Factors Influencing Testes Size in the Cape Ground Squirrel (*Xerus inauris*).

by

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A thesis submitted to the Department of Biological Sciences, University of Manitoba,

in partial fulfilment of the requirements for the course

BIOL 4100 (Honours Thesis)

for the degree of

B.Sc. Hons (Biological Sciences): Ecology & Environmental Biology

Abstract

When males compete through sperm competition, selection favours an increased investment in sperm production to streghten the male's probability of siring offspring when females multiply mate. This increased investment in sperm quantity results in large testes relative to the species' size. Thus, testes size can be used as a measure of reproductive fitness in males experiencing intense sperm competition. Despite this relationship, studies investigating testes size have focused on interspecific variation, leaving the factors driving intraspecific variation in testes size an underexplored area of study. I investigated the influence of age, body condition, rainfall, parasite abundance and testosterone concentration on the testes size of Cape ground squirrels, a sciurid known to be under intense sperm competition. Data was collected from males trapped on SA Lombard nature reserve in South Africa between the years 2013-2024 and the influence of each factor on testes mass was assessed using linear regression. Testes mass was found to increase with both age and body condition and body condition also increased with age. While the energetic demands of spermatogenesis appear to shape the relationship between body condition and testes size, declining oxidative stress with age and a terminal investment strategy may drive enhanced testes investment in older individuals. Parasite abundance negatively affected testes size suggesting parasites remove resources from their host that would otherwise be allocated to reproduction. Rainfall and testosterone concentration did not affect testes size. The lack of relationships between rainfall and testes size may have occurred due to the limitations of this study, while testosterone's lack of impact on testes size may be caused by the low testosterone demands of a year-round breeding strategy. These findings aid in our understanding of the factors influencing reproductive success in males experiencing sperm competition and the selective forces shaping intraspecific testes size variation.

Acknowledgements

I would like to thank my advisor, Dr. Jane Waterman, for her unwavering support and guidance during this project and for allowing me to use her long-term dataset for my analyses. I would also like to extend my gratitude to my advisory committee, including Dr. Gail Davoren and Dr. Collin Garroway, for their thought provoking questions and constructive advice throughout the development of my thesis. Additionally, I am grateful for the funding provided by the Faculty of Science Undergraduate Summer Research Award (USRA), which allowed me to travel to South Africa to collect additional data for this project. Finally, I would like to express my gratitude to all those who have contributed to Dr. Waterman's dataset over the years and to the staff on SA Lombard Nature Reserve for their endless support in the field.

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Introduction

An organism's fitness can be equated to its ability to survive and produce viable offspring (Darwin, 1859). To increase their probability of successful reproduction, organisms employ strategies that vary by the selection pressures they experience (Orbach, 2019; Simmons & Buzatto, 2014). In mating systems where females are promiscuous, mating with more than one male during a breeding event, males increasingly invest in sperm production and/or sperm quality allowing them to mate with more than one female in short periods and strengthening the chance of successful fertilization (Lüpold et al., 2020). This increased investment in sperm results in large testes relative to the species' size (Kenagy & Trombulak, 1986; Lüpold et al., 2020). Due to this relationship, testes size can be used as a measure of reproductive fitness in species where the sperm of different males compete to fertilize the egg of a single female (sperm competition). While the factors influencing relative testes size between species have been explored repeatedly, the factors influencing intraspecific variation in testes size are less understood (Firman et al., 2022; Kenagy & Trombulak, 1986; Pitcher et al., 2005; Ribble & Millar, 1992; Schulte-Hostedde & Millar, 2004). Thus, exploring what factors influence testes size intraspecifically is an important and underexplored area of study.

The Cape ground squirrel (*Xerus inauris*), a highly social sciurid residing in arid areas of southern Africa, is a species with intense sperm competition (Waterman, 1995; Manjerovic et al. 2008). Males of this species have the largest testes-to-body mass ratios of any sciurid, do not engage in pre-copulatory male-male competition for access to females, and females multiply mate (Manjerovic et al., 2008; Waterman, 1998). Additionally, Cape ground squirrels are year-round breeders providing an ideal opportunity to study the factors influencing testes size. Many previous studies investigating these relationships have focused on seasonal breeders where testes size fluctuates with the offset and onset of the breeding season (Gupta et al., 2000; Richard et al., 2017; Sarasa et al., 2010). This relationship can confound the effects of other variables on testes size, making year-round breeders an ideal model for studying intraspecific variation. Cape ground squirrel males also exhibit alternative mating tactics when they reach maturity; they can either stay at home with their natal group at maturity (natal males) or leave their natal groups and join bands of dispersed males (band males) (Scantlebury et al., 2008; Waterman, 1995). While testes size does not differ between these two tactics in general (Scantlebury et al., 2008), differences in male susceptibility to adverse environmental conditions between tactics have been demonstrated (O'Brien et al., 2021; Scantlebury et al., 2008). Therefore, testes size may be differentially impacted between males exhibiting alternative reproductive strategies in years with poor environmental conditions.

Resource abundance may be one such factor differentially impacting the testes size of Cape ground squirrel males. To maintain their large testes, males must have access to sufficient resources to allocate to the growth and maintenance of their testes. Body condition reflects an organism's energy reserves and is a reliable predictor of testicular size in live animals (Ouchene-Khelifi & Ouchene, 2021; Strong & Sherry, 2000). In the arid habitat of this herbivorous species, food abundance varies with varying rainfall (O'Brien et al., 2021). The body condition of band males is negatively impacted during years of low rainfall, while natal males are shielded from adverse environmental effects, with little variance in body condition with varying rainfall (O'Brien et al., 2021). This relationship indicates band males have fewer intrinsic resources to devote to reproduction during years of low rainfall, therefore testes size and reproductive success may be impacted during these years.

The abundance of parasites may also influence resource allocation for reproduction. Adult Cape ground squirrel males are particularly prone to infection by ectoparasites (fleas, ticks, and lice) and natal and band males exhibit similar parasite loads (Hillegass et al., 2008). Parasitic infection causes an immune response that is energetically costly (Lochmiller & Deerenberg, 2000). This increasing demand for reallocation of resources to the immune system could result in lower resource availability for reproduction, a theory that is corroborated by studies demonstrating a negative relationship between parasitic infection and reproduction, including testes size, in other mammals (Bindseil & Hau, 1991; Boonstra et al., 1980; Sarasa et al., 2011; Smith, 1977).

However, following the Immunocompetence Handicap Hypothesis (ICHH), the influence of testosterone would be expected to confound this relationship. There is some evidence that increased testosterone levels result in a suppressed immune system (Folstad & Karter, 1992). The ICHH states there is a trade-off between investment in the immune system and investment in reproduction due to the duel effects of the androgen testosterone working as both an immune system suppressant and a stimulator of male sexual traits (Folstad & Karter, 1992). Testosterone is an androgen crucial for the production of sperm and increases in circulating testosterone levels are associated with increased testes size (Glick, 1979; Oduwole et al., 2021; Preston et al., 2012). Following the ICHH, we would expect high levels of testosterone to be associated with large testes size, which would reduce the individual's immunity to parasitic infection and result in high degrees of infection for males with large testes. However, in Cape ground squirrels, increased testosterone concentrations do not affect the immune response (O'Brien et al., 2018), allowing us to investigate the effects of parasitic infection on male reproductive fitness without the influence of testosterone confounding results.

Testes size variation within species can also be attributed to age. In humans, testes size shows a clear pattern with age, whereby testes size increases most profoundly during puberty, increasing to a peak size at around 30 years of age, where testes size remains fairly constant until it declines at around age 60 (Yang et al., 2011). These early patterns of testicular increases with age are mirrored in other mammalian taxa (Ariyaratne & Chamindrani Mendis-Handagama, 2000; Berger et al., 1982; Glick, 1979; Ouchene-Khelifi & Ouchene, 2021), as well as a decline in testes size and testicular function in old individuals (Hayward et al., 2015; Wang et al., 1993). While no significant difference has been found between the testes size of the younger natal males and older band males in Cape ground squirrels (Scantlebury et al., 2008), a thorough investigation of how testes size changes with age across both tactics is yet to be conducted.

My study aims to uncover the factors influencing testes size in Cape ground squirrel males to shed light on how male reproductive success is influenced by various extrinsic and intrinsic factors. I hypothesize that extrinsic resource availability influences resource allocation to reproduction in males of both tactics (H1). I predict that testes size will increase with precipitation only in band males whose body condition depends on extrinsic resource availability (HIP1) and testes size will increase with increasing body condition across males of either reproductive tactic (H1P2). The investment of resources in reproduction is also expected to be influenced by the resource demand of other physiological processes. I hypothesize that parasitic infection causes a shift in resource allocation from reproduction to the immune system (H2) and predict that testes size will decrease with increasing total ectoparasite abundance across all males (H2P1). Testes size has also been documented to fluctuate with age across mammalian taxa. I hypothesize that testes size is governed by age-related processes (H3) and predict that, like other mammals, testes size will increase to some threshold size attained during adulthood and decline in old individuals (H3P1). Finally, due to the importance of testosterone in spermatogenesis, I hypothesize that testes size is determined by testosterone concentration (H4) and predict that testes size will increase with increasing circulating testosterone concentration (H4P1). Investigating these relationships will help uncover the factors influencing reproductive success in males experiencing intense sperm competition and contribute to our understanding of the selective pressures driving intraspecific variation in testes size (for a list of hypotheses and predictions see Table 1).

Materials and Methods

Study site- I used a long-term dataset, set as well as new data, collected from S.A. Lombard Nature Reserve, approximately 18km from Bloemhof, South Africa (27°35'S, 35°23'E). This population has been studied since 2002, excluding the years 2008-2010 and 2020. This 3,660 ha site receives over 500 mm of average annual rainfall with the majority of rainfall occurring between the summer months from November to April (Herzig-Straschil, 1977; O'Brien et al., 2021).

Trapping- I trapped squirrels using Tomahawk live traps (Tomahawk Live Trap Inc. WI, USA: 15 x 15 x 50 cm) baited with peanut butter and bird seed from May through July (Waterman, 1995). I checked traps at least every 1-2 hours between 8:30 am and 5:30 pm. Upon the first capture of an individual, a permanent passive indicator transponder (PIT) tag (AVID Inc., Norco, CA, U.S.A or Shenzhen XCC RFID Technology Co., Ltd. China) was placed under the skin and the squirrel received a unique identification number (Hillegass et al., 2008). For temporary long-distance identification, squirrels were marked dorsally using hair dye (Rodol D; Lowenstein and Sons Inc., New York, NY, U.S.A.; Waterman, 1996). Once trapped, I transferred the animals to cone-shaped handling bags to collect measurements (Koprowski, 2002). Only adult males were included in my study and I determined maturity by scrotal descent, which occurs at approximately 8-10 months (Waterman, 1996). I distinguished the reproductive tactic of males (band or natal) through their sleeping arrangements, as natal males continue to sleep with their natal group, while band males sleep alone or with other adult males (Waterman, 1995). To determine sleeping arrangements, I observed the morning emergence of squirrels from their burrows from an observation tower or hides placed on top of vehicles (O'Brien et al., 2021; Warrington et al., 2022). To distinguish squirrels, I used 10 x 50 binoculars and 15 – 45 x 60 spotting scopes to detect the unique dye marks of the individuals (O'Brien et al, 2021). I then categorized adult males who consistently emerged with family groups as natal males and males who emerged alone or with other adult males as band males. For males who were not observed during these sleeping arrangement observations, I used the locations of trapping to determine reproductive tactic (Warrington et al. 2022). Band males change their sleeping locations regularly, while natal males and their families continue to sleep in the same location (Waterman, 1995). Males who were consistently trapped at their natal burrow were then assigned a natal tactic while males who were trapped at various locations across the reserve were assigned a band tactic (Warrington et al., 2022).

Testes mass- I measured testis' length and width (mm) using digital callipers and calculated the volume using the ellipsoid volume formula ($\frac{\frac{4}{3}p}{8}$ * width * depth * length). Since testis density is approximately equal to 1.0 g/ml across mammalian taxa (França & Godinho, 2003; Handelsman & Staraj, 1985; Morais et al., 2014), I converted testes volume (mm^3) to ml for both right and left testicles, which is assumed to equal testes mass (g), and combined the mass of each testicle for overall testes mass. I then averaged testes mass for males assessed more than once in a trapping season to decrease the effects of measurement error between observations. These averages were calculated between 2 and 11 measurements, with the majority of averages obtained from 2-4 measurements. Testes size for males only observed once in a trapping period were unaltered yielding 358 averages and 584 single measures of testes mass (942 observations from 538 males).

Body condition- To obtain a relative measure of individual body condition, I recorded body mass (g) using Pesola spring scales (Pesola AG, Baar, Switzerland), and measured spine length (mm) with a tape measure from the occipital condyles to the end of the caudal vertebrae. I subtracted testes mass from body mass to avoid autocorrelation in later regression models. Both spine length and body mass were averaged for males assessed more than once in a trapping season yielding 358 averages and 584 single measures of mass and spine length for 942 observations from 538 males. Mass was then regressed on spine length using an ordinary least squares regression and the residuals of this model were used as an index of body condition (Schulte-Hostedde et al., 2005a).

Precipitation- Monthly precipitation (mm) was recorded using a rain gauge located on S.A. Lombard Nature Reserve and totalled for the wet season months (November to April). The wet season occurs before the trapping period (May to July) and total precipitation during these months is assumed to reflect primary productivity and thus food abundance during the trapping period (O’Brien et al. 2021).

Parasites- I determined the abundance of ectoparasites by combing the left, right, and central planes of the dorsal area a total of three times with a metal flea comb (Hillegass et al., 2008), and by inspecting the inner thighs and groin area (O’Brien et al., 2018). I then placed removed parasites in 70% ethanol to be counted (Hillegass et al., 2008) and recorded the total parasite abundance for each individual. Parasite abundance was then averaged for males assessed more than once in a trapping season yielding 181 averages and 578 single measures (759 observations from 541 males).

Age- Since males are known to reach maturity at approximately 8-10 months (Waterman, 1996), I estimated the age of males (in years) from the first instance of trapping after scrotal descent. Age was only used for males who were trapped prior to scrotal descent, who were new natal adult males not previously observed in the group, or who were observed as juveniles in burrows before trapping as adult males to ensure the accuracy of the age estimates. For band males who dispersed onto the study site after reaching maturity, I recorded age (in years) as a measure of tenure on site that was calculated from the first year they were trapped on the study site.

Testosterone- To access circulating testosterone concentrations, I collected blood samples from the femoral vein and separated plasma and red blood cells through centrifugation (O'Brien et al., 2021; Scantlebury et al., 2008). Plasma testosterone concentration was then measured using a commercially available assay kit (Coat-a-Count TKTT1, Diagnostic Products Corporation, Los Angeles, California) in the lab of Dr. Nigel Bennett, University of Pretoria. The kit assay can determine testosterone concentrations of 10 to 1600 nmol/l. Cross-reactivity of the Coat-a-Count testosterone antibody was 16% with 11-ketotestosterone, b5% with dihydrotestosterone and 19-hydroxyandrostenedione; and less than 1% with other steroids tested (Scantlebury et al. 2008; O'Brien et al. 2018). The assay was validated by testing for parallelism between serial dilutions of plasma (obtained from an individual with high testosterone concentrations) and the standard curve. The curves were parallel and not significantly different from the reference preparation (see Scantlebury et al. 2008 and O'Brien et al. 2018 for more details).

Data analysis- I performed all analyses using R studio software version 2024.09.0 (R Core Team, 2021) and the significance of each relationship was determined using an alpha value of 0.05 (for a full list of models see Table 2). The data set included observations from the years 2013-2024 excluding the year 2020 and consisted of 538 unique individuals.

To access H1P1, I used a linear mixed model with a Gaussian distribution. I used the testes mass (g) of band males as the dependent variable, with total wet season precipitation (November to April) as a fixed effect and ID (TagID) as a random effect. TagID was used as a random effect to control for measurements taken on the same squirrels throughout the study period to ensure the independence of the data. A total of 234 observations from 103 band males were assessed and consisted of 94 averages and 140 single measures. I also conducted this model using natal males' testes mass (g), to assess the prediction that only the testes size of band males responds to variation in precipitation. A total of 305 observations from 205 natal males were assessed and consisted of 154 averages and 151 single measures. To investigate H1P2, I used the same models as the analysis for H1P1, only I replaced wet season precipitation with body condition as the fixed factor in the model. This model was also conducted with all males in the data set regardless of tactic. A total of 942 observations from 538 males were assessed and consisted of 358 averages and 584 single measures.

I investigated H2P1 using a linear mixed model. Testes mass (g) was used as the dependent variable in the model, with parasite abundance as the fixed factor, and TagID as a random effect. Due to a violation of the assumption of normally distributed residuals in this model, which could not be resolved by transforming the data, all averaged data were replaced with the original single measures resulting in a total of 1391 observations from 537 males.

To explore H3P1 I use a linear mixed model with a fitted quadratic term as I expected a non-linear relationship. I ran a separate analysis for males of known ages and males who dispersed onto the site after scrotal descent with testes mass (g) as the dependent variable in the model, age or tenure on site as the fixed factor, and TagID as the random factor. A total of 239 males were accurately aged and the model included 439 observations consisting of 215 averages and 224 single measures. Tenure on site was reported for a total of 298 males and this analysis included 480 observations containing 122 averages and 358 single measures.

Finally, I assessed H4P1 using a linear mixed model with a Gaussian distribution. Testes mass (g) was used as the dependent variable in the model, with plasma testosterone concentration as the fixed factor, and TagID as the random factor. A total of 77 plasma samples were assessed from 71 individual males and these observations contained 32 averages and 45 single measures. Testosterone concentrations were not assessed in 2024. To meet the assumptions of linear regression, testes mass was square root transformed in all models excluding those testing H1P1 and H1P4. A cube root transformation of testes mass was applied for the H1P4 analysis.

Results

I found no significant linear relationship between total wet season precipitation and testes mass for either natal males ($\beta = 0.0006 \pm 0.0016$, $P = 0.72$, $n = 205$, Figure 1A) or band males (H1P1, $\beta = 0.0017 \pm 0.0022$, $P = 0.45$, $n = 103$, Figure 1B). However, the body condition of all males had a significant positive effect on testes size (H1P2) ($\beta = 0.0032 \pm 0.0002$, $P < 0.0001$, $n = 538$, figure 2) and this relationship remained significant for both natal and band males (natal: $\beta = 0.0184 \pm 0.0030$, $P < 0.0001$, $n = 205$; band: $\beta = 0.0161 \pm 0.0038$, $P < 0.0001$, $n = 103$, respectively).

Age had a significant negative quadratic relationship with testes size (H3P1) (Linear term: β_1= 0.242 ± 0.060, P < 0.0001, Quadratic term: β_2= -0.021 ± 0.009, P = 0.02, n = 239, figure 3A). However, a linear regression without a quadratic term yielded a significant positive linear relationship between log-transformed age and testes mass (β = 0.283 ± 0.040, P < 0.0001, n = 239, figure 3B). An AIC analysis was performed to determine the model with the best fit for assessing the effect of age on testes size. The linear model exhibited the best fit when comparing ΔAIC values for each model (linear ΔAIC: 0.00 quadratic ΔAIC:12.29). For males who could not be accurately aged, a positive linear relationship was also significant between testes size and tenure on site (β = 0.093 ± 0.017, P < 0.0001, n = 298, figure 4).

To assess whether age and body condition were related, the effect of age on body condition was modelled using a linear regression analysis. Age had a significant positive effect on body condition ($\beta = 11.86 \pm 1.44$, P < 0.0001, n = 237, Figure 5). Due to the potential for age to confound the effects of body condition on testes size, I analysed the effect of body condition on testes size within age categories with observations from at least 30 males (ages 1, 2, 3 and 4). Body condition had a significant positive effect on testes size for males aged 1-3 (age 1: β = 0.018 ± 0.005, P = 0.0001, n = 164, age 2: β = 0.011 ± 0.005, P = 0.03, n = 112, age 3: β = 0.015 ± 0.007, P = 0.04, n = 63) and the effect for 4-year-old males was non-significant (β = 0.011 ± 0.010, P = 0.30, n = 44).

When assessing the impact of ectoparasites on testes (H2P1), a significant negative relationship between parasite abundance and testes mass was observed (β = -0.0053 ± 0.0025 P = 0.03, n = 537, Figure 6). Finally, no significant relationship between circulating testosterone concentration and testes mass (H4P1) was observed (β = -0.0003 ± 0.0004, P = 0.52, n = 71, Figure 7).

Discussion

As predicted, testes size increased with increasing body condition, regardless of the reproductive tactic exhibited by the male. Also, the positive relationship between these variables persisted within age groups of 1- to 3-year-old males, although the effect for 4-year-old males was non-significant (possibly due to a small sample size). Since producing sperm is energetically costly (Olsson et al., 1997), the larger testes size of males in better body condition is to be expected and this relationship has also been documented for other small mammals (Schulte-Hostedde, Millar, et al., 2005b). These findings emphasize the potential fitness benefits associated with better body condition in males under intense sperm competition. Since male testes size can be correlated to sperm quantity (Lüpold, 2013), and a larger quantity of sperm is positively associated with successful fertilization (Hale et al., 2008; Pace et al., 1981; Sorci et al., 2023), males in better body condition may benefit from an increased chance of successful fertilization during copulation events. In contrast, males in poor body condition may suffer from a low probability of successful fertilization due to low sperm quantity. These relationships are consistent with a previous study on *X. inauris* males that found males in better body condition sired more offspring (Warrington et al., 2024). Together, these results highlight the importance of body condition as a key determinant of reproductive success in male Cape ground squirrels. By investing in greater sperm production, males with better body condition may gain a competitive advantage, ultimately increasing their likelihood of paternity under intense sperm competition.

Another result of this study that indicates the costly nature of sperm production is the effect of parasite abundance on testes size. As predicted, testes size decreased with increasing ectoparasite abundance. The energetic costs of parasitic infection are two-fold: first, parasites remove resources from their host by feeding on them directly (Khokhlova et al., 2002) and second, parasitic infection causes an energetically costly immune response (Lochmiller & Deerenberg, 2000; Møller et al., 1994). These energetic costs and the reproductive costs of parasitic infection observed in this study may indicate that the increasing energetic demands associated with increased parasite abundance reduce the availability of resources that would otherwise be invested in reproduction. A similar relationship has been observed in female Cape ground squirrels where the removal of ectoparasites resulted in increased reproductive success (Hillegass et al., 2010). Interestingly, females of this species did not increase their body mass when their parasites were removed, suggesting these parasites remove resources that would otherwise be invested in reproduction instead of the female's body mass (Hillegass et al., 2010). Male Cape ground squirrels appear to exhibit the same relationship between reproductive investment and parasitic infection. However, it should be noted that the analysis of this relationship yielded a relatively large associated error. This high error may be explained by the confounding influence of other environmental variables. Allander (1998) found that the effects of ectoparasites on the body condition of great tit (*Parus major*) nestlings were only significant in certain years, suggesting other environmental variables may offset or increase the effects of parasitism. Thus, a thorough analysis of how parasite abundance impacts testes size under different environmental conditions is needed to further investigate this relationship.

In contrast to the predicted plateau and decline in testes size in ageing individuals, the change in testes size with age was more accurately explained by a linear model demonstrating increasing testes size through age. This positive linear trend aligns with findings from a previous study showing that older *X. inauris* males sired more offspring than their younger counterparts (Warrington et al., 2024). This observed relationship may be explained by the terminal investment hypothesis. This hypothesis predicts that organisms will increasingly invest in reproduction as they age and the opportunities to pass on their genes decline (Clutton-Brock, 1984). There has been some support for this hypothesis throughout various animal taxa including ungulates, rodents, birds, amphibians, and insects (Bercovitch et al., 2009; Brannelly et al., 2016; Thanda Win et al., 2013; Velando et al., 2006; Weil et al., 2006). My study may also support the terminal investment hypothesis with increasing testes size throughout adulthood suggesting that males increasingly invest in sperm quantity as they age to maximize their fitness as their reproductive value declines. However, my findings may be explained by the lifespan of Cape ground squirrels. The pattern of testes size increasing into middle age, stabilizing, and then declining in old age is documented in long-lived humans (Yang et al., 2011). The oldest squirrels in this study were 7 years old, but this species has been documented to live up to 11 years in captivity (Weigl, 2005). Thus, the longevity of these squirrels in the wild may be too short to see the effects of senescence causing declines in testes size. Despite the lack of data for older individuals, the relationship between testes size and age is clear for younger adults, demonstrating the importance of age in shaping variation in male reproductive success.

Additionally, the relationship between age and testes size may be attributed to a decline in oxidative stress in aging *X. inauris* species. Oxidative stress is the imbalance between the reactive oxygen species and neutralizing antioxidants in the body (Santos et al., 2024). As individuals age, these reactive oxygen species accumulate impairing physiological function and lead to cellular damage (Hamilton et al., 2001; Santos et al., 2024). Following oxidative stress theory, we would expect testes size and body condition to decline in older individuals as physiological function becomes increasingly impaired by oxidative damage. This theory is corroborated by a study on deer mice (*Peromyscus maniculatus*), where increased oxidative stress was associated with both smaller testes mass and poorer body condition (Rodríguez-

Estival et al., 2016). However, in *X. inarius* species, oxidative stress declines rather than increases with age (Jacobs et al., 2024). This decline in oxidative stress through age may result in less cellular damage accumulation in older individuals, reducing the impairment of physiological functions usually associated with aging (Gunes et al., 2016; Santos et al., 2024). Thus, the increasing body condition and testes mass with age in male Cape ground squirrels could be attributed to unknown physiological mechanisms reducing oxidative damage in older individuals.

Unlike the effects of body condition and age, wet season precipitation did not have a positive effect on testes size. While a lack of a relationship between testes size and rainfall was expected for natal males, band male testes size was predicted to increase with increasing precipitation due to the impact of precipitation on their body condition (O'Brien et al., 2021). The two major assumptions when investigating this relationship were 1: the difference in wet season precipitation across the study period was large enough to cause a significant change in food abundance, thus impacting resource consumption and 2: wet season precipitation (November-April) reflects food abundance during the trapping period (May-September). A study by O'Brien et al (2021) can be used to address the first assumption of this analysis. Throughout their study an increase in yearly rainfall from 336-551 mm resulted in a significant increase in band male body condition, suggesting a change in resource availability within this precipitation range (O'Brien et al., 2021). My study observed a wet season precipitation range of 354.6-644.3 mm demonstrating a 74.7 mm larger range than O'Brien's. Thus, the lack of a relationship between rainfall and testes size does not appear to be due to a limited precipitation range. To address the second assumption, relationships between precipitation and primary productivity in other semi-arid grasslands can be used. Swemmer et al found precipitation event size rather than precipitation amount more accurately reflected plant productivity in a semi-arid grassland (2007). Here, rainfall events larger than 25 mm were less effective than smaller rainfall events at promoting plant growth suggesting there is some threshold of precipitation beyond which these plants cannot efficiently utilize water to promote growth (Swemmer et al., 2007). Thus, the total wet season precipitation used in this study may not reflect food availability in the trapping period when large precipitation events contribute disproportionately to total wet season precipitation. To further investigate the relationship between testes size and resource abundance, I suggest future studies use reliable predictors of plant productivity such as a normalized difference vegetation index (Box et al., 1989).

Finally, testosterone had no relationship to testes size in the Cape ground squirrel. A similar lack of a relationship between these two variables was demonstrated in these males by O'Brien et al. (2018). These researchers discovered an immune challenge negatively affected circulating testosterone concentration, but scrotal size remained unaffected (O'Brien et al., 2018). This lack of a relationship may be explained by the low testosterone requirements of maintaining spermatogenesis. In mice (*Mus musculus*), the initiation of spermatogenesis requires a larger testosterone input than the maintenance of this process, allowing testes mass to be maintained at low testosterone concentrations over multiple weeks (Handelsman et al., 1999). Seasonal breeders experience periods of testicular regression where spermatogenesis is decreased or inactivated, and recrudescence in which spermatogenesis must be reinitiated (Bueno et al., 2024). In contrast, Cape ground squirrels breed throughout the year, maintaining spermatogenesis year-round (Waterman, 1998). The low testosterone demand of sperm production maintenance versus sperm production reinitiation may allow year-round breeders to maintain their testes size at low testosterone concentrations. Thus, the lack of a relationship between testosterone and testes size in my study may be a result of the breeding strategy of the Cape ground squirrel.

In conclusion, this study has demonstrated that both age and body condition influence intraspecific variation in testes size leading to enhanced reproductive success for older males and males in better condition. While the energetic demands of spermatogenesis appear to shape the relationship between body condition and testes size, declining oxidative stress with age and a terminal investment strategy may drive enhanced testes investment in older individuals. Additionally, increasing parasite abundance appears to reduce resource allocation to reproduction, although the extent to which parasites impact reproductive investment should be further investigated by considering potentially confounding environmental variables. The lack of a relationship observed between testes size and circulating testosterone concentration may be indicative of the year-round breeding strategy of these males allowing large testes size to be maintained at low levels of testosterone. Similarly, wet season precipitation did not affect testes size. The lack of a relationship between testes size and rainfall in this study may be due to a threshold in the amount of water that can be used in primary production at any one time and future studies should aim to use more reliable predictors of above-ground plant biomass. Overall, this study aids in our understanding of the factors influencing reproductive success in males experiencing sperm competition and helps bridge the gap in our knowledge of the selective forces shaping intraspecific testes size variation.

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Tables and Figures:

Table 1. Hypotheses and predictions regarding the influence of various factors on the testes size of the Cape ground squirrel (*Xerus inauris*).

Hypotheses	**Predictions**
H1: Extrinsic resource abundance influences resource allocation to reproduction.	**H1P1**: Only band male testes mass will increase with increasing precipitation. **H1P2**: Testes mass will increase with increasing body condition.
H2: Parasitic infection causes a shift in resource allocation from reproduction to the immune system.	**H2P1**: Testes mass will decrease with increasing ectoparasite abundance.
H3: Adult testes size is influenced by age-related processes.	**H3P1**: Testes size will increase to some threshold size attained during adulthood and decline in old-aged individuals.
H4: Circulating testosterone levels influence testes size through sperm production.	**H4P1**: Testes size will increase with increasing testosterone concentration

Table 2. The models and associated predictions for studying the influence of various factors on the testes size of the Cape ground squirrel (*Xerus inauris*).

Prediction	**Model**	**Response variable**	**Fixed effect**	**Random effect**	**Band and natal males differentiated**
H1P1	LMM (Gaussian distribution)	Testes Mass (g)	Precipitation (mm)	Tag ID	Yes
H1P2	LMM (Gaussian distribution)	Testes Mass (g)	Body condition	TagID	Yes
H2P1	LMM (Gaussian distribution)	Testes Mass (g)	Parasite abundance	TagID	No
H3P1	LMM (Quadratic expression)	Testes Mass (g)	Age (all males of known age) Tenure on site (band males of unknown age)	TagID	Yes- tenure on site contains only band males
H4P1	LMM (Gaussian distribution)	Testes Mass (g)	Plasma testosterone concentration	TagID	No

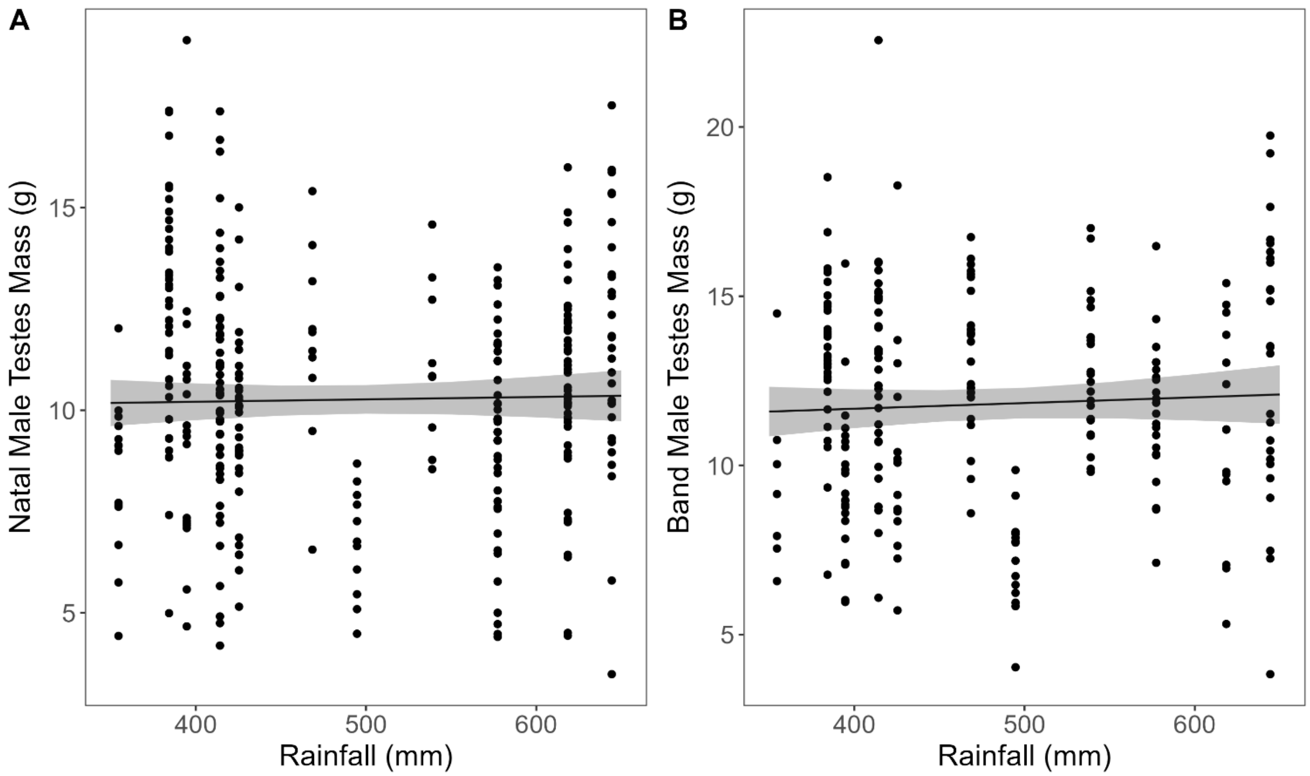

Figure 1. The effect of total wet season precipitation (mm)(November-April) on the testes mass (g) of natal (A) and band (B) males. Testes mass was averaged for individuals trapped more than once per trapping season resulting in 154 averages and 151 single measures (305 observations from 205 males) for natal males and 94 averages and 140 single measurements (234 observations from 103 males) for band males for the years 2013-2024, excluding the year 2020. The shaded area represents the 95% confidence interval.

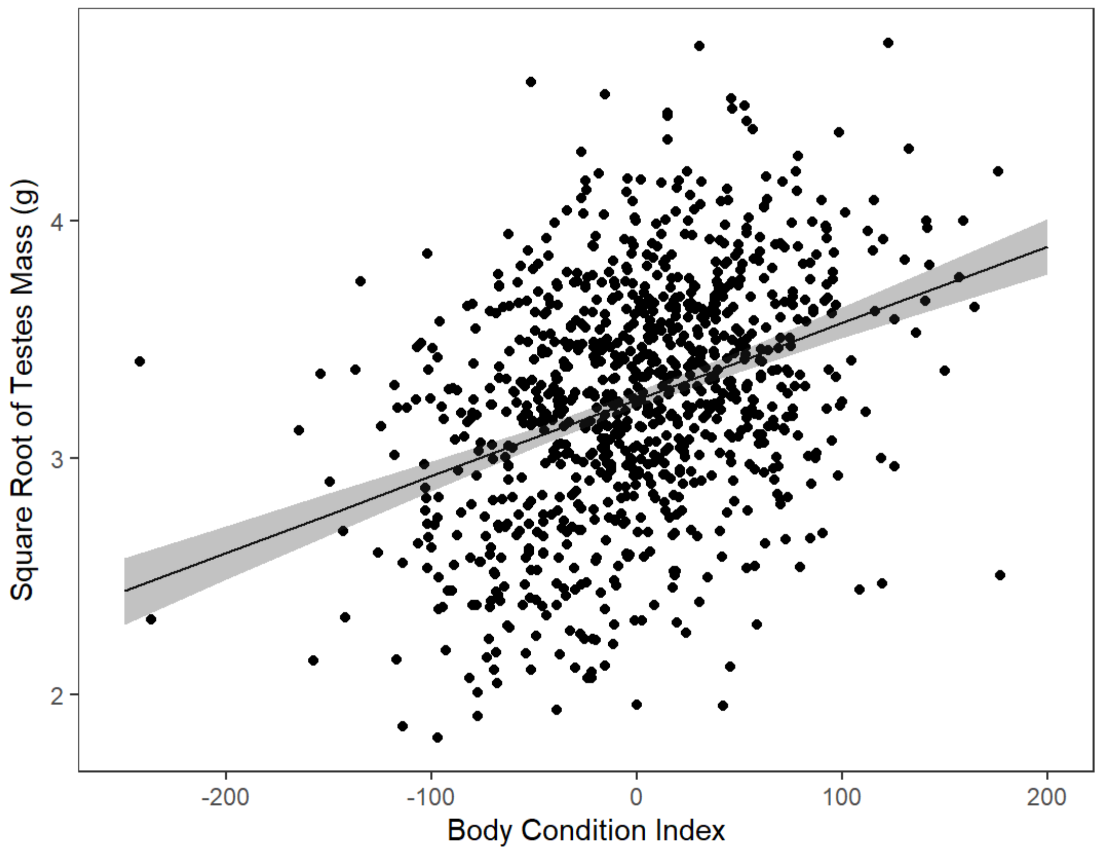

Figure 2. The effect of Cape ground squirrel body condition on their testes mass (g). Body condition was assessed by regressing body mass on spine length and testes mass was averaged for individuals observed more than once during a trapping period for a total of 358 averages and 584 single measures (942 observations from 538 males) throughout the years 2013-2024, excluding the year 2020. The shaded area represents the 95% confidence interval.

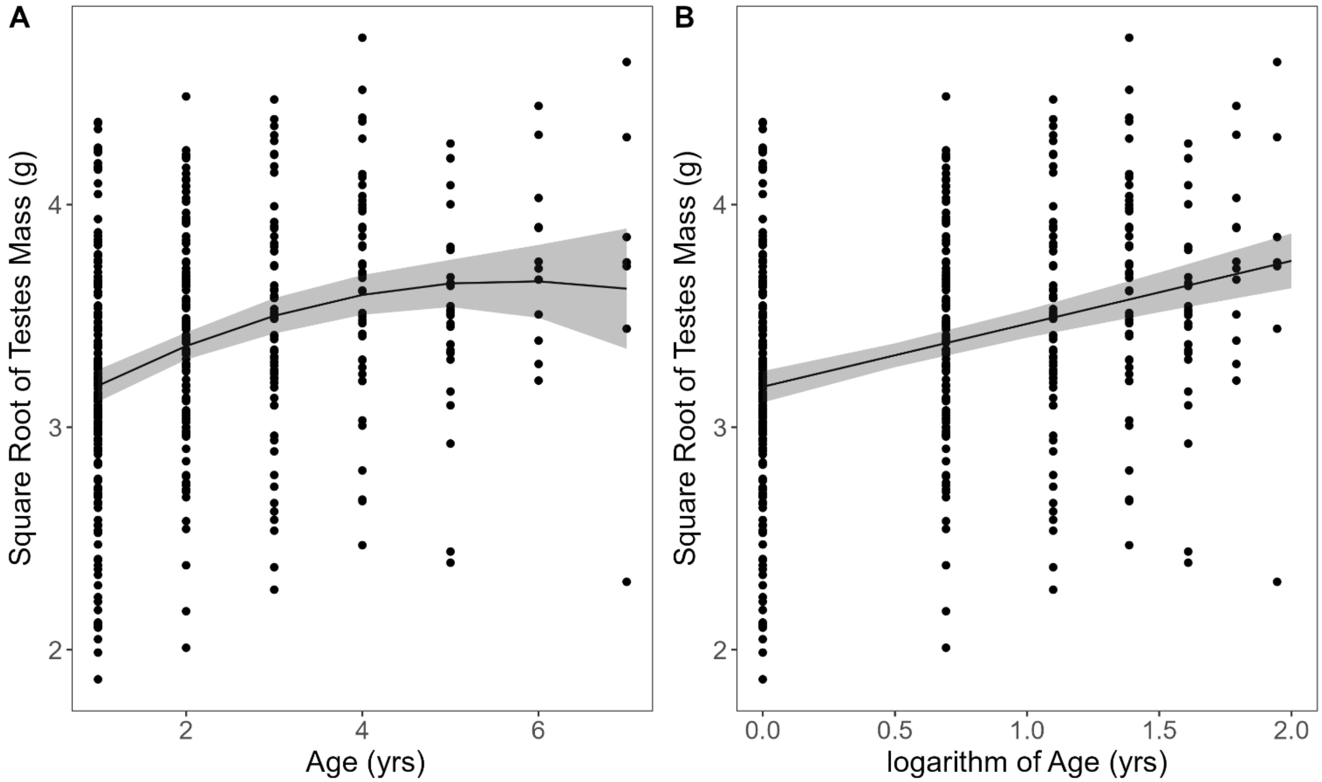

Figure 3. The influence of male Cape ground squirrel age (yrs) on their testes mass (g) using quadratic (A) and linear (B) regression. Testes mass was averaged for individuals caught more than once during the trapping season for a total of 215 averages and 224 single measures (439 observations from 239 males) for the years 2013-2024, excluding the year 2020. The shaded area represents the 95% confidence interval.

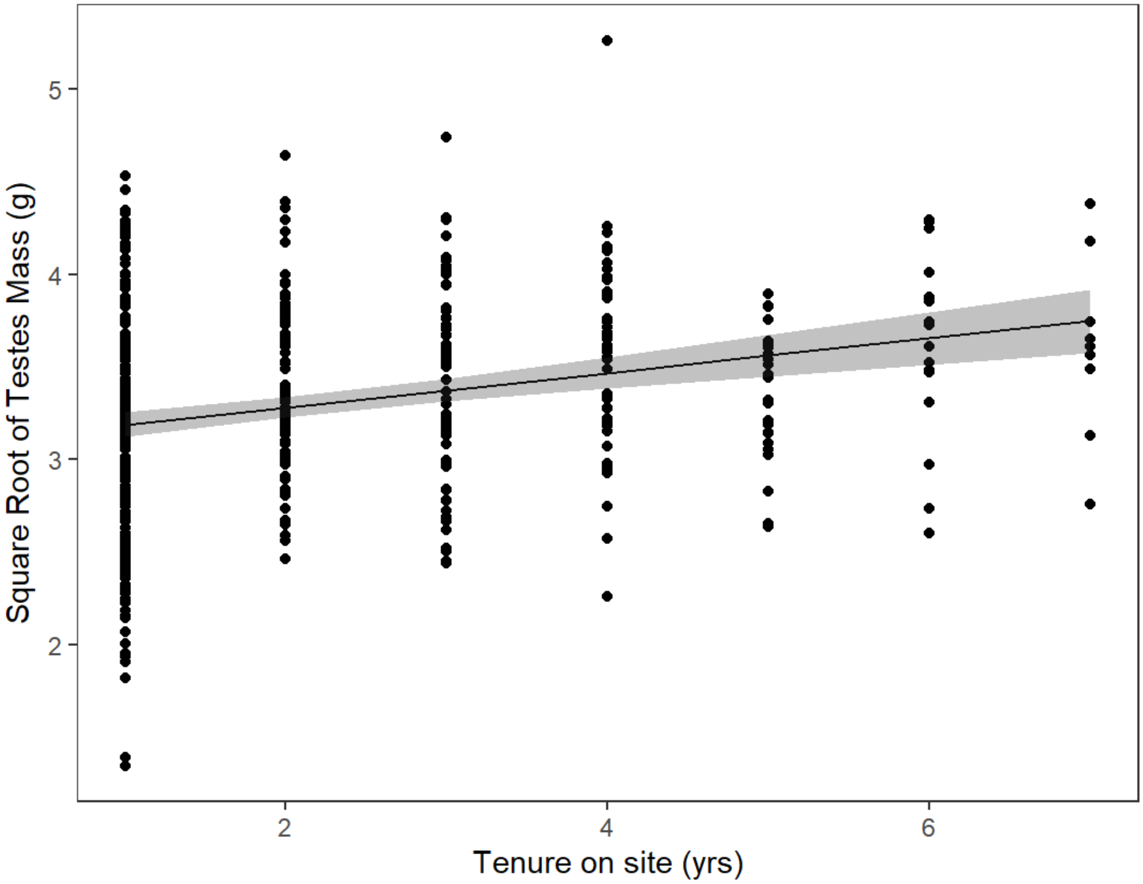

Figure 4. The influence of male Cape ground squirrel tenure on site (yrs) on their testes mass (g). Testes mass was averaged for individuals measured more than once during a trapping season resulting in 122 averages and 358 single observations (480 observations from 298 males) for the years 2013-2024, excluding the year 2020. Tenure on site was estimated from the first instance of trapping as an adult, excluding individuals trapped prior to sexual maturation. The shaded area represents the 95% confidence interval.

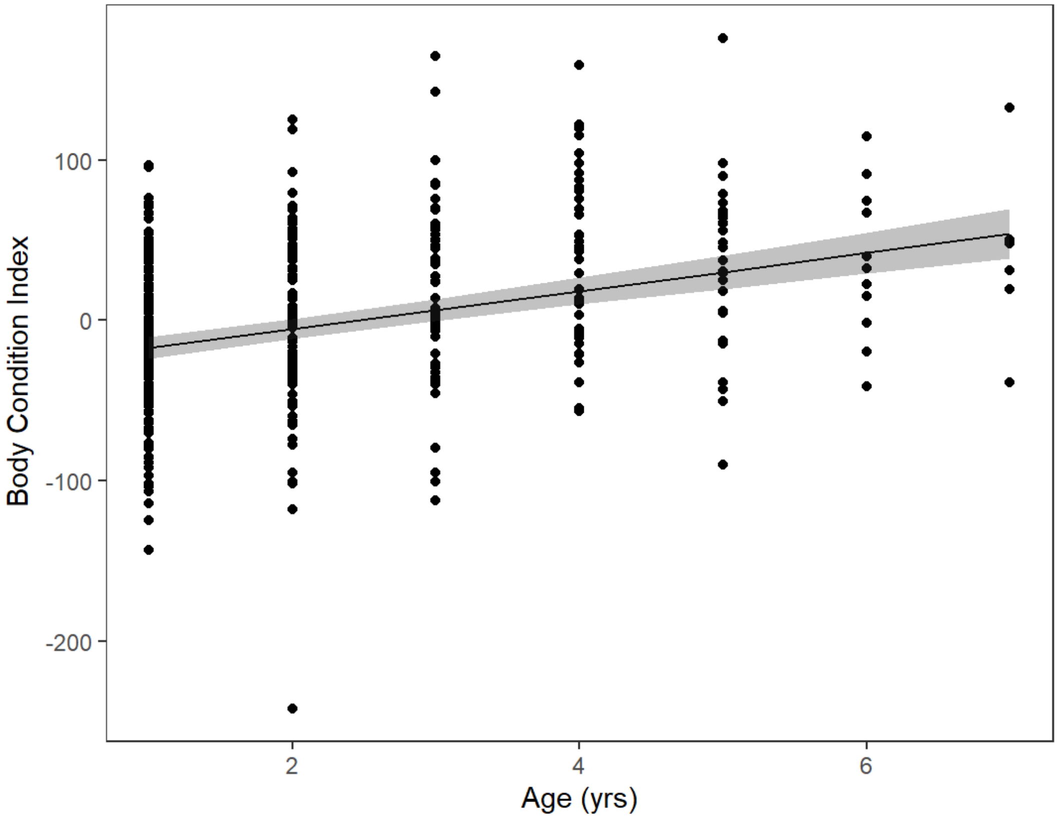

Figure 5. The influence of male Cape ground squirrel age (yrs) on their relative body condition. A body condition index was obtained from the residuals of a body mass on spine length linear regression. Body mass and spine length were averaged for individuals assessed more than once during a trapping season resulting in 203 averages and 226 single observations (429 observations from 237 males) for the years 2013-2024, excluding the year 2020. The shaded area represents the 95% confidence interval.

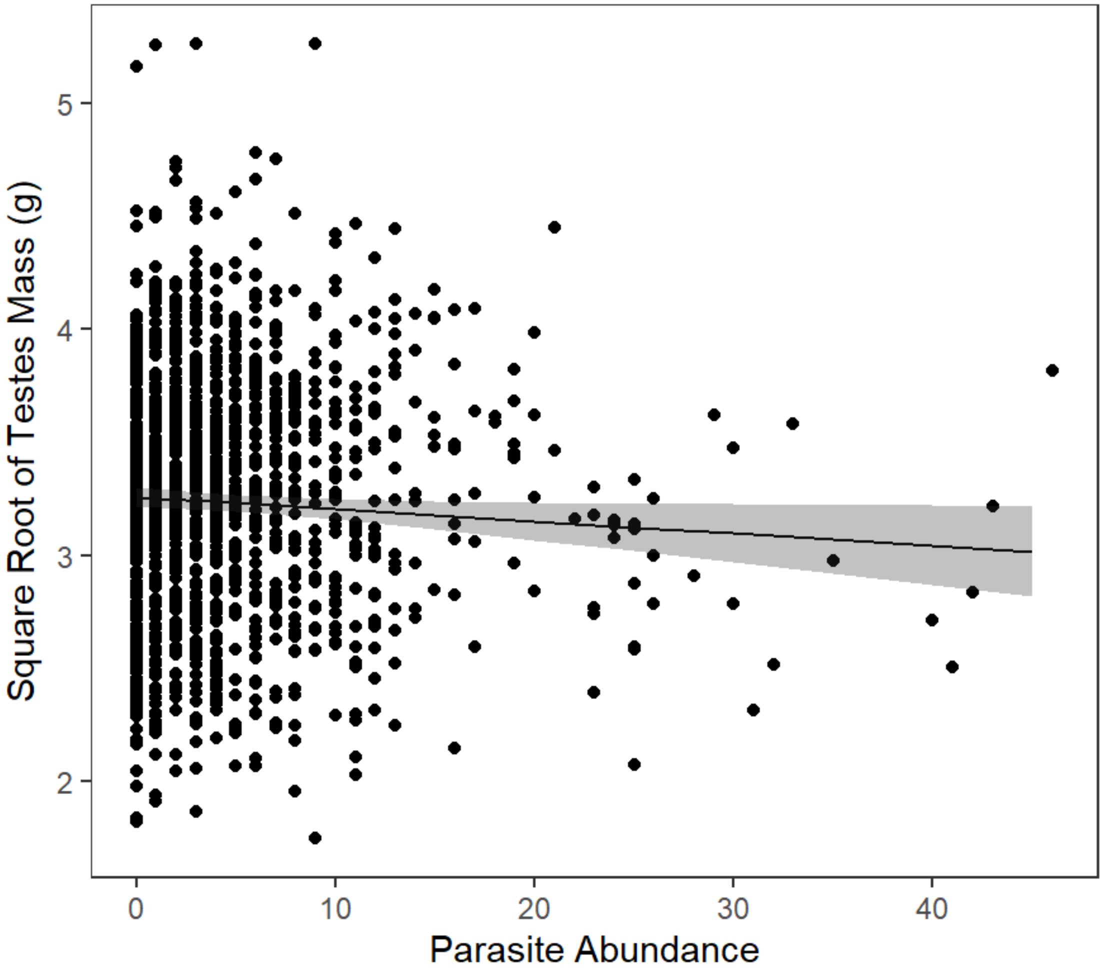

Figure 6. The relationship between parasite abundance and testes mass (g) of the Cape ground squirrel. Males were assessed between the years 2013-2024, excluding the year 2020 yielding 1391 observations from 537 males over 11 years. A square root transformation was applied to testes mass to meet the normality assumption of linear regression. The shaded area represents the 95% confidence interval.

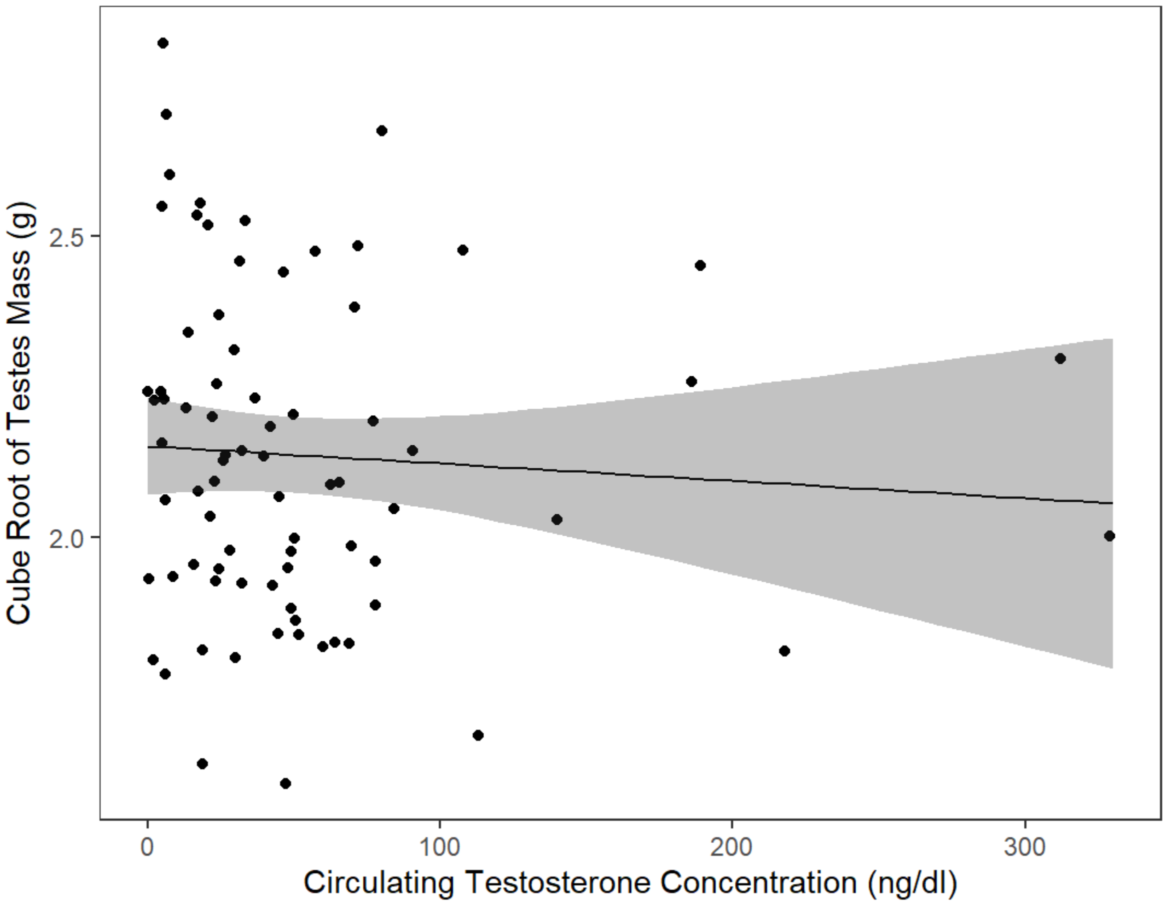

Figure 7. The effect of circulating testosterone concentration (ng/dl) on the average testes mass (g) of the Cape ground squirrel. Testes mass was averaged for individuals assessed more than once during a trapping season for a total of 32 averages and 45 single measures (77 observations from 71 males) for the years 2013, 2015, 2021, and 2023. Testosterone concentration was estimated from blood samples collected from the femoral artery. The shaded area represents the 95% confidence interval.