confederation of Hunters Associations of South Africa (CHASA) deemed it necessary to develop a wholly South African measuring system for hunting

trophies. The same as with the other systems only official measurers, in this case trained by CHASA, may take measurements (in inches) for registration (Van Rooyen & Van Rooyen, 2002). Trophies are however restricted to African species hunted by CHASA members only. Horn length is determined for the longest horn, while horn circumference is measured from the thickest horn. The distance between horn tips is also measured, but the position attained on the trophy list is determined by the length of the longest horn.

Measurements from 24 trophy individuals were also acquired in the Free State, permitting comparisons with Roland Ward, SCI and CHASA trophy records (Table 4.6). The minimum requirements for blesbok (41.9 cm) and bontebok (35.6 cm) are standard for both the Rowland Ward and CHASA systems. Record measurements pertaining to both blesbok (52.4 cm) and bontebok (47.5 cm) are highest for the SCI record system, while the lowest record measurements are registered with CHASA. By comparison, the blesbok record is a massive 10.0 cm longer than that of bontebok and exceeds the maximum blesbok horn length obtained in the Free State with 6.4 cm (*vide* Table 4.1). Mean HL of blesbok determined from SCI records (Cornus, 2005) measured almost 7.5 cm more compared to Rowland Ward (Ward, 1998), whereas both systems yielded equal mean HC measurements (Table 4.6). In relation, the means obtained from trophy blesbok hunted at Gariep Dam Nature Reserve met the minimum requirements of Rowland Ward as well as CHASA. Mean HL of these individuals measured 4.5 cm less than the SCI mean, but measured 2.9 cm longer compared to Rowland Ward means. Horn circumference means were more or less the same in comparison with SCI, but roughly 1.0 cm more compared to Rowland Ward.

During 2006, trophy bontebok reached high market values in the region of R8 000 - R10 000. In general, such steep prices limit bontebok trophy hunting to overseas hunters and for that reason very few bontebok are taken as trophies compared to the high number of blesbok hunted annually. Measurements could therefore only be obtained from two trophy specimens. The mean HL established for bontebok in the province were found to be lower than both SCI and Rowland Ward means (Table 4.6). Ward (1998) also specified two other measurements for a single bontebok individual, namely live weight (90.7 kg) and shoulder height (93.3 cm). By comparison, a mean weight of 80.0 kg and mean shoulder height of 91.4 cm was determined for the Free State trophy specimens.

**Table 4.6** Various criteria to determine trophy quality of blesbok and bontebok according to three trophy measuring systems as well as measurements of trophy animals obtained in the Free State Province. Measurements are in cm.

SYSTEM	HORN MEASUREMENTS											
	Minimum Requirements		Record Measurements		Mean Horn Length				Mean Horn Circumference			
	Blesbok	Bontebok	Blesbok	Bontebok	Blesbok	<i>n</i>	Bontebok	<i>n</i>	Blesbok	<i>n</i>	Bontebok	<i>n</i>
Rowland Ward	41.9	35.6	52.4	42.5	39.2	39	38.7	20	16.3	39	16.3	20
	16.5	14.0	20.6	16.8								
SCI			59.4	47.5	46.6	38	41.3	37	17.7	38	16.9	37
	39.0	36.0	21.6	18.7								
CHASA	41.9	35.6	49.9	39.4								
	16.5	14.0	19.6	15.5								
Free State					42.1	22	36.9	2	17.6	22	16.1	2



It is thus probable that these individuals received supplementary artificial feed that led to increased weight, given that weight measurements were 20.0 - 30.0 kg above the norm. Artificial feeding products can possibly also lead to increased horn development (in bases and length) and cause animals to reach sexual maturity at an earlier age (Schmidt & Snyman, 2002).

Given that morphometric measurements were determined from a very large sample size ( $n = 562$ ), the obtained data can be considered a good indication of blesbok representative of the Free State. Blesbok demonstrate low levels of sexual dimorphism and noticeable differences between the sexes were mainly limited to live weight and the different horn parameters. Gender, age, social status and various environmental factors influencing habitat quality are all potentially important factors affecting variability of body measurements.



Chapter 5:

# Activity Patterns



## 5 ACTIVITY PATTERNS



The survival of a species is dependant on its ability to adapt to a particular ecological niche. The nature of the niche is partially dependant on the environment, but also on the morphology, physiology and behaviour of the species which sequentially establishes the utilisation capabilities of the animal concerning that environment (Delany & Happold, 1979). Behavioural adaptations enable an individual to utilise these environmental components that are vital for its survival, hence behaviour largely determines the ecological characteristics of a species and thus niche selection. Environmental conditions of habitats differ regionally, each consisting of its own characteristics of flora, resources, weather conditions and seasonality and these effects can modify or alter the expression of the behavioural characteristics of the species so that a single species may show different behavioural patterns in different environmental conditions (Leuthold, 1977).

Furthermore, it is widely accepted that a variety of behavioural activities performed by animals are not uniformly distributed in time since there are rather distinctive phases in which certain activities are outweighed by others (Leuthold, 1977). The activity pattern is thus considered descriptive of the particular sequence of these phases and is an important component of eco-ethology. According to Aschoff (1964), daily activity patterns result from a complex trade-off between optimal foraging time, social activities and interactions with several properties of the environment. In relation, Leuthold (1977) recognised the adaptive value of activity patterns and suggested that the activity pattern of any given animal could considerably vary on a daily basis. This implies that the amount of time spent daily on each activity would not be constant, nor would the time of day when it was carried out (David, 1973). As a result, activity patterns reflect the animal's behavioural adjustment to daily and seasonal variation in the environment and the latter variation is primarily governed by the accessibility and quality of food and/or water (Van Hoven, 2002b; Ryan & Jordaan, 2005). It is therefore considered vital for an organism's survival that several

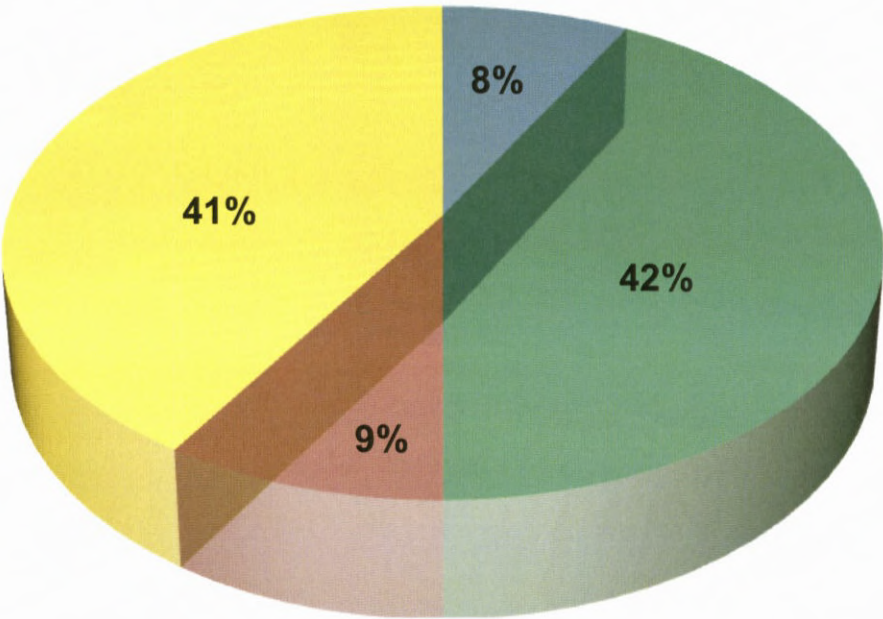
physiological functions and behaviours demonstrate not only diurnal, but also seasonal variations (Meijer *et al.*, 2007). Norton (1981) recognised the applicability of activity pattern quantification in different areas and seasons as a useful method to gain sufficient knowledge of how each species interacts with its environment, given that information on the capacity to adapt to varying conditions provides the needed scientific basis for practical management decisions on matters such as suitability of habitat for translocations and optimum seasons for culling or capturing.

## 5.1 TIME BUDGET

### 5.1.1 General time budget

Behavioural data from bontebok were used to quantify general time budget and diurnal activity patterns. All observational data were grouped together, allowing comparisons with other animal species, but most importantly with blesbok in the Free State (Van Aswegen, 1994).

The time budget of an individual constitutes the allocation of a certain amount of time an animal devotes each day to different activities and is a common measurement in behavioural ecology studies, representing a major aspect of the temporal behaviour of animals. An analysis of how bontebok distribute their time among various activities seems to be an important precursor to understanding the interrelations between ecological influences and behaviour. Therefore behavioural states are divided into basic daily components which include grazing, moving (i.e. walking, running, play, fighting), lying and standing. Due to the variable total number of observational recordings throughout the study period the results are presented on a percentage basis. The first two categories are regarded as active phases and the last two mentioned categories are considered inactive phases, thus active time was determined as a proportion of total time of daylight hours by subtracting periods spent inactive. Time devoted to each activity category is shown in Figure 5.1 and it is evident that an equal amount of time was spent on active (50%) and inactive phases (50%). Overall, grazing and lying were the major activities embarked upon and these categories represented more than three quarters (83%) of total observed activity. Less than one fifth of their time budget was diverted to the remaining categories, namely standing (9%) and moving (8%).



**Figure 5.1** Time budget of diurnal activity patterns of bontebok in the Free State Province.



These findings deviate slightly from the results obtained for blesbok by Van Aswegen (1994). This author reported that blesbok allocated more time to lying (40.4%) than to grazing (35.5%), while standing (16.5%) and moving (7.7%) represented nearly a quarter of the general time budget. By comparison, blesbok and bontebok spent almost equal amounts of time on lying and moving, whereas bontebok allocated more time (6.5%) to the grazing category and less time (7.5%) to standing activity. Even though comparison of blesbok and bontebok time budgets indicate some dissimilarities, the same underlying factors that influence each of the behavioural categories should be applicable to both subspecies.

Evolutionary theory hypothesises that the definitive objective of a living organism is to capitalise on its inclusive fitness and an important underlying objective must be to obtain optimal food intake, to comply with the nutrient demands of not only survival, but also growth and reproduction (Gordon, 2003). Therefore, as a means to meet individual nutritional and energy demands, an organism is obliged to dedicate a certain portion of its overall activity time budget to feeding. Variations in the amount of time that different species allocate to feeding behaviour are therefore related to diverse nutritional requirements. Furthermore, feeding behaviour is subject to several interrelationships of species' adaptations in grouping with influential factors such as food availability, the manner in which food is gathered (browsing/grazing) the relation between body size and the level of selectivity, mobility as well as physiology of the digestive tract (ruminant/non-ruminants) (Leuthold, 1977; Van Hoven, 2002b). It is thus apparent that an animal's anatomy and physiology undoubtedly influences food choices and that characteristics of the plant material utilised as food, in turn, are one of the primary underlying factors. In addition, the habitat of the related study area influences food resources in terms of quantity and quality, in turn also affecting specific feeding behaviour of a species (Van Hoven, 2002b).

Body size is an important entry point for understanding species' adaptation seeing as it bears upon nearly all aspects of the animal's biology. Numerous physiological and ecological traits of animals scale significantly with body size and keeping in mind that these traits influences behaviour, it is not unforeseen that behavioural patterns should also scale in certain aspects with body size (Peters, 1983). To accentuate the relation of body size and feeding time budgets related to the present study,



dissimilarity between grazers and browsers will foremost be considered. First, the classification of feeding habits regarding herbivores can be approached either in terms of the anatomy of the digestive system or it can be based on the feeding style of the animal. Pertaining to the latter, Jarman (1974) attempted to classify the feeding styles of some bovids along a continuum ranging from grazers to browsers. This classification relates a variety of morphological traits and ecological influences to the different feeding styles of ungulates. Species can appropriately be assigned to a feeding category based on selectivity of food items, which is generally conversely related to body size. Numerous ungulates display a notable degree of evolutionary convergence in view of feeding ecology. Subsequently it has been observed that browsers are prone to be ungulates of small size found in closed habitats, whereas grazers are more often than not larger in size, inhabiting more open areas (Jarman, 1974).

Following Jarman (1974), the foremost variation with regards to the utilisation of grasses or browse concerns the growth form and dispersion of plant material across a landscape. Grasses grow in thick, continuous carpets, while dicotyledonous plants are sparsely distributed and not as readily accessible. Consequently, browsers have to move about more in search of food items and are further constrained by the lesser amount of food per bite that they can ingest. Given the elevated energy expenditure associated with the browsing feeding style, a high-quality diet is typical of browsers. By comparison, grazers generally forage on plant foliage of lower nutritional value, necessitating bulky food intakes to obtain adequate nutrition (Estes, 1992). The circumstances for larger grazers thus deviate from those prevailing for other trophic categories (i.e. browsers) and signify food utilisation of larger ungulates as less effective compared to smaller species.

A definite correlation between body size, diet and food quantity is thus evident, but how does this relate to feeding time budgets? Mysterud (1998) accordingly established that the amount of time ungulates assign to grazing increase in agreement with body weight. For instance, klipspringers (*Oreotragus oreotragus*) have a small body size and allocate 20 - 30% of the day to feeding (Norton, 1981). Larger species, for example plains zebra (*Equus quagga*) demonstrate a 30 - 40% increase in their feeding time budget of around 60% (Twine, 2002). Given that bontebok are regarded as medium-sized antelopes the results obtained from this study show a positive association with time

spent feeding and body size as bontebok show a 10 - 20% increase compared to the smaller klipspringer.

Metabolic rate is another variable related to body size and also influences the type of grazing as well as the frequency of feeding behaviour (Van Hoven, 2002b). Basal metabolic rate scales allometrically with body size (weight), hence antelopes of small body size are subject to higher specific metabolic costs compared to larger herbivores (Lovegrove, 1993). As an example, small species such as the common duiker (*Sylvicapra grimmia*) and steenbok (*Raphicerus campestris*) require more protein per energy unit of body weight than larger species in order to meet their metabolic requirements. Body size can thus be considered a significant determinant of a number of ecological and behavioural traits.

Differences between grazers and browsers also extend beyond diet selection. Differentiation between these two feeding styles also include specialisation within the digestive tract that may permit grazers and browsers to extract nutrients more efficiently from the plant materials included in their respective diets. Subsequently, anatomical differences between ruminants and non-ruminants have several functional correlates. Owen-Smith & Novellie (1982) established that certain factors controlling the rates of ingestion and digestive passage are associated with nutrient availabilities, while several factors in turn influence the conversion of plant material into the needed energy to sustain vital functions of herbivores (Van Hoven, 2002b). These effects are consequently expected to vary amongst species. The abovementioned factors are accordingly influenced by not only the selectivity axes, but also by morphological specialisations within the gastro-intestinal tract of ruminants and non-ruminants (Gordon, 2003). As previously inferred, browsing herbivores feed selectively and their choices of food items are greatly influenced by the need to minimise cellulose intake. Browser diets encompass vegetation that offers more short-chain carbohydrates and lower cellulose contents, therefore grasses are very seldom utilised (Lovegrove, 1993). On the other hand, the majority of larger southern African herbivores are grazing ruminants and the main difficulty they are faced with is the extraction of adequate energy and sustenance from food resources that are easily available, but of low nutritional value (Estes, 1992). Since grasses mainly consist of low protein and high contents of slowly-digestible plant fibers (Lovegrove, 1993), it is vital that the digestive

system of ruminants are capable of converting cellulose into digestible carbohydrates. Various anatomical features of the digestive systems of grazer and browser ruminants are therefore accordingly adapted to allow for the utmost efficient utilisation of food. One such feature is the sizes of the stomach compartments in relation to one another and to body weight and are particular to either grazer or browser ruminants (Van Hoven, 2002a). Bulk eaters of larger body size are commonly characterised by voluminous rumens and longer intestines that allows large intakes of fairly fibrous foods (Mentis & Duke, 1976), while browser ruminants of smaller body size tend to have rumens of smaller capacity. Ruminants are also referred to as foregut fermenters since the ingested plant material is subjected to microbial fermentation in the foregut prior to gastric digestion. All ruminants, except for the small rumen of concentrate feeders, have a dense inner wall lining of papillae to maximise nutrient absorption. This process offers the advantage of more sufficient utilisation of plant fiber, however thorough digestion of cellulose is a lengthy procedure and the more fibrous the food the longer the digestive process. Estes (1992) commented that digestion can take as long as four days from ingestion to excretion in some species.

The order of digestion in non-ruminants (hindgut fermenters) is the exact opposite to that of ruminants as microbial digestion does not precede protein removal, digestion and assimilation (Van Hooven, 2002a). Following passage of forage through the stomach, fermentation takes place in the large intestine and in the caecum (Estes, 1992) and the process thus consists of unrestricted through-flow. Although digestion in a hindgut fermenter is less effective than in a ruminant of comparable size, double the amount of food can pass through its digestive tract in an equal period of time (Ben-Shahar & Coe, 1992; Lovegrove, 1993). They can thereby manage to obtain adequate sustenance from vegetation too tough and fibrous for ruminants to process. Time spent feeding and digestive processing are consequently regarded as important constraints given that due to the bulky, low-quality nature of their food and a slower digestion process bontebok must spend a good deal of their active time foraging. In addition, environmental influences as well as various physiological factors such as stress, pregnancy, lactation and age not only affect the extent of feeding, but also affect feeding behaviour in terms of preference of food selection and the quantity of food that is ingested (Van Hoven, 2002b).

Lying also represents a dominant category of the overall time budget (*vide* Fig. 5.1), but distinct variations amongst different species have been observed in several studies. In accordance with several of these studies, the total amount of time spent lying for bontebok (41%) are relatively lower compared to values obtained for other antelope species. Kok (1975) established that red hartebeest (*A. buselaphus*) spend substantially more time (61%) lying down compared to bontebok, whilst black wildebeest (*C. gnou*) lie down for 45.6% of the day (Vrahimis, 1990). These dissimilarities could plainly be due to behavioural differences of different species, though it is probable that other influences also affect lying time budgets. One such factor could be linked with certain aspects of rumination. Animals ruminate whilst in a relaxed state, either by lying down or in a standing position as this enable unrestricted movement of the stomach compartments (Estes, 1992). Forage is swallowed prior to mastication and when sufficient food has been ingested, it is regurgitated in small amounts and only then chewed with periodic side-to-side jaw movements. Adequate grinding of the food promotes full extraction of nutrients before it is again swallowed for further digestion. Given that observational distances were often very large it was not always possible to discern when bontebok were indeed ruminating. Nevertheless, as the lying category comprised 32% more of the day compared to standing (9%) (*vide* Fig. 5.1) it is assumed that bontebok predominantly ruminated in a lying position. Typical of ruminants, bontebok lay down by first kneeling on their forelegs and then lowering the rear end. They rose in reverse order, raising the rear end first. David (1973) did not differentiate between rumination whilst lying and standing, but he did determine that rumination represented more or less 22% of year-round bontebok activities. Furthermore, Eltringham (1979) commented that ruminators spend more or less equal amounts of time ruminating as they do feeding and this relates to the similar time budgets of feeding and lying exhibited in this study. Considering the amount of time spent on lying, differences between grazer and browser ruminants are yet again an influential factor that deserves attention. Concentrate selectors tend to feed and digest in relatively short bouts and consequently more time is spent on grazing and less on ruminating (Van Hoven, 2002b). The Damara dik-dik (*Madoqua kirkii*) has a fermentation rate faster than that of the vast majority of African ungulates and reduces the time that food is retained in the rumen to between 12 and 20 hours (Lovegrove, 1993), whereas bulk feeders (i.e. bontebok) have larger capacities and slower digestion rates and therefore intervals between foraging bouts tend to be longer. Leuthold (1977) also commented on the possible influences of a range of climatological factors pertaining to

different study areas that in turn affect several aspects of behaviour. In view of that, various authors (Bigalke, 1972; Kok, 1975; Belovsky & Slade, 1986; Nicholson & Husband, 1992; Somers, 1997; Owen-Smith, 1998) suggested temperature to be a further likely influence on activity, for instance a decrease in activity (associated with an increase in lying) is expected at higher temperatures and an increase in inactivity at low ambient temperatures. These probable influences are discussed in more detail later in the chapter.

Standing behaviour is not considered an outstanding activity as it merely represents less than one tenth of the total time budget (*vide* Fig. 5.1). Standing individuals were observed during transitions between grazing and lying and before setting out to a different area. Following grazing, individuals did not lie down right away, but lingered in the standing position for a short period before lying down. Standing individuals were also noted when the herd rose after a resting period and individuals remained standing for a short while before setting out to the next grazing area. Random disturbances, individuals partaking in grooming behaviour and females attending to their calves also added to this category. If suddenly disturbed, alarmed animals assumed a particular standing posture whilst constantly looking at the disturbance (Fig. 5.2) and subsequent to fleeing for some distance they once again stood and stared at the source of interruption. In view of this, it is a possibility that the blesbok herd observed by Van Aswegen (1994) experienced more disturbances during the day which is reflected in their increased time budget for standing activity (16.5%). Grooming and nursing behaviour were also noted when individuals were in a standing position. Whilst lying, some individuals got up at random to groom or perform other maintenance activities. Self-grooming entailed scraping their coats with their teeth (Fig. 5.3), nibbling with their lips and grooming certain areas with the muzzle. Horns were used to scratch the back or rump, while the hindfeet were used to scratch the head, ears and upper forelegs (Fig. 5.3). Lactating females also rose at intermittent times to allow suckling.

Bontebok spend a small portion of the day moving (*vide* Fig. 5.1). Localised movements were observed during the daily search for food resources and other requirements and animals walking from one grazing or resting area to the next were accountable for the greatest amount of time spent on this category. Individuals were observed to nod their heads with commencement of walking (Fig. 5.4)



**Figure 5.2** When alert and tense, bontebok hold the neck stiffly erect with the ears pricked and turned forward.





**Figure 5.3** When self-grooming, individuals **A**, scrape their coats with their lower incisors or **B**, scratch themselves with the hindfoot.

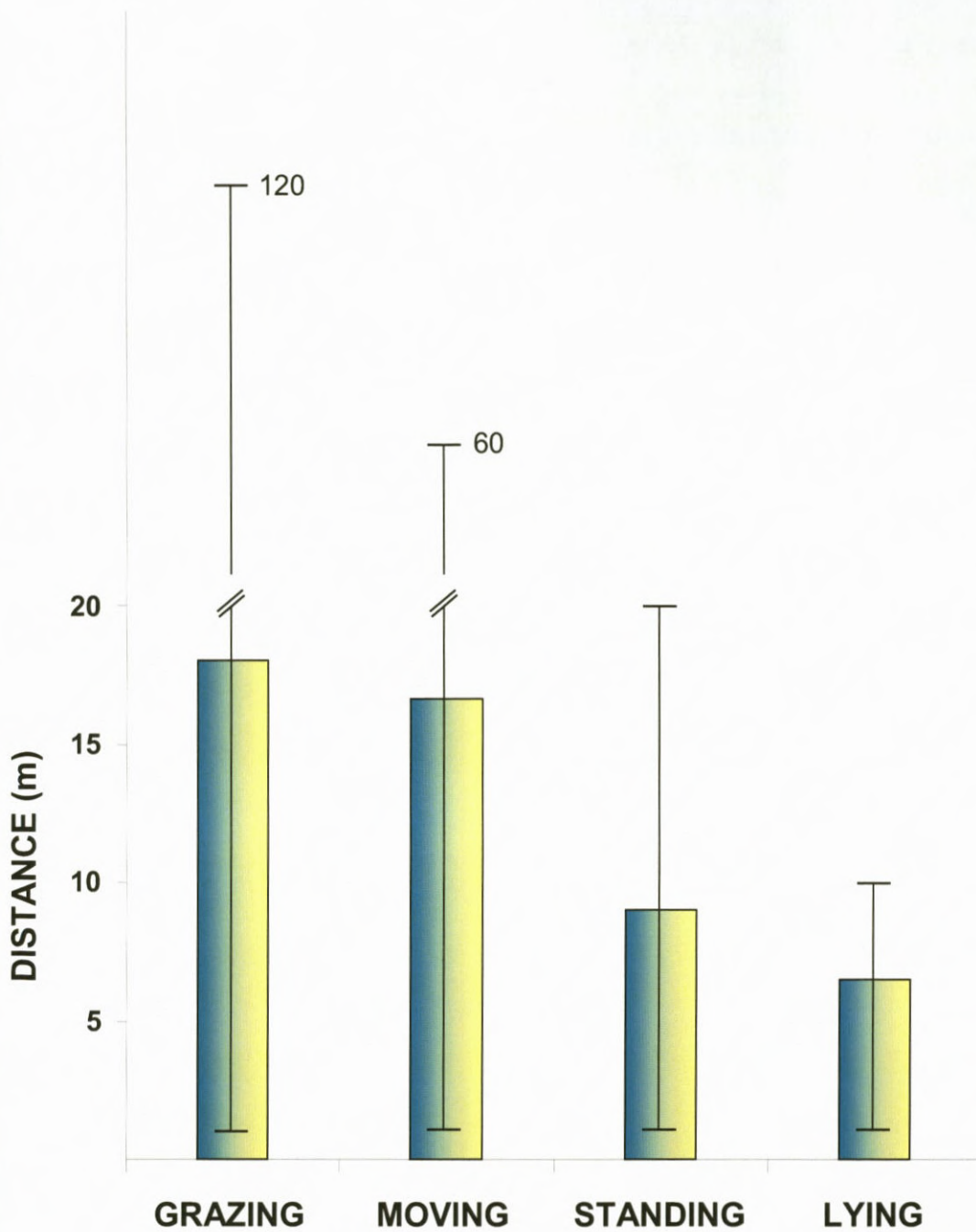


**Figure 5.4** A male bontebok walking with head low. The head is lowered towards the ground and positioned below shoulder level.

whilst following each other in single file. These movements were sometimes random and dependant on the location of sufficient resources, though the herd habitually moved to the same grazing or resting areas on different days. In relation to other aspects representative of this category, running behaviour was seldom observed and was mostly noted if the herd was suddenly distressed and moved off to a different area. Occasionally one individual was also seen to chase another member of the herd, but only for a short period of time. Regularly this individual was identified as the territorial male and this behaviour serves to keep the herd together (Lynch, 1974). They normally ran in tight, repeated circles for a short period of time before returning to the remainder of the herd. During such behaviour chasing rarely exceeded distances of 20 - 50 m and was of low intensity. Likewise, social interactions regarding individuals engaging in play did not represent a remarkable component of movement as it was observed to an even lesser extent than running. Play fighting between young members of the herd occurred at erratic times and only lasted for a few minutes.

Herd-forming ungulates live mainly in fairly open habitats. Species exploiting the higher productivity of open habitats attain higher densities and usually form groups to aid in protection against predators. Blesbok are known to form large congregations of 30 - 200 animals or more, depending on population density and rutting activities (Lynch, 1983). Bontebok are known to form noticeably smaller herds ranging from 10 - 30 individuals (Bigalke, 1955). It was observed that the herd acted as a relatively coordinated unit throughout the day and Figure 5.5 shows the mean, minimum and maximum distances between herd members during the different activities. In general, mean nearest-neighbour distances between herd members ranged from 7 - 18 m, lying being the category with the smallest individual spacing and the mean distances between animals increased from standing to moving to grazing behaviour. Conversely, minimum distances between individuals were 1 m for all activities as it was not uncommon for two or three bontebok to be positioned right next to each other regardless of the activity at any given time. Delany & Happold (1979) stated that the "follower" behaviour pattern of precocial young is characteristic of most herd-forming ungulates. Young gain protection from adults and calves thus stayed in very close proximity of their mothers, distances between them almost never exceeded 3 m. Hence, non-parametric testing did not support significant minimum distance differences for the various categories (Chi-square (3) = 3.273,  $p < 0.01$ ). Maximum distances between individuals ranged over greater distances (10 - 120 m) and maximal





**Figure 5.5** Mean spacing in a bontebok herd during four different activities. Minimum and maximum distances are also indicated for each category.

spatial proximity between animals in the herd was mainly associated with grazing and moving activities. Individuals did not remain in one spot when feeding and the general tendency of the herd was to disperse as individuals slowly moved forward until the herd was scattered over a vast area (Fig. 5.6). Furthermore, when the group set out to a different grazing or resting area the leading individual could be up to 40 m in front of the rest of the herd. Spatial proximity was least during inactive periods of lying (Fig. 5.7) or standing and members of the group usually came closer together showing a clustered pattern of distribution, while others were scattered in between. Parametric testing supported the results of greatest maximum distances between individuals that were found to be most prominent during grazing activity, followed by moving, then standing and ultimately lying as Chi-square (3) = 14.955,  $p < 0.01$  proved to be statistically significant.

### **5.1.2 Time budgets of social groups**

Du Plessis (1968) remarked that owing to the presence of horns in both sexes as well as similarities in other respects, only the experienced observer can sex a herd of blesbok on open range and David (1973) found the same to be true of bontebok. However, close herd observational distances were viable at the second study area and allowed for precise differentiation between the territorial ram, females and juvenile herd members as well as yearlings. In order to investigate time budgets of social groups as a possible function of sex and age, data were sorted accordingly and pooled for all observations.

As portrayed in Figure 5.8 the optimal allocation of time to the respective activity categories differ between social groups. The most noteworthy difference between grazing time budgets of the respective groups relates to females and territorial males. Females spent the most time grazing (41%) and territorial males allocated the least amount of time to this essential category (31%). With regards to lying, juveniles spent nearly half of their time budget on this inactive category and territorial males were least observed to be lying down (36%). However, territorial rams exhibited much higher standing (19%) and moving (14%) activity compared to any of the other social classes.

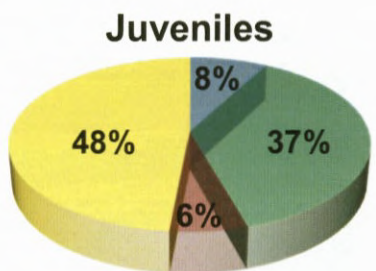
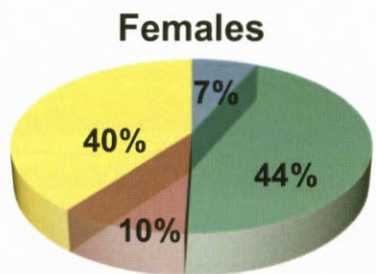
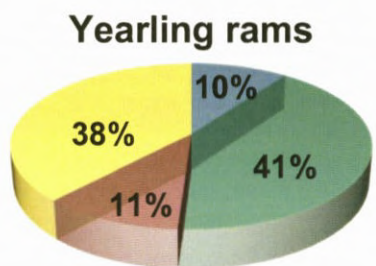
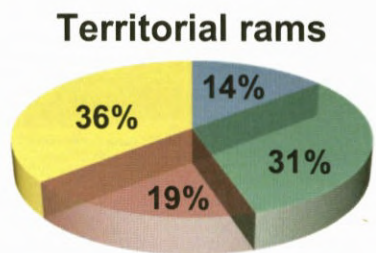


**Figure 5.6** Individual spacing between herd members of grazing bontebok.





**Figure 5.7** Individual spacing between herd members of resting bontebok.



**Figure 5.8** Time budget of diurnal activities of bontebok social groups in the central Free State.

The Z-test was applied to compare time budgets of two separate data sets consisting of two social groups each. Juveniles were not considered in the statistical analysis given that time budgets of young are most likely a reflection of the mothers' activities (Leuthold, 1977) and are therefore discussed separately. First, territorial rams and females were compared to determine if there were any significant divergences for time budgets of each behavioural category. The results coincide with the differences shown in Figure 5.8 with territorial rams significantly lower on grazing (31% vs. 44%:  $Z = -5.248$ ,  $p < 0.001$ ) and lying (36% vs. 40%:  $Z = -7.732$ ,  $p < 0.001$ ) and significantly higher on standing (19% vs. 10%:  $Z = 14.670$ ,  $p < 0.001$ ) and moving (14% vs. 7%:  $Z = 5.391$ ,  $p < 0.001$ ).

Findings of the present study for grazing time budgets of social groups were in contrast to the findings of Vrahimis (1990) whom reported that territorial black wildebeest bulls (43.2%) and bachelor herds (42.0%) allocate more time to grazing than females (39.6%). Various contributing factors have been put forward in support of territorial males having greater grazing time budgets and these include larger body size, energetic costs of territorial and mating behaviours as well as lower-quality diets. Nevertheless, findings of the present study are congruent with those of blesbok in the Free State (Van Aswegen, 1994). In this regard, blesbok territorial rams also spent the least amount of time on this activity (28.4%). Juvenile blesbok spent 31.8% of their time budget grazing, while non-territorial rams and ewes allocated 34.9% and 38.4%, respectively to this behavioural category. Grazing time budget differences of bontebok social groups (Fig. 5.8) are thus remarkably consistent with that of blesbok. However pertaining to the latter, slightly lower time budget percentages were evident for all behavioural categories.

With focus on the time budget discrepancies between the adult genders, it is apparent that females exhibit a 13% higher grazing time budget (Fig. 5.8) and similar findings of females grazing more than males have been reported by several authors. Garroway & Broders (2005) have commented on the decline of male ungulates' physical condition during the mating season as they spend less time feeding and a number of other ungulate studies have also confirmed that females spend more time feeding than males (Norton, 1981; Owen-Smith, 1994; Ruckstuhl, 1998). Most importantly these findings were also supported by the results obtained by David (1973) for bontebok in the Western Cape Province. This author commented that it is logical to assume that foraging

behaviour of the sexes would show variations at different times of the year, as was observed in the present study. He accordingly established that males partaking in rutting activities during the peak rutting season (January to mid-March) allocated less time to feeding and more time to other activities associated with territorial or mating behaviour. He also found that females that were in a certain state of reproduction spent an added 10.2 % of the day feeding on a year round average. Individuals in late pregnancy or early lactation with high reproductive costs need to maximise daily energy gain through increased feeding. Nutritional demands on pregnant females would increase throughout gestation into lactation and Owen-Smith & Novellie (1982) remarked that energy requirements could be raised by as much as 50% during peak lactation periods. Taking into consideration that female bontebok normally have one offspring a year, have a gestation period of around eight months (David, 1973) and then lactate for more or less four months, their nutritional requirements should thus be greater than a males' throughout the 12 months of the year in order to replace the reserves that have been utilised during these energy costly periods.

A small difference (4%) between lying time budgets of territorial males and females is shown in Figure 5.8. Time budgets of blesbok territorial rams and females also differed by a small percentage (5.6%), however territorial rams (43.9%) spent more time on lying than females (38.3%) (Van Aswegen, 1994). The same factors, namely reproductive costs of pregnancy and lactation that influence grazing behaviour of females, might also be relevant to bontebok females spending more time lying. Grazing for longer periods of time would necessitate ruminating more often and as formerly mentioned, it seems as if bontebok mainly ruminate whilst lying down. In addition, being pregnant or having a calf at heel could also contribute to females spending more time resting (Vrahimis, 1990).

The significant increase in time spent standing by male territorial rams (19%) (*vide* Fig. 5.8), can mainly be ascribed to their involvement in behaviours pertaining to territoriality. When the bontebok herd was considered in its entirety (*vide* Fig. 5.1), standing behaviour was primarily associated with disturbances or transitions between the two main activities (grazing and lying). However, new dimensions are added to standing behaviour of territorial ungulate males. Behaviours associated with social status advertisement (i.e. static-optic advertising, demonstrative defaecation) and agonistic

behaviour (dominance displays) come into play. The territorial male was regularly seen standing in an alert posture, often on an elevated area, which serves to visually advertise his presence. Whilst standing in this proud posture he would constantly scan the area. A standing position offers a better view of the surrounding area and the territorial ram was persistently observed to be looking in the direction of the yearling rams when visible in the vicinity of the territory periphery. Demonstrative defaecation was recognisable from a distance on several occasions and is basically an overly exaggerated form of the normal defaecation posture of the other social groups. This distinct behaviour was seen in the absence and also in the presence of yearling rams. Differences of standing time budgets between blesbok social groups were not as prominent (Van Aswegen, 1994). Standing time budgets were nearly equal for non-territorial rams (15.4%) and ewes (15.3%) and territorial rams allocated only 16.4% of their time budgets to standing. Overall, blesbok territorial rams were not the social group that spent the most time standing as was found for bontebok. According to Van Aswegen (1994), the social group that yielded the highest standing time budget (18.6%) was juveniles.

Territorial males not only moved about more than females, but also significantly more compared to all the other social classes (*vide* Fig. 5.8). Blesbok territorial rams yielded a similar trend (Van Aswegen, 1994). Territorial rams (11.3%) also moved more than not only females (8.1%), but also more than non-territorial rams (9.7%) and juveniles (6.3%). For territorial rams, this category includes not only moving to a different area for grazing or lying, but also territory inspection, intra- and inter-specific agonistic interactions as well as sexual behaviour. It was observed that the bontebok territorial ram increased his daily proportion of walking in the form of patrolling the territory. Females that were lying down were often harassed and chased up by the male so that he could smell her vulva to establish reproductive status. However, chasing behaviour was not only limited to adult male-female interactions. On one occasion the territorial ram aggressively chased after a juvenile in an attempt to separate them, thus allowing the male access to the female.

Mating behaviour was divided into two categories, namely a mating approach and a mating display and nearly identical behaviours have been described for blesbok (Du Plessis, 1968). These two behaviours are essentially one sexual display, but are separated on the basis of performance intensity (David, 1973; Lynch, 1974). The mating approach was characterised by the male approaching from a



distance with a stiff-legged gallop with the head in an upright position, usually when returning to the herd after territory inspection. Mating display behaviour was observed when the male was with the other herd members and was performed with an outstretched neck and horns laid back. Given that the territorial male's approach is rather sudden, the mating display has to be performed in a non-threatening manner (Lynch, 1974). This is accomplished by holding the head horizontally, known as a lowstretch display, which makes the horns as unobtrusive as possible (Estes, 1992). This type of behaviour was observed after the male chased up a lying female or when approaching a female that was grazing or standing near him. Even within these categories, some features were differentiated on the basis of low or high intensity. Ears positioned downwards or back and the tail in a horizontal position was viewed as low intensity, while ears in a horizontal position and the tail vertically curled over the back represented high intensity behaviour. Females usually responded with evasive behaviour by walking forward a few steps and this was often associated with intermittent grazing. If the male persisted and again advanced towards an unreceptive female, a response known as a mating circle was observed. This entailed the male and female circling around each other, a few or several times, in a head-to-tail orientation while the females' head was in a lowered position.

With the second comparison, male social groups (territorial and yearling rams) were evaluated to determine if there were any significant time budget divergences for each behavioural category. The results again coincide with the differences shown in Figure 5.8 and the exact same categorical dissimilarities evident from the first social group comparison was found when comparing territorial rams with yearling males. Hence, territorial ram time budgets are significantly lower on grazing (31% vs. 41%:  $Z = -4.327$ ,  $p < 0.001$ ) and lying (36% vs. 38%:  $Z = -7.910$ ,  $p < 0.001$ ) and significantly higher on standing (19% vs. 11%:  $Z = 11.649$ ,  $p < 0.001$ ) and moving (14% vs. 10%:  $Z = 2.256$ ,  $p < 0.05$ ).

In view of grazing, yearlings are expected to spend more time feeding than adult males as their energetic needs are driven by body growth. Also, the constraints on time budgets associated with sexual behaviour are not yet applicable to these individuals as they only reach sexual maturity at three years of age. Hence, they can afford to spend more time on essential activities such as grazing and lying. However, at the onset of observations a single yearling female was associated with the yearling males for a few weeks. During this time period, innate courtship behaviour of high intensity

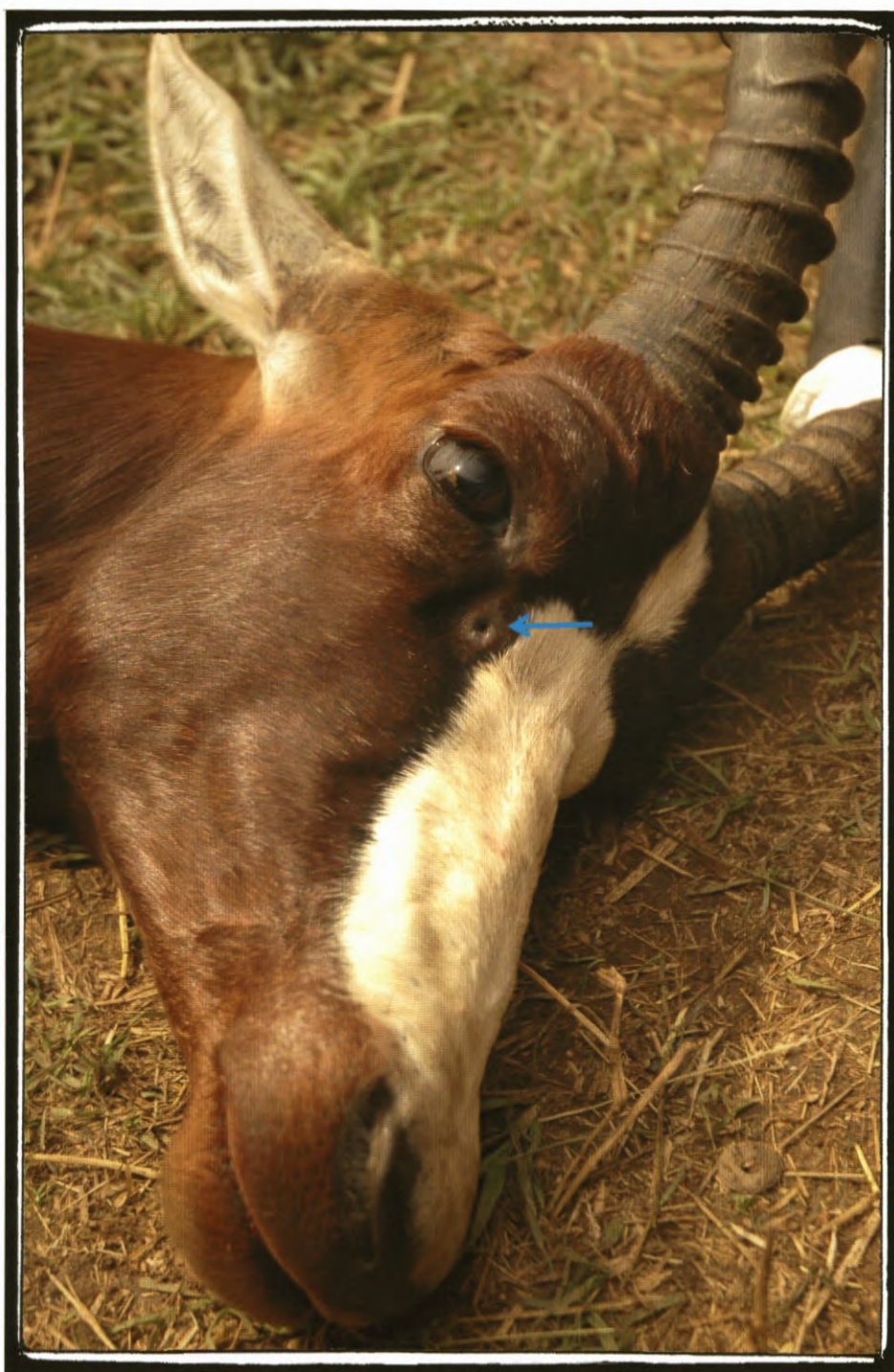
was frequently observed, but never advanced to copulation, though an attempt to mount the yearling female was seen on one occasion.

Considering standing and moving, time budget differences are mainly associated with territorial behaviour and intra-specific agonistic encounters. Pertaining to the latter, this term encompasses all behaviours (i.e. aggression, threat, intimidation, defence, appeasement and submission) associated with conflict situations (Leuthold, 1977). Chasing was observed several times and varied in different circumstances, but generally chasing events between the territorial male and immature males were of low intensity and at a canter pace over distances of  $< 50$  m. Apart from chasing, agonistic behaviour in the form of challenge rituals were also documented. A challenge ritual consists of several basic steps, often performed in a particular order (David, 1973). Following David (1973) and Lynch (1974), a typical bontebok or blesbok challenge ritual commonly encompasses a look about approach, lateral presentation, mutual anus smelling (also referred to as the greeting ceremony), horn clashing, displacement activities and grazing behaviour. Also, at any time between these steps an individual might defaecate, perform glandular weaving or displacement activities (Lynch, 1974). The latter refers to activities (i.e. grooming or grazing) that appear unbecoming when performed during agonistic encounters (Estes, 1992).

In the majority of cases, meetings between the territorial ram and a yearling ram took place within his own territory and he generally responded by approaching the yearling. The intruding yearling (often closely followed by a second yearling ram) also moved towards the approaching male in a slow manner. He frequently stopped and stood in the head-low position (submissive display), before commencing the approach. This was repeated until the yearling was in close proximity of the ram. At close quarters the two males met perpendicularly and stood in a lateral position. According to Lynch (1974) lateral presentation serves to accentuate body size. They either remained stationary in this position for several minutes or mutual anus smelling in an opposite parallel position was witnessed. The challenge ritual was commonly terminated by one individual walking off or mock-grazing.

According to David (1973) yearling rams have a passive nature and he never observed a challenge that was initiated by a non-territorial ram. Observations of the present study were not congruent with his findings. On one occasion, a yearling male invaded the territory of the dominant ram to engage in a challenge ritual. Upon seeing the main herd approaching, the yearling male performed a low intensity display walk (tail in horizontal position) towards the herd. The display walk was carried out over a distance of roughly 10 m and thereafter he ran directly towards the herd until he was a short distance (2 m) from the territorial male. They stood facing each other directly for a few minutes until the yearling ram again advanced towards the stationary territorial male. The two orientated in a parallel position before the territorial male moved a short distance away to face the intruding male directly. This position is more threatening than presenting the side of the body (Estes, 1992). As the main herd approached, they orientated in a tail-to-head position and mutual anus smelling was observed. The yearling male suddenly directed his attention to an adult female from the main herd and chased her at full speed over a short distance (20 m). Thereafter he changed to high intensity display running and trotted after the female with his tail in a vertical position. This behaviour enticed the territorial ram to aggressively chase the yearling away from the main herd. High-speed chasing persisted over some distance (50 m) and throughout the chase the yearling ran with his tail in a vertical position. After the chase, both males slowly walked back towards the main herd and again the yearling was observed to perform high intensity display walk behaviour. As the two males came closer, the main herd became very uneasy and scattered in different directions. This led to a second chasing incident over a greater distance (100 m) and the territorial ram even attempted to horn the yearling from behind. The territorial ram persisted to chase the yearling for a further 50 m before returning to herd. The chase was probably terminated when the territorial ram chased the yearling beyond the border of his territory.

Furthermore, glandular weaving was observed on two occasions for the yearling rams, but was never witnessed for the territorial ram during the observational period. The yearling was seen waving his muzzle in the grass in a characteristic manner and this behaviour has been linked with transfer of pre-orbital gland secretions in other ungulate species. Bontebok have conspicuous pre-orbital glands that are larger in males than in females and secrete a sticky substance (Fig. 5.9). David (1973), however, found no evidence of pre-orbital gland secretions in bontebok territories in Bontebok



**Figure 5.9** Pre-orbital gland (arrow) of a male bontebok, situated below the eye.

National Park. Nonetheless, findings from the present study seem to point towards the possibility that this behaviour pattern might indeed have a demarcating function, but this is not believed to be the case by the above-mentioned author.

It is also worth mentioning that an inter-specific agonistic encounter was witnessed between a yearling male and a territorial tsessebe. Following an aggressive approach by the tsessebe, the two encircled each before assuming a lateral presentation position. The bontebok stamped with his forefoot, whereas the tsessebe defaecated in a demonstrative manner. This was followed by the bontebok performing displacement scratching. A second tsessebe male approached from a distance of 30 - 40 m, enticing the first tsessebe to charge and chase after the yearling. Subsequent to the chase, they stood motionless facing each other directly. Displacement scratching was noted a second time and at this point the encounter escalated to physical horn clashing with the tsessebe in a knelt position. Horn clashing was interrupted by the second tsessebe and both tsessebés chased after the yearling (50 m), while the bontebok ran with his tail in a vertical position. At cessation of the chase, the bontebok returned to the yearling group and the entire group took off in an opposite direction. According to Leuthold (1977), aggressive fighting between different species is rarely witnessed in the wild, but occasionally occurs in captive situations where animals are commonly kept in mixed species assemblages, as was the case at Emoya Wildlife Estate. Also, based on observations of the present study, it appears as if inter-specific encounters are of a more aggressive nature compared to the witnessed intra-specific encounters.

Lastly, the proportion of time devoted by juveniles to each activity category is also shown in Figure 5.8. The most significant feature of juveniles is the lying category, representing nearly half (48%) of the overall diurnal time budget. The second-most noticeable activity is grazing (37%), while the remaining two categories represent less than 15% of the daily time budget. Following Vrahimis (1990), juveniles are expected to spend a substantial part of the day lying down seeing that it is not only part of the behavioural developmental process, but is probably also related to predator avoidance. Furthermore, grazing is not their only means of obtaining ample nutrition as their diet is also supplemented with milk from the mother. Consequently, they exhibit deviant lying time budgets as they can afford to spend such a substantial amount of time being inactive. Most of the standing



(6%) and moving (8%) behaviour can be ascribed to the aforementioned "follower" behaviour pattern. When in a standing position, juveniles would always stand in close proximity to their mothers and in instances where the herd moved to a different area they would also follow them closely. In view of the latter, play among juvenile individuals also contributed to this category.

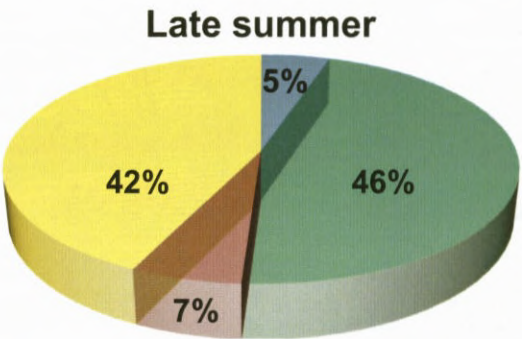
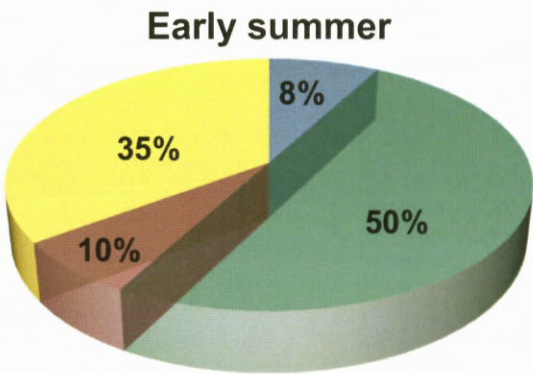
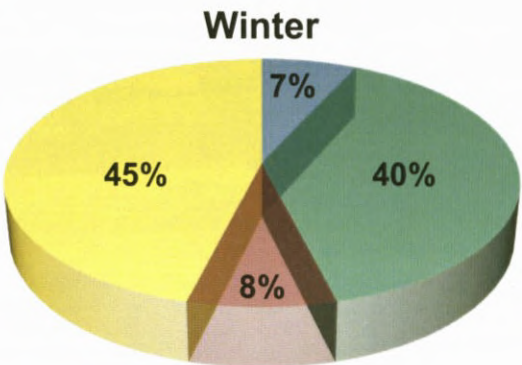
These findings seem to point out that the amount of time invested by herd members in each of these activities varied within the subspecies according to age as well as sex, but seeing that all herd members must carry out their activities within limitations imposed by daily cycles, time is limited to all of them. Though, pertaining to the dissimilarities between the social groupings, it seems that time allocated to essential activities across the diurnal period are likely influenced by conflicting demands (i.e. territorial and mating behaviour, lactation/pregnancy or growth) resulting in activity trade-offs.

### **5.1.3 Seasonal variation of time budget**

To establish seasonal variations of time budgets, statistical analysis of selected activity data was carried out relating to the various climatological seasons namely, winter (May - August), early summer (September - December) and late summer (January - April).

The amount of time dedicated to different activities on a seasonal basis is displayed in Figure 5.10. Throughout all seasons, lying and grazing comprises 85 - 89% of the time budget. Modal activity during the winter months is lying and in summer modal activity that bontebok engage in is grazing behaviour. Lying is the most prevailing behaviour during winter (45%) and late summer (42%), however there is a substantial difference noticeable when comparing lying in early summer (35%) with late summer. Hence, a steady increase from early summer to late summer to winter is obvious and the same seasonal progression (early summer: 37%; late summer 42.5%; winter: 45.5%) was observed for blesbok in the Free State (Van Aswegen, 1994).

The ability to extract nutrients from poor-quality food probably influences activity patterns to some extent during this season (Leuthold, 1977). As formerly mentioned, bontebok ruminate whilst lying down, thus one can assume that longer rumination bouts as well as the total amount of time



**Figure 5.10** Seasonal variation of time budget of diurnal activities of bontebok in the Free State Province.

spent ruminating helps to maximise nutrient extraction from the low-quality forage. Furthermore, an increase in lying behaviour during winter could also be of thermoregulatory significance. It is a possibility that being in a lying posture could serve as a behavioural strategy to decrease convective heat loss.

An opposite seasonal trend is evident for grazing behaviour (Fig. 5.10). With regards to the lying category, more time is allocated to grazing in summer (combination of early and late summer) than in winter and feeding behaviour shows an increase from winter (40%), late summer (46%) to early summer (50%). According to McNaughton (1984) grazers generally alter their feeding patterns relative to the vegetation's productivity potential. It would thus seem that grazing time changes most adaptively in response to changing nutrient requirements and forage depletion. David (1973), however, observed that bontebok spent the most time (70%) feeding during the winter season. Despite habitat and seasonal influences, this percentage is considerably higher compared to other ungulate studies. David (1973) ascribed the great increase in grazing behaviour during the winter months mainly to the influence of temperature and stated that bontebok grazed for longer during this season as the weather is cooler. Yet, in relation to the findings of this study, congruent results were obtained by Van Aswegen (1994) seeing that blesbok also displayed shorter feeding periods during winter (23.5%) compared to early (35.8%) or late (40.6%) summer. Eltringham (1977) accordingly associated the winter decline of grazing behaviour with the high fibre content of dry forage, necessitating longer digestion periods. More time was allocated to grazing during summer and Du Plessis (1968) suggested that a bigger selection of palatable grasses of higher nutritional value are available during these months and in all probability leads to increased feeding behaviour.

No outstanding seasonal variation trend is evident for standing behaviour (Fig. 5.10). Early summer represents the most observed standing individuals (10%), while standing was observed to a lesser extent during late summer (7%) and winter (8%). These results did not concur with the findings for blesbok in the Free State. According to Van Aswegen (1994), blesbok spent the most time standing in winter (25.9%) and standing even outweighed grazing (23.4%) during this season. Similar time budgets were evident for late summer (8.9%), but early summer was also associated with a much higher standing time budget (19%). The author ascribed this to the seasonal differences of

grazing behaviour seeing that as grazing increased, standing decreased and vice versa. In relation to the present study, the above-mentioned correlation between grazing and standing could only be linked with early summer and not with any of the other seasons. Furthermore, it is also probable that seasonal ambient temperatures influence the length of standing periods and that this category is noteworthy in terms of behavioural thermoregulatory adaptations (Leuthold, 1977).

It is not unanticipated that moving shows an increase during summer (early and late summer) compared to winter since the increase in grazing from winter to summer appears to be associated with the winter to summer increase of walking behaviour (*vide* Fig. 5.10). Blesbok demonstrated a similar pattern of less walking during winter (5.2%) compared to early (8.2%) and late (8.1%) summer (Van Aswegen, 1994). However, when comparing winter (7%) to late summer (5%), bontebok moving activity seems somewhat random. It was expected that more moving individuals would be observed during late summer in accordance with the increase in grazing activity.

Overall moving and standing activities were not as prevalent, hardly varied across the seasons and represented infrequent behavioural categories, but foregoing results of lying and grazing were much more prevalent and showed seasonal reciprocal variability. Even so, difference in the patterns of activities across the seasons were found to be statistically significant (Chi-square (6) = 40.177,  $p < 0.001$ ), but with moderately weak effect size (Cramér's  $V = 0.08$ ).

Also, when the active phases are grouped together (grazing and moving) a seasonal increase in activity is evident from winter (47%), late summer (51%) to early summer (58%) (*vide* Fig. 5.10). Conversely, results obtained from grouping the inactive phases (lying and standing) together, shows a seasonal decrease in herd activity from early summer (42%) to late summer (49%) to winter (53%). The time budget for total activity was thus characterised by winter being the season of minimal activity with longer resting bouts. This could likely be attributed to the cessation of behaviours associated with mating and/or low daily food intake on account of the low digestibility of forage during winter. Pertaining to the later, associated higher levels of fibre intake requires an increase in rumination time and may actively compete with the amount of time allocated to foraging, consequently reducing available feeding time. On the contrary, summer months are characterised by

higher levels of activity and foraging allocation was increased, both by spending more time per day active and by devoting a higher proportion of active time to feeding.

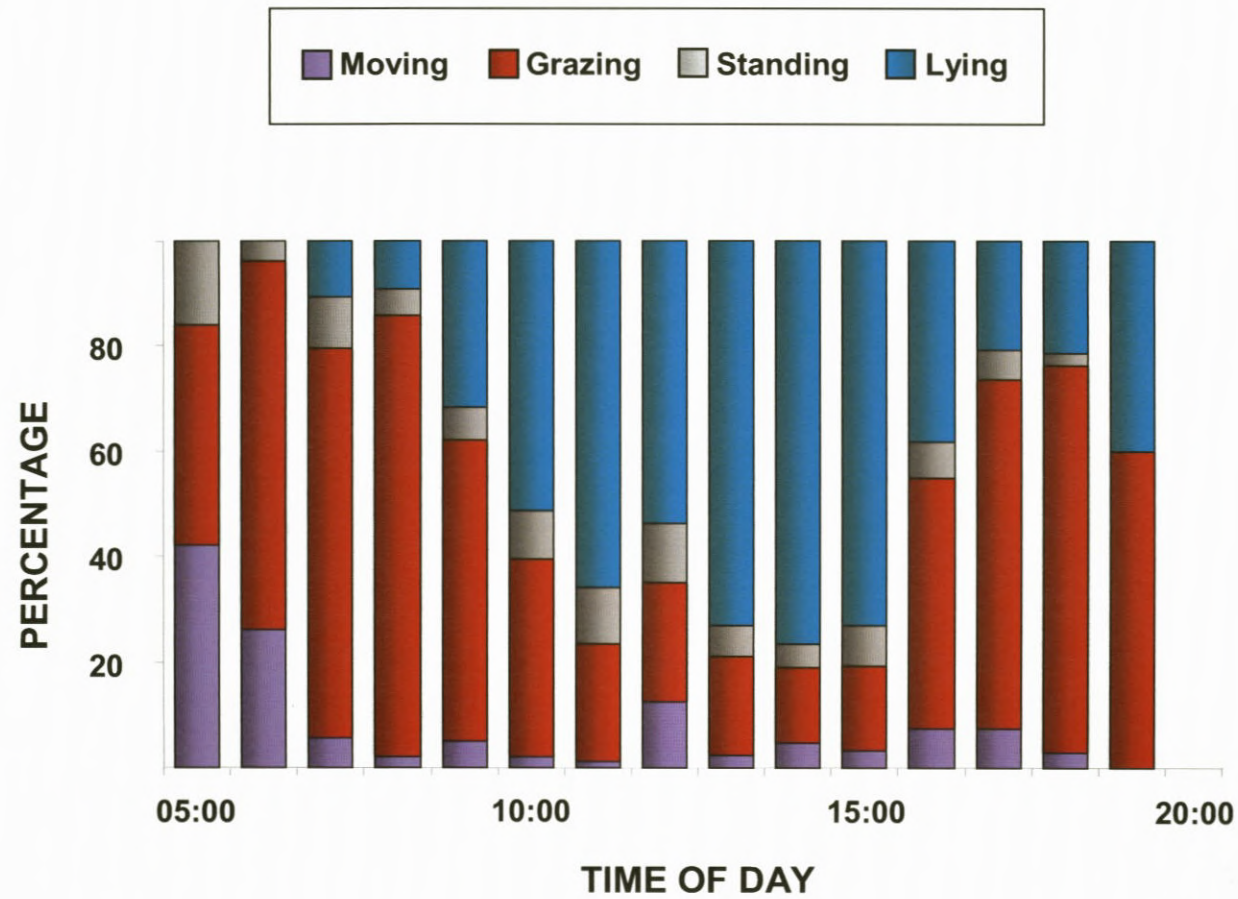
## 5.2 DIURNAL PATTERN

### 5.2.1 General diurnal pattern

Some species, for instance bushbuck (*T. scriptus*), lesser kudu (*T. imberbis*) and reedbuck (*Redunca arundinum*) are mainly nocturnal. The majority of bovids, however, are more active during daylight hours especially early and late in the day and most animals remain relatively inactive during the hottest hours (Estes, 1992). Even though individual herd members do not follow the exact same routine on a daily basis, a definite and constant daily activity pattern is evident when the herd is considered as a whole and for discussion purposes data collected were grouped into hourly intervals allowing different activities to be expressed as a percentage for every hour of the day.

Figure 5.11 illustrates the overall diurnal activity pattern of bontebok throughout the study period. Examination of their daily activity shows relatively rigid grazing and resting patterns and high levels of within-group synchrony is very prominent for these behaviours. It was not unusual for the majority of the herd (70 - 80%) to simultaneously engage in feeding or lying at certain stages during the day and occasionally all herd members would be grazing or lying down. Contagion, also known as social facilitation, may possibly be a causative factor with regards to synchronisation of activities (Lynch, 1974). This featured strongly in bontebok seeing that during certain periods, the entire bontebok herd would be feeding, but after a while some individuals lay down. Shortly thereafter more individuals followed there example, though the remainder of the herd continued to graze. On the other hand, some of the lying individuals occasionally opted to rise and again commenced with grazing. A short period later, some of the grazing individuals lay down and this was repeated until ultimately all members of the herd lay down. Thus, changing from one activity to another consisted of various increasing and decreasing steps and transitions between activities were not immediate. Nonetheless, the activities were rather closely coordinated. It appears as if a herd can only be coherent if herd members remain in the same area at the same time and for this to occur it may be





**Figure 5.11** Diurnal activities of bontebok in the Free State Province.

necessary for them to engage in the same activity. In view of this, synchronising activities with other herd members could be viewed as an adaptation that contributes to maintaining group cohesion and group stability, ensuring that individuals stay together as a herd (Jarman, 1974; Lynch, 1974).

Regardless of substantial deviation amongst individuals, recurrent temporal patterns of animal activity appear to be species-specific (Aschoff, 1964), hence Figure 5.11 also shows a relative persistence of rhythmic behaviour for bontebok. Different behaviours relating to activity and inactivity are not equally distributed over the day, but follow a somewhat distinct pattern. In general, the time of day bontebok were most active is shown as a double peaked pattern during the early morning (05:00 - 09:00) and the late afternoon (16:00 - 19:00), while the remainder of the day (10:00 - 15:00) was mainly represented by a quiescent period (Fig. 5.11). Several authors (Kok 1975; Norton, 1981; Doi *et al.*, 1983; Ryan & Jordaan, 2005) observed similar active and inactive phases for red hartebeest, klipspringer, defassa waterbuck (*Kobus defassa defassa*) and African buffalo (*Syncerus caffer*).

Prominent active phases are accordingly associated with the two major daytime periods allocated to grazing (Fig. 5.11). These early morning and late afternoon feeding peaks (bimodal feeding pattern) were found to be consistent with the findings of a range of other ungulate species, including African buffalo (Ryan & Jordan, 2005), black wildebeest (Vrahimis, 1990), bushbuck (Dankwa-Wiredu & Euler, 2002), impala (Klein & Fairall, 1986), klipspringer (Norton, 1981), red hartebeest (Kok, 1975), springbok (Bigalke, 1972) and tsessebe (Huntley, 1972). A prominent bimodal feeding pattern has also previously been ascertained for bontebok (David, 1973) as well as blesbok (Van Aswegen, 1994).

A considerable amount of feeding was noted from the onset of observations (05:00) and grazing increased substantially during the early morning (Fig. 5.11). It was only from late morning (10:00) onwards that most herd members lay down. The second peak of bontebok grazing activity was noticeable during the late afternoon (16:00 - 19:00) when the majority of the group once again commenced with feeding until after sunset. By comparison, feeding was more pronounced during the early morning grazing bout than during the afternoon. Leuthold (1977) commented that grasses contain higher moisture content during the early morning. As a result, feeding during this time of day

can be considered an essential behavioural adaptation that is beneficial in terms of higher food quality as well as higher moisture intake (Leuthold, 1977). The observed bimodal pattern was also found to be in accordance with the findings of Eltringham (1979) whom concluded that grazing behaviour is not observed by chance, but is somewhat limited to particular periods of the day. However, apart from the obvious peaks of intensive feeding, Leuthold (1977) suggested that grazing behaviour should rather be considered as a continuous process of alternation between feeding and ruminating. Van Aswegen (1994) accordingly observed that in the absence of any disturbances the regular change between feeding and ruminating was fairly marked for blesbok. Lengthy periods of inactivity are thus prohibited as food intake alternated with short rest periods is necessary to ensure an ongoing process. This probably provides an adequate explanation why food consumption in a herd were never entirely ceased, even in cases where the majority of the individuals were resting there was always at least one grazing individual (*vide* Fig. 5.11).

The abovementioned factors provide ample explanations regarding the observed grazing rhythm, but it is an added likelihood that these rhythms are also responses to a combination of regularly changing external environmental stimuli as well as endogenous physiological factors. Aschoff (1964) suggested that rhythmicity is brought on by cyclic factors in the natural environment referred to as synchronisers, though caution must be applied in the interpretation of correlations between animal behaviour and particular environmental stimuli when based on field observations. The activity peaks for example, have been referred to as dawn and dusk effects since they usually occur at the onset of sunrise and sunset and are presumably related to the changes in light intensity (Aschoff, 1964). As previously mentioned some authors suggested that inactivity is related to high ambient temperatures, though the associations of activity peaks as a response to dim illumination or the inactive period as a response to high temperature can only be ascertained under controlled conditions. Aschoff (1964) thus commented that late afternoon activity and the distinct early morning activity are considered coincident with dim illumination as no concurrent environmental stimulus could be specified as the proximate cause. The observed rhythm could therefore be regarded as primarily endogenous but with external correlates and within the framework of these innate physiological cycles, there are environmental patterns that cause synchrony between bontebok activity and the factors to which they respond. Activity is thus influenced by environmental conditions which can either suppress or

accentuate some aspects of the pattern, but the environmental conditions do not produce the basic pattern anew each day (Leuthold, 1977). An already existent pattern is merely modified and these activity patterns have proven to be useful in providing some indication of environmental relationships of the subspecies.

Given that a simple foraging-resting-foraging activity pattern is evident (*vide* Fig. 5.11), lying was also found to be a well pronounced activity and quite the opposite of grazing, the greatest concentration of lying behaviour was noticeable around mid-day. None or very few individuals lay directly after sunrise or shortly before sunset as these represented periods of intensive feeding. Consequently, lying showed a progressive increase from morning (09:00) to later afternoon hours (13:00 - 15:00). Spearman's rank order correlation coefficient showed a high negative correlation ( $r = -0.93$ ) between grazing and lying, thus supporting the significance of the inversely related pattern of these two categories that is displayed in Fig. 5.11.

Observed alternations between grazing and rumination whilst lying appear to be greatly associated with the digestive physiology of ruminators. Although many factors determine activity patterns, the daily cycle of feeding and resting/ruminating seems to be largely dictated by the dynamics of rumen repletion and depletion. When enough forage has been obtained and the rumen is filled, grazing is terminated to allow time for rumination. As previously referred to, sufficient mastication of food is imperative for nutrient assimilation and affects the amount of nutrients that are extracted from a given quantity of ingested forage. Ample rumination time is thus a vital aspect of the general diurnal pattern.

Figure 5.11 indicates that standing and moving behaviour does not constitute distinctive features of the daytime activity pattern, nonetheless the most prominent standing peak was noted during the first observational hour (05:00). Even though the herd were located a half hour prior to commencement of observations, some were still alarmed by the observer and frequently discontinued grazing to look up in the direction of the earlier disturbance. Nevertheless, the likelihood of observing some bontebok in the standing position throughout the day was reasonably low. Rumination might also be a contributing influence on this category as associated with grazing

and lying. Even though it was assumed that most rumination took place whilst lying, it is important to consider that some rumination probably also took place in a standing position, albeit to a lesser extent. Given that lying was more pronounced during the middle of the day, rumination early and late in the day could probably be associated with standing behaviour.

The moving category includes a variety of activities which did not feature strongly in the general diurnal activity pattern, apart from walking individuals (*vide* Fig. 5.11). During the course of the day, the entire group frequently moved from one area of the enclosure to another and accordingly some moving behaviour was noted at almost every hour of the day. Peaks in moving activity were more often associated with grazing peaks than lying peaks and increased activity during the first couple of daylight hours (05:00 - 06:00) conformed to field observations that bontebok normally moved some distance from the area where they were located at dawn, when setting out to the first grazing site of the day. No moving individuals were noted just prior to cessation of observations (18:00 - 19:00). As dusk approached the majority of bontebok were still grazing, but a third of the herd had already lain down. With reference to standing, a prominent moving pattern is also lacking (*vide* Fig. 5.11).

### 5.2.2 Diurnal pattern of social groups

Diurnal activity patterns of bontebok social groups are shown in Figure 5.12. For discussion purposes time of day is split into three periods of five-hour intervals, namely morning (05:00 - 10:00), mid-day (10:00 - 15:00) and afternoon (15:00 - 20:00). Congruent with the general diurnal pattern (*vide* Fig. 5.11), a typical bimodal pattern was evident for all social groups, reaching higher peaks during the early morning hours (05:00 - 07:00) compared to the late afternoon feeding bouts (17:00 - 19:00), while the mid-day period was mainly associated with inactive behaviour.

Comparing the diurnal pattern of grazing activity of all social groups, across the three time periods, revealed significant (Chi-square (6) = 23.859,  $p < 0.01$ ), but weak differences as shown by Cramér's  $V$  (0.067). Females engaged in moderate to high grazing activity throughout every hour of the diurnal period and a similar pattern is reflected in the results for juveniles. This complies with the prior mention of juvenile activity patterns being a reflection of that of the mothers'. Furthermore,



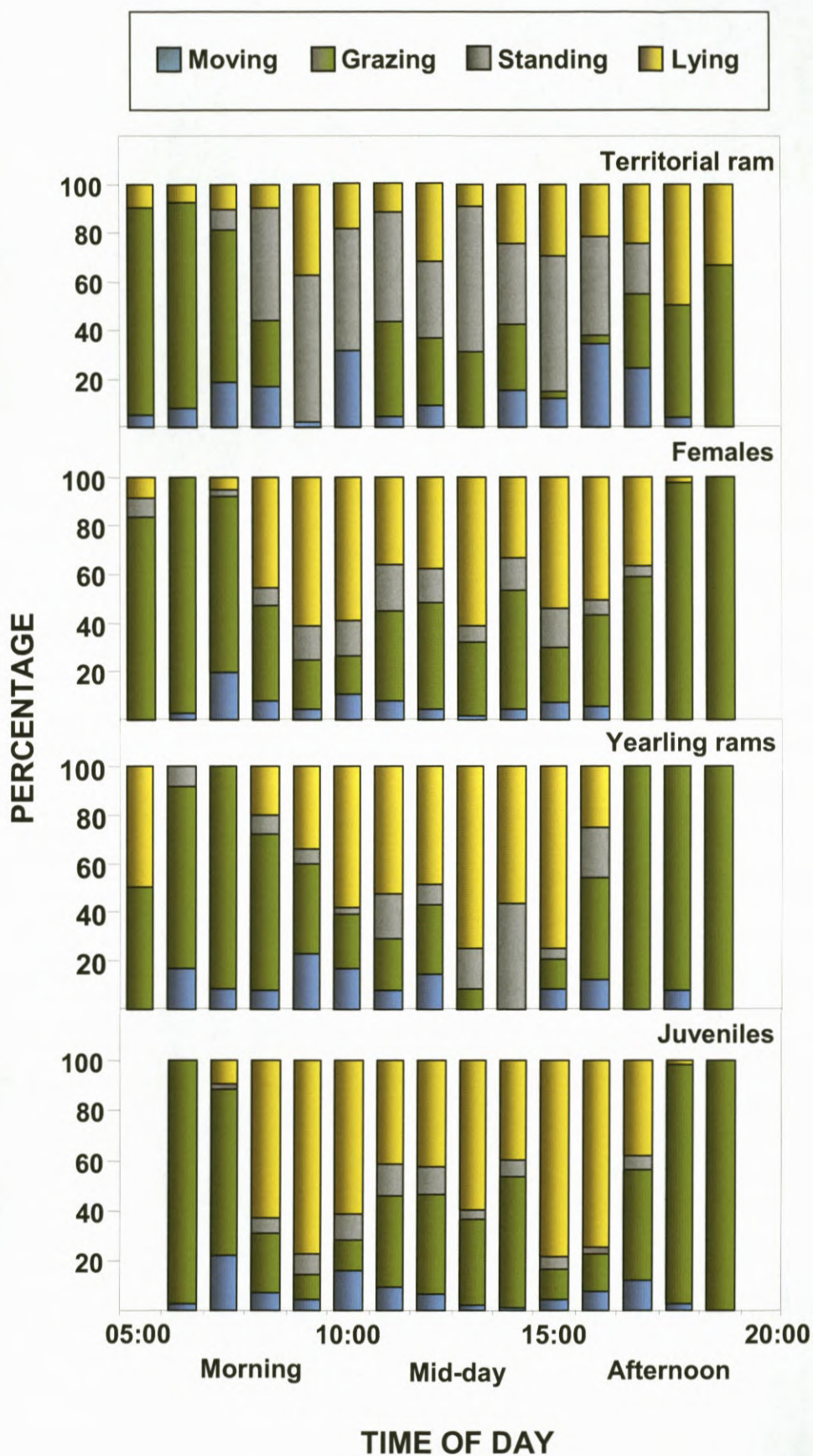


Figure 5.12 Diurnal activities of bontebok social groups in the central Free State.



these results also reflect on the social class time budgets (*vide* Fig. 5.8). Females were found to spend the most time grazing (*vide* Fig. 5.8) and this is accordingly displayed in the diurnal pattern (Fig. 5.12). Apart from early morning and late afternoon grazing bouts of territorial rams, grazing activity was also noticeable during some hours of the mid-day period (11:00 - 14:00) (Fig. 5.12). Of all the social groups, the latter has the least prominent grazing pattern. Yearlings show a similar pattern to females and this can most likely be related to the fact that they also have higher energy demands, but in this case energy demands are associated with growth. Compared to territorial rams, non-territoriality of yearlings probably allow them to spend more time during the day on grazing behaviour.

All social groups, apart from territorial rams, showed a significant increase in lying behaviour during the mid-day period (Chi-square (6) = 0.01,  $p < 0.001$ ; Cramér's  $V = 0.104$ ). Territorial rams, on the other hand, exhibited a fairly even distribution of lying behaviour throughout the day (Fig. 5.12).

When examining the diurnal standing pattern of the social groups, this behavioural category is most outstanding for territorial rams (Fig. 5.12). Compared to the general diurnal pattern (*vide* 5.11), lying did not feature as the main activity during mid-day as standing behaviour was nearly noticeable throughout all hours of the day. These results are, in part, contrary to the findings of several studies (Jarman, 1977; Vrahimis, 1990; Van Aswegen, 1994). These authors observed that most standing activity took place during the morning hours and to a lesser extent during the afternoon, hence standing activity reduced as the day progressed. As formerly mentioned, territorial behaviour is a major contributing factor that underlies the more prominent standing activity displayed by territorial bontebok rams. The other social groups showed no definite pattern and standing took place irregularly during the day, which is compliant with previous findings. Despite major variation between territorial rams and the other social groups, these findings were not significantly different (Chi-square (6) = 6.458).

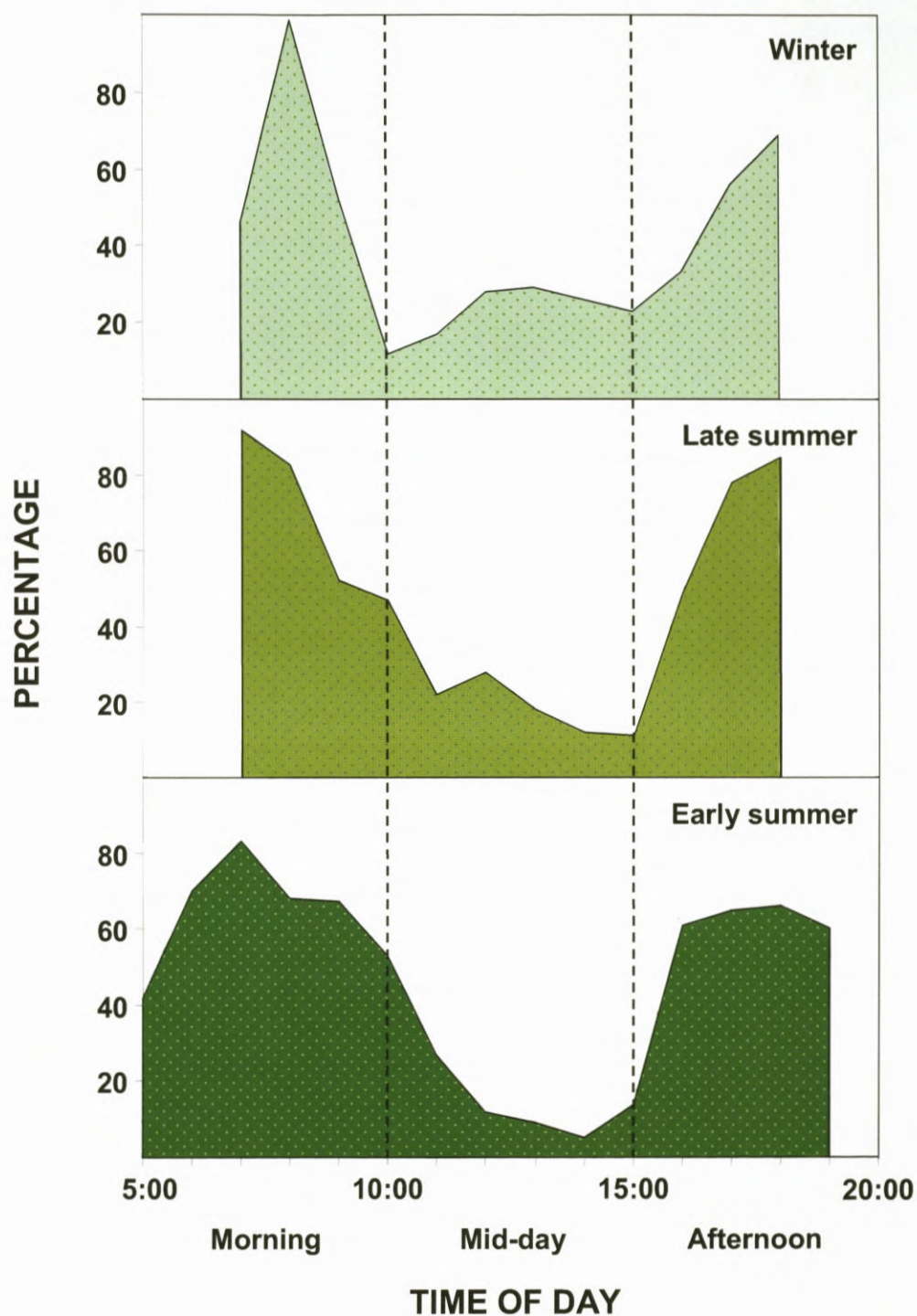
Moving and standing activities show similar results seeing that moving behaviour is also more outstanding for the territorial ram compared to the erratic patterns of females, yearling rams and juveniles (Fig. 5.12). These dissimilarities can also be explained at the hand of territorial behaviour as

challenge rituals, sexual displays and territory patrol contributed to this activity category. In contrast to the standing category, moving comparisons were of statistical significance (Chi-square (6) = 20.871,  $p < 0.05$ ), but with weak effect size (Cramér's  $V = 0.141$ ).

### 5.2.3 Seasonal variation of diurnal pattern

The foraging responses of bontebok over a complete seasonal cycle is demonstrated in Figure 5.13. Grazing occurred in regular pronounced phases irrespective of season, but weather conditions and the state of vegetation possibly influenced the timing and extent of these peaks. The more intensive peak represented morning feeding and was most pronounced around sunrise, whilst grazing in the afternoon was resumed rather suddenly (15:00) and steadily progressed until a second feeding activity peak was reached around sunset. Some grazing was also noticed during mid-day as some animals continued to feed every so often throughout the daylight hours. In general, six to nine hours (depending on season) of diurnal activity was allocated to grazing. Seasonal variation of time spent grazing throughout the day were of significance (Chi-square (4) = 18.467,  $p < 0.001$ ), though the correlation was weak (Cramér's  $V = 0.102$ ). Foraging responses to the different seasons are thus likely ascribed to the covariance of food abundance and food quality over the seasonal cycle.

Average utilisation percentages of different veld components by bontebok have been found to consist of 90 - 97.5% grasses (and some herbs) (Van Hoven, 2002b; Luyt, 2005), hence bontebok are considered almost exclusive grazers with a preference for short grass veld (Stuart & Stuart, 2000). Table 5.1 shows that the grass component was largely represented by carrot grass (*Tragus racemosus*), followed by Lehmann's love grass (*Eragrostis lehmanniana*), three-awn grass (*Aristida congesta*) and drop seed grass (*Sporobolus fimbriatus*). Red grass constituted a mere 6.4% of the basal plant species. A combination of pioneer, subclimax and climax stage grass species were thus present. Carrot grass and three-awn grass are considered pioneer grasses, whereas Lehmann's love grass and drop seed grass represent subclimax to climax species (Roberts, 1973). The climax stage is the best stage for grazing and soil protection and is only represented by red grass. All of these grasses, aside from red grass, are associated with disturbed areas and increase due to the disturbing effect of overgrazing (Roberts, 1973). The presence of these particular grasses and the high



**Figure 5.13** Seasonal variation of grazing behaviour of bontebok in the Free State Province.

**Table 5.1** Summary of the frequency (*f*) and percentage of the botanical composition of plant species of the study areas.

PLANT SPECIES	Plains		Outcrops	
	<i>f</i>	%	<i>f</i>	%
<b>Grasses</b>				
<i>Aristida congesta</i>	65	8.3	12	5.5
<i>A. diffusa</i>	39	5.0	48	21.8
<i>Digitaria tricholaenoides</i>	12	1.5		
<i>Eragrostis lehmanniana</i>	83	10.7	30	13.6
<i>E. racemosa</i>	24	3.1		
<i>E. obtusa</i>	15	2.0		
<i>E. rigidior</i>	31	4.0	8	3.6
<i>Sporobolus fimbriatus</i>	58	7.4	5	2.3
<i>Themeda triandra</i>	50	6.4	2	0.9
<i>Tragus racemosus</i>	119	15.3	26	11.8
<b>Shrubs</b>				
<i>Chrysocoma ciliata</i>	19	2.4	4	1.8
<i>Cuspidia cernua</i>	5	0.6		
<i>Eriocephalus decussatus</i>			3	1.4
<i>E. ericoides</i>	14	1.8		
<i>Felicia muricata</i>	27	3.5		
<i>Helichrysum lucilioides</i>			15	6.8
<i>H. zeyheri</i>	28	3.6	8	3.6
<i>Lepidium africanum</i>	4	0.5	1	0.5
<i>Lycium hirsutum</i>	5	0.6		
<i>Melolobium candicans</i>			9	4.1
<i>Pentzia globosa</i>	56	7.2	24	10.9
<i>P. spinescens</i>	16	2.0		
<i>Rosenia humilis</i>	107	13.7	25	11.4
<i>Trichodiadema setuliferum</i>	3	0.4		
<b>Total</b>	<b>780</b>	<b>100</b>	<b>220</b>	<b>100</b>

percentage of karroid shrubs (Table 5.1) are thus representative of dry veld conditions and indicate that the study areas can be viewed primarily as overutilised pioneer's veld. The overgrazed state of the environmental surroundings and state of the habitat during different seasons should thus influence food intake in terms of time contributed to feeding, digestive processing as well as metabolic requirements for energy and specific nutrients.

As previously conferred, the respective time budgets of grazing behaviour showed a seasonal progression from winter to summer (*vide* Fig. 5.10), thus seasonal variation of the diurnal feeding pattern (*vide* Fig. 5.13) is discussed accordingly. During winter, grazing behaviour was noted from the onset of recordings (07:00) and during this time the herd appeared to feed rather selectively and would spread out in all directions whilst grazing. Feeding suddenly declined over the morning period (08:00 - 10:00) and limited grazing behaviour was observed over mid-day. During the remainder of the afternoon, grazing steadily increased again in the late afternoon (16:00) and utmost feeding behaviour was again observed at the end of the recording session (18:00).

Seasonal changes with regards to food resources are rather dramatic and Weiss & Laties (1961) commented that animals vary their ingestive behaviour to compensate for deficiency states during harsh conditions. Veld deterioration was noticeable in the heavily used areas and was particularly prominent during the dry winter months. Grasses dry out consistently within a relatively short period of time following the cessation of rains (Bigalke, 1972) and the combination of low ambient temperatures and regular frost experienced in the Free State, not only decreases the ability of food to supply the needed energy, sufficient protein, minerals and vitamins in terms of availability, but also decreases the palatability of grasses.

Grasses vary considerably in their grazing value and Table 5.2 shows the ecological status and grazing values of some grass species from the study areas. It is clear that the variety of increaser 2 grass species, which are of low nutritional value, constituted slightly more than three quarters of the basal plant coverage, whereas decreaser grass species represented a mere quarter of the basal plants (Table 5.2). These palatable climax grasses of high grazing value would have been abundant if the veld was in good condition, but they decrease in number when the veld is overgrazed or undergrazed.

**Table 5.2** Frequency, percentage and ecological status of grass species from the study areas. Estimated grazing value is indicated on a scale of 0 (poor) to 10 (excellent). Adapted from Fourie & Visagie (1985).

GRASS SPECIES	<i>f</i>	%	ECOLOGICAL STATUS	MEAN SCORE	GRAZING VALUE
<i>Aristida congesta</i>	65	13.1	Increaser 2c	1	1
<i>A. diffusa</i>	39	7.9	Increaser 2c	1	1
<i>Digitaria tricholaenoides</i>	12	2.4	Decreaser	10	10
<i>Eragrostis lehmanniana</i>	83	16.7	Increaser 2a	5	4
<i>E. racemosa</i>	24	4.8	Increaser 2b	4	2
<i>E. obtusa</i>	15	3.0	Increaser 2c	1	2
<i>E. rigidior</i>	31	6.3	Increaser 2c	1	1
<i>Sporobolus fimbriatus</i>	58	11.7	Decreaser	10	7
<i>Themeda triandra</i>	50	10.1	Decreaser	10	10
<i>Tragus racemosus</i>	119	24.0	Increaser 2c	1	1
<b>Total</b>	<b>496</b>	<b>100</b>			



Palatability (digestibility and nutritional value) and production (quantity of leaf material produced) are the two key factors affecting the grazing value of grasses. Some authors (Kutilek, 1979; Owen-Smith & Novellie, 1982) view the crude protein value as the most important contributor to nutritional value and is directly related to digestible energy availability. Nutritional value is in turn determined by fibre content (mainly lignin and cellulose). Van Hoven (2002b) stated that a crude protein content of 5% in vegetation is required for African ungulates. Bontebok are thus hard-pressed during winter to meet maintenance energy and protein requirements since forage resources (Table 5.3) contain less than 5% crude protein and only 26 - 28% digestibility. Therefore, the limit imposed by digestive processes or when the point is reached where it is no longer profitable to exert unnecessary energy, probably resulted in less time allocated to foraging. Bontebok thus appeared to effectively adjust their energy expenditure to compensate for the seasonal decrease in the quality of forage. The balance of nutrient demand and supply is also reflected in seasonal changes in body weight and it is not uncommon for ungulates to lose a considerable percentage of their utmost autumn weight during winter (Owen-Smith, 1994). Additionally, thermoregulatory efficiency possibly also affects grazing (Belovsky & Slade, 1986; Leuthold, 1977) as certain times of day present excessive heat losses in turn associated with increased metabolic rate. Activity would thus be limited during these times to conserve energy. What's more, winter conditions are also expected to affect the nutritional requirements of different age groups. Calves would be more susceptible to winter conditions than adults, resulting from the need to direct energy towards growth and their small body size would probably be associated with an increased cost of thermoregulation (Vrahimis, 1990).

During early summer (*vide* Fig. 5.13), herd members grazed for a longer period of time (05:00 - 10:00) compared to the other seasons. Lengthier observational sessions (14 hours) compared to 11 hours of available daylight during winter and late summer, presumably contributed to the longer hours spent grazing in the morning. In relation, Aschoff (1964) stated that the duration of the circadian period is considered to be a monotonic function of light intensity for many animals and an increase in light intensity leads to a shortened circadian period which simultaneously lengthens the duration of activity in diurnal mammalian species. The mid-day period was characterised by minimal feeding activity, in particular during the hottest hours of the day (12:00 - 15:00). From the onset of the afternoon period, the majority of bontebok would once again commence with feeding activity.

**Table 5.3** The nutritional value of some of the grass species during winter and summer, measured as the percentage crude protein and percentage digestibility. Adapted from Van Rooyen (2002b). Increaser (I); Decreaser (D).

GRASS SPECIES	% CRUDE PROTEIN		% DIGESTIBILITY	
	Winter	Summer	Winter	Summer
<i>Aristida congesta</i> I	3.8	6.1	26.5	45.5
<i>A. diffusa</i> I		4.0		
<i>Eragrostis lehmanniana</i> I	3.0	7.9	28.0	55.0
<i>E. rigidior</i> I	3.1	4.6	26.0	53.0
<i>Sporobolus fimbriatus</i> D	4.2	9.5		
<i>Themeda triandra</i> D	3.3	7.4		

The number of individuals seen grazing became less as sunset steadily approached (18:00), but a substantial amount of feeding behaviour was still noticed at dusk (19:00). Commencement of the afternoon feeding bout is possibly correlated with cooler temperatures in the afternoon, whereas the slight decline of grazing behaviour subsequent to sunset is probably coincident with the change in light intensity when grazing gradually terminates after dusk.

In contrast to the other seasons utmost feeding was noticed from the onset of observations (07:00) during late summer, followed by rather abrupt hourly declines for the remainder of the morning that continued until the end of the mid-day period (*vide* Fig. 5.13). The general routine pattern was disrupted by some individuals that grazed at intermittent intervals throughout the day. In contrast to the other seasonal patterns, nearly the same amount of grazing behaviour was observed during the last (18:00) and first observational sessions (07:00).

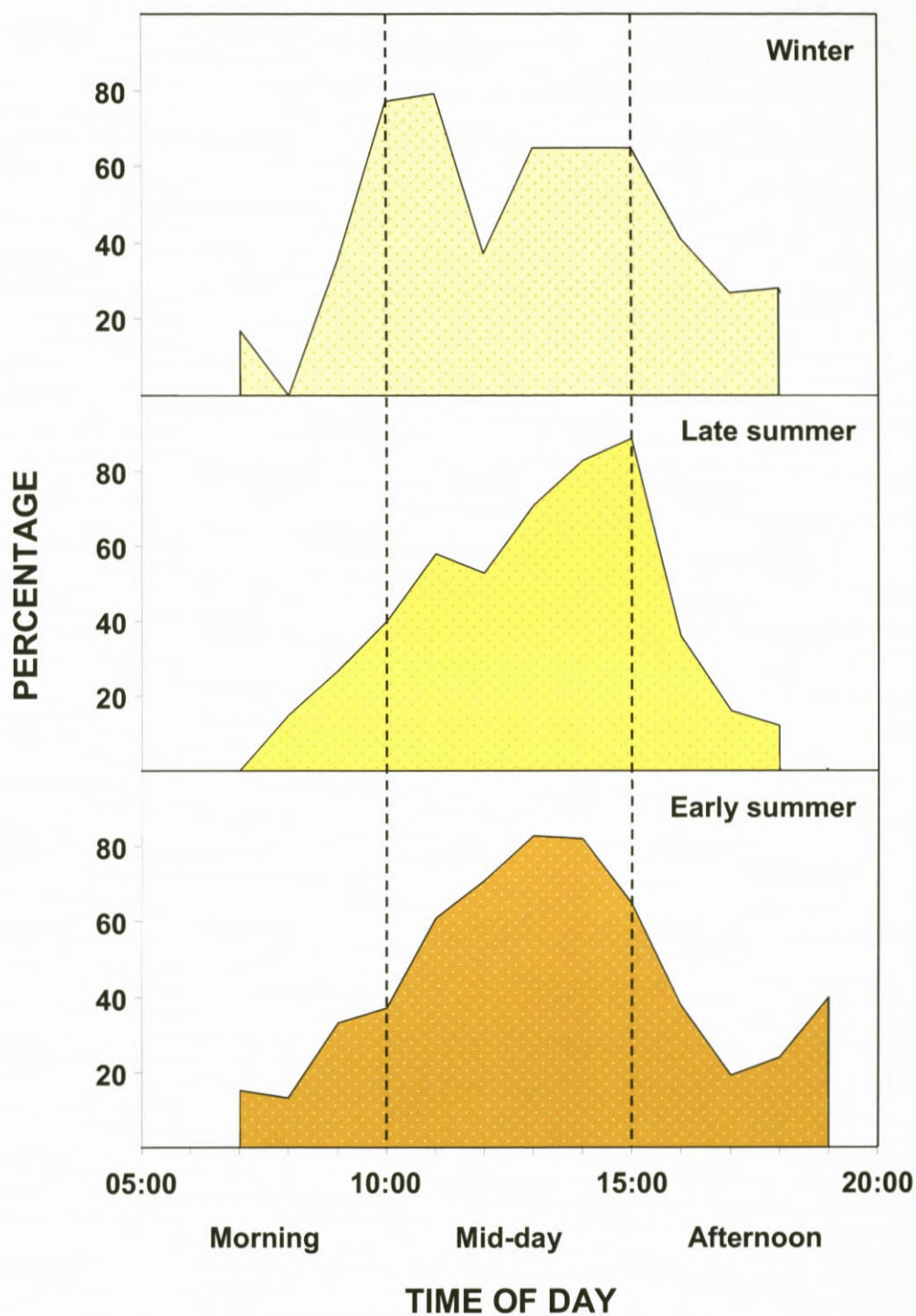
Provided that comparable climatic conditions prevailed during early and late summer, influential factors were expected to have similar affects on grazing patterns. The summer months represent more favourable environmental conditions and young plant material with higher protein content is readily available following summer rains (Taylor, 2006). Hence, bontebok had no problem allocating adequate food in terms of both quality and quantity. Table 5.3 shows that all of the grass species indicated, irrespective of increaser or decreaser status, almost double in terms of crude protein content from winter to summer. This vegetation recovery allows animals to replace the body reserves lost during winter and feeding sequentially increased over the summer months. Even though the percentage digestibility is only indicated for three species of low grazing value, digestibility still nearly doubled from winter to summer. A similar seasonal increase should also apply to the more palatable grasses and the higher diet quality permits more rapid digestive processing thereby increasing the opportunity to feed more frequently. Regarding thermoregulatory efficiency, various authors (Belovsky & Slade, 1986; Nicholson & Husband, 1992; Owen-Smith, 1998; Twine, 2002) suggested that for an animal to maintain its heat balance under high temperature conditions it may have to reduce feeding activity during the hottest hours of the day. For that reason, ruminant species are expected to take advantage of these energetically costly hours to lie down and ruminate rather

than forage and even though bontebok grazed interruptedly throughout the day during all seasons, the least amount of grazing individuals were noted during mid-day over the summer months.

Seasonal variation pertaining to lying behaviour is displayed in Figure 5.14. As previously conferred, this behaviour was most prominent during the middle of the day (*vide* Fig. 5.11) and a seasonal increase was evident from winter to summer (*vide* Fig. 5.10). Distinct lying periods across the seasons and seasonal variation of this behavioural category was accordingly found to be highly significant (Chi-square (4) = 55.354,  $p < 0.001$ ). Given that seasonal changes concerning grazing and lying time budgets were largely reciprocal, it is therefore expected that seasonal variations of grazing and lying diurnal patterns should be affected in a similar manner.

The general pattern revealed an increase of lying behaviour during the morning until a major lying bout was noted during mid-day, followed by less lying during the afternoon (Fig. 5.14). Very few lying animals were noticed during the early morning and late afternoon, but there were always some lying individuals up until observations ceased. Similar trends were observed throughout all seasons with only a few inconsistent increases or decreases of lying behaviour throughout the day.

During winter, it was expected that cold early morning ambient temperatures would most likely inhibit activity at these times, but few lying individuals were observed (Fig. 5.14). On the contrary, feeding behaviour was most prominent during these hours (*vide* Fig. 5.13). Following Schmidt-Nielsen (1997), the output of endogenous heat production is dependent on the work level of activity, hence active feeding would thus contribute to increasing heat production when ambient temperatures are low. A major bout of inactivity was observed during the morning (10:00 - 11:00) when the majority of individuals lay down (Fig. 5.14) and evidently an outstanding decline in grazing activity was noticeable at precisely the same time (*vide* Fig. 5.13). The sudden plunge at midday during winter and early summer were also consistent with the small peaks noticed for grazing activity (*vide* Fig. 5.13). Regardless of the reciprocal pattern associated with these two categories, the concentration of lying behaviour over the mid-day period appears to be of thermoregulatory importance, regardless of seasonal variation. The extended rest in the sun during winter would tend to minimise loss of body



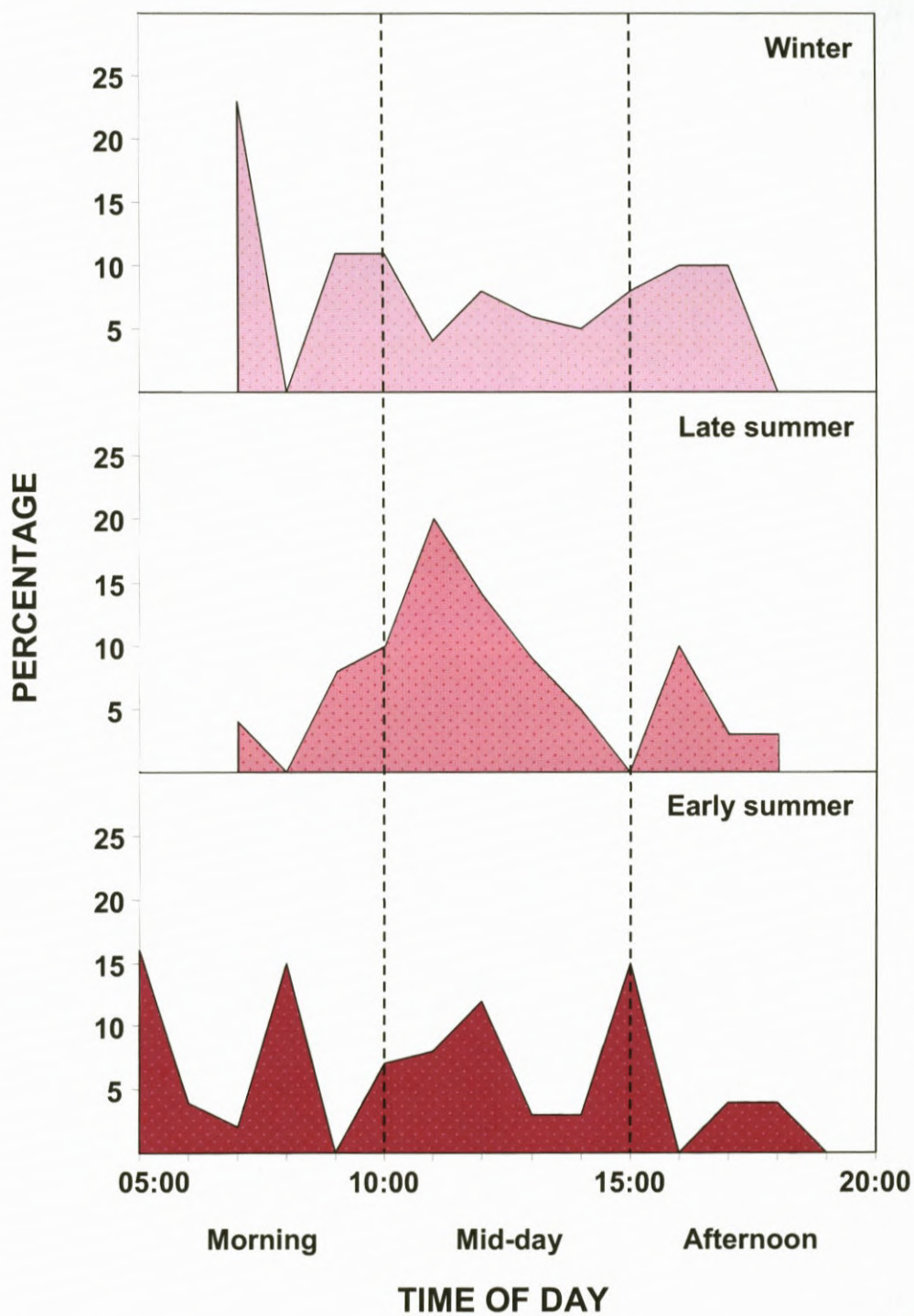
**Figure 5.14** Seasonal variation of lying behaviour of bontebok in the Free State Province.

heat, whereas excessive heat gain during summer would be prevented by remaining inactive during periods of high ambient temperatures over mid-day.

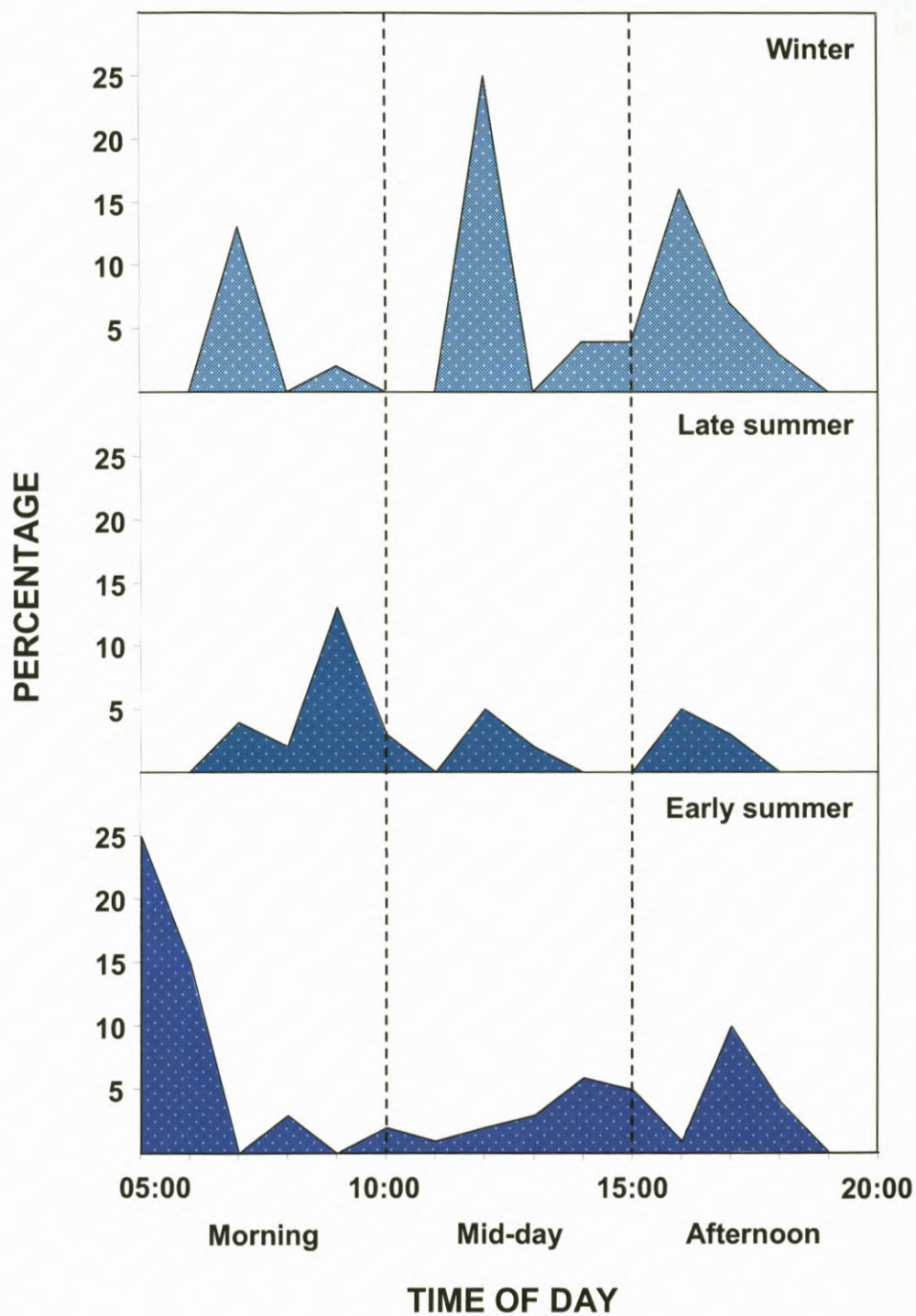
Compared to the abovementioned categories, there is no exceptional seasonal rhythmicity regarding standing (Fig. 5.15) and moving (Fig. 5.16) behaviour as both activities exhibit fragmented behaviour. Notwithstanding the irregularity of this behavioural category, seasonal differences for standing was statistically significant at the 0.1% confidence level (Chi-square (4) = 55.354), but again a weak effect size was evident (Cramér's  $V = 0.155$ ). Since standing was mainly associated with transitions between lying and grazing, comparisons are made accordingly. It is therefore assumed that regardless of the observed randomness, standing noticed during the morning and afternoon periods were mainly linked to grazing activity, whilst standing behaviour noticed during the mid-day period was generally associated with lying behaviour. The duration of standing between activities varied considerably, but relatively short periods of less than five minutes were most frequent. Hence, even though an increase in standing almost always preceded or followed lying periods, this is not persistently obvious as data was grouped per hour. Apart from the association with transitional periods, individuals would also occasionally interrupt grazing or moving activities to promptly scan their surroundings. Furthermore, random disturbances, individuals that rose at certain times to self-groom or that rose simply to change their lying positions, all contributed to the observed diurnal patterns of standing behaviour.

The results obtained for moving shows no consistent seasonal pattern (Fig. 5.16). Nevertheless, seasonal variation of moving behaviour was statistically significant (Chi-square (4) = 32.963,  $p < 0.001$ ), but with fairly weak effect size (Cramér's  $V = 0.283$ ). More movement was noticed in the morning during the summer months, but activity shifted to later in the day during winter when two separate peaks were noticeable. The first moving peak was at midday and the second, smaller peak was during the afternoon (16:00). As previously commented on, moving activity was generally associated with movements to and from feeding and resting sites during the morning and afternoon. In relation, a small grazing peak was also observed at midday (*vide* Fig. 5.13), but a more noteworthy decline was noticed for lying behaviour (*vide* Fig. 5.14). Nevertheless, sudden movement observed at this time was rather inconsistent pertaining to both grazing and lying and was probably due to an





**Figure 5.15** Seasonal variation of standing behaviour of bontebok in the Free State Province.



**Figure 5.16** Seasonal variation of moving behaviour of bontebok in the Free State Province.

unexpected disturbance that caused the herd to suddenly rise and move to another area. On the contrary, noteworthy moving activity in the afternoon (Fig. 5.16) appeared to be consistent with commencement of grazing activity at the exact same time (*vide* Fig. 5.13). A further noteworthy increase in moving behaviour was observed at dawn during early summer as longer daylight hours allowed for longer grazing sessions and the herd set out earlier to the first feeding area.

It should be noted that Chi-square test results should be interpreted with caution as it assumes identical observations, which may not be so if the same herd is being observed repeatedly. There could be inherent dependence in the observations and controversy of the independence of observation assumption of the Chi-square test may lead to type 1 errors. Although, in the present study significant chi-squares were obtained on the herd observed repeatedly, these significant differences were consistently weak. As a result, significant differences derived through Chi-square may not in fact reflect true differences. However, the analysis of frequency in repeated measure designs is complex and beyond the scope of the present study.

In summary, time budgets covering daylight hours obtained for bontebok were very similar to the time budgets determined by Van Aswegen (1994) for blesbok in the Free State. Different behaviours were not evenly distributed during the day, but followed a distinct pattern for both subspecies, considering the two outstanding activities observed for blesbok were also lying and grazing.

Activity budgets appears to be moderately flexible in terms of seasonal adjustment, but also appears to be relatively fixed in terms of the necessity to forage and ruminate at certain intervals and consequently this was displayed in phases of increased activity during the early morning and the late afternoon, with a long period of inactivity during mid-day. These phases varied in relative duration over the daily cycle, hence the daily activity schedule and time budgets for activities were outcomes of the abovementioned pattern. The presence of pronounced variation in environmental conditions across seasons seems to account for the marked intra-annual variation of the majority of activity categories. Furthermore, the obtained results suggests that time budgeting appears to play an important role in balancing individual energy reserves and that diurnal patterns are presumably representative of the basic responses to physiological processes (i.e. feeding, digestion, rumination)

and environmental stimuli (sunrise/sunset, temperature, available vegetation). Seasonal variation of bontebok behaviour found for this study conforms to that of blesbok as determined by both Lynch (1974) and Van Aswegen (1994). These seasonal differences of diurnal patterns provides an indication of behavioural adaptations resulting from selection for strategies by which bontebok exploit temporal and spatial niches in relation to varying environmental factors.

### **5.3 BEHAVIOURAL THERMOREGULATION**

According to Schmidt-Nielsen (1997) many animal species must maintain body temperature within fairly narrow limits. Ungulates are expected to sufficiently regulate heat exchange with the environment over a wide range of thermal conditions by not only responding physiologically, but by also responding behaviourally to weather-related factors (Weiss & Laties, 1961; Garroway & Broders, 2005). The latter is thus expected to influence ungulate activity patterns primarily in terms of thermoregulatory behaviour as well as movements (Leuthold, 1977). Though, in the opinion of Davenport (1985), behavioural thermoregulation beyond shade seeking and sunbathing does not play a role in larger mammals.

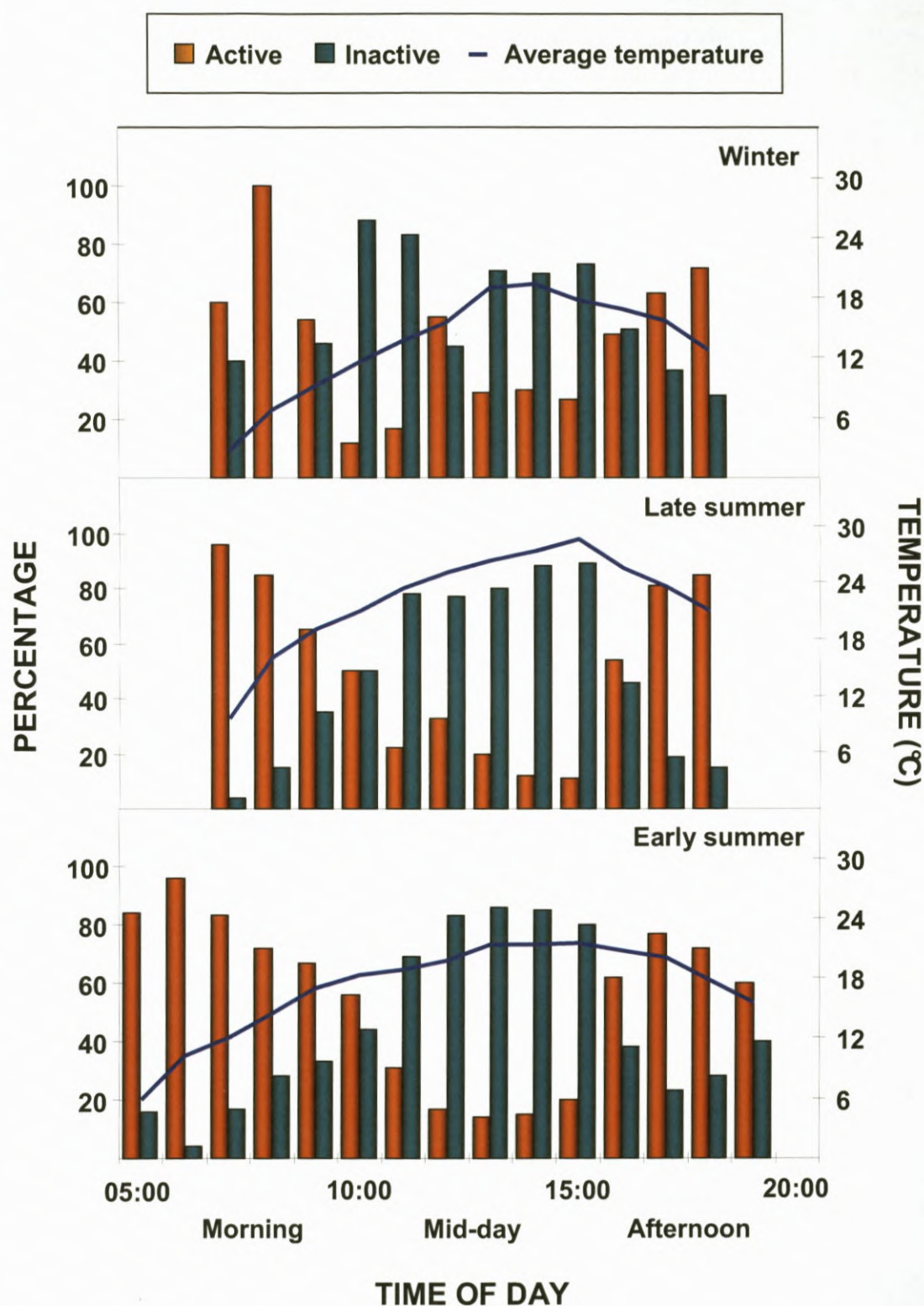
Within their natural distribution range, bontebok occur in areas of the Cape fynbos zone that consists of a mixture of grassland and low scrubs (Stuart & Stuart, 2000). Within their habitat they are in need of short grass, permanent water and some shelter (Apps, 2000). Given that they are active during the day, it is known that they exploit the thermal advantages of resting in shade during the hottest hours of the day (Apps, 2000). The shelter provided by trees or bushes found in their natural habitat provides refuge from solar radiation, yet permitting radiative heat loss. However, the study areas in the Free State consist mainly of open grassland habitats, with limited shelter from the elements. In general, therefore, bontebok are more exposed to direct sunlight and other varying climatic conditions in the Free State than in their natural habitat. The observations were thus directed towards the question of how modification of activity periods and changes in body orientation contribute to avoiding thermal stress in an environment that offers minimal shade.

### 5.3.1 Thermal environment and activity

Body temperature of most resting mammals is normally within the range of 36 - 38°C (Delany & Happold, 1979) and it is vital that body temperature must be maintained within these limits regardless of the environmental ambient temperature. The methods of temperature variation and the necessity for precise regulation vary according to the species and the environment in which it lives. One of the tactics that some animals employ to regulate body temperature when exposed to unfavourable ambient temperatures is to seek shelter in a more favourable microclimate (Delany & Happold, 1979). However, depending on the environment, this strategy is not always possible and animals need to employ alternative methods to reduce the effects of stress resulting from low or high ambient temperatures. This can be achieved through strategies concerning behaviour and adjustment of activity patterns such as time of rest, activity cycles as well as seasonal and daily changes in behaviour.

Seasonal comparisons of active (grazing; moving) and inactive (lying; standing) behaviour pertaining to influences of ambient temperature are shown in Figure 5.17. The southern region of the Free State experiences extremely cold winters and during the winter months, mean temperature ranged from below 5°C to around 20°C. Individuals were active during the first few hours following sunrise (07:00 - 09:00) when mean ambient temperature remained below 10°C, but steadily increased as the morning progressed (Fig. 5.17). More than half of the herd engaged in active behaviour at the onset of recordings, while the mid-day period (10:00 - 15:00) was generally spent being inactive, apart from an abrupt moving increase at midday brought on by sudden movement of the herd. Inactivity was noticeable during late morning (10:00 - 11:00) and remained fairly constant throughout the remainder of the mid-day period (13:00 - 15:00) when warmer ambient temperatures were recorded. Almost equal amounts of individuals engaged in active and inactive behaviour during the late afternoon (16:00) and activity once again increased till around sunset (18:00). The Spearman's ranking order correlation coefficient was determined to ascertain if there is a significant correlation between ambient temperature and the percentage of time spent active during the day, but a significant relation was concluded as lacking ( $r = -0.406$ ).





**Figure 5.17** Seasonal influence of temperature on active and inactive behaviour of bontebok in the Free State Province.



Observations commenced a good deal earlier during early summer (Fig. 5.17) and mean ambient temperatures increased from around 6°C (05:00) until a maximum of 22°C was reached over mid-day (13:00 - 15:00). Active behaviour was most prevalent during the morning (05:00 - 10:00) and afternoon (16:00 - 19:00), while the majority of individuals (70 - 85%) were inactive during the hottest hours of the day (11:00 - 15:00). Data obtained during the early summer months were statistically significant ( $r = -0.795$ ) at the 5% confidence level.

The late summer activity trend is characterised by a sharp peak subsequent to sunrise, a steady decrease over the next couple of hours with the lowest point during mid-day and a secondary afternoon peak (Fig. 5.17). Utmost activity was evident during the first (07:00) and last (18:00) observations, whilst uppermost inactivity was noted during the first hour of the afternoon period. Mean ambient temperatures ranged from 10°C to nearly 30°C during daylight hours and the highest ambient temperature recorded (29.1°C) was accordingly associated with the time of day when most individuals were inactive (90%).

A general trend of pronounced morning activity (as temperature increased), lengthy and rather even inactivity over mid-day (as temperature increased to the maximum temperature of the day) and a secondary increase in activity (as temperature decreased) in the afternoon was clear. However, time of peak activity, magnitude of activity and duration of rest periods differed between seasons. Active phases during winter stretched over a three hour period following sunset as well as three hours prior to dusk. Magnitude of activity was greatest (55 - 100%) in the morning and slightly less (50 - 75%) in the afternoon. The inactive phase stretched over a five-hour period when the majority of bontebok (79 - 90%) rested.

Given the extended observational period and more favourable environmental conditions during early summer, time spent active doubled from winter to early summer during the morning phase. The duration of the afternoon active phase was also slightly longer and resting over mid-day for an extended period was again evident. The late summer months are considered a difficult period for heat stress and the obtained results showed that high ambient temperatures tended to depress the proportion of the day that bontebok spent active as more time was allocated to inactive behaviour.

Higher ambient temperatures restricted active phases to the morning hours and a shorter bout of activity was evident in the afternoon (*vide* Fig. 5.17). Unlike the other seasons, the utmost activity peak was observed at the onset of recordings since temperatures sharply increased throughout the day. The negative association of temperature and activity was most outstanding during this season and temperature appeared to be a significant factor causing a decrease in both grazing and moving behaviour ( $r = -0.825$ ,  $p < 0.05$ ).

Endogenous heat constitutes a large part of the heat load on mammals (Schmidt-Nielsen, 1997), hence decreasing behaviours that increase heat production would thus be an effective method to reduce heat stress behaviourally. The intensity and duration of activities in response to the warmer weather were moderate and the activity pattern accordingly shifted to the cooler hours in the early morning and late afternoon during hot weather (*vide* Fig. 5.17). Influences of high ambient temperature on activities have also been observed in other studies. Dankwa-Wiredu & Euler (2002) established that bushbuck (*T. scriptus*) were most active at temperatures below 30°C, when the temperature exceeded 31°C they opted to retreat to the bushes to rest and ruminate. Klein & Fairall (1986) also found that there was an existent relationship between ambient temperature and activity in blesbok. Greatest activity occurred within a temperature range of 22 - 31°C, when temperatures exceeded 31°C individuals were inclined to remaining inactive. Behavioural thermoregulation thus appears to play an important role in aiding bontebok to avoid thermal stress. It appears that minimal activity coincident with the daily maximum of temperature might be of survival value since Schmidt-Nielsen (1997) commented that active phases increases thermal load through exposure to solar radiation and the higher the ambient temperature, the more difficult it is to dissipate heat.

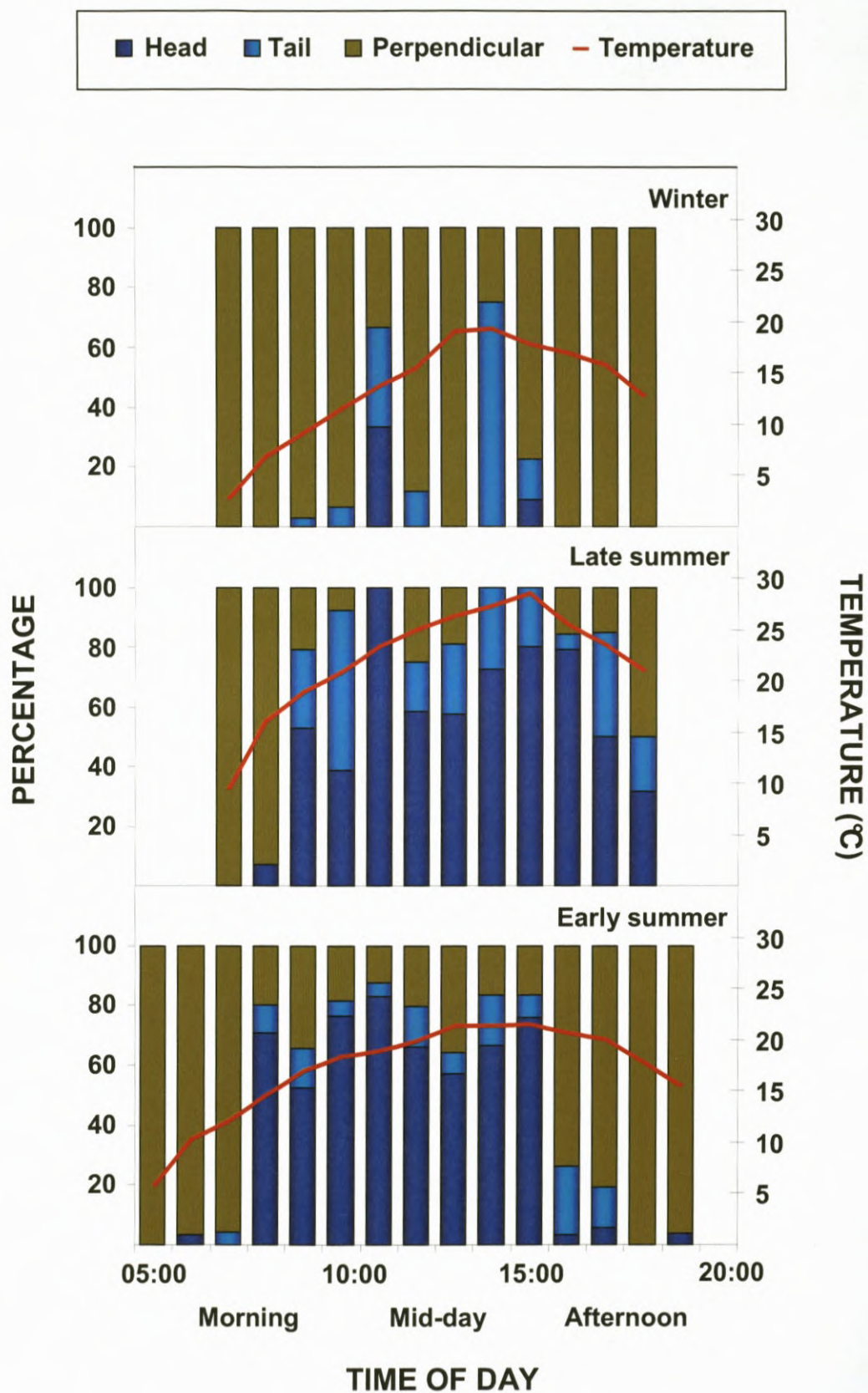
Although similar active and inactive tendencies prevailed, connotations of these patterns also vary in terms of seasonality. Inactivity for the duration of the mid-day period during winter allows animals to warm up by basking in the sun when ambient temperatures are warmer, whereas limiting activity during the hottest parts of the day during the summer months prevents contribution to thermal load, both because of the heat generated by muscular work and by exposing the animal to radiant energy. Cycles in daily body temperature are the result of proximate changes in activity levels combined with endogenous physiological rhythms that contribute to conserving energy during the

daytime when individuals are relatively inactive (Belovsky & Slade, 1986). Nevertheless, the obtained results still give some idea to the importance of behavioural thermoregulation as a mechanism for maintaining homeostasis. Average daily ambient temperature is not a complete description of the thermal environment and variation in bontebok activity and inactivity was probably caused by combined effects of various factors. However, daily ambient temperature and season appears to be important variables in predicting the probability of activity as well as inactivity.

### 5.3.2 Body orientation

The complete thermal environment is defined by ambient temperature, short-wave and long-wave radiation, wind velocity and humidity (Finch, 1972; Maloney *et al.*, 2005a). The effective thermal environment probably varies unpredictably in terms of postural adjustments of the animal, though it is assumed that air temperature and solar radiation were adequate indices of ambient conditions. To assess the preferred body orientation of bontebok concerning incident sun exposure, the angle between orientation of the sun and the long body axis were divided into two general categories. These consisted of perpendicular or parallel orientation, the latter was subdivided into animals directly facing the sun or with their tails directed towards the sun.

During the winter observational period bontebok showed a general tendency to orientate perpendicular with regards to incident solar radiation regardless of time of day (Fig. 5.18), hence there was an insignificant relation based on the Spearman's rank correlation between ambient temperature and the percentage of bontebok that displayed perpendicular orientation ( $r = -0.137$ ). Nevertheless, perpendicular orientation was particularly evident during the cold morning hours (07:00 - 10:00) when the majority of observed individuals preferred this orientation. Figure 5.18 shows that over the next few hours mean ambient temperatures ranged between 14°C - 19°C and during this time a somewhat random pattern is displayed. At 11:00 the majority of the herd orientated themselves in a parallel position, however almost equal amounts of individuals were either facing the sun, standing with their tails towards the sun or were positioned laterally. Over the next couple of hours the majority of herd members orientated perpendicular, whereas when temperatures



**Figure 5.18** Preferred body orientation in relation to incident sun exposure of a bontebok herd during the 2006/07 study period in the southern Free State.

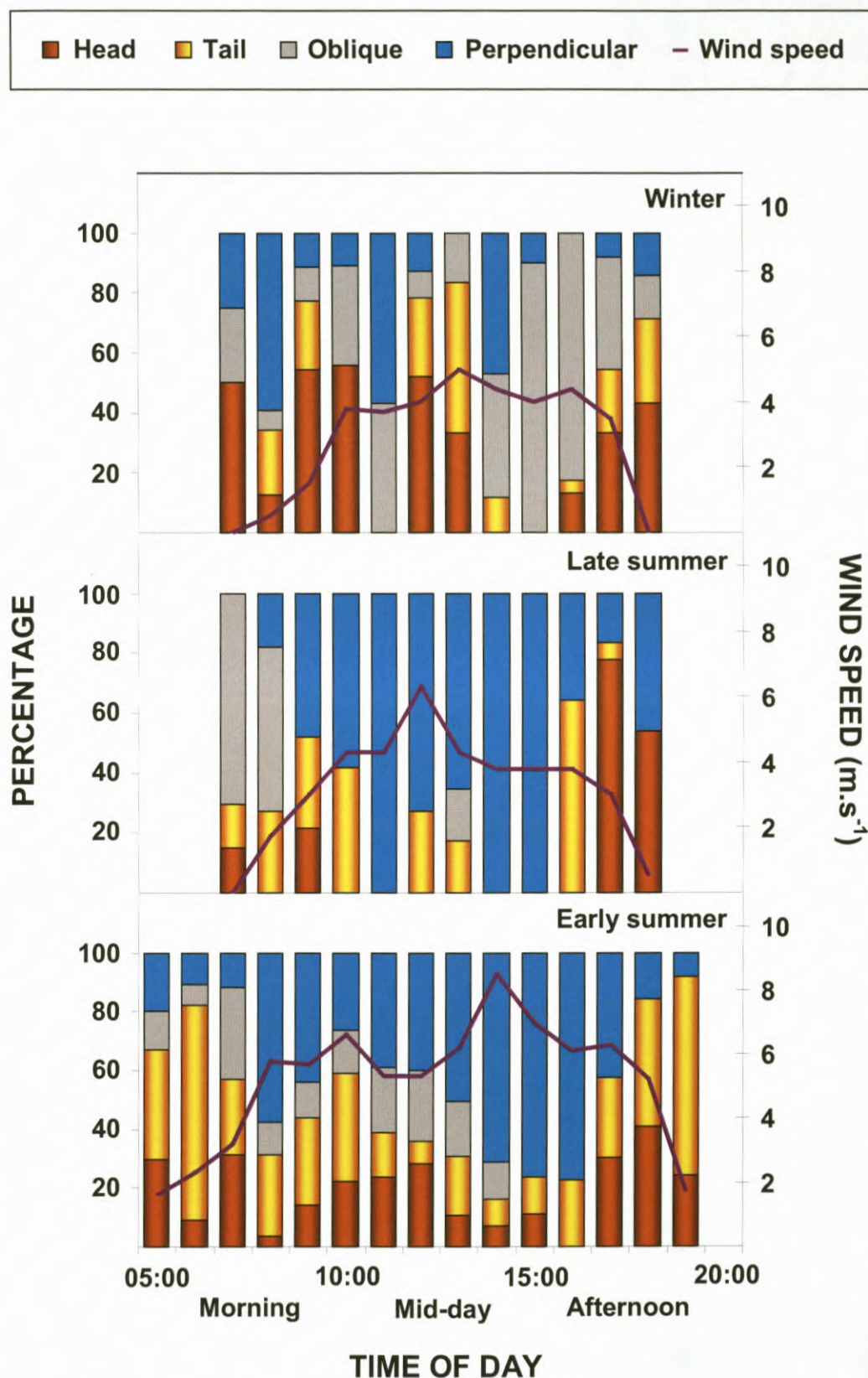
were high (19.3°C) nearly three quarters of observed bontebok stood with their tails towards the sun. Temperatures declined during the afternoon and bontebok once again tended to orientate laterally.

During early summer, for the first few hours following sunrise (05:00 - 08:00) at fairly low mean ambient temperatures (6°C - 12°C) bontebok were inclined to a perpendicular body orientation (Fig. 5.18). Throughout the remainder of the morning hours and the mid-day period, the majority of the herd were observed to be facing the sun. Very few animals directed their tails towards the sun, or demonstrated a broadside position. Temperatures steadily decreased during the afternoon hours and accordingly the majority of bontebok again preferred a perpendicular orientation. Preferred body orientation pertaining to this seasonal category was significant ( $r = -0.590$ ) at the 5% confidence level.

In general, the late summer season shows the most prominent pattern in terms of preferred parallel orientation (Fig. 5.18). Temperatures were relatively mild (9.5°C - 16°C) during the early morning (07:00 - 08:00) when nearly all herd members were prone to a perpendicular orientation. Temperatures increased over mid-day until the maximum temperature (29°C) was reached during the early afternoon (15:00). Throughout this period parallel orientation was favoured, particularly regarding animals that opted to stand directly facing the sun. The only exception was noted during the morning (10:00) when more than half of the herd members stood with their tails directed towards the sun, compared to fewer individuals (39%) that stood facing the sun. Unlike winter and early summer, the majority of herd members did not change to a perpendicular position during the late afternoon when mean ambient temperature started to decline. Only prior to dusk (18:00) when the mean ambient temperature had declined (21°C), did the majority change from a parallel to a lateral orientation. Observations were statistically significant as a negative correlation between the two variables was evident ( $r = -0.662$ ,  $p < 0.05$ ).

In order to determine the favoured body orientation of bontebok regarding the influence of wind (Fig. 5.19), the angle between wind direction and the long body axis were divided into three general categories. These consisted of perpendicular (90°; 270°), oblique (45°; 135°; 225°; 315°) or parallel orientation. The latter category was again subdivided into animals directly facing the sun (0°) or with their tails (180°) directed towards the sun.





**Figure 5.19** Preferred body orientation in relation to wind direction of a bontebok herd during the 2006/07 study period in the southern Free State.



Figure 5.19 shows no outstanding preferred body orientation regarding wind direction during winter. Accordingly, the obtained results were found to be insignificant ( $r = -0.220$ ). During the morning period most of the observed animals stood facing the wind, however more than half of these individuals were observed in a broadside position shortly after sunrise (08:00). At this time, wind speed was low ( $0.5 \text{ m.s}^{-1}$ ), but increased over the morning period ( $4 \text{ m.s}^{-1}$ ). Subsequent to a slight decrease in wind speed, all visible individuals preferred either a perpendicular (57%) or an oblique (43%) orientation (Fig. 5.19). A slight increase in wind speed, later during the day, was associated with the majority directly facing the wind. However, when maximum wind speeds ( $5 \text{ m.s}^{-1}$ ) were reached half of the visible animals (50%) stood with their rumps towards the wind and less bontebok (33%) directly faced the wind. A further decrease in wind speed during the early afternoon (Fig. 5.19) was associated with almost equal amounts of animals standing at an oblique or perpendicular angle (14:00), while the majority (83 - 90%) favoured an oblique orientation later in the afternoon (15:00 - 16:00). Prior to dusk, wind speed would sharply decrease, though more than half of the herd (54 - 71%) were inclined to a parallel orientation.

During early summer, wind speed was measurable from the onset of recordings and varied from more or less  $2.0 - 8.5 \text{ m.s}^{-1}$  throughout the day (Fig. 5.19). During the early morning hours (05:00 - 07:00) the majority of animals (57 - 83%) stood at a parallel angle, generally directing their tails towards the wind. Within the following couple of hours wind speed increased considerably ( $6 \text{ m.s}^{-1}$ ) and the majority of animals orientated themselves in a broadside position. An further increase in wind speed (10:00) was associated with more than half (60%) of herd members standing at a parallel angle. The remainder of individuals stood at a perpendicular angle and within this category, the majority stood with their rumps towards the wind (37%), while the others stood facing the wind. During the next couple of hours all of the orientation categories were evident (Fig. 5.19). Several individuals orientated in a parallel position, however more individuals stood facing the wind. Nearly half showed a perpendicular orientation, while more or less a quarter of individuals were positioned at an oblique angle. Shortly after midday till the early afternoon (13:00 - 16:00), the general tendency was for animals to orientate themselves either at a  $90^\circ$  or  $270^\circ$  angle with regards to wind direction. The highest wind speed ( $8.5 \text{ m.s}^{-1}$ ) was recorded over mid-day (14:00) when nearly three quarters of animals were positioned in a perpendicular manner. Average wind speed steadily

decreased during the late afternoon (17:00 - 19:00) and individuals once again opted for a parallel orientation, particularly standing with their rumps directed towards the wind (*vide* Fig. 5.19). Compared to winter, the relation between wind speed and orientation in early summer was negatively correlated ( $r = -0.781$ ,  $p < 0.05$ ).

During the observational period in late summer (*vide* Fig. 5.19), bontebok showed a somewhat different body orientation tendency concerning wind direction. Contrasting to a preferred parallel orientation during early summer, the majority of herd members (55 - 70%) were noted in an oblique position subsequent to sunrise (07:00 - 08:00). During the next observational hour almost equal amounts of individuals were observed in a perpendicular (48%) or parallel (52%) position. The latter category was represented by more animals standing with their tails towards the wind and fewer individuals stood facing the wind. For the remainder of the morning period and throughout the mid-day period the general tendency was for animals to position themselves at a perpendicular angle. In terms of parallel orientation, some animals were observed with their tails towards the wind during late morning (10:00) and again over the middle of the day (12:00 - 13:00) when mean wind speed ranged between 4 - 6  $\text{m.s}^{-1}$ . During the late afternoon (16:00 - 18:00), wind speed sharply declined and this was associated with animals favouring a parallel orientation. Several herd members stood with their tails towards the wind, but as wind speed increased during this period the majority of bontebok in a parallel position favoured a head towards the wind orientation. Wind speed declined sharply (0.5  $\text{m.s}^{-1}$ ) just before sunset when slightly more individuals (54%) were facing the wind, while the remainder (46%) were positioned at a perpendicular angle. Findings were statistically significant ( $r = -0.710$ ) at the 5% confidence level.

Unfavourable environmental conditions that prevail during the winter months may possibly add to the metabolic cost of thermoregulation (Garroway & Broders, 2005). Bontebok thus orientated at a perpendicular angle making full use of exposure to the sun's rays when ambient temperatures were relatively low. With the gradual increase in temperature the majority of animals were at certain times inclined to a parallel position either facing the sun or with their tails directed towards the sun. In contrast to the findings for black wildebeest (Vrahimis & Kok, 1992) and blue wildebeest (Berry *et al.*, 1983), the majority of bontebok individuals opted to stand with their rumps towards the sun instead

of a preferred head towards the sun orientation. A considerable amount of animals were also noted in a perpendicular orientation during the mid-day period, however there was a more distinct tendency of observed perpendicular orientation as ambient temperature decreased during the late afternoon. Generally, wind speed was relatively low during winter and appeared to have a lesser effect on body orientation than did sun angle. However, when temperatures were fairly mild and wind speed gradually increased over the mid-day period, animals orientated parallel and commonly favoured a tail towards the wind position.

During early and late summer bontebok also tended to present the long axis of their bodies to the sun during the early morning hours when temperatures were still relatively low to mild. For the duration of the mid-day period when temperatures were fairly high, the majority of observed animals showed a definite preference in terms of directly facing the sun. Cooler temperatures during the afternoon were again associated with a preferred perpendicular position, however this was not the case during late summer given that ambient temperatures were still relatively high and animals thus favoured a parallel position until shortly before sunset. Berry *et al.* (1983) commented that the analysis of data concerning sun angle during the summer months should be made carefully since sun altitude is high during these periods. When the sun is close to an overhead angle the incoming radiation impinges on the animal at angles of incidence close to  $0^\circ$  and the surface area that is presented to incident solar radiation becomes essentially independent of orientation (Riemerschmid, 1943). It would thus seem that the response to sun angle was therefore of greatest importance during the early morning and late afternoon. Early summer was associated with relatively high wind speeds and animals stood with their tails towards the wind during the early morning. A broadside orientation was favoured over mid-day when the majority of bontebok were positioned in an oblique or perpendicular angle, the latter was however the commonly preferred position. Wind speed decreased during the late afternoon and individuals once again stood with their rumps directed towards the wind. During late summer observed individuals preferred an oblique or perpendicular orientation from the onset of recordings. Mid-day, however, was again the time of day when a perpendicular angle was most evident. Parallel orientation was observed during the late afternoon, but the majority of animals did not position their rumps towards the wind and preferred to stand directly facing the wind.

The exact manner in which heat load affects the behaviour of animals varies between species, partly as a function of thermoregulatory mechanisms or morphological traits such as body size and characteristics of the coat (Wronski *et al.*, 2006). In view of this radiation, convection and evaporation are means of heat loss that are directly related to body size regarding the surface area of the body (Schmidt-Nielsen, 1997). Conduction is usually insignificant, but in combination with convection, it is a very important form of heat loss (Walsberg, 1983). The rate of conduction is not only a function of air temperature and ambient air movement, but also of body surface area. Postural changes affect body surface area, hence alternating the exposed body surface area would thus be an effective thermoregulatory mechanism. Taken together, the results suggest that bontebok act to avoid heat loss in the morning and afternoon. A broadside orientation in relation to sun angle during the early morning hours and late afternoon could possibly be interpreted as a means of seeking insolation by orientating and exposing their bodies in such a way as to facilitate heat uptake. This would possibly reduce the requirements for metabolic heat production. Wind was also considered an important ecological factor because of its influence on thermoregulation. In the winter months, wind slightly added to the cooling effect on the low air temperatures and individuals thus exposed their smallest surface of the body to air movement.

Under environmental conditions typical of summer in the southern Free State, the most important avenues of heat gain for bontebok were possibly solar radiation and thermal radiation emitted by ground surfaces given that air temperature and solar radiation are substantial components of the heat load borne by an animal. Conversely, during the summer months the main priority in dealing with problems of high radiant heat load concerns reducing heat gained from the environment and promoting heat loss (Stevenson, 1985; Schmidt-Nielsen, 1997). These authors postulated that a reduction of direct solar influx is unmistakably important in reducing excessive heat load and is accomplished by remaining in shade when radiation influx is highest and by grazing at night and at dusk and dawn. Although bontebok conform to the latter by having high intensity feeding phases at dusk and dawn (*vide* Fig. 5.13), they were unable to avoid ambient extremes and alternative behavioural mechanisms were observed during summer. Bontebok similar to blesbok, adopt a very distinctive standing posture (Fig. 5.20) during hot summer days (Du Plessis, 1968; David, 1973; Lynch, 1973) and orientate either with their heads or tails towards the sun. This parallel orientation



**Figure 5.20** An adult bontebok orientated towards the sun. The muzzle is close to the ground and the horns point slightly forward.

possibly contributes to thermoregulation as the smallest surface area is exposed to incident radiation. This reduction in heat gain would possibly reduce the requirement for evaporative cooling. David (1973) suggested that keeping the muzzle close to the ground could be advantageous in terms of convection currents bringing in cooler air closer to the ground. Individuals did not always orientate in the same direction, however, David (1973) stated that bontebok preferred facing the sun to any other direction and this was also observed during this study.

The fact that a fairly large percentage of observed bontebok were lying down over mid-day (*vide* Fig. 5.14) might also be of importance in terms of postural adaptations. Jarman (1977) proposed that lying down, rather than standing, would lessen the intake of reflected radiation from the ground during the summer months. Maloney *et al.* (2005b), however, suggested that specific body orientation whilst lying down almost certainly makes little difference to the surface area intercepting incident solar radiation as the animal presents its entire side to the sky. On the other hand, cold winds during winter can diminish activity to the point of many animals lying down for extended periods. This would then rather aid to conserve heat as less body surface is exposed to the wind. A good deal of bontebok also tended to turn their backs to the wind, which presumably has a similar effect. In addition to heat-loss, Leuthold (1977) commented that wind is also important to ungulates as a vector of scents and sounds providing them with information about the environment.

Conversely, breezes during the summer months were of higher velocity and here the effect was beneficial rather than adverse as wind contributed to relieve stress under hot conditions. As the sun angle cannot be avoided over certain hours of the mid-day period, individuals orientate themselves at an oblique or perpendicular angle, thus allowing the greatest amount of airflow over the body surface (Vrahimis & Kok, 1992).

As aforementioned, heat load is also affected by physical properties of the coat. Various authors (Finch, 1972; Walsberg, 1983; Rogowitz, 1990; Walsberg & Wolf, 1995) have recognised that it is a valuable part of the overall strategy to minimise or maximise heat absorption during the course of the day. The amount of heat that is absorbed through the body surface is determined by the density and colour of the fur as these factors affect the absorption and reflectivity of radiant heat (Delany &



Happold, 1979). Postural adjustments would change the angle of incidence of both sunlight and wind striking the coat and would probably influence the degree to which both sunlight and wind penetrate the coat. Animals may thus orientate in such a manner as to differentially expose body regions to vary insulation and colouration influences. The total resistance of the coat to heat transfer is determined from the resistance of the air trapped within the fur and also the resistance of the boundary layer of still air above the surface of the coat (Schmidt-Nielsen, 1997). Hofmeyr (1985) investigated the effect of wind speed on insulation of the coat. He determined that bontebok have a relatively thin pelage depth of around 4.0 mm and at a wind speed of only  $2 \text{ m.s}^{-1}$  the resistance of the boundary layer was significantly reduced. Given that average wind velocity fluctuated from  $0.5 - 8.5 \text{ m.s}^{-1}$  during the study period (*vide* Fig. 5.19), it seems that wind would have a pronounced effect on the thermal insulation of the coat. Apart from thickness of the pelage, the colour of the coat is also involved in thermal adaptation. The fractional absorptivity of the animal's surface, which is one component of its colouration, determines the proportion of intercepted sunlight and generates heat (Walsberg & Wolf, 1995). Riemerschmid (1943) accordingly demonstrated that cattle with light-coloured coats reflected more solar radiation than cattle with darker coloured coats. Hofmeyr (1985) found that reflectance values of different ungulate pelages increased with a decreasing angle of incidence and the mean absorptance for direct solar radiation varies across the coat. Bontebok have darker fur on their dorsum areas than on their ventrum areas. The saddle-area of bontebok have a value of 0.62, while the darker areas on the rump and sides have a value of 0.79 and the distinctive white belly has an average absorptance of 0.28 (Hofmeyr, 1985). Estes (1992) suggested that white rumps of ungulates may be used in thermoregulation since exposing the less absorptive white areas to solar radiation could minimise radiative heat gain. Springbok also exploit the colouration of their pelage by presenting the white rump which has a high reflectance, towards incident radiation (Hofmeyr & Louw, 1987). Hofmeyr (1985) determined that at wind speeds below  $2 \text{ m.s}^{-1}$ , the most important factor influencing surface temperature would thus be the absorptance of solar radiation. It is therefore expected that the dark brown surface should have a much higher temperature than the white surface and the reddish brown fur of the saddle area should demonstrate a heat load intermediate between that of the dark brown and white pelage (Hofmeyr, 1985). Taking this into consideration, the dark thin coat would thus be an advantage for sun basking on cold sunny days and would reduce the metabolic demands for thermoregulation. Conversely, the relatively low

absorptance value associated with a bontebok coat would reduce environmental heat load during hot conditions.

The thermal insulation of the coat is affected by forced convection and it has been established that conductivity increases with an increasing wind speed. Hofmeyr (1985) determined that the heat load of the reddish brown and white areas of a bontebok coat became more or less equal when wind speed increased to 2 - 4 m.s<sup>-1</sup>. Even so, at low wind speeds, the lower absorptance of the white fur outweighed the difference in penetrance. At higher wind speeds, however, the heat load on the different coloured areas was greatly reduced and the difference in absorptance became less important (Hofmeyr, 1985). Thus coat colour, thickness and density must all be taken into consideration when assessing the capabilities of the pelage to provide protection from heat gain.

Individuals commonly combine a number of thermoregulatory strategies to control body temperature. Ungulates demonstrate the variety of strategies commonly utilised and show how size, activity patterns and environmental requirements modify the methods of temperature regulation (Leuthold, 1977). The findings indicate that bontebok behaviour did vary with ambient thermal conditions in a manner implying that animals adopt strategies to behaviourally adjust energy exchange with the environment. It seems that there is an interaction between solar radiation and wind speed and that these variables jointly affect bontebok body orientation and can be viewed as a two-phase response to both sun and wind. Comparisons across seasons showed that parallel orientation occurred more during the summer months, and less in winter and early summer than during the late summer season. During the day, bontebok in an open habitat were more likely to orientate in a parallel orientation than in a broadside orientation, as ambient temperature and solar radiation increased or when wind speed decreased. It appears as if sun angle influences body orientation during early morning and late afternoon, while wind plays a role over midday when the sun is almost directly overhead. The extent of this reorientation process is thus dependant both on solar radiation and on wind velocity. Blesbok exhibited the same strategies to behaviourally adjust energy exchange with the environment (Van Aswegen, 1994). Thus, avoidance behaviour involving altering of activity periods as well as changing body surface areas that are exposed to thermal fluxes

appears to be the primary components of the behavioural thermoregulation strategy of both bontebok and blesbok.

To conclude, the acquired results highlight several behavioural similarities between bontebok and blesbok and this was not unforeseen owing to the close relation of the subspecies' and the comparable habitats of the study areas. The findings of this study are thus relevant to management of bontebok in the Free State and to further behavioural studies of the subspecies. Pertaining to consistencies between certain aspects of bontebok and blesbok behaviour, these can be considered indicative that behavioural differences are not sufficient to avoid hybridisation incidents. The occurrence of hybridisation between blesbok and bontebok specifically in the Free State can therefore not only be ascribed primarily to their relatively recent phylogenetic divergence and overlapping breeding seasons, but also as a result of the convergent behaviour observed in comparable habitats. Consequently, an effective preventative measure to consider relating to the hybridisation threat is removal of all bontebok stock from the natural range of blesbok. It would be in the best interest for conservation of the remaining pure populations that introductions of both subspecies should rather be restricted to areas in accordance with natural distribution ranges and habitat suitability.

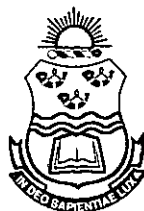


Chapter 6:

# STR Markers



## 6 STR MARKERS



### 6.1 STR MARKER ATTRIBUTES

Genetic molecular markers have allowed the opportunity to view the natural biological world from new and exciting perspectives and have become an indispensable means for conservation biologists. Relevant to the present study, detection of hybrid individuals has customarily relied upon applying morphological methodology. Though, these methods are considered as biased, presume that phenotypical differences have a genetic basis and lack the ability to identify hybrid individuals accurately beyond the first generation ( $F_1$ ) (Latch *et al.*, 2006). Therefore, use of current molecular genetic markers provides advanced techniques to differentiate hybrids without bias and with a high degree of resolution (Mock *et al.*, 2002; Cronin, 2003).

A genetic marker can be described as a segment of DNA (short or long sequence) or a gene with an identifiable location on a chromosome that can be employed to identify individuals, subspecies and species, but how would one define the "ideal" genetic marker? According to Queller *et al.* (1993) the ideal genetic marker would be user-friendly, conveniently discernable, highly variable and also infer high quality data for a large quantity of scorable loci. The quality of data made available by a genetic marker is thus contingent on how constantly and impartially the products can be scored, on how correctly they reveal the primary genetic variation and on how representative they are. No genetic marker fully satisfies all these criteria, but STR markers come exceedingly close to fitting this "ideal" model as this class of modern molecular markers have numerous attributes that sets them apart from certain other marker systems.

STR markers are abundantly dispersed throughout eukaryotic genomes. STR markers represent specific sequences of DNA consisting of tandemly repeated units of mononucleotide, dinucleotide and other short oligonucleotide motifs. The majority of microsatellites (30 - 67%) found are dinucleotides and in the genome of vertebrates, (AC)<sub>n</sub> is the most common dinucleotide motif (Toth *et al.*, 2000). It is 2.3-fold more numerous than (AT)<sub>n</sub>, the second most general type of dinucleotide, while higher order STR classes such as tri-, tetra-, penta- and hexanucleotides, are about 1.5-fold less frequent in genomic DNA of vertebrates (Toth *et al.*, 2000). A repeated simple sequence motif is expected to be present every 10 kb of DNA sequence (Tautz, 1989) and occur in protein-encoding as well as non-coding regions (Toth *et al.*, 2000), making it relatively easy to locate anywhere in the genome. By comparison though, it appears as if they are more concentrated in non-coding regions (particularly di- and tetranucleotide motifs) (Metzgar *et al.*, 2000; Chistiakov *et al.*, 2006) and less common in coding DNA, varying between 9 - 15% in vertebrates (Moran, 1993; Van Lith & Van Zutphen, 1996). Taking these features into consideration it can be concluded that genetic markers plentiful in distinct areas would thus be less informative compared to STR markers. Also, microsatellite loci found in non-coding regions are uninfluenced by selection and can consequently be used to measure neutral genetic variation at a locus, thus making them very useful in studies of hybridisation.

The most significant characteristic of STR markers is the hypermutability and consequent hypervariability in species and populations (Chistiakov *et al.*, 2006). The number of STR tandem repeats can vary in a sequence and many such variants (alleles) can be present in a population (Zane *et al.*, 1995; Mueller & Wolfenbarger, 1999). Following DeYoung *et al.* (2003), microsatellites boast higher mutation rates ( $10^{-2}$  -  $10^{-6}$  per locus per generation) compared to other neutral regions of DNA (Ellegren, 2000). The process of slipped-strand mispairing and subsequent errors during DNA replication, repair or recombination is known as DNA slippage and has been named the predominant mutation mechanism underlying microsatellite variability (Schlötter & Tautz, 1992). DNA slippage causes the loss or gain of one or more microsatellite repeat units and variation in the number of these repeats represents many different allelic states at individual loci. Hypermutability thus results in high information content of inter- and intra-specific polymorphism (Queller *et al.*, 1993). Compared to allozymes, the overall average number of alleles per loci has been found to be almost three-fold more



(Engel *et al.*, 1996). High polymorphism makes available significant data with which to distinguish, social groups, populations and individuals at a range of spatial scales (Anderson *et al.*, 2006).

A further attribute is that microsatellites are co-dominant and inherited in a Mendelian fashion. These markers are also of small size, allowing easy amplification via the PCR process to produce an adequate amount of DNA that can be visualised on polyacrylamide gels. Multiplexing of loci lessens the quantity of gel lanes and thus also the time and cost invested to obtain genotypes. Multiplexing of STR markers are also made possible by labelling primers with different colours of fluorescent labels.

The discovery that microsatellite loci are conserved across mammalian genomes (Moore *et al.*, 1991) raised the question of whether primers designed to amplify repeats in one species will amplify loci in other species. With the advances in PCR technology, primers that flank microsatellite loci are straightforward and quick to use, but the development of species-specific primers is a fairly time-consuming and costly process. The ability to use cross-species primers would therefore eliminate the time and expense involved in isolating microsatellite markers for every species of interest, which requires the construction and screening of genomic DNA libraries, DNA sequencing and design of primers. Subsequently, it has been well documented that conservation of the flanking sequences of long, uninterrupted microsatellite repeats across taxa, does indeed permit use of the same loci in closely related species (Moore *et al.*, 1991; Vaiman *et al.*, 1994; Slate *et al.*, 1998; Zane *et al.*, 2002; Kim *et al.*, 2004; Couch *et al.*, 2006). The capability to amplify STR markers from domestic animals for studies of wildlife species has proven to be beneficial since genetic loci from numerous domestic animals have been isolated and mapped to precise chromosomal locations. Furthermore, it has also been established that primers designed for use in one species of artiodactyl are capable of producing amplified microsatellites products in other artiodactyls (Arevalo *et al.*, 1994; Bhebbhe *et al.*, 1994; Holder *et al.*, 1994). This may occur within a family as shown for the Bovidae or between families as shown for species of Bovidae and Cervidae (Peppin *et al.*, 1995; Engel *et al.*, 1996; Roed, 1998; Van Hooft *et al.*, 1999).

Management of genetic diversity at the species (and subspecies) level has gained substantial importance in recent years (Bowen, 1999). Also, the abundance and wide genome coverage,

multi-allelic nature, high information content of polymorphisms, co-dominant mode of inheritance, amenability to PCR technology and high reproducibility features of STR markers have provided the foundation for their successful application in several studies that focussed on hybridisation between several animal species (Roy *et al.*, 1994; Beaumont *et al.*, 2001; Pierpaoli *et al.*, 2003; Grobler *et al.*, 2005; Funk *et al.*, 2007). STR markers have also been drawn on to assess hybridisation events below the species level and this served as a further incentive to make use of STR markers for the present study (Lorenzen & Siegmund, 2004; Lorenzon *et al.*, 2006).

## 6.2 DNA PROFILING OF *D. pygargus*

Summary of the genotypes obtained are presented in Table 6.1 for both blesbok and bontebok. Allelic variation ranged from one (MAF46) to six (OarFCB304) alleles per locus, with an average allelic diversity of 2.9. Comparison of the microsatellite loci between blesbok and bontebok revealed that all microsatellite loci except MAF46 were polymorphic in both subspecies. The MAF46 locus was monomorphic for allele 95 in bontebok, but polymorphic for blesbok which had three alleles present. In general, the overall size ranges of alleles detected for each locus was fairly similar for blesbok and bontebok, but a tendency towards a difference in size range was evident in MAF46, OarCFB304 and TGLA53 loci. Nonetheless, unique alleles were observed in all five loci that were screened in the present study. The results indicated that 41% of the alleles were found to be unique for blesbok, while bontebok exhibited 27% unique alleles. The other 32% was shared between the two subspecies. The locus CSSM18 had two alleles (192 and 198) that were unique to blesbok, with no unique alleles present for bontebok. As formerly mentioned MAF46 was found to be monomorphic for bontebok, but yielded two unique alleles for blesbok, namely 97 and 103. The locus OarCP26 had two alleles (120 and 124) unique to blesbok and also two alleles (126 and 128) unique to bontebok. At the locus OarFCB304, two alleles (135 and 139) were present in blesbok that were absent in bontebok, while alleles 145, 147 and 154 were restricted to bontebok. The locus TGLA53 had one unique allele for each subspecies, namely 142 (blesbok) and 154 (bontebok).

**Table 6.1** Genotyping analyses for five microsatellite loci indicating allelic variation and observed size ranges.

LOCUS	BLESBOK ( <i>n</i> = 12)			BONTEBOK ( <i>n</i> = 28)		
	No. of alleles	Allele sizes (bp)	Size range (bp)	No. of alleles	Allele sizes (bp)	Size range (bp)
CSSM18	4	190 192 196 198	190 - 198	2	190 196	190 - 196
MAF46	3	95 97 103	95 - 103	1	95	95
OarCP26	3	120 122 124	120 - 124	3	122 126 128	122 - 128
OarCFB304	3	135 139 141	135 - 141	4	141 145 147 154	141 - 154
TGLA53	3	142 150 152	142 - 152	3	150 152 154	150 - 154

Overall, the observed level of polymorphism was lower than the expected averages reported in the literature. According to Engel *et al.* (1996) microsatellites yield much higher levels of polymorphism in artiodactyls and according to this author as many as 14 alleles can be expected per locus. Here, an average number of 2.9 alleles per locus were reported. Since the microsatellites used in this study were originally developed for other species, there are a few possible explanations for the moderate microsatellite diversity of blesbok and bontebok. Lower observed polymorphism could have been the result, by part, of ascertainment bias. This concept refers to the implied predisposition of microsatellites to be less variable in species other than the focal species (Ellegren *et al.*, 1995). Several studies have shown that higher polymorphism and overall larger alleles were present in the focal species for which the primers were originally designed (Ellegren *et al.*, 1995; Rubinsztein *et al.*, 1995). This may partly be ascribed to a larger sample size for the focal species and also by unchecked variation in flanking regions (Jarne & Lagoda, 1996). Given that microsatellite loci are commonly selected for their attribute of being highly polymorphic, they are expected to exhibit larger repeats in the focal species than in a related species (Jarne & Lagoda, 1996).

Variation provided by the five microsatellite loci in this study indicated that the ovine markers amplified at a higher success rate and yielded higher polymorphism than the bovine primers. These findings were supported by the results of Flagstad *et al.* (1999) for the closely related hartebeest as well as findings by Van der Walt (2002) pertaining to *D. pygargus*. Following Matthee & Robinson (1999), the close relationship of alcelaphines to caprine species rather than to bovine species may partially explain the superior amplification results generated by the ovine primers.

Given that shared alleles were present at all of the screened loci, none could be identified as possible diagnostic markers for blesbok/bontebok hybrids. A substantial number of alleles shared between *D. pygargus* subspecies were not unexpected given that divergence from a common ancestor was estimated to have occurred about 1 million years ago. From an evolutionary perspective this time period is viewed as fairly recent. In addition, all samples were collected from Free State populations and seeing that the extent of admixture between the subspecies is unknown in the province, the influence of past hybridisation events on allelic diversity cannot be overlooked. It should also be pointed out that even greater success at amplification of these loci may be achieved by

varying the PCR conditions when required to conclude whether amplification products can be produced.

### 6.3 GENETIC STUDIES DONE

The allelic variation at three (MAF46, OarCP26, and TGLA53) of the five studied loci was compared to results obtained for the same three loci in a related study described in Van der Walt (2002) and is presented in Table 6.2. Findings of the current study corroborate previous genetic evaluations by this author. Of the 12 alleles found for blesbok and bontebok at MAF46, OarCP26 and TGLA53 in this study, eight have previously been reported as occurring in *D. pygargus* by Van der Walt (2002). Comparisons of each individual locus indicated similarities as well as discrepancies for scored loci. For MAF46 an added two unique alleles (93 and 107) for blesbok were found that were absent in this study, while allele 95 was also found to be monomorphic for bontebok. However, a second monomorphic allele (101) for bontebok was detected by Van der Walt (2002). Both studies scored allele 124 at the OarCP26 locus as well as alleles 152 and 154 at TGLA53, while Van der Walt reported a new finding for allele 144 (TGLA53).

Furthermore, on the basis of the studies' comparison, allelic variability of unique loci identified in the present study declined dramatically. The following alleles, namely 103 (MAF46), 126 and 128 (OarCP26) and 154 (TGLA53), that were found to be private for either blesbok or bontebok were eliminated as unique alleles given that comparison of the results of these two studies rendered them as shared alleles. It has previously been recognised that the issue of identifying diagnostic alleles is confounded by the fact that some parental populations may have overlapping sets of alleles (due to constraints on repeat number and back mutations) and there may be very limited numbers of private alleles that are found in only one or the other contributing population, as was the findings for this study (Nauta & Weissing, 1996; Kumar *et al.*, 2002). Van der Walt (2002) also stated that it was probable that any unique genetic variation that had evolved in the allopatric populations had been lost through hybridisation. Nevertheless, the observed allele size range for some loci was not in coincidence with those reported by Van der Walt (2002), so the following can possibly be considered new alleles for these loci: 120 (OarCP26) and 142 (TGLA53). It should be noted that no

**Table 6.2** Allele size ranges comparison for three loci obtained for blesbok and bontebok.

LOCUS			VAN DER WALT, 2002	
	BLESBOK	BONTEBOK	BLESBOK	BONTEBOK
	Allele sizes (bp)		Allele sizes (bp)	
MAF46	95	95	93	95
	97		95	101
	103		97	103
			103	
			107	
OarCP26	120	122	122	126
	122	126	124	128
	124	128	126	
			128	
			130	
TGLA53	142	150	144	152
	150	152	152	154
	152	154	154	



definite conclusions can be made based on these comparative results as the genotyping process between the two studies was not standardised.

#### 6.4 HYBRID IDENTIFICATION

As shown by the present study, assessment of allele sizes allows distinction between the alleles that are shared between taxa and the alleles that are unique to each taxon. If unique alleles are found to be present, they can be utilised as a tool to detect admixture among closely related taxa. The final step for identifying definite ancestry-informative alleles would thus require detailed studies of carefully selected pure blesbok, pure bontebok and confirmed hybrid populations to ascertain the assignment capabilities of promising loci. It should be noted, though, that the usefulness of STR markers is determined by the capabilities of statistical theories and associated methods which are used to analyse the conferred data (Chambers & MacAvoy, 2000). The modern development of statistical assignment tests to determine population membership of certain individuals (e.g. hybrid or pure taxa) through likelihood or Bayesian approaches has greatly advanced the facilitation of markers, such as STR markers, to assess matters of hybridisation (Latch *et al.*, 2006). Software programmes such as Structure (Pritchard *et al.*, 2000; Falush *et al.*, 2003) and Newhybrid (Anderson & Thompson, 2002) enable researchers to characterise parental populations, assign individuals to these populations, detect admixed individuals and estimate admixture of specific individuals based on allele frequency data. The ideal would be to sample all individuals from the population being tested, but this would be constrained by limitations of sample collection normally associated with these kinds of studies. Also, testing all members of large populations would involve high analysis costs that would make this alternative method less practical.

The analysis of the significance of variation at microsatellite loci within and between populations of blesbok and bontebok must be carefully considered and the choice of loci for hybridisation control should be made with caution. Rigorous assessment using larger sample sizes from different populations as well as a larger number of polymorphic microsatellite loci are highly recommended. It is also imperative to eliminate loci with null alleles and to avoid loci with high mutation rates. None of these factors were taken into consideration in the present study. Nevertheless, in spite of its

shortcomings, the present investigation illustrates that STR markers could prove to be powerful tools for future hybridisation analysis in *D. pygargus*.

In summary, genetic diversity can be viewed as the clay of evolution. The plight of *D. pygargus* in South Africa demonstrates that resolving anthropogenically-driven hybridisation and assuring viable blesbok and bontebok populations for the future are largely overlapping pursuits. This highlights the importance of maintaining the genetic integrity and diversity of both subspecies with a managerial programme for the identification and elimination of hybrids. In view of developing an alternative hybrid identification technique, analysis of DNA studies of populations offers the advantage of assaying the genotype rather than the phenotype. Categorisation capabilities of individuals and populations based on individual genotypes holds great potential for more accurate assessment of introgressive hybridisation of *D. pygargus*. This study, evaluating the ability of selected primers to amplify STR loci at the specific level, is preliminary to such a definitive study. Data obtained not only confirmed that informative polymorphic microsatellites can be cross-amplified between closely related representatives of Artiodactyla, but were also testimony of noteworthy differences in microsatellite allelic variation in *D. pygargus*. These findings thus represent baseline data that could assist future molecular studies aiming to identify alleles that would substantiate discrimination between pure and hybrid taxa. This information would not only augment conservation decisions, but also lend insight into the extent of introgressive hybridisation affecting *D. pygargus* populations.



Chapter 7:

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# Summary/Opsomming



## SUMMARY

Two subspecies of *Damaliscus pygargus*, blesbok (*D. p. phillipsi*) and bontebok (*D. p. pygargus*), are recognised based on phenotypical and native geographical distribution differences. Past introductions of bontebok lead to extensive hybridisation that produced numerous fertile hybrids in the Free State. The present study aimed at investigating behaviour of introduced herds by quantifying activity budgets and patterns to examine diurnal and seasonal effects on certain behavioural aspects, allowing comparisons with blesbok. A secondary objective was to determine amplification of ungulate microsatellite primers in *D. pygargus*. Activity data on bontebok totalling 1 098 hours were collected during 2006/2007 and 2009 in the Free State. Observational measurements involved instantaneous scan sampling as well as focal animal sampling. Blesbok and bontebok hair samples were also collected for genetic analysis. Time budgets revealed that equal amounts of time (50%) were spent on active and inactive phases. Grazing and lying were the major activities identified and represented more than three quarters (83%) of total observed activities. Less than one fifth of their time was diverted to standing (9%) and moving (8%). Behaviour of bontebok deviated slightly from blesbok as blesbok allocated more time to lying (40.4%) than to grazing (35.5%), while standing (16.5%) and moving (7.7%) represented nearly a quarter of the general time budget. The subspecies spent almost equal amounts of time on lying and moving, whereas bontebok allocated more time (6.5%) to grazing and less time (7.5%) to standing. Differences between bontebok social groups indicated that females spent the most time grazing (41%) and territorial males the least (31%). Juveniles spent nearly half of their time budget on lying and territorial males were least observed to be lying down (36%). However, territorial rams exhibited much higher standing (19%) and moving (14%) activity compared to any of the other social classes. Comparisons with blesbok social groups indicated similarities as well as discrepancies. Territorial rams also spent the least time grazing, but spent the most time lying. Blesbok juveniles allocated the most time to standing, however territorial rams also spent the most time on moving behaviour. Bontebok exhibited the same diurnal pattern as blesbok. A typical bimodal feeding pattern with principal grazing bouts in the early morning and late afternoon and a reciprocal pattern of lying activity around mid-day were observed. Adverse weather conditions had a seasonal effect on the basic pattern,



while grazing and lying time budgets showed significant seasonal differences. Noteworthy differences were thus found regarding bontebok behaviour categories among subsets classified by time of day, season, gender and age. Consistencies between certain behavioural aspects of bontebok and blesbok can be considered indicative that behavioural differences are insufficient to prevent hybridisation incidents. The occurrence of past hybridisation events can therefore not only be ascribed primarily to their relatively recent phylogenetic divergence and overlapping breeding seasons, but also as a result of the convergent behaviour observed in comparable habitats. Following screening of a preliminary panel of 72 ungulate microsatellite loci a concluding bovine and ovine panel of five microsatellite loci was identified. Disregarding MAF46 monomorphic pattern for *D. p. pygargus*, microsatellite loci showed moderate levels of polymorphism. Allelic variation ranged from one (MAF46) to six (OarFCB304) alleles per locus, with an average allelic diversity of 2.9. Alleles per locus per population ranged from one to four. Data obtained not only confirmed that informative polymorphic microsatellites can be cross-amplified between closely related representatives of Artiodactyla, but were also testimony of noteworthy differences in microsatellite allelic variation in *D. pygargus*. These findings thus represent baseline data that could assist future molecular studies aiming to identify alleles that would substantiate discrimination between pure and hybrid taxa.

**Keywords:** *Damaliscus pygargus*, translocation, subspecies, hybridisation, morphometrics, activity time budget, diurnal pattern, microsatellite markers, alleles, genotype.

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## OPSOMMING

Twee subspesies van *Damaliscus pygargus*, blesbok (*D. p. phillipsi*) en bontebok (*D. p. pygargus*), word erken op grond van fenotipiese en inheemse geografiese verskille in verspreiding. Bontebok translokasies in die verlede het gelei tot uitgebreide verbastering en gevolglik is daar talle vrugbare hibriede in die Vrystaat voortgebring. Die huidige studie het gepoog om gedrag van ingevoerde troppe te ondersoek deur aktiwiteitsbesteding en patrone te kwantifiseer om sodanig daaglikse en seisoenale effekte op sekere gedragsaspekte te ondersoek, wat vergelykings met blesbok moontlik gemaak het. 'n Sekondêre doelwit was om te bepaal of hoefdier-mikrosatelliet merkers kan amplifiseer in *D. pygargus*. In totaal is 1 098 ure se data van bontebok aktiwiteite verkry gedurende 2006/2007 en 2009 in die Vrystaat. Waarnemings is uitgevoer deur gebruik te maak van die momentskandeermetode sowel as die fokusdier-tegniek. Blesbok en bontebok haarmonsters is ook ingesamel vir genetiese analises. Tydsbegrotings het uitgewys dat gelyke hoeveelhede tyd (50%) op aktiewe en onaktiewe fases spandeer word. Wei en lê gedrag is as die hoof aktiwiteite geïdentifiseer en het meer as 'n driekwart (83%) van die totale waargenome aktiwiteite verteenwoordig. Minder as 'n vyfde van hul tyd is afgestaan aan staan (9%) en beweging (8%). Gedrag van bontebokke het effens afgewyk van blesbok, aangesien blesbokke meer tyd bestee het aan lê (40.4%) as aan weiding (35.5%), terwyl staan (16.5%) en beweging (7.7%) byna 'n kwart van hul algemene tydsbegroting verteenwoordig het. Die subspesies het byna gelyke hoeveelhede tyd spandeer aan lê en beweging, terwyl bontebokke meer tyd (6.5%) aan weiding toegestaan het en minder tyd (7.5%) aan staan. Verskille tussen bontebok sosiale groepe het aangedui dat ooie die meeste wei (41%) en territoriale ramme die minste (31%). Lammers het byna die helfde van hul tydsbegroting bestee aan lê en territoriale ramme (36%) was die minste waargeneem om te lê. Territoriale ramme het baie hoër staan (19%) en beweging (14%) aktiwiteite getoon as enige van die ander sosiale groepe. Vergelykings met blesbok sosiale groepe het ooreenkomste sowel as verskille aangedui. Territoriale ramme het ook die minste tyd aan weiding bestee, maar het die meeste tyd bestee aan lê. Blesboklammers het meer tyd toegestaan aan staan, maar territoriale ramme het ook die meeste tyd op bewegingsgedrag spandeer. Bontebokke het dieselfde daaglikse patroon as blesbokke getoon. 'n Tipiese bimodale voedingspatroon met die hoof weidingspieke in die vroeë oggend en laatmiddag en

'n wedersydse patroon van lê-aktiwiteit gedurende die middel van die dag is waargeneem. Ongunstige weerstoestande het 'n seisoenale uitwerking op die basiese patroon getoon, terwyl wei en lê tydsbegrotings beduidende seisoenale verskille getoon het. Noemenswaardige verskille is dus gevind met betrekking tot gedragskategorieë geklassifiseer deur tyd van die dag, seisoen, geslag en ouderdom. Konsekwenheid tussed sekere gedragsaspekte van bontebokke en blesbokke kan beskou word as 'n aanduiding dat gedragsverskille onvoldoende is om hibridisasie gebeurte te voorkom. Die voorkoms van hibridisasie gevalle in die verlede kan dus nie alleenlik toegeskryf word aan hul relatiewe onlangse filogenetiese divergensie en gedeeltelike oorvleueling van hul teelseisoene nie, maar ook as gevolg van konvergerende gedrag wat waargeneem word in soortgelyke habitats. Nadat 'n voorlopige paneel van 72 hoefdier-mikrosatelliet merkers getoets was is 'n finale bees en skaap paneel bestaande uit vyf mikrosatelliet merkers geïdentifiseer. Uitsluitend MAF46 wat monomorfies is vir *D. pygargus*, is matige vlakke van polimorfisme getoon. Alleliese variasie het gewissel van een (MAF46) tot ses (OarFCB304) allele per lokus, met 'n gemiddelde alleliese diversiteit van 2.9. Allele per lokus per bevolking het gewissel van een tot vier. Verkrygte data bevestig nie net dat insiggewende polimorfiese mikrosatelliete kan kruis-amplifiseer tussen naverwante verteenwoordigers van Artiodactyla nie, maar is ook getuigenis van noemenswaardige verskille van mikrosatelliet alleliese variasie profiele in *D. pygargus*. Hierdie bevindinge verteenwoordig dus basis data wat kan bydra tot toekomstige molekulêre studies met die doel om allele wat diskriminasie tussen suiwer en hibried taksa staaf, te identifiseer.

**Sleutelwoorde:** *Damaliscus pygargus*, translokasie, subspesies, verbastering, morfometrie, aktiwiteit tydsbegroting, daaglikse patroon, mikrosatelliet merkers, allele, genotipe.

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