Association between horn size, anthropometrical parameters and scrotal circumference of gemsbok bulls (*Oryx gazella*)

By

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Declaration

I, the undersigned, declare that the dissertation, hereby submitted for the degree MSc (Agric.) Animal Science: Production Physiology, at the University of Pretoria, is my own work and has not previously been submitted by me or another individual for a degree at this or any other tertiary institution.

S. Grünschloss
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Summary

Commercial game breeding practices place immense pressure on the selection of antelope species with long horns and improved genetics in order to maximise their monetary values. Current hunting policies are aimed at the longest-horned individuals within a population and the longevity of such individuals is drastically reduced. Eliminating long-horned animals from natural populations will shift their dynamics toward young and inexperienced males carrying short horns. In order to either support or dissuade current gaming industry directives, the genuine influence of extra-long horns on the male antelope's fertility and overall masculine appearance should be thoroughly investigated. The objective of this study was therefore to determine the correlation between horn size and scrotal circumference of gemsbok bulls. The gemsbok population sampled in this study was periodically subjected to culling. Bulls (n = 144) were harvested by professional hunters on a random seeing-equals-shooting basis. However, due to continuous growth and the onset of senescence, respectively, males younger than 3 years and older than 10 years were excluded, reducing n to 106 animals. Individually marked carcases were categorised into either age group 1 (3 years ≤ x ≤ 5 years) or age group 2 (5 years < x ≤ 7 years), weighed and the relevant body and horn parameters recorded. These parameters were determined using a measuring tape and comprised of the following: heart girth, body length, face length, neck circumference, cannon bone length, cannon bone circumference, scrotal circumference (SC) and shoulder circumference, as well as horn lengths, apex lengths (distance between last ring and tip of horn), horn ring number, long horn (long horn), total horn (sum of right and left horns), tip-to-tip, and horn base circumferences. The influence of the different age groups on individual body and horn parameters was analysed and the only non-significant difference in means observed for body measurements was for cannon bone circumference (CBC). This observation meant that bone circumference did not increase, irrespective of age. With regards to horn measurements only horn rings left (HRL) showed to be significantly different ($P \leq 0.05$) between the age groups, indicating higher ring numbers for older bulls. All the parameters were then correlated with SC to test the perceptions that male appearance and weaponry would be significantly impacted by changes in scrotal size. Heart girth, body length, face length, neck circumference, and shoulder circumference were found to be greatly influenced by SC ($P \leq 0.001$) in young bulls (age group 1), suggesting that any developmental change in one would significantly affect the other. These strong relationships also implied that SC gave a good indication as to a gemsbok bull's growth and physical conformation up to the age of 5 years, and that bulls exhibiting large testes would have increased body weights and larger anthropometric measures. It appeared, however, that all relevant growth parameters reached their maximum points as they showed no further increase in bulls older than 6 years (age group 2). Looking at age group 2, cannon bone length was the only parameter significantly ($P \leq 0.01$) correlated with SC. This finding suggested that significant bone growth only occurred after a specific threshold for SC was achieved. Both apex lengths for age group 1 were highly significant ($P \leq 0.001$), but negative in association with SC, suggesting a reduction in apex lengths with scrotal enlargement. These correlation coefficients allowed for the calculation of relevant parameter regressions per age group. Body weight, heart girth, body length, and face length (age group 1) increased linearly with SC, as well as cannon bone length (age group 2). Left horn length, total horn, and long horn for age group 1 increased simultaneously in correlation with SC until a circumference of approximately 24 cm was reached. An increase in ring number was hinted at when both apex lengths decreased for age group 1. The opposite was observed for age group 2: ring numbers decreased significantly ($P \leq 0.05$) as SC increased which suggested a reduction in overall horn lengths for older bulls due to honing or breakage during aggressive displays. Therefore, bulls with the largest scrotums were often the most dominant and did not exhibit the longest horns. However, no significant negative impacts of larger-than-average weapons masculinity and SC was observed in young gemsbok bulls while, a negative effect was noted in males older than 6 years, so H_1 was conditionally accepted in the present study area. Since the animals sampled in this study were a free-roaming population, they were not subjected to intensive breeding protocols for trophy horns. There is a need for more research on the effects of horn size on the fertility and masculinity of male antelope in populations with intensive selection pressure for horn size.

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LIST OF ABBREVIATIONS

BAG	Bushmanland Arid Grassland
BL	Body length
BW	Body weight
CA	Cyproterone acetate
CBC	Cannon bone circumference
CBC	Cannon bone circumference
CBL	Cannon bone length
DSP	Daily sperm production
FA	Fluctuating asymmetry
FL	Face length
FSH	Follicular stimulating hormone
GH	Growth hormone
GHRH	Growth hormone releasing hormone
GTRH	Gonadotrophin releasing hormone
HG	Heart girth
HRL	Horn rings left
IGF-I	Insulin-like growth factor I
LH	Luteinising hormone
n	Effective population size
SE	Standard error
NC	Neck circumference
SC	Scrotal circumference
SHC	Shoulder circumference
STs	Seminiferous tubules
T	Testosterone

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction and motivation

Having large weaponry appears to be advantageous to the survival of male bovids and cervids. Santiago-Moreno *et al.* (2012) found that access to oestrous females were mostly obtained by bulls and rams which exhibited the longest and most symmetrical horns as these animals had greater competitive abilities.

During a study performed by Tabbaa *et al.* (2006), in which he investigated factors affecting scrotal circumference and semen characteristics of Awassi rams, similar results were recorded. It was found that horned males were much heavier and had better semen quality than their polled counterparts.

In addition it was found that buffalo bulls with larger horns had stronger immune systems and were less likely to be infected by parasites (Ezenwa & Jolles, 2008), and that the presence of horns or antlers significantly affected the fertility of bucks and rams, with incidences of intersexuality associated with polledness (Al-Ghalban *et al*., 2004).

Because weapons are such prominent features of successful mating rituals, horns and antlers are regarded as honest indicators of male competitive ability and quality, such as body weight and sperm production (Ezenwa & Jolles, 2008; Santiago-Moreno *et al*., 2012).

An animal's ability to survive within its environment is reflected by a measurement of fluctuating asymmetry (FA) which is defined by Møller & Pomiankowski (1993): "Fluctuating asymmetry occurs when an individual is unable to undergo identical development on both sides of a bilaterally symmetrical trait". To put it simpler, FA refers to a deviation from perfect bilateral symmetry caused by developmental instability, environmental stressors, and genetic problems during development. In other words, the more perfectly symmetrical an organism is, the better it has been able to handle developmental stress and therefore has more developmental stability.

Fluctuating asymmetry is used in resource competition, as well as in sexual selection. This means that the higher the FA, the less symmetrical or attractive an individual will appear and mates with a low FA should be selected. Due to this negative correlation between FA and the size of horns, Møller & Pomiankowski (1993) concluded that weapons offer a reliable projection of individual quality.

During a study conducted on gemsbok (*Oryx gazella*; Linnaeus, 1758) in Namibia's Etosha National Park, Møller *et al.* (1996) attempted to determine the relationship between fitness traits and horn FA. They found that individuals of both sexes, that carried symmetrical horns, were in better condition and were therefore more likely to win aggressive interactions than asymmetric individuals. They also learned that bulls living as part of the herd had less symmetric horns (higher FA) than single, territorial males, making them less likely candidates for mating as females found them less attractive (Møller *et al*., 1996).

A correlation between testosterone (T) increase and horn growth was investigated as the development of large weapons was thought to be primarily under the control of this androgen (Bartoš *et al.*, 2009). Insulin-like growth factor I (IGF-I), an important contributing hormone in the growth process, was also suspected.

After investigating the effects of testosterone and IGF-I on antler growth in red deer, Suttie *et al.* (1985) found controversial results, which Bartoš *et al.* (2009) explained was caused by the differences in the ages of subjects used.

Antler growth for immature bucks occurred during the time of accelerated body growth. As these young males exhibited low levels of testosterone, IGF-I was the primary hormone correlated with body growth, more strongly than with antler growth (Bartoš *et al.*, 2009). In mature males the primary hormone associated with growth was testosterone, which led Bartoš *et al.* (2009) to make the same conclusion as they did for IGF-I: body growth rather than horn growth was associated with increased testosterone levels in dominant males (Bartoš *et al.*, 2009).

This finding was supported by a study conducted by Santiago-Moreno *et al.* (2012), in which they investigated the role of testosterone and photoperiod on seasonal changes in horn growth and sperm variables in the Iberian ibex.

Most horned mammals are seasonal breeders, with greatest activity during the cooler months of autumn. Santiago-Moreno *et al.* (2012) postulated that high levels of testosterone stimulated an endocrine signal that suppressed horn growth during the breeding season. Their hypothesis was rejected as they discovered that horn growth during the mating period was primarily regulated by day length. This meant that the periods during which horn growth was inhibited (breeding season), the highest levels of testosterone was produced.

Horns and antlers are rich in minerals. These minerals include calcium, magnesium, sodium, potassium, zinc, iron, and silicon (Landete-Castillejos *et al.*, 2007). The amount of daily energy required to grow and maintain horns and antlers was found by Toïgo *et al.* (2013) to be quite significant, leading to the expectation of high social and physiological costs. By growing large weaponry early in life, the future growth and survival of the animal was jeopardised (Toïgo *et al.*, 2013). Theories regarding potential reproductive and growth problems were thought to be associated with large horns, supported by the fact that bulls and rams experienced a proposed natural selection pressure which prevented them from developing too elaborate horns (Bro-Jørgensen, 2007).

The objective of this research project was to determine whether the presence of larger-than-average weaponry negatively influenced gemsbok bull secondary sexual characteristics such as scrotal circumference and horn parameters, directly affecting its masculinity and associated survival abilities.

The aim of this project was to test the following hypotheses.

- H_0: There is an observable negative impact on gemsbok bull scrotal circumference and masculinity parameters associated with larger-than-average horn sizes.

- H_1: There is no observable negative influence on gemsbok bull scrotal circumference and masculinity parameters associated with larger-than-average horn sizes.

1.2 Factors that affect horn growth in game

1.2.1 Resource availability

During a 30 year study conducted by Festa-Bianchet *et al.* (2004) in Alberta, Canada, the variation of resource allocation between horn and body growth of bighorn rams were investigated. They found that horns became progressively smaller as ram mass decreased. This reduction in horn length was also recorded during periods of intense drought and in areas where habitat loss was severe. Taking into account the influence of age, these observations were to be expected as younger males carried smaller horns than mature rams and horn size decreased when resources was scarce. Horn growth was therefore affected by a complex interaction of resource quality and availability, body weight, and age (Festa-Bianchet *et al*., 2004).

Older rams seemed to be impacted differently, as found by Bunnell (1978), who studied variation in horn growth of Dall sheep rams in Alaska, Canada. Rams showed greater horn growth at low rather than high resource availability (Bunnell, 1978). The same observations were made by Festa-Bianchet *et al.* (2004). Yearly horn growth of bighorn rams, as a result of resource scarcity, only decreased up to the age of 5 years, after which horns were grown more efficiently.

During good years in terms of resource quality and availability, horn and body growth across all age-classes were uniformly enhanced, whereas only horn growth of young rams (body growth continued albeit at a reduced rate) was suppressed during periods of resource scarcity (Bunnell, 1978). It was therefore concluded that ram age had a significant influence on horn and body growth resource allocation.

1.2.2 Energy allocation

"The production of antlers requires energy above that for maintenance of basal functions, and is especially demanding of minerals". Stewart *et al.* (2000) made this statement after thoroughly studying antler size: body mass ratio in moose. They found that mature bulls, at great expense to body condition, invested more successfully in reproduction and antler growth than young males.

However, less energy was found to be directed towards antler growth by immature and old bulls, because of the necessity to complete body growth, and due to the onset of senescence, respectively (Stewart *et al*., 2000). It can therefore be concluded that young males delayed investment in breeding until they were physically mature and capable of competing (well developed weapons) for oestrus females in order to maximise their mating success (Stewart *et al*., 2000). Festa-Bianchet *et al.* (2004) made a similar claim: young rams only improved their reproductive success after they had sufficiently invested in horn growth.

1.2.3 Population density

Another factor that appeared to have impacted horn lengths was population density. This affect was demonstrated by bighorn herds when they developed smaller and thinner horns with a concomitant increase in their numbers (Jorgensen *et al*., 1998). Crosmary *et al*. (2013) obtained similar results for impala and greater kudu. However, only when resource availability was significantly reduced as a result of high numbers did population density became effective as a limiting factor of horn growth (Crosmary *et al*., 2013; Jorgensen *et al*., 1998).

1.2.4 Testes size

At any stage of development the removal of the testes had an immediate inhibiting effect on horn growth in herdwick sheep (Marshall & Hammond, 1914). Testes size for mammalian species was found to have a direct correlation with the amount of spermatogenic tissue it contained, the rate at which sperm was produced and, therefore, with the male secondary sexual characteristics including weaponry and masculine appearance (Musaddin *et al*., 1982; Preston *et al*., 2012).

Møller (1989) found that testes size was positively correlated with sperm reserves (number of sperm in the epididymis) and sperm production. Mammal species with large testes exhibited large sperm reserves, high sperm production rates, a greater proportion of motile sperm, and a large number of sperm per ejaculate (Møller, 1989). Harvey & Harcourt (1984) observed a higher number of seminiferous tubule: connective tissue ratio associated with larger testes which allowed for the accommodation of large sperm reserves.

Similar results regarding testes size and sperm quality were recorded for *Mirza zaza* lemurs (Rode-Margono *et al*., 2015) and fish species (Stockley *et al*., 1997).

Hemicastration in adult rabbits did not change the development of the remaining testis in terms of its daily sperm production, number of Sertoli cells, or testicular weight (Thompson & Berndtson, 1993). This finding supported observations made by Marshall & Hammond (1914) that when only one testis was removed for herdwick rams, the other still provided sufficient stimuli for horn growth to continue, albeit at a slower rate.

However, as was previously mentioned, testosterone appeared to affect body growth rather than horn development, which contradicts these findings by Marshall & Hammond (1914). As horn growth was most rapid during periods of low testosterone levels, it can be postulated that castration (no testosterone production) will result in the longest horns.

During a study conducted by Simmons & Emlen (2006) on horned beetles it was found that a trade-off existed between testes size and weapon growth. Male beetles that were prevented from growing horns had larger testes compared to their horned counterparts (Simmons & Emlen, 2006). The possibility of such a trade-off existing for antelope species will be investigated in this research project, as the selection for longest horns in the game industry is suspected by this author to negatively influence scrotal circumference and masculinity in male antelope.

1.3 The impacts of long horns and trophy hunting on population dynamics

1.3.1 Longevity

Reproductive success of male antelope is intricately linked to the size of their weapons, which determine their position in the dominance hierarchy. Possible negative impacts of long horns on the male's fitness are thought to exist, although to what degree is still uncertain.

Robinson *et al*. (2006) found evidence of a trade-off between longevity and breeding success in large-horned Soay rams. They postulated that rapid horn growth may be counterbalanced by a reduction in longevity, because horns are costly to produce and maintain. Substantiating this theory was evidence provided by Bergeron *et al*. (2008) and Toïgo *et al*. (2013) after they investigated the correlation between horn length and longevity in Soay and ibex rams, respectively. Both studies indicated that rapid horn growth at a young age significantly compromised survival of these individuals.

When horn sizes and longevity were compared, Callaway (2013) only found 61 % of long-horned Soay rams to have survived the harsh winter conditions, as opposed to the 75 % of small-horned males. Therefore, longevity of dominant males (thought to also be the most fertile) appeared to be significantly shorter than the average individual.

Reasons for this reduced longevity were unclear. Toïgo *et al*. (2013) theorised that long-horned males experienced a greater pressure on their daily energy reserves, with large amounts directed towards reproduction, even at a young age. However, this train of thought contradicts previous theories that young males will only direct energy towards reproduction when they have fully matured. Many contradictions revolve around the possible detrimental effects of long horns on male antelope fitness and the aspects comprising it (masculinity and secondary sexual characteristics), making further research imperative.

1.3.2 Trophy hunting

Loehr *et al*. (2006) suggested trophy hunting as a reason for shorter life-spans in long-horned rams. They compared horn lengths of thin-horn rams in the Yukon, Canada, between hunted and non-harvested populations and observed a decrease in longevity which they associated with rapid horn growth. They theorised that hunting policies, based on horn curl, had negative implications for fast-growing, long-horned rams as these policies resulted in long-horned rams being shot at an earlier age than small-horned males (Loehr *et al*., 2006).

These findings were substantiated by Festa-Bianchet *et al*. (2014) when they conducted a similar study on bighorn sheep and found a concomitant increase in age with a decrease in horn size associated with trophy hunting. They found, over a period of 18 years, an average increase of 4 years in the age of harvested males with an associated decrease in horn size.

Similar results were obtained by Crosmary *et al*. (2013), who recorded a 4 % and a 6 % decline in the horn lengths of impala and sable antelope, respectively. However, the opposite were found for greater kudu:

bull ages and horn lengths (by 14 %) increased. These unexpected results were ascribed to the reduced hunting pressure experienced by the species during the study period (Crosmary *et al*., 2013).

As was predicted, rams that conformed to predetermined hunting thresholds (larger individuals with long horns), were harvested at a young age, leading to a decline in the available trophy rams within the population (Festa-Bianchet *et al*., 2014). This morphologically-biased hunting explained the negative relationship between longevity and growth rate as was demonstrated in harvested populations, whereas reduced life-spans were attributed to natural mortalities in non-harvested herds (Loehr *et al*., 2006). These findings suggested both direct and indirect mortality costs associated with rapid growth and large weapons (Loehr *et al*., 2006).

The stability of populations was significantly influenced by the removal of long-horned Soay rams, shifting their dynamics toward herds dominated by young, inexperienced males (Robinson *et al*., 2006). Crosmary *et al*. (2013) came to the same conclusion as "...harvesting shifts the surviving male segment towards younger age classes".

Greater kudu and sable antelope bulls showed an average of 4 years at which horn growth was fully completed, making them breeding-competent (capable of monopolizing mating opportunities) by age 5 (Crosmary *et al*., 2013). Assuming they would have had immediate success in gaining access to their own ewe herds, this meant that bulls became potential hunting trophies at least an entire year before they reached the appropriate social status which would have enabled them to reproduce (Crosmary *et al*., 2013).

This reduced longevity observed for trophy males cancelled their potential for enhanced competitive abilities and mating successes, offering inferior rams equal or even greater opportunities for procreation (Festa-Bianchet *et al*., 2014). Crosmary *et al*. (2013) thought that morphological changes were induced in the population due to this dominance by small-horned rams, which included lower genetic variability and reduced fitness.

The negative impacts of trophy hunting, however, remain equivocal as no evidence of this depressed survivorship was found by Murphy *et al*. (1990) when they compared non-harvested and harvested Dall sheep populations in Alaska.

Dominant bulls and rams, associated with the largest weapons, generally produced the best quality sperm (Santiago-Moreno *et al*., 2012). However, due to the high percentage of sperm abnormalities, which was recorded for these males, Santiago-Moreno *et al*. (2012) associated it with rapid horn growth.

Horns and antlers were theorised to comprise 15 % of the animal's total body mass, totalling 1 % of its energy budget in maintenance, and functioning as major sources of heat loss (Festa-Bianchet *et al*., 2004). This concept of heat regulation led Brø-Jorgensen (2007) to believe that small-horns dominated in cold climates, and long-horns in warm climates, in order to facilitate the loss of excess body heat. Picard *et al*. (1994) first made this link when they stated that "... the metabolic costs of possessing large horns in cold climates may impose constraints on morphology and sexual selection". Antlers, which are shed during active breeding periods, were excluded from this assumption.

"If selection acts continuously upon a trait it will presumably favour certain genotypes at the expense of others and hence result in the depletion of genetic variation underlying that trait". Robinson *et al.* (2006) made this statement after realising the potential negative impacts of inbreeding on the genetic stability of game populations. Inbreeding was found to be a real and definite threat to the sustainability of game herds as it led to decreased body weights, slower growth rates and poorer muscular development in male breeder animals (Du Plessis *et al*., 2006). As game was fenced in since the 1980's, and considering intensive game breeding practices, inbreeding can be considered a significant influencing aspect on game genotypes (Du Plessis *et al*., 2006).

1.4 Hormonal influences on body and horn growth

1.4.1 The gonadal and somatotrophic axes

Growth was determined by the sum totals of linear anthropometric measures and body weight gains inYankasa rams (Akpa *et al*., 2006). Both these parameters were found to be significantly influenced by age and testes size, the latter of which was attributed to the combined physiological effects of the (1) gonadal and (2) somatotrophic axes (Akpa *et al*., 2006).

Explanations of these axes were provided as follows:

(1) Testosterone, the male sexual androgen, was produced by the gonads, more specifically, the Leydig cells found in the testes, initiating the development of reproductive organs and sperm in the mature male individual (Society for Endocrinology, 22 Apex Court, Woodlands, Bradley Stoke, Bristol BS32 4JT, UK, www.endocrinology.org). In response to gonadotrophin-releasing-hormone (GTRH) from the hypothalamus, luteinising hormone (LH) was produced and secreted by the activated pituitary gland. After reaching the gonads through the bloodstream, LH triggered the production of testosterone (Society for Endocrinology, 22 Apex Court, Woodlands, Bradley Stoke, Bristol BS32 4JT, UK, www.endocrinology.org).

High levels of testosterone fed back to the hypothalamus in order to inhibit the release of GTRH. This suppressed level of GTRH terminated the production of LH, decreasing the secretion of testosterone. When these low levels of testosterone were detected, the cycle was reactivated (Society for Endocrinology, 22 Apex Court, Woodlands, Bradley Stoke, Bristol BS32 4JT, UK, www.endocrinology.org).

(2) The hypothalamus released growth-hormone-releasing-hormone (GHRH), stimulating the pituitary gland to release growth hormone (GH) into the bloodstream, initiating and sustaining growth processes throughout the body (Society for Endocrinology, 22 Apex Court, Woodlands, Bradley Stoke, Bristol BS32 4JT, UK, www.endocrinology.org). Insulin-like growth factor I (IGF-I) was produced and secreted by the liver in response to GH, and was associated with several metabolic and growth actions, including horn production and maintenance (Rizvi *et al*., 2006).

The levels of IGF-I, GH, and GHRH were regulated by each other. In order to terminate the release of GHRH, IGF-I and GH acted upon the hypothalamus. Somatostatin was secreted by this negative feedback pathway and prevented the release of GH from the pituitary gland and of GHRH from the hypothalamus (Society for endocrinology, 22 Apex Court, Woodlands, Bradley Stoke, Bristol BS32 4JT, UK, www.endocrinology.org).

1.4.2 The influence of testosterone and insulin-like growth factor 1 (IGF-I) on horn development

When Suttie *et al.* (1985, 1988) compared the increase in antler growth with seasonal variations of hormones in red deer, they found IGF-I plasma concentrations to be considerably high during the velvet antler growth period. They concluded that this information was sufficient to classify IGF-I as an antler-stimulating hormone.

Although IGF-I was found to be positively correlated with GH, its specific effect on weapon growth was still considered to be inferior to that of testosterone and its derivatives (Bartoš *et al.*, 2009). This uncertainty regarding the importance of IGF-I on horn and antler growth was enhanced when data recorded by Blake *et al.* (1998) and Bartoš *et al.* (2000) contradicted conclusions made by Suttie (1985, 1988). These researchers performed experiments on reindeer and castrated fellow deer, respectively, and observed that antlers continued to grow during the last 2 months of the growth period, when IGF-I levels had significantly decreased. Insulin-like growth factor I, therefore, had a much lesser impact on antler growth than was initially suspected.

Admittedly, ages of trail animals differed greatly, which might have led to inconsistencies in results (Bartoš *et al.*, 2009). It is therefore recommended to perform future horn growth studies on mature males, which will exhibit finished body growth and limited GH secretion.

Bartoš *et al.* (2009) followed their own advice and set about measuring blood concentrations of IGF-I and testosterone of 2-year-old and adult red deer stags. They predicted that, rather than having a direct impact on weapon growth IGF-I secretion only accelerated body growth, and would thus be most prevalent in young stags. They measured antler lengths at 1 week intervals and discovered that, in adult males, antler growth was primarily regulated by testosterone (Bartoš *et al.*, 2009). A possible effect of IGF-Ion antler growth was only detected for the 2-year-olds, as they did not yet produce sufficient LH and testosterone at this age (Bartoš *et al.*, 2009).

1.5 Histological functions of testicular tissue and the impacts on daily sperm production

1.5.1 Leydig cells

Spermatogenesis refers to “all the processes involved in the production of gametes” and occurs in two morphologically distinguishable compartments of the testis: the seminiferous tubules (STs) and the interstitial compartment between the STs (Weinbauer *et al.*, 2010).

Neaves *et al.* (1987) discovered that Leydig cells, situated within interstitial tissue of the testes, were responsible for testosterone secretion. Testosterone, as the primary male sexual androgen, is imperative for the stimulation of spermatogenesis (Sertoli cells) within seminiferous tubules (Neaves *et al*., 1987). A possible relationship between the number of Leydig cells and the rate of daily sperm production (DSP) was expected and investigated by Neaves *et al*. (1987) in human males. Only older men were included in their study as young men was found to exhibit a functional excess of Leydig cells.

Results obtained were disappointing as no correlation was detected after the effects of age were removed (Neaves *et al*., 1987). Although there was an observable decline in the number of Leydig cells associated with age, the DSP was maintained due to an increase in cell size (greater cytoplasm volume). This suggested compensatory testosterone production by the enlarged Leydig cells (Neaves *et al*., 1987).

Although performed on humans, histological similarities allow for the same conclusion as Neaves *et al*. (1987) to be made for game species.

A major constraint to the production and accumulation of spermatogonia in antelope species is their seasonal patterns of reproduction (Asher *et al*., 2000). These alternating periods of fertility are reflected by drastic changes in semen characteristics, directly influencing testicular function and size (Asher *et al*., 2000). Comparing testes weights of adult rock hyrax during the breeding and non-breeding seasons, Neaves (1972) recorded weights of up to 5 times larger during the rutting season. This increase in testis size was found to be the result of enlarged STs "with the simultaneous stimulation of spermatogenic activity" (Neaves, 1972).

Asher *et al*. (2000) observed semen completely devoid of spermatogonia for male deer during the antler-regeneration period (summer/spring). This aspermatogenesis suggested inactive Leydig cells outside the breeding season (Neaves, 1972). Microscopic evaluations of non-breeding hyrax males indicated no significant change in Leydig cell numbers, but rather cell shrinkage, due to cytoplasmic hypotrophy, was observed (Neaves, 1972). Associated with this Leydig cell hypotrophy outside the mating season was low plasma testosterone levels and involuted testes (Asher *et al*., 2000).

To further underline the significant influence of mating period on semen parameters, Paris *et al*. (2005) compared these measurements between breeding and non-breeding wallaby males. They recorded greatest ejaculate coagulation and volume (highest levels of testosterone secretion as a result of Leydig cell enlargement), as well as highest sperm motility during the breeding period. Asher *et al*. (2000) was further supported by Paris e*t al*. (2005) when they also observed reduced spermatogenesis and testicular regression for non-breeding males.

1.5.2 Sertoli cells

The most important cells of the STs are the Sertoli cells which function as the site of spermatogenesis (Weinbauer *et al*., 2010), facilitating the development of germ cells to mature spermatozoa by controlling their immediate environment i.e. the seminiferous tubules (Griswold, 1998).

Johnson *et al*. (1994) conducted a study in stallions in an attempt to establish whether the number of Sertoli cells was positively associated with DSP, and also to determine a possible relationship between DSP and paired testicular weight.

Because Sertoli cells were identified as the site of spermatogenesis, their relationship with DSP was established as early as the development of the most primitive spermatogonia (Johnson *et al.*, 1994).In adult horses, Johnson *et al.* (1994) found that the number of Sertoli cells influenced the number of germ cells, which in turn, were significantly correlated to testicular size and DSP. Sharpe *et al.* (2003) substantiated this finding by stating that "... the number of Sertoli cells will determine the number of germ cells that can be supported through spermatogenesis and will hence numerically determine the extent of sperm production, a factor with obvious bearing on fertility".

When Griswold (1998) limited the proliferation of Sertoli cells during testicular development in rats, a simultaneous reduction in testis size and spermatogenic output was observed. Rats with larger than normal testes were produced when thyroid hormone function was inhibited (proliferation of Sertoli cells was increased). These findings not only supported results recorded by Johnson *et al.* (1994), it also proved that the maximum number of germ cells supported by Sertoli cells remains constant within a species (Griswold, 1998). In other words, the number of germ cells is directly determined by the number of functional Sertoli cells (Griswold, 1998).

The relationship between DSP and Sertoli cells was established for multiple species including horses (Johnson *et al.*, 1994), rats (Griswold, 1998), and humans (Sharpe *et al.*, 2003). Horses, similar to game species, were seasonal breeders and the correlations between DSP, testicular weight and Sertoli cell numbers were only determined by Johnson *et al.*(1994) after seasonal variations were considered.

Stallions regulated spermatogenesis by seasonally altering the number of primitive spermatogonia. The number of germ cells was found to be twice their numbers during the breeding season, mimicking changes in testicular size and the number of Sertoli cells (Johnson *et al.*, 1994).This decline in Sertoli cell numbers was believed to cause spermatogonia degeneration during non-breeding seasons (Johnson *et al.*, 1994).A similar decline of spermatogonia due to high levels of germ cell degeneration was observed for rams following long-day periods characteristic of the non-breeding season (Ortavant, 1958). Neaves (1987) established that the increase in testis weight observed during the breeding season was due to enlarged STs.

Weinbauer *et al.* (2010) stated that a higher number of Sertoli cells directly increased testes size and led to greater DSP. This statement was found to be correct when Johnson *et al.* (1994) linked the number of Sertoli cells to the rate of spermatogenesis and the number of primitive spermatogonia. The amount of testicular cells was therefore positively correlated with testicular weight, which in turn, was significantly and positively associated with DSP (Johnson *et al.*, 1994).

In adult men, the failure of Sertoli cells to exhibit normal maturation and proliferation during the neonatal stage, led to testicular disorders as such occurrences predetermined Sertoli cell behaviour and function in adulthood (Sharpe *et al.*, 2003).

Additional hormones involved in sexual maturity had to be mentioned at this point namely luteinizing hormone (LH), follicular stimulating hormone (FSH), and thyroid hormones. Both LH and FSH were secreted by the pituitary gland into the bloodstream after stimulation from GTRH (Society for

Endocrinology, 22 Apex Court, Woodlands, Bradley Stoke, Bristol BS32 4JT, UK, www.endocrinology.org). Luteinizing hormone stimulated testosterone production by Leydig cells, whereas FSH initiated and maintained spermatogenesis within Sertoli cells as well as the rate of Sertoli cell proliferation (Society for Endocrinology, 22 Apex Court, Woodlands, Bradley Stoke, Bristol BS32 4JT, UK, www.endocrinology.org). Suppression of FSH led to a reduction in Sertoli cell numbers (Sharpe *et al*., 2003).

Final Sertoli cell numbers varied according to the level of activation of thyroid hormones, decreasing when these hormones were over-active (hyperthyroidism) and increasing when they underperformed (hypothyroidism) in the rhesus monkey (Sharpe *et al*., 2003). However, the importance of thyroid hormones across all species in reaching healthy Sertoli cell numbers was uncertain (Sharpe *et al*., 2003).

One fact that was found by Sharpe *et al*. (2003) to be proved explicably through animal studies was that testes size differences among species were due to differences in their Sertoli cells numbers. This finding showed that the assumption made by Johnson *et al*. (1994) that changes in testicular cell numbers contributed significantly to associated testicular weight to be correct.

A linear relationship between Sertoli cell number and DSP existed (Sharpe *et al*., 2003).

1.5.3 Sperm number and testicular weight

Several studies have found high correlations of testis parameters with scrotal circumference (SC) in bulls (Boyd & Van Demark, 1957) and rams (Lino, 1972) of which the most significant were testes diameter and weight, as well as the number of sperm (Ortavant, 1958).

Knight (1977) wanted to substantiate these observations and conducted a study on 25 Romney and 67 Merino rams at the University of Australia over a 3-year period. He recorded sperm number, testes weight, live weight, and scrotal volume in order to determine the strength of correlations between these parameters. He found similar results as previous studies and established associations between live weight and testes weight with total number of sperm in the testes. For both species, scrotal volume was significantly correlated with testes weight, but only moderately associated with the number of sperm in the testes (Knight, 1977).

By producing and storing many sperm, males are capable of performing more successful copulations (Møller, 1989). Studies performed on nine different mammalian species indicated that, despite body size, reserves held sperm equivalent to approximately 21 ejaculates (Møller, 1989). Møller (1989) found that sperm reserves (number of sperm in the epididymes) and sperm production rates were positively correlated with the number of sperm per ejaculate and testes weight.

As was previously mentioned, this increase in testes weight is related to the enlargement of STs which inadvertently contain more Sertoli cells and larger Leydig cells in order to support higher sperm production (Møller, 1989). Møller (1989) observed that mammalian species with large testes (positively correlated with body weight) exhibited relatively large sperm reserves as well as high sperm production rates.

The type of breeding system of a species determined testes sizes. This phenomenon was discovered for mammals (Kenagy & Trombulak, 1986; Gomendio & Roldan, 1991), primates (Møller, 1989), and fishes (Stockley, 1997). Species practicing polygyny experienced sperm competition (gametes of multiple males compete to fertilize a single ovum) and carried larger testes than their monogamous relatives in order to support increased spermatogenesis. These enlarged testes were directly associated with increased sperm reserves and sperm production rates (Stockley, 1997; Gage & Freckleton 2003).

According to Gage & Freckleton (2003), positive associations existed between sperm morphometry (total sperm lengths) and relative testes mass across 83 mammalian species. This meant that males with larger testes produced sperm with longer flagella, which enabled them to swim faster (high motility) and enhance fertilization (Gomendio & Roldan, 1991; Stockley, 1997; Gage & Freckleton, 2003). However, sperm longevity was suspected of being compromised by the increased demands placed on mitochondria in the mid-piece for greater thrusting forces by these longer flagella (Gage & Freckleton, 2003).

The most important observation made by these researchers, with regards to this research project, was that SC gave a good indication of sperm number influencing male secondary sexual characteristics such as masculinity and horn size.

1.6 The influence of castration on body conformation

Horn and bone growth is controlled by testosterone secretion, although to what degree, still remains uncertain (Bartoš *et al.*, 2009). Evidence provided by Bartoš *et al.* (2009) that the influence of testosterone varied between growth stages allowed for some insight.

In an attempt to determine the impact of testosterone on antler and bone growth and calcification, Bubenik *et al.* (2005) used the anti-androgenic compound cyproterone acetate (CA) in a study on white-tailed deer. Cyproterone acetate reversibly blocked the effect of testosterone through competitive inhibition and antler and bone calcification was expected to be inhibited.

Results were surprising as antlers showed continued growth throughout the year whereas bone mineralization and spermatogenesis were completely inhibited by only a minute amount of CA (Bubenik *et al.*, 2005). Experimental stags exhibited body appearances typical to that of castrates, had high plasma GH levels, and lower testosterone levels compared to the controls (Bubenik *et al.*, 2005).

The expression of many male associated traits was linked to testosterone, including mate-seeking behaviour and aggression, horn growth, and masculinity (Preston *et al.*, 2012). Preston *et al.* (2012) conducted a study on the differences in meat quality produced by bulls and steers and found smaller neck and shoulder muscle measurements for castrates.

1.7 Characteristics of the gemsbok (*Oryx gazella*, Linnaeus, 1758)

Gemsbok is one of three subspecies from the Bovidae family and is associated with arid regions of Southern Africa (NSE, 1995). Compared to the Arabian or white oryx (*Oryx leucoryx*, Linnaeus, 1758), and the scimitar (*Oryx dammah*), the gemsbok is the largest of the oryx species (NSE, 1995).

The gemsbok is identified by its tan colour and distinguishable black markings in the face, ears, legs, and back. It exhibits a black tail and mane, and well defined neck and shoulder muscles (NSE, 1995).

The lance-like, slightly curved horns are ridged and are considered a grand prize by game hunters (Møller *et al*., 1996). Møller *et al*. (1996) found that although bulls had thicker horns, their lengths were similar for both sexes. Compared to its body size, horn base circumference was small and Møller *et al*. (1996) suspected that this could have contributed to the high breakage percentage observed for the species.

Head clashes, as a defensive mechanism against predators and the rite of passage to oestrus females, often resulted in severe injury and even death (Møller *et al*., 1996).

The species exhibits little sexual dimorphism with average body weight and shoulder-height measuring approximately 190 kg and 1.2 m, respectively (NSE, 1995). Horn lengths vary between 0.6 and 1.4 m (NSE, 1995). Gemsbok lives in herds of up to 50 animals but bulls also thrive in solitude (NSE, 1995). This antelope feeds on shrubs and grasses and can go prolonged periods with little water (NSE, 1995).

The social hierarchy usually consists of evenly-spaced and dominant bulls, each occupying a territory of a few square-kilometres which includes his herd (Møller *et al*., 1996).

Gemsbok females reach maturity at the age of approximately 24 months (Vié, 1996). They are polyestrous (multiple estrous cycles during the breeding season) and have a mean cycle length of 26 days (Vié, 1996; Engel, 2004). Single calves, weighing between 9 and 14 kg are born throughout the year following a nine-month (±270 day) gestation period (Engel, 2004). The inter-calving period of the species varies from 260 to 295 days and calves are weaned at 3 months (Engel, 2004). Bulls, similar to females, reach puberty at the age of 2 years (Engel, 2004).

Reproductive traits of gemsbok do not appear to be affected by season with mating occurring between receptive females and the dominant bull (NSE, 1995).

CHAPTER 2

MATERIALS AND METHODS

2.1 Study area

The study was conducted at Glen Lyon game ranch (latitude: -28.8000°; longitude: 22.4333°) in the Northern Cape Province of South Africa over a period of 10 months, from October 2014 to July 2015.

The game ranch is situated near the wine-producing town of Groblershoop in the remote Kalahari region of South Africa, about 2 km from the Orange River.

Average annual rainfall is low (approximately 108 mm/year) and the region receives most of its rainfall during autumn, specifically the month of March (Figure 1). During winter (June, July), temperatures may drop to 2°C and rain is not likely (Figure 2). Because the game ranch is situated in the Kalahari, high temperatures of up to 33°C are common during the summer (2015, L. Dlamini, Pers. Comm., South African Weather Service, Private Bag X097, Pretoria, 0001).

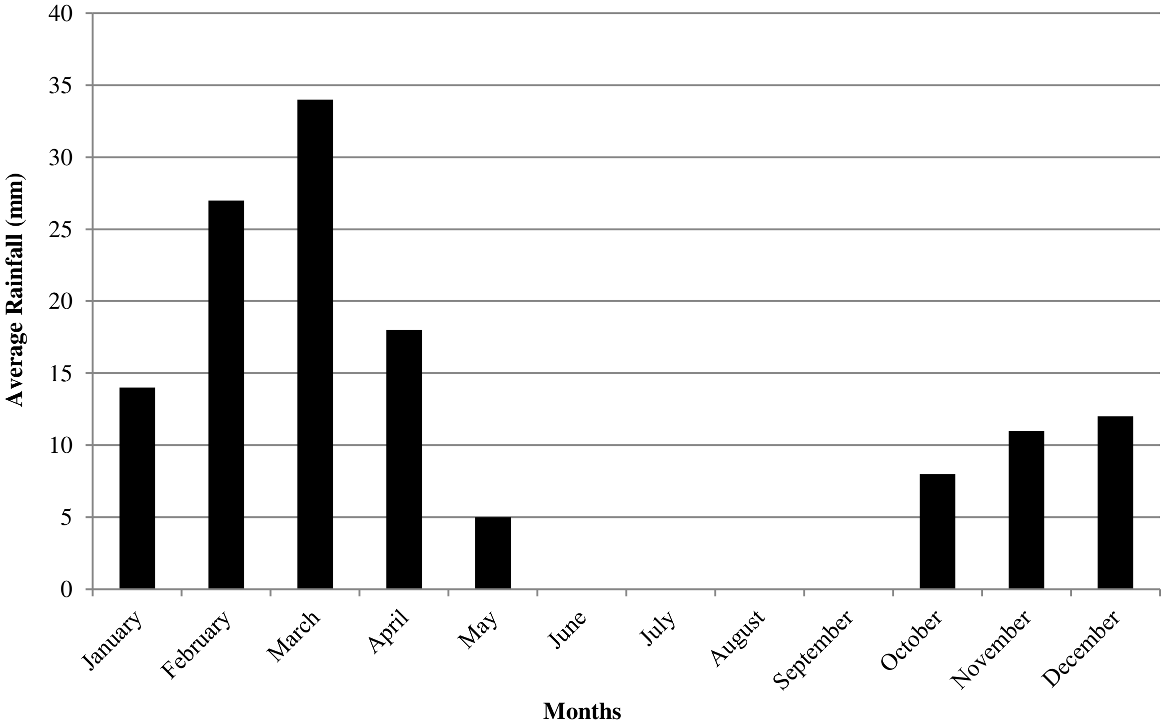

Figure 1 Average rainfall data of the study area (adapted from information supplied by the South African Weather Service).

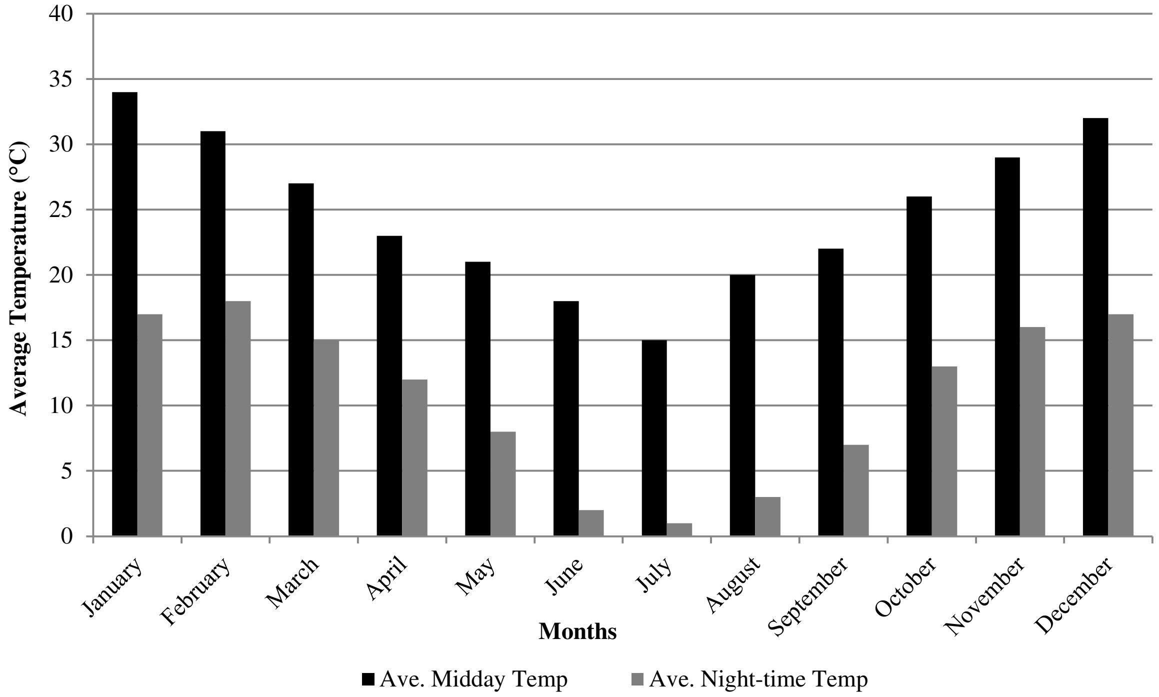

Figure 2 Average temperature data of the study area (adapted from information supplied by the South African Weather Service).

Rainfall and temperature data of Glen Lyon were recorded during the study period and found to closely adhere to the climatic description of the area (Figure 3).

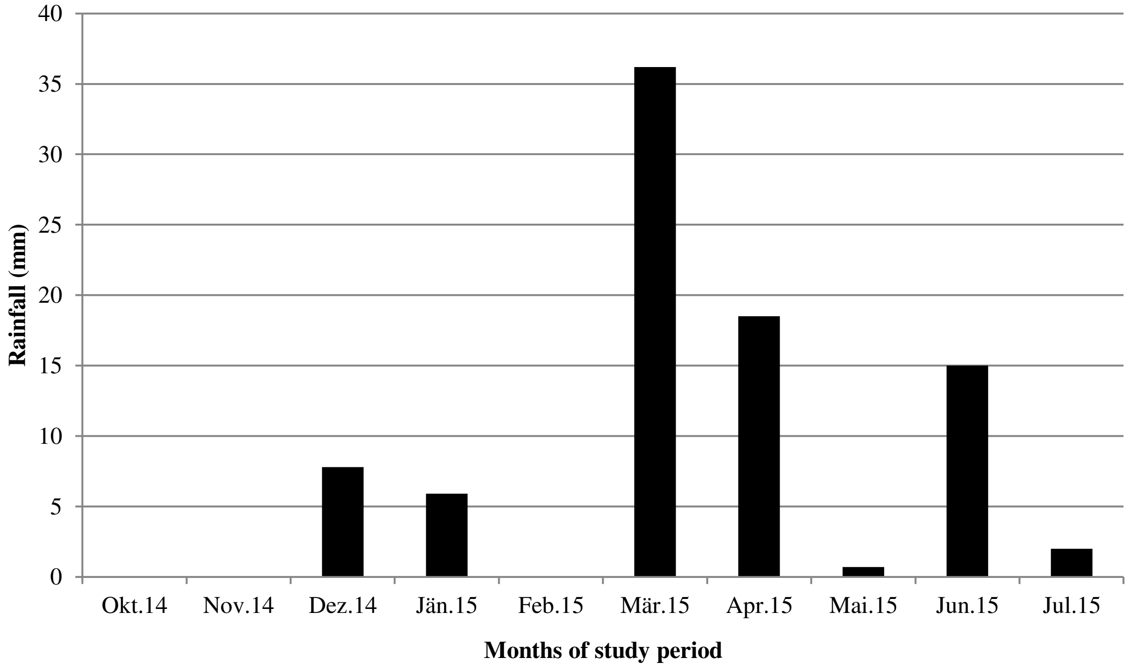

Figure 3 Monthly rainfall data, in mm, recorded during the study for the period of October 2014 to July 2015.

2.2 Animals

Glen Lyon is a privately-owned 60 000 hectare game farm, keeping several game species including roan and sable antelope. However, springbuck and gemsbok dominated in numbers as these were the species best adapted to the environment. Aerial surveys of the game ranch at the start of 2014 estimated the total gemsbok population at 2000 individuals. No large predators were present during the time of the research project.

Management of Glen Lyon introduced a one-week-per-month culling program at the start of the year 2014 in order to mimic the role of the predator. This culling program controlled animal numbers by eliminating old and diseased individuals and by fulfilling the terms of a contract with a factory in Kimberley for a monthly 15 tons of meat.

Culling of game was done by professional hunters. Ordinarily both sexes of selected species (wildebeest, eland, gemsbok, springbuck, rooihartbees) were harvested. However, for the purpose of this research study in order to obtain as high a number of subjects as possible, hunters focused on gemsbok bulls.

2.3 Vegetation

Bioregions as defined by Mucina & Rutherford (2014) were used to describe vegetation of the study area. According to these ecologists, Glen Lyon is located within three vegetation units, namely the Gordonia Duneveld (SVkd1), the Lower Gariep Broken Veld (NKb1), and the Bushmanland Arid Grassland (NKb3) bioregions (Figure 4).

A bioregion is an area whose borders are defined by naturally occurring biological (characteristic fauna and flora) and topographic features which influence the function of entire ecosystems (Mucina & Rutherford, 2014).

(1) Gordonia Duneveld (SVkd1): Distributed in the Northern Cape Province, Gordonia Duneveld consists mostly of dunes and comprises the largest part of the Kgalagadi Transfrontier Park. Dominant species on dune crests and slopes are *A. mellifera* and *R. trichotomum*, respectively (Mucina & Rutherford, 2014).

(2) Lower Gariep Broken Veld (NKb1): Stretching from the Boegoeberg in the Northern Cape, LGBV includes a series of koppies and ridges around Groblershoop and into Namibia. Vegetation is sparse and dominated by shrubs and low trees such as *A. mellifera* and *Aloe dichotoma* var *dichotoma* (Mucina & Rutherford, 2014).

(3) Bushmanland Arid Grassland (NKb3): Borders with desert vegetation in the north and the Namaqualand hills in the west. White grasses (*Stipagrostis* species) are the most common species occurring on the plains of BAG (Mucina & Rutherford, 2014).

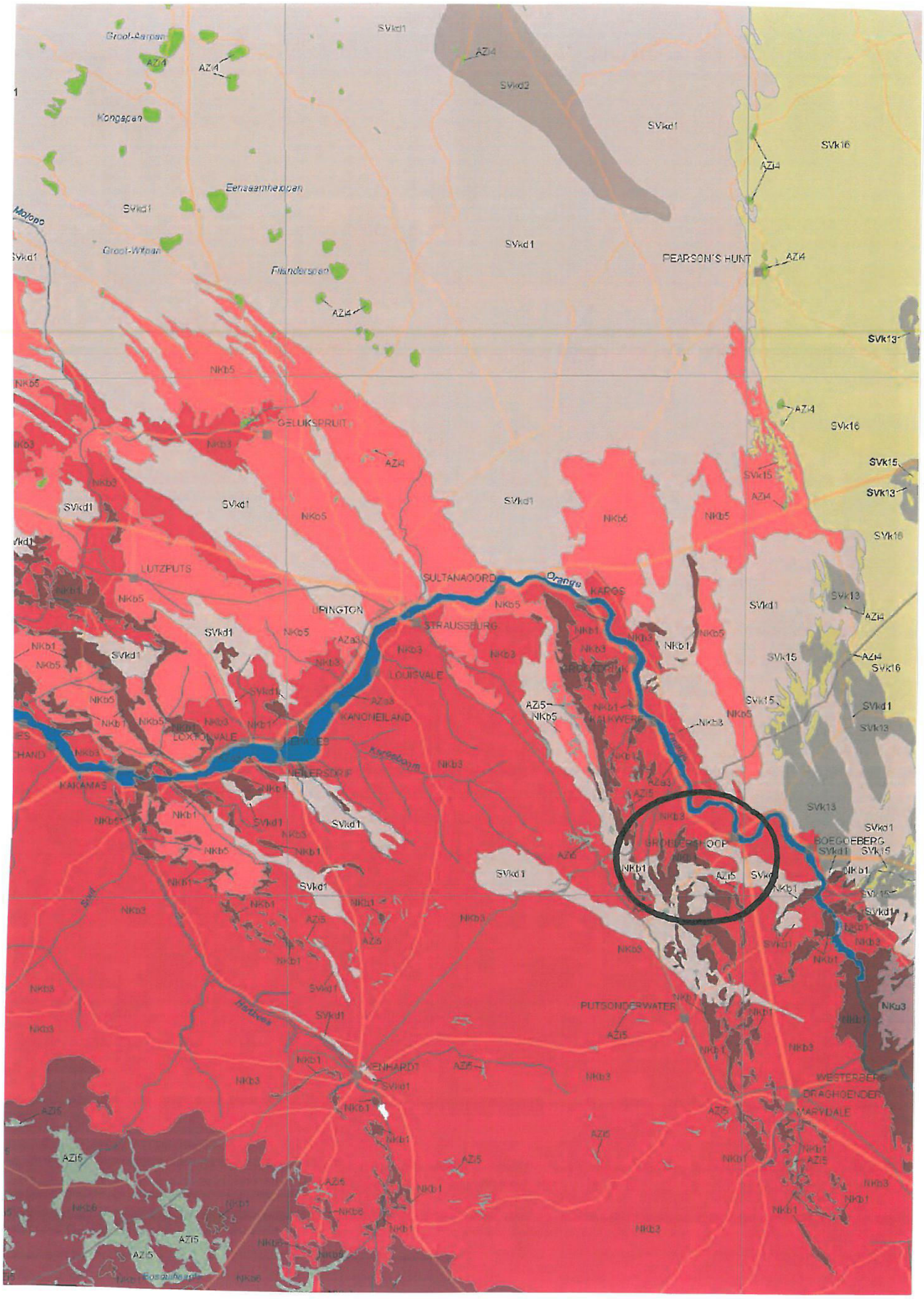

Figure 4 Vegetation types of the study area (adapted from Mucina & Rutherford, 2014).

Photos taken at different parts of Glen Lyon showed that all three vegetation types were present and that they were quite easily discernible (Figure 5).

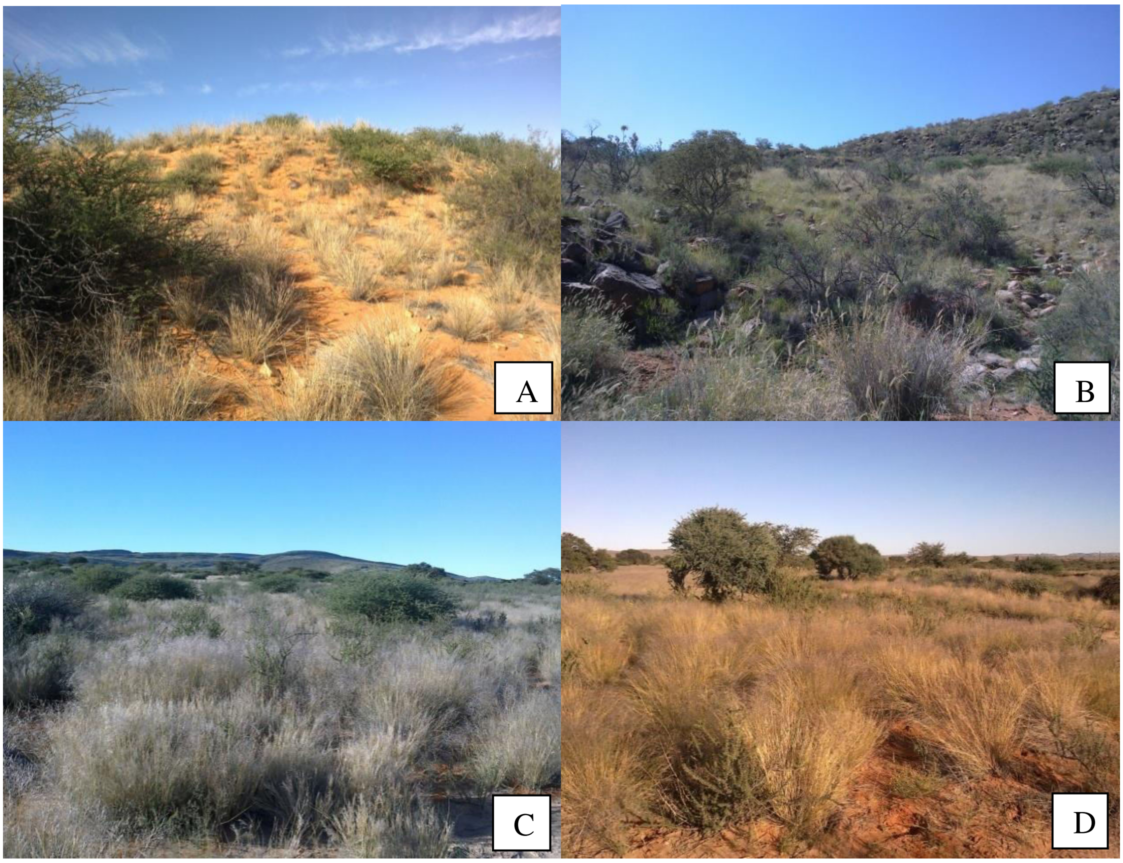

Figure 5 The vegetation types of the study area. A: sandy dunes of Gordonia Duneveld; B: rocky outcrops and koppies of Lower Gariep Broken Veld; C and D: plains of Bushmanland Arid Grassland.

2.4 Ethics approval

This research project, reference number EC049-15, conformed to the requirements of the University of Pretoria's Animal Use and Care Committee.

2.5 Field procedures

As explained in section 2.2, game was periodically harvested in order for Glen Lyon to fulfil in a 15 ton/month meat contract with a packing factory in Kimberley. Both sexes of selected species (wildebeest, eland, waterbuck, springbuck, gemsbok, and rooihartbees) were culled. However, for the purpose of this project, hunters mainly focused on gemsbok bulls.

From sunrise until midday during the hunting period (4 days/month), gemsbok was searched for along main roads while hunters stood on the back of pickup trucks in camouflage. The camouflage allowed for on-foot tracking when necessary.

During the summer months, all activities were ceased at about 13h00 in order to avoid the heat and to allow hunters to rest. Hunting was then again resumed from 14h00 and usually continued until 20h00. Over the course of the study a total of 144 gemsbok bulls were culled, measured and recorded. Each carcass was individually marked (Figure 6) and its age determined according to dentition. Males younger than 3 years were excluded from harvesting whereas obviously sick and injured bulls were eliminated from the study.

As soon as possible after an animal was shot, the carcass was transported to the on-site abattoir where the following parameters were measured: body weight (kg), horn length, horn base circumference, scrotal circumference, neck circumference, and heart girth. Body length, face length, cannon bone length and cannon bone circumference were also measured.

All parameters were recorded in cm, except for body weight which was noted in kg.

These parameters were then correlated with scrotal circumference (SC) and the significance of these correlations established. If a correlation existed, the significance level indicated whether SC was positively or negatively influenced by the relevant parameter.

Intensity of the black colour in the face and along the midriff gave a good indication of gemsbok bull age and for this reason photos of individual males were taken.

General observations such as the presence of ticks, broken horns, hairless patches, and overall condition were recorded. Due to the absence of large predators, bulls tended to be older than their natural longevity.

Figure 6 A: Each bull was individually marked; B: carcasses were hung from a scale to determine weight and to simplify the measuring of parameters.

2.6 Collection of data

2.6.1 Horn parameters

Horn length was measured at the front of the horn, following the midline for its entire length (direct distance from the base of the horn to the tip), measuring at the level where the rings were most pronounced (Figure 7).

Horn base circumference was measured as close to the skull as possible, just above the hairline (Figure 7).

The tip-to-tip distance, the apex length (distance from the first ring to the tip of the horn), and the number of rings, were also determined.

Secondary horn growth (fine ridges at the base of the horn) seen in older bulls were not counted but their presence recorded. These parameters were noted for both horns.

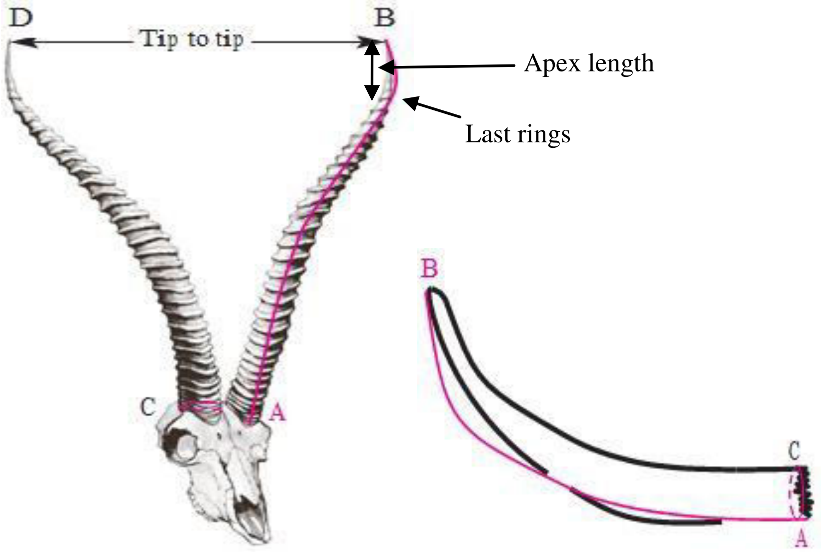

Figure 7 Measuring horn length from base to tip (A-B), horn base circumference (C), and (B-D) tip-to-tip distance (Rowland Ward).

2.6.2 Scrotal circumference

After it was certain that both testes were properly down in the scrotal sac, scrotal circumference could be recorded. Scrotal circumference was measured horizontally, in the middle of the scrotum (Figure 8). In order to ensure accurate data, this measurement was taken by the researcher herself, at relatively the same position for all individuals.

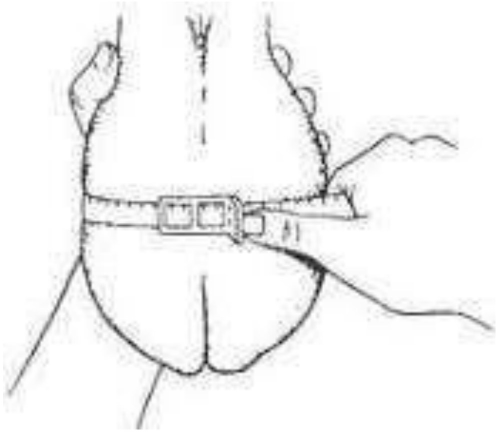

Figure 8 Measuring scrotal circumference (SC) in the middle of the scrotum.

2.6.3 Anthropometric measures

Neck circumference was measured proximal to the shoulders, at about a third of the total neck length (Figure 9.2). By placing the measuring tape directly behind the front legs heart girth could be measured. The tape was then traced around the body so that its two points met again at the spine of the bull, at the midsection of the hump (Figure 9.1).

Body length was recorded from the distal tip of the scapula along the midriff to the pin bone (Figure 9.2). Face length was taken from the point midway between the horn bases, where they met the skull, to the tip of the upper lip (Figure 9.2)

Cannon bone length of the right front leg was measured from the middle of the knee, along the cannon bone, to the cannon bone/pastern bone junction. The level just above the sesamoid bone was used to determine cannon bone circumference (Figure 9.2).

2.7 Statistical analysis

Before being recorded in excel spread sheets, data was carefully scrutinized for errors and the summary statistics (means, standard deviations, outliers) calculated using the GLM model and Analysis of Variance (ANOVA). Data was then tested for normal distribution and analysed by means of multi-factorial analyses of variance.

Pearson correlation coefficients between all the dependent variables were determined in SAS (Statistical Analysis System, 2015). Linear and quadratic relationships between the independent variables (x1 x2...) and the dependent variables (y1 y2...) were determined by means of the GLM model (Statistical Analysis System, 2015). Fischer’s test was then used to determine the significance of the differences between means (Samuels, 1989).

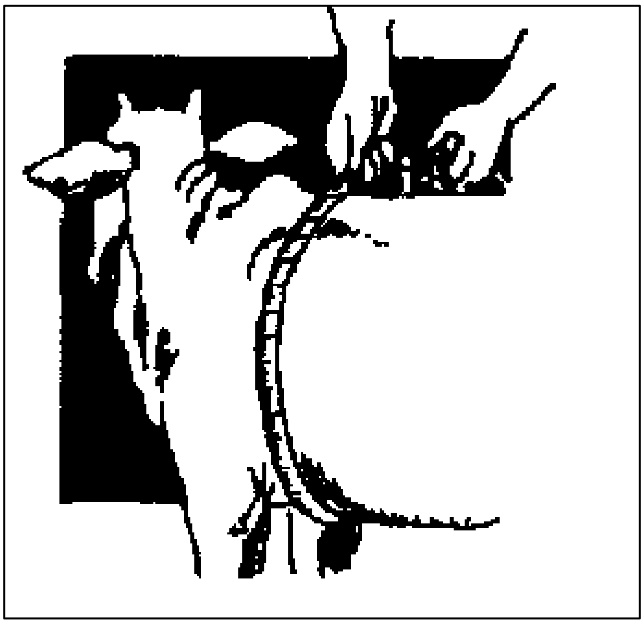

Figure 9.1 Measuring heart girth behind the front legs and around the body.

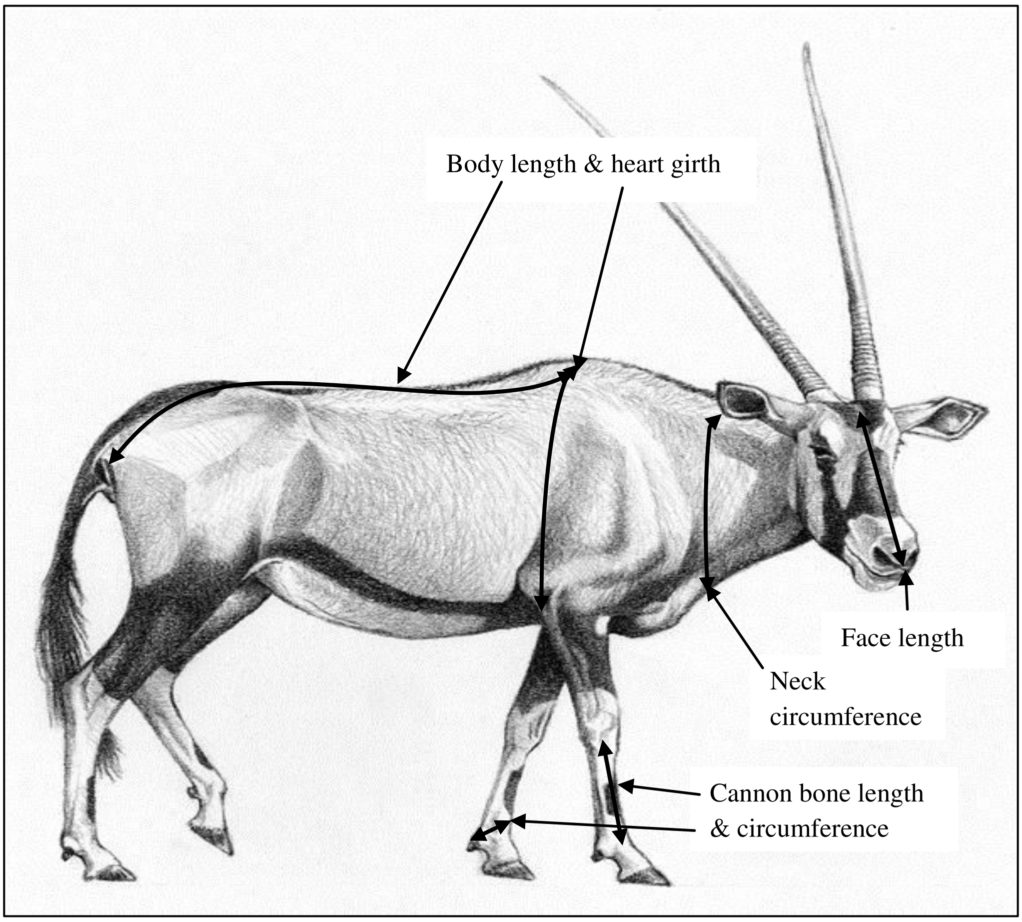

Figure 9.2 Measuring neck circumference, heart girth, body length, face length, cannon bone length, and cannon bone circumference.

CHAPTER 3

RESULTS AND DISCUSSION

Scrotal circumference was included as a measure of one of the important secondary sexual characteristics associated with male fertility, because the size of the scrotum was directly linked to testis size and the number of spermatozoa in previous studies (Musaddin *et al*., 1994; Møller, 1989; Preston *et al*., 2012).

3.1 Results from analysis of the complete data set

One-hundred-and-forty-four (n=144) gemsbok bulls between the ages of 3 and <10 years were harvested, measured, and recorded. Parameters measured were categorised into body and horn measurements (Tables 1 and 2).

Statistical analysis of this complete data set allowed for the average, minimum and maximum values of each parameter to be determined (ANOVA). Differences in effective population numbers (n) occurred when a specific parameter for an individual bull was accidentally skipped or, in the case of horn measurements, due to breakage.

Table 1 Means and deviations of body measurements of gemsbok bulls (calculated for the complete data set)

Parameter	Effective population number (n)	Population mean	Parameter minimum	Parameter maximum
Age (years)	144	5.3	3.0	8.5
Body Weight (kg)	144	184.6	103.4	235.4
Heart Girth (cm)	144	139.2	82.1	187.3
Body Length (cm)	144	99.6	83.5	133.5
Face Length (cm)	144	36.9	31.9	40.3
Scrotal Circumference (cm)	144	23.8	13.0	28.9
Neck Circumference (cm)	144	78.6	57.1	107.1
Cannon Bone Length (cm)	144	25.9	22.2	29.1
Cannon Bone Circumference (cm)	144	16.6	14.7	19.6
Shoulder Circumference (cm)	143	45.3	37.7	51.1

Table 2 Means and deviations of horn measurements of gemsbok bulls (calculated for the complete data set)

Parameter	Effective sample number (n)	Sample mean	Parameter minimum	Parameter maximum
Right Horn Length (cm)	143	82.4	60.3	99.0
Left Horn Length (cm)	143	82.4	58.0	97.9
Longhorn (cm)	141	83.5	60.3	99.0
Total Horn (cm)	142	164.8	118.8	196.9
Right Apex (cm)	142	43.2	15.9	62.1
Left Apex (cm)	142	42.7	16.0	59.6
Tip-to-tip (cm)	141	39.9	24.9	58.7
Rings Left	143	21.8	5.0	34.0
Horn Base Circumference Left (cm)	144	18.5	15.9	20.4
Horn Base Circumference Right (cm)	143	18.5	15.7	20.4

Table 2 indicates little or no differences between the values of parallel horn measurements. For example, the population average of horn length is similar for both right and left (82.4 cm) horn lengths with small differences in their minimum and maximum values.

3.2 Effect of age on horn and body measurements

As an animal ages there is an associated change in its body dimensions, as well as increases in its body weight and size. Initially, when the animal is young, growth rate takes place at a fast and increasing rate (self-accelerating phase), until a maximum point is reached (inflection point) where little or no growth occurs (growth plateau). After this plateau is achieved growth rate will start to decrease at an increasing rate (self-retarding phase).

This pattern of growth was explained by Hammond (1955) through a sigmoidal curve (Figure 10).

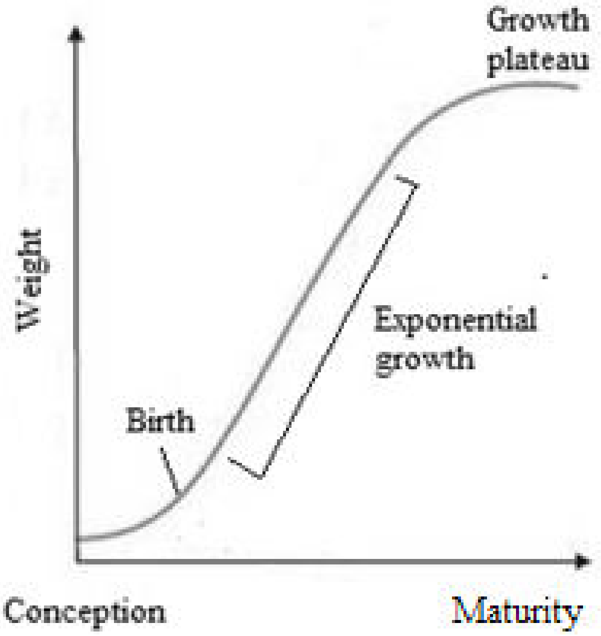

Figure 10 The sigmoidal growth curve (Hammond, 1955).

The concept of age-related growth became important in this research project with regards to changes in hormone secretions (Bartoš *et al.*, 2009), body growth parameters (Bunnell, 1978; Bitro *et al.*, 2002; Akpa *et al.*, 2006; Du Plessis *et al.*, 2006), and associated behaviours (Jennings *et al.*, 2004; Bonenfant *et al.*, 2009). Young animals still in the initial stage of the self-accelerating phase had to be excluded from the study as well as males already in the self-retarding phase. By eliminating these animals from the study, n was reduced to 106.

Gemsbok bulls in the accelerated and retarded growth phases were identified as younger than 3 years (only two permanent teeth) and older than 8 years (secondary horn growth, severely worn permanent teeth), respectively. This age group classification was established after frequency analysis of the complete data set indicated large differences between maximum and minimum values of individual parameters (Tables 1 and 2).

3.3 Effect of different age groups on body and horn measurements

In order to keep animals in the different growth phases separate, gemsbok bulls were divided into age groups 1 and 2, based on frequency distribution analysis. Age group 1 (n=68) consisted of bulls between the ages of 4 and 5 years, whereas 6 to 7-year-old males were categorised into group 2 (n=38).

The influence of the different age groups on individual body measurements (Table 3) and horn parameters (Table 4) was analysed in order to determine the significance of the differences between means.

Table 3 The influence of different age groups on body measurements of gemsbok bulls (means and SE)

Anthropometric measure	**Age group 1**	**Age group 2**
Body Weight (kg)	183.46^{a} (2.81)	199.23^{b} (3.96)
Heart Girth (cm)	139.69^{a} (0.83)	143.18^{b} (1.18)
Body Length (cm)	99.02^{a} (0.79)	102.44^{b} (1.12)
Face Length (cm)	36.78^{a} (0.21)	37.58^{b} (0.29)
Scrotal Circumference (cm)	23.39^{a} (0.32)	25.48^{b} (0.45)
Neck Circumference (cm)	78.04^{a} (0.82)	83.74^{b} (1.16)
Cannon Bone Length (cm)	25.78^{a} (0.16)	26.36^{b} (0.22)
Cannon Bone Circumference (cm)	16.62^{a} (0.12)	16.86^{a} (0.17)
Shoulder Circumference (cm)	45.59^{a} (0.24)	46.65^{b} (0.34)

^{a,b} Row means with different superscript letters differ ($P \leq 0.05$)

Reaching maturity inevitably results in increased measurements of anthropometric measures associated with older bulls, and therefore explains the observed differences between age groups 1 and 2 (Table 3). However, the extend of such differences between age groups varies depending on body measurements. For example, body weight shows a difference of about 16 kg between age groups 1 and 2, whereas SC only varies by approximately 2 cm (Table 3). Shoulder circumference, face length, and cannon bone length show similar small, but definite ($P<0.05$), age group differences. These small variations indicate earlier maturity and rapid growth rates associated with the relevant measurements (Table 3).

The only difference between the age groups for body measurements that is not significant is observed for cannon bone circumference (CBC). This observation indicates similar CBC for bulls in both age classes, suggesting that bone circumference does not increase after the age of 4 years.

In horses, bone growth is extremely rapid. According to Lawrence (2016), a 6-month old foal have already achieved 84 % of its mature height and mineralized 69 % of its bone content, with a thoroughbred yearling standing 15 hands (94 % of its adult height) tall and having completed 76 % of its maximum bone mineralization. Lawrence (2016) found no increase in CBC beyond the age of 5 years for multiple horse breeds.

Working with horses as a benchmark for bone growth, it is not far-fetched to assume that cannon bone circumference of gemsbok bulls will increase little, if any, after the ages of 4 to 5 years.

Table 4 The influence of different age groups on horn measurements of gemsbok bulls (means and SE)

Horn parameter	Age group 1	Age group 2
Right Horn Length (cm)	83.25^a (0.86)	82.79^a (1.21)
Left Horn Length (cm)	83.48^a (0.90)	83.09^a (1.27)
Longhorn (cm)	84.19^a (0.86)	84.31^a (1.19)
Total Horn (cm)	166.75^a (1.74)	165.88^a (2.43)
Right Apex (cm)	44.93^a (0.95)	82.79^a (1.21)
Left Apex (cm)	44.23^a (0.99)	41.36^a (1.36)
Tip-to-tip (cm)	39.66^a (0.71)	40.44^a (0.99)
Number Rings Left	21.01^a (0.57)	24.51^b (0.80)
Horn Base Circumference Left (cm)	18.63^a (0.11)	18.75^a (0.15)
Horn Base Circumference Right (cm)	18.68^a (0.11)	18.76^a (0.15)

^{a,b} Row means with different superscript letters differ ($P<0.05$)

Only one significant difference ($P\leq0.05$), namely number of horn rings left (HRL), was recorded between the age groups for horn measurements. This result for HRL was expected, because as bulls aged, the number of ridges increased. Due to the number of animals and the time constraint allocated to sampling, only the number of left horn rings was recorded. However, results for horn rings right (HRR) similar to HRL did not seem unlikely.

3.4 Influence of scrotal circumference on individual parameters

The purpose of this research project was to determine whether the presence of long horns decreased overall masculinity and fertility in gemsbok bulls. Fertility in the present research study was defined in terms of scrotal size. Because it was found in previous studies that testes size had a direct correlation with the amount of spermatogenic tissue it contained and therefore the rate at which sperm was produced (Musaddin *et al*., 1982; Møller, 1989; Preston *et al*., 2012), SC was used as an indirect measure of potential sperm production.

Because testosterone production in the testes regulated male secondary characteristics, it was assumed that male appearance and weaponry would be significantly impacted by changes in scrotal size.

Table 5 Correlation coefficients between scrotal circumference (SC) and anthropometric measures for the complete data set

Anthropometric measure	**SC of complete data set**
Age (years)	0.48***
Body Weight (kg)	0.66***
Heart Girth (cm)	0.54***
Body Length (cm)	0.49***
Face Length (cm)	0.45***
Neck Circumference (cm)	0.56***
Cannon Bone Length(cm)	0.17^{NS}
Cannon Bone Circumference (cm)	0.22^{NS}
Shoulder Circumference (cm)	0.38***

NS = non-significant; * *P*≤0.05; ** *P*≤0.01; *** *P*≤0.001

The observed high positive association (*P*<0.001) of scrotal circumference with age and linear body measurements suggests that any developmental change in one characteristic would significantly affect the other. These strong relationships also implies that SC provides a good indication of a gemsbok bull's growth and physical conformation up to the age of 5 years, and that bulls exhibiting large SC will have increased body weights and larger anthropometric measures.

Table 6 Correlation coefficients between scrotal circumference (SC) and anthropometric measures for each age group

Anthropometric measure	**SC of Age group 1**	**SC of Age group 2**
Age (years)	0.35**	0.23^{NS}
Body Weight (kg)	0.65**	0.13^{NS}
Heart Girth (cm)	0.46***	0.05^{NS}
Body Length (cm)	0.49***	-0.07^{NS}
Face Length (cm)	0.35**	0.05^{NS}
Neck Circumference (cm)	0.54***	0.06^{NS}
Cannon Bone Length(cm)	-0.08^{NS}	0.52**
Cannon Bone Circumference (cm)	0.14^{NS}	0.17^{NS}
Shoulder Circumference (cm)	0.60***	0.02^{NS}

NS = non-significant; * *P*≤0.05; ** *P*≤0.01; *** *P*≤0.001

Scrotal circumference is significantly and positively correlated with most anthropometric measures of age group 1 (Table 6), reflecting an increase in size associated with age and BW (*P*≤0.01).

Because heart girth, body length, face length, neck circumference, and shoulder circumference are parameters of growth they are greatly influenced by SC ($P \leq 0.001$) in young bulls (Table 6). These results indicate that bulls with larger SC exhibited heavier weights, thicker necks, and longer bodies. Al-Ghalban *et al*. (2004) and Tabbaa *et al*. (2006) both recorded significantly ($P<0.01$) heavier weights for Damascus and Awassi rams associated with larger SC. Adult males exhibited significantly larger SC than their immature counterparts (Al-Ghalban *et al*., 2004; Tabbaa *et al*., 2006), reflecting results obtained by this research study.

Further supporting the present research project, was Akpa *et al*. (2006) reporting all measured characteristics (age, body weight, heart girth, horn length, and SC) to be significantly ($P<0.01$) and positively correlated for young rams.

It appears, however, that all relevant growth parameters reach their maximum points as they showed no further increase in bulls older than 6 years (age group 2). If age group 2 is considered, cannon bone length is the only parameter which correlates significantly ($P \leq 0.01$) with SC (Table 6). This finding suggests that significant bone growth only occurs after a specific threshold for SC is achieved. Little research has been conducted on the cannon bone as a growth parameter in species other than the horse, which exhibited completed bone growth at an early age (Lawrence, 2016). Fourie *et al*. (2002) found no significant correlations of cannon bone circumference and cannon bone length with SC.

Data recorded by Akpa *et al*. (2006) on the relationship between testicular and horn development with body growth parameters in Yankasa rams, indicated that although SC and linear body measurements increased with age, this increase occurred at a faster rate for young rams compared to mature males. This reduced growth in older males was also observed in the present research study.

Table 7 Correlation coefficients between scrotal circumference (SC) and horn parameters for the complete data set

Horn parameter	**SC of complete data set**
Right Horn Length (cm)	0.19^{NS}
Left Horn Length (cm)	0.17^{NS}
Longhorn (cm)	0.21^{NS}
Total Horn (cm)	0.19^{NS}
Right Apex (cm)	-0.24^{NS}
Left Apex (cm)	-0.27^{NS}
Tip-to-tip (cm)	-0.14^{NS}
Rings Left	0.61***
Horn Base Circumference Left (cm)	0.24^{NS}
Horn Base Circumference Right (cm)	0.26^{NS}

NS = non-significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Studies that relate scrotal development with horn growth are almost non-existing, making this research project imperative.

The only significant correlation ($P \leq 0.001$) of horn parameters with SC for the complete data set is shown for number of rings left. The overall non-significance of horn measurements in association with SC indicates that scrotal size has no effect on horn measurements.

Taking into account the influence of age, correlations might become more pronounced.

Table 8 Correlation coefficients between scrotal circumference (SC) and horn parameters for each age group

Horn parameter	SC of Age group 1	SC of Age group 2
Right Horn Length (cm)	0.22^{NS}	-0.02^{NS}
Left Horn Length (cm)	0.29*	-0.02^{NS}
Longhorn (cm)	0.28*	-0.07^{NS}
Total Horn (cm)	0.26*	-0.02^{NS}
Right Apex (cm)	-0.39***	0.30^{NS}
Left Apex (cm)	-0.38***	0.18^{NS}
Tip-to-tip (cm)	-0.09^{NS}	-0.12^{NS}
Rings Left	0.70***	-0.37*
Horn Base Circumference Left (cm)	0.22^{NS}	0.24^{NS}
Horn Base Circumference Right (cm)	0.20^{NS}	0.18^{NS}

NS = non-significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Table 8 indicates several significant correlations of horn measurements with SC. The significance levels for age group 1 differ between parameters, and the apex measurements, although highly significant ($P \leq 0.001$), indicate negative associations with SC. This negative correlation between apex length and SC suggests a significant ($P \leq 0.001$) increase in ring number as SC increased.

Bratte *et al.* (1999) observed that average horn length for Yankasa rams increased with age. Because BW and SC were found to be highly and significantly ($P \leq 0.01$) correlated, horn length would also be expected to increase in relation with SC. This expectation was confirmed when a definite, albeit small ($P \leq 0.05$), increases in horn length left, long horn, and total horn in correlation with SC were observed for gemsbok bulls. The correlation coefficient of 0.933 ($P < 0.01$) of average horn length with SC, obtained by Akpa *et al.* (2006), provided further supporting evidence about the influence of SC on horn development.

For age group 2, only ring number of the left horn shows a significant relationship ($P \leq 0.05$) with SC. This level of significance, however, is less pronounced than for age group 1, as well as negative, indicating horn breakage or honing.

By determining the coefficients of body and horn parameters with SC, the irrelevant correlations were eliminated. The quadratic and linear equations of only the significant correlations were determined, and the most relevant formula for each parameter used to calculate regressions for both age groups (Tables 8 and 9).

Table 9 The most significant equation of individual anthropometric measures that were used to calculate regressions with scrotal circumference (SC)

Age group 1	
Anthropometric measure (y)	**Equation**
Body Weight	y = 5.04x + 65.53
Heart Girth	y = 1.19x + 111.97
Body Length	y = 1.00x + 75.60
Face Length	y = 0.21x + 31.79
Neck Circumference	y = 1.32x + 47.23
Shoulder Circumference	y = 0.43x + 35.57
Age group 2	
Cannon Bone Length	y = 0.47x + 14.30

All significant anthropometric measures for both age groups indicate linear regressions with SC, meaning the quadratic equations all tested non-significant in correlation with SC. For age group 1 body weight (Figure 11.1), heart girth (Figure 11.2), body length (Figure 11.3), and face length (Figure 11.4) increase significantly and linearly as scrotal size increased. These pronounced positive associations (*P*<0.001) between SC and linear body measurements indicate that any developmental change in one characteristic will significantly affect the other, and that SC is a good predictor of a gemsbok bull's growth and physical conformation up to the age of 5 years.

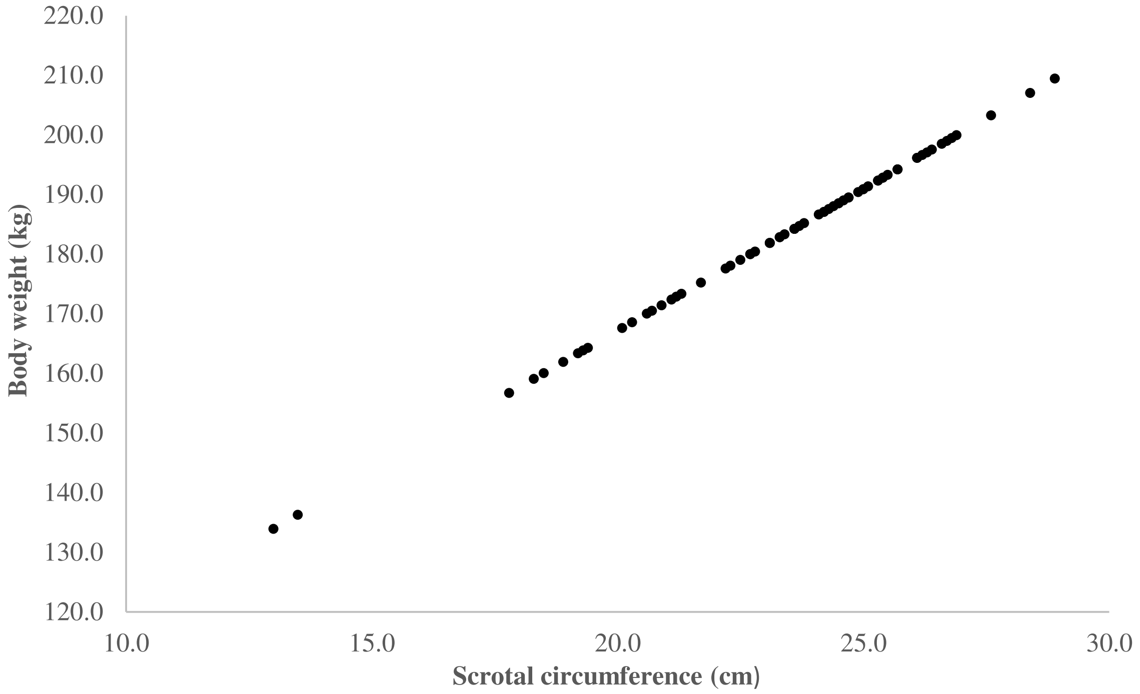

Figure 11.1 Regression of scrotal circumference with body weight for age group 1

In young bulls, shoulder circumference, face length, neck circumference, body length, and heart girth (all defined as parameters of growth) are greatly influenced by SC ($P \leq 0.001$), implying that bulls with larger SC exhibited heavier weights, thicker necks, and longer bodies.

Referring to previous studies, it was found that both Al-Ghalban *et al.* (2004) and Tabbaa *et al.* (2006) recorded heavier weights in correlation with larger SC in Damascus and Awassi rams, respectively. Studies conducted by Akpa *et al.* (2006) provided further supporting results when they indicated all measured characteristics (age, body weight, heart girth, horn length, and SC) to be significantly ($P < 0.01$) and positively correlated for young rams.

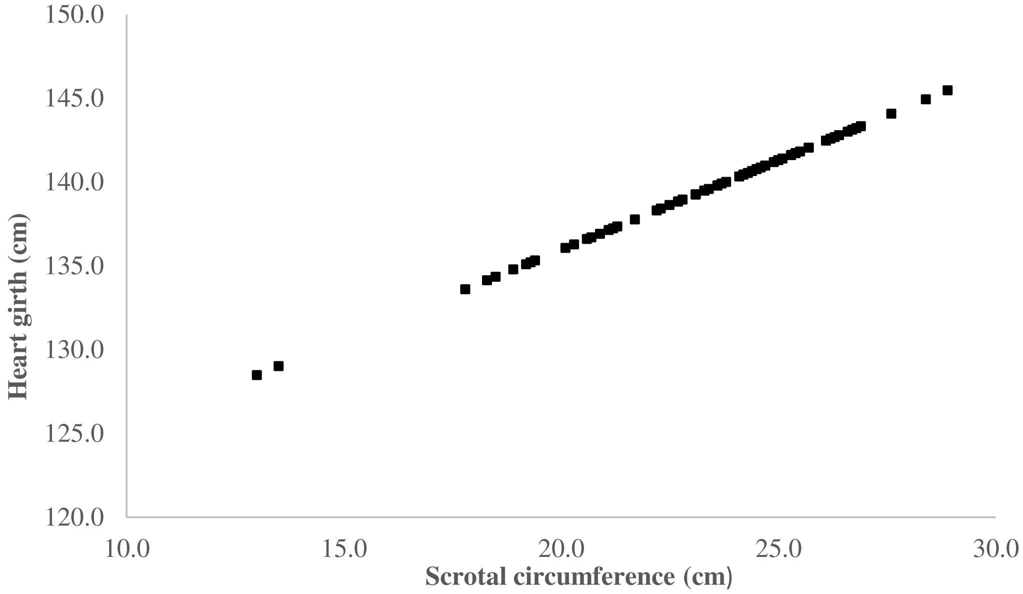

Figure 11.2 Regression of scrotal circumference with heart girth for age group 1.

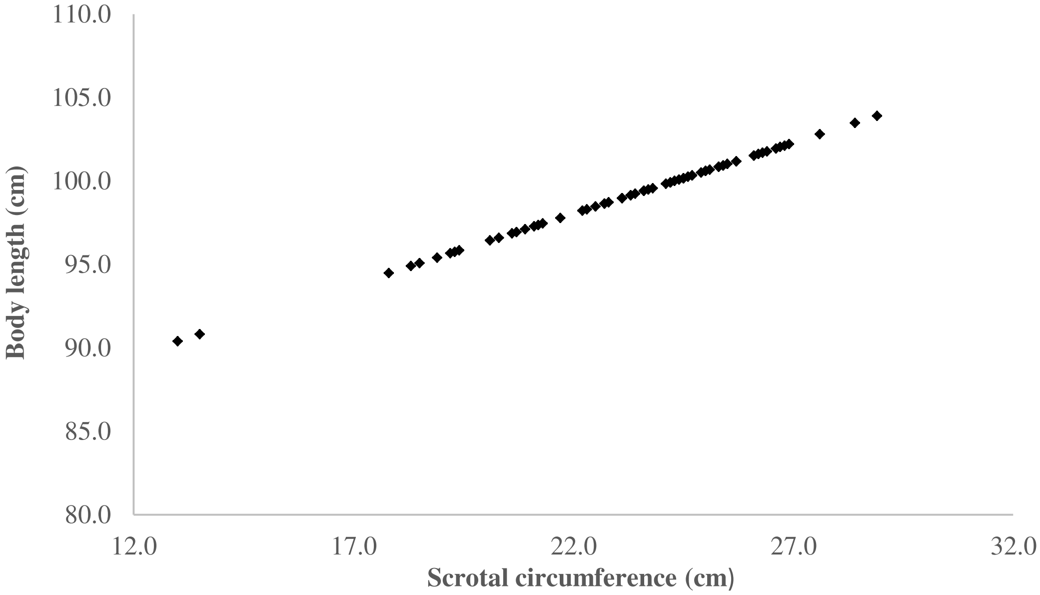

Figure 11.3 Regression of scrotal circumference with body length for age group 1.

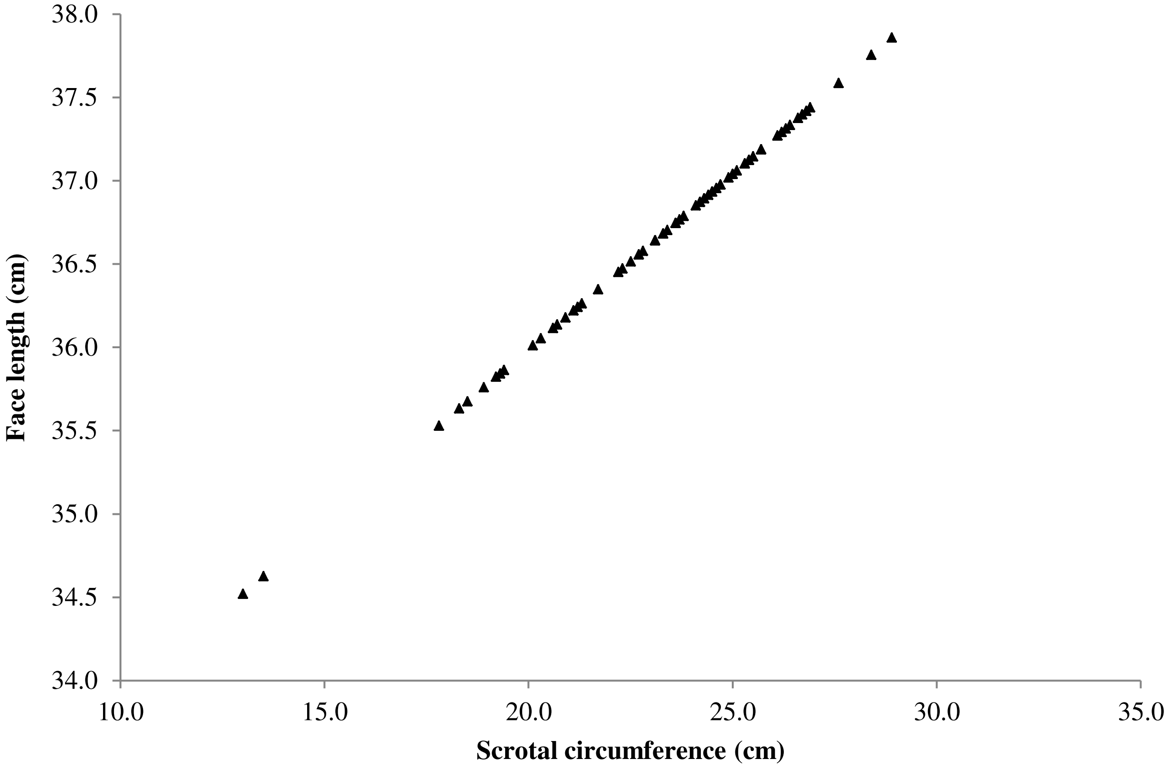

Figure 11.4 Regression of scrotal circumference with face length for age group 1.

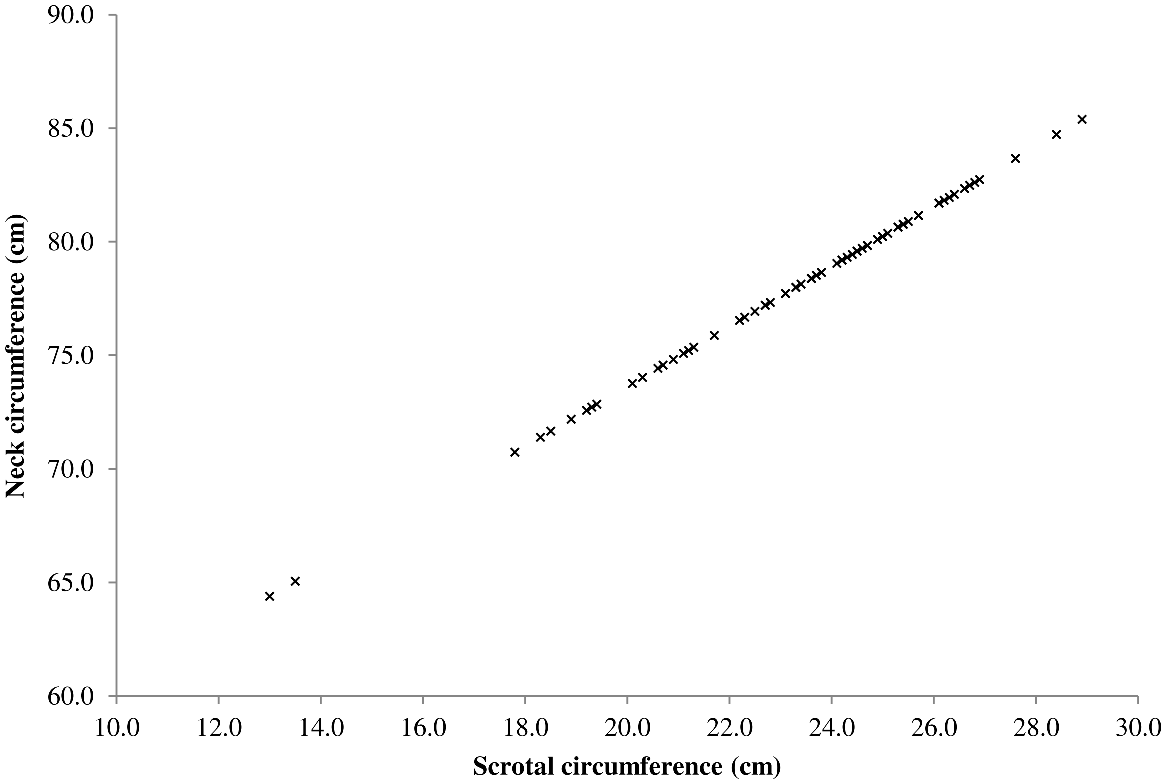

Figure 11.5 Regression of scrotal circumference with neck circumference for age group 1.

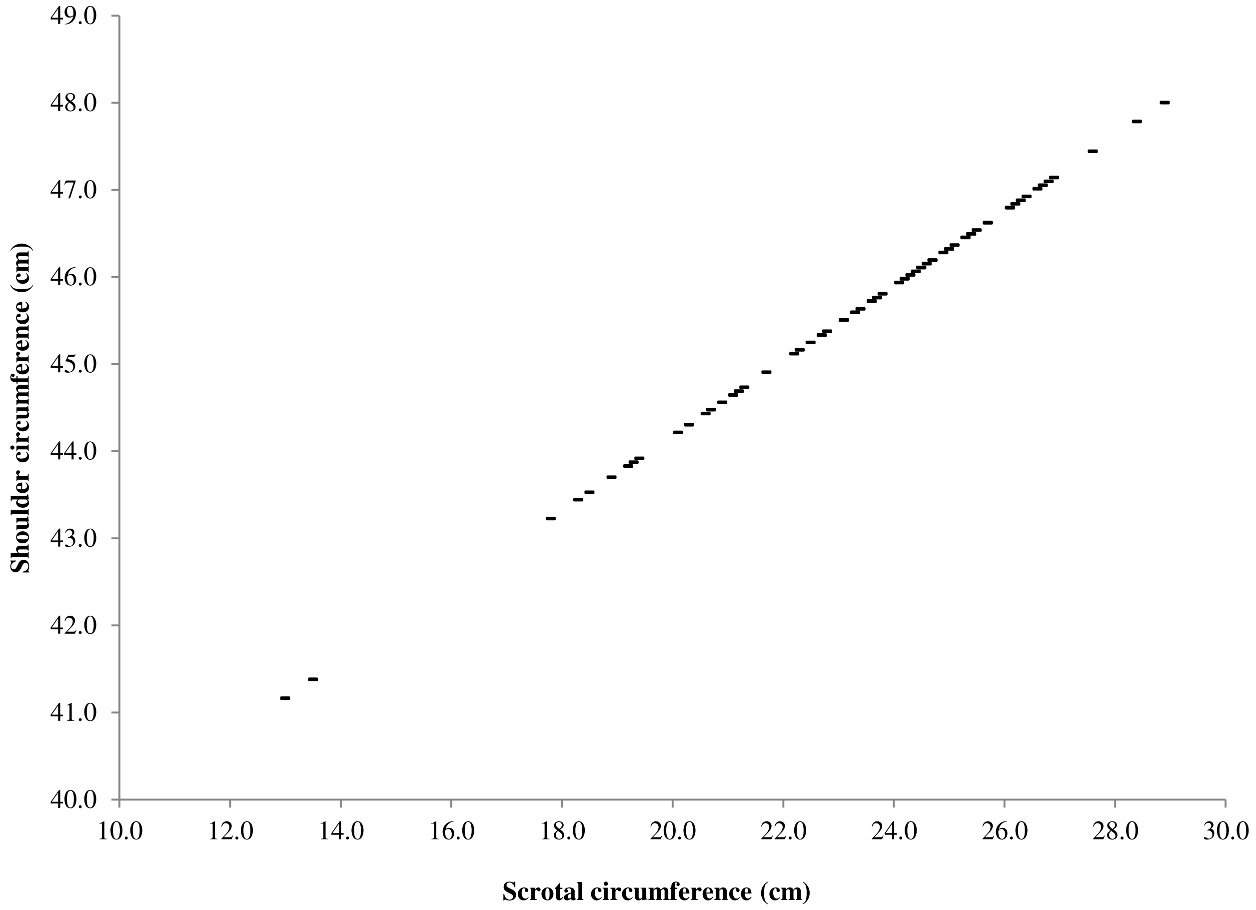

Figure 11.6 Regression of scrotal circumference with shoulder circumference for age group 1.

It appears, however, that all relevant growth parameters reach their maximum points as they show no further increase in bulls older than 6 years (age group 2). If age group 2 is considered, cannon bone length is the only parameter significantly ($P \leq 0.01$) correlated with SC. This finding suggests that significant cannon bone growth only occurs after a specific threshold for SC (24 cm) is achieved. Age group 2 exhibits a rapidly increasing and linear regression for CBL associated with SC (Figure 11.7).

In species other than the horse (completed bone growth at an early age), research with regards to the cannon bone as a growth parameter, was negligible. Fourie *et al.* (2002) also found no significant correlations between cannon bone circumference and cannon bone length with SC in the Dorper ram.

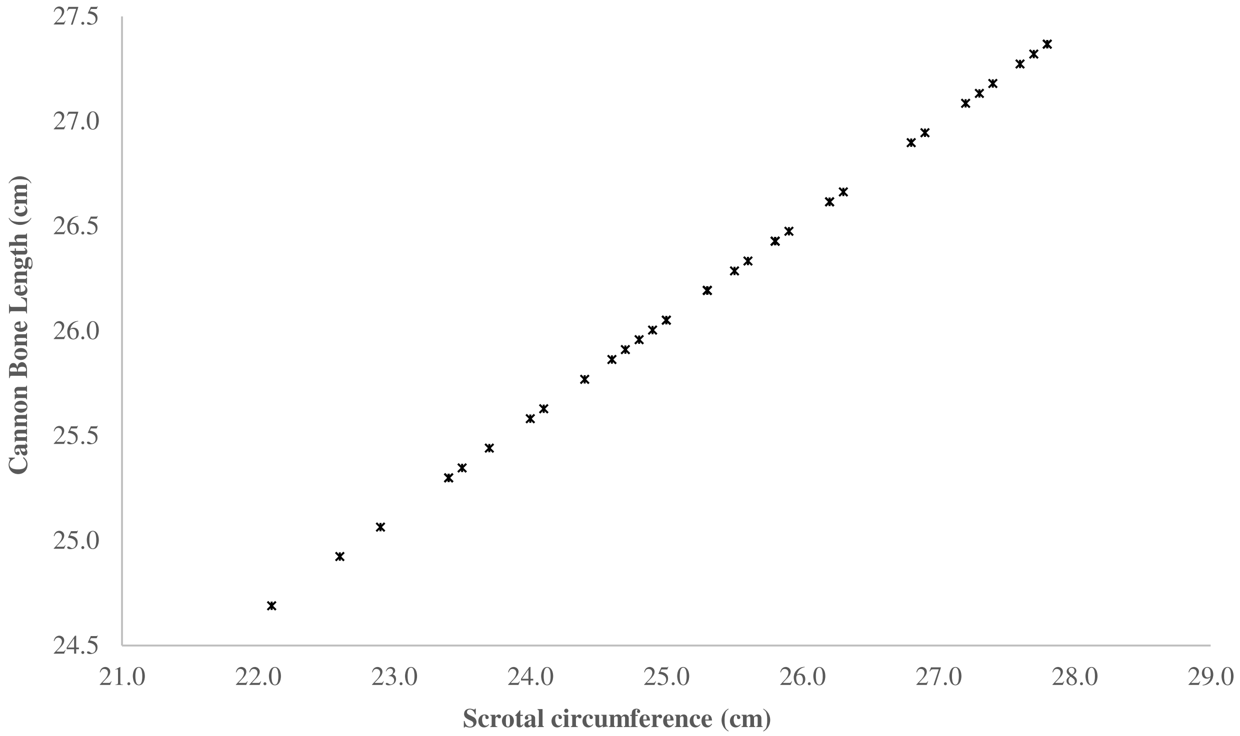

Figure 11.7 Regression of scrotal circumference with cannon bone length for age group 2.

Table 10 The most significant equation of individual horn parameters that were used to calculate regressions with scrotal circumference (SC)

Age group 1	
Horn parameter (y)	**Equation**
Left Horn Length	$y = -0.16x^2 + 7.56x - 4.6$
Longhorn	$y = -0.16x^2 + 7.35x - 0.94$
Total horn	$y = -0.32x^2 + 15.18x -7.72$
Right Apex	$y = -0.92x + 66.46$
Left Apex	$y = -0.94x + 66.34$
Rings Left	$y = 1.19x – 6.75$
Age group 2	
Rings Left	$y = -0.88x + 46.87$

According to Figures 12.1, 12.2, and 12.3, left horn length, long horn, and total horn for age group 1 increase concomitantly with SC until the latter reaches approximately 24 cm. After this inflection point is reached, associations between horn measurements and SC exhibit negative correlations i.e. a curvilinear response (Figures 12.1, 12.2, and 12.3). These negative associations in young bulls suggest the onset of sexual maturity and the participation in aggressive displays in order to win territories and gain access to oestrus females.

Average horn length for Yankasa rams were also found to increase with age (Bratte *et al*., 1999). Because BW and SC were found to be highly and significantly ($P \leq 0.01$) correlated, horn length would also be expected to increase in relation with SC. This expectation was confirmed when a definite, albeit small ($P \leq 0.05$) increase in horn length left, long horn, and total horn in correlation with SC was observed for gemsbok bulls.

Further supporting evidence with regards to the influence of SC on horn development was supplied by Akpa *et al.* (2006) who recorded a correlation coefficient of 0.933 ($P<0.01$) between average horn length and SC in Yankasa rams.

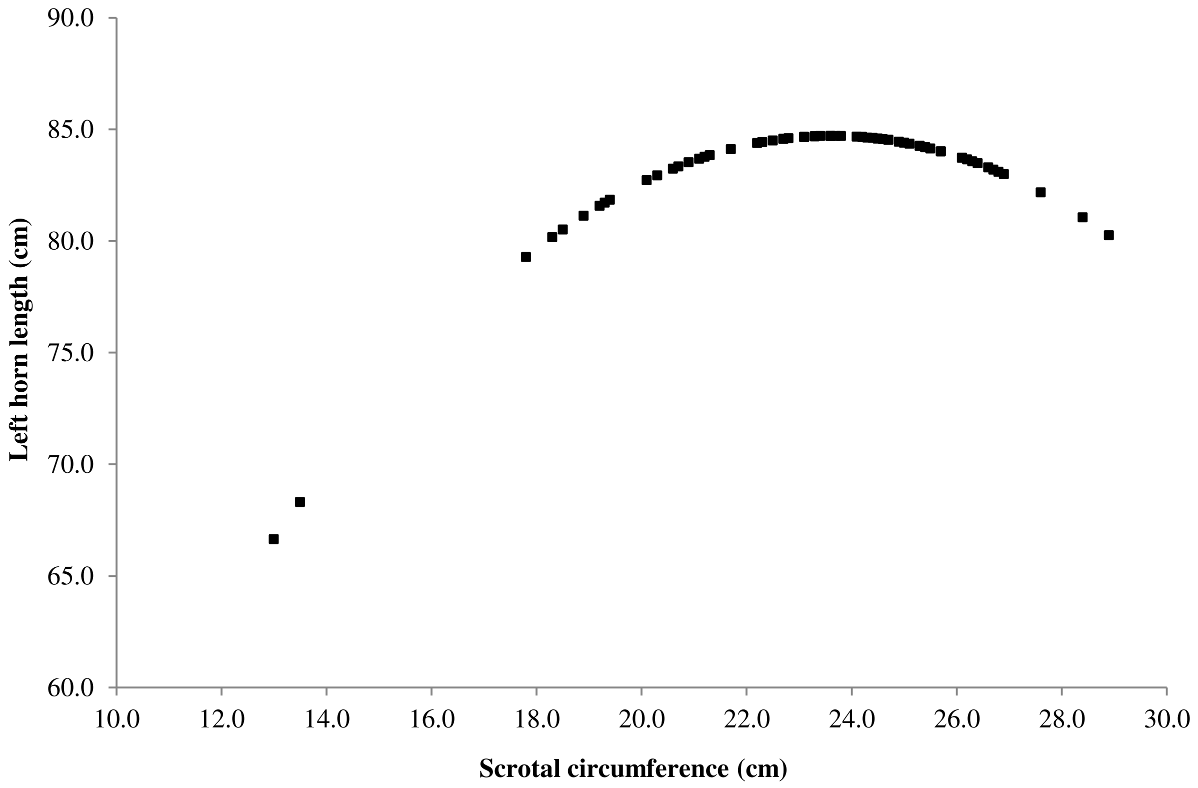

Figure 12.1 Regression of scrotal circumference with left horn length for age group 1.

Both apex left and apex right for age group 1 declined as SC increased, indicating an increase in ring number (Figures 12.4, 12.5, and 12.6). Scrotal circumference showed a negative trend in association with total horn length exceeding 172 cm (Figure 12.3).

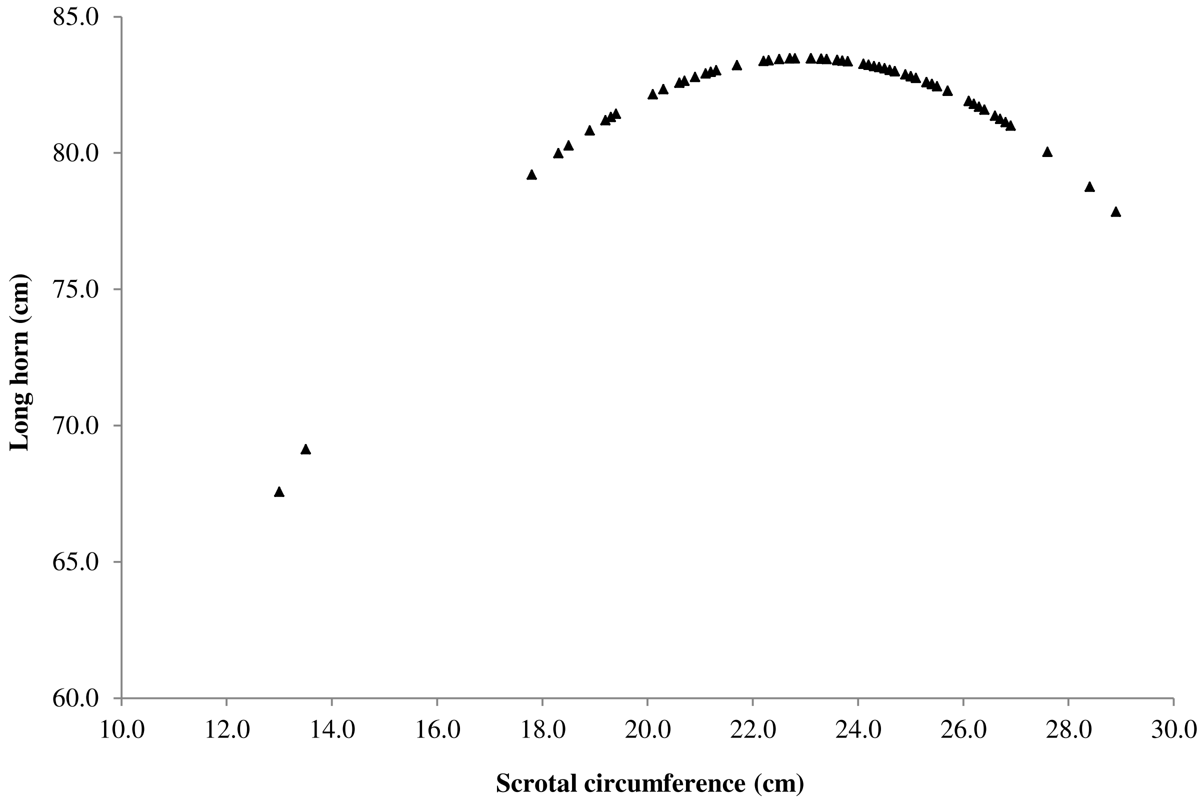

Figure 12.2 Regression of scrotal circumference with long horn for age group 1.

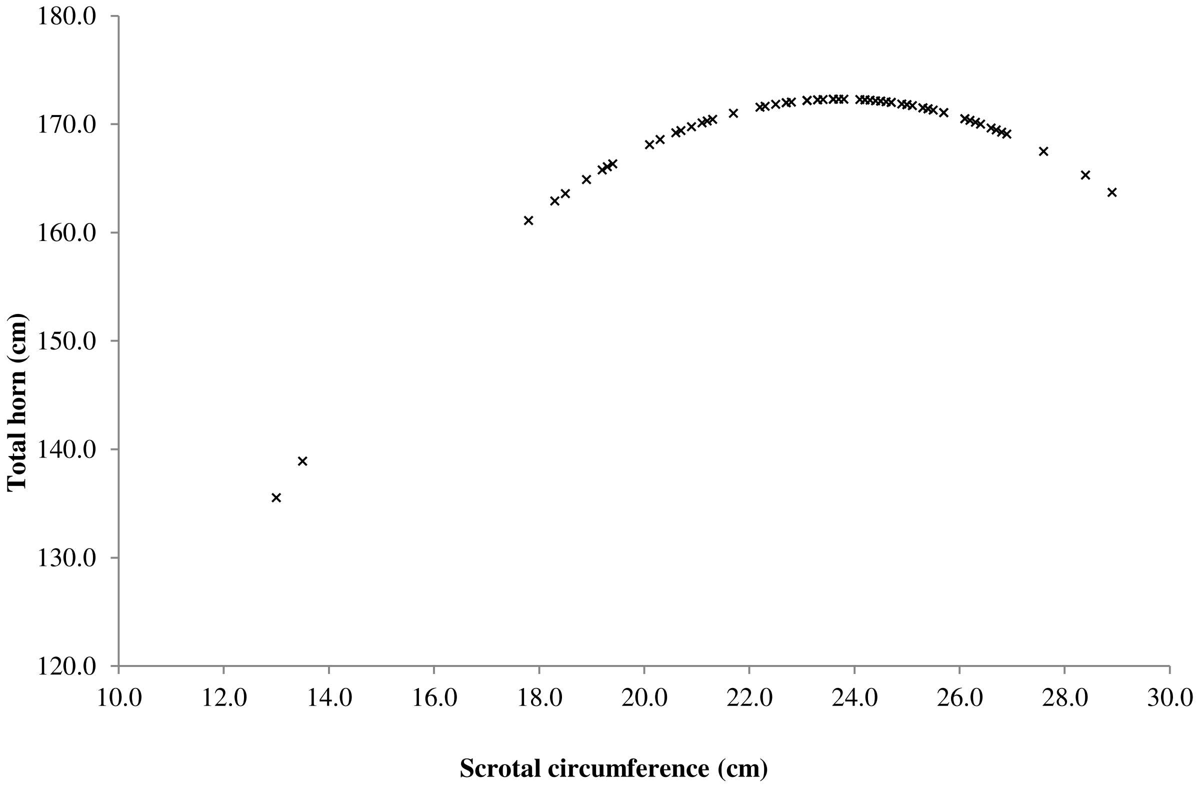

Figure 12.3 Regression of scrotal circumference with total horn for age group 1.

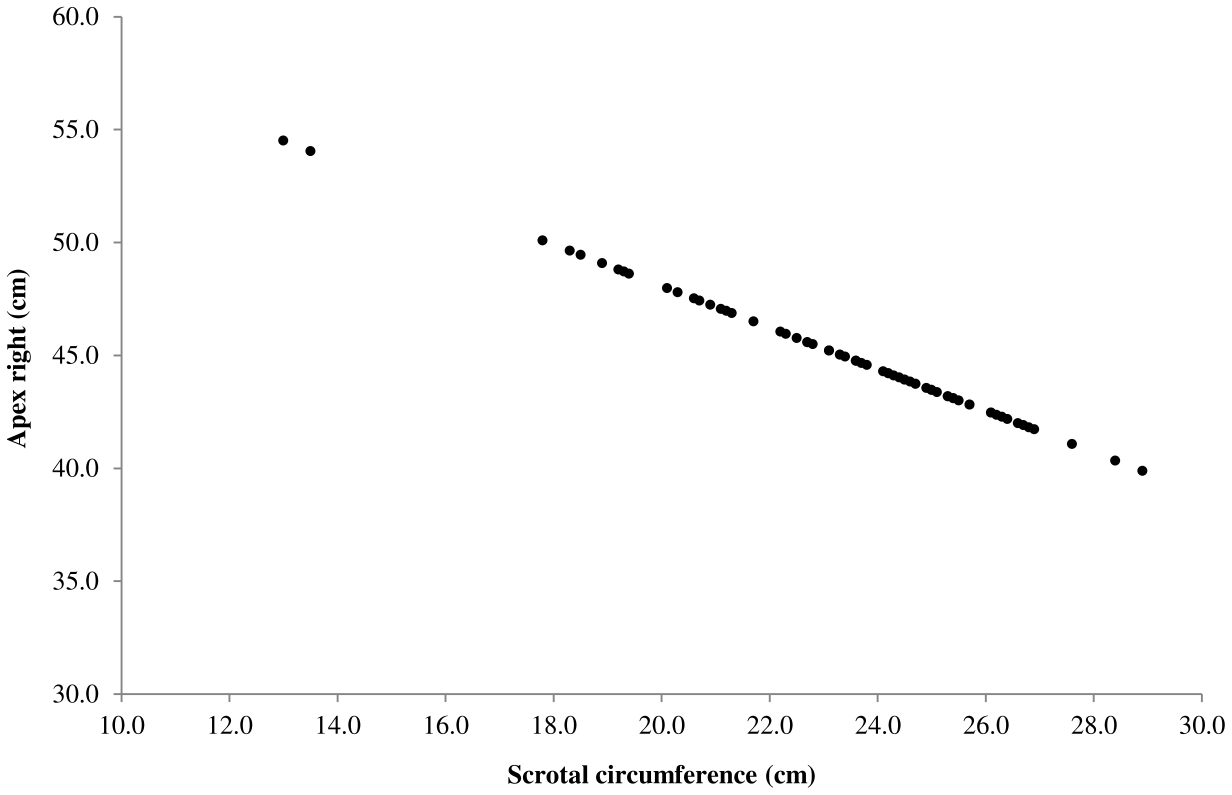

Figure 12.4 Regression of scrotal circumference with apex right for age group 1.

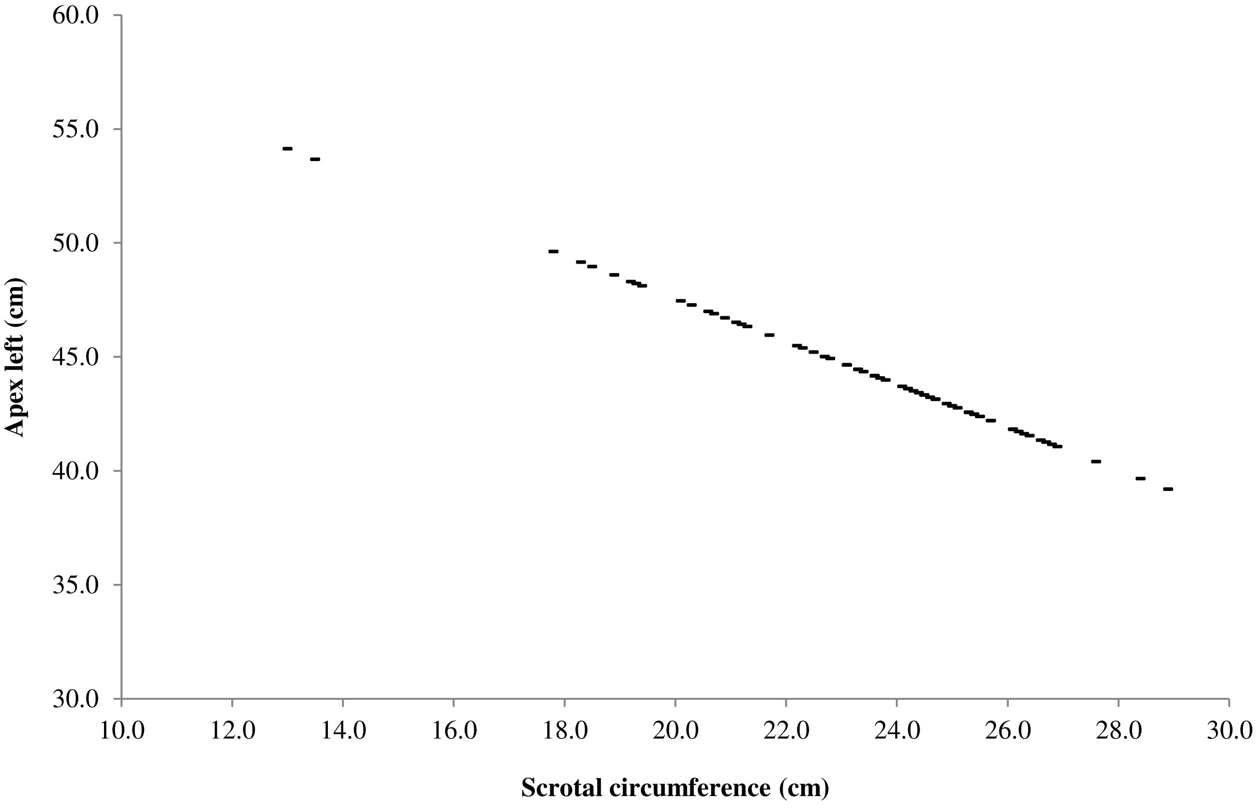

Figure 12.5 Regression of scrotal circumference with apex left for age group 1.

The number of rings for age group 2 of both horns decreases significantly as SC increases (Figures 12.6 and 12.7). This reduction in ring number for older gemsbok bulls suggests a decrease in their horn lengths due to honing or breakage during aggressive displays. Thus, bulls with the largest scrotums are the most dominant and do not exhibit the longest horns.

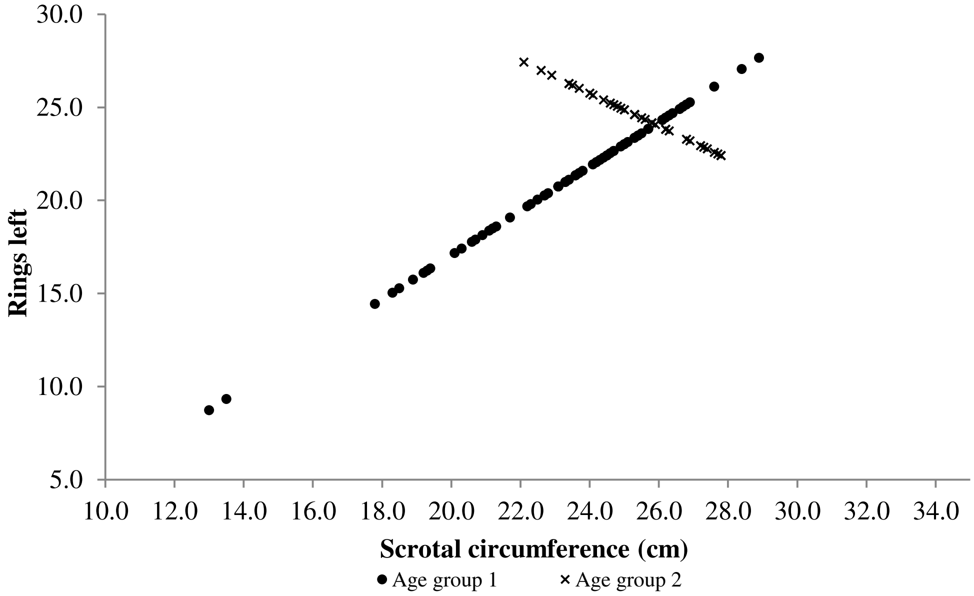

Figure 12.6 Regression of scrotal circumference with rings left for age groups 1 and 2.

Rings of the left horn, for age group 2, indicates a significant relationship ($P \leq 0.05$) with SC. This level of significance, however, is less pronounced than for age group 1 ($P \leq 0.001$), as well as negative, indicating a reduction in horn ring number most likely due to horn breakage and/or honing.

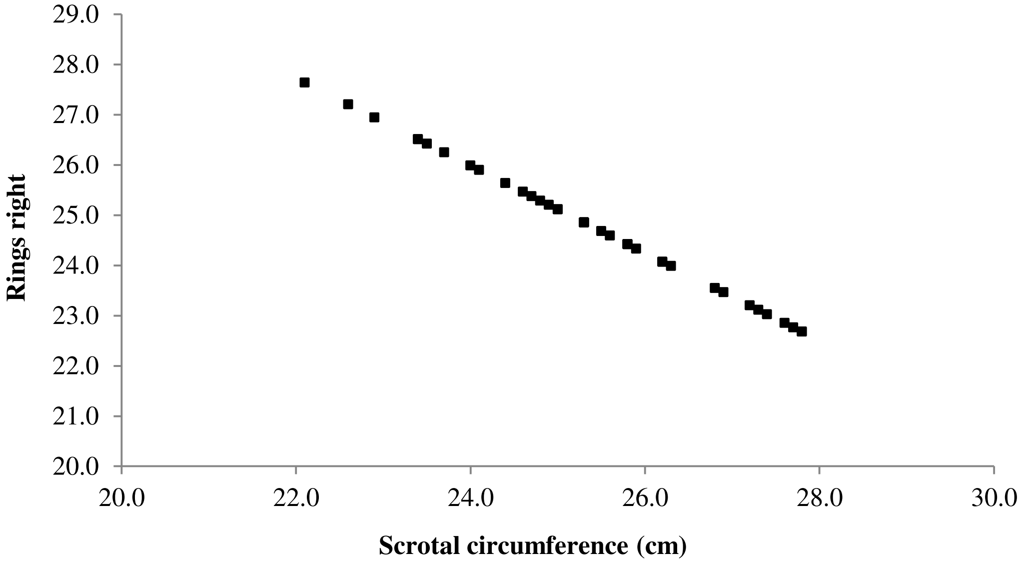

Figure 12.7 Regressions of scrotal circumference with rings right for age group 2.

CHAPTER 4

CONCLUSION

The competitive ability and quality, such as body weight and sperm production, of male antelope were significantly influenced by the presence of weapons. Because scrotal circumference gave a direct indication of testes size and potential sperm production, horns or antlers served as true indicators of male fertility. Therefore, bulls and rams with the largest testes were often assumed to be the most fertile and the most dominant within their populations.

The objective of this research project was to determine whether these male secondary sexual characteristics were negatively impacted by elaborate horns in gemsbok bulls. A definite trade-off between large weapons and longevity was observed for several antelope species, mostly ascribed to trophy hunting, but also due to suspected high maintenance requirements of larger-than-average horns.

Most anthropometric measures differed significantly between age groups 1 and 2, simultaneously increasing with age until maturity was reached (age group 2). This was expected as these increases are typical of growth. However, the result for CBC did not adhere to expectations as it showed similar measurements for bulls in both age classes. Bone circumference reached its maximum by the age of 4 years.

For horn measurements, only the left number of horn rings was significant with age group 2, reflecting an increase in the number of horn ridges associated with age. Indirectly, this observation indicated a reduction in apex length.

These findings for age groups were quite standard and did not indicate any negative relationships between weapon size and male survivability and the influence of SC was investigated next.

Fertility was defined in terms of testes size due to its direct correlation with the amount of spermatogenic tissue it contained and its sperm production potential. In the present study, SC was measured as an indirect indicator of testes capacity and potential sperm production. For age group 1, body weight, face length, heart girth, body length, neck circumference, and shoulder circumference all tested significant and positive in association with SC. In other words, as the animal grew, its masculinity, fertility, and competitive ability increased until growth was suspended at maturity (age group 2). Cannon bone length was the only parameter for age group 2 that correlated significantly with SC, indicating completed bone growth (length and density) by the age of 4 years.

Several significant relationships of horn measurements for age group 1 with SC were observed. Left horn length, long horn, total horn and number of rings left increased in response to scrotal growth. Both right and left apex decreased with an increase in SC, reflecting higher horn ring numbers.

For age group 2, only rings left showed a significant, but negative correlation with SC. This low significance level was indicative of a slower horn growth rate.

No significant negative impacts of larger-than-average weapons on gemsbok bull masculinity and SC were observed for young bulls. However, for older bulls, several negative correlations were observed. Data therefore shows that it is acceptable to select young bulls for horn length, but selecting for horn length in older bulls (older than 6 years) is risky.

H_1 was conditionally accepted as it was found to be true for young gemsbok bulls, but not true for older males (beyond inflection point of 24 rings, 172 cm total horn length, or 24 cm SC).

It must be taken into account that animals used in this study formed part of a free-roaming population, not subjected to intensive breeding protocols for trophy horns, and did not carry larger-than-average weapons. When these observations are taken into account, the need for further studies become imperative in order to determine the true effects of selection for horn size on the fertility and masculinity of male antelope under such intensive breeding pressures.

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