The effects of rainfall and sociality on host-parasite dynamics in the common mole-rat, *Cryptomys hottentotus hottentotus* (Lesson 1812)

by

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Declaration

I, Elizabeth Archer, declare that the thesis, which I hereby submit for the degree of MSc Zoology at the University of Pretoria, is my own work and has not been previously submitted by me for a degree at this or any other tertiary institution.

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Summary

There are many factors which may influence the distribution of parasites and often parasites are not evenly distributed amongst their hosts. The development and survival of ectoparasites is usually dependent on abiotic factors such as temperature and rainfall. Therefore differences in ectoparasite load between host populations and seasonal fluctuations in abundance are often found to be associated with climatic variations. However, biotic (host-related) factors have also been found to play a role in these differential infection rates. Host social aggregations during the breeding season have been shown to enhance horizontal transfer of ectoparasites due to increased host density. As a consequence, the question arises as to whether an increase in parasitism comes as a cost to social species. Recently, a number of studies have started to assess whether particular individuals within social groups tend to suffer higher parasitic burdens as a consequence of their social status. Some of these studies have employed the analysis of testosterone and glucocorticoid concentrations in the host in relation to these factors. Both of these steroid hormones may have immunosuppressive effects when their circulating concentrations increase. Additionally, each can vary with the seasons and the social environment. To date universal trends have not been reported for either abiotic or biotic factors in association with parasite load. Therefore, this study aimed to provide a comprehensive report of the ectoparasitic fauna associated with the social, subterranean rodent *Cryptomys hottentotus hottentotus.* Differences in parasite prevalence and abundance were analysed across two habitats at the extremes of a rainfall gradient within which the host species is found. Once the inventory had been compiled, the impact of abiotic and biotic factors was assessed across the two populations to determine which was the most influential on parasite dynamics. The study also attempted to assess whether parasitism was costly to sociality and if certain members of the colony suffered greater parasitism due to their social status. Testosterone and glucocorticoid concentrations were also measured and compared against factors such as season, breeding status and parasitic infection. The ectoparasite communities were very similar between populations, inferring that the subterranean environment has led to specialisation of ectoparasites on *C. h. hottentotus.* The confinement of being underground also appears to have made the ectoparasite community more dependent on the host. Seasonal increases in parasite prevalence and abundance are thought to be linked to increased host dispersal and social interactions that occur during peak

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rainfall. However, there were distinct differences in parasite load between locations. Additionally, few common patterns were found between separate parasite taxa. Group size either did not influence or reduced mite abundance, suggesting sociality is beneficial to the host. However, differences between hosts of different social status were more difficult to assess, particularly as this may differ between locations. It is unlikely that any differences in parasite burden observed between individuals are a direct result of immunosuppression from steroid hormones. In conclusion there are many factors which may influence the host-parasite relationship simultaneously and therefore generate unique circumstances within each host population.

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General Introduction

Parasites present a challenge to the fitness of their host and they can be detrimental to both their survival (Combes 2001) and reproductive output (Hillegass *et al.* 2010; Gooderham & Schulte-Hostedde 2011). Due to their ubiquitous distribution, parasites can infest all free-living animals which in turn have to adapt to deal with these invasions (Combes 2001; Roberts & Janovy Jr. 2005). Macroparasites such as ectoparasites are often unevenly distributed amongst hosts with a few individuals suffering from much higher parasite burdens than others, even within the same host population (Shaw & Dobson 1995; Rózsa *et al*. 2000; Wilson *et al*. 2001). Furthermore, parasite populations tend to fluctuate both spatially and temporally, exhibiting seasonal peaks and troughs (Wilson *et al.* 2001). Consequently, a large number of studies have been carried out to assess why some hosts suffer higher parasite loads than others. From these studies, it has been deduced that a significant element of differences in parasitic infections is the level of host exposure (Poulin 2007; Bordes *et al*. 2010).

An initial point of consideration is the number of parasites within a habitat that could infect a host. The abundance and prevalence of ectoparasites on a specific host species tends to be heavily influenced by the abiotic factors associated with the habitat because of the exposure these parasites have to external influences (Korallo-Vinarskaya *et al*. 2009). Variations in ectoparasite burdens between populations of the same host, and the overall range within which a parasite species is present, has been found to be linked to differences in temperature, humidity and rainfall (Fabiyi 1996; Krasnov *et al*. 1997, 2008; Vinarski *et al*. 2007; Korallo-Vinarskaya *et al*. 2009). Accordingly temporal variations in parasite infection are attributed to seasonal fluctuations in weather conditions (Merino & Potti 1996). Variations in ectoparasitic arthropod abundance are thought mostly to be caused by the impact that abiotic factors can impose on the development and survival of arthropods (Marshall 1981). Raised temperatures, for instance, tend to increase production of eggs and development of pre-adult phases and can lead to increased ectoparasite abundances (Lehane 2005; Krasnov 2008). However, the impact of the environment differs among parasites depending upon their life history strategies (Bordes *et al*. 2010). In particular, species that spend more time off the host, such as ticks and fleas, tend to be more susceptible to changes in the surrounding environment (Krasnov *et al*. 2011). Potential hosts would be less likely

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to be infected by ticks when rainfall is low as questing ticks are more liable to increased desiccation (Horak *et al*. 1995). Yet, abiotic factors are less likely to affect the transmission of lice in a similar fashion, as the entire lifecycle is completed on the host (Singh *et al*. 2009). Different ectoparasitic species can have different tolerances to external conditions (Lehane 2005), which can produce varying patterns in parasite dynamics. Studies on the influence of rainfall (and humidity) have produced extremely varied results amongst parasite species, perhaps as a result of differences in adaptation across ectoparasite taxa (Krasnov *et al*. 2008). Within a host population, different ectoparasite taxa (i.e. ticks, lice or mites) may reach peak abundances at different times of the year depending on their preference to various weather conditions (Merino & Potti 1996). One example that illustrates such differences is a study on striped-mice (*Rhabdomys pumilo*) in South Africa. Fleas, mites and lice were more prevalent and abundant on the host in the wet winter season, whereas ticks displayed opposing preferences (Matthee *et al*. 2007). Even at lower taxonomic levels there may not be a general trend; studies investigating gamasid mites across various small mammal hosts have not shown common patterns between precipitation and mite abundance (Krasnov *et al*. 2008; Linardi & Krasnov 2013). Furthermore, rainfall does not appear to have a general influence on arthropod development such as that seen with temperature (Marshall 1981; Krasnov 2008). As a result, no common pattern of ectoparasite prevalence and abundance and the amount rainfall has been presented thus far.

In addition to abiotic effects, biotic (host-related) factors can affect parasite burden amongst host individuals, though none of these appear to be universal for host-parasite relationships (Poulin 2007). Behavioural differences between individuals in a host population can result in individual variation in parasite exposure (Wilson *et al*. 2001). One of the few biotic factors that has been shown to display a common trend in vertebrate hosts is that males tend to have much higher ectoparasite loads (Schalk & Forbes 1997). Several factors may cause this. Sexual size dimorphism, which is common in many species, may result in males being larger than females. It has been proposed that larger individuals can cope with higher parasite abundances and males in such species are found to harbour greater parasite loads (Moore & Wilson 2002; Harrison *et al*. 2010). Nevertheless behavioural differences may also impact infestations; males often have larger home ranges than females, which increases the chance of parasite exposure (Nunn *et al*. 2003; Lindenfors *et al*. 2007). Increased mobility within a home range can also increase exposure and potentially raise rates of horizontal transmission through more frequent social contact (Grear *et al*. 2009). Seasonal

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variation in exposure has been credited to changes in behaviour during the breeding season. Aggressive behaviour associated with intra-sexual competition, particularly in males, is said to contribute to horizontal transmission (Klein 2000). Territorial behaviour or mate guarding is also positively correlated with increased parasite loads (Mooring *et al*. 1996; Negro *et al*. 2010). In impala (*Aepyceros melampus*), an increase in tick load in territorial males was attributed to a distinct drop in grooming behaviour as a result of increased territorial behaviour (Mooring *et al*. 1996). Additionally, many free-living species aggregate during the breeding season to reproduce, and increased host density has been shown to have positive effects on transmission between individuals via increased proximity of hosts (Anderson & May 1979; Arneberg *et al*. 1998). The breeding season of the host has been shown to lead to an increase in ectoparasite burdens (Marshall 1981). This is illustrated by a study on bats, where increased density of host females during the breeding season led to one of the few published cases of host female-biased parasite infection (Christe *et al*. 2007).

Despite the clear indications provided in earlier studies of positive correlations between parasite infection and population density, there is much ambiguity surrounding the influence of social behaviour of gregarious species on ectoparasite numbers. It has been suggested that social behaviour can increase parasite exposure due to the increased density and proximity of hosts, thus increasing the chances for horizontal transmission (Alexander 1974). There are studies that support Alexander’s claim (Ezenwa 2004; Godfrey *et al*. 2009) and there is evidence of larger social groups suffering higher parasite loads (Freeland 1979). However, results have also been found to differ between different ectoparasite taxa infecting the same host (Monello & Gompper 2010). In two studies involving a meta-analysis there has been an overall positive correlation between group size and parasite load or risk of infection, although differences between taxa have been attributed to the form of transmission utilised by the parasite (Cote & Poulin 1995; Rifkin *et al*. 2012). Both studies found that parasites transmitted by close contact had positive correlations with group size. Although the prevalence and intensity of parasites that actively search out hosts had a negative correlation to group size in one study (Cote & Poulin 1995), no pattern was found between group size and parasite risk in the other (Rifkin *et al*. 2012). Conversely there is also evidence that suggests parasitism is not a cost of sociality, but sociality may be beneficial (Snaith *et al*. 2008; Viljoen *et al*. 2011; Burger *et al*. 2012). Results from a study into gregarious invertebrates suggests that the isolation of social groups from others in the

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population may be detrimental to parasite abundances, leading to larger groups having fewer parasites as opposed to increased numbers seen in other studies (Wilson *et al*. 2003). The pressure of parasitism associated with social groups could have also selected for the evolution of anti-parasite behaviour such as allogrooming (Loehle 1995; Bordes *et al*. 2007). In addition to the mixed positive and negative correlations, some studies indicate no correlation between social group size and parasite infection (Arnold & Lichtenstein 1993; Hillegass *et al*. 2008).

Overall there is no definitive conclusion as to whether larger social groups suffer higher parasite burdens. It has also been found that aggressive territorial males have higher parasite loads (Mooring *et al*. 1996; Negro *et al*. 2010), and individuals of higher status in social groups have higher parasite burdens, meaning it is possible that maintaining this position could be costly in terms of parasite infection (Muehlenbein & Watts 2010). Aggression is often utilised to maintain higher social rank (e.g. breeding status in species with reproductive skew or dominant individuals in a hierarchy; Creel *et al*. 1993; Clarke & Faulkes 1997) and this could increase the chances for parasite transmission and therefore parasite exposure (Klein 2000). It is posited that with more energy and time devoted to aggressive behaviour an individual is less likely to self-groom (Mooring *et al*. 1996). Nevertheless, there are situations whereby social status has no correlation to parasite infection (Clough *et al*. 2010; Benavides *et al*. 2012), or a negative relationship exists between the two (Fugazzola & Stancampiano 2012). Although self-grooming may be reduced by aggressive behaviour, allogrooming is often most frequently carried out on higher ranking aggressive individuals to aid maintenance of social stability (Madden & Clutton-Brock 2009; Newton-Fisher & Lee 2011). A bias in allogrooming recipients has been shown to reduce tick loads in dominant individuals in comparison to their lower ranking counterparts in troops of baboons (*Papio cynocephalus*; Akinyi *et al*. 2013). Therefore there is no conclusive evidence to suggest that maintenance of high social status increases parasite burden.

Ectoparasites and host physiology

Though differences in parasite exposure play a major role in the variation in infection between individuals, another key factor that needs to be taken into account is the susceptibility of the host to parasites (Wilson *et al*. 2001). Trade-offs between physiological

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demands (e.g. immune system, reproductive system) are constantly occurring within a host due to the limited amount of resources an individual possesses (Stearns 1989). Parasites create additional burdens for the host by increasing energetic costs (Scantlebury *et al*. 2010). Therefore hosts must adapt in order to maintain fitness (Combes 2001). However, there are times when host susceptibility to parasitic infection increases (Wilson *et al*. 2001; Møller *et al*. 2003). For example, seasonal fluctuations in parasite abundance can be due to increased energy being redirected to reproduction, thus immune function may decrease (Møller *et al*. 2003; Martin 2009). Female immune function can be reduced during gestation and lactation due to the large energy requirements associated with these reproductive processes (Altizer *et al*. 2006). This has been demonstrated clearly in studies on bats, where the individuals with the highest ectoparasite loads were females that were pregnant or lactating (Pearce & O'Shea 2007; Sundari *et al*. 2012). Seasonal variation in males on the other hand is often connected to circulating testosterone. In addition to its role in male physiological sexual development, circulating testosterone concentration rises during breeding to mediate secondary sexual characteristics for mate display and social aggressive behaviour (Roberts *et al*. 2004). However this androgen also can act as an immunosuppressant (Klein 2000). Based on its immunosuppressant properties, it is predicted that testosterone concentrations would be positively correlated to parasite burden. Male bias in parasite load connected to testosterone concentrations has been shown in a few vertebrate classes (Folstad & Karter 1992; Klein 2000; Hoby *et al*. 2006). Further studies in male small mammals and lizards with testosterone implants have indicated that elevated testosterone levels may result in increased parasite loads (Salvador *et al*. 1995; Hughes & Randolph 2001). However, as with correlations between parasite load and other factors, relationships with different parasite taxa are not always the same (Hoby *et al*. 2006; Fuxjager *et al*. 2011; Pollock *et al*. 2012). Further, negative correlations between testosterone and parasite infection are reported in the literature (Pena *et al*. 2007; Ezenwa *et al*. 2012; Lutermann *et al*. 2012). It has been suggested that parasites may reduce the reproductive functionality of their hosts (Morales *et al*. 1996). Alternatively the hosts may be forced to increase immune function to respond to infection by reducing reproductive efforts (trade-off), thus lowering testosterone concentrations (Lutermann *et al*. 2012).

The seasonal peak in testosterone during the breeding season is also known to mediate social aggressive behaviour during reproductive competition (Wingfield *et al*. 1990; Hirschenhauser & Oliveira 2006). For instance, male lizards (*Psammodromus algirus*) with

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testosterone implants have been shown to behave more aggressively during the reproductive period (Salvador *et al.* 1995). Furthermore territorial male fur seals (*Arctocephalus forsteri*) displayed increased aggression rates correlated to testosterone concentrations (Negro *et al.* 2010). Though most of the studies involving seasonal aggression are confined to males, it has been demonstrated in several social vertebrates that testosterone is higher in both males and females of higher social status (Creel *et al.* 1993; Clarke & Faulkes 1997; Shargal *et al.* 2008; Muehlenbein & Watts 2010; van Kesteren *et al.* 2012; Lutermann *et al.* 2013). Yet there are studies on males from some species that indicate that this is not always the case (Ostner *et al.* 2008). Increased aggression could increase parasite exposure (Klein 2000). Both the study on lizards and fur seals revealed that males with higher testosterone also had higher parasite loads (Salvador *et al.* 1995; Negro *et al.* 2010). However, the topic of testosterone, social status and parasite loads is not well documented. A study on male chimpanzees (*Pan troglodytes*) found that both testosterone and social dominance rank were positively correlated to parasite species richness (Muehlenbein & Watts 2010). On the other hand there have been papers on other primates that showed no link between testosterone and social status, and no correlation between status and parasite infection (Clough *et al.* 2010; Benavides *et al.* 2012). One of these studies did indicate a negative correlation between parasite load and testosterone associated with seasonal differences (Clough *et al.* 2010). Although work is starting to emerge that demonstrates testosterone in higher status females is greater than those of lower rank, virtually no work has been done on how this links to female parasite load.

Interestingly glucocorticoids (cortisol and corticosterone), which are associated with physiological preservation of allostasis (the ability to maintain stability through change; McEwen 1998), can show similar responses to some factors as those seen with testosterone concentrations. Circulating glucocorticoid concentrations increase in response to ‘stressors’ such as predation or low food availability. Increased levels of glucocorticoids can aid the release of stored energy and enable the individual to react appropriately to survive (Sapolsky *et al.* 2000; Romero 2004). Raised glucocorticoid levels generally do not last for extended periods of time, though extended elevation of glucocorticoids can be detrimental to the individual (Sapolsky *et al.* 2000; Romero 2004). However, seasonal baseline fluctuations in glucocorticoids have been found in vertebrates for the majority of species studied and the peak is most commonly associated with the breeding season (Romero 2002). As with testosterone, glucocorticoids may be immunosuppressive (Sapolsky *et al.*

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2000). Further it has been suggested that parasites may act as chronic stressors and are therefore likely to elevate glucocorticoid levels (Frandsen 1987). Experimental manipulations of parasite exposure or infection have led to increased glucocorticoids in vertebrates (Fleming 1997; Bowers *et al*. 2000; Lobato *et al*. 2008). Yet there appears to be just as many studies in the literature that indicate that parasitic infection may have little impact on glucocorticoid levels (Frandsen 1987; Grutter & Pankhurst 2000; Monello *et al*. 2010). Although it is not certain exactly how glucocorticoids play a role here, immune function is known to increase in response to acute stressors before decreasing once a stressor becomes chronic (Martin 2009). This could account for differences found between experimental studies in the literature. Natural infections could therefore be considered chronic stressors. In wild social primates, there have been several studies that show positive correlations between gastrointestinal parasite infection and cortisol levels (Clough *et al*. 2010; Muehlenbein & Watts 2010; Setchell *et al*. 2010), supporting the theory that natural parasite infections may be a chronic stressor to their host. In a study on red-fronted lemurs (*Eulemur fulvus rufus*), peak nematode infection occurred just following peak cortisol levels which were reached during the breeding season (Clough *et al*. 2010). However, parasite responses to glucocorticoids differ in studies involving multiple parasite taxa (Hoby *et al*. 2006; Fuxjager *et al*. 2011). Moreover, other studies show no correlation between glucocorticoid levels and natural parasitic infection (Pena *et al*. 2007; Eggert *et al*. 2010; Lutermann *et al*. 2012). Alternatively, it has been found that parasites only became significantly linked to glucocorticoids when levels were already raised due to other chronic stressors (Quillfeldt *et al*. 2004; Pedersen & Greives 2008).

Though glucocorticoids are not as clearly linked to aggression as testosterone is (Hanley & Stamps 2002; Creel 2005), they have been shown to be correlated with social status (Sapolsky 2005). Some studies indicate that glucocorticoids concentrations are greater in individuals of higher social status (Clarke & Faulkes 1997; Creel 2005; van Kesteren *et al*. 2012), whereas the opposite has been shown in others (Sapolsky 2005). Studies in primates have indicated that glucocorticoid levels do not differ according to an individual's social status (Ostner *et al*. 2008; Clough *et al*. 2010) and that patterns may change relative to social stability (Setchell *et al*. 2010). It has been inferred that glucocorticoids will be higher in certain individuals depending on social status only if the costs of maintaining allostasis are higher in either higher or lower status individuals (Goymann & Wingfield 2004). Therefore it appears that the impact of social status on 'stress' hormones may vary between

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species and even temporally within a social group. Studies that have analysed the impact of social status on glucocorticoid levels in the context of parasites in wild populations are extremely scarce. Cortisol levels have been positively linked to parasite infection in primates where social rank is only correlated to cortisol concentrations during times of social instability (Setchell *et al*. 2010). Elsewhere parasites have been positively correlated to cortisol levels where there is no influence of social status (Clough *et al*. 2010; Muehlenbein & Watts 2010). Thus it remains unclear whether the influence of parasitic infection could vary amongst members of social groups depending on variation in host glucocorticoid levels pertaining to social status.

Study animal

The common mole-rat, *Cryptomys hottentotus hottentotus* (Lesson 1812), belongs to one of the six genera within the Bathyergidae (Rodentia: Hystricomorpha) family (Kock *et al*. 2006). This is a particularly interesting family of rodents which are endemic to Africa and exhibit an array of social structures, from completely solitary species through to social colonies and the only two eusocial mammal species in the world (Bennett & Faulkes 2000). All of these animals are subterranean and the majority never come above ground. As herbivorous species, the bathyergids generally feed upon underground geophytes that provide them with water in addition to energy and nutrients (Bennett & Faulkes 2000). Whilst foraging for food resources the mole-rats create a burrow system, within which they are protected from extreme weather conditions and most potential predators (Thomas *et al*. 2012). *C. h. hottentotus* occurs in colonies of between 2-14 animals with each colony containing between one and two breeding males and a single reproductive female (Spinks *et al*. 1999; Bishop *et al*. 2004). Many of the non-breeding members of the colony are offspring of the breeding individuals, but immigrants may also be present in colonies (Bishop *et al*. 2004). *C. h. hottentotus* is one of two social mole-rat species that are known to breed seasonally (Janse van Rensburg *et al*. 2002). The breeding season lasts from September through to November (southern hemisphere late winter/early spring) (Spinks *et al*. 1997, 1999). Although there are a few individuals that physically reproduce, all adult members of the colony are physiologically able to reproduce throughout the year. Reproductive skew is likely to be partially maintained through inbreeding avoidance. In addition, breeding animals, in particular the breeding females, may maintain the dominant

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position within the colony through aggressive behaviour (Spinks *et al*. 1997, 1999). Due to the subterranean environment, most non-breeders are only able to disperse from the natal colony during times of high rainfall (Spinks *et al*. 1997). *C. h. hottentotus* has a widespread distribution and is found throughout the western and central parts of South Africa, from the south western coast of the Western Cape to the most northerly part of the border in the Northern Cape of South Africa (Bennett & Faulkes 2000). This broad distribution enables the study to focus on two populations at different extremes of a rainfall gradient along the west coast. *C. h. hottentotus* in the more southern mesic areas is much more densely distributed and thus dispersal and immigration is a more frequent occurrence (Spinks *et al*. 1998). Furthermore geophytes are much more uniformly distributed, unlike the clumped and widely spread food resources found in more arid areas further north (Spinks *et al*. 2000). As the habitats along the west coast of South Africa are winter rainfall areas, breeding for populations of common mole-rats in this area occur subsequent to peak rainfall. It is during the time of highest precipitation that they are most likely to disperse (Spinks *et al*. 1999).

Previous work on Bathyergidae and overall aim of study

The ectoparasites that infect *C. h. hottentotus* are not well documented. Although previous research has identified some ectoparasite species (De Graaff 1964; 1981), no work has been carried out to look at the parasite community dynamics. Indeed few parasite studies have been carried out on other host species in the family Bathyergidae, though results from previous work indicates that ecto- and endoparasites vary seasonally in solitary and social species (Viljoen *et al*. 2011; Lutermann & Bennett 2012). Seasonal fluctuations of parasites have not yet been linked to a specific seasonal factor in mole-rat species. There is also evidence from a very closely related species (*C. h. pretoriae*) showing that group size is an important determinant of ectoparasite abundance, although this relationship was negative (Viljoen *et al*. 2011). Furthermore, the specific colony identity was significantly linked to ectoparasite abundances, inferring a level of isolation between colonies influencing parasite population dynamics. However, there appears to be no significant difference in parasite load between individuals with respect to sex, or between breeding and non-breeding host individuals (Viljoen *et al*. 2011). A further study also analysed the influence of natural parasite and simulated infections on *C. h. pretoriae.* The results suggested that these hosts

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are able to adapt their physiology in order to deal with parasite infections, but further infection may cause a noticeable increase in physiological stress (Lutermann *et al*. 2012). However, this study did not look at the differences between the host sex or the breeding status of individuals, so it is unknown if a difference occurs between different members in a colony.

The wide range of habitat and seasonal fluctuations in climatic factors (e.g. rainfall and temperature) associated with its habitat makes *C. h. hottentotus* a good study animal to assess how abiotic factors can influence ectoparasite dynamics. Moreover, it provided a clearer picture as to whether biotic or abiotic factors have a more significant impact on fluctuation in ectoparasite populations. The social behaviour of the host also allowed further insight into whether parasitism is a cost to social groups as a whole. Despite seasonal breeding patterns, *C. h. hottentotus* maintain the physiological ability to breed year-round, regardless of social status (Spinks *et al*. 1997, 1999, 2000a). Additionally, the social status of ‘breeder’ and ‘non-breeder’ is mostly maintained throughout the year (Bennett & Faulkes 2000). It was therefore possible to tease apart the variation in testosterone and cortisol levels between breeders and non-breeders and see how this then varied in relation to seasonal changes associated with social interactions prior to the reproductive period and parasite infection. Therefore it was possible to infer whether parasites may impact individuals in a social group differently depending on their social (in this case, breeding) status.

Thesis chapters

Each chapter presented in this thesis was aimed at analysing a different aspect of the host-parasite relationship. Chapter 1 investigates the ectoparasite community associated with *C. h. hottentotus*. Much of the literature that identifies parasites infecting the common mole-rat is relatively old, thus comparisons were made both with previous work and on a recent study on a closely related species. The aim of this chapter was to provide the most extensive review of ectoparasites of a member of the Bathyergidae. The analysis included looking at different habitats across the distributional range of *C. h. hottentotus*, and at seasonal variations within these habitats. Once a general picture of the ectoparasite community had been assessed, the aim was to investigate which factors influenced ectoparasite population dynamics. In Chapter 2, the focus was directed at the influence of

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abiotic (i.e. rainfall and temperature) and biotic factors (host sex, breeding status, body mass and colony size) on infestation. Chapter 3 investigated the host-parasite relationship in relation to host physiology. The impact of ectoparasite infection on host testosterone and cortisol concentrations was analysed. In addition to parasite burdens the effect of season and biotic factors considered in Chapter 2 were included in the analyses. Overall, this thesis provides a description of the parasite community infecting *C. h. hottentotus.* Additionally it investigates how parasite loads change with climate, and investigates why specific individuals of *C. h. hottentotus* suffer from higher parasitism than others and how this may impact the host.

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Chapter 1

Ectoparasite burdens of the common mole-rat (*Cryptomys hottentotus hottentotus*) from the Western and Northern Cape Provinces of South Africa

Abstract

The African mole-rat family (Bathyergidae) are subterranean rodents that display a range of sociality from large colonies of eusocial species to completely solitary species. The common mole-rat (*Cryptomys hottentotus hottentotus*) is a species of one of the social genera and has a relatively wide distribution compared to other members of this rodent family. Despite the large amount of research that has been carried out on *C. h. hottentotus*, very little is known about the associated ectoparasites. Recent taxonomic revisions surrounding certain *Cryptomys* spp., including the common mole-rat, make it unclear from the earlier literature which ectoparasites infect *C. h. hottentotus.* This study looked at 268 common mole-rats from two sites from the edges of their distributional range in winter and summer in an attempt to get an overall picture of the ectoparasite community that infects *C. h. hottentotus* and to look at certain aspects of the parasite distribution. Three mite species (*Androlaelaps scapularis, A. capensis* and *Radfordia ensifera*) were found to regularly infect this bathyergid with one flea (*Cryptopsylla ingrami*) and one louse species (*Eulinognathus hilli*) that are both possibly specialised to *C. h. hottentotus.* The results indicate that all of the parasite populations had aggregated distributions amongst the individual hosts and that they had uneven sex ratios except in the case of the lice. Clear seasonal patterns were found across all taxa, with each species being more prevalent and abundant on the host in the rainy winter season. Further study would be needed to determine whether this is due to abiotic factors such as increased rainfall or changes in host behaviour due to dispersal prior to the breeding season.

A revision of this chapter has been submitted to The Journal of Parasitology.

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Introduction

There are vast arrays of parasitic species spread across the five kingdoms, from prokaryotic bacteria, through fungi and protists, up to plants and animals (Roberts & Janovy Jr. 2005). Each has adapted in its own way to infecting the necessary hosts whether they live inside (endoparasites) or outside (ectoparasites) the host (Combes 2001; Roberts & Janovy Jr. 2005). Due to the degree of specialisation in some parasites, wild populations of hosts are often infected with more than one species and may harbour a community of parasitic organisms (Roberts & Janovy Jr. 2005). The spread of the parasite community across a population of hosts is influenced by many factors and it differs with different forms of parasites (Combes 2001; Wilson *et al*., 2001; Roberts & Janovy Jr. 2005). Macroparasite species (large parasites that generally do not multiply within their definitive host, Combes 2001; Roberts & Janovy Jr. 2005), tend to have aggregated population structures, with only a few individuals within the host population carrying the majority of the parasites (Rózsa *et al*. 2000; Roberts & Janovy Jr. 2005; Poulin 2007). Reasons for this heterogeneity can be influenced by various factors; including host immune function (Wilson *et al*. 2001), host sex (Schalk & Forbes 1997) and host behaviour (Wilson *et al*. 2001). There is also a degree of seasonal variation with both endo- and ectoparasites (Merino & Potti 1996; Wilson *et al*. 2001; Altizer *et al*. 2006). however, the influences of the environment on parasite populations tends to be more pronounced in ectoparasites and ectoparasite assemblages can differ between habitats (Krasnov *et al*. 1998, 2006).

The impact of abiotic factors such as temperature, rainfall or humidity on ectoparasite communities often varies with the different parasite taxa (Marshall 1981; Wilson *et al*. 2001). Parasites that have developmental stages that live off the host are particularly susceptible to effects associated with the surrounding habitat (Krasnov *et al*. 2011) However, there does not appear to be a set pattern for any specific taxon (Bordes *et al*. 2010). For example, several studies have also shown that lower levels of humidity can reduce the abundances for fleas, ticks and lice (Cooke & Skewes 1988; Basu & Bandyopadhyay 2004) however, the opposite has also been found for lice (Fabiyi 1996). Gamasid mites on the other hand are known to be largely influenced by the surrounding environment (Korallo-Vinarskaya *et al*. 2009). In particular their abundances are closely correlated to temperature and rainfall patterns although reactions can vary from species to species (Krasnov *et al*. 2008). Seasonal changes in parasite abundances are not always

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thought to be a direct result of abiotic fluctuations; seasonal fluctuations of abiotic factors lead to alterations in the behaviour of host populations (Wilson *et al*. 2001). The breeding season of host species can lead to increased population densities that enhance potential for horizontal transmission of parasites. Furthermore, changes in hormonal balances of the host during the reproductive period can alter immune function and therefore susceptibility to parasitic infection (Schalk & Forbes 1997; McCurdy *et al*. 1998; Wilson *et al.* 2001). Since the breeding season for many host species coincides with times of higher resource availability, it makes sense that many ectoparasites align their reproductive period with that of their hosts when resources may be much more available to them (Marshall 1981; Christe *et al*. 2000).

The members of the African mole-rat family (Bathyergidae) are subterranean rodents of the Hystricomorpha that display a range of social systems, from solitary living species through to social and even eusocial species (Bennett & Faulkes 2000). Since the naked mole-rat, *Heterocephalus glaber* was described as the first eusocial mammal (Jarvis 1981) much research has been conducted upon various bathyergid species (Bennett & Faulkes 2000). One such species that has been extensively studied is the common mole-rat, *Cryptomys hottentotus hottentotus*, which is known to be relatively geographically widespread compared to other members of the family (Bennett & Faulkes 2000; Spinks *et al.* 2000). Whilst extensive data has been collected on the reproduction (Spinks *et al*. 1997, 1999, 2000a), foraging behaviour (Davies & Jarvis 1986; Spinks *et al*. 1999a; Robb *et al*. 2012) and genetics (Bishop *et al*. 2004, 2007) of *C. h. hottentotus*, studies regarding its parasite fauna are scarce (De Graaff 1964).

Much of the work that has been done on the parasitology of African mole-rats was carried out decades ago and quite often there were few specimens of the parasite species found (De Graaff 1964). To make matters more complicated, the taxonomy and classification of the family Bathyergidae has been revised repeatedly since this earlier work. For example, the genus *Cryptomys* has been split into two genera (*Cryptomys* and *Fukomys*, Faulkes *et al*. 1997, 2004; Van Daele *et al*. 2007; but see Ingram *et al*. 2004 for a differing opinion regarding taxonomic names). Additionally, the species *Cryptomys hottentotus* has been split into several subspecies (De Graaff 1981; Bennett & Faulkes 2000), many of which are thought to have sufficient genetic differences to be classed as separate species (Faulkes *et al*. 1997, 2004; Bennett & Faulkes 2000), and may yet still change their scientific names.

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Thus it is not always certain which host species was referred to when earlier work discusses '*Cryptomys hottentotus*'.

Some of the previous work on this rodent family has recovered no ectoparasites (Shortridge 1934; Scharff *et al*. 1997). In contrast, other studies have found several ectoparasite species on bathyergid hosts including mole-rats that were previously referred to as *C. hottentotus*, ranging from fleas, to mites and ticks, to anopluran lice (summarised in De Graaff 1964, 1981). Work carried out before 1980 suggests that mites are the most speciose ectoparasites found on *C. hottentotus*; three of these mite species previously found belong to the genus *Androlaelaps* (Mesostigmata: Laelapidae); *A. scapularis* (Bateman 1960), *A. capensis* (Bedford 1932), and *A. marshalli* (De Graaff 1964). Also part of the Laelapidae family, *Macronyssus bacoti* (De Graaff 1964, 1981), and *Myonyssoides capensis* of the Ascaidae family (Bedford 1932) have been reported from *C. hottentotus*. One of the myobiid (Prostigmata) fur mites, *Cryptomyobia rotundata*, has been collected from the pelage of *C. hottentotus* in addition to three trombiculid mites; *Gahrliepia nana, Acomatacarus polydiscum* and *Scoutedenichia crocidurae* (Zumpt 1961). One species of sucking louse, *Eulinognathus hilli* (Anoplura), has been collected from *C. hottentotus* (Bedford 1932) and it is possible that this is the type host for this ectoparasite (Ledger 1980). Only one tick species has been reported from any members of the Bathyergidae family, *Ixodes alluaudi* (Theiler 1962). Fleas (Siphonaptera) collected from *C. hottentotus* on the other hand appear to be almost as diverse as the mites with a total of eight species having been recovered (De Graaff 1981). These species are from three different siphonapteran families. Of the Hystrichopsyllidae, there are *Ctenophthalmus edwarsi, C. ansorgei* and *Dinopsylla zuluensis*, although the latter identification has been questioned (De Graaff 1964, 1981). Furthermore, four species from the family Pulicidae; *Procamopsylla creusae, Xenopsylla philoxera, X. piriei* and *X. georychi* have been reported. Finally there is one species from the family Chimaeropsyllidae, *Cryptopsylla ingrami*, which is thought to be specific to *C. hottentotus* (De Meillon *et al*. 1961; Zumpt 1966; De Graaff 1981).

The most recent study on bathyergid parasites was carried out by Viljoen *et al.* (2011) who reported mites and a louse species on colonies of the highveld mole-rat, *C. h. pretoriae*. This study appears to be the largest done on a specific species of mole-rat so far, having looked at 88 individuals. The louse (*Linognathus* sp.) was not very prevalent and was also the least abundant of the ectoparasites (Viljoen *et al*. 2011). The same three *Androlaelaps*

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species (*A. scapularis*, *A. capensis* and *A. marshalli*), that have previously been collected from *C. hottentotus* were also recorded from the highveld mole-rat with *A. scapularis* being the most prevalent and abundant (Viljoen *et al.* 2011). The aim of the current study was to document the composition and structure of the ectoparasite community of *C. h. hottentotus.* In order to account for potential differences in ectoparasite communities caused by geographical range and habitat differences, mole-rats were sampled from two sites at opposite ends of its distributional range along a rainfall gradient; Darling, Western Cape and Kamieskroon, Northern Cape. Furthermore, the host animals were caught in both summer and winter to account for seasonal differences in ectoparasite assemblage. Developmental stages and sex ratios of the parasite populations were documented and patterns in prevalence, abundance and aggregation were analysed for each species, particularly for seasonal differences.

Materials and Methods

Study sites and animal capture

This project was given ethical clearance by the University of Pretoria (AUCC number EC005-11) and permits were granted by Cape Nature Conservation, Western Cape, and Northern Cape and Gauteng Provincial Governments. Individuals of *C. h. hottentotus* were captured from two sites between February 2011 and August 2012, with 2-3 week trapping sessions occurring at each site. Trapping sessions were carried out in both summer (from January to March) and winter (from June to August) each year, resulting in a total of 22 weeks of field work. The first site, Pedroskloof Farm, was a semi-arid location 25km outside of Kamieskroon, Northern Cape in the Kamiesberg Mountains (30.13^{o}S, 17.57^{o}E). This area has an average rainfall of 284.5mm per annum with the majority of this falling in winter (average 2009-2012, SA Weather Service). The habitat has soft soil with a high sand content (Romañach 2005) and rocky outcrops. The fields in which animals were caught were used occasionally for grazing livestock. As part of the Namaqualand area there are also lots of dwarf shrub land plants, particularly small succulents typical of the succulent Karoo habitat (Milton *et al.* 1997). The second site, Waylands Farm, was situated approximately 440km south just outside of Darling, Western Cape (33.25^{o}S, 18.25^{o}E) in part of the lowland Renosterveld fynbos area (Hagenah & Bennett 2013). This more mesic area has an average rainfall of 411.6mm per annum with most of it also falling in winter

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(average 2009-2012, SA Weather Service). The farmland at this site is used for sheep and cattle grazing and tends to consist of a much harder clay-based soil although there are sandy areas (Romañach 2005; Hagenah & Bennett 2013), supporting grassland that mostly consisted of rye grass, *Lolium* spp. (Thomas *et al*. 2009).

The position of burrow systems was identified by the presence of mounds that mole-rats produce above ground to clear out their tunnels. Tunnels were uncovered by digging up these mounds and Hickman live traps (1979) baited with sweet potato were placed in the opening. To prevent trapping the same colony in more than one location, trap sites were kept at least 30m apart from one another. Once set, traps were checked just before dawn, then every 2-3 hours throughout the day with one last check after dark. Any animals caught were kept in plastic crates (41 x 28 x 25cm minimum) with air holes in the lid and at least a 3cm layer of soil. Members of the same colony were housed together and fed sweet potato daily. An entire colony was thought to be caught when there had been no activity at a trap site for three consecutive days after the last mole-rat had been trapped (Spinks *et al*. 1998).

Ectoparasite collection and identification

Individual hosts were euthanised with an overdose of halothane and washed thoroughly using 100ml of soapy water in a 22 x 7 x 5cm tub to remove any ectoparasites. Washing the host has been used for collecting ectoparasites from mole-rats previously (Viljoen *et al*. 2011). The washing process involved kneading through the fur with the fingertips all the way along and around the body length 5 times, before washing the head and the posterior end. Then individuals were moved back and forth through the water, first on their front and then on their back (10 times each side), in order to remove any ectoparasites that remained caught in the fur. This wash-water was then transferred to a labelled screw-top container until processed. The wash tub was then rinsed using a wash bottle and this water added to the sample. Each wash sample was analysed under a binocular dissection microscope. Using a 3ml Pasteur pipette, a small amount of the sample was placed into a petri dish at a time. Any ectoparasites found were placed into Eppendorf tubes containing 70% ethanol for storage. The petri dish was rinsed thoroughly between samples and the pipette was discarded to avoid contamination between samples. Mites, fleas and lice were retrieved from various individuals and initial counts were made for each. No ticks were found in any of the samples.

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Mites were mounted for identification following Krantz and Walter (2009). Each was placed in a cavity slide with 85% lactic acid and held over a spirit burner until it became transparent. A small drop of polyvinyl alcohol (PVA) was then added to the slide for each mite. The mites were placed into the PVA with either the dorsal or the ventral side facing up, so that a coverslip could be placed over it. The coverslip was placed gently over the sample and pressed down slightly before the slide was left to dry. Once dry, the coverslips were ringed with clear nail varnish and put into an oven set between 30^{o}C and 40^{o}C for at least 48 hours. Fleas and lice were not permanently mounted for identification. Each specimen was cleared sufficiently to see the reproductive organs by being placed in 15% KOH solution for a minimum of 20 hours. Once ready the specimen was washed in tap water and placed in 10% acetic acid for 3 minutes to remove any remaining KOH, before being washed 3 times in distilled water. These were then stored in 70% ethanol until identified and sexed. For identification, each specimen was placed temporarily on a slide by pipetting it onto the slide with a few drops of 70% ethanol and placing a cover slip over it. Ectoparasites were identified with the use of identifications handbooks and keys (Till 1963; Ledger 1980; Segerman 1995; Baker 1999). The mounting and identification process also made it possible to corroborate the initial counts made during the collection and to determine the proportions of the developmental stages (larvae, nymphs, male and female adults) collected for each parasite species.

Statistical analyses

There are various ways of measuring how parasite populations are spread throughout a host population. One of the most commonly used measures is the prevalence, which is defined as the proportion of hosts in a population infected by a specific parasite species (Bush *et al*. 1997; Rózsa *et al*. 2000). This statistic is usually displayed alongside average abundance or average intensity; the former is the average number of a specific parasite that infects an individual from a host population, whereas the latter is the average number of a parasite species infecting an individual of the infected members of a host population (Bush *et al.* 1997; Rózsa *et al.* 2000). The total number of parasite species infecting each individual mole-rat was calculated. Calculations were made for prevalence, mean abundance and median intensity. Although the data was non-normal and median abundance should have been calculated, median abundances for data similar to a negative binomial distribution

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tend to be zero (Rózsa *et al.* 2000). Therefore mean abundance was calculated for statistical analyses but median intensities were also calculated to give an idea of general infection levels for hosts carrying parasites. The program QP3.0 (Rózsa *et al.* 2000) was utilised for calculating the prevalences, mean abundance and median intensity. Various indices are also used to describe the extent of aggregation in a parasite population, although each method has its own shortcomings. The simplest one is the variance to mean ratio (s^2/m, Poulin 1993; Rózsa *et al.* 2000), which works on the principle that an aggregated population will have a higher variance than the mean of that same population. Thus a ratio that is higher than unity indicates that there is an aggregated distribution (Rózsa *et al.* 2000). Another popular method is to compare the distribution of the parasite population to the negative binomial distribution, since the typical over-dispersion produced by aggregation produces a similar spread of data. This tests for significant difference between an observed population and a theoretical aggregated distribution (Bliss & Fisher 1953; Poulin 1993; Rózsa *et al.* 2000). The exponent produced (k) tends towards zero the closer to the theoretical distribution the observed population is (Bliss & Fisher 1953; Poulin 1993). Both of these methods tend to vary with host sample size and mean number of parasites. In fact both have been said to not actually measure what is theoretically described as aggregation (Poulin 1993). A final method is to calculate the index of dispersion (D) by comparing the population to a uniform distribution. The index displays a uniform distribution at zero and tends towards one the more aggregated the observed population becomes (Poulin 1993; Rózsa *et al.* 2000). The main problem with this calculation is that although it measures what is theoretically known as aggregation, the index does not differentiate well between a negative binomial and a random distribution (Poulin 1993). The program QP3.0 was used to calculate the variance to mean ratios, the index of discrepancy (D) and the exponent of the negative binomial distribution (k). All three indices were calculated due to the differences in their weaknesses as a measure of parasite aggregation (Poulin 1993). Tests for significant differences between the prevalences and abundances for each parasite species and parasite diversity between seasons were carried out. χ^2 –tests were used for the prevalences and Mann-Whitney-U tests were employed for the abundances and parasite diversity. In addition, to test for possible sex-biases within particular parasite species χ^2 –tests were utilised. Tests were considered significant at $P \leq 0.05$. All statistical analyses were carried out using SPSS (IBM SPSS statistics version 21, 2012).

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Results

Ectoparasites were collected from 268 animals from 58 colonies (Table 1.1). In total 4810 individual parasites were collected, with an overall prevalence of ectoparasitic infection of 78.4%. There were six ectoparasite species recorded; four mite species, one flea species and one sucking louse species (Table 1.2). The majority of host animals were infected with one or two ectoparasite species (26.9% and 27.6% respectively), with a slightly smaller proportion (18.3%) harbouring three species. Only 4.9% carried four ectoparasite species and 0.7% was infected with five species. As well as being the most speciose, mites were the most abundant parasite taxon. The mesostigmatic mite *Androlaelaps scapularis* was particularly abundant, comprising 78.3% of all the parasites collected (Table 1.3). There was also one other species of the same genus however, it was not as prevalent or abundant as the other Acari. In fact, *A. capensis* was always found on hosts also harbouring *A. scapularis* except on one occasion. The second most prevalent species was also a mite; *Radfordia ensifera* (Table 1.3). This prostigmatic mite was more prevalent than the majority of the other ectoparasite species seen but it should be noted that the mean abundance was only 1.29 (Table 1.3). The median intensity of *R. ensifera* indicated that typical levels of infection with this mite were not particularly high (average of 2 individual parasites to an infected host, Table 1.3). These numbers are very similar to that of *A. capensis* (Table 1.3). A single chigger larva (Trombiculidae) was found on one *C. h. hottentotus* in Kamieskroon. Of the remaining ectoparasite taxa, the flea *Cryptopsylla ingrami* was most prevalent (Table 1.3). The louse *Eulinognathus hilli* was only collected from Kamieskroon and 98.6% of these were collected from one specific winter trip. Despite this, *E. hilli* was the second most abundant ectoparasite found. The median intensity for this species was higher than for all other ectoparasite species (Table 1.3).

Table 1.1: Summary of host numbers of *C. h. hottentotus* captured at both sites.

	Kamieskroon		**Darling**
Summer	20		54
Winter	104		90
Overall	124		144
Total		**268**	

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Table 1.2: List of ectoparasitic species found on *C. h. hottentotus* in Darling, W.C. and Kamieskroon, N.C.

Ectoparasite	**Order**	**Suborder/Superfamily**	**Family**	**Species**
Mites	Parasitiformes	Mesostigmata	Laelapidae	*Androlaelaps scapularis* (Berlese 1918)
				Androlaelaps capensis (Hirst 1916)
		Prostigmata	Trombiculidae	Unidentified chigger larva
			Myobiidae	*Radfordia ensifera* (Poppe 1896)
Fleas	Siphonaptera	Hystrichopsylloidea	Chimaeropsyllidae	*Cryptopsylla ingrami* (De Meillon 1938)
Lice	Phthiraptera	Anoplura	Polyplacidae	*Eulinognathus hilli* (Bedford 1929)

Table 1.3: The prevalence, mean abundance, median intensity and aggregation indices for ectoparasite species infecting *C. h. hottentotus*. The exponents of the negative binomial that did not significantly differ from theoretical distribution are indicated with a *.

	A. scapularis	***A. capensis***	***R. ensifera***	***C. ingrami***	***E. hilli***
Prevalence (%)	73.1	21.6	43.3	16.0	8.2
95% CI	64.7 - 78.2	16.9 - 27.0	34.5 – 46.3	12.1 - 21.0	5.4 – 12.1
Mean abundance	14.05	0.72	1.29	0.28	1.59
95% CI	11.28–17.07	0.45 – 1.02	1.05-1.57	0.20 – 0.38	0.88 – 2.41
Median intensity	9	1	2	1	14
Exact CI	8 - 12	1 - 2	2 - 3	1 - 2	10 - 22
Aggregation indices:					
Variance:mean (s^2/m)	44.92	9.07	4.45	2.33	37.94
Index of discrepancy (*D*)	0.707	0.895	0.766	0.886	0.951
Negative binomial (*k*)	0.357*	0.126	0.325*	0.196*	0.020

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The variance to mean ratios displayed in Table 1.3 suggest that all of the species' populations had an aggregated distribution typical of parasites (Rózsa *et al.* 2000; Roberts & Janovy Jr. 2005; Poulin 2007). In particular *A. scapularis* was noted to have the highest ratio, followed by *E. hilli*. *C. ingrami* had the least aggregated population and were never found in high numbers (usually 1 or 2 would be found on an infected host). The index of discrepancy indicates that all of the parasite species had a non-uniform distribution (Table 1.3). In fact, the most prevalent species (*A. scapularis* and *R. ensifera*) had the lowest index numbers and therefore the slightly more uniform distribution. The exponents of the negative binomial distribution are very low suggesting the parasite populations were all aggregated. However, two species (*A. capensis* and *E. hilli*) deviated significantly from the negative binomial distribution.

Table 1.4: A summary of the proportions of developmental stages and the sex ratios of the parasite species found infecting *C. h. hottentotus*. Sex ratio that were significantly different are marked with a *.

	Total	**Male (%)**	**Female (%)**	**Sex ratio**	**χ^2 test statistic**	***P*-value (0.05)**	**Nymph (%)**	**Larvae (%)**
Parasitiformes								
A. scapularis	3766	6.58	54.77	1:8.33	1432.81	<0.001*	37.89	0.71
A. capensis	194	54.12	37.11	1:0.69	6.15	0.016*	7.73	0.00
R. ensifera	347	55.91	32.85	1:0.59	20.78	<0.001*	7.49	2.59
Siphonaptera								
C. ingrami	76	64.47	32.89	1:0.51	7.78	0.007*	-	-
Phthiraptera								
E. hilli	427	17.10	21.08	1:1.23	1.77	0.210	58.08	-

The adults of *A. scapularis* exhibited a significant female sex bias (Table 1.4). Additionally a large percentage of nymph stages were found although there were very few larvae. With *A. capensis* and *R. ensifera* the sex bias was significantly male-biased (Table 1.4). Nymphal stages represented only a small proportion of the specimens collected of these mite species and few larvae were found for *R. ensifera*. No larval stages of *A. capensis* were collected. *C. ingrami* was also found to have a significant male sex bias (Table 1.4). There was no

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significant difference between the proportion of male and female *E. hilli*, but there was a distinct difference between the number of nymphs and adults with almost 60% of the specimens being juvenile stages (Table 1.4).

Table 1.5: The prevalence and mean abundance of ectoparasites in summer and winter with tests for statistical differences.

	Prevalence (%)		**χ^2 test**		**Mean abundance**		**Mann-Whitney-U**	
	Summer	**Winter**	**Statistic**	***P*-value**	**Summer**	**Winter**	**Statistic**	***P*-value**
A. scapularis	44.6	80.4	42.38	<0.001	3.41	18.11	7.00	<0.001
A. capensis	1.4	29.4	24.82	<0.001	0.01	0.99	4.97	<0.001
R. ensifera	16.2	49.5	24.64	<0.001	0.35	1.65	5.06	<0.001
C. ingrami	0.0	22.2	19.54	<0.001	-	0.39	4.39	<0.001
E. hilli	1.4	10.8	6.83	0.012	0.08	2.17	2.54	0.011

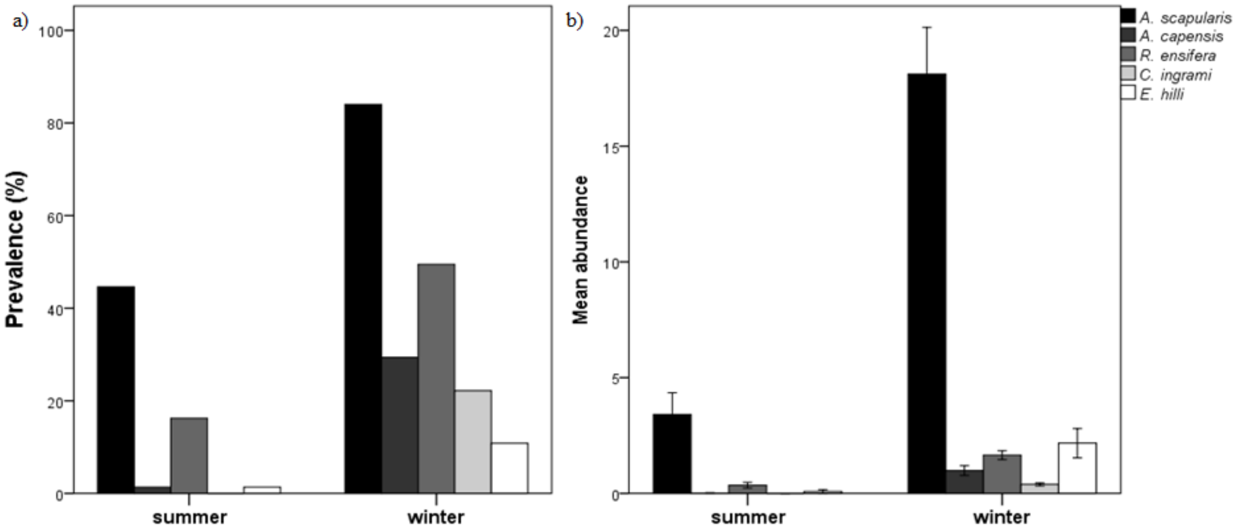

Figure 1.1: The variation in the a) prevalence (number of infected *C. h. hottentotus*) and b) mean abundance (average number of parasites infecting *C. h. hottentotus*) per species of ectoparasite. Error bars indicate ±std. error of the mean.

All of the ectoparasite species found had similar seasonal patterns and were significantly more prevalent and abundant in winter (Figure 1.1, Table 1.5). In fact, *C. ingrami* was only

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found in winter, and *A. capensis* and *E. hilli* were almost exclusive to the winter collections. Due to the larger abundances of parasites in winter host animals tend to harbour more parasite species during this season (Mann-Whitney-U; U = 8.41 *P* = <0.001, Figure 1.2).

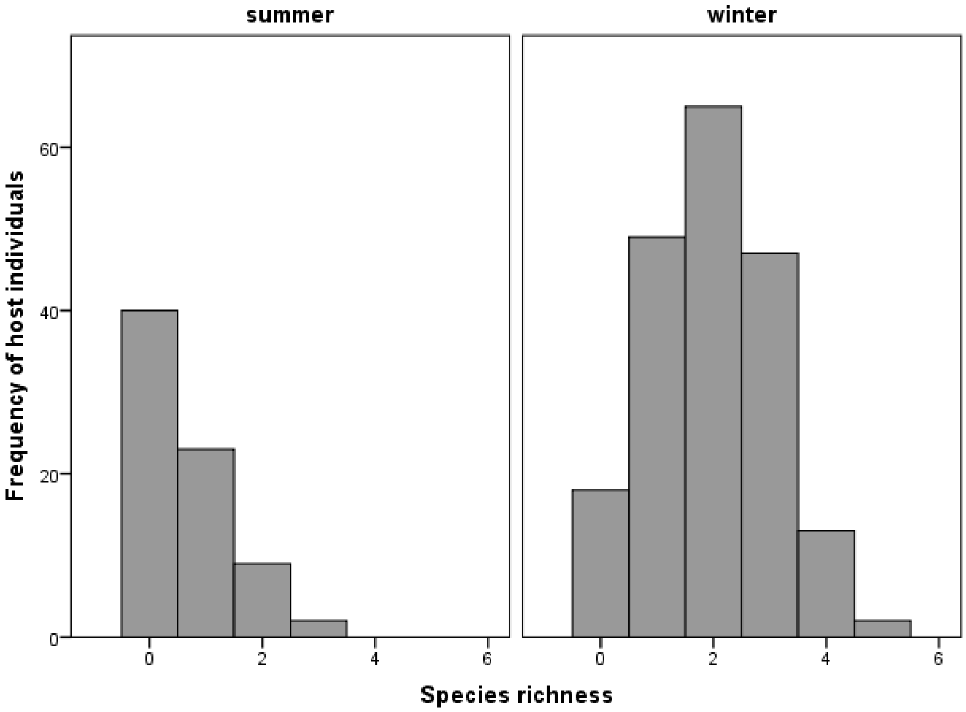

Figure 1.2: The variation in the number of parasite species infecting individuals of *C. h. hottentotus* in summer and winter.

Discussion

Overall there were few species of ectoparasite found on *C. h. hottentotus*, and most of those that were collected have been collected in earlier studies on mole-rats previously referred to as *C. hottentotus* (De Graaff 1964, 1981). The low number of species found however, does appear to be a typical pattern based on previous studies investigating the ectoparasites of various bathyergid species (De Graaff 1964, 1981; Viljoen *et al.* 2011). Though washing the host is not a common method for collecting ectoparasites from rodents, this method has proven effective in previous mole-rat studies for recovering all typical ectoparasites (Viljoen *et al.* 2011; Lutermann *pers. comm.*). Furthermore, the large host sample size and seasonal sampling should ensure that the results produced from this study give a good representation of ectoparasite infections in this species.

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As with the previous studies, mites were the most speciose (De Graaff 1964, 1981). This also seems to be the case for the closely related highveld mole-rat (*C. h. pretoriae*) in more recent investigations where mites were the most prevalent and abundant. In particular the most prevalent species was *A. scapularis* (Viljoen *et al.* 2011), which correlates with findings from this study. The less prevalent species of laelaptid mite obtained, *A. capensis*, was also collected from *C. h. pretoriae* alongside *A. marshalli* (Viljoen *et al.* 2011). However this latter species was not collected from *C. h. hottentotus* in the current study. Earlier work has indicated that *A. marshalli* infected *C. hottentotus* in areas of Kruger National Park (De Graaff 1964) and Viljoen *et al.* (2011) collected this particular species from *C. h. pretoriae* in the Tshwane region of South Africa. These habitats are both much farther east than the populations of *C. h. hottentotus* collected in this study, which could suggest that this particular parasite does not occur in the western parts of South Africa, although further study would be needed to confirm this hypothesis. *A. capensis* has also been found on the Cape mole-rat (*Georychus capensis*), whilst *A. scapularis* has been collected from the Cape dune mole-rat (*Bathyergus suillus*; Zumpt 1961; De Graaff 1981). Both of these species live sympatrically with *C. h. hottentotus* in south-western parts of South Africa, including Darling where part of this research was carried out (Bennett and Faulkes 2000). Additionally *A. cryptomius* has been collected from *G. capensis* (Zumpt 1961), though this mite may be specific to its host (Till 1963). Though clearly not host specific, both *A. scapularis* and *A. capensis* have only been collected from bathyergid hosts, except for one observation of *A. capensis* on white-tailed rats (*Mystromys albicaudatus*) in the Eastern Cape (Till 1963). *R. ensifera* on the other hand has not been previously recorded on any member of the Bathyergidae family (although another fur mite of the Myobiidae family has been documented; Zumpt 1961). This mite species, like other members of its family, appears to be a generalist ectoparasite of rodents and other small mammals and has a worldwide distribution (Fain *et al*. 1980; Reboucas *et al*. 1985; Baker 1999; Zenner & Regnault 1999; Soliman *et al*. 2001), particularly on laboratory animals (Reboucas *et al.* 1986; Oku *et al.* 1994; Zenner & Regnault 1999). This was the most prevalent ectoparasite after *A. scapularis* although there were usually few individuals infecting a single host. One chigger larva was collected from the entire sample population; there is a possibility that this was an accidental infection. However, trombiculid larvae have been found on the pelage of bathyergids before, including on *C. hottentotus* (De Graaff 1981). The other ectoparasitic species appear to be specialist parasites; the flea *C. ingrami* and the louse *E. hilli* have only been recorded on *C. hottentotus* before now (Zumpt 1961;

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Ledger 1980; Durden 1991; Segerman 1995). *C. ingrami* in particular seems to be particularly well adapted to living within the burrow systems of its host as it is the only member of the Chimaeropsyllidae family that has no eyes (Segerman 1995). This could be a similar adaptation to that of their hosts which have lost most of their visual capabilities (Stein 2000; Bennett & Faulkes 2000). Other flea species that infect subterranean rodents have also been documented to have no eyes, such as *Pulex sinoculus* which are found on pocket gophers (Geomyidae) and fleas of the genus *Dinopsylla* that also infect bathyergids (Hafner *et al.* 2000). The full geographical range of *C. ingrami* and *E. hilli* is largely unknown, particularly in the case of *E. hilli* which has only been recorded from a small sample of mole-rats in the Natal region on the east coast of South Africa (Ledger 1980; Durden 1991). It has now been collected from the western part of the country at a similar latitude (Kamieskroon), however this species did not occur further south in Darling. A very closely related species of louse, *E. lawrensis*, has been collected from the Cape dune mole-rat, (*Bathyergus suillus*), which also occurs in Darling, and this particular louse is also thought to be specific to its mole-rat host (Ledger 1980). *C. ingrami* on the other hand has been found along several sites on the western side of the country previously. Although, this would be the furthest recorded occurrence north since the most northerly point of identification previously was Calvinia, Northern Cape (Segerman 1995). Neither the louse or the flea were particularly prevalent, however the average intensity of louse infection was very high in comparison to other species.

The aggregation indices mostly suggest that the ectoparasite populations are all aggregated. The most commonly used method is the exponent of the negative binomial (Wilson *et al.* 2001) but it is this method that holds the most confusing results. Previous studies have indicated that almost all macroparasites have a distribution comparable to that of the negative binomial distribution (Shaw & Dobson 1995; Shaw *et al.* 1998) and that the exponents calculated for the vast majority of observed parasite populations are less than one (Shaw *et al.* 1998). Although this is the case for all parasite species analysed here, *A. capensis* and *E. hilli* were significantly different from the theoretical distribution. However it has been said that the negative binomial is not as reliable for parasites of a low prevalence (Scott 1987), which was the case for both *A. capensis* and *E. hilli*. The variance to mean ratio has been suggested as a more reliable calculation for samples where many of the hosts harbour no parasites and is much better for direct comparisons between samples of varying means (Scott 1987). The variance to mean ratios also suggest that the ectoparasites all had

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an aggregated distribution throughout the host population, as the variance was not equal to that of the mean. *A. scapularis* and *E. hilli* have very high ratios in comparison to the other species, with the flea *C. ingrami* having the lowest. The index of discrepancy has also been put forward as a good way of comparing aggregation between various ectoparasite populations (Poulin 1993) and all of the indices displayed are closer to one than zero, suggesting that each has an aggregated population. However, there is conflict regarding which species seems to be the most overdispersed. Poulin (1993) expected indices to decrease with increased prevalence, which is the case with these results. Scott (1987) described the variance to mean ratio to be a good measurement for the extent of aggregation and would therefore depict the length of the highly infected 'tail' of the population. The species in this study that appear to have the largest tail are also those with large overall abundances. This suggests that the extremity of the tail may increase with overall abundance in this case, although it should be noted that the variance to mean ratio is closely related to the mean number of parasites (Poulin 1993).

Aggregation of parasites could have implications for the sex ratios of parasitic species, since it is thought that the formation of 'host islands' or infrapopulations can lead to inbreeding (Clayton & Drown 2001; Pap *et al*. 2013), and this would increase with aggregation. Marshall (1981) claimed that inbreeding causes the prevalence of female biases recorded in ectoparasites based on local mate competition theory, which states that females are more likely to produce more female offspring during times of inbreeding to reduce sexual competition between males (Hamilton 1967). Since aggregation of a parasite population is thought to decrease with the prevalence of the species (Poulin 1993), it is reasonable that it has also been suggested that more prevalent species are likely to have more equal sex ratios, particularly in species that need close host interactions for transmission such as lice (Pap *et al.* 2013). This however, was not supported by our study since the most prevalent mite, *A. scapularis*, had a very significant female sex bias and the anopluran louse, *E. hilli*, had an almost equal adult sex ratio despite being the least prevalent parasite. Furthermore, the other three ectoparasite species analysed (*A. capensis, R. ensifera* and *C. ingrami*) displayed a significant male bias. Mites of the Laelapidae such as *A. scapularis* are known to have reproductive systems based on haplodiploidy, which tend to produce a high female bias (Norton *et al.* 1993). This could explain the female bias seen in *A. scapularis*, although it is interesting that *A. capensis* does not comply with this pattern. There are two possible explanations for the male sex bias found; the first is that it is

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more costly to produce females. Sex ratio theory suggests that the numbers of each sex in a population will be equal unless it is more costly to produce one sex compared to the other (Fisher 1930; Strohm & Linsenmair 1997). In all three species with a male sex bias, the females are larger than the males (Till 1963; Baker 1999; Segerman 1995) thus males would be less costly to produce. This has already been suggested for other flea species (Krasnov *et al*. 2008a). The second possibility is that the male is the dispersing sex for these ectoparasite species, which means that producing more males reduces competition amongst females that do not disperse so far (Clark 1978), although dispersal in either sex would be difficult for these parasites due to the fossorial lifestyle of their host.

There were clear seasonal patterns apparent in all five ectoparasite species found in the current study. Seasonal differences for abundance and prevalence were significant for all parasite species with each being more prevalent and abundant in the rainy winter season. Similar patterns were also found in *C. h. pretoriae* where the ectoparasite taxa found were more abundant in the wetter months (Viljoen *et al.* 2011). There could be various reasons for this, for example abiotic factors can influence the life cycle of the host. Some flea species in particular are known to be adapted to survive in colder conditions (Krasnov *et al*. 1997). Fleas from this study were only collected in winter when their survival rates are likely to be best. Anopluran lice are also known to suffer under higher temperatures and lower humidity (Cooke & Skewes 1988; Fabiyi 1996), therefore it was surprising to only find them in the warmer and drier site. However, this species was found almost exclusively in winter when humidity would be higher, which may account for this observation. Mites are also known to be influenced by their surrounding environment (Vinarski *et al.* 2007), particularly temperature and humidity (Krasnov *et al*. 2008; Korallo-Vinarskaya *et al*. 2009). There are not always set patterns for this taxa however, as different species have varying preferences to higher or lower temperatures and differing tolerances of lower humidity (Krasnov *et al.* 2008). Viljoen and colleagues (2011) did find that mites on *C. h. pretoriae* were more abundant in the wetter and warmer months (summer in this particular case) and one suggestion was the increase in humidity. However, it is important to consider that *C. h. hottentotus* spends almost all of its life below the surface of the ground meaning that the influence of abiotic factors may well be buffered by the burrow system of the host (Roper *et al*. 2001; Carpenter-Kling 2012). This would be more pronounced in Darling where the burrow systems tend to be deeper than that of Kamieskroon (E. Archer, pers.

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obs.). Rainfall however would still be likely to influence both host and parasite even underground as it would seep through the soil.

In fact, rainfall influences the social and foraging behaviour of the host; during the rainy months when the soil is softer *C. h. hottentotus* increases digging activities in the search of food and also for mates from other colonies. This is also peak dispersal time for the host (Spinks *et al.* 1999a, 2000). There is a possibility that the ectoparasites infecting the common mole-rat have coincided their breeding season with the dispersal of the host. Many other ectoparasitic species align their breeding with that of the host in order to enhance their chances of transmission (Marshall 1981; Christe *et al*. 2000; Wilson *et al.* 2001). However, optimal chances for ectoparasites on *C. h. hottentotus* to disperse to other colonies would be during peak host dispersal due to the restrictions of the subterranean environment. Rates of inter-colonial interactions for the host species greatly increase just prior to the breeding season (Spinks *et al.* 1999), which will allow individual parasites to transmit to other colonies of hosts and outbreed. It could be said that this would be more influential for species that are more dependent on their hosts, in this case *E. hilli* and the mites that are generally less mobile than fleas (Marshall 1981). However, *C. ingrami* is possibly specialised to this host (Segerman 1995) and could be as limited to the movements of the host as the other ectoparasites due to its burrow system habitat. If this was the case, it might suggest that *C. ingrami* spends more time off host in the nesting material outside of their own breeding season (Krasnov *et al*. 1997) resulting in the observed decrease of flea abundance during summer.

In conclusion, as in other studies on ectoparasites of bathyergids, mites are the most speciose ectoparasites. Those of the Laelapidae family were particularly prevalent, though one of the myobiid fur mites, *R. ensifera* was collected from a bathyergid for the first time. The *Androlaelaps* mites may be specialised to the Bathyergidae family. We also confirmed the presence of two ectoparasites that are potentially specific to *Cryptomys* spp.; *C. ingrami* and *E. hilli.* All of the species populations were aggregated amongst the host population and although the majority of the parasites had a significant sex bias, most of them were male-biased. Additionally, bias was not related to prevalence, suggesting that inbreeding is not the major cause of sex bias in these parasite species. All species of parasites were more abundant in winter when the rainfall is higher, however further study would be needed to ascertain whether this is due to abiotic influences or behavioural changes in the host.

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Chapter 2

Contributions of abiotic and biotic factors to variation in ectoparasite burdens in *Cryptomys hottentotus hottentotus*

Abstract

The interactions between a host and the parasites infecting it are very complex due to the large number of factors that can influence this relationship. Biotic factors associated with the host are known to mediate parasite numbers via differences in exposure and susceptibility, whilst the surrounding habitat can alter parasite numbers directly or through its action on the behaviour of the host. This study attempted to look at this broad picture by assessing the impact of abiotic and biotic factors on the ectoparasite assemblages infecting the common mole-rat (*Cryptomys hottentotus hottentotus*). Colonies of this widely distributed social bathyergid were captured from two locations at either end of a rainfall gradient in the western coastal region of South Africa. The impacts of location, rainfall, temperature, host sex, host breeding status, host body mass and colony size on parasite prevalence and abundance were analysed. Overall the abiotic factors had the largest impact. Surprisingly, the more arid location had higher parasitic burdens than the more mesic habitat, but the seasonal increase in abundance in each location was closely linked to higher rainfall. Temperature had little effect. The patterns associated with biotic factors were much more varied; however the patterns could be linked to differences in the parasite life cycles and could even be used to make inferences about the amount of time the parasite may spend on or off-host. Colony size appeared to be more influential on parasites that may be more dependent on their host. Body mass, sex and breeding status of the host appeared to have impacts on the prevalence and abundance of the different parasitic species. However, these three factors are closely linked in *C. h. hottentotus* and increased parasitism on male and breeding individuals may be a sign of reduced immune function.

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Introduction

Macroparasites are not evenly distributed amongst host individuals in a population and various factors have been suggested to explain the aggregated populations of parasites on hosts (Rózsa *et al*. 2000; Wilson *et al*. 2001; Roberts & Janovy Jr. 2005). Abiotic factors, such as the surrounding habitat and temperature are known to influence the life cycles of parasites directly (Stromberg 1997; Altizer *et al*. 2006; Krasnov 2008) and consequently may influence how many parasites are in the community and the risk of a host becoming infected (Altizer *et al*. 2006). One such factor that has been widely suggested is the effect of rainfall on parasite distribution, which may lead to variation between parasite communities in different habitats (Fabiyi 1996; Krasnov *et al*. 2008; Froeschke *et al*. 2010), or fluctuations in prevalence or abundance of specific parasite species in a population (Wilson *et al*. 2001; Altizer *et al*. 2006; Krasnov *et al*. 2008; Thurber *et al*. 2011). Temperature has also been shown to impact the geographical distributions (Cattadori *et al*. 1999; Salkeld *et al*. 2008) and the temporal variation (Stromberg 1997; Waller *et al*. 2004; Cattadori *et al.* 2005; Altizer *et al*. 2006) of parasite populations.

Ectoparasite abundance and prevalence in particular is thought to be hugely impacted by abiotic factors (Fabiyi 1996; Krasnov *et al*. 1997, 2008, 2008a; Vinarski *et al*. 2007; Korallo-Vinarskaya *et al*. 2009). However, the associations between various ectoparasitic taxa and rainfall and temperature patterns vary (Matthee *et al*. 2007; Krasnov *et al.* 2008). Flea prevalence and abundance for example have been shown to increase in the rainy season (Merino & Potti 1996; Matthee *et al*. 2007), although in these cases the increase in rainfall was associated with a decrease in temperature which is also known to have a positive effect on flea abundances (Krasnov *et al*. 1997). There have been variable results from studies on ticks; increased prevalence has been associated with warmer and drier months on small mammals (Matthee *et al*. 2007) as well as colder and wetter months (Oorebeek & Kleindorfer 2008). High humidity, associated with high rainfall in tropical habitats, has also been shown to have a positive effect on tick populations found on hosts (Basu & Bandyopadhyay 2004). It has been indicated that ectoparasites, such as fleas and ticks, which have part of their life-cycles off-host are more susceptible to abiotic factors (Krasnov *et al*. 2011) and that different developmental stages may display variations in their responses to these factors (Needham & Teel 1991; Horak *et al*. 1995; Benoit & Denlinger 2010; Lutermann *et al*. 2012). Fleas are known to spend more time on or off-host with alterations in temperature, often remaining in the nesting material and entering

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reproductive diapause when conditions are not favourable (Krasnov *et al*. 1997). Furthermore the survival rates of pre-imaginal fleas can be determined by very small fluctuations in temperature (Krasnov *et al*. 2001). The interactions between ectoparasitic mite species and humidity and/or rainfall are just as variable as those regarding ticks (Ugbomoiko & Obiamiwe 1991; Frances *et al*. 1999; Krasnov *et al*. 2008; Møller 2010). Accordingly, a study that was carried out to look for common patterns between gamasid mites and climatic factors across various locations concluded that the only re-occurring pattern to be found was an increase in species diversity in relation to increasing rainfall (Krasnov *et al*. 2008). Abiotic factors have been found to be less influential on parasitic lice since their entire life cycle occurs almost exclusively on the host (Lareschi & Krasnov 2010; Møller 2010). However, increases in humidity have been found to be detrimental to various species of lice (Singh *et al*. 2009), and the duration and level of humidity may determine the habitat in which certain lice species are able to survive and reproduce (Fabiyi 1996).

In addition to the effects of abiotic factors, there are also host related factors that may influence parasite distribution, such as host sex (Schalk & Forbes 1997; Soliman *et al.* 2001; Krasnov *et al*. 2005; Perez-Orella & Schulte-Hostedde 2005; Christe *et al*. 2007; Hillegass *et al*. 2008), reproduction (Christe *et al*. 2000; Grear *et al*. 2009; Pollock *et al*. 2012) and behaviour (Wilson *et al.* 2001; Stewart & Macdonald 2003; Altizer *et al*. 2006; Bordes *et al*. 2007). Particularly among mammals, there tends to be a male bias in parasitism (Schalk & Forbes 1997; Soliman *et al.* 2001; Krasnov *et al*. 2005; Perez-Orella & Schulte-Hostedde 2005; but see Christe *et al*. 2007; Hillegass *et al*. 2008). One frequently suggested hypothesis is that androgens such as testosterone reduce immune function leading to higher susceptibility to parasites in males (Folstad & Karter 1992; Schalk & Forbes 1997; Perez-Orella & Schulte-Hostedde 2005). Additionally some behavioural differences between the sexes have also been proposed to explain sex biased parasitism, including differences in mobility or home range (Combes 2001; Krasnov *et al*. 2005; Hillegass *et al*. 2008; Grear *et al*. 2009, Scantlebury *et al*. 2010) or variation in grooming behaviour (Mooring *et al*. 1996; Christe *et al*. 2007). Intra-sexual differences in parasitism have furthermore been reported between breeding and non-breeding hosts (Christe *et al*. 2000; Carpenter-Kling 2012). A host only has limited resources and the increase in energy consumption during reproduction may result in the host having less to expend into the immune system and hence reduced immune responses (Møller *et al*. 2003).

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In particular, breeding females are known to suffer from higher parasite burdens during the breeding season and with gestation (Christe *et al*. 2000, 2007; Pollock *et al*. 2012). Parasitic infection during the breeding season may also put a further drain on resources and reduce reproductive success (Hillegass *et al*. 2010).

Several studies have taken particular interest in the impact of social behaviour on parasite burdens. The majority of hypotheses surrounding these studies refer to Alexander (1974) who stated that increased host densities such as those associated with social groups would increase the potential for horizontal transmission of parasites. This would create a 'cost' of group living. It has been demonstrated that host density is important for horizontal transmission (Mohr & Adams 1963; Arneberg *et al*. 1998) and several studies have shown that an increase in group size can lead to increased parasite burden (Cote & Poulin 1995; Godfrey *et al*. 2009; Clough *et al*. 2010; Rifkin *et al*. 2012). This can also occur when host animals congregate for the breeding season (Wilson *et al*. 2001; Christe *et al*. 2007). However there are also some studies that have shown that this is not always the case (Bordes *et al*. 2007; Hillegass *et al*. 2008; Snaith *et al*. 2008). In particular there are very mixed results for ectoparasite studies. There is evidence for increased infection risk in larger groups (Cote & Poulin 1995; Godfrey *et al*. 2009). On the other hand there are many studies that show no link between social group size and ectoparasite load (Arnold & Lichtenstein 1993; Van Vuren 1996; Hillegass *et al*. 2008). Further, there are studies that show a negative correlation between parasite load and sociality (Bordes *et al*. 2007; Viljoen *et al*. 2011). Several hypotheses have been proposed to explain these inconsistencies; one that particularly applies to ectoparasites suggests that behavioural defences against parasites, such as grooming, tend to increase in frequency in social groups (Loehle 1995). It has also been suggested that social groups create isolated islands that prevent transmission of parasites to other groups, although this can lead to increased parasite burden in larger groups (Freeland 1979) or filter out parasite infection more rapidly with less inter-group interactions (Wilson *et al*. 2003). Discrepancies seen in results between studies in different parasite taxa may also be due to differences in the method of transmission adopted by the parasite to get from one host to the next (Cote & Poulin 1995; Rifkin *et al*. 2012). For example, ectoparasites with a free living parasitic stage are known to display dynamics more similar to that of a predator if they actively seek out their host. Thus group sizes may not have as strong an influence on such species in comparison to those which have their entire life cycle on-host (Cote & Poulin 1995). It has further been shown that relationships

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vary with ectoparasitic taxa on the same host species (Monello & Gompper 2010). A study on aggregation and contact rates in racoons (*Procyon lotor*) indicated that increased host densities had no influence on flea abundances. However, tick abundances increased and lice displayed the opposite pattern. Differences in parasite life-history, particularly those associated with the mode of transmission, alters how host aggregation influences parasite dynamics (Loehle 1995; Monello & Gompper 2010; Rifkin *et al*. 2012).

The aim of this study was to look at the influence of geographic location, rainfall, temperature and group size on ectoparasite prevalence and abundance on common mole-rats (*Cryptomys hottentotus hottentotus*). This subterranean rodent is a member of the African mole-rat family (Hystricomorpha: Bathyergidae). This family consists of subterranean species that mostly feed upon tubers and bulbs underground (Bennett & Faulkes 2000). *C. h. hottentotus* has a relatively large distributional range compared to other species within the family (Bennett & Faulkes 2000). Like other members of the genus, *C. h. hottentotus* is social and lives in colonies of up to 14 individuals with a single breeding female and one, perhaps two, breeding males (Bennett 1989; Bishop *et al*. 2004). Breeding individuals tend to be larger than their non-breeding counterparts (Bennett 1992; Spinks *et al*. 1997, 1999). Unlike most other social bathyergid species, *C. h. hottentotus* has a breeding season, which occurs during the months following peak rainfall (Spinks *et al*. 1999). Mole-rat species usually disperse during the period of most rainfall, as this is when it is least costly for them to do so (Thomas *et al*. 2012). In the case of *C. h. hottentotus* found along the west coast of South Africa this occurs in the months preceding the breeding season (June to August; Spinks *et al*. 2000). Dispersal patterns do differ between mesic and arid habitats however, as individuals in more arid areas leave their familial colonies less frequently (Spinks *et al*. 2000). Consequently recruitment is significantly reduced in such colonies in comparison to their counterparts in more mesic habitats (Spinks *et al*. 2000). Populations in arid habitats are less dense due to a more clumped spread of food resources within areas of lower rainfall. With food resources being sparser and other colonies not being in close proximity to one another, the costs of dispersal are much higher in arid habitats for *C. h. hottentotus* (Spinks *et al*. 2000). Despite the differences in dispersal, colonies in mesic and arid habitats are on average the same size (Spinks *et al*. 2000a). Breeding activity ensues throughout September to early November (Spinks *et al*. 1999). Few animals in the colony breed, but all male individuals are physiologically able to reproduce (Spinks *et al*. 1997). Non-breeding females are also physiologically able to breed

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and all females are potentially able to reproduce at any time in the year (Spinks *et al*. 1999). The broad geographical distribution of the species enabled this study to focus on two *C. h. hottentotus* populations along a rainfall gradient in South Africa. Comparisons of parasite populations in relation to abiotic factors were therefore made both between and within habitats. Seasonal collections were undertaken to cover the full range of rainfall for each site. Although rainfall, temperature and colony size are the main focus of this study as these are expected to influence parasite prevalence and abundance, host related factors such as host sex, body size, and breeding status were also considered for the analyses. Previous work on this species and other social bathyergid species has revealed that ectoparasite prevalence and abundance tends to increase during the wet season whether the habitat is found in a summer- or winter-rainfall area (Viljoen *et al*. 2011; Carpenter-Kling 2012; see Chapter 1). Therefore it was predicted that these seasonal increases could be due to rainfall as opposed to temperature. Both prevalence and abundance of ectoparasites was expected to increase with increasing rainfall, on both a geographic scale and within location. Although there are a number of questions surrounding the influence of gregarious living, several studies still show that ectoparasite prevalence or abundance may increase with group size (Cote & Poulin 1995; Godfrey *et al*. 2009, but see Bordes *et al*. 2007 and Viljoen *et al*. 2011). Additionally, a recent meta-analysis maintained that larger social groups suffer higher parasite risk (Rifkin *et al*. 2012). Thus it was predicted that parasite burden would increase on individuals in larger colonies. Further, it was predicted that this increase would be more pronounced in ectoparasite species that spend the entirety of their life cycle on the host.

Materials and Methods

Study sites and animal capture

Trapping was carried out at two sites approximately 440km apart on the western coast of South Africa between February 2011 and August 2012. Darling, Western Cape (33.25^{o}S, 18.25^{o}E) was the more mesic site with approximately 411.6mm of rainfall per annum (average 2009-2012, SA Weather Service). The habitat in this area was typical of the Renosterveld fynbos region (Hagenah & Bennett 2013) although the farmland on which the mole-rats were caught was used for grazing cattle and the majority of the vegetation was grassland dominated by *Lolium* spp. (rye grass, Thomas *et al*. 2009). The soil here was

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mainly clay-based with areas containing a higher sand content (Romañach 2005; Hagenah & Bennett 2013). The second site was in Kamieskroon, Northern Cape (30.13^{o}S, 17.57^{o}E). Located in the Namaqualand habitat, Kamieskroon was a much more arid area with an average annual rainfall of 284.5mm (average 2009-2012, SA Weather Service). The soil was much softer with a higher sand content, which supported a low vegetation biomass (Romañach 2005) of primarily small succulents found within the succulent Karoo habitat (Milton *et al*. 1997). The land was also used for livestock although not as frequently as that in Darling.

Trapping sessions occurred at both sites during summer (January-March) and winter (June-August) in order to cover the range of rainfall at each site. The aim was to catch ten complete colonies from each site during each season. Colonies of *C. h. hottentotus* were captured using live traps (Hickman 1979) with sweet potato as bait. Traps were checked frequently (every two to three hours) from first light until just after dark. To ensure that trap sites were allocated to a single colony, traps were placed at least 30m apart. An entire colony was considered captured when no activity was recorded at a trap site for three days after the last animal capture (Spinks *et al*. 1998). Once captured, individuals of the same colony were housed together in plastic crates no smaller than 41 x 28 x 25cm, provided with at least a 3cm layer of soil and air holes in the lid. Sweet potato was used to feed the mole-rats once a day.

Processing host animals

Each individual was euthanised with an overdose of halothane. Immediately after expiration the animals were sexed and weighed with scales (SNUG-III precision balance, Jadever) accurate to 0.05g. At this point adults were subdivided into breeders and non-breeders. The female breeder of the colony was identified by the presence of nipples, whereas the male breeder(s) were identified as the heaviest males (Bennett 1992; Rosenthal *et al*. 1992). Then each individual was washed thoroughly in 100ml of soapy water in a 22cm x 7cm x 5cm tub to remove any ectoparasites. The washing process involved kneading through the fur with the fingertips all the way along and around the body length five times, before washing the head and the posterior end. They were then moved back and forth through the water ten times, first on their front and then on their back, in order to remove any ectoparasites that remained caught in the fur. The sample was transferred to an appropriate screw-top

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container until ready to be processed. Ectoparasites were subsequently collected, identified and counted as described in the previous chapter.

Statistical analyses

The body mass of *C. h. hottentotus* is associated with its sex and breeding status (Bennett 1992; Rosenthal *et al*. 1992; Spinks *et al*. 1997, 1999), and also with its habitat (Spinks *et al*. 2000). After a log10 transformation the body mass data was normally distributed (Kolmogorov-Smirnov; 0.045 $P = 0.20$). Thus a general linear model (LM) was utilised to test for any significant impact these factors may have had on the body mass of the host. This was carried out in R2.15.1 (R Core Team 2012) with the use of the 'lm' function from the stats package (R Core Team 2012). A Mann-Whitney-U comparison was also carried out to test for any difference in colony size between the two locations using SPSS (IBM SPSS version 21 2012). Temperature and rainfall data for each location was provided by the South African Weather Service. Previous work concerning ectoparasites showed that rainfall over longer periods of time and not just the weather conditions preceding capture were most relevant to the parasite species in question (Lutermann *et al*. 2012). Therefore several measurements of rainfall were used to see which was most relevant to the parasites in this study. The total rainfall during the month of capture was calculated from the South African Weather Service data. In addition, the cumulative rainfall for intervals of two, three and six months were calculated. This produced four total rainfall calculations to be compared in statistical analyses. Average temperature for the month of capture was calculated. Prevalence, the number of host species infected, and abundance, the mean number of parasites an individual host carried (Bush *et al*. 1997), was calculated for each season at each location. Due to the non-parametric nature of the data of each parasite species, generalised linear mixed effects models (GLMMs) were used to analyse the importance of abiotic and biotic factors on the ectoparasites found on *C. h. hottentotus.* An information-theoretic (IT) approach was employed based on Akaike information criterion (AIC; Akaike 1973) calculations (Burnham & Anderson 2002). This method allowed several hypotheses to be investigated at once and was used to indicate predictors that were most influential on the parasite data and warranted further investigation (Garamszegi 2011). GLMMs containing the predictors could be compared directly to one another via the AIC statistic to see which has the best fit to the data in relation to the others (Burnham &

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Anderson 2002; Burnham *et al*. 2011; Garamszegi 2011; Symonds & Moussalli 2011). Analyses were carried out using R 2.15.1. The ‘lmer’ function in the lme4 package (Bates *et al*. 2012) was employed for models fitted to binomial distribution for parasite prevalences. Parasite abundances were compared to a negative binomial distribution using the ‘glmmadmb’ function from the glmmADMB package (Fournier *et al*. 2012). Due to the lower prevalence of *R. ensifera* mites, zero inflation was indicated in the abundance models. Additionally, the less prevalent *Androlaelaps* mite species was always found, with one exception, coexisting on hosts infected by the other *Androlaelaps* species (see previous chapter). Consequently, data for this genus were combined for analyses.

Colony ID was defined as a random effect in all GLMMs. Predictors for the GLMMs were entered into the model as fixed effects. These were location, host sex and breeding status (i.e. breeder and non-breeder). Body mass and colony size were included as covariates. If any incomplete colonies were captured, the data from individuals in these colonies were excluded from the models. Incomplete colonies consisted of social groups that did not include all members as it was not possible to capture them all. To further investigate the influence of abiotic factors on seasonal variation revealed in these ectoparasites (see previous chapter), average temperature and total rainfall were also added as covariates. Each measure of total rainfall was included in the GLMMs as separate predictors until it was clear which appeared most frequently in the models with the lowest AIC using cumulative w_i (see below). When the most appropriate measure of rainfall had been selected, the models containing the other totals were removed. Since highly complicated models are unlikely to be statistically viable (Burnham & Anderson 2002; Symonds & Moussalli 2011) and there was a large number of predictor variables to include, all possible models with all possible combinations were run with up to 4 predictors. Both 2 and 3-way interactions between categorical variables were included. The models described above were only carried out on *Androlaelaps* and *R. ensifera* data.

Once the AIC values had been calculated in R 2.15.1, a corrected AIC value (AICc) was calculated manually since the sample size was considered small. When using these analyses a small sample size is defined as the number of samples divided by the number of parameters in the most complicated model being less than 40 (Burnham & Anderson 2002; Symonds & Moussalli 2011). The ΔAIC values were calculated as the difference between the model in question and the best fitting model (i.e. model with the lowest AIC value, Burnham & Anderson 2002). In addition, the Akaike weight (w_i) was calculated and only

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models that account for a total of 95% of cumulative w_i (Burnham & Anderson 2002; Symonds & Moussalli 2011) will be reported here. Following further advice from Symonds and Moussalli (2011), the evidence ratio (ER) was calculated to show how many times less likely each model was compared to the one with the lowest AIC. Although many GLMMs were run, there is a possibility that leaving out the most complicated models may lead to a small amount of bias in the data and making the best model alone not necessarily reliable (Garamszegi 2011). Therefore the cumulative w_i for each predictor was also calculated by summing the weights of models including a specific predictor, which can be used to infer the significance of the predictor in question. The cumulative w_i could also be expressed as a percentage once multiplied by 100 (Burnham & Anderson 2002; Symonds & Moussalli 2011). Although cumulative w_i of above 90% are generally considered high (Symonds & Moussalli 2011), predictors that accumulated w_i above 70% were considered for further analysis and discussion here. This calculation was also used to select the best measurement of rainfall for the ectoparasites analysed by selecting the total that had the highest cumulative w_i most frequently. GLMMs containing the other rainfall measurements were removed from analyses before the final ERs and cumulative w_i were calculated. The significance of colony identity was analysed separately with the use of generalised linear models (GLMs), also carried out in R2.15.1. The GLMs contained colony identity as a predictor and were compared to both the abundance and prevalence of each mite group. The stats package was utilised for comparing colony ID to mite prevalences via the ‘glm’ function. The MASS package on the other hand was employed for the ‘glm.nb’ function for abundance models (Venables & Ripley 2002).

The low prevalence and/or abundance of fleas and lice (see results) precluded the use of similarly complex GLMMs for these parasite taxa. Therefore much simpler GLMMs were run for these species from smaller data sets to analyse the biotic factors that may influence the prevalence or abundance of these taxa. The flea data were analysed using only the data from winter trapping sessions (see results section and previous chapter). GLMMs included location, host sex and breeding status as predictors, with body mass as a covariate. In the case of the lice which were only found in one site and mostly one season, the data only included that of the hosts from Kamieskroon in the winter trapping sessions (see results section and previous chapter). Therefore the same models were employed as for the fleas but location was not a necessary predictor. Both sets of GLMMs for lice and fleas also

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included data from incomplete colonies of hosts since colony size was not included in the analyses.

Results

In total, 268 *C. h. hottentotus* were collected during this study (Table 2.1). Only one collection could be conducted in Kamieskroon in the summer months. In addition, two collection trips were carried out for each site in winter and also for Darling in summer. Significant differences in the body mass of these animals were found between the two locations (Table 2.2), with common mole-rats tending to be larger in Darling (Figure 2.1). Males also had a significantly higher body mass compared to females, whilst breeders of both sexes were significantly larger than their non-breeding counterparts (Table 2.2, Figure 2.1). Of the total number of mole-rats collected, 151 were from complete colonies. There was no significant difference in the size of colonies between the two locations (average colony size, mean ±SD; Darling = 5.9 ±2.7, and Kamieskroon = 5.6 ±2.1. Mann-Whitney-U; U = 0.998, *P* = 0.318).

Table 2.1: Total number of individuals for each sex captured at each location per season. The percentage of breeding animals of each sex is included.

		Kamieskroon		Darling	
Animals caught:		**Male (% breeders)**	**Female (% breeders)**	**Male (% breeders)**	**Female (% breeders)**
	Summer	10 (30.0)	10 (20.0)	34 (26.5)	20 (30.0)
	Winter	50 (31.8)	54 (31.5)	39 (43.6)	51 (23.5)
	Overall	60	64	73	71
	Total	**124**		**144**	

A total of five species of ectoparasite were found to regularly infect *C. h. hottentotus*. Three mite species, *Androlaelaps scapularis, A. capensis,* and *Radfordia ensifera* were collected, of which those of the genus *Androlaelaps* were most abundant and prevalent (Table 2.3). In addition, there was one species of flea, *Cryptopsylla ingrami*, which was not very prevalent or abundant. It was also only collected during the winter months (Table 2.3). Almost

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Table 2.2: Linear model (LM) results displaying the significance of sex, breeding status and location in the variation of body mass for *C. h. hottentotus*. Based on log10 transformation of body mass data. Interactions between factors are denoted by ‘x’. Significant predictors are indicated using *.

	Estimate	**Std. Error**	***P*-value**
Location	- 0.31 *	0.06	< 0.001
Sex	0.13 *	0.06	< 0.001
Breeding status	0.31 *	0.07	< 0.001
Location x Sex	0.09	0.08	0.583
Location x Breeding status	0.12	0.10	0.703
Sex x Breeding status	0.20	0.10	0.073
Location x Sex x Breeding status	-0.15	0.15	0.302

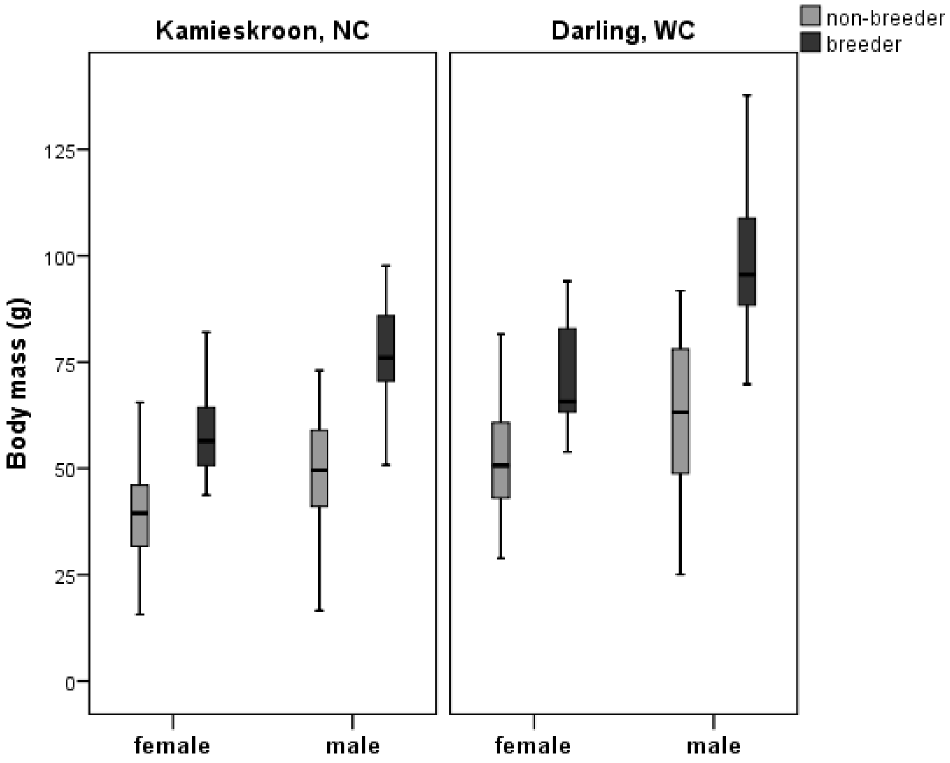

Figure 2.1: Differences in body mass of *C. h. hottentotus* between male and females of different breeding statuses in two locations. The middle bar, the box and the whiskers refer to the median, interquartile range and full range respectively.

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Table 2.3: The prevalence and abundance of particular genera of ectoparasites infecting *C. h. hottentotus* at each location during each season.

		***Androlaelaps* spp.**	***Radfordia ensifera***	***Cryptopsylla ingrami***	***Eulinognathus hilli***
Prevalence %					
Darling	summer	29.09	5.45	0.00	-
	winter	78.89	33.33	7.78	-
Kamieskroon	summer	85.00	45.00	0.00	5.00
	winter	89.42	63.46	34.62	20.19
Abundance					
Darling	summer	84	5	0	-
	winter	1139	86	12	-
Kamieskroon	summer	169	21	0	6
	winter	2567	235	64	421

exclusive to a single field collection and site (i.e. Kamieskroon) was the one louse species, *Eulinognathus hilli*. This anopluran louse was less prevalent than fleas however, a larger number of them were collected (Table 2.3). Nevertheless it is apparent from the data that mites were the most prevalent and abundant ectoparasites at both locations.

Table 2.3 shows that all parasite species were more prevalent and abundant in the more xeric site, Kamieskroon. Although the temperature averages were relatively similar between the two locations at times of capture, Darling generally had higher rainfall and also had more extreme fluctuations between summer and winter (7.2-182.0mm in comparison to Kamieskroon which had a range of 19.0–86.7mm, Fig. 2.2). In every case the abundance of each species was at least doubled in Kamieskroon compared to the corresponding season in Darling (Table 2.3). All species were also more prevalent and abundant in winter. Furthermore the variation in prevalence between seasons was more pronounced in the Darling populations (Table 2.3).

The GLMMs for the mite taxa indicate that the predictors used influenced the prevalence and abundance data, as the intercept model had a much larger AICc for both *Androlaelaps* spp. and *R. ensifera*. However the best fitting GLMM for the prevalence and abundance of *Androlaelaps* spp. was much more distinct than those for *R. ensifera*. The best fitting

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Table 2.4: The 95% confidence set of GLMMs for the abundance of *Androlaelaps* spp. Contains the numbers of parameters in the model (*k*), corrected Akaike value (AICc), difference between model and best fitting model (ΔAICc), Akaike weight (w_i) and evidence ratio (ER). The intercept model is included for reference.

Model	***k***	**AICc**	**ΔAICc**	w_i	**ER**
location x breeding status + colony size + rainfall	7	815.8028	0.000	0.559	-
location + body mass + colony size + rainfall	6	818.1078	2.305	0.177	3.166
location + breeding status + colony size + rainfall	6	820.8078	5.005	0.046	12.213
location x breeding status + body mass + rainfall	7	821.1768	5.374	0.038	14.688
location + colony size + rainfall	5	821.185	5.382	0.038	14.748
location + sex + colony size + rainfall	6	821.3278	5.525	0.035	15.840
location x breeding status + rainfall	6	822.2458	6.443	0.022	25.067
location + temperature + colony size + rainfall	6	822.7558	6.953	0.017	32.347
location x sex + colony size + rainfall	7	823.4308	7.628	0.012	45.331
location x breeding status + temperature + rainfall	7	823.5128	7.710	0.012	47.229
(intercept)	2	852.2233	36.421	6.9×10^{-9}	8.1×10^{7}

The '+' symbol indicates where predictors were added as main interactions. Any predictors connected by 'x' were employed in the model as a two-way (or three-way where three predictors are connected in this way) interaction term as well as separate main interactions.

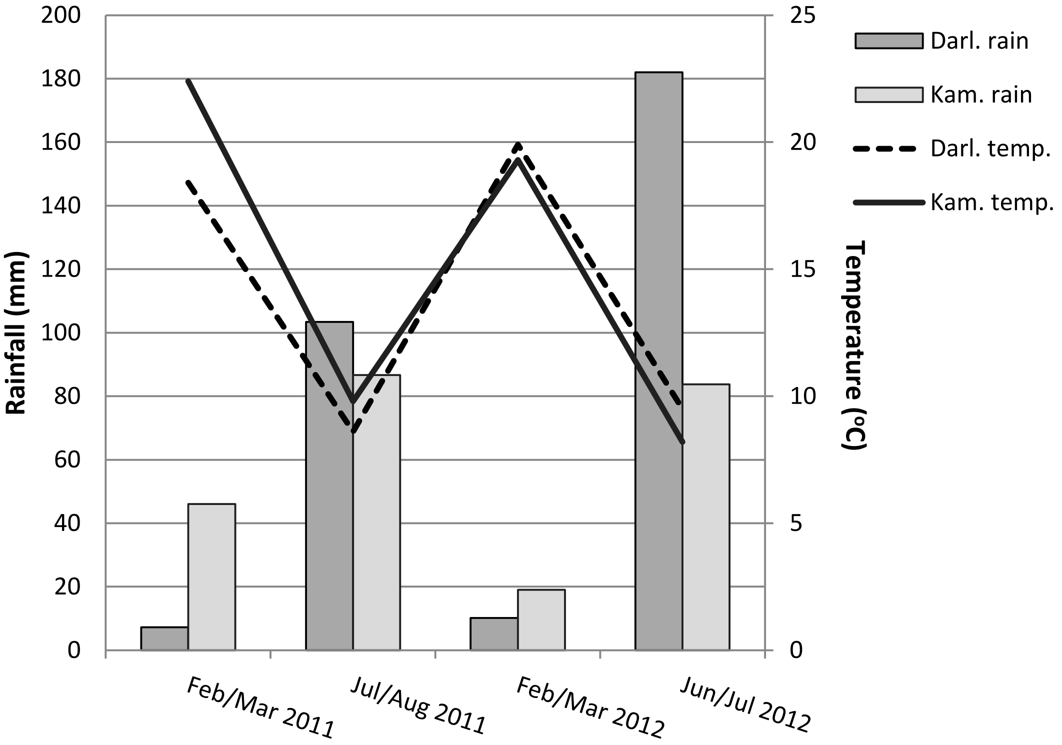

Figure 2.2: The temperature and rainfall variations at each location. (Abbreviation in the legend: Darl. refers to Darling, W.C. and Kam. refers to Kamieskroon, N.C.).

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GLMMs for the abundance and prevalence of *Androlaelaps* spp. had a w_i of 56% and 33% respectively (Tables 2.4 and 2.5), whereas the w_i of the best fitting GLMMs for *R. ensifera* were much lower (9% for abundance, 5% for prevalence, tables were placed in Appendix I due to size; Tables I and II). Furthermore, the next best-fitting model for *Androlaelaps* spp. abundance was more than three times less likely to fit the data (Table 2.4). The GLMMs for the influence of location and biotic factors on the flea *C. ingrami* were much more certain than those of the *Androlaelaps* spp. The best fitting models had higher w_i than those of *R. ensifera* (56% for abundance, 21% for prevalence, Tables 2.7-2.8). Although in the case of the abundance of these fleas, only the predictors in the top 2 models had any impact since the intercept model appears third in the list (Table 2.7). The best fitting model for the biotic predictors on *E. hilli* abundance was also relatively high (51%, Table 2.9). Yet the same predictors seemed to have little influence on the prevalence of these lice as the intercept

Table 2.5: The 95% confidence set of GLMMs for the prevalence of *Androlaelaps* spp. Contains number of parameters in the model (*k*), corrected Akaike value (AICc), difference between model and best fitting model (ΔAICc), Akaike weight (w_i) and evidence ratio (ER). The intercept model is included for reference.

Model	***k***	**AICc**	**ΔAICc**	w_i	**ER**
location + temperature + colony size + rainfall	6	104.340	0.000	0.332	-
location + sex + colony size + rainfall	6	104.975	0.635	0.241	1.374
location + body mass + colony size + rainfall	6	106.776	2.436	0.098	3.380
location x sex + colony size + rainfall	7	107.079	2.630	0.089	3.725
location + colony size + rainfall	5	107.165	2.918	0.077	4.301
location + breeding status + colony size + rainfall	6	108.991	4.651	0.032	10.232
location + temperature + body mass + rainfall	6	109.653	5.313	0.023	14.248
location x breeding status + colony size + rainfall	7	110.266	5.817	0.018	18.333
location x season + sex + colony size	7	111.558	7.218	0.009	36.922
location + season + sex + colony size	6	111.989	7.742	0.007	47.995
location + temperature + sex + rainfall	6	112.264	8.016	0.006	55.050
location x season + body mass + colony size	7	112.656	8.409	0.005	66.974
location + body mass + rainfall	5	112.932	8.592	0.005	73.413
location + sex + rainfall	5	113.442	9.102	0.003	94.746
location + season + body mass + colony size	6	113.599	9.150	0.003	97.018
location x season + colony size	6	113.627	9.287	0.003	103.907
(intercept)	2	127.145	23.081	3.2×10^{-6}	1.0×10^{5}

The '+' symbol indicates where predictors were added as main interactions. Any predictors connected by 'x' were employed in the model as a two-way (or three-way where three predictors are connected in this way) interaction term as well as separate main interactions.

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Table 2.6: The cumulative weight (w_i) of the predictors from the IT-based inference GLMMs for all parasite taxa. Predictors are split into abiotic and biotic factors. Those that appear in the best fit models are indicated using ~ and w_i above 70% are in bold.

	Androlaelaps **spp.**		***R. ensifera***		***C. ingrami***		***E. hilli***	
	Prev.	Abun.	Prev.	Abun.	Prev.	Abun.	Prev.	Abun.
location	**0.990** ~	**0.999** ~	**0.997** ~	**0.997** ~	**0.995** ~	**0.778** ~	**-**	**-**
rainfall	**0.979** ~	**0.983** ~	0.498	**0.815** ~	**-**	**-**	**-**	**-**
temperature	0.393 ~	0.048	0.249	0.287	-	-	-	-
colony size	**0.913** ~	**0.900** ~	0.158	0.256	-	-	-	-
sex	0.376	0.064	0.548 ~	0.258	**0.773** ~	0.046	0.271	**0.916** ~
br. status	0.077	**0.705** ~	0.450 ~	0.317	0.391	**0.855** ~	0.277	0.427
body mass	0.150	0.229	0.455 ~	0.373 ~	0.321	0.076	0.265	**0.802** ~

model fitted the data most accurately out of the candidate GLMMs (w_i of 39%), and each model containing a single predictor were all around 2.7 times less likely to be the best fitting model (Table 2.10).

The differences in parasite populations between the locations in the abundance and prevalence data were reflected in the results from the inference modelling for the mite genera. Location appeared in every model within the 95% confidence set of models for abundances and prevalence of both mite genera (Tables 2.4 and 2.5, see also Appendix I, Tables I and II). Furthermore the cumulative w_i for location in each set of models never dropped below 99% (Table 2.6). Location also had a cumulative w_i of above 75% for both the abundance and prevalence of *C. ingrami* (Table 2.6). Similarly, location was very important to *E. hilli* as it was only found within one habitat.

The two month rainfall total (total rainfall over the month of trapping and the month preceding it) had the highest cumulative w_i more often out of the various sets of GLMMs for the mite data and was retained for further analysis. The higher GLMMs for the abundance of *Androlaelaps* spp. indicate that rainfall had a large influence, particularly on its abundance as this factor appeared in all of the models within the 95% confidence set (Table 2.4). Rainfall had a cumulative w_i of 98% for both the abundance and prevalence for *Androlaelaps* mites (Table 2.6). Temperature, unlike rainfall, did not appear to be as

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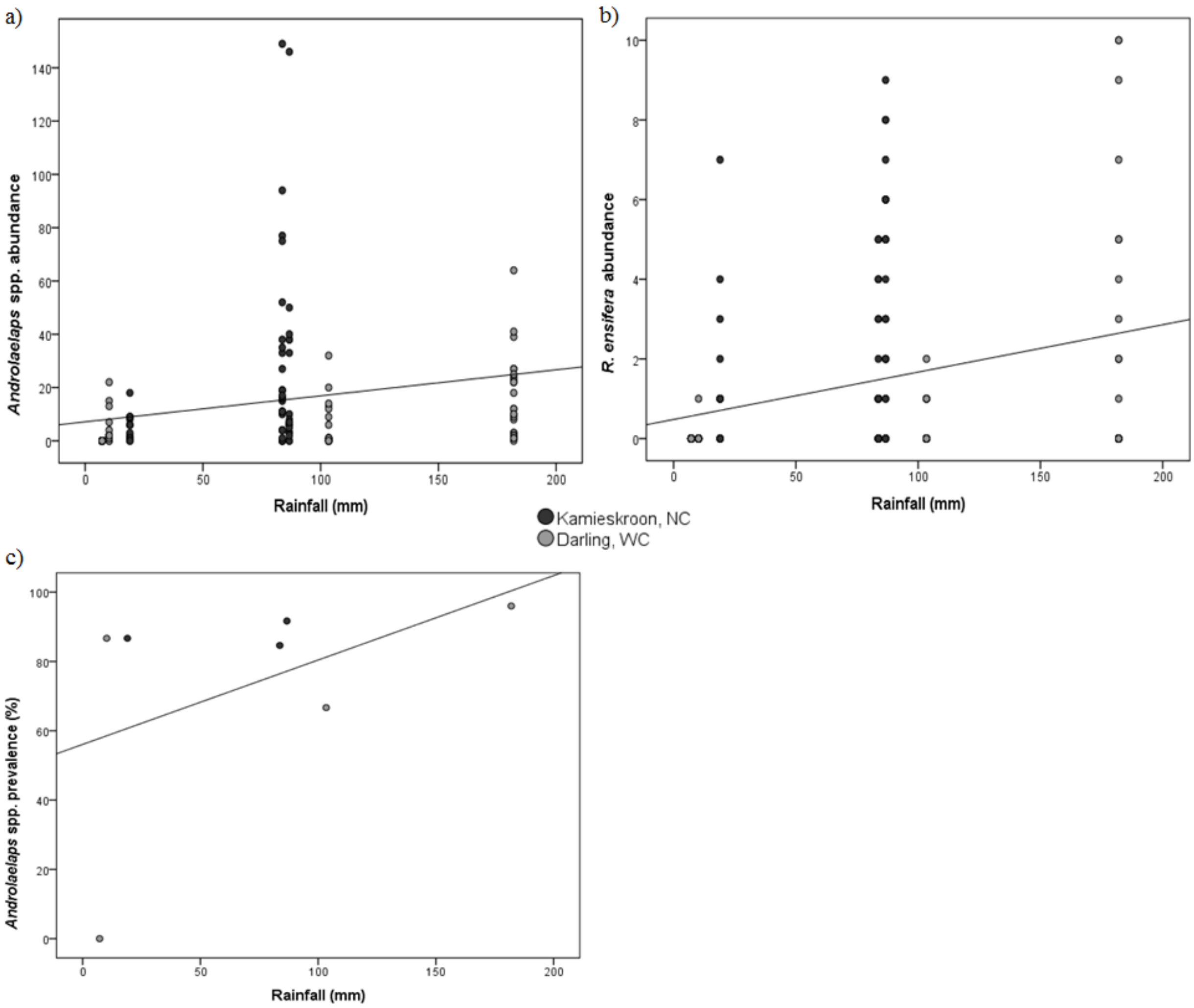

Figure 2.3: Variation in mite load in relation to total rainfall during the month of capture and the preceding month. a) *Androlaelaps* spp. abundance; b) *R. ensifera* abundance; c) *Androlaelaps* spp. prevalence. Data points have been separated by location.

Table 2.7: The 95% confidence set of GLMMs for the abundance of *C. ingrami*. Contains the number of parameters in the model (*k*), corrected Akaike value (AICc), difference between model and best fitting model (ΔAICc), Akaike weight (w_i) and evidence ratio (ER). The intercept model was part of this set.

Models	*k*	AICc	ΔAICc	w_i	ER
location + breeding status	4	278.669	0.000	0.559	-
location x breeding status	5	280.551	1.882	0.218	2.563
(intercept)	2	282.318	3.649	0.090	6.200
body mass	3	284.120	5.452	0.037	15.270
breeding status	3	284.126	5.458	0.037	15.316
sex + body mass	4	285.571	6.902	0.018	31.532

The '+' symbol indicates where predictors were added as main interactions. Any predictors connected by 'x' were employed in the model as a two-way (or three-way where three predictors are connected in this way) interaction term as well as separate main interactions.

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Table 2.8: The 95% confidence set of GLMMs for the prevalence of *C. ingrami*. Contains the number of parameters in the model (*k*), corrected Akaike value (AICc), difference between model and best fitting model (ΔAICc), Akaike weight (w_i) and evidence ratio (ER). The intercept model is included for reference.

Models	***k***	**AICc**	**ΔAICc**	w_i	**ER**
location x sex	5	169.743	0.000	0.208	-
location + sex	4	170.420	0.677	0.148	1.403
location	3	171.476	1.732	0.087	2.378
location x sex + breeding status	6	171.476	1.733	0.087	2.378
location x sex + body mass	6	171.881	2.138	0.071	2.912
location + sex + breeding status	5	172.048	2.305	0.066	3.166
location + sex + body mass	5	172.486	2.742	0.053	3.940
location + body mass	4	173.121	3.378	0.038	5.413
location + breeding status	4	173.134	3.391	0.038	5.448
location x sex + breeding status + body mass	7	173.340	3.597	0.034	6.039
location	5	173.400	3.657	0.033	6.223
location	6	173.770	4.027	0.028	7.490
location x breeding status + sex	6	174.057	4.313	0.024	8.641
location + sex + breeding status + body mass	6	174.058	4.315	0.024	8.648
location x breeding status + body mass	6	175.148	5.405	0.014	14.917
(intercept)	2	180.482	10.739	0.001	214.720

The '+' symbol indicates where predictors were added as main interactions. Any predictors connected by 'x' were employed in the model as a two-way (or three-way where three predictors are connected in this way) interaction term as well as separate main interactions.

influential on the abundance or prevalence of *Androlaelaps* spp. present on *C. h. hottentotus*. Although temperature appeared in the best fitting model for the prevalence of *Androlaelaps* spp. (Table 2.5), the w_i was below 40% (Table 2.6). This suggests that the cumulative w_i provided a more accurate indication of the predictors that were most influential on the data than the best fitting model alone. The abundance of *R. ensifera* was also largely influenced by rainfall (cumulative w_i of 82%), though temperature also appeared to not have a distinct impact on the abundance or prevalence of this species (Table 2.6). Despite the increased prevalence and abundance of parasites in the more arid location, rainfall had a positive influence on the abundances of *Androlaelaps* and *R. ensifera* mites (estimates ±SE; 0.017 ±0.004 and 0.015 ±0.006 respectively, Figures 2.3a and b). This pattern was repeated with the prevalence of *Androlaelaps* spp. (estimate ±SE; 0.049 ±0.017, Figure 2.3c). *C. ingrami* was only present in winter months, and the same was mostly the case for *E. hilli*. This suggests that abiotic factors do play an important role for the

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dynamics of these species, although it could not be explicitly tested which specific abiotic factors were significant.

Table 2.9: The 95% confidence set of GLMMs for the abundance of *E. hilli*. Contains the number of parameters in the model (*k*), corrected Akaike value (AICc), difference between model and best fitting model (ΔAICc), Akaike weight (w_i) and evidence ratio (ER). The intercept model is included for reference.

Models	***k***	**AICc**	**ΔAICc**	w_i	**ER**
sex + body mass	4	200.100	0.000	0.509	-
sex + breeding status + body mass	5	202.129	2.029	0.185	2.758
sex + breeding status	4	203.875	3.774	0.077	6.600
sex x breeding status + body mass	6	203.999	3.899	0.072	7.024
breeding status	3	204.796	4.696	0.049	10.465
Sex	3	204.883	4.783	0.047	10.930
(intercept)	2	289.384	89.284	$2.1\text{x}10^{-20}$	$2.4\text{x}10^{19}$

The '+' symbol indicates where predictors were added as main interactions. Any predictors connected by 'x' were employed in the model as a two-way (or three-way where three predictors are connected in this way) interaction term as well as separate main interactions.

Table 2.10: The 95% confidence set of GLMMs for the prevalence of *E. hilli*. Contains the number of parameters in the model (*k*), corrected Akaike value (AICc), difference between model and best fitting model (ΔAICc), Akaike weight (w_i) and evidence ratio (ER). The intercept model was part of this set.

Models	***k***	**AICc**	**ΔAIC**	w_i	**ER**
(intercept)	2	29.499	0.000	0.393	-
body mass	3	31.502	2.003	0.144	2.723
breeding status	3	31.532	2.033	0.142	2.764
sex	3	31.627	2.128	0.136	2.898
sex + body mass	4	33.642	4.143	0.050	7.938
breeding status + body mass	4	33.665	4.166	0.049	8.027
sex + breeding status	4	33.705	4.206	0.048	8.192

The '+' symbol indicates where predictors were added as main interactions. Any predictors connected by 'x' were employed in the model as a two-way (or three-way where three predictors are connected in this way) interaction term as well as separate main interactions.

Biotic factors did not appear to be as influential on the mite populations. Social factors appeared to play a role and colony size appeared in the best fitting GLMMs for both abundance and prevalence of *Androlaelaps* spp. (Tables 2.4 and 2.5). The cumulative w_i for

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this predictor was around 90% (Table 2.6). When analysed, the abundance of *Androlaelaps* mites was highest on individuals in smaller colonies of around three to six animals compared to larger colonies (Figure 2.4a). This pattern is repeated with the prevalence of the mites of this genus (Figure 2.4b). The breeding status of the host individuals also appeared to have an effect on the abundances of *Androlaelaps* spp. (cumulative w_i 71%, Table 2.6). It was found that breeding individuals of *C. h. hottentotus* tended to have higher abundances of *Androlaelaps* mites (Figure 2.5a). *R. ensifera* on the other hand was not influenced by biotic factors. Host colony size did not appear to affect the prevalence or abundance of *R. ensifera* (Appendix I, Tables I-II, cumulative w_i of 16% for prevalence and 26% for abundance, Table 2.6). Generalised linear models however, did indicate that the colony identity is a significant predictor for both the presence (*Androlaelaps* spp., estimate ±SE; 0.104 ±0.017, P = <0.001; *R. ensifera*, estimate ±SE; 0.030 ±0.010, P = 0.003), and abundance (*Androlaelaps* spp., estimate ±SE; 0.040 ±0.008, P = <0.0001; *R. ensifera*, estimate ±SE; 0.030 ±0.007, P = 0.015) of both mite taxa.

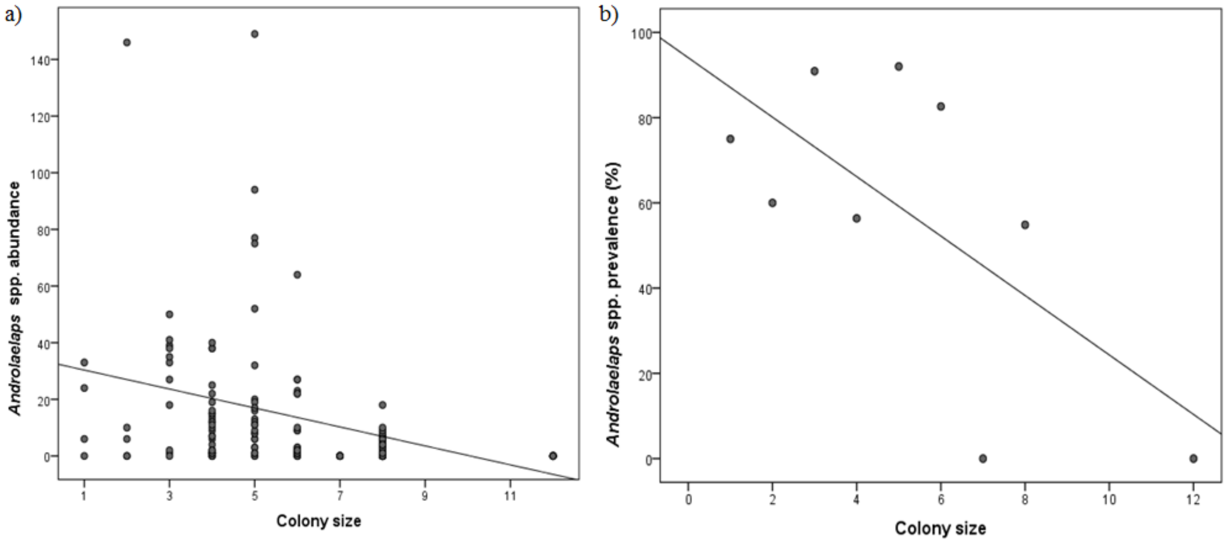

Figure 2.4: The variation of *Androlaelaps* spp. a) abundance and b) prevalence on *C. h. hottentotus* based on the colony size of the host.

The breeding status of the host individuals also appeared to have an effect on the abundances *C. ingrami*. Breeding status was calculated to have cumulative w_i of 86% in relation to flea abundance (Table 2.6). However, unlike *Androlaelaps* spp., breeding individuals were infected by fewer *C. ingrami* than their non-breeding counterparts, though

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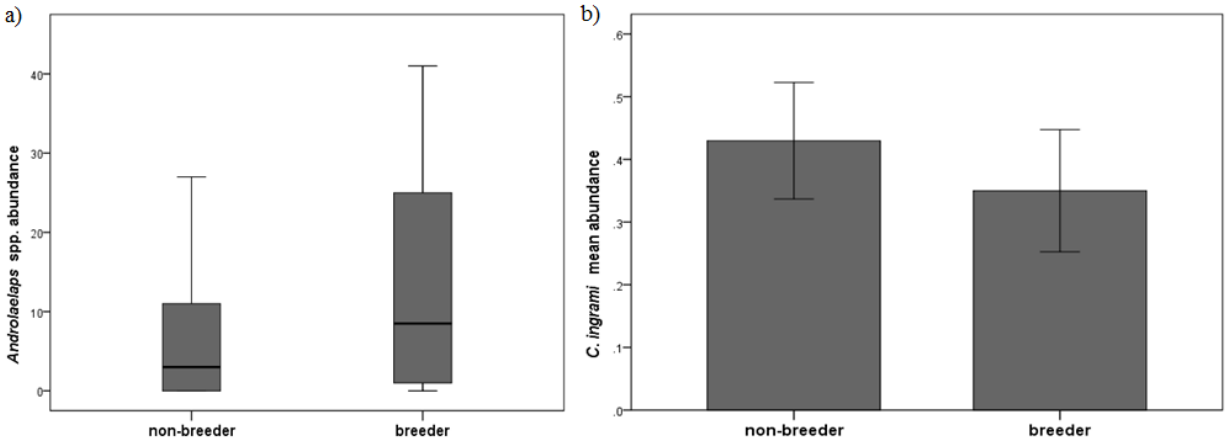

Figure 2.5: Differences in parasite burden based on host breeding status; a) the variation in abundance of *Androlaelaps* spp. on *C. h. hottentotus*. The middle bar, the box and the whiskers refer to the median, interquartile range and full range respectively, b) the variation in *C. ingrami* abundance on *C. h. hottentotus*. Error bars indicate ±std. error of the mean.

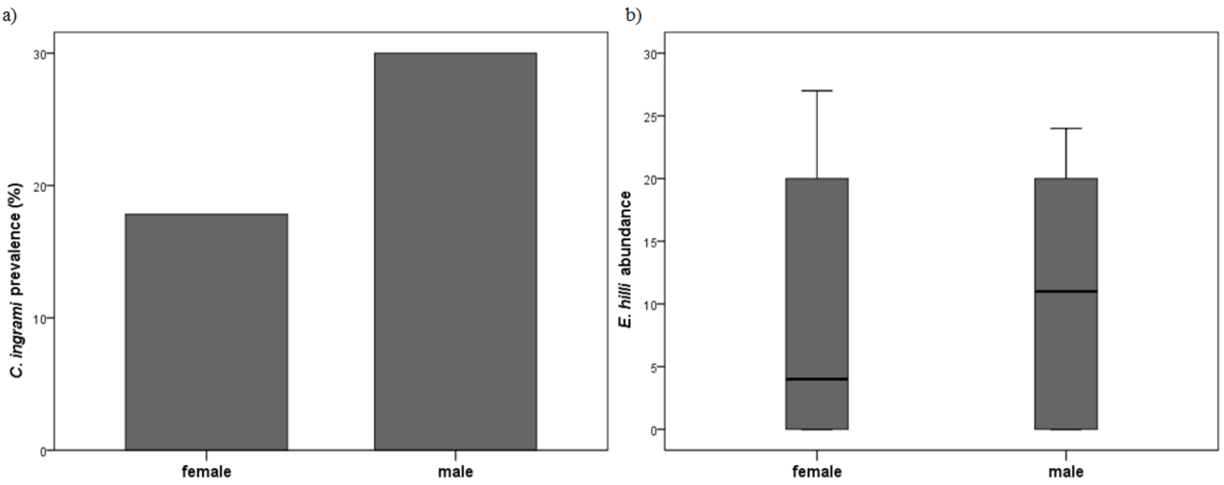

Figure 2.6: Host sex bias in a) *C. ingrami* prevalence on *C. h. hottentotus* in winter, and b) *E. hilli* abundance in Kamieskroon Jul/Aug 2011. The middle bar, the box and the whiskers refer to the median, interquartile range and full range respectively.

the difference does not appear to be as distinct as in the case of the mites (Figure 2.5b). Host sex was indicated as a significant predictor on the prevalence of *C. ingrami* with a cumulative w_i of 77% (Table 2.6). *C. ingrami* were found on male hosts more frequently than that of females (Figure 2.6a). Host sex was also found to have a very high cumulative w_i in relation to louse abundance (92%), alongside host body mass (80%, Table 2.6). *E. hilli*

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were, on average, more abundant on male hosts though the ranges of abundances were similar across both sexes (Figure 2.6b). Body mass generally had a positive effect on *E. hilli* abundance (Figure 2.7). Additionally, none of the biotic factors were indicated as a good predictor for the variation in *E. hilli* prevalence as the intercept model had the lowest AICc (Table 2.10).

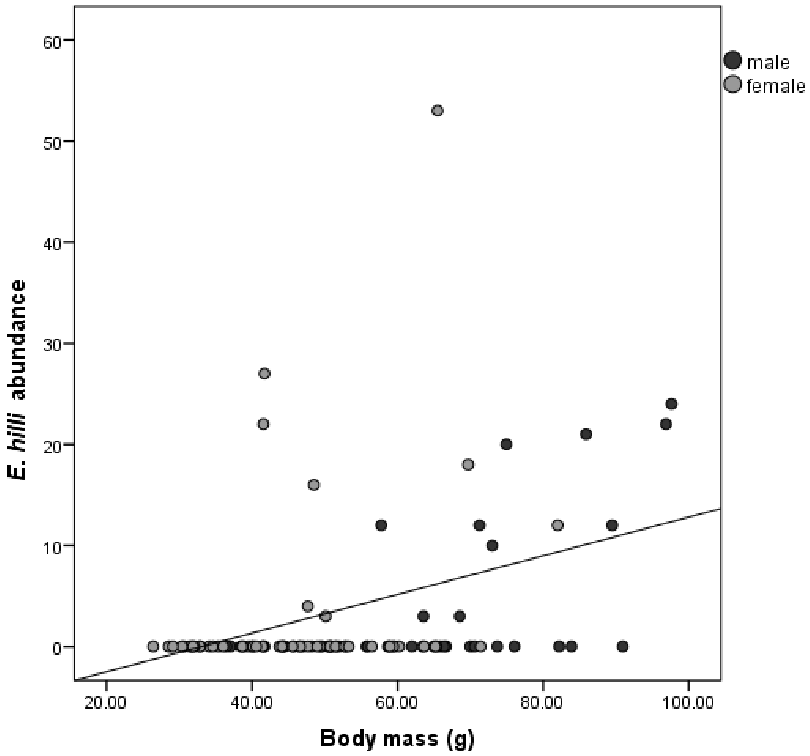

Figure 2.7: The variation in *E. hilli* abundance in relation to the body mass of the host *C. h. hottentotus* in winter months from Kamieskroon. Data points are distinguished by sex.

Discussion

The results of this study reflect the variation of the impact of abiotic and biotic factors on different ectoparasite taxa found in the literature. In particular, the dissimilarities in the influence of biotic factors on the different parasite populations associated with *C. h. hottentotus* were quite pronounced. However some clear patterns across taxa were apparent with respect to abiotic factors.

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Variation at the geographic scale

Location was indicated as a very important factor influencing both prevalence and abundance of all parasites that occurred in both communities. Contrary to expectations it was the *C. h. hottentotus* population in the arid habitat which were infected by a more prevalent and abundant ectoparasite community. This was also the site that had one more ectoparasitic species. It has been suggested that parasites may vary in abundance and richness across latitudinal distances, as many free-living species increase in diversity in latitudes closer to the equator (Bordes *et al*. 2010). However, there is increasing evidence that latitudinal distances are not enough to explain variation in abundance and species diversity of parasites (reviewed in Bordes *et al*. 2010; but see Poulin 2010). Studies looking at differences in ectoparasite assemblages across host populations have indicated that the distribution of parasites has developed through the co-evolution of host and parasite (Vinarski *et al*. 2007), and that the abundance of a parasite on a specific host is a trait specific to that host-parasite relationship (Krasnov *et al*. 2006; Korallo-Vinarskaya *et al*. 2009). Variations in abundance between host populations however, are most likely to be due to biotic differences between the populations or abiotic variations associated with the local habitats (Krasnov *et al*. 2006; Vinarski *et al*. 2007; Korallo-Vinarskaya *et al*. 2009). Yet it would be expected that the Kamieskroon population would harbour less ectoparasites when considering known host-related differences between the two populations. Decreased host population density is thought to lead to lower parasite burdens due to the reduced transmission opportunities available for parasites (Mohr & Adams 1963; Arneberg *et al*. 1998). Food resources for bathyergids in arid habitats are more patchy and widespread than in more mesic locations. Thus common mole-rat populations in more xeric habitats are less densely populated (Spinks *et al*. 2000). On the other hand it has been shown that parasites could influence growth rates and that smaller individuals can host larger parasite abundances (Perez-Orella & Schulte-Hostedde 2005). The Kamieskroon population had a lower average body mass than their counterparts in Darling; this is supported in another comparison of *C. h. hottentotus* populations in an arid and mesic habitat (Spinks *et al*. 2000a). The authors suggested this was an adaptation to life in an arid environment, since the animals were in good condition. However it could be possible that they have less energy to provide for growth due to increased parasite burdens (Perez-Orella & Schulte-Hostedde 2005). The work carried out on body mass and parasite burden has produced varied results

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though (Morand & Poulin 1998; Poulin & George-Nascimento 2007), so this interpretation may not be the most likely.

Several studies have revealed that the distribution of ectoparasites can be influenced across habitats by differences in abiotic factors between these habitats (Fabiyi 1996; Krasnov *et al*. 1997, 2008; Vinarski *et al*. 2007; Korallo-Vinarskaya *et al*. 2009). Although temperature varied little between habitats when collections were made at both locations during the same season, differences of one or two degrees Celsius can make a significant difference to the arthropod lifecycle (Marshall 1981; Krasnov *et al*. 2001; Lehane 2005; Krasnov 2008). Additionally more long term effects may have been induced to produce the differences between parasite populations at these locations. It has been shown that both fleas and mites tend to exist in higher abundances in areas of consistently higher temperatures, assuming that the temperatures do not get too high (Linardi & Krasnov 2013). Rainfall may play some role in this phenomenon; although the average annual rainfall in Kamieskroon is much lower than that at Darling and the fluctuation in rainfall is not as great as that seen in the more mesic region of Darling. Total rainfall during the summer months was lower in Darling, which may allow prevalence and abundance of parasites to decrease further. With more moisture in the ground throughout the summer in Kamieskroon the ectoparasite populations may be able to propagate more effectively and thus accumulate in much higher abundances in more favourable conditions. Humidity, influenced by rainfall, is thought to impact on the development of fleas and lice (Marshall 1981; Krasnov 2008), and the distribution of lice has been found to be due to differences in humidity in different habitats (Fabiyi 1996). There is also a possibility that the difference in parasite burden between habitats is associated with factors that have not been considered in this study. Regardless of the differences in the assemblages found on the two *C. h. hottentotus* populations, almost all of the parasite species found were shared between the two habitats. This reflects the co-evolution of host and ectoparasite seen in other studies (Vinarski *et al*. 2007; Krasnov *et al*. 2008; Korallo-Vinarskaya *et al*. 2009).

Abiotic factors: variation at the local scale

As expected, the abundance of both mite taxa increased in association with an increase in rainfall. In addition, the prevalence of *Androlaelaps* spp. was also positively linked to total rainfall. The AIC w_i indicate that this was a very strong predictor. Furthermore, the seasonal

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fluctuations in *C. ingrami* were associated with the wet winter season and *E. hilli* was almost exclusively found during the trapping session with the highest rainfall at that location (although it should be noted the variation between winter 2011 and winter 2012 was around 3mm). Despite this clear pattern across all five species the influence of temperature cannot be ruled out for the lice and fleas as this could not be assessed. A study on the highveld mole-rat (*C. h. pretoriae*) has revealed that the three *Androlaelaps* species and an anopluran louse (*Linognathus* sp.) were also more prevalent and abundant in the rainy season (Viljoen *et al*. 2011). The same pattern was also shown with *Androlaelaps* mites on the Damaraland mole-rat (*F. damarensis*) from three geographically distinct habitats (Carpenter-Kling 2012). *C h. pretoriae* and *F. damarensis* occur in summer rainfall regions, meaning temperature increases with rainfall (Bennett & Faulkes 2000; Viljoen *et al*. 2011; Carpenter-Kling 2012) unlike the habitats *C. h. hottentotus* inhabited in this study. The mites collected from both these previous studies included the two *Androlaelaps* spp. found on *C. h. hottentotus* (Viljoen *et al*. 2011; Carpenter-Kling 2012). This provides further evidence that the fluctuations in prevalence in abundance in the *Androlaelaps* mites are due to rainfall as opposed to temperature.

The positive influence of rainfall on parasites may result from an increase in the humidity of the host burrow system, which can influence the developmental stages of parasitic arthropods (Marshall 1981). Egg development of lice and mites in turn may be influenced by relative humidity, although optimal levels vary between species (Murray 1963; Nordenfors *et al*. 1999; Cueto *et al*. 2006; Halbritter & Mullens 2011). It is also well documented that low humidity largely reduces life span and decreases developmental rates of all stages in flea life cycles (Krasnov 2008). However the relative humidity in the burrow systems of other mole-rat species are high throughout the day (Sumbera *et al*. 2004; Bennett *et al*. 2009; Carpenter-Kling 2012) and fluctuations between seasons are very low (Sumbera *et al*. 2004; Carpenter-Kling 2012). It may therefore be unlikely that rainfall and in turn humidity influence the life cycles of the ectoparasites directly. An alternative explanation is that these parasite species have synchronised their breeding season with the dispersal of their host. Many ectoparasite species are known to adjust their breeding cycle to fit that of the host (Marshall 1981; Christe *et al*. 2000). Aggregation of individuals during this time can lead to increased opportunities for horizontal transmission and the potential to infect hosts with a naïve immune system (Christe *et al*. 2000). However, the only opportunity the ectoparasite community infecting *C. h. hottentotus* will have to

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disperse outside a host colony is when the host does itself, due to the limitations of the burrow system. Furthermore increased inter-colonial interactions may bring more host-dependent parasites such as some mites and lice into the colony through increased host contact for horizontal transmission (Freeland 1979). On the other hand the increase in ectoparasite abundance may be a consequence of the increased foraging activity that occurs during periods of higher rainfall (Spinks *et al*. 2000), meaning that the host hypothetically would have less time to perform grooming activities that reduce parasite burdens (Yamada & Urabe 2007).

Biotic factors: host sociality

Colony size was a significant predictor for the prevalence and abundance of *Androlaelaps* spp. Contrary to the hypothesis, the relationship between colony size and *Androlaelaps* spp. burden was negative. Fewer large colonies were captured which could have biased the data. Nevertheless, this pattern corresponds with the work of Viljoen *et al*. (2011) who found a similarly negative relationship between laelaptid mites and colony size for *C. h. pretoriae*. Reduction of ectoparasite load in larger groups of other mammalian hosts is also present in the literature (Mooring & Hart 1992; Bordes *et al*. 2007). Formation of social groups may lead to increased anti-parasite behaviours such as allogrooming to reduce the cost of potential increased parasite load (Loehle 1995; Sanchez-Villagra *et al*. 1998; Bordes *et al*. 2007; Hillegass *et al*. 2010). In particular such behaviour is thought to be beneficial for closely related familial groups (Loehle 1995). Alternatively larger colonies could result from lower dispersal rates in the host, leading to increased isolation of the parasites associated with such colonies. Previous study does suggest that colony size does not differ between sites despite differing dispersal rates (Spinks *et al.* 2000). However, this pattern could still occur on a local scale. This could lead to parasite infection being reduced, assuming that the benefit of less inter-colonial interaction outweighs that of potential increased transmission within the colony (Wilson *et al*. 2003). This hypothesis is supported by the current study as colony identity was significantly linked to parasite abundance and prevalence for both mite taxa. In addition, colony identity was also a significant predictor for *C. ingrami* and *E. hilli* abundances. Colony identity was found to be a significant predictor for *Androlaelaps* spp. burden on two other social bathyergid species (Viljoen *et al*. 2011; Carpenter-Kling 2012). It would appear that a subterranean habitat provides a

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distinctive barrier not only for host dispersal, but for parasite transmission in social mole-rats. Without further investigations, neither theory can be disputed.

Variation in the influence of colony size on parasites that differ in their dependence on the host was much harder to assess. *C. ingrami* prevalences were too low to carry out GLMMs on thus it was impossible to make comparisons with the one parasite taxa that is known not to spend the entire lifecycle on the host (Lehane 2005; Krasnov 2008). Nevertheless, there are indications that *R. ensifera* may spend time off host in the nesting material (Donelly 2011), even if the majority of the life cycle is thought to take place on the host (Oku *et al*. 1994). If this is the case, then it makes sense that colony size was not significantly linked to the abundance and prevalence of *R. ensifera*. Two studies have shown that fleas were not influenced by sociality of the host, whereas lice infecting the same host species had a negative association with the level of sociality (Van Vuren 1996; Monello & Gompper 2010). This was thought to be due to differences between the dependence on the parasite of the host and modes of transmission (Monello and Gompper 2010). Though transmission of both mites on *C. h. hottentotus* is thought to be direct contact (Oku *et al*. 1994; Dowling 2006), the myobiid mites may be similar to fleas and spend time within the nesting material. Thus they may be able to avoid anti-parasitic behaviour such as grooming more easily. The impact of colony size on *R. ensifera* could be reduced compared to that of *Androlaelaps* spp. The differences in the dependency of the two mite taxa on *C. h. hottentotus* are currently unknown however, as neither have been studied closely in regards to time spent on or off-host. The significance of colony identity for all the ectoparasites infecting the common mole-rat does infer that all of these species are dependent on host contact for transmission, which is unsurprising due to the restrictions of the burrow systems.

Biotic factors: host life-history traits

A distinct bias in parasitism by *Androlaelaps* spp. on breeding hosts was found. Increased parasitism in breeding animals has been found as a result of reduced immune function (Christe *et al*. 2000). There are different explanations for this; one is that the stress of breeding can lead to reduced immune function and thus lead to higher parasite loads at that time (Christe *et al*. 2000). Despite the fact that capture was not carried out in the peak breeding season for *C. h. hottentotus*, a few pregnant females (a total of three across the two

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years) were found. It is possible that social aggression in breeding individuals occurs during peak dispersal, which may increase parasitic load (Klein 2000). Alternatively there have been studies indicating increased parasite burden in animals with higher testosterone (Salvador *et al.* 1995; Hughes & Randolph 2001; Perez-Orella & Schulte-Hostedde 2005; Scantlebury *et al.* 2010). Evidence suggests that increased testosterone levels can lead to depressed immunity in certain individuals due to the negative effect of androgens on the immune system (Folstad & Karter 1992). Male and female breeding individuals of *C. h. hottentotus* have higher levels of testosterone than non-breeding individuals (Bennett 1989; Spinks *et al*. 1999). Therefore it is possible that breeding males and females may have depressed immunity due to higher circulating testosterone levels (Klein 2000).

Testosterone-induced immune depression could also explain the male-bias in parasitism found for *E. hilli* abundance and *C. ingrami* prevalence. Increased testosterone levels in male hosts have been attributed to sex-biased parasitism in several host-parasite relationships (Salvador *et al*. 1995; Hughes *et al*. 2001; Perez-Orella and Schulte-Hostedde 2005), including some involving fleas and lice (Hillegass *et al*. 2008; Khokhlova *et al*. 2010; Scantlebury *et al*. 2010). Flea feeding times and reproductive success can be improved by preferentially feeding from male hosts, which has been ascribed to differences in immune function between the sexes (Khokhlova *et al*. 2009). On the other hand *C. ingrami* appears to be more abundant on non-breeding individuals, which potentially refutes this hypothesis in the case of fleas. Sex-biased parasitism as a result of testosterone levels has not only been linked to immune function; increased testosterone levels can lead to behavioural differences including greater home range sizes in host species. This has been shown to increase potential host contact for horizontal transmission (Grear *et al*. 2009), or generally increase exposure to more parasites through increased home range (Hillegass *et al*. 2008). However, the host burrow system makes this unlikely in the study species. Testosterone has been shown to reduce self-grooming behaviour in some animals (Mooring *et al*. 2006; but see Leonard *et al*. 2005) thus lice and fleas may survive longer on male hosts due to decreased grooming rates. Conversely, breeding individuals within social groups (i.e. those highest in a dominance hierarchy) often receive higher levels of allogrooming (Madden & Clutton-Brock 2009; Newton-Fisher & Lee 2011), which may explain the bias of *C. ingrami* present on non-breeding hosts.

A further alternative is sexual dimorphism in size that can lead to one sex having higher parasite burdens than the other due to larger body mass (Moore & Wilson 2002; Harrison *et*

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al. 2010). Male *C. h. hottentotus* in this study were larger than the females. Though correlations between body mass and parasite infection have been mixed (Morand & Poulin 1998; Poulin & George-Nascimento 2007), body mass was positively linked to *E. hilli* abundance suggesting larger *C. h. hottentotus* can support higher numbers of lice. This may be a better explanation for *E. hilli* host-sex bias, since the range of *E. hilli* abundance was very similar between the sexes although the average was higher in males. Even within each sex, the abundance of lice infecting a host tended to increase with its body mass, although the few females that suffered from much higher abundances appear not to be the largest of the group. From our results, the largest females are the breeding individuals which may be groomed by other members of the colony more frequently thus leading to lower *E. hilli* abundances in the largest females. It appears that the aggregation in abundance of this ectoparasite could be due to a balance between host size and behaviour. Host sex-bias in parasites has been demonstrated in other small mammal species (Christe *et al*. 2000; Perez-Orella & Schulte-Hostedde 2005; Harrison *et al*. 2010; Scantlebury *et al*. 2010). Despite the large number of species that have been shown to suffer male-biased parasitism due to males being larger than females there are studies that show a negative (Perez-Orella & Schulte-Hostedde 2005) or non-significant (Krasnov *et al*. 2005; Poulin 2013) relationship between body mass and parasite abundance. Furthermore it has been shown that parasite-bias on male hosts may not be related to body mass at all (Scantlebury *et al*. 2010). Consequently the body mass argument may hold true for lice on *C. h. hottentotus*, but is questionable for other species within this parasite community.

Conclusions

In conclusion, the abiotic factors appear to have had a more consistent effect than biotic factors on the ectoparasite community of *C. h. hottentotus.* In particular rainfall seemed to have a significant impact on the seasonal variation of the prevalence and abundance of these species. Nevertheless the constraints of infecting a subterranean host mean it is probable these species are particularly dependent on the movements of the host. Therefore the ectoparasites are influenced by the amount of rainfall because the host depends on increased moisture in the soil for dispersal. Although this could not be tested for all the parasites present, the similar seasonal fluctuations despite the difference in life-cycles and host dependence suggest this is the case for all species. Influences of biotic factors on the

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other hand were not as repeatable across the parasite taxa. The results of this study do indicate that higher mite burden is not a cost of sociality for bathyergid species, perhaps due to the effective isolating barrier created by living underground. Yet for both social and host-related factors associated with the individual, it seems that the life-history of the parasite is important in considering the differences in aggregation between various parasite taxa. It is therefore imperative that studies are carried out on the life history of parasite species where they are unknown.

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Appendix I

Contains the 95% confidence interval tables for *R. ensifera*, as these were too large to fit in the results section.

Table I: The 95% confidence set of GLMMs for the abundance of *R. ensifera*. Contains the number of parameters in the model (*k*), corrected Akaike value (AICc), difference between model and best fitting model (ΔAICc), Akaike weight (w_i) and evidence ratio (ER). The intercept model is included for reference.

Model	***k***	**AICc**	**ΔAIC**	w_i	**ER**
location + body mass + rainfall	5	348.421	0.000	0.091	
location + rainfall	4	348.396	0.052	0.089	1.026
location + body mass + colony size + rainfall	6	349.472	0.958	0.056	1.614
location + breeding status + rainfall	5	349.499	1.078	0.053	1.714
location + temperature + body mass + rainfall	6	349.642	1.128	0.052	1.758
location + colony size + rainfall	5	349.581	1.160	0.051	1.786
location + temperature + rainfall	5	349.749	1.328	0.047	1.943
location + sex + body mass + rainfall	6	350.242	1.728	0.038	2.373
location + sex + rainfall	5	350.291	1.870	0.036	2.547
location + breeding status + body mass + colony size	6	350.508	1.994	0.034	2.710
location + breeding status + colony size + rainfall	6	350.588	2.074	0.032	2.821
location + temperature + breeding status + rainfall	6	350.784	2.270	0.029	3.111
location + temperature + colony size + rainfall	6	350.922	2.408	0.027	3.333
location + sex + breeding status + rainfall	6	351.490	2.976	0.021	4.428
location + sex + colony size + rainfall	6	351.510	2.996	0.020	4.473
location x breeding status + rainfall	6	351.544	3.030	0.020	4.549
location + temperature + sex + rainfall	6	351.678	3.164	0.019	4.865
location + temperature	4	352.010	3.666	0.015	6.253
location x sex + body mass + rainfall	7	352.345	3.722	0.014	6.430
location + temperature + body mass	5	352.177	3.756	0.014	6.540
location x sex + rainfall	6	352.382	3.868	0.013	6.917
location x breeding status + body mass + rainfall	7	352.613	3.990	0.012	7.352
location x breeding status + colony size + rainfall	7	352.667	4.044	0.012	7.553
location	3	352.377	4.094	0.012	7.745
location + body mass	4	352.444	4.100	0.012	7.768
location x breeding status + temperature + rainfall	7	352.857	4.234	0.011	8.306
location + breeding status + temperature	5	353.091	4.670	0.009	10.329
location + sex x breeding status + rainfall	7	353.537	4.914	0.008	11.670
location x sex + breeding status + rainfall	7	353.567	4.944	0.008	11.846
location + breeding status	4	353.290	4.946	0.008	11.858
location x breeding status + sex + rainfall	7	353.575	4.952	0.008	11.894
location x sex + colony size + rainfall	7	353.619	4.996	0.007	12.158
location x sex + temperature + rainfall	7	353.787	5.164	0.007	13.224
location + temperature + colony size	5	353.607	5.186	0.007	13.370

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location + temperature + body mass + rainfall	6	353.700	5.186	0.007	13.370
location + temperature + sex	5	353.899	5.478	0.006	15.472
location + temperature + sex + body mass	6	354.036	5.522	0.006	15.816
location + temperature + breeding status + body mass	6	354.252	5.738	0.005	17.619
location + body mass + colony size	5	354.191	5.770	0.005	17.904
location + sex + body mass	5	354.205	5.784	0.005	18.029
location + colony size	4	354.180	5.836	0.005	18.504
location + sex	4	354.286	5.942	0.005	19.511
location + breeding status + body mass	5	354.479	6.058	0.004	20.677
location + temperature + breeding status + colony size	6	354.630	6.116	0.004	21.285
location + temperature + sex + breeding status	6	355.080	6.566	0.003	26.656
location + breeding status + colony size	5	355.045	6.624	0.003	27.440
location x breeding status + temperature	6	355.140	6.626	0.003	27.467
(intercept)	2	362.349	14.112	7.8x10^{-5}	1159.797

The '+' symbol indicates where predictors were added as main interactions. Any predictors connected by 'x' were employed in the model as a two-way (or three-way where three predictors are connected in this way) interaction term as well as separate main interactions.

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Table II: The 95% confidence set of GLMMs for the prevalence of *R. ensifera*. Contains the number of parameters in the model (k), corrected Akaike value (AICc), difference between model and best fitting model (ΔAICc), Akaike weight (w_i) and evidence ratio (ER). The intercept model is included for reference.

Model	**k**	**AICc**	**ΔAIC**	**w_i**	**ER**
location x sex x breeding status + body mass	10	134.941	0.000	0.053	
location + rainfall	4	135.209	0.971	0.033	1.625
location x sex x breeding status + rainfall	10	136.142	1.200	0.029	1.822
location + body mass + rainfall	5	135.738	1.423	0.027	1.998
location x sex + body mass + rainfall	7	136.220	1.704	0.026	2.037
location x sex + rainfall	6	136.273	1.865	0.026	2.047
location + breeding status + body mass + rainfall	6	136.298	1.891	0.023	2.344
location x sex + breeding status + body mass	7	136.749	2.233	0.022	2.449
location + temperature	4	136.916	2.679	0.021	2.511
location x sex x breeding status	9	137.541	2.758	0.021	2.541
location + temperature + rainfall	5	137.189	2.875	0.021	2.574
location x sex + body mass	6	137.285	2.877	0.020	2.615
location+ colony size + rainfall	5	137.205	2.891	0.017	3.054
location	3	137.138	2.962	0.017	3.078
location + breeding status + rainfall	5	137.280	2.965	0.015	3.507
location + sex + rainfall	5	137.284	2.969	0.014	3.723
location x sex x breeding status + temp	10	137.970	3.029	0.014	3.817
location + sex + body mass + rainfall	6	137.499	3.092	0.014	3.844
location + temperature + body mass + rainfall	6	137.581	3.174	0.013	3.971
location x breeding status + body mass + rainfall	7	137.759	3.243	0.013	4.210
location + body mass + colony size + rainfall	6	137.685	3.278	0.013	4.215
location x sex	5	137.648	3.334	0.013	4.243
location + temperature + body mass	5	137.685	3.371	0.012	4.279
location + body mass	4	137.665	3.427	0.012	4.398
location x sex + temperature	6	137.860	3.453	0.012	4.404
location x sex + temperature + body mass	7	138.040	3.524	0.012	4.413
location x sex + temperature + rainfall	7	138.264	3.748	0.012	4.547
location x sex + colony size + rainfall	7	138.328	3.812	0.011	4.692
location x sex + breeding status + rainfall	7	138.360	3.844	0.011	4.889
location + temperature + breeding status + body mass	6	138.309	3.901	0.011	4.946
location + breeding status + body mass	5	138.316	4.001	0.011	5.060
location x breeding status + rainfall	6	138.951	4.543	0.010	5.150
location x sex x breeding status + colony size	10	139.578	4.637	0.010	5.295
location + temperature + breeding status	5	138.979	4.664	0.010	5.394
location + temperature + colony size	5	138.979	4.665	0.010	5.442
location + temperature + sex	5	138.990	4.675	0.010	5.521
location + sex + breeding status + body mass	6	139.202	4.795	0.010	5.549
location + temperature + colony size + rainfall	6	139.206	4.798	0.010	5.563
location + temperature + breeding status + rainfall	6	139.279	4.872	0.009	5.620
location + temperature + sex + rainfall	6	139.281	4.874	0.009	5.803
location x sex + body mass + colony size	7	139.393	4.877	0.009	5.825

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location+ breeding status + colony size + rainfall	6	139.295	4.888	0.008	6.434
location + sex + colony size + rainfall	6	139.297	4.890	0.008	6.513
location + sex + body mass	5	139.225	4.911	0.008	6.644
location + colony size	4	139.182	4.944	0.008	6.725
location + sex	4	139.196	4.958	0.008	6.756
location + breeding status	4	139.198	4.961	0.008	6.813
location + sex + breeding status + rainfall	6	139.369	4.962	0.008	6.833
location + temperature + sex + body mass	6	139.495	5.087	0.008	6.978
location x breeding status + body mass	6	139.605	5.197	0.008	7.021
location x breeding status + temperature + body mass	7	139.783	5.267	0.008	7.034
location x sex + colony size	6	139.711	5.303	0.008	7.052
location x sex + breeding status	6	139.736	5.328	0.007	7.394
location + temperature + body mass + colony size	6	139.746	5.339	0.006	8.223
location x sex + temperature + breeding status	7	139.938	5.422	0.006	8.897
location + body mass + colony size	5	139.739	5.424	0.005	9.695
location x sex + temperature + colony size	7	139.965	5.449	0.005	9.726
location x breeding status + sex + body mass	7	140.377	5.861	0.005	9.981
location + breeding status + body mass + colony size	6	140.388	5.981	0.005	10.160
location x breeding status + temperature	6	140.664	6.257	0.005	10.239
location x breeding status + temperature + rainfall	7	140.944	6.428	0.005	10.297
location x breeding status	5	140.756	6.441	0.005	10.301
(intercept)	2	148.224	14.093	7.4x10^{-5}	1149.29

The '+' symbol indicates where predictors were added as main interactions. Any predictors connected by 'x' were employed in the model as a two-way (or three-way where three predictors are connected in this way) interaction term as well as separate main interactions.

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Chapter 3

Effects of ectoparasitic infections on the androgen and glucocorticoid levels of the common mole-rat in relation to differences in habitat, season, host sex and status

Abstract

In recent years, research into the relationship between host physiology and parasite infection has increased, both to analyse the impact that infection has on the host and to deduce the mechanisms that promote parasite aggregation. Glucocorticoids (e.g. cortisol and corticosterone) and testosterone have been central in the investigation, due to the former having a fundamental role in the stress response and the fact that each is involved in the general realm of immunosuppression. However, several factors that are important to parasite population dynamics also have significant influences on these hormones within the host. For example, testosterone and glucocorticoids have been found to fluctuate seasonally, a pattern that has also been shown with parasites. Therefore the influence a parasitic infection has on host endocrinology, and vice versa, should be analysed whilst considering the variation caused by factors that may also influence parasite dynamics and host endocrinology. *Cryptomys hottentotus hottentotus* is a subterranean rodent that lives in social colonies that display reproductive skew. Two populations from differing habitats were captured to analyse the impact the various ectoparasite taxa had on cortisol and testosterone concentrations of the host. Other factors such as season, host sex, social (breeding) status, body mass and age were included in the analyses. The results indicated that the relationship between ectoparasites and androgen levels of their subterranean host were not very strong in comparison to the effects of sex and breeding status of the host. However, differences between the habitats (e.g. amount of rainfall, distribution of food resources) appear to have created fundamental differences between the populations as the pressures placed on the host differ across the distributional range. There was less seasonal fluctuation in hormones than anticipated. Cortisol concentrations were positively correlated

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to flea (*Cryptopsylla ingrami*) abundance though only within a more stressful (arid) habitat. Infection of *Androlaelaps* mites on the other hand had a negative impact on testosterone concentrations in the population inhabiting the mesic site. Overall, increased cortisol concentrations appeared to be much more closely tied to stressors that impacted energy consumption whereas testosterone appeared to be associated with social aggression. Future studies analysing the relationship between parasitic infection and host endocrinology should consider potential confounding factors, such as differences associated with habitat, season, host sex and social status as they appear to have great bearing on the true impact that parasites may have on their host.

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Introduction

An individual has a limited amount of energy with which it can function. Accordingly, its various physiological demands (e.g. reproductive function, immune system) are within a constant trade-off situation with one another so that the individual can function effectively (Stearns 1989). The endocrine system plays an integral role in mediating physiological systems. For instance, testosterone is not just important for the development of reproductive organs in males, but also for channelling resources into reproduction and secondary sexual characteristics involved with breeding success (Klein 2000; Roberts *et al.* 2004). Testosterone has also been linked to territoriality or male-male aggression for mates during the breeding season (challenge hypothesis; Wingfield *et al.* 1990). In social groups, increased testosterone may be correlated with aggression utilised to maintain dominant behaviour (Creel *et al.* 1993). However, it has been suggested that raised testosterone concentrations can increase susceptibility and/or exposure to parasitic infections (Klein 2000). It has been posited that males with higher testosterone levels would expend more effort on reproductive display and/or aggression, but would suffer increased parasitism due to immune depression (immunocompetence handicap hypothesis or ICHH; Folstad & Karter 1992). Testosterone is also thought to increase host exposure to parasites through behavioural mechanisms. For example mobility and home range are often positively correlated with testosterone titres in vertebrates (Grear *et al.* 2009), which may consequently increase chances of contact with conspecifics. An increase in the frequency of contact raises the potential for horizontal transfer of parasites between hosts (Grear *et al.* 2009; Godfrey *et al.* 2010). Similarly aggressive encounters can also provide potential opportunities for parasites to transfer to a new host (Klein 2000). There are studies that indicate that parasite load is positively correlated to host testosterone as anticipated by these findings (Salvador *et al.* 1995; Mooring *et al.* 1996; Hughes & Randolph 2001; Muehlenbein & Watts 2010). However, not all associations between testosterone and parasite burden are positive. Negative correlations between parasite load and testosterone concentrations in the host have been suggested to be due to a trade-off between parasite resistance and reproductive effort; either the host adapts to parasite infection by applying more energy to immune function in comparison to breeding (Lutermann *et al.* 2012) or parasites may depress reproductive function in their host (Morales *et al.* 1996; Pena *et al.* 2007). In some studies testosterone has even been found to be immuno-enhancing (Evans *et al.* 2000; Ezenwa *et al.* 2012). Additionally, testosterone can influence certain aspects of

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immunity differently (Klein 2000; Sapolsky *et al*. 2000; Fuxjager *et al*. 2011) and different parasite taxa may go through intra-specific competition (Pollock *et al*. 2012). Hence different relationships between parasite taxa and steroid hormones may be observed on a single host species (Hoby *et al*. 2006; Fuxjager *et al.* 2011; Ezenwa *et al*. 2012; Pollock *et al*. 2012).

Interactions between parasite infection and glucocorticoids may be even more complex. Glucocorticoids play a significant role in sustaining allostasis (defined as 'stability through change' and considered part of homeostasis; McEwen 1998). Glucocorticoids such as cortisol and corticosterone are secreted via the hypothalamic-pituitary-adrenal (HPA) axis in response to adverse external stressors, such as predation and extreme weather conditions (Sapolsky *et al.* 2000; Romero 2004). They direct energy away from non-essential processes and make it available for evading potential stressors, for instance releasing more glucose for the muscles to enable escape from predatory attack (McEwen 1998; Sapolsky *et al*. 2000; Romero 2004). Once the stressor has passed, glucocorticoids return to baseline levels via negative feedback (Sapolsky *et al*. 2000; Romero 2004). Although acute stress responses are beneficial, chronically elevated levels can disrupt the negative feedback loop such that glucocorticoid levels remain above baseline levels. Thus response to subsequent stressors is not necessarily as effective and constant elevations in glucocorticoids may be detrimental to immune function (Romero 2004). Parasites potentially represent a chronic stressor (Frandsen 1987), or may be able to infect a host more intensely if chronic levels of glucocorticoids are already circulating through a host's blood stream (Quillfeldt *et al*. 2004; Dubansky *et al*. 2011). Though there are systems where anticipated positive influences occur (Fleming 1997; Bowers *et al.* 2000; Dubansky *et al*. 2011), there are also systems that display non-significant results, or results opposing expectations (Grutter & Pankhurst 2000; Haond *et al*. 2003; Pena *et al*. 2007). As with testosterone, there are studies that have discovered different reactions from various parasite taxa infecting one host species (Hoby *et al*. 2006; Fuxjager *et al*. 2011). Research into parasite infection and glucocorticoids have also taken into account that stress responses can decrease circulating testosterone in order to repress reproductive function. A study on house sparrows (*Passer domesticus*) found that testosterone produced an enhancing effect on antibody production when corticosterone (primary glucocorticoid in reptiles and birds) was controlled for, inferring that glucocorticoids mediate immunocompetence (stress-mediated ICHH; Evans *et al*. 2000). Though glucocorticoids are known to have depressive effects on testosterone secretion

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(Bambino & Hsueh 1981), correlations between testosterone and glucocorticoids in the literature do not always exist or may be positive as opposed to negative (Poiani *et al*. 2000; Berger *et al*. 2005). Few studies have analysed this hypothesis directly with parasitic infection however, there is some support for the stress-mediated ICHH in such work (Bortolotti *et al*. 2009; Cárdenas *et al*. 2012).

Parasite abundance is not only tied to host endocrinology. Seasonal fluctuations in weather conditions (Krasnov *et al*. 2005; Matthee *et al*. 2007), and differences between hosts based on their sex (Schalk & Forbes 1997; Krasnov *et al*. 2005; Hillegass *et al*. 2008), and social status (Muehlenbein & Watts 2010) are all known to influence parasite prevalence and abundance. Interestingly, each of these factors is likely to influence testosterone and glucocorticoid concentrations in the host as well. For example, seasonal peaks in parasite abundance amongst a host population are common (Krasnov *et al*. 2005; Matthee *et al*. 2007; Viljoen *et al*. 2011). One possible reason for this is that macroparasites align breeding with that of their host to take full advantage of transmission opportunities from close host contact (Marshall 1981, Christe *et al*. 2000). Alternatively the increase in parasite abundances has also been attributed to seasonal increases in testosterone (Salvador *et al*. 1995; Mooring *et al*. 1996; Negro *et al*. 2010). Particularly in males, testosterone increases following intra-sexual encounters resulting from competitive aggression during the breeding season (Wingfield *et al*. 1990, 1997). Though much of the original work was carried out on birds, there is also evidence for this in mammals and other vertebrates (Hirschenhauser & Oliveira 2006) and is not exclusive to males (Shargal *et al*. 2008; Lutermann *et al*. 2013). Glucocorticoids on the other hand can peak following adverse conditions, particularly annual patterns that involve low food availability (Huber *et al*. 2003; Hoby *et al*. 2006; Pedersen & Greives 2008). One such example is the winters in temperate regions, where deciduous plants are food sources and glucocorticoids may be released to aid survival through winter (Huber *et al*. 2003; Hoby *et al*. 2006). However the majority of seasonal peaks of glucocorticoids in most vertebrates are associated with the breeding season (Romero 2002). This pattern is not as distinct in mammals as it is in other vertebrate classes (Romero 2002). Though some recent work on social primates indicate that cortisol increases during the mating season (Ostner *et al*. 2008; Clough *et al*. 2010), work in small mammals specify that cortisol reaches peak either prior to, or after the breeding season (Boswell *et al*. 1994; Kenagy & Place 2000; Place & Kenagy 2000; Soto-Gamboa *et al*. 2005; but see Vera *et al*. 2013). The mechanisms behind seasonal

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fluctuations in glucocorticoids are not fully understood (Romero 2002). In some birds glucocorticoids have been directly linked to aggressive behaviour and thought to be linked to the challenge hypothesis (Sorenson *et al*. 1997; Meddle *et al*. 2002). It has been posited that these seasonal peaks are due to the increased need the individual possesses for energy resources stored in the body, such as breeding which is energetically expensive (Romero 2002). An alternative explanation is that basal corticosteroid concentrations increase for anticipated events where it may aid responses to stressors. Though chronic elevation of cortisol can be harmful, a slight increase in concentrations may help to prepare stress responses when stressors are predictable (Sapolsky *et al*. 2000), such as intra-sexual competition for the breeding season (Romero 2002).

Due to testosterone playing an integral role in sexual development and intra-sexual competition in males, this androgen has been implicated in male-biased parasitism (Folstad & Karter 1992; Salvador *et al*. 1995). Glucocorticoids generally display the opposite trend (Reeder & Kramer 2005) though it has not been implicated as a mechanism for female bias in parasitism where such situations exist. On the other hand, both of these steroids have not been well analysed in the context of individual status within social groups and how this compares with parasite load. Within true social groups there tends to be a form of hierarchy (Kutsukake 2008). This may be a few individuals that have higher status and obtain the rights to breed or get access to better food resources compared to subordinates (Creel *et al*. 1993; Kutsukake & Clutton-Brock 2006). Those of higher status tend to have higher individual fitness than others, though this position may have to be maintained via costly and stressful behaviours such as aggression (Ellis 1995).

Studies that have covered the impact social status has on parasite infection and testosterone and glucocorticoids have considered a range of social structures (Clough *et al*. 2010, Negro *et al*. 2010, Muehlenbein & Watts 2010; Setchell *et al*. 2010). Though a relationship does not always exist, it has generally been found that individuals of a higher social status or those that exhibit more agonistic behaviour (i.e. territorial males) have higher testosterone levels compared to others in the group (Buhl *et al*. 1978; Creel *et al*. 1992; Clarke & Faulkes 1997; Soto-Gamboa *et al*. 2005; Muehlenbein & Watts 2010; Negro *et al*. 2010). This relationship may only exist during times of social instability (Higham *et al*. 2013). This is likely to be due to aggressive behaviour that is necessary to maintain a higher status within the group (Creel *et al*. 1993). Though testosterone has also been linked to aggression in female vertebrates (Shargal *et al*. 2008), social status has been shown to have negative

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effects on testosterone concentrations of female members (Koren & Geffen 2009) as well as positive effects (Shargal *et al*. 2008; Lutermann *et al*. 2013). However social correlates to testosterone concentrations in females are extremely underrepresented in the literature (Lutermann *et al*. 2013). Therefore studies involving parasite and hormonal correlates to social status have exclusively been carried out on males. Territorial males in New Zealand fur seals have been found to have higher intestinal parasite loads than transient competitors, which was positively correlated to testosterone (Negro *et al*. 2010). Similarly, impala (*Aepyceros melampus*) that defend harems during the breeding season have higher tick loads than bachelor males. Though this was linked to reduced auto- and allogrooming rates in territorial males, the authors did note that grooming behaviour had been negatively correlated to testosterone concentrations in other ungulates (Mooring *et al*. 1996). Dominant individuals in chimpanzee (*Pan troglodytes*) hierarchies have also been found to possess higher numbers of parasite species, both of which were also correlated to testosterone concentrations in the host (Muehlenbein & Watts 2010). This has not held true for all primate species (Ostner *et al*. 2008; Clough *et al*. 2010; Benavides *et al*. 2012). Testosterone also appears to be unrelated to rank in laboratory mice (*Mus musculus*) and unaffected by experimental parasitic infection of trichostrongyloid nematodes (Barnard *et al*. 1998).

The influence of glucocorticoids on social status and parasite load (and vice versa) is still dominated by studies on males. In an experiment by Barnard *et al*. (1998), both low-ranking and high-ranking male mice naturally dropped glucocorticoid levels after being placed within a novel social environment. However, this changed once some subjects had been experimentally infected with parasites. Glucocorticoid levels in infected low-ranking mice did not drop as significantly as control subjects, whereas the more aggressive high-ranking individuals actually suffered a rise in glucocorticoids as a consequence of parasitic burden (Barnard *et al*. 1998). Links between parasite infection and individual cortisol levels have also been indicated in social primates though social status was not indicative of cortisol levels (Muehlenbein & Watts 2010; Setchell *et al*. 2010). Nevertheless, cortisol has been observed to be higher in territorial males, potentially due to the energetic costs associated with defending territory or females (Soto-Gamboa *et al*. 2005). Yet unlike testosterone, cortisol has been found to be higher in individuals of higher (Clarke & Faulkes 1997; Creel 2005) and lower (Virgin & Sapolsky 1997; Young *et al*. 2006) social status. It has been suggested that the influence of social status is only secondary to the cost of

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maintaining allostasis and thus depends on the situation in question (Goymann & Wingfield 2004). Where there is no difference in sustaining allostasis between individuals of different status, there will be no significant correlation between glucocorticoids and social status (Goymann & Wingfield 2004). Who suffers higher cortisol concentrations may additionally depend on social stability (Sapolsky 2005). In spotted hyaenas (*Crocuta crocuta*), lower ranking females had higher faecal corticosterone levels, though this pattern was lost during times of social stress (Goymann *et al.* 2001). Correspondingly in mandrills (*Mandrillus sphinx*), subordinates have higher cortisol levels when the social group was stable, though the pattern reversed when the social environment become unstable (Setchell *et al*. 2010). In rhesus macaques (*Macaca mulatta*), cortisol only became positively correlated to dominance when aggression increased due to competition for social status (Higham *et al*. 2013).

With several factors influencing cortisol and testosterone that are simultaneously connected to parasite dynamics, it raises a question as to how great an impact the parasites truly have on endocrine fluctuations and vice versa. Though studies are increasingly including confounding factors (e.g. Quillfeldt *et al*. 2004; Setchell *et al*. 2010), much of the earlier work looked for direct associations between testosterone or cortisol to parasite load without considering environmental and host-related aspects. Studies assessing the variation in testosterone and cortisol concentrations in light of parasitic infection in mammals with reproductive skew (where individuals can be classed as breeders and non-breeders) are also seriously lacking. Therefore this study aimed to improve on current knowledge in these areas. The effects of parasite infection on cortisol and testosterone levels in the common mole-rat (*Cryptomys hottentotus hottentotus*) were analysed. The common mole-rat is a herbivorous cooperatively breeding rodent that inhabits subterranean burrow systems that are constructed whilst foraging for bulbs and tubers (Spinks *et al*. 1999; Bennett & Faulkes 2000). A colony consists of one breeding female and between one or two breeding males with several non-breeding helpers (Spinks *et al*. 2000; Bishop *et al*. 2004). The majority of the non-breeders are offspring of the breeding individuals, though there are also unrelated individuals that have been accepted into the colony (Spinks *et al*. 1999; Bishop *et al*. 2004). Non-breeding individuals are physiologically able to breed (Spinks *et al*. 1997, 1999, 2000a). Thus reproductive skew in colonies is likely to be maintained via a combination of inbreeding avoidance (Spinks *et al.* 1999) and possibly social aggression (e.g. Creel *et al.* 1993). Unlike most social cryptomids, *C. h. hottentotus* is a seasonal breeder (Spinks *et al.*

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1997, 1999). The breeding season occurs between September and November, which is subsequent to annual peak rainfall (Spinks *et al*. 1997, 1999). Data from the wet season was collected during peak rainfall and prior to the breeding season.

C. h. hottentotus has been well studied in terms of its ecology. In more arid environments, food resources are more spread out than in mesic regions. Since the majority of the rain falls within a small number of months, peak rainfall leads to a greater increase in foraging activity in *C. h. hottentotus* from arid areas compared to more mesic habitats (Spinks *et al*. 2000). Further the lack of rainfall puts higher constraints on dispersal in arid habitats (Spinks *et al*. 2000b). However, less work has been carried out to investigate the concentrations of testosterone and cortisol in *C. h. hottentotus*. Testosterone levels are higher in breeding females during the reproductive season (Spinks *et al*. 1999), which has been seen in other female bathyergids (Lutermann *et al*. 2013). Additionally, it has been shown that breeding males have higher testosterone levels than non-breeding males (Bennett 1989) though seasonal fluctuations have not been measured. Males of highveld mole-rats (*C. h. pretoriae*), the only other known social and seasonal breeding species in the Bathyergidae, do not display a seasonal peak in testosterone (Janse van Rensburg *et al*. 2002), which means male common mole-rats may not display the same pattern as females. Males of solitary mole-rat species, which are all seasonal breeders, have provided mixed results in this regard (Herbst *et al*. 2004; Hart *et al*. 2006; Oosthuizen & Bennett 2009). Cortisol concentrations in bathyergids on the other hand have received little attention making predictions more difficult. It has been found that cortisol and testosterone concentrations are positively correlated to social rank in the linear hierarchy of the naked mole-rat (*Heterocephalus glaber*), where breeding individuals are at the top of the linear hierarchy (Clarke & Faulkes 1997). Though the social hierarchy is almost linear in *C. h. hottentotus*, the division of labour is not as distinct as that seen in *H. glaber* (Bennett 1989; Rosenthal *et al*. 1992). Furthermore, female non-breeding Damaraland mole-rats (*Fukomys damarensis*) have been shown to increase urinary cortisol concentrations during the wet season (Young *et al*. 2010). This was suggested to be due to increased foraging activity and therefore higher energetic demands. Alternatively, the increase in cortisol may be the result of receiving elevated levels of aggression from the breeding female that were potentially linked to maintaining breeding monopoly or forcing dispersal (Young *et al*. 2010). Only one study has been carried out with relation to hormone levels in mole-rats and parasite infection. This research indicated that circulating cortisol in *C. h. pretoriae* only increases

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with the initial infection, but does not remain chronically elevated as this could be detrimental to the host (Lutermann *et al*. 2012). Analyses from the previous chapter did indicate that *C. h. hottentotus* in the arid habitat suffered from higher parasite loads than individuals in the mesic site. In addition to parasites, the impact of season, host sex and breeding (social) status on testosterone and cortisol were also considered, along with the age and body mass of the host. C. *h. hottentotus* were collected from two different locations (one arid, one mesic) in both the dry and the wet season. Two mole-rat populations were used to assess if patterns remained constant over different habitats that vary in the extent of environmental (i.e. food availability and parasite burden) and social stressors (challenges to breeding monopoly).

From this information there were five hypotheses made at the beginning of this study. Firstly, it was predicted that cortisol concentrations would be higher in the Kamieskroon *C. h. hottentotus* population. The second was that cortisol and testosterone would show a significant increase during the wet season. Thirdly, it was expected that testosterone concentrations would be higher in males and cortisol would be higher in females, as these are the patterns that are generally seen in the literature. Further it is predicted that breeding individuals would have higher testosterone and cortisol concentrations compared to their non-breeding counterparts. The fourth prediction was that ectoparasites infecting *C. h. hottentotus* should have little impact on the cortisol concentrations of their host within a specific location. Season and host sex should have a larger impact on cortisol concentrations. Finally it was hypothesised that significant relationships between parasite abundances and testosterone concentrations in the host populations would be positive.

Materials and Methods

Study sites and animal capture

Trapping was carried out at two sites approximately 440km apart on the western coast of South Africa between February 2011 and August 2012; Darling, Western Cape (33.25^{o}S, 18.25^{o}E) and Kamieskroon, Northern Cape (30.13^{o}S, 17.57^{o}E). Darling has a much higher annual rainfall average of 411.6mm per annum (average 2009-2012, SA Weather Service) in comparison to Kamieskroon, which has approximately 284.5mm per annum (average 2009-2012, SA Weather Service). Land use was mostly for livestock at both sites, however

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grazing was much more frequent in Darling. Trapping sessions occurred at both sites during summer (January-March) and winter (June-August) so that seasonal variation in hormonal profiles could be assessed. Colonies of *Cryptomys hottentotus hottentotus* were captured using live traps (Hickman 1979) with sweet potato as bait. Traps were checked every two to three hours from first light until just after dark. Trap sites were kept to a minimum of 30m apart to ensure that a single trap site was assigned to one colony. An entire colony was considered captured when no activity was recorded at a trap site for three consecutive days after the last animal capture (Spinks *et al*. 1998). Captured mole-rats were kept in plastic crates with other members of their colony and fed sweet potato once a day until ready to be processed. The plastic crates were a minimum of 41 x 28 x 25cm in dimension with air holes in the lids and contained a 3cm layer of soil.

Processing host animals and sample collection

Each individual was euthanised with an overdose of halothane. Immediately after expiration the animals were sexed and weighed with scales (SNUG-III precision balance, Jadever) accurate to 0.05g. Individuals were assigned a breeding status (breeder or non-breeder); breeding males were identified as the heaviest males (Bennett 1992; Rosenthal *et al*. 1992). The breeding female was recognised by the presence of nipples. Individuals were also put into an age class (juvenile or adult). Juveniles were defined as being less than 30g in body mass and not having a full, thick pelage such as that found in adults.

A 22cm x 7cm x 5cm tub with 100ml soapy water was used to wash the individual mole-rat for ectoparasite removal. The washing process and ectoparasite identification were executed as specified in the previous two chapters. The individual was subsequently dissected along the ventral surface to expose the thoracic cavity. The rib cage was cut open and the heart was punctured so that the blood could be collected intra-cardially using a heparinised syringe. Where possible a minimum of 1.5ml of blood was collected. All of these procedures were executed quickly to ensure the blood did not clot prior to collection. Blood was placed into a 2ml Eppendorf tube and placed into a miniature centrifuge (Spectrafuge Mini Centrifuge, Labnet International, Inc.) and spun for 15 minutes at 6000 rpm to separate out the plasma. Plasma was pipetted off with a 200µl Gilson pipette set to take up 100µl and placed into a labelled Eppendorf tube to be stored at -20^{o}C.

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Hormonal assays

Radioimmunoassays were performed for duplicate samples per individual using Count-A-Coat kits (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) for measuring cortisol and testosterone, respectively. Both kits were highly specific to their respective steroid hormone (sensitivity for cortisol, 5.5nmol/l; testosterone, 0.14nmol/l). Cross-reactivity of the antibody for cortisol was less than 1% except for prednisone (2.6%), 11-deoxycortisol (11.4%) and prednisolone (76%). For testosterone, the cross-reactivity of the antibody was also below 1% for the majority of naturally occurring steroids. Exceptions included methyltestosterone (1.7%), 5α-dihydrotestosterone (<3.5%), 11-ketotestosterone (16%) and 19-nortestosterone (22%). For cortisol assays duplicate sets of calibrators ranging from 0 (maximum binding) to 1380nmol/l were prepared in coated propylene tubes with 25µl calibrator and 1ml of iodinated (^{125}I) cortisol. An extra duplicate of the zero calibrator was pipetted into non-specific binding propylene tubes and four uncoated control tubes without sample or calibrator were also prepared. 25µl of each plasma sample was placed into two specific antibody coated propylene tubes with 1ml ^{125}I cortisol. The control tubes were set aside whilst calibrators and samples were incubated at 37^{o}C for 45 minutes. All tubes were then inverted to void excess label and drained thoroughly before being placed in the Gamma counter with the controls to be counted for one minute. A similar method was employed for the testosterone assays. Duplicate calibrators (ranging from maximum binding to 1600nmol/l) were set up using 50µl and 1ml of ^{125}I testosterone. Then controls were set up as with cortisol assay before duplicate sample tubes (50µl sample and 1ml of ^{125}I testosterone) were set up in specific anti-body coated tubes and incubated for 3 hours at 37^{o}C. All tubes were then inverted to void excess label and drained thoroughly before being placed in the Gamma counter with the controls to be counted for one minute. The validations of both assays were tested via a comparison of a serial dilution curve of the plasma (from 1:1 to 1:16) with the standard calibration curve. The data was transformed via a log-logit transformation (Chard 1987), and the curves were compared via an ANCOVA using SPSS (IBM SPSS version 21 2012). The slopes were found not to differ significantly from one another (cortisol, $F = 0.003$, $P = 0.961$; testosterone, $F = 2.552$, $P = 0.154$).

Statistical analysis

The previous chapter revealed a distinct difference in parasite burden between locations.

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Each population of *C. h. hottentotus* was considered separately, i.e. separate analyses were run for testosterone concentrations in the Darling population and in the Kamieskroon population. The same was done for the cortisol data. This meant that any potential variation caused by the differences between habitats was not masked by combining the data. The mean and standard error for each hormone within each population was calculated. Due to the non-normal distribution of data (Kolmogorov-Smirnov; cortisol 0.156, P = <0.001, testosterone 0.269, P = <0.001), Mann-Whitney-U tests were employed to check for differences in cortisol and testosterone concentrations between sites. Additionally, Spearman's rho correlations were used for any possible connection between the cortisol and testosterone levels of an individual in both locations. The abovementioned tests were carried out using SPSS (IBM SPSS Statistics version 21, 2012). Generalised linear mixed effects models (GLMMs) were subsequently utilised to assess the causes of variation in testosterone and cortisol levels in *C. h. hottentotus.* An information-theoretic (IT) approach was used to indicate which predictors may have a substantial impact on hormone concentrations and would be worthy of further consideration (Garamszegi 2011). This involved running all possible GLMMs with the set of predictors used (Burnham & Anderson 2002) and using the Akaike's information criterion (AIC) to make direct comparisons between models (Burnham & Anderson 2002). The AIC values are a way of inferring how well the predictors in a model fit the response variable; the lower the value the better the fit (Akaike 1973). Fixed effect predictors included were host sex, breeding status, age (only in the case of Kamieskroon as no juveniles were captured in Darling) and the season (summer or winter). Body mass of the host was included as a covariate along with parasite abundances. Due to the low likelihood of very complex models being statistically accurate (Burnham & Anderson 2002; Symonds & Moussalli 2011), the most complicated GLMMs included four main predictors and interactions no larger than 3-way interactions. All models included colony ID as a random effect. Four sets of models were run in total. The models were run using a Gamma distribution and a log link function. All models and their corresponding AIC values were calculated using R2.15.1 (R Core Team 2012), with the 'glmmadmb' function from the glmmADMB package (Fournier *et al.* 2012).

Once all AIC values had been calculated, the corrected AIC for small sample sizes (AICc) was calculated manually. Then models were placed into order of increasing AICc. From this a ΔAICc showing the difference between the specific model and the best-fitting model

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was calculated and subsequently the model weight (w_i) could be calculated (Burnham & Anderson 2002; Symonds & Moussalli 2011). Bias may be created from not using all possible models (Garamszegi 2011) so referring to the top models may not be sufficient. Therefore the cumulative $w_{i,}$ was calculated to evaluate model fit. Any predictors that had a cumulative w_i of >70% were considered for further investigation. Evidence ratios (ER) were also calculated for model comparison (Symonds & Moussalli 2011). More detailed descriptions of these calculations are provided in Chapter 2.

Results

A total of 268 *C. h. hottentotus* were captured (144 from Darling, 124 from Kamieskroon) throughout the course of this study. Of these, there were five individuals where full hormonal data was not available, therefore, 263 were used in the analyses (Table 3.1). Five ectoparasite species were found; three mite species (*Androlaelaps scapularis, A. capensis, Radfordia ensifera*), one flea species (*Cryptopsylla ingrami*) and one sucking louse species (*Eulinognathus hilli*). For the purpose of the analyses, the data from the two *Androlaelaps* spp. were combined as they were likely to fill a very similar niche, being adapted mostly to bathyergid hosts (Till 1963). Further, the less prevalent species was only present on hosts already infected with the other species (see Chapter 2). All four parasite taxa were collected from both populations of *C. h. hottentotus* except *E. hilli*, which was only found in

Table 3.1: The *C. h. hottentotus* captured from which plasma cortisol and testosterone samples were collected.

			Kamieskroon		**Darling**	
Animals			**Summer**	**Winter**	**Summer**	**Winter**
caught:	Adults:	male	7	50	32	39
		female	5	54	19	50
	Breeders (%):	male	42.9	31.8	28.1	43.6
		female	20.0	31.5	26.3	24.0
	Juveniles		7	0	0	0
	Overall		19	104	51	89
	Total		**123**		**140**	

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Table 3.2: The percentage prevalence, mean abundance and standard error of mean abundance of the four parasite taxa collected from each location.

	Kamieskroon			**Darling**		
	Prevalence (%)	**Mean abundance**	**S.E.**	**Prevalence (%)**	**Mean abundance**	**S.E.**
Androlaelaps spp.	88.6	22.24	2.97	60.7	8.70	1.30
R. ensifera	60.2	2.02	0.25	22.9	0.64	0.15
C. ingrami	29.3	0.52	0.10	5.0	0.09	0.034
E. hilli	17.9	3.47	0.98	-	-	-

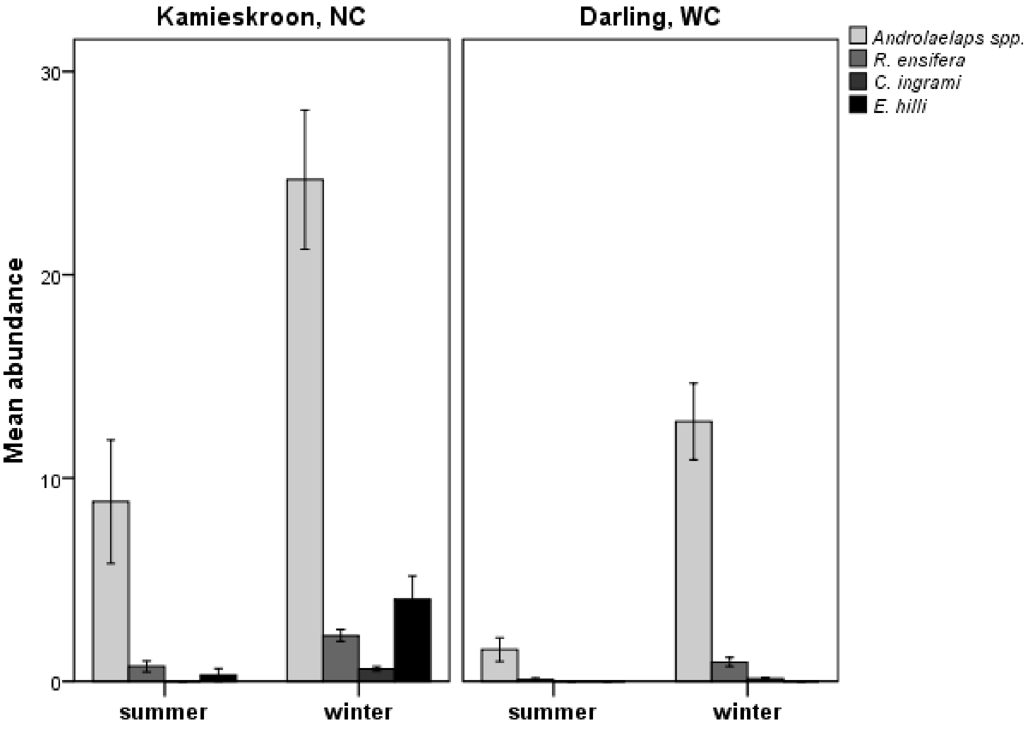

Figure 3.1: Variation in abundance of the four parasite taxa at each location between summer (dry) and winter (wet). Error bars indicate ±std. error of the mean.

Kamieskroon (Table 3.2). Additionally, the ectoparasites that were collected from both locations were more prevalent and abundant in Kamieskroon. All parasites were more abundant in the high rainfall winter season (Figure 3.1).

There was no correlation between testosterone or cortisol levels from either location (Darling, $R_S = -0.103$, $n = 263$, $P = 0.228$; Kamieskroon, $R_S = -0.021$, $n = 263$, $P = 0.821$).

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However a significant difference was found between circulating levels of testosterone (Mann-Whitney-U; U = 2.439, *P* = 0.015) and cortisol (Mann-Whitney-U; U = 2.951, *P* = 0.003) between both locations. Plasma cortisol concentrations were, on average, higher in Kamieskroon (mean and ±SE; 131.10 ±10.62 nmol/l compared to Darling, 98.68 ±7.73) whereas average testosterone levels were higher in Darling (mean and ±SE; 6.52 ±0.77 nmol/l compared to Kamieskroon, 5.64 ±0.97).

Table 3.3: Cumulative AIC weight (w_i) for the predictors in GLMMs for inference modelling. Percentages above 70% are highlighted in bold.

	Cortisol		**Testosterone**	
	Darling	**Kamieskroon**	**Darling**	**Kamieskroon**
season	0.319	**0.763**	0.300	0.401
sex	**0.709**	**0.999**	**0.915**	**0.991**
breeding status	**0.833**	0.250	**0.999**	**0.928**
age	-	0.151	-	0.031
body mass	**0.777**	0.149	0.276	0.693
Androlaelaps spp.	0.225	0.229	**0.830**	0.185
R. ensifera	0.176	0.196	0.192	0.454
C. ingrami	0.182	**0.760**	0.128	0.027
E. hilli	-	0.115	-	0.240

The predictors used in the GLMMs appeared to influence the concentrations of both circulating testosterone and cortisol, as the intercept model had much larger AICc values than the majority of the GLMMs run for each set (Appendix Tables III-VI). However, the impact of predictors varied between the different hormones, and even between sites. A cumulative percentage of 76% was calculated for the influence of season on circulating cortisol in *C. h. hottentotus* found at Kamieskroon. A large increase in concentrations of cortisol was found in individuals caught in winter at this site (Figure 3.2). Season was not a key predictor for testosterone or cortisol concentrations in the Darling population. Sex on the other hand was a significant predictor for both plasma hormone concentrations in both locations as was predicted (Table 3.3). Females had a much larger range and mean circulating cortisol concentration was higher than for males in both populations (Figure

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3.3a). Conversely males possessed a higher mean circulating testosterone concentration than females (Figure 3.3b). Breeding status also appeared to be a significant predictor for

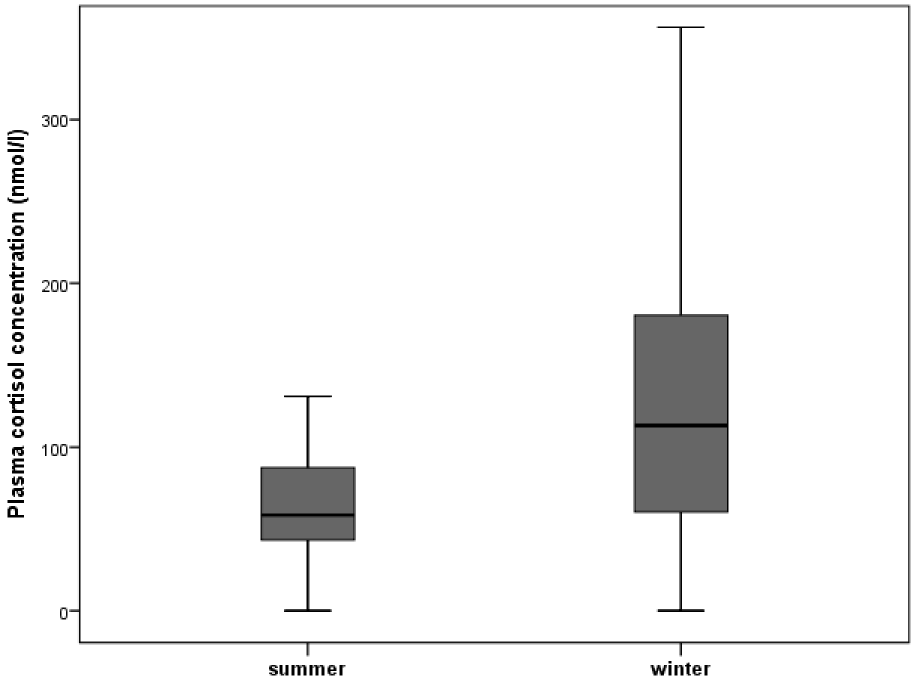

Figure 3.2: The variation in circulating plasma cortisol concentrations of *C. h. hottentotus* between seasons at Kamieskroon. The middle bar, the box and the whiskers refer to the median, interquartile range and full range respectively.

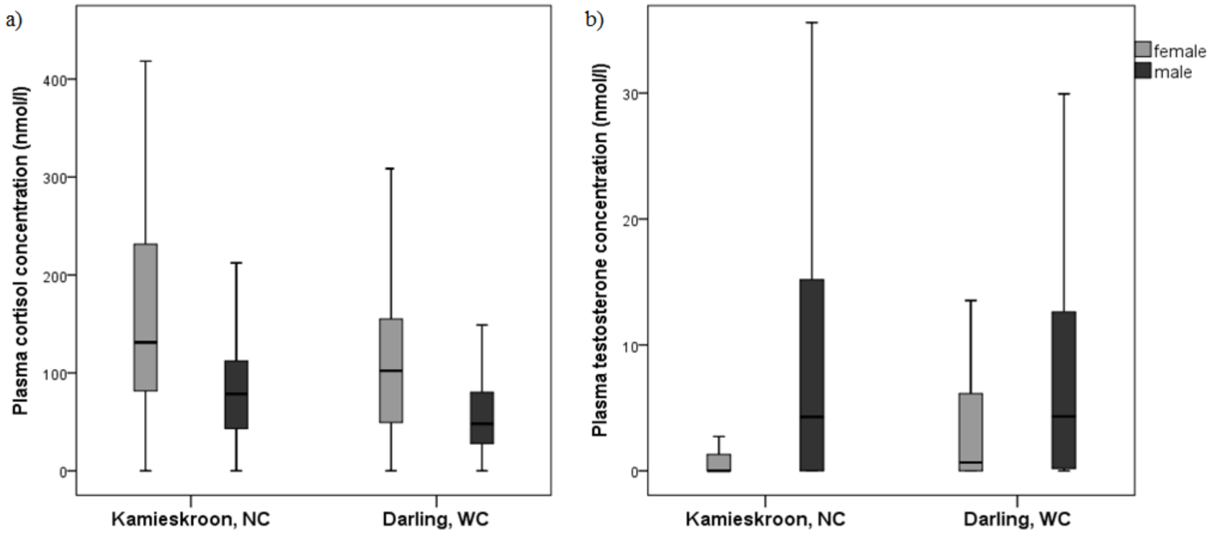

Figure 3.3: The variation in circulating plasma hormone concentrations between the sexes of two populations of *C. h. hottentotus*. a) cortisol and b) testosterone. The middle bar, the box and the whiskers refer to the median, interquartile range and full range respectively.

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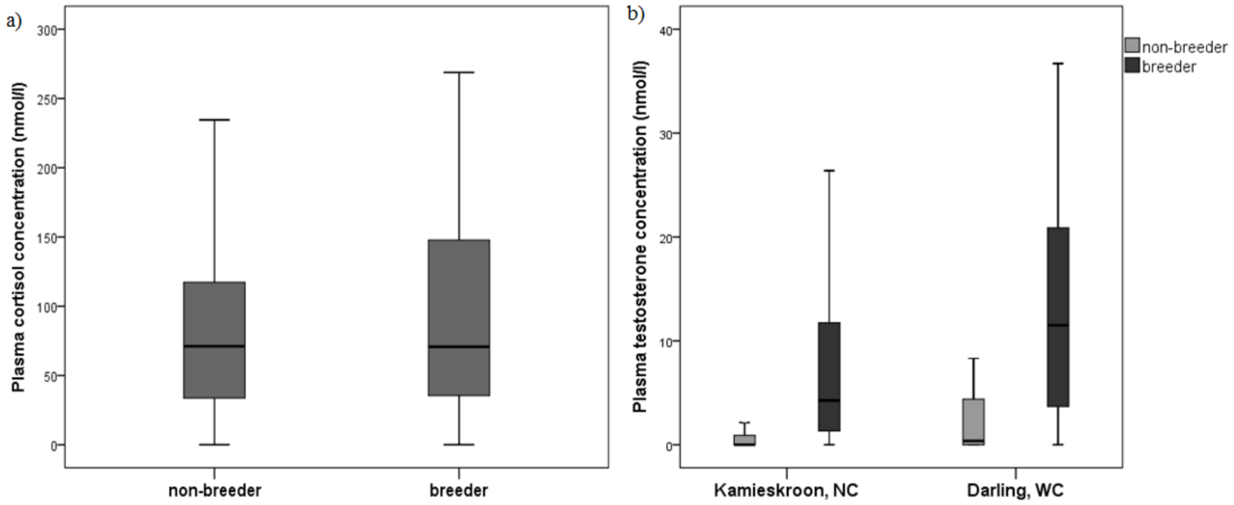

Figure 3.4: The variation in circulating plasma hormone concentration between breeders and non-breeders from *C. h. hottentotus* at Darling. The middle bar, the box and the whiskers refer to the median, interquartile range and full range respectively.

both hormones, except for circulating cortisol levels at the Kamieskroon population (Table 3.3). The difference in cortisol concentrations found in breeding and non-breeding individuals in Darling was not very distinct. Breeding individuals however, did have a greater range and possessed higher concentrations than their non-breeding counterparts (Figure 3.4a). The difference in circulating plasma testosterone between individual hosts of each breeding status was much more distinct, with a significantly larger mean concentration in breeding individuals within both populations (Figure 3.4b). Body mass did not appear to be as influential on hormone levels in either population except in Darling, where smaller animals tended to have higher circulating cortisol concentrations (Figure 3.5). Age was not a significant predictor for either hormone (Table 3.3).

As predicted, ectoparasites did not have a particularly strong impact on host cortisol concentrations. However, the flea *C. ingrami* appeared to have a significant influence on the variation in cortisol levels of their host at Kamieskroon but not Darling (AIC w_i 76% compared to 18%, Table 3.3). Higher cortisol levels were associated with higher flea abundances (Figure 3.6a). In contrast, more significant relationships were expected between ectoparasites and host testosterone concentrations. The abundance of *R. ensifera* was not an important predictor of hormone concentrations for either population. Moreover, *E. hilli* had little impression on the hormonal concentrations of the host in Kamieskroon (Table 3.3). However, testosterone levels in Darling, but not Kamieskroon, were negatively correlated to *Androlaelaps* spp. abundance (AIC w_i 83% compared to 19%, Table 3, Figure 3.6b).

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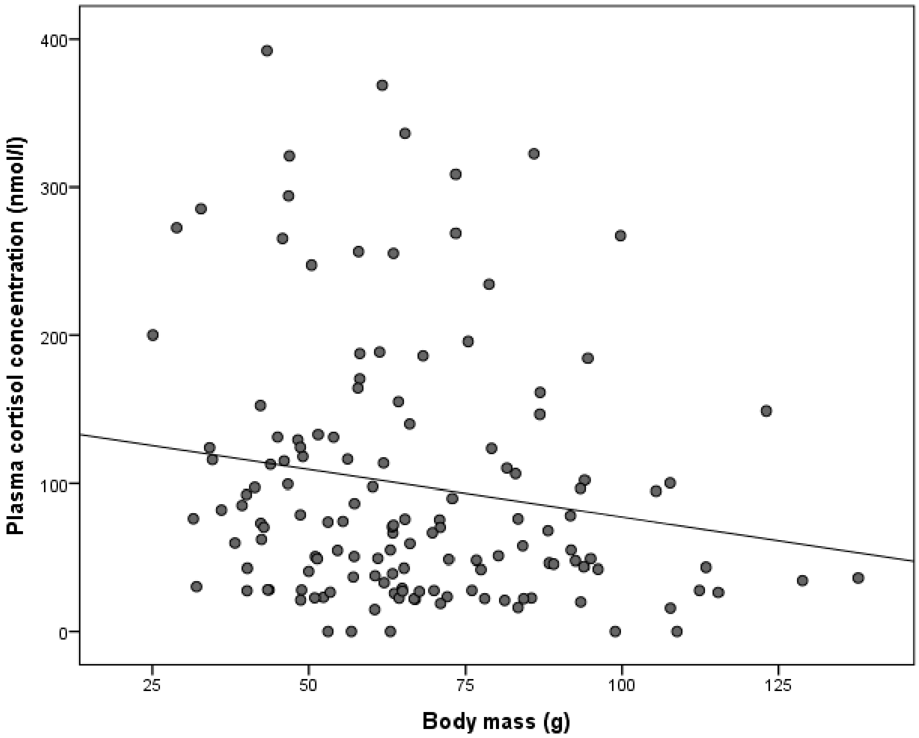

Figure 3.5: The relationship between body mass and circulating cortisol levels of *C. h. hottentotus* at Darling.

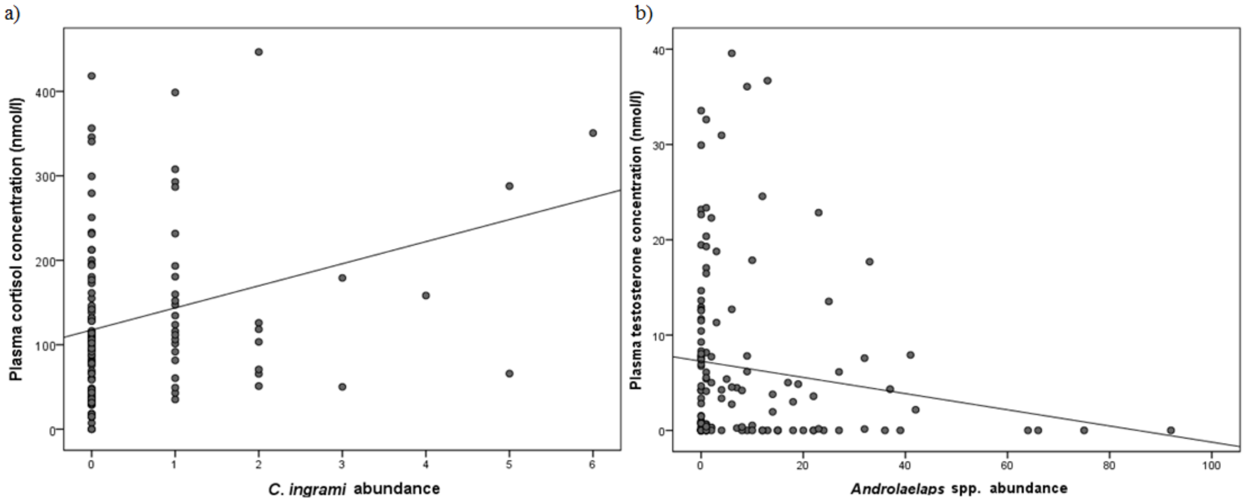

Figure 3.6: Correlations between host plasma hormone concentrations and parasite abundances. a) *C. ingrami* abundance and plasma cortisol concentrations of *C. h. hottentotus* in Kamieskroon; b) *Androlaelaps* spp. abundance and plasma testosterone concentrations in Darling.

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Discussion

In this study most hypotheses put forward to explain relationships between hormones and ectoparasite infection were not found to hold for *C. h. hottentotus*. The results indicated that the prediction that cortisol would be higher in the arid habitat was held. Nevertheless testosterone levels were higher in Darling, which was not anticipated. The effects of season were not as influential as expected. Our prediction that host sex would be a significant predictor of steroid hormones held true across both populations. Though breeding status was also a significant predictor for testosterone in both populations as expected, this factor was only important for cortisol levels of the Darling population of hosts. Overall, the impact of ectoparasites on host endocrinology was relatively small. It was hypothesised that there would be positive associations between some ectoparasite species and testosterone concentrations, yet the only significant relationship found (*Androlaelaps* spp.) was negative. Further this was only true for the host population in Darling. Although it was thought that no ectoparasites would influence cortisol concentrations, *Cryptopsylla ingrami* had a significant positive influence on cortisol concentrations of their hosts at Kamieskroon.

Differences between host populations

Perhaps the most intriguing finding in the current study was the distinct differences between the two host populations. The challenges associated with the arid habitat were illustrated by the higher average cortisol concentrations in the Kamieskroon population. It has been demonstrated that stressors from the surrounding environment can lead to raised glucocorticoid levels (Quillfeldt *et al*. 2004; Hoby *et al*. 2006), particularly when food is low and stored energy in the body may need to be released (Pride 2005; Hoby *et al*. 2006; Pedersen & Greives 2008). A comparison by Spinks *et al.* (2000) showed that *C. h. hottentotus* in drier habitats have to dig longer burrow systems in order to gain access to food resources. Their counterparts in a more mesic area do not have to dig as far between underground tubers or bulbs as they are more uniformly distributed within their habitat. Additionally more frequent rainfall allows them to forage for longer periods throughout the year (Spinks *et al*. 2000). It has already been reported that *C. h. hottentotus* in arid regions tend to have a smaller body mass (Spinks *et al*. 2000; see Chapter 2). This was thought to be an adaptation to lower food availability (Spinks *et al.* 2000). The most plausible

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explanation may be that the *C. h. hottentotus* in Kamieskroon have also adapted glucocorticoid levels to the greater energy expenditure required to reach food resources and the longer time scale within which they may have to survive on food stores. Similar effects have been described in a comparative study of female hyaenas in two populations, whereas those inhabiting an area with more sparse food resources had higher glucocorticoid concentrations (Goymann *et al*. 2001). Alternatively but not mutually exclusive, higher ectoparasite loads associated with the more arid environment (see Chapter 2) may account for the higher circulating cortisol concentrations in the Kamieskroon population. The statistical analysis suggests that ectoparasites did not account for temporal and between individual variation in cortisol levels within populations. However, it is possible that a long term difference between parasite abundances in the two populations may have contributed to the cortisol differences. An increase in parasitic load could lead to increased energy being taken from the host (Combes 2001). Consequently, in a similar way to which the *C. h. hottentotus* may have adapted to less readily available food in their habitat, baseline cortisol concentrations may have increased due to increased pressure on the host (Sapolsky *et al*. 2000).

Conversely, testosterone concentrations were greater in the Darling population. This could be an indication of the antagonistic role glucocorticoids can have on testosterone secretions (Bambino & Hsueh 1981) on a long term scale, as cortisol concentrations were generally lower in *C. h. hottentotus* from Darling. On the other hand the greater testosterone concentrations in Darling could be related to increased social interactions in the mesic habitat. Restrictions on immigration and dispersal are much lower in Darling due to higher rainfall and a more densely distributed population (Spinks *et al*. 2000b). As a result, higher numbers of unrelated individuals tend to be found in colonies inhabiting mesic habitats (Bishop *et al*. 2004). Increased aggression due to circulating testosterone is thought to play a role in maintenance of higher social status in both males and females (Creel *et al*. 1992; Clarke & Faulkes 1997; Klein 2000; Shargal *et al*. 2008; Muehlenbein & Watts 2010). Lutermann *et al*. (2013) have previously suggested that testosterone may play a fundamental role in maintaining female reproductive skew in bathyergids based on their study of females from two mole-rat species. The *C. h. hottentotus* captured from Darling appeared certainly more aggressive (E. Archer, pers. obs.). Thus increased testosterone concentrations may be an adaptation to increased social instability (Wingfield *et al*. 1990). Though it has been shown that cortisol can increase with socially unstable environments

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(Sapolsky 2005), work on three species of social Bathyergidae found that dyadic encounters between mole-rats from other colonies do not initiate a significant increase in cortisol levels, even when agonistic behaviour occurred (Ganem & Bennett 2004). Similarly, previous work on social degus found that increased cortisol is not linked to social factors (Ebensperger *et al*. 2011). Therefore it could be suggested that cortisol is not linked to social aggression in this species.

Seasonal differences

Cortisol was only significantly higher during the wet season in the Kamieskroon population. Significant elevations in cortisol and other glucocorticoids have been found in some social mammals approaching their breeding season (Sands & Creel 2004; Clough *et al*. 2010) though this trend is far from ubiquitous (Creel *et al*. 1992, 1993; van Kesteren *et al*. 2012; Higham *et al*. 2013). It is not entirely certain why seasonal glucocorticoid fluctuations in vertebrates exist (Romero 2002). Even where seasonal patterns are seen in social groups it is not always associated with an increase in aggressive behaviour as seen with testosterone (Creel 2005). As previously mentioned increased cortisol also does not appear to increase due to social aggression in social mole-rat species (Ganem & Bennett 2004). Seasonal increases in cortisol associated with the wet season have been found in the subordinates of the bathyergid *F. damarensis,* despite the fact that this species breeds year-round (Young *et al*. 2010). As has been found in the *C. h. hottentotus* population from Kamieskroon, *F. damarensis* inhabit arid areas, though peak rainfall occurs in the summer (Bennett & Faulkes 2000; Young *et al*. 2010). In both cases cortisol increases when constraints on foraging and dispersal are least (Bennett & Faulkes 2000; Spinks *et al*. 2000b), which is potentially an adaptation to increased energy consumption associated with digging activity (Young *et al*. 2010). In the Darling population studied here, foraging and dispersal are not likely to be restricted to such a small time window and food resources are much more evenly dispersed (Spinks *et al*. 2000b), such that the same adaptations seen in the Kamieskroon population or in *F. damarensis* would not be necessary. Young *et al*. (2010) also suggested that breeding female Damaraland mole-rats may increase aggression towards non-breeding individuals due to increased pressure on the breeding monopoly, or to promote dispersal in subordinates. Though it is possible that pressures on maintaining a higher social status may increase during the wet season, this was associated with changes in

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reproductive physiology of subordinates in *F. damarensis* (Young *et al*. 2010). Reproductive physiology of non-breeding *C. h. hottentotus* is maintained throughout the year (Spinks *et al.* 2000a).

With respect to testosterone concentrations, there is no evidence of a significant increase in *C. h. hottentotus* during the winter rainfall period in either population. This is contrary to Spinks *et al*. (1999) who found that breeding female testosterone levels increased during the breeding season. Non-breeding individuals of *C. h. hottentotus* are not physiologically suppressed and all members of the colony have been shown to be able to reproduce at any time of the year (Bennett *et al*. 1997; Spinks *et al*. 1997; 1999; 2000a; de Bruin *et al*. 2012). Therefore testosterone levels would not increase for activating physiological mechanisms needed for seasonal reproduction. Additionally, average testosterone has been shown to increase in females in two aseasonal breeding mole-rat species during the wet season inferring a link to seasonal social aggressive behaviour (Lutermann *et al*. 2013). Although data from winter was collected prior to peak breeding activity, high rainfall prompts dispersal in *C. h. hottentotus* thus inter-colonial encounters are likely to increase (Spinks *et al*. 2000, 2000b). It would be predicted that testosterone concentration would increase in response to elevated frequencies of social interaction in accordance with the challenge hypothesis (Wingfield *et al*. 1990; Hirschenhauser & Oliveira 2006). However, the lack of a seasonal trend seems common amongst male bathyergids even when the species breeds seasonally (Janse van Rensburg *et al*. 2002; Oosthuizen & Bennett 2009). Furthermore, some social carnivores (dwarf mongooses, African wild dogs and Ethiopian wolves) also do not display seasonal peaks in testosterone during breeding (Creel *et al*. 1992, 1993; van Kesteren *et al*. 2012). Interestingly the canids do not display increased glucocorticoid concentrations during breeding season either (Creel *et al*. 1992; van Kesteren *et al*. 2012). Creel *et al*. (1993) suggested that it is too costly for individuals of both high and low social status to decrease testosterone concentrations outside of breeding season due to ‘sneaky’ mating behaviour and social aggression occurring throughout the year (1993). Similar to dwarf mongooses which also have social systems with reproductive skew (Creel *et al*. 1993), it is possible that social aggression and suppression occur throughout the year in order to maintain social status in *C. h. hottentotus*. In addition to social aggression, seasonal testosterone fluctuations have also been suggested to be correlated to parasite dynamics in other species due to the differences in immune function and changes to host behaviour (Salvador *et al*. 1995; Møller *et al*. 2003; Grear *et al*. 2009). All ectoparasite species were

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more abundant in the wet season prior to breeding (see previous chapter). However as significant changes in testosterone throughout the seasons are not apparent, it seems seasonal increases in abundance of ectoparasites on *C. h. hottentotus* are not linked to immunosuppressive effects or behavioural alterations caused by testosterone.

Influence of host-related factors

As is seen with most mammals, males had higher testosterone concentrations whereas the females had higher cortisol concentrations (Reeder & Kramer 2005). Additionally, breeding individuals had higher testosterone concentrations in both populations, which was expected. Individuals of higher social status in social systems are frequently found to have increased testosterone concentrations in comparison to those of lower status (Creel *et al*. 1992; Muehlenbein & Watts 2010; van Kesteren *et al*. 2012) and it is thought to be associated with increased aggression rates (Creel *et al*. 1993). Increased testosterone has also been seen in breeding individuals of *Heterocephalus glaber* in both sexes (Clarke & Faulkes 1997) and male Ansell's mole-rats (*F. anselli*; de Bruin *et al*. 2012) as well as females of Natal mole-rat (*C. h. natalensis*) and *F. damarensis* (Lutermann *et al*. 2013) in the Bathyergidae. Breeding individuals had higher cortisol concentrations, but only in Darling. A review on work in primates indicates that in social groups with cooperative breeding, it is the higher ranking individuals that usually have higher cortisol levels (Sapolsky 2005). However, this is clearly not the case here otherwise the trend would be the same across both populations. Glucocorticoid levels should only differ between high and low ranking individuals in a social group when the cost of maintaining allostasis is greater for individuals of either status (Goymann & Wingfield 2004). Based on the results here, it appears that there was no significant difference in costs between breeding and non-breeding individuals in Kamieskroon. However, breeding individuals in the colonies caught from Darling appeared to incur higher costs in maintaining allostasis. It is possible that higher environmental stress associated with the arid habitat in Kamieskroon may mask any differences between breeding status as the increased energy requirements are equally costly on an individual's fitness. Alternatively, this difference could be due to the variation in the cost of maintaining higher social (breeding) status between the two populations. Frequency of dispersal and immigration in colonies of *C. h. hottentotus* in arid areas is greatly reduced relative to populations in areas of higher rainfall, thus colonies are more stable (Spinks *et*

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al. 2000b). In mesic areas such as Darling, colony conflict is greatly increased as the number of foreign conspecifics in a colony tends to be larger in such habitats (Spinks *et al*. 2000b; Bishop *et al.* 2004). In primates, dominant individuals in a social group may only suffer higher cortisol levels when the social environment becomes unstable (Sapolsky 2005; Setchell *et al.* 2010; Higham *et al*. 2013). However, the results discussed here so far and previous work on Bathyergidae infers that cortisol is not directly associated with aggression (Ganem & Bennett 2004). Consequently the increased cortisol levels in breeding individuals from Darling are likely to be a consequence of higher energy consumption linked to social aggression required to maintain higher social status (Goymann & Wingfield 2004). Body mass also appeared to be a significant factor for cortisol concentrations in the Darling population. Sex and breeding status of *C. h. hottentotus* are correlated to the mass of the animal (Spinks *et al*. 1997, 1999; also see previous chapter). It could be this factor accumulated a higher statistical weight since few other factors influenced cortisol concentrations of *C. h. hottentotus* in Darling other than sex and breeding status.

The influence of ectoparasites

In relation to site, season and host factors the abundance of ectoparasites appeared to have very little or no influence on steroid hormone concentrations in *C. h. hottentotus*. Previous studies investigating the relationships between cortisol concentrations and parasitic infection have provided very mixed results (e.g. Grutter & Pankhurst 2000; Haond *et al*. 2003; Chapman *et al*. 2006; Hoby *et al*. 2006; Pena *et al*. 2007; Fuxjager *et al*. 2011). Perhaps this is due to the large number of stressors that can impact an individual's HPA axis at any one time in its natural environment (Romero 2004). The fact that negative effects are associated with chronically raised glucocorticoid levels is well documented (McEwen 1998; Sapolsky *et al*. 2000; Romero 2004). As hosts and their parasites co-evolve, hosts must adapt to infection or remove it entirely in order to persist (Combes 2001). It would be evolutionary unstable for the host to develop physiological responses to parasitic infection that are likely to cause damage to itself. Consequently, natural levels of parasitic infection are not necessarily going to correlate to chronic secretions of glucocorticoids. This is illustrated in a study by Lutermann *et al*. (2012) on *C. h. pretoriae*. Natural cestode infection already occurring in captured mole-rats was not correlated to host cortisol concentrations. However a novel infection led to increased cortisol levels as part of

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the initial stress response (Lutermann *et al*. 2012). Ectoparasite abundances are also largely influenced by the same external environment as the host itself (Vinarski *et al*. 2007; Korallo-Vinarskaya *et al*. 2009) thus strong correlations with the host physiology may be less likely compared to some endoparasites. However, this cannot be quantified here as endoparasitic infection was not analysed. As with other studies considering several parasites (e.g. Fuxjager *et al*. 2011) the patterns between ectoparasite abundance and cortisol concentrations in *C. h. hottentotus* varied with different parasite taxa. For this study there was not a significant relationship in most cases, yet a positive correlation was found between the flea *C. ingrami* and circulating cortisol. It has been suggested that such differential effects could be due to either immunosuppression from cortisol only influencing certain arms of the immune system or due to different life-strategies employed by parasites (Hoby *et al*. 2006; Fuxjager *et al*. 2011). It is possible that *C. ingrami* initiates a stress response in comparison to the other ectoparasites because its feeding method causes more damage to the host. It has also been suggested that some *Androlaelaps* spp. supplement their parasitic food source via predation meaning they may not cause as large a pressure on the immune system for example (Dowling 2006). However, based on the data collected here it is very difficult to assess whether an infection by a certain kind of parasite causes more damage than another but this possibility should be addressed in future studies. It is possible that potential parasite effects have been masked by the already elevated baseline cortisol levels in the Kamieskroon population due to the stress associated with the habitat. A study in birds has shown that parasite infection may only correlate to corticosterone levels when they are already raised from unfavourable weather conditions (Quillfeldt *et al*. 2004). Additionally, a study in wild mice inferred that annual population crashes were caused by the combined effect of low food resources and parasite infection, though neither factor could account for the crash alone (Pedersen & Greives 2008).

The lack of positive relationships between ectoparasites and testosterone infer that testosterone is not causing significant immunosuppression such as Folstad and Karter (1992) discussed in their original ICHH hypothesis. This is unsurprising since increasing evidence suggests that the ICHH is not that common in mammal hosts (Roberts *et al*. 2004). Furthermore, the lack of positive correlations also indicate that male-biased parasitism seen in some ectoparasite species infecting the common mole-rat (see previous chapter) are not due to differences in testosterone levels between the sexes, as seen in other vertebrates (e.g. Salvador *et al*. 1995; Hughes & Randolph 2001; Hoby *et al*. 2006). Only

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Androlaelaps mites appeared to have a negative effect on testosterone concentrations or vice versa. Such differences between parasite taxa and their connection with testosterone where multiple taxa were studied have been seen (Hoby *et al*. 2006; Fuxjager *et al*. 2011; Ezenwa *et al.* 2012; Pollock *et al*. 2012). However, the fact that this correlation is only present in Darling is puzzling. *Androlaelaps* abundances were greater in Kamieskroon, thus the expectation would be that the same trend should have been more pronounced in this population. As indicated earlier there were potential behavioural differences associated with testosterone between the two populations, which may account for the variation. Alternatively, higher status individuals in social groups tend to receive more allogrooming from other individuals (Schino 2001; Kutsukake & Clutton-Brock 2006). In meerkats, this behaviour is associated with placating more aggressive individuals of higher status in addition to removal of ectoparasites (Madden & Clutton-Brock 2009). This is mere speculation however, as aggression and grooming behaviour was not measured in the current study.

Conclusions

The correlation between ectoparasites and host hormone concentrations were almost non-existent in comparison to other factors. Instead in *C. h. hottentotus*, elevations in cortisol appear to be mostly related to potential increased energy consumption associated with stressful situations. These may be due to an expected increase in foraging activity or dispersal indicated by environmental cues i.e. rainfall, or increased stress from elevated aggression, stemming from potential challenges to the breeding monopoly. The former suggestion ties in with ‘preparative’ levels of glucocorticoids, which was suggested by Romero (2002) to explain seasonal fluctuations, whereas the latter is suggestive of the ‘energy mobilisation’ hypothesis. Testosterone on the other hand did not conform to the expectations of the challenge hypothesis, though potentially being linked to aggressive behaviour and social instability. As with other social species, such aggression is likely to exist all through the year, thus it would be costly to lower testosterone concentrations. This is most noticeable in breeding *C. h. hottentotus* which maintain elevated testosterone levels in comparison to their non-breeding counterparts. The differences between the two populations indicate a need to consider a broader picture and may account for the substantial differences in correlation between ectoparasites and host physiology between

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the two populations. Whether parasite abundance was significantly correlated to hormonal concentrations depended greatly on the environmental and social factors already influencing the host populations. Future studies should consider a larger range of factors that could influence hormone concentrations in addition to parasite challenge before investigating mechanisms that may elucidate the relationship between host physiology and parasite infection.

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Appendix II

Contains the 95% confidence interval tables calculated from the GLMMs analysed for cortisol and testosterone concentrations of *C. h. hottentotus* in each location.

Table III: The 95% confidence set of GLMMs for *C. h. hottentotus* cortisol concentrations from the Darling population. Contains number of parameters in the model (*k*), corrected Akaike value (AICc), difference between model and best fitting model (ΔAICc), Akaike weight (w_i) and evidence ratio (ER). The intercept model is included for reference.

Models	***k***	**AICc**	**ΔAIC**	w_i	**ER**
sex + breeding status + body mass	5	1446.704	0.000	0.107	-
breeding status + body mass	4	1447.955	1.251	0.057	1.870
sex + breeding status + body mass + *Androlaelaps*	6	1448.079	1.375	0.054	1.989
season + sex + breeding status + body mass	6	1448.115	1.411	0.053	2.025
sex x breeding status + body mass	6	1448.589	1.885	0.042	2.567
season + breeding status + body mass	5	1448.612	1.908	0.041	2.596
sex + breeding status + body mass + *Cryptopsylla*	6	1448.723	2.019	0.039	2.745
sex + breeding status + body mass + *Radfordia*	6	1448.837	2.133	0.037	2.906
breeding status + body mass + *Androlaelaps*	5	1449.318	2.614	0.029	3.695
sex	3	1449.451	2.747	0.027	3.949
season x sex + breeding status + body mass	7	1449.689	2.985	0.024	4.447
breeding status + body mass + *Cryptopsylla*	5	1449.724	3.020	0.024	4.527
breeding status + body mass + *Radfordia*	5	1449.846	3.142	0.022	4.811
sex x breeding status + *Androlaelaps*	7	1450.035	3.331	0.020	5.287
season + sex x breeding status + body mass	7	1450.043	3.339	0.020	5.308
season x breeding status + sex + body mass	7	1450.259	3.555	0.018	5.914
sex + breeding status	4	1450.365	3.661	0.017	6.238
season + breeding status + body mass + *Androlaelaps*	6	1450.519	3.815	0.016	6.738
season + breeding status + body mass + *Cryptopsylla*	6	1450.637	3.933	0.015	7.147
sex x breeding status + body mass + *Cryptopsylla*	7	1450.645	3.941	0.015	7.173
season x breeding status + body mass	6	1450.705	4.001	0.014	7.394
sex x breeding status + body mass + *Radfordia*	7	1450.757	4.053	0.014	7.586
season + breeding status + body mass + *Radfordia*	6	1450.757	4.053	0.014	7.589
sex + body mass	4	1450.847	4.143	0.013	7.939
sex x breeding status	5	1450.852	4.148	0.013	7.957
sex + *Androlaelaps*	4	1451.057	4.353	0.012	8.817
breeding status+ body mass+ *Androlaelaps* + *Cryptopsylla*	6	1451.233	4.529	0.011	9.628
breeding status + body mass + *Androlaelaps*+ *Radfordia*	6	1451.383	4.679	0.010	10.378
season + sex	4	1451.501	4.797	0.010	11.009
sex + *Radfordia*	4	1451.547	4.843	0.009	11.265
sex + *Cryptopsylla*	4	1451.551	4.847	0.009	11.288
breeding status + body mass + *Radfordia* + *Cryptopsylla*	6	1451.687	4.983	0.009	12.082

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sex + breeding status + *Androlaelaps*	5	1451.956	5.252	0.008	13.818
sex + body mass + *Androlaelaps*	5	1452.440	5.736	0.006	17.602
season + sex + breeding status	5	1452.462	5.758	0.006	17.796
sex+ breeding status + *Cryptopsylla*	5	1452.486	5.782	0.006	18.011
sex x breeding status + *Androlaelaps*	6	1452.501	5.797	0.006	18.151
sex + breeding status + *Radfordia*	5	1452.502	5.798	0.006	18.156
season x breeding status + body mass + *Androlaelaps*	7	1452.623	5.919	0.006	19.284
season x breeding status + body mass + *Cryptopsylla*	7	1452.757	6.053	0.005	20.620
season + sex + body mass	5	1452.794	6.090	0.005	21.010
season x breeding status + body mass + *Radfordia*	7	1452.883	6.179	0.005	21.961
season + sex x breeding status	6	1452.921	6.217	0.005	22.392
sex + body mass + *Radfordia*	5	1452.968	6.264	0.005	22.920
sex + body mass + *Cryptopsylla*	5	1452.976	6.272	0.005	23.012
sex x breeding status + *Cryptopsylla*	6	1452.979	6.275	0.005	23.051
sex x breeding status + *Radfordia*	6	1453.007	6.303	0.005	23.376
season x sex	5	1453.088	6.384	0.004	24.337
sex + *Androlaelaps* + *Cryptopsylla*	5	1453.214	6.510	0.004	25.920
season + sex + *Androlaelaps*	5	1453.216	6.512	0.004	25.946
sex + *Androlaelaps* + *Radfordia*	5	1453.216	6.512	0.004	25.946
season + sex + *Radfordia*	5	1453.648	6.944	0.003	32.201
season + sex + *Cryptopsylla*	5	1453.650	6.946	0.003	32.233
sex + *Radfordia + Cryptopsylla*	5	1453.684	6.980	0.003	32.786
sex + breeding status + *Androlaelaps* + Cryptopsylla	6	1454.137	7.433	0.003	41.129
season + sex + breeding status + *Androlaelaps*	6	1454.151	7.447	0.003	41.418
sex + breeding status + *Androlaelaps* + *Radfordia*	6	1454.151	7.447	0.003	41.418
season x sex + breeding status	6	1454.165	7.461	0.003	41.709
season x sex + body mass	6	1454.323	7.619	0.002	45.137
season + sex + body mass + *Androlaelaps*	6	1454.577	7.873	0.002	51.250
season + sex + breeding status + *Cryptopsylla*	6	1454.631	7.927	0.002	52.652
sex + body mass + *Androlaelaps* + *Cryptopsylla*	6	1454.631	7.927	0.002	52.652
sex + body mass + *Androlaelaps* + *Radfordia*	6	1454.633	7.929	0.002	52.705
season + sex + breeding status + *Radfordia*	6	1454.647	7.943	0.002	53.075
season x breeding status + sex	6	1454.651	7.947	0.002	53.181
sex + breeding status + *Radfordia* + *Cryptopsylla*	6	1454.659	7.955	0.002	53.395
(intercept)	2	1456.184	9.480	0.001	114.420

The '+' symbol indicates where predictors were added as main interactions. Any predictors connected by 'x' were employed in the model as a two-way (or three-way where three predictors are connected in this way) interaction term as well as separate main interactions.

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Table IV: The 95% confidence set of GLMMs for *C. h. hottentotus* cortisol concentrations from the Kamieskroon population. Contains number of parameters in the model (*k*), corrected Akaike value (AICc), difference between model and best fitting model (ΔAICc), Akaike weight (w_i) and evidence ratio (ER). The intercept model is included for reference.

Models	***k***	**AICc**	**ΔAIC**	w_i	**ER**
season + sex + *Cryptopsylla*	5	1348.197	0.000	0.078	-
season + sex + *Radfordia* + *Cryptopsylla*	6	1348.730	0.533	0.060	1.305
season + sex + breeding status + *Cryptopsylla*	6	1349.006	0.809	0.052	1.499
season + sex + *Androlaelaps* + *Cryptopsylla*	6	1349.032	0.835	0.051	1.518
season x sex + *Cryptopsylla*	6	1349.382	1.185	0.043	1.809
season + sex + body mass + *Cryptopsylla*	6	1349.802	1.605	0.035	2.231
season x sex + *Radfordia* + *Cryptopsylla*	7	1349.842	1.645	0.034	2.276
season x sex + breeding status + *Cryptopsylla*	7	1350.346	2.149	0.027	2.929
season + sex + *Cryptopsylla* + *Eulinognathus*	6	1350.402	2.205	0.026	3.012
season x sex + *Androlaelaps* + *Cryptopsylla*	7	1350.412	2.215	0.026	3.027
season + age + sex	6	1350.418	2.221	0.026	3.036
season x sex + body mass + *Cryptopsylla*	7	1350.838	2.641	0.021	3.745
season + sex x breeding status + *Cryptopsylla*	7	1350.958	2.761	0.020	3.977
season x breeding status + sex + *Cryptopsylla*	7	1351.074	2.877	0.018	4.214
sex + *Cryptopsylla*	4	1351.253	3.057	0.017	4.610
sex + *Androlaelaps* + *Cryptopsylla*	5	1351.373	3.176	0.016	4.894
season + sex	4	1351.417	3.221	0.016	5.004
season + sex + *Androlaelaps*	5	1351.537	3.340	0.015	5.312
sex + breeding status + *Cryptopsylla*	5	1351.583	3.386	0.014	5.436
season x sex + age + *Cryptopsylla*	7	1351.592	3.395	0.014	5.460
season x sex + *Cryptopsylla* + *Eulinognathus*	7	1351.614	3.417	0.014	5.521
sex + body mass + *Cryptopsylla*	5	1351.725	3.528	0.013	5.836
season + sex + *Androlaelaps* + *Radfordia*	6	1352.414	4.217	0.009	8.236
age + sex + *Cryptopsylla*	5	1352.423	4.226	0.009	8.273
season + sex x age + *Cryptopsylla*	7	1352.424	4.227	0.009	8.277
sex + body mass + *Androlaelaps* + *Cryptopsylla*	6	1352.562	4.365	0.009	8.869
season + sex + *Radfordia*	5	1352.617	4.420	0.009	9.116
season + sex + *Androlaelaps* + *Eulinognathus*	6	1352.630	4.433	0.008	9.176
sex + Androlaelaps + *Radfordia* + *Cryptopsylla*	6	1352.660	4.463	0.008	9.314
sex + body mass + *Radfordia* + *Cryptopsylla*	6	1352.668	4.471	0.008	9.352
sex + breeding status + *Androlaelaps* + *Cryptopsylla*	6	1352.684	4.487	0.008	9.427
season x sex	5	1352.823	4.626	0.008	10.105
sex + *Radfordia* + *Cryptopsylla*	5	1352.865	4.668	0.008	10.319
season + sex + breeding status	5	1352.881	4.684	0.007	10.402
age + sex + *Androlaelaps* + *Cryptopsylla*	6	1352.904	4.707	0.007	10.523
sex + breeding status + *Radfordia* + *Cryptopsylla*	6	1353.060	4.863	0.007	11.376
age + sex + *Cryptopsylla*	6	1353.112	4.915	0.007	11.676
season x sex *Androlaelaps*	6	1353.124	4.927	0.007	11.746
season + sex + body mass	5	1353.263	5.066	0.006	12.591
sex + *Androlaelaps* + *Cryptopsylla* + *Eulinognathus*	6	1353.348	5.151	0.006	13.139

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sex + *Cryptopsylla* + *Eulinognathus*	5	1353.367	5.170	0.006	13.263
sex x breeder + *Cryptopsylla*	6	1353.482	5.285	0.006	14.049
season + sex + *Eulinognathus*	5	1353.499	5.302	0.005	14.168
sex + breeding status + body mass + *Cryptopsylla*	6	1353.556	5.359	0.005	14.579
season + sex + breeding status + *Androlaelaps*	6	1353.574	5.377	0.005	14.710
season + age + sex	5	1353.595	5.398	0.005	14.865
season + sex + body mass + *Androlaelaps*	6	1353.664	5.467	0.005	15.387
age + sex + body mass + *Cryptopsylla*	6	1353.712	5.515	0.005	15.761
season + age + sex *Androlaelaps*	6	1353.714	5.517	0.005	15.777
age + sex + *Radfordia* + *Cryptopsylla*	6	1353.714	-5.517	0.005	15.777
sex + breeding status + *Cryptopsylla* + *Eulinognathus*	6	1353.806	5.609	0.005	16.520
sex + body mass + *Cryptopsylla* + *Eulinognathus*	6	1353.942	5.745	0.004	17.682
season x sex + *Androlaelaps* + *Radfordia*	7	1353.998	5.801	0.004	18.183
season x sex + *Radfordia*	6	1354.010	5.813	0.004	18.294
season + sex + breeding status + *Radfordia*	6	1354.104	5.907	0.004	19.174
season x sex + *Androlaelaps* + *Eulinognathus*	7	1354.230	6.033	0.004	20.420
season + sex + body mass + *Radfordia*	6	1354.276	6.079	0.004	20.896
age x sex + *Cryptopsylla*	6	1354.338	6.141	0.004	21.554
season x sex + breeding status	6	1354.414	6.217	0.003	22.389
season x sex + body mass	6	1354.600	6.403	0.003	24.571
age + sex + *Cryptopsylla* + *Eulinognathus*	6	1354.628	6.431	0.003	24.917
sex x breeding status + *Androlaelaps* + *Cryptopsylla*	7	1354.644	6.447	0.003	25.116
sex + *Radfordia* + *Cryptopsylla* + *Eulinognathus*	6	1354.658	6.461	0.003	25.294
season + sex x breeding status	6	1354.734	6.537	0.003	26.273
sex + *Androlaelaps*	4	1354.823	6.627	0.003	27.476
season + sex + *Radfordia* + *Eulinognathus*	6	1354.834	6.637	0.003	27.620
season + age + sex + *Radfordia*	6	1354.840	6.643	0.003	27.703
age x sex + *Androlaelaps* + *Cryptopsylla*	7	1354.846	6.649	0.003	27.785
season + sex + breeding status + *Eulinognathus*	6	1354.880	6.683	0.003	28.263
sex x breeding status + *Radfordia* + *Cryptopsylla*	7	1354.888	6.691	0.003	28.375
season x sex + *Eulinognathus*	6	1354.920	6.723	0.003	28.834
season x breeding status + sex	6	1354.986	6.789	0.003	29.801
season x sex + age	6	1354.988	6.791	0.003	29.831
age + sex x breeding status	7	1355.020	6.823	0.003	30.310
age x sex + breeding status + *Cryptopsylla*	7	1355.048	6.851	0.003	30.738
season + age + sex + breeding status	6	1355.062	6.865	0.003	30.955
season + sex + breeding status + body mass	6	1355.104	6.907	0.002	31.612
season x sex + body mass + *Androlaelaps*	7	1355.226	7.029	0.002	33.599
season x sex + breeding status + *Androlaelaps*	7	1355.230	7.033	0.002	33.666
season x sex + age + *Androlaelaps*	7	1355.274	7.077	0.002	34.415
season + sex + body mass + *Eulinognathus*	6	1355.288	7.091	0.002	34.659
sex x breeding status + body mass + *Eulinognathus*	7	1355.354	7.157	0.002	35.819
season + age + sex + body mass	6	1355.354	7.157	0.002	35.821
age x sex + body mass + *Cryptopsylla*	7	1355.464	7.267	0.002	37.845
season + sex x breeding status + *Androlaelaps*	7	1355.470	7.273	0.002	37.958
season x sex + body mass + *Radfordia*	7	1355.534	7.337	0.002	39.193

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season x sex + breeding status + *Radfordia*	7	1355.630	7.433	0.002	41.120
season + age x sex	6	1355.638	7.441	0.002	41.287
age x sex + *Radfordia* + *Cryptopsylla*	7	1355.644	7.447	0.002	41.409
season x breeding status + sex + *Androlaelaps*	7	1355.682	7.485	0.002	42.203
season + age + sex + *Eulinognathus*	6	1355.718	7.521	0.002	42.972
sex x breeding status + *Cryptopsylla* + *Eulinognathus*	7	1355.746	7.549	0.002	43.575
sex	3	1355.746	7.550	0.002	43.594
season + age x sex + *Androlaelaps*	7	1355.772	7.575	0.002	44.146
season + sex x breeding status + *Radfordia*	7	1355.860	7.663	0.002	46.131
(intercept)	2	1368.117	19.921	$3.7\text{x}10^{-6}$	$2.1\text{x}10^{4}$

The ‘+’ symbol indicates where predictors were added as main interactions. Any predictors connected by ‘x’ were employed in the model as a two-way (or three-way where three predictors are connected in this way) interaction term as well as separate main interactions.

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Table V: The 95% confidence set of GLMMs for *C. h. hottentotus* testosterone concentrations from the Darling population. Contains number of parameters in the model (k), corrected Akaike value (AICc), difference between model and best fitting model (ΔAICc), Akaike weight (w_i) and evidence ratio (ER). The intercept model is included for reference.

Models	***k***	**AICc**	**ΔAIC**	w_i	**ER**
sex x breeding status + body mass + *Androlaelaps*	7	727.009	0.000	0.135	
sex x breeding status	6	727.011	0.003	0.135	1.001
sex x breeding status + *Androlaelaps* + *Radfordia*	7	727.597	0.588	0.101	1.342
sex + breeding status + *Androlaelaps*	5	728.672	1.663	0.059	2.297
season x breeding status + sex + *Androlaelaps*	7	728.725	1.716	0.057	2.358
season x sex x breeding status + *Androlaelaps*	10	728.741	1.733	0.057	2.378
sex x breeding status + *Androlaelaps* + *Cryptopsylla*	7	728.953	1.944	0.051	2.643
season + sex x breeding status + *Androlaelaps*	7	729.169	2.160	0.046	2.945
sex + breeding status + *Androlaelaps* + *Radfordia*	6	729.671	2.663	0.036	3.786
sex + breeding status + body mass + *Androlaelaps*	6	730.003	2.995	0.030	4.470
sex + breeding status + *Androlaelaps* + *Cryptopsylla*	6	730.429	3.421	0.024	5.531
season + sex + breeding status + *Androlaelaps*	6	730.865	3.857	0.020	6.879
sex x breeding status	5	731.400	4.391	0.015	8.986
breeding status + body mass + *Androlaelaps*	5	731.462	4.453	0.015	9.269
season x breeding status + body mass + *Androlaelaps*	7	731.607	4.598	0.014	9.964
season x sex + breeding status + *Androlaelaps*	7	731.855	4.846	0.012	11.280
sex + breeding status	4	731.991	4.983	0.011	12.079
season x breeding status + sex	6	732.137	5.129	0.010	12.993
sex x breeding status + body mass	6	732.139	5.131	0.010	13.006
breeding status + body mass + *Androlaelaps* + *Radfordia*	6	732.415	5.407	0.009	14.931
breeding status+ body mass + *Androlaelaps* + *Cryptopsylla*	6	732.825	5.817	0.007	18.328
sex x breeding status + *Cryptopsylla*	6	733.105	6.097	0.006	21.082
sex x breeding status + *Radfordia*	6	733.157	6.149	0.006	21.637
season + breeding status + *Androlaelaps*	6	733.333	6.325	0.006	23.628
season + sex x breeding status	6	733.335	6.327	0.006	23.651
season x breeding status + body mass	7	733.375	6.366	0.006	24.119
sex + breeding status + *Cryptopsylla*	5	733.530	6.521	0.005	26.068
season x breeding status + *Radfordia*	7	733.549	6.540	0.005	26.311
sex + breeding status + body mass	5	733.592	6.583	0.005	26.889
sex x breeding status + body mass + *Cryptopsylla*	7	733.657	6.648	0.005	27.771
season + sex x breeding status + body mass	7	733.695	6.686	0.005	28.304
season + sex + breeding status	5	733.746	6.737	0.005	29.041
season x breeding status + sex	7	733.805	6.796	0.005	29.904
sex x breeding status + body mass + *Radfordia*	7	733.859	6.850	0.004	30.723
sex + breeding status + *Radfordia*	5	733.860	6.851	0.004	30.745
season x sex x breeding status	9	733.962	6.953	0.004	32.347
breeding status + *Androlaelaps*	4	734.097	7.089	0.004	34.620

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season x sex x breeding status + body mass	10	734.371	7.363	0.003	39.701
season x breeding status + body mass	6	734.745	7.737	0.003	47.867
sex x breeding status + *Radfordia* + *Cryptopsylla*	7	734.803	7.794	0.003	49.254
season + sex x breeding status	7	734.865	7.856	0.003	50.805
season x breeding status + *Androlaelaps*	6	734.901	7.893	0.003	51.750
season x sex + breeding status	6	734.913	7.905	0.003	52.061
(intercept)	2	785.670	58.661	2.4x10^{-14}	5.4x10^{12}

The '+' symbol indicates where predictors were added as main interactions. Any predictors connected by 'x' were employed in the model as a two-way (or three-way where three predictors are connected in this way) interaction term as well as separate main interactions.

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Table VI: The 95% confidence set of GLMMs for *C. h. hottentotus* testosterone concentrations from the Kamieskroon population. Contains number of parameters in the model (*k*), corrected Akaike value (AICc), difference between model and best fitting model (ΔAICc), Akaike weight (w_i) and evidence ratio (ER). The intercept model is included for reference.

Models	***k***	**AICc**	**ΔAICc**	w_i	**ER**
season x sex x breeding status + body mass	10	530.600	0.000	0.172	-
sex x breeding status + *Radfordia*	7	531.629	1.029	0.103	1.672
sex + breeding status + *Radfordia*	6	532.081	1.481	0.082	2.097
season x sex x breeding status + *Radfordia*	10	532.226	1.626	0.076	2.255
sex x breeding status + body mass + *Eulinognathus*	7	532.259	1.659	0.075	2.292
sex x breeding status + body mass + *Androlaelaps*	7	533.023	2.423	0.051	3.358
sex + breeding status + body mass + *Eulinognathus*	6	533.051	2.451	0.050	3.406
season x breeding status + sex + body mass	7	533.887	3.287	0.033	5.172
sex + breeding status + body mass + *Androlaelaps*	6	534.029	3.429	0.031	5.555
season x breeding status + sex + *Radfordia*	7	534.275	3.675	0.027	6.279
season x sex x breeding status + *Eulinognathus*	10	534.724	4.124	0.022	7.862
sex + breeding status + *Androlaelaps* + Radfordia	6	534.779	4.179	0.021	8.082
sex + body mass + *Androlaelaps* + *Radfordia*	6	534.983	4.383	0.019	8.950
sex + breeding status + *Radfordia* + *Eulinognathus*	6	535.075	4.475	0.018	9.371
sex x breeding status + *Androlaelaps* + *Radfordia*	7	535.267	4.667	0.017	10.312
sex x breeding status + *Radfordia* + *Eulinognathus*	7	535.503	4.903	0.015	11.603
season x sex + breeding status + Radfordia	7	535.989	5.389	0.012	14.795
sex + breeding status + *Radfordia*	5	536.514	5.914	0.009	19.243
season + sex + breeding status + *Radfordia*	6	536.745	6.145	0.008	21.600
sex x breeding status + *Radfordia*	6	536.903	6.303	0.007	23.375
sex + body mass + *Androlaelaps* + *Eulinognathus*	6	537.027	6.427	0.007	24.870
sex + body mass + *Androlaelaps*	5	537.048	6.448	0.007	25.132
season x sex x breeding status + *Androlaelaps*	10	537.070	6.470	0.007	25.406
season x breeding status + *Eulinognathus*	7	537.085	6.485	0.007	25.592
season x breeding status + *Radfordia*	7	537.121	6.521	0.007	26.056
sex + body mass + *Radfordia* + *Eulinognathus*	6	537.363	6.763	0.006	29.420
sex + breeding status + *Radfordia* + *Cryptopsylla*	6	537.423	6.823	0.006	30.316
sex + body mass + *Eulinognathus*	5	537.526	6.926	0.005	31.917
sex x breeding status + body mass	6	537.803	7.203	0.005	36.659
age + sex + body mass + *Androlaelaps*	6	538.131	7.531	0.004	43.193
sex x breeding status + *Radfordia* + *Cryptopsylla*	7	538.135	7.535	0.004	43.262
age + sex + *breeding status* + *Radfordia*	6	538.215	7.615	0.004	45.045
sex + breeding status + *Cryptopsylla* + *Eulinognathus*	6	538.635	8.035	0.003	55.572
age + sex x breeding status + *Radfordia*	7	538.691	8.091	0.003	57.126
sex + breeding status + body mass	5	538.896	8.296	0.003	63.315
season + sex x breeding status + body mass	7	538.929	8.329	0.003	64.345
sex + breeding status + *Eulinognathus*	5	538.998	8.398	0.003	66.628

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sex x breeding status + *Eulinognathus*	6	539.041	8.441	0.003	68.079
sex x breeding status + *Cryptopsylla* + *Eulinognathus*	7	539.075	8.475	0.002	69.218
age x sex + breeding status + *Radfordia*	7	539.097	8.497	0.002	69.984
season + sex + body mass + *Androlaelaps*	6	539.109	8.509	0.002	70.434
age + sex + body mass + *Eulinognathus*	6	539.133	8.533	0.002	71.284
sex + body mass + *Cryptopsylla* + *Eulinognathus*	6	539.151	8.551	0.002	71.928
season + sex x breeding status + *Eulinognathus*	7	539.229	8.629	0.002	74.759
sex + body mass + *Androlaelaps* + *Cryptopsylla*	6	539.231	8.631	0.002	74.864
season + sex + body mass + *Eulinognathus*	6	539.261	8.661	0.002	75.995
(intercept)	2	606.293	75.693	6.2x10^{-18}	2.7x10^{16}

The '+' symbol indicates where predictors were added as main interactions. Any predictors connected by 'x' were employed in the model as a two-way (or three-way where three predictors are connected in this way) interaction term as well as separate main interactions.

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General Discussion

Overall, this study aimed to assess whether abiotic or biotic factors had the most significant impact on ectoparasites infecting *Cryptomys hottentotus hottentotus*. In particular, the impact of climatic factors and social group size were considered. The study additionally aimed to look at how different individuals suffered varying parasite loads depending on their social status and how this was related to an individual's circulating hormone levels. Finally, hormonal correlates were considered in terms of habitat and seasonal differences in ectoparasite load.

It would be expected that abiotic factors have the largest impact on ectoparasite dynamics (Fabiyi 1996; Krasnov *et al.* 1997; Korallo-Vinarskaya *et al.* 2009). The largest influence on both the host and ectoparasites was the subterranean environment, which appears to have had great bearing on the adaptation of the ectoparasites. Overall, the small number of ectoparasites found in both habitats indicates that the subterranean environment may provide some protection from exposure to many of the ectoparasites found on rodents above ground. Few recent studies appear to have been carried out on rodents in the Northern Cape Province of South Africa. A study on springhares (*Pedetes capensis*) found only seven ectoparasite species, though this occurred in an arid savannah habitat and quite far from Kamieskroon (Anderson & Kok 2003). However, a study on four-striped mice (*Rhabdomys pumilo*), which also took place in similar lowland fynbos habitat and surrounding agricultural land in the Western Cape of South Africa, found 33 ectoparasite species (Matthee *et al*. 2007). This difference could be partially due to the larger number of locations sampled and the larger number of hosts studied (Matthee *et al*. 2007). However, a further study also analysed another 41 individuals of *R. pumilo* and seven individuals of vlei rats (*Otomys irroratus*) in various habitats within a nature reserve in the Western Cape Province and found 21 and 19 ectoparasite species respectively (Matthee *et al*. 2010). Recent work on a bathyergid species of the same genus as *C. h. hottentotus* also indicates that few ectoparasites are associated with individuals from this family of subterranean rodents. Four ectoparasite species were found on the highveld mole-rat (*C. h. pretoriae*) suggesting the subterranean environment may provide protection from more generalist parasites (Viljoen *et al*. 2011). It has been proposed that the restrictions of burrow systems would result in the ectoparasites of subterranean mammals displaying higher levels of co-

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speciation due to the adaptations required for parasites to survive (Hafner *et al*. 2000). One example of this is the close adaptation of chewing lice to their hosts, as has been demonstrated in the pocket gopher (Geomyidae; Hafner *et al*. 1994). Yet such close co-speciation is not demonstrated in all systems (Hafner *et al*. 2000). The majority of species found here appear to have specialised to the Bathyergidae if not *C. h. hottentotus*. Intriguingly, the population dynamics of the only generalist parasite found (*R. ensifera*) was not largely impacted by any biotic factors. Thus it appears that the subterranean environment may have protected the hosts from most generalist parasites, but has resulted in a few species of ectoparasite evolving closely with them over time.

The level of specialisation may be more distinct in this subterranean host-parasite system, but general patterns demonstrated by most macroparasites were still observed. For example, all parasite species were aggregated amongst the host populations, a pattern that is seen across all macroparasites (Shaw & Dobson 1995; Rózsa *et al*. 2000; Wilson *et al*. 2001). Most parasites were found among both host populations despite the great difference in overall parasite load between the two habitats. Ectoparasites tend to be more heavily influenced by the surrounding environment due to their exposure to factors such as rainfall and temperature (Fabiyi 1996; Krasnov *et al*. 1997, 2008; Matthee *et al*. 2007; Korallo-Vinarskaya *et al*. 2009). Therefore it might be expected that the parasite communities would differ greatly between the two host populations. Nevertheless studies on gamasid mites across multiple hosts and habitats indicate that host species were the most significant predictor for the presence and abundance of a parasite species, due to close co-evolution of host and parasite (Vinarski *et al.* 2007; Krasnov *et al.* 2008). Despite their need for close contact between hosts for transmission mite assemblages were not found to be largely influenced by geographic distance (Vinarski *et al.* 2007). Further, abiotic influences associated with the habitat came second to the impact of host species (Krasnov *et al*. 2008). Due to the high level of specialisation found in the ectoparasitic fauna associated with *C. h. hottentotus*, it is highly probable that such close co-adaptation exists here. Nevertheless it is clear that the surrounding habitat can alter the relationship between a host species and its ectoparasite fauna. The variation in prevalence and abundance between locations were most likely to be a result of contrasts in abiotic factors as shown in previous studies (Fabiyi 1996; Korallo-Vinarskaya *et al*. 2009). Yet differences in rainfall and distribution of food resources between arid and mesic habitats are known to create variations in foraging and dispersal behaviour in *C. h. hottentotus* (Spinks *et al*. 2000, 2000a; Bishop *et al*. 2004).

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This was supported by the differences of the hormonal profiles between host populations. For example, the cortisol concentrations of *C. h. hottentotus* were higher in the arid habitat, where parasites were more abundant. However, it is not certain whether abiotic factors influence the parasite dynamics directly, or whether the differences in the host due to abiotic factors subsequently lead to variations in parasite load. For instance, it could be that parasite abundances are high in the arid region due to climatic factors such as temperature, which could increase survival and developmental rates for ectoparasites (Linardi & Krasnov 2013). Alternatively, abiotic factors have led to increased cortisol concentrations in the host which may have led to higher parasite loads via a less effective immune system (Dubansky *et al*. 2011). It has also been demonstrated that parasites may only have a significant influence on cortisol levels of the host when abiotic factors have already lead to increased cortisol concentrations (Quillfeldt *et al*. 2004). Further study would be required to determine how these factors influence one another.

Seasonal fluctuations were also found in both locations, with the prevalence and abundance of all parasite species increasing during the winter rains. Seasonal peaks in ectoparasite abundance and prevalence are extremely common (Wilson *et al*. 2001; Altizer *et al.* 2006). As with differences in habitat, seasonal fluctuations in abiotic factors not only influence the dynamics of the parasite community, but also the dynamics of the host population. However, the expected seasonal fluctuations in testosterone were not found in the host and thus did not conform to the challenge hypothesis (Wingfield *et al*. 1990). Additionally, there were no common positive trends across populations between parasite abundance and steroid hormone concentrations in the host. Therefore it appears that fluctuations in prevalence and abundance were not associated with immunosuppression from increased androgens or glucocorticoids as seen in several host males above ground (Folstad & Karter 1992; Salvador *et al*. 1995; Berger *et al*. 2005; Clough *et al*. 2010). Fluctuations in ectoparasitic species that infect hosts above ground are often found to be due to abiotic factors such as temperature or rainfall (Merino & Potti 1996; Altizer *et al*. 2006). Although the differences in mite abundance and prevalence between the two seasons were closely correlated to rainfall, it is thought that the potentially high humidity within the burrow system (Sumbera *et al*. 2004), would make the direct effects of rainfall unlikely. Similarly, the burrow creates a buffer from temperature fluctuations, thus this was thought to explain why temperature appeared not be significant within a habitat (Sumbera *et al*. 2004). Peak rainfall is an important time for *C. h. hottentotus,* as it enables more effective foraging and

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the ability to disperse prior to breeding (Spinks *et al*. 2000). Host social interactions do increase during this time providing an opportunity for ectoparasites to disperse and maintain outbreeding events themselves. One of the suggestions for increased parasitism during host breeding season is the increased proximity of hosts during breeding (Marshall 1981; Christe *et al*. 2000). Therefore it is plausible that the ectoparasites here have adapted to the subterranean lifestyle of their host by reproducing during host dispersal.

The influence of biotic factors on the other hand varied depending on the parasite taxa. This is commonly found in studies that analyse parasitic taxa on a single host species separately (Hillegass *et al*. 2008; Monello & Gompper 2010; Krasnov & Matthee 2010; Scantlebury *et al*. 2010). The differences in life history and strategies of parasites are likely to be the cause of this variation (Monello & Gompper 2010). Not all parasite taxa could be studied for effects of group size. But from analyses on mites, it appears that living within social groups does not appear to be a cost to the host. This is further supported by a study on energetics in Natal mole-rats (*C. h. natalensis*), where larger colonies had smaller abundances of gastrointestinal cestodes. It was also found that energetic costs of endoparasites were only noticeable in infected individuals when abundances were high (Lutermann *et al.* 2013). In this study, the abundance and prevalence of *R. ensifera* was not significantly influenced by colony size. *Androlaelaps* mites on the other hand, were found in smaller abundances in larger colonies. Three meta-analyses of the parasite literature have shown that parasitic burden generally increases in relation to group size in social species (Cote & Poulin 1995; Rifkin *et al*. 2012; Patterson & Ruckstuhl 2013), though one did indicate that this did not always apply to mammals (Rifkin *et al*. 2012). Each of these studies indicates that these correlations are strongest for parasite species that are transmitted by direct contact, such as mites (Cote & Poulin 1995; Rifkin *et al*. 2012; Patterson & Ruckstuhl 2013). The latest of these studies also posited that the mobility of the host has a significant influence on how colony size and parasite load are related. Patterson and Ruckstuhl (2013) showed that sedentary species (i.e. species with a nest or a burrow that they regularly return to) tend to suffer higher parasitism as a result of group size than more mobile hosts. This may be the case for social vertebrates as a whole and certainly appears to be the case for fleas infesting two prairie dogs (*Cynomys* spp.; Hoogland 1979). One study on fleas in European badger (*Meles meles*) also indicated a positive relationship though only at the larger social group level. No significant correlation was found between the number of individuals within a sett group and flea abundance (Johnson *et al*. 2004). However the majority of studies on

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ectoparasites infecting small mammals with nesting burrows indicate no influence of group size (Arnold & Lichtenstein 1993; Van Vuren 1996; Hillegass *et al*. 2008; Burger *et al.* 2012). In the case of yellow-bellied marmots (*Marmota flaviventris*), lice were less prevalent in colonial individuals than those that were solitary (Van Vuren 1996). Further, the negative relationship between *Androlaelaps* spp. abundance and colony size has also been seen in another cryptomid species, *C. h. pretoriae* (Viljoen *et al*. 2011). This suggests that the pressure of increased parasitism in social burrow systems has led to behavioural adaptations in several species (Loehle 1995; Johnson *et al*. 2004). For example, the use of several nests within a burrow or sett has been observed to prevent parasite infections increasing rapidly (Butler & Roper 1996; Roper *et al*. 2002). Anti-parasitic behaviour such as allogrooming has been attributed to non-significant and negative correlations in several studies (Sanchez-Villagra *et al*. 1998; Johnson *et al*. 2004; Bordes *et al*. 2007). However, isolation between colonies would be strong due to the constraints of living underground, thus this may also play a role (Viljoen *et al*. 2011). Isolation between social groups has been demonstrated to be effective in reducing parasite abundances within populations of gregarious invertebrates (Wilson *et al*. 2003).

Further evidence that parasite taxa respond differently to biotic factors was indicated by host differences between host sex and social status. Male-biased parasitism was found in *C. ingrami* and *E. hilli* for instance, though *Androlaelaps* spp. and *R. ensifera* appeared to have no significant preference for hosts of a certain sex. Additionally, the results observed in this study indicate that multiple mechanisms associated with these two biotic factors may be interacting with the differences in parasite life history. Male-biased parasitism, which is common in many macroparasites infecting vertebrates that move above ground but there are several potential reasons why this may occur (Schalk & Forbes 1997; Perez-Orella & Schulte-Hostedde 2005; Krasnov *et al*. 2005; Harrison *et al*. 2010). Male-biased parasitism may be a common pattern between two ectoparasite taxa found on *C. h. hottentotus* but there appears to be different mechanisms acting on each species. Although it is not certain what factor contributes to male-biased parasitism in *C. ingrami*, it could be inferred from the hormonal study that this is not associated with immunosuppression or behavioural traits mediated by increased testosterone in males. The correlation between flea abundance and testosterone concentrations of the host were not particularly strong when factors such as season and host sex were taken into account. Similar conclusions could be made for *E. hilli*, as the abundance of this louse species was not related to testosterone levels of the host.

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However, in the case of *E. hilli*, a significant correlation between its abundance with body size indicated the male bias was associated with sexual size dimorphism in the species. This appears to be quite common in several vertebrates (Moore & Wilson 2002). The prevalence or abundance of *C. ingrami* on the other hand was not related to the body mass of the host. There are studies that indicate that body mass is not always the reason for sex-biased parasitism (Perez-Orella & Schulte-Hostedde 2005; Poulin 2013), including a study on fleas of various rodent hosts (Krasnov *et al*. 2005). The entire lifecycle of lice occurs on the host (Ledger 1980), which makes it likely that body size is more important to this species than the flea, which will not stay on the host permanently (Krasnov 2008). *E. hilli* did sometimes reach quite large intensities and it is possible that intraspecific competition may arise if this occurred on a smaller host. Additionally, the handful of individuals that suffered the greatest abundance of *E. hilli* appeared to be the largest females, despite the average abundance being higher on males. Therefore there may be multiple mechanisms influencing the aggregation in abundance of *E. hilli*, as body mass cannot be the only cause.

Though sociality may be a benefit to the group as a whole, it is more difficult to tell whether certain individuals suffer higher parasitism due to their social status. Previous studies show individuals of both higher social status (Ezenwa 2004; Muehlenbein & Watts 2010) and lower status (Zuk *et al*. 1998; Fugazzola & Stancampiano 2012) may suffer higher parasite burdens. In a previous study on bathyergids, Viljoen *et al.* (2011) indicated that *Androlaelaps* spp. abundance and prevalence was not significantly different between breeding and non-breeding individuals in *C. h. pretoriae.* This was suggested to be a result of equal exposure within a colony to the parasites within its burrow system (Viljoen *et al*. 2011). However, this study suggests that differences in exposure or susceptibility may differ between breeders and non-breeders, though the results differed between parasite taxa. Breeding individuals of *C. h. hottentotus* suffered higher abundances of *Androlaelaps* spp., though non-breeding individuals had higher abundances of *C. ingrami*. The mechanisms behind the differences in parasite load found between breeders and non-breeders of *C. h. hottentotus* are uncertain. It could be inferred that this difference may be a result of behavioural differences between breeders and non-breeders in this species creating differences in exposure (e.g. differences in grooming rates, Mooring *et al*. 1996), as the hormonal data did not fit the bias of parasitism on breeding and non-breeding individuals. For example, increased testosterone has been attributed to larger parasite burdens in higher ranking males in both social and communal breeding species (Muehlenbein & Watts 2010;

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Negro *et al*. 2010). However, the abundance of *Androlaelaps* spp. was negatively correlated to testosterone concentrations of the host, despite breeding individuals generally having higher testosterone levels. This suggests that breeding individuals in this population do not suffer higher parasitism as a result of immunosuppression, or behavioural differences associated with elevated testosterone that increases exposure. Furthermore the significant relationship between parasite and host hormone data was not constant across both populations. The relationship between testosterone and *Androlaelaps* mites was only significant in the mesic habitat. *C. ingrami* significantly led to increased cortisol concentrations in their host in the arid habitat, which infers that these fleas incur a cost to their host at this site. Yet the non-breeding individuals did not have significantly different cortisol levels from those of higher status at this site. Therefore immunosuppression from raised glucocorticoids is not a cost of lower social status either. However, these conclusions are speculative, as it is difficult to draw conclusions from comparing results between the second and third chapters. The former was aimed at looking for general patterns in parasite dynamics across the populations, whereas the latter considered each population separately looking for differences. With host sex-bias in parasitism, there are many possible mechanisms that could influence whether higher or lower social status is more costly in terms of parasitism. As has been shown with hormonal differences here, host sex-bias can alter between a specific host and parasite species across habitats (Krasnov & Matthee 2010). Conclusions made from host-parasite systems within one habitat may not be applicable to the same host species and its parasites in another. Therefore, the hypothesis that hosts adapt to *Androlaelaps* spp. infection by reducing testosterone levels in order to increase immunological function (Lutermann *et al*. 2012) cannot be assumed for all populations of *C. h. hottentotus.*

In summary, this study indicates just how complex relationships between hosts and parasites are. The social and subterranean lifestyle of the host has created an environment that has proven beneficial to the host in terms of parasitic infection. The ectoparasites that have adapted to the subterranean environment may be more dependent on their hosts than the majority of ectoparasites on the surface. However, similar to host-parasite systems above ground, there are multiple factors impacting both host and parasite dynamics at any one time. Specifically biotic factors such as host sex and social status create differences in both physiology and behaviour, which are all likely to influence an individual's parasite load differently. Hypothetically, a female of higher social status may increase exposure by

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aggressive behaviour, and higher testosterone levels may also increase susceptibility. Additionally, increased body size of higher ranking individuals presents the potential for her to carry higher parasite abundances. As a consequence, she is also more likely to receive higher rates of allogrooming from other members of the colony in order to pacify her. This would reduce her overall parasite load. Further, as part of a large social group, these grooming rates are higher and overall parasite exposure is relatively low. Which of these factors are most significant within the host-parasite relationship is likely to depend on how each one interacts with the life history of the parasite in question. Finally, differences in abiotic and biotic factors associated with habitat are likely to alter the relationship between host and parasite. Overall, general patterns in parasite ecology are likely to be difficult to find (Poulin 2007). Perhaps future studies should consider how host-parasite relationships may change between different habitats and what may be the cause of these differences.

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