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Drivers of bat fly diversity and prevalence of six *Rhinolophus* bat species in southern Africa

by

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As the candidate's supervisor I have/have not approved this thesis/dissertation for submission

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Abstract

Demographic and ecological characteristics render obligate cave roosting bats highly susceptible to infestation by ectoparasites. However, the patterns and factors of ectoparasite loads among bat host species are understudied, particularly in the Old World. I tested predictions of habitat heterogeneity, host sex, body size hypotheses to explain parasitic bat fly (Streblidae and Nycteribiidae) abundance, morphospecies richness and prevalence on six *Rhinolophus* bat species in southern Africa. I sampled and classified 930 bat flies to six morphospecies (3 streblids and 3 nycteribiids) captured on 333 bats at 20 sites in eight biomes. In support of the habitat heterogeneity hypothesis, there were significantly positive relationships between habitat heterogeneity and bat fly abundance, morphospecies richness and prevalence. In support of the host body size hypothesis, there were significantly positive relationships between host body condition and bat fly abundance, morphospecies richness and prevalence. By contrast, there was little evidence that parasitic flies preferred either male or female bats. Recursive partitioning analysis showed that the most significant predictor of bat fly abundance and morphospecies richness was habitat heterogeneity, specifically the number of land cover classes surrounding bat roosts. My results suggest that land use and biome characteristics at the meso-scale, and to a lesser degree biotic processes at the local scale, mediate bat fly abundance and morphospecies richness on rhinolophid bats. Specifically, structurally heterogeneous and complex habitats increase the number of niches available for bat species as well as their prey, which, in turn, may favour diverse bat fly populations. Thus, factors responsible for driving bat diversity may also drive bat fly diversity. Future studies should focus on other families of cave-roosting bats, as well as endoparasites, to better understand the mechanisms responsible for ectoparasitism in Old World bats.

Preface

The field and lab work described in this dissertation was carried out in the School of Life Sciences, University of KwaZulu-Natal, Westville campus, from July 2014 to December 2016, under the supervision of Prof. M.C Schoeman.

This study represents original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

Declaration

I, ………………………………………. declare that

1. The research reported in this dissertation, except where otherwise indicated, is my original research.

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Chapter 1. Introduction

Bats (order Chiroptera) comprise more than 20 % of total mammal species richness (Bordes et al., 2008), with 25 % of global bat species richness represented in Africa and Madagascar (Monadjem et al., 2010). Bats are important for a wide range of ecological processes and ecosystem services including pollination, seed dispersal and control of insect populations (Patterson et al., 2008a). Thus, understanding the mechanisms that may impact their population dynamics and individual fitness is of paramount importance. Studies in bat community ecology set in the Old World have primarily focused on predator-prey interactions (e.g. Allotonic Frequency Hypothesis; Jacobs, 2000; Schoeman and Jacobs, 2003; Schoeman and Jacobs, 2011), habitat-driven selection (Willig and Moulton, 1989), body size (e.g. Allometric Hypothesis; Stoffberg et al., 2011; Taylor et al., 2012), facilitation and competition (Findley and Black, 1983; Aguirre, 2002; Schoeman and Jacobs, 2008). Few studies have investigated bat-parasite interactions, particularly in the Old World. Understanding the drivers of bat ectoparasite diversity may reveal new insights into the factors that shape bat population and community structures, allowing for the development and implementation of conservation and management strategies.

1.1. Bat ectoparasites

Bats are hosts to a number of different ectoparasites including mites and ticks (Acari), fleas (Siphonaptrea), lice (Thichodectidae), bat bugs (Cimicidae) and bat flies (Nycteribiidae and Streblidae) (Zahn and Rupp, 2004; Lourenço and Palmeirim, 2008; Billeter et al., 2012). Studies on bat-parasite interactions have increased markedly in the last two decades, particularly in the New World (Ter Hofstede and Fenton, 2005; Patterson et al., 2008a; Dick et al., 2009; Camilotti et al., 2010; de Souza Aguiar and Antonini, 2011). However, there have been comparatively few studies conducted on African bat-ectoparasite interactions, and these studies are either taxonomic (Jobling, 1936; Maa, 1965), brief technical reports (Whitaker and Mumford, 1978), evaluate the potential of bat flies as viral vectors between hosts in the roost (Billeter et al., 2012; Kamani et al., 2014), or reviews of host-bat fly associations (Shapiro et al., 2016).

1.1.1. Bat flies

Bat flies are classified into two morphologically distinct families: Nycteribiidae and Streblidae. However, the Streblidae family is paraphyletic, between members of the Old and New World groups (Figure 1), where Old World streblids are at a molecular level more similar to members of the monophyletic Nycteribiidae family than to New World streblids (Dittmar et al., 2006).

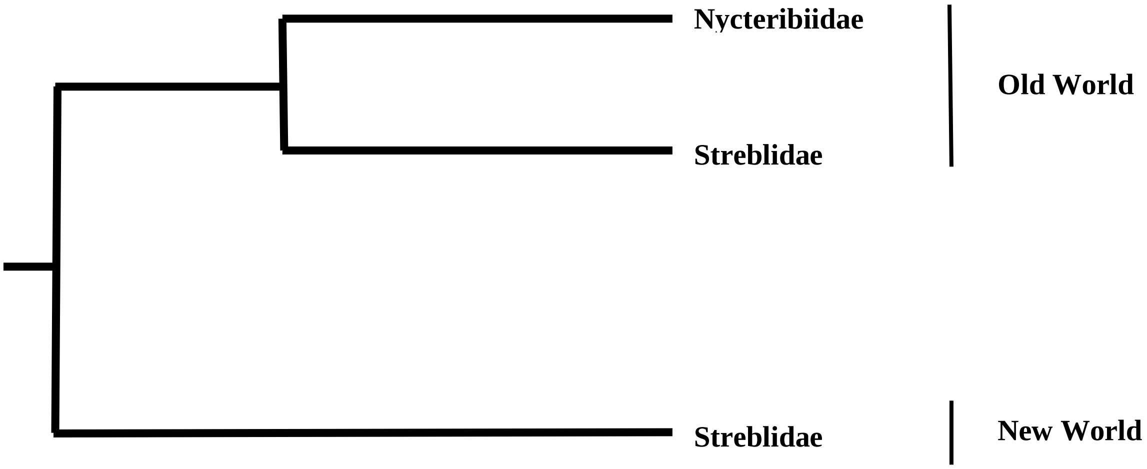

Figure 1 Simplified phylogeny of Old World and New World bat flies (Dittmar et al., 2006)

Nycteribiids are represented by ± 275 species worldwide, occurring mainly in the eastern hemisphere. The highest diversity is in the Malaysian subregion of the Oriental Region, the hypothesised origin of the family (Dick and Patterson, 2006; Billeter et al., 2012). Members of this family have also radiated in other biogeographical areas such as the Australian, Palaeartic and Ethiopian subregions (Dittmar et al., 2006). Nycteribiids are wingless, with dorsoventrally flattened thoraxes, reduced eyes and spider-like in appearance. Nycteribiids are found predominantly within the fur of the bats, both on the dorsal and ventral surfaces (Dick and Patterson, 2006).

By contrast, Streblidae comprise ± 227 species worldwide, with ~70 % of the species in the western hemisphere. There have been at least two separate dispersal events from their origin in the Neotropics across the New World (Dick and Patterson, 2006; Billeter et al., 2012). Streblids range from laterally compressed (resembling fleas in appearance) to dorsoventrally flattened or completely uncompressed, and their wings range from absent to fully developed (Dick and Patterson, 2006). Individuals with fully developed wings are weak flyers (Dick and Patterson, 2006). Streblids are found mainly around the joints of the wing and tail membranes of bats. Limited dispersal capabilities of both bat fly families means that parasite prevalence and abundance are largely governed by the bats' behavioural and ecological characteristics.

1.1.2. Bat fly life histories

Bat flies are specialised blood feeders on bats and often exhibit high host specificity for a single bat species or genus (Ter Hofstede and Fenton, 2005; Dick and Patterson, 2007; Patterson et al., 2007; Bordes et al., 2008). The flies stay attached to the fur and/or on the wing and tail membranes of bats using a number of specialised appendages, including ctenidia and tarsal claws (Dick and Patterson, 2006). Males spend their entire adult life stage (60 - 90 days) on their hosts, whereas females exhibit viviparous puparity and leave their hosts every 10 days to deposit a single larvae in the 3rd in-star on the roost walls. The larvae pupate immediately after they are deposited and emerge from the puparium within 3-4 weeks (Dick and Patterson, 2006). Newly emerged adults require their first blood meals within a few hours to survive, thus making host roost fidelity vitally important for bat fly survival (Dick, 2007).

1.1.3. Impact of parasite on host

Hosts and parasites are in an evolutionary arms race where parasites are favoured to detect and utilise hosts, whereas hosts are favoured to avoid and remove parasites (Reckardt and Kerth, 2006). High ectoparasite loads can lower the bat's immunocompetence (Bordes et al., 2008). Low immunocompetence renders the host more susceptible to diseases and poor body condition which negatively impacts growth and development, and ultimately fitness (Reckardt and Kerth, 2006; Young et al., 2015).

Host specific parasites that have co-evolved with their hosts are often able to avoid initial detection through, for example, reduced immune responses to their presence, whereas more generalist and opportunistic parasites often evoke a greater initial immune response from the host (Giorgi et al., 2004; Dick and Patterson, 2007). Host specificity arises from three main mechanisms (Giorgi et al., 2004; de Vasconcelos et al., 2016): (i) encounter, which may mediate the number of host species it may come into contact with over its life history due to the dispersal capabilities of the parasite; (ii) compatibility, which would mediate the evolution of host preference through variability in the fitness gains acquired from different host species; and (iii) reproductive, through differences in reproductive success after colonisation of a new host species. The host can avoid detection by parasites using various behavioural strategies such as switching roosts and grooming (Reckardt and Kerth, 2007; Krasnov et al., 2012).

1.2. Factors influencing bat fly abundance and prevalence

1.2.1. Bat defences

Bats use different behavioural and immunological characteristics to reduce bat fly abundance. Bat flies need to bite through the skin of the bat to feed on their blood. As foreign saliva enters the blood stream of the bat this should trigger an immune response alerting the bat to its presence. However, the degree of host specificity (immune mimicry; Dick, 2007) will influence the intensity of the immune response. If the parasite-host association evolved in high host specificity, often bat flies circumvent the bats' immune responses by using similar immune-signalling molecules that allow the bat flies to exploit the bat with minimal reaction to their presence (Dick and Patterson, 2007). When an immune response is triggered, the bat will begin grooming the affected area (Dick and Patterson, 2007). Grooming is the main mechanism for bat fly removal. Grooming agitates the bat fly to leave the bat, or it causes mortality by fatally wounding the bat fly or by eating it, yet this has been rarely documented (Ter Hofstede and Fenton, 2005). Energy used in grooming could decrease the fitness of the bat more than fitness gained by removing the ectoparasite (Ter Hofstede and Fenton, 2005; Reckardt and Kerth, 2006). Bat flies are highly maneuverable and hard to dislodge (Dick and Patterson, 2006).

Alternatively, bats may reduce bat fly abundance by switching roosts (Ter Hofstede and Fenton, 2005; Dittmar et al., 2009). Bat flies prefer warm humid roosts because they increase reproduction and pupae development. Before winter bats often switch to roosts with lower temperatures (Reckardt and Kerth, 2006; Bordes et al., 2008). This is particularly important for breeding female bats which utilise spermatogenesis during winter (Sharifi et al., 2013). Females store sperm within their reproductive tract before winter and delay fertilisation until favourable conditions return around spring. This survival strategy is favoured by selection because insects are low in calcium, therefore, females requires high prey availability for the development of their offspring to avoid sacrificing too much calcium from her own bones (Kwiecinski et al., 1987; Barclay, 1994). Bats also significantly reduce their activity and enter hibernation or deep torpor in colder roosts in winter to cope with low availability of prey resources (Park et al., 2000). High ectoparasite abundance in winter could increase the bat's physiological and behavioural responses and cause it to exit torpor more frequently, severely compromising its chances of survival (Horáček et al., 2014). On the other hand, during torpor bats reduce their body temperatures and the amount of blood flowing to the surface of their skin (Currie et al., 2015) which results in less available food resources for bat flies. Coupled with the reduced developmental success of the free living pupae stages of the life cycle, cooler roost sites induce physiological responses in the bat flies to reduce or halt energetically costly reproductive activity (Reckardt and Kerth, 2006; Lourenço and Palmeirim, 2008).

1.2.2. Bat roost characteristics

Bat species inhabit different types of roosts, such as permanent and temporary roost. Permanent or long-term roosts such as caves, tunnels, houses and mines are highly durable. These permanent roosts give excellent protection from abiotic, environmental factors and biotic factors such as predation (Kunz, 1982). However, these roost parameters often provide favourable micro-habitat availability for bat flies because of high bat densities and reduced spacing between them, which in turn, increases bat fly diversity (Patterson et al., 2007). Conversely, temporary roosts such as leaf tents, and spaces under bark and foliage are often more common in the landscape than permanent roosts, yet they are less durable and provide less protection from environmental elements and, predators, hence resulting in less stable living conditions. These roost attributes lead to reduced roost fidelity by bats, and as a result, reduced bat fly diversity because there is a high likelihood that bats may switch roosts before the new adult emerges from the puparium. Similar types of roosts may differ in their structural properties (protection and durability), and spatial properties (occupancy, colony size) (Patterson et al., 2007).

Parasite transmission between host individuals occur through two pathways, vertical and horizontal transfer (Clayton and Tompkins, 1994). Vertical transfer is the dispersal of parasites from an adult to its offspring (Clayton and Tompkins, 1994). In bats this occurs often in the maternity colonies between females and their pups (Webber et al., 2015). Horizontal transfer is the dispersal of parasites between members of the bat colony and where prolonged direct contact is not required for the transfer to take place (Ter Hofstede and Fenton, 2005). Horizontal transfer commonly occurs within roosts as well as during swarming events (Webber et al., 2015). Bats roosting in large colonies at sites with high roost temperatures have high parasite abundance because of high rates of horizontal transfer between bat individuals, and concomitant positive effects on the reproductive success of parasites (Marshall, 1982; Christe et al., 2007; Sharifi et al., 2008).

1.2.3. Habitat heterogeneity hypothesis

Environmental variables can have both direct and indirect effects on parasite diversity. Direct effects include fluctuations in parasite reproductive success and developmental rates. Indirect effects include fluctuations in host density, which in turn changes food resources and habitat availability to the parasites. Environmental heterogeneity is characterised by variables such as vegetation type and structure, precipitation, altitude and climate. There is a positive relationship between environmental heterogeneity and small mammal diversity, as well as their ectoparasite richness and abundance (Thamm et al., 2009; Halos et al., 2010).

1.2.3.1. Structural diversity of vegetation

There is a significant positive relationship between bat diversity and structural environmental heterogeneity and vegetation diversity in southern Africa (Qian et al., 2009; Schoeman et al., 2013), which in turn, may be correlated with bat fly diversity (Lourenço et al., 2016). High structural diversity of habitats and diversity of vegetation favours high bat diversity because there are more available niches, particularly roosts and food to exploit (de la Peña-Cuéllar et al., 2015). Further, biomes with higher vegetative structure such as Albany Thicket, Forest, Fynbos and Savanna in southern Africa are associated with higher insect diversity than habitats with lower vegetative structure such as Grassland, Desert, Nama-Karoo and Succulent-Karoo (Lawton, 1983; Tews et al., 2004).

Few studies have investigated the influence of vegetation heterogeneity on bat ectoparasites. Brazilian deciduous forests were found to have lower bat fly prevalence and diversity when compared to other Brazilian forest types, being attributed to differences in biotic and abiotic conditions such as; low bat species richness; lack of durable roosts; and low winter temperatures impacted the streblid species richness (Camilotti et al., 2010). The positive relationship between vegetative structure and vertebrate diversity is important to parasites with multiple blood feeding life cycles, such as *Ixodes* ticks (Thamm et al., 2009). Additionally, increased host diversity leads to increased chances of host encounters, and hence increased parasite abundance (Thamm et al., 2009; Halos et al., 2010). Ground cover is positively correlated with flea abundance, because cover keeps the soil moist and provides a humid environment which favours flea development. By contrast, Lourenço et al. (2016) found no relationship between vegetation diversity and mite diversity. Instead, mite diversity was driven primarily by the presence and abundance of bats rather than external factors.

1.2.4. Host sex hypothesis

Sex of host affects parasite abundance and prevalence in vertebrates. Specifically, male mammals often have higher parasite abundance than females because they disperse over longer distances rendering them more vulnerable and likely to encounter a wider diversity of parasites (Kowalski et al., 2015). Further, increased contact time between male bats during the breeding season, especially at swarming sites or leks, may facilitate horizontal transfer of ectoparasites between individuals (Webber et al., 2015). Additionally, the exhibition of secondary sexual traits by males in polygamous mating systems may be associated with high parasite loads, because the energy required for the development of these traits lowers the amount of energy available for grooming and immunocompetence (Kowalski et al., 2015; Young et al., 2015). Further, increased testosterone levels may also decrease immune defences (Schalk and Forbes, 1997; Christe et al., 2007; Kowalski et al., 2015). Decreased immune defences increase both parasite abundance and prevalence. Consequently, low immunocompetence typically occurs during reproductive periods (Zahn and Rupp, 2004). Studies in the New World have demonstrated male host preference by bat flies. For example, behavioural characteristics such as roosting in colonial harems increase the desirability of male hosts to bat flies (Komeno and Linhares, 1999). Similarly, male bats that switched between roost colonies and multiple harems were favoured by bat flies (Komeno and Linhares, 1999). Presently there are no data on the preference of bat flies for males in the Old World.

By contrast, female bats also have lower immune defences during the reproductive season, specifically when they are pregnant or rear young (Zahn and Rupp, 2004; Christe et al., 2007). Additionally, females often congregate in maternity colonies, which enables vertical and horizontal transfer of ectoparasites among individuals (Zahn and Rupp, 2004; Lucan, 2006; Christe et al., 2007; Patterson et al., 2008a; Presley and Willig, 2008). Further, females bat are often larger than males, which my favour higher parasite abundance (see Section 1.2.5). Moreover, bat flies and other ectoparasites may favour female hosts because the ectoparasites then have access to juvenile bats which have underdeveloped immune defences (Christe et al., 2007). Additionally, grooming in juveniles is relatively inefficient (Lourenço et al., 2016). Ectoparasites with limited dispersal capabilities and that spend their entire life cycle on the bats, such as mites, show distinct female bias on European Vespertilionidae bats (Zahn and Rupp, 2004; Lucan, 2006; Christe et al., 2007). Similarly, ectoparasites that have life stages free of bats, such as bat flies in the Neotropics, also appear to prefer female bats (Patterson et al., 2008a; Presley and Willig, 2008).

1.2.5. Body size and condition hypotheses

The body of the host provides the primary habitat of the resident ectoparasite, providing not only food and habitat but also a stable living environment in which to survive and reproduce. Two distinct microhabitats on bats are their fur and membranous areas, and each independently govern the number and composition of ectoparasites (Patterson et al., 2008a; Presley, 2011). Body size of small mammals is positively correlated to the abundance and richness of their ectoparasites (Young et al., 2015). This habitat is firstly characterised by surface area (i.e. body size) of the host. Increased size often means more microhabitats and resources available to the parasites. Larger bat species may also be more easily located by ectoparasites than smaller hosts (Patterson et al., 2008a).

Bats with good body conditions may provide more nutrient-rich blood meals for resident parasites and hence be favoured by parasites. In support, bat mites prefer bats with good body conditions (Christe et al., 2003). However, as parasite abundance increases, bat body condition should decrease because of increased bites for blood meals and increased energetic outputs on parasite avoidance (Lourenço and Palmeirim, 2008).

1.3. Rhinolophidae as model taxa

Taxonomic diversity, and demographic and ecological characteristics make Rhinolophidae a good model taxa to test predictions from hypotheses on host-parasite interactions (Ter Hofstede and Fenton, 2005; Patterson et al., 2007). Bats are classified into two distinct infraorders: the Pteropodiformes and Vespertilioniformes (Jones and Teeling, 2006). Molecular evidence shows that the two insectivorous bat families Rhinolophidae and Hipposideridae are more closely related to fruit bats (Pteropodidae) than to other echolocating insectivorous bats (Bogdanowicz et al., 1999; Eick et al., 2005; Jones and Teeling, 2006). Rhinolophidae are an Old World family of bats comprising a single genus (*Rhinolophus*) with 71 species (Maree and Grant, 1997; Monadjem et al., 2010; Taylor et al., 2012). The family originated ca. 39 million years ago (MYA) probably in the tropical forests of South East Asia (Teeling et al., 2005). Extant rhinolophids are widespread in Africa, Europe, the Middle East, Australia and Asia (Bogdanowicz and Owen, 1992; Maree and Grant, 1997).

Rhinolophidae are recognisable by their specialised nosed leaf which resembles a horseshoe (Monadjem et al., 2010; Patrick et al., 2013). Rhinolophidae and Hipposideridae emit high duty cycle echolocation calls (HDC) (Obrist et al., 1993). Sound is generated in the larynx, and emitted through the nasal leaf. HDC bats are able to simultaneously emit a pulse and receive echoes, utilising Doppler shift compensation (DSC) where the emitted pulse and returning echo have different frequencies (Jones and Teeling, 2006). HDC calls are characterised by a long duration (50-80 ms) constant frequency (CF) part, and frequency modulated components at the beginning and end of the call (Schnitzler and Denzinger, 2011). The CF component of the call coupled with DSC allow Rhinolophidae bats to detect and classify fluttering insects with a high degree of accuracy though amplitude modulations in the retuning echo. These modulations are formed by the wingbeats of the insects (Schnitzler et al., 1983; Jones and Teeling, 2006; Denzinger and Schnitzler, 2014). When the wings are perpendicular to the emitted call an acoustic glint is produced. From these glints the bat is able to ascertain the speed and direction of flight of their prey (Neuweiler et al., 1980; Schnitzler and Denzinger, 2011). This echolocation system coupled with short and broad wings make rhinolophids highly adept at foraging in cluttered habitats (Bontadina et al., 2002). Moreover, most rhinolophid bats use high echolocation frequencies that are more directional and provide higher resolutions in cluttered environments than low frequencies (Neuweiler et al., 1980). These high frequencies are usually outside the best hearing frequencies (20 – 60 kHz) that have evolved in many insect prey (Fenton and Fullard, 1979), coupled with reduced avoidance success within cluttered habitats due to short detection distances (Jacobs et al., 2008), may explain why most southern African *Rhinolophus* bats are lepidopteran specialists (Schoeman and Jacobs, 2003; 2011).

Southern African *Rhinolophus* bats and their ectoparasites provide a particularly interesting test case. *Rhinolophus* is the most species rich genus of bats in southern Africa (Monadjem et al., 2010). with at least 15 known species occurring in the region, however this number is most certainly an under representation due to the number of cryptic species being discovered within the genus (Taylor et al., 2012). Body size of these species range from the small *R. swinnyi* (FA: 37-44mm W: 5-8g) to the large *R. cohenae* (FA: 61-67mm, W: 20-38.5g) (Monadjem et al., 2010; Taylor et al., 2012). Most southern African rhinolophids are obligate cave, mine, tunnel and cavern dwellers where they form social congregations ranging between 30 – 1000s. Finally, molecular evidence shows that many southern African *Rhinolophus* species are adapted to distinct biogeographic regions (Maree and Grant, 1997; Stoffberg et al., 2010; Stoffberg et al., 2012; Taylor et al., 2012; Jacobs et al., 2013)

Currently, there are few studies on the patterns and environmental correlates of bat fly diversity in rhinolophids, with most studies evaluating only a single species (Sharifi et al., 2013). Sharifi et al. (2013) demonstrated seasonal changes in bat fly (genus: *Eyndhovenia*) abundance on *R. mehelyi* in Mahidasht caves of western Iran. Further, bat flies preferred females during the gestation period and males during the reproductive season. Other studies on the bat flies of *Rhinolophus* species are limited to brief taxonomic reports (Kim et al., 2012; Shapiro et al., 2016).

1.4. Aim, Objectives, Hypotheses and Predictions

The aim of this study was to investigate environmental, demographic and biotic drivers of bat fly diversity of six *Rhinolophus* bat species – *R. clivosus*, *R. damarensis*, *R. capensis*, *R. simulator*, *R. denti* and *R. swinnyi* – at local and regional spatial scales in southern Africa.

The objectives were to:

1. Sample bat flies from the families Nycteribiidae and Streblidae on *Rhinolophus* bats at 20 sites in eight biomes during wet and dry seasons.
2. Identify bat flies to morphospecies, estimate ectoparasite abundance, richness and prevalence.
3. Estimate completeness of sampling effort using species richness estimators.
4. Investigate drivers of ectoparasite co-occurrence patterns at local and regional spatial scales based on three hypotheses.

The following three hypotheses were tested:

1. The habitat heterogeneity hypothesis proposes a positive relationship between habitat heterogeneity and bat fly diversity. I predicted that bat species that roost in heterogeneous habitats should have a greater abundance, richness and prevalence of bat flies than those that roost in homogenous habitats.
2. The host sex hypothesis proposes that the prevalence, and diversity of bat flies is driven by sex and reproductive season. I predicted that prevalence and abundance of bat flies should be higher on female and juvenile bats before and after gestation (September – March), whereas prevalence and abundance of bat flies should be higher on males during and after the mating season (April – August).
3. The host body size hypothesis proposes that large bats harbour greater abundance, richness and diversity of bat flies than small bats. I predicted that parasite abundance, richness and prevalence should be higher on large rhinolophid species (*R. clivosus* and *R. capensis*) than smaller rhinolophids (*R. swinnyi* and *R. denti*). As an alternative to body size, I predicted that parasite abundance, richness and prevalence will be higher on individuals with higher body condition.

Chapter 2. Materials and Methods

2.1. Study sites

Bat and bat flies were sampled from 20 sites in the Eastern, Northern, and Western Cape and KwaZulu-Natal provinces of South Africa from January 2014 until March 2016. These sites represent eight biomes in southern Africa, namely Albany Thicket, Desert, Forest, Fynbos, Grassland, Nama-Karoo, Savanna and Succulent Karoo (Figure 1, www.sanbi.org). Sampling was conducted seasonally (dry and wet seasons) to control for seasonal variations in parasite abundance.

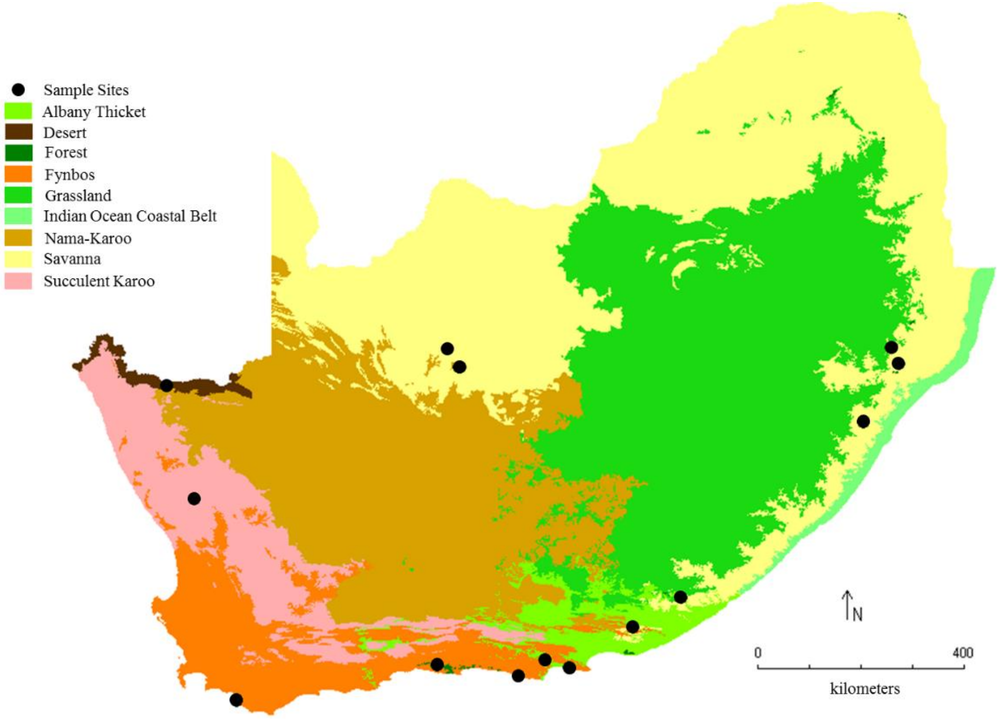

Figure 1 Sample sites and biomes in southern Africa. Map created from data sourced from www.sanbi.org

2.1.1. Albany thicket

The Albany Thicket is located in the semi-arid regions of the Western and Eastern Cape provinces and is one of two biomes endemic to South Africa (Hoare et al., 2006). This biome receives year round rainfall, with mean annual rainfall ranging from 250 – 900 mm (Nocita et al., 2011). The vegetation is characterised by dense Mediterranean type shrubs, short trees and succulents (Hoare et al., 2006). Historically the vegetation of the biome was shaped by high abundances of megafauna, including elephants (*Loxadonta africana*) and black rhinoceros (*Diceros bicornis*) (Hoare et al., 2006).

2.1.2. Fynbos

The Fynbos biome is located in south-west South Africa. Fynbos is the smallest of the six floral kingdoms of the world covering ~0.04 % of the earth's surface, but one of the richest in species (Witkowski and Mitchell, 1987; Cowling et al., 1999). The altitude of the biome ranges from sea level to 1 100 m. Fynbos is characterised by nutrient poor soil and Mediterranean-type climate where rainfall falls primarily in winter (Richardson et al., 1989; Cowling et al., 1999; Rebelo et al., 2006). It receives on average annual rainfall of 480 mm. Fynbos is broadly subdivided into two major fire prone vegetation types, the Cape Floristic Kingdom (represented by mountainous and lowland Fynbos) and the Cape Paleotropical Floristic Kingdom (represented by the Renosterveld and Strandveld) (Witkowski and Mitchell, 1987). Fynbos vegetation is characterised by thin-leaved evergreen shrubs, with the presence of restios (Campbell, 1986). Renosterveld is dominated by the shrub *Elytropappus rhinocerotis* (renosterbos), which was eaten by the now locally extinct black rhinoceros (*D. bicornis*).

2.1.3. Savanna

The Savanna biome is the largest biome in South Africa, covering an area of 399 600 km^2 (Rutherford et al., 2006a; Shackleton et al., 2007). The Savanna biome receives summer rainfall ranging from 200 mm in the west Kalahari region to 500 – 750 mm elsewhere (Rutherford et al., 2006a). The vegetation in the biome is relatively species poor with increasing diversity of trees and large shrubs from west to east across South Africa (Rutherford et al., 2006a). The Savanna biome is variable in its vegetative properties: certain areas are grass dominated with interspersed woody shrubs and trees, whereas other areas are dominated by dense stands of woody trees and large shrubs (Beerling and Osborne, 2006).

2.1.4. Grassland

The Grassland biome extends from the inland areas of the east coast westward up the escarpment of KwaZulu-Natal and the Eastern Cape provinces, and dominates the central plateau. The biome is characterised by summer rainfall with mean annual rainfall varying geographically from 400 – 1500 mm (Rutherford and Westfall, 1994; Bond et al., 2003). During the winter months the productivity of the region is markedly low. The biome exhibits high forb diversity, yet is structurally simple, and is dominated by grass species (Rutherford et al., 2006b). Woody species are present only in a few areas (Bond et al., 2003).

2.1.5. Forest

The Forest biome is dominated by large multi-layered vegetation characterised by large evergreen to semi-deciduous trees (Rutherford et al., 2006b), such as Yellowwood (*Podocarpus folius*), Stinkwood (*Ocotea bullata*) and Cherrywood (*Pterocelastrus tricuspidatus*). The forest biome is the smallest biome in South Africa covering a geographic area of ~ 350 000 ha (Shackleton et al., 2007). The Knysna forest covers an area ~ 558 km^2, located from Mossel Bay in the west to Van Stradens River Canyon (west of Port Elizabeth) in the east. This low altitude (20 - 600 m) forest receives year round rainfall with a mean annual average between 1000 – 1500 m (Midgley et al., 1995).

2.1.6. Succulent Karoo

The Succulent Karoo stretches from the west coast and extends southwards and eastwards where it borders the Fynbos biome and Albany Thicket and Nama-Karoo biomes, respectively (Mucina et al., 2006a). It covers an area of 111 000 km^2, making it the fourth largest biome in South Africa. Altitude ranges from sea level to 1 500 m. The area receives very little rainfall that falls predominantly in the winter months in the north, and in the spring and autumn months in the south, with an annual average of 150 mm (Mucina et al., 2006a; Hoffman et al., 2009). The vegetation of the biome is dominated with dwarf leafed-succulent shrubs and geophytes (Hoffman et al., 2009).

2.1.7. Nama-Karoo

The Nama-Karoo biome is located on the western parts of South Africa on the central plateau, and extends northwards into Namibia (Mucina et al., 2006b). This arid biome covers 248 284 km^2 and has a variable mean annual rainfall ranging from 70 mm in the north bordering the Desert biome up to 500 mm in the southeast bordering the Albany Thicket biome (Beukes et al., 2002; Mucina et al., 2006b). The majority of the rainfall is received during late summer. The vegetation is dominated by short (< 1 m in height) shrubs, mixed grasses, succulents and forbs (Dean, 1997; Mucina et al., 2006b).

2.1.8. Desert

The only true desert biome in South Africa is located from the west coast eastward along the perennial Orange River on the border with Namibia (Rutherford et al., 2006b). The biome does not extend more than 30 km south of the river. Annual average rainfall ranges between 45 - 80 mm peaking in late summer to early autumn. The vegetation comprises mainly small grass species with a few succulent shrub species located in the drainage lines (Rutherford et al., 2006b).

2.2. Bat captures and phenotypic measurements

Bats were caught in their roosts (caves, mines, tunnels) during the day using hand-nets. Bats were immediately placed singularly in cloth bags. I identified bats to species using the species identification matrices in Monadjem et al. (2010). Additionally, I recorded the echolocation calls (resting frequency (RF) of handheld bats with an Echo Meter EM3 Ultrasonic Detector and Recorder (Wildlife Acoustics Inc., USA, version 1.0.0, www.wildlifeacoustics.com) because rhinolophid species have distinct RF frequencies (Monadjem et al., 2010). RF was recorded with the hand-held bat ca. 30 cm away from the microphone to eliminate variability in the peak frequency of the emitted echolocation call due to Doppler shift compensation by the bat during flight (Stoffberg et al., 2012). I measured the frequency of maximum energy in the constant frequency part of the pulse from the power spectrum. Finally, two 3 mm biopsy samples were collected from the wing membranes of each bat and stored in Eppendorf tubes filled with 99 % Ethanol (Wilkinson et al., 1997) for future genetic analyses.

First, ectoparasites were removed from each captured bat (Section 2.3). Bats were aged based on the fusion of digital epiphyseal cartilages of the 5th finger (Kunz and Anthony, 1982), and sexed. Bat forearm length (to the nearest 0.5 mm) was measured with callipers, and body mass (to the nearest 0.5 g) with a Pesola scale. The extended right wing of each captured bat was photographed with a

Canon 600D camera (Canon Inc., Tokyo, Japan) on graph paper, ensuring that the camera was positioned at 90° above the wing. I calibrated the wing images with the dimensions of the graph paper, and measured wing length and area (sensu Schoeman and Jacobs, 2008) using Sigma Scan software (version 5, Aspire Software International, USA).

2.3. Bat fly collection and phenotypic measurements

Bat flies were removed from the fur and wing and tail membranes using forceps, and immediately placed into 1.5 ml Eppendorf vials containing 99 % Ethanol. Body length of bat flies was measured using dial callipers with a micrometer gauge (to the nearest 0.1 mm) from the tip of the head to the tip of the abdomen. Bat flies were classified to morphospecies (sensu Oliver and Beattie, 1996) using a dissecting microscope by examining morphological differences between individuals. Thereafter, I constructed the dichotomous key below to aid identification.

2.3.1. Bat fly morphospecies dichotomous key

1.1. Wing present	Streblidae………...	(1.3.)
1.2. Wings absent	Nycteribiidae……..	(1.5.)
1.3. Body size > 2 mm	Yes………………	Streblidae Morphospecies 1
	No………………..	(1.4.)
1.4. Abdomen round in shape	Yes……………...	Streblidae Morphospecies 2
	No………………..	Streblidae Morphospecies 3
1.5. Body size < 3.5 mm	Yes………………	Nycteribiidae Morphospecies 1
	No………………..	(1.6.)
1.6. Double row of hairs on abdomen (dorsal view)	Yes……………...	Nycteribiidae Morphospecies 2
	No………………..	Nycteribiidae Morphospecies 3

Bat flies were sexed using the following characteristics (pers. comm. C. Dick). Nycteribiidae males exhibited two visible external claspers at the tip of the ventral end of the abdomen (Figure 2a) whereas females had no visible structures on the ventral end of the abdomen (Figure 2b). Streblidae males had genitalia comprising paired postgonites and the aedeagus that resembles a single thin, spine which is often vertically curved (Figure 3).

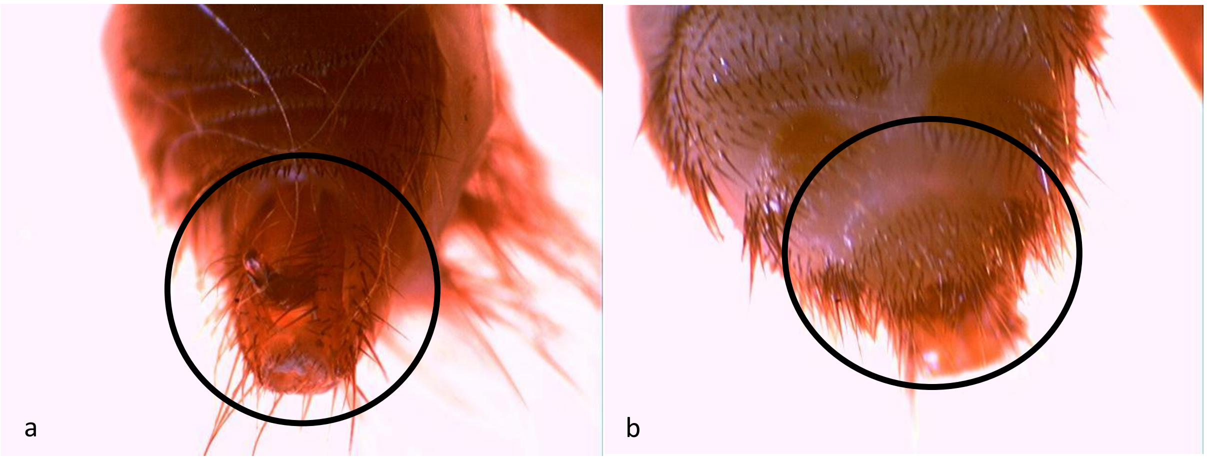

Figure 2 a. Male Nycteribiidae, showing external claspers at the ventral tip of the abdomen within circle; b. Female Nycteribiidae, showing no external claspers at the ventral tip of the abdomen. Magnification X 40.

Figure 3 Male Streblidae, showing spine-like structure of paired postgonites and aedeagus within circle. Magnification X 40.

2.4. Statistical analyses

In this dissertation, parasite prevalence refers to the percentage of hosts sampled carrying one or more bat fly. Morphospecies richness refers to the number of bat fly morphospecies collected from a sampled host population or species. Abundance refers to the number of bat flies found on a single host individual. Average abundance is a measure of the average number of a bat fly family or morphospecies collected from a sampled host population or species including individuals carrying no parasites.

All statistical analyses were performed in R (v.3.2.2.0, R Core Team, 2015).

2.4.1. Habitat heterogeneity

I tested the habitat heterogeneity hypothesis, using number of land cover classes and Enhanced Vegetation Index (EVI) as proxies of habitat heterogeneity.

2.4.1.1. EVI

Vegetation indices have been widely used to quantify variation in vegetation structure. Data collected at local scale in the field is often difficult to interpret and compare at regional or global scales because of different methodologies employed. Conversely, satellite imagery show real time variation in vegetation distribution and diversity (Waring et al., 2006). The Normalised Difference Vegetation Index (NDVI) correlates significantly with vegetation structure and complexity (Huete et al., 2002). However, NDVI may be sensitive to bare soil and vegetation such as forests that have high densities and Leaf Area Indices (LAI) which may cause NDVI to become saturated (Huete et al., 2002; Waring et al., 2006). Thus I used the Enhanced Vegetative Index (EVI) to minimise these shortcomings (Waring et al., 2006). EVI calculates the photosynthetic potential using the formula:

$$EVI = G(NIR-RED)/(NIR+C1\times RED-C2\times BLUE+L),$$

Where G (the gain factor) is equal to 2.5, NIR is the near infrared light absorbed by the green leaves of the plant, RED is the visible red light reflected of the vegetation back to the satellite, C1 and C2 are coefficients of the aerosol resistance term equal to 6 and 7.5 respectively, L accounts for canopy adjustment as the NIR non-linearly passes through the canopy transferring red radiant energy and

BLUE is the amount of blue light reflected (Huete et al., 2002; Waring et al., 2006). Values of EVI range from 0 to 1, where values close to 0 correspond to an absence of vegetation and values close to 1 correspond to dense vegetation.

To calculate EVI, I used the Moderate Resolution Imaging Spectroradiometer (MODIS) Vegetation Indices dataset from NASA's TERRA and AQUA satellites, with a resolution of 1 km from 2000 to present. I extracted the annual mean EVI values from a 10 km radius around each sampling site using DIVA-GIS (version 7.5.0) software (Hijmans et al., 2005). A 10 km radius was used because rhinolophids forage up to 10 km from their roost each night (Goiti et al., 2006).

I investigated the relationship between EVI values around roosts and bat fly presence/absence using a single effect logistic regression, and bat fly morphospecies richness and abundance using non-parametric kernel regressions with a Poisson distribution because the data failed the assumptions of linearity. Models were constructed for bat fly families combined, within each bat fly family and morphospecies.

2.4.1.2. Number of land cover classes

I also quantified heterogeneity of habitat complexity based on the number of land cover classes surrounding each roost site (Schoeman et al., 2013). To do this, I constructed a 10 km radius around each roost site using DIVA-GIS (version 7.5.0) software and counted the number of different land cover types based on the vegetation land cover data set from the Food and Agriculture Organisation of the United Nations (FAO, www.fao.org).

I investigated the relationship between the number of land cover classes around roosts and bat fly presence/absence using a single effect logistic regression, and bat fly morphospecies richness and abundance using non-parametric kernel regressions with a Poisson distribution. Models were constructed for bat fly families combined, and within each bat fly family and morphospecies.

2.4.2. Host sex hypothesis

I investigated the relationships between sex and bat fly presence/absence using single effect logistic regression. Models were constructed for bat fly families combined, and within each bat fly family and morphospecies.

I tested whether parasite abundance and morphospecies richness were significantly different between host sexes using Generalised Linear Mixed Models (GLMM) with host species, host sex and season as fixed factors and site as a random factor. GLMMs were also constructed for each host species individually with host sex and season as fixed factors and site as a random factor. To enhance normality parasite abundance and morphospecies richness were square root transformed, and GLMMs are highly robust with respects to the assumptions of normality (Hedeker, 2005; Bolker et al., 2009). Models were constructed for bat fly families combined, and within each bat fly family and morphospecies.

2.4.3. Body size and condition hypothesis

I investigated the relationships between body size (quantified with forearm length because this measure does not change daily or seasonally like body weight) or body condition (body condition = host forearm length/host weight (Lucan, 2006)) and abundance and morphospecies richness using GLMMs with host species, host forearm, host body condition and season as fixed factors and site as a random factor. Models were constructed for bat fly families combined, and within each bat fly family and morphospecies. To improve normality parasite abundance and morphospecies richness were square root transformed.

2.4.4. Relative importance of the three hypotheses

To investigate which of the variables associated with the above three hypotheses was the best overall predictor of bat fly morphospecies richness and abundance, I performed a recursive partitioning analysis (RPA, Zeileis et al., 2008), using the ctree function in the party package in R (v.3.2.2.0, R Core Team, 2015). RPA use conditional inference procedures, which provide an unbiased way to determine the best predictors variables of the data (Hothorn et al., 2006). The advantages of using a RPA are: (i) the analysis is robust even if the data do not fulfill assumptions of parametric tests and spatial autocorrelation; (ii) RPA can deal with large datasets with many predictor variables and without overfitting (Hothorn et al., 2006; Zeileis et al., 2008). The dependent variables were bat fly morphospecies richness and abundance, and the independent variables were habitat heterogeneity, land cover, host sex, forearm length and host body condition.

Chapter 3. Results

In total I captured 333 bats classified to six *Rhinolophus* species at 20 sites: *R. clivosus* (n = 156), *R. capensis* (n = 96), *R. damarensis* (n = 23), *R. denti* (n = 21), *R. simulator* (n = 15) and *R. swinnyi* (n = 22). I collected 939 bat fly specimens from two families: Streblidae (n = 833) and Nycteribiidae (n = 126). Individuals within each family were classified to three morphospecies: Streblidae morphospecies 1 (n = 598), Streblidae morphospecies 2 (n = 15), Streblidae morphospecies 3 (n = 220), Nycterbiidae morphospecies 1 (n = 98), Nycterbiidae morphospecies 2 (n = 15) and Nycterbiidae morphospecies 3 (n = 13). Bat flies were collected from 233 bats of the 333 captured bats (70 %), whereas prevalence on bat species ranged from 21.7 – 81.8 % (Appendix A, Table 1). There was a significantly positive relationship between *Rhinolophus* abundace and bat fly abundance ($p < 0.0001$, Figure 4).

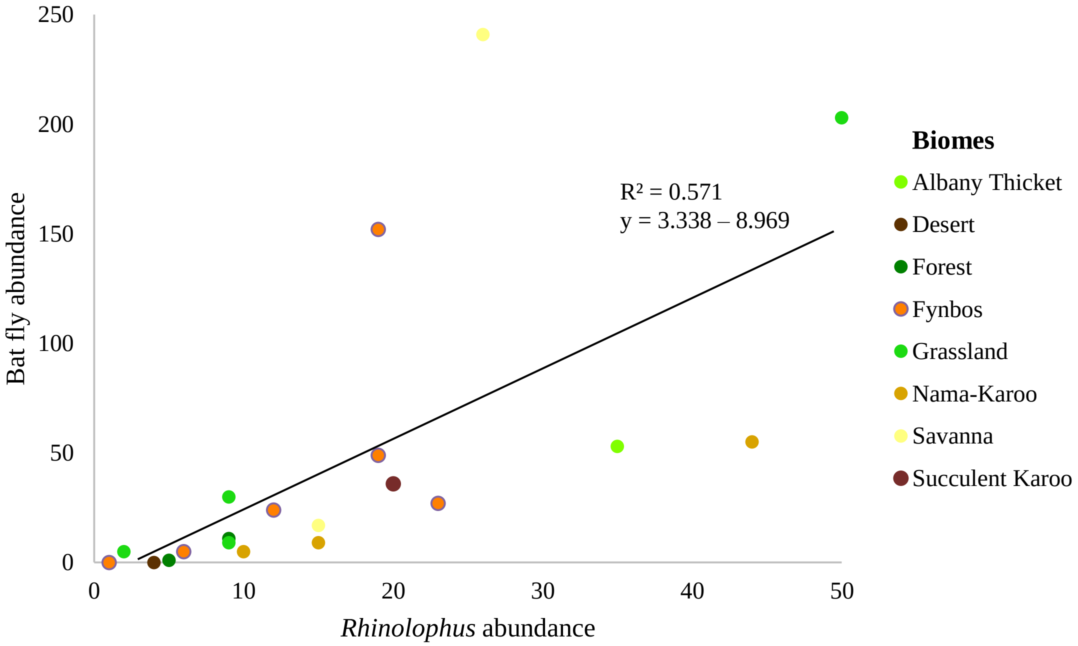

Figure 4 Relationship between bat fly abundance and bat abundance across 20 sample sites representing 8 biomes in southern Africa.

Bat fly morphospecies exhibited different distributions. Streblidae morphospecies 1 and Nycteribiidae morphospecies 1 were collected from all roost sites except Suikerhoek mine in the Desert biome. Nycteribiidae morphospecies 2 and 3 were collected from sites in KwaZulu-Natal (Babanango Valley Lodge), Eastern Cape (Table Farm & Phillips Tunnel), Western Cape (De

Kelders cave 2) and Northern Cape (Hopefield Farm Mine 1 & 2). By contrast, Streblidae morphospecies 2 were collected only from Hopefield Farm in the Nama-Karoo biome (Northern Cape), whereas Streblidae morphospecies 3 was collected at two KwaZulu-Natal locations (Doornhoek Mine and Mooiplaas Mine). Lowest prevalence of bat flies were recorded in the Desert biome (Suikerhoek Mine – 0 %) and the Forest biome (Forest Edge Knysna – 10 % and Sandile's Rest Cave – 25 %), the highest prevalence was recorded in the Savanna biome (Mooiplaas Mine – 100 %) (Appendix A Table 1). However all bat fly species collected were considered to be oligoxenous (i.e. occurred on more than one host), except Streblidae morphospecies 2 that was found on *R. denti* only (i.e. monoxenous).

There were significant differences in total bat fly abundance ($F_{(19,313)} = 10.46$, $p < 0.001$) and total morphospecies richness ($F_{(19,313)} = 6.32$, $p < 0.001$) among the roost sites. Post-hoc Tukey tests revealed that three sites, Babanango Valley Lodge Mine, Mooiplaas Mine and De Kelders Cave 2, had significantly higher bat fly abundances and morphospecies richness than Blink Klip Grotte, Babanango Valley Lodge, Doornhoek Mine, Forest Edge Knysna, Hopefield Farm Mine 1, Hopefield Farm Mine 2, Phillips Tunnel, Sleepy Hollow Mine, Suikerhoek Mine and Table Farm Grahamstown (all P values < 0.05; Figure 5a, d). Similarly, at a family level, Streblidae abundance ($F_{(19,313)} = 9.34$, $p < 0.001$) and morphospecies richness ($F_{(19,313)} = 5.34$, $p < 0.001$), were significanty higher among the above mentioned roost sites (Figure 5b, e). Nycteribiidae abundance ($F_{(19,313)} = 4.26$, $p < 0.001$) and morphospecies richness ($F_{(19,313)} = 4.05$, $p < 0.001$) were significantly different among sites. Post-hoc Tukey-tests showed that both abundance and morphospecies richness were significantly higher at Babanango Valley Lodge Mine than Blink Klip Grotte, De Kelders Cave 1, Doornhoek Mine, Forest Edge Knysna, Hopefield Farm Mine 1, Klaasies River Cave, Phillips Tunnel, Sleepy Hollow Mine, and Table Farm Grahamstown (all $p < 0.05$; Figure 5c, f).

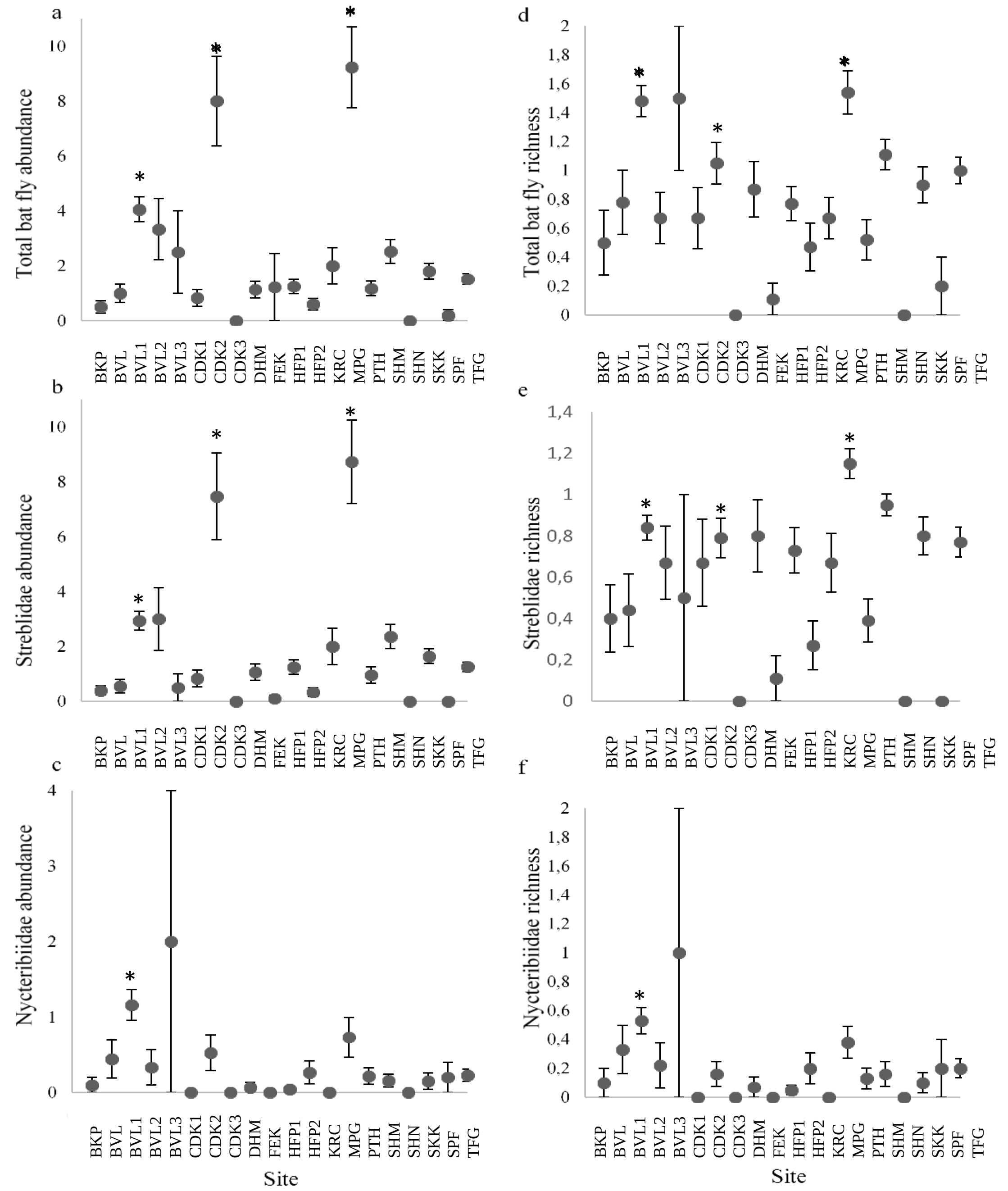

Figure 5 Mean (± SD)a. total bat fly abundance; b. total bat fly morphospecies richness; c. Streblidae abundance; d. Streblidae morphospecies richness; e. Nycteribiidae abundance; f. Nycteribiidae morphospecies richness at roost sites. BKP = Blink Klip Grotte, BVL = Babanango Valley Lodge, BVL1 = Babanango Valley Lodge Mine, BVL2 = Babanango Valley Lodge Tunnel 1, BVL3 = Babanango Valley Lodge Tunnel 2, CDK1 = De Kelders Cave 1, CDK2 = De Kelders Cave 2, CDK3 = De Kelders Cave 3, DHM = Doornhoek Mine, FEK = Forest Edge Knysna, HFP1 = Hopefield Farm Mine 1; HFP2 = Hopefield Farm Mine 2, KRC = Klaasies River Cave, MPG = Mooiplaas Mine, PTH = Phillips Tunnel, SHM = Sleepy Hollow Mine, SHN = Suikerhoek Mine, TFG =Table Farm Grahamstown. Significant differences among sites (p<0.05) are denoted by the *.

Total bat fly abundance ($F_{(5,327)} = 11.34$, $p < 0.001$) and total morphospecies richness ($F_{(5,327)} = 7.02$, $p < 0.001$) differed significantly among host species. Three species had significantly high bat fly abundance (Figure 6a): Post-hoc Tukey tests showed that fly abundance was significantly higher on *R. capensis* than *R. damarensis* ($p < 0.001$); *R. clivosus* than *R. capensis* ($p < 0.001$), *R. damarensis* ($p < 0.001$), *R. denti* ($p < 0.05$) and *R. simulator* ($p < 0.05$); and *R. swinnyi* than *R. capensis* ($p < 0.001$), *R. damarensis* ($p < 0.001$), *R. denti* ($p < 0.001$). Aditionally, morphospecies richness on *R. damarensis* was significantly lower than all other *Rhinolophus* hosts (all $p < 0.05$). Morphospecies richness was higher on *R. clivosus* than *R. capensis* ($p < 0.01$).

At a family level, Streblidae abundance ($F_{(5,327)} = 10.21$, $p < 0.001$), morphospecies richness ($F_{(5,327)} = 6.96$, $p < 0.001$), Nycteribiidae abundance ($F_{(5,327)} = 6.86$, $p < 0.001$) and morphospecies richness ($F_{(5,327)} = 6.05$, $p < 0.001$) differed among the host species. Streblidae abundance and morphospecies richness on *R. damarensis* was significantly lower than on all other *Rhinolophus* hosts (all $p < 0.05$), with the exception of *R. simulator* (Figure 6b, e). *R. clivosus* harboured higher Streblidae abundance than *R. capensis*, whereas Streblidae abundance was higher on *R. swinnyi* than *R. capensis* ($p < 0.001$), *R. clivosus* ($p < 0.05$), *R. denti* ($p < 0.01$) and *R. simulator* ($p < 0.01$). Nycteribiidae abundance and morphspecies richness was significantly higher on *R. clivosus* than *R. capensis* ($p < 0.001$), *R. denti* ($p < 0.001$), *R. simulator* ($p < 0.05$) and *R. swinnyi* ($p < 0.05$) (Figure 6c, f).

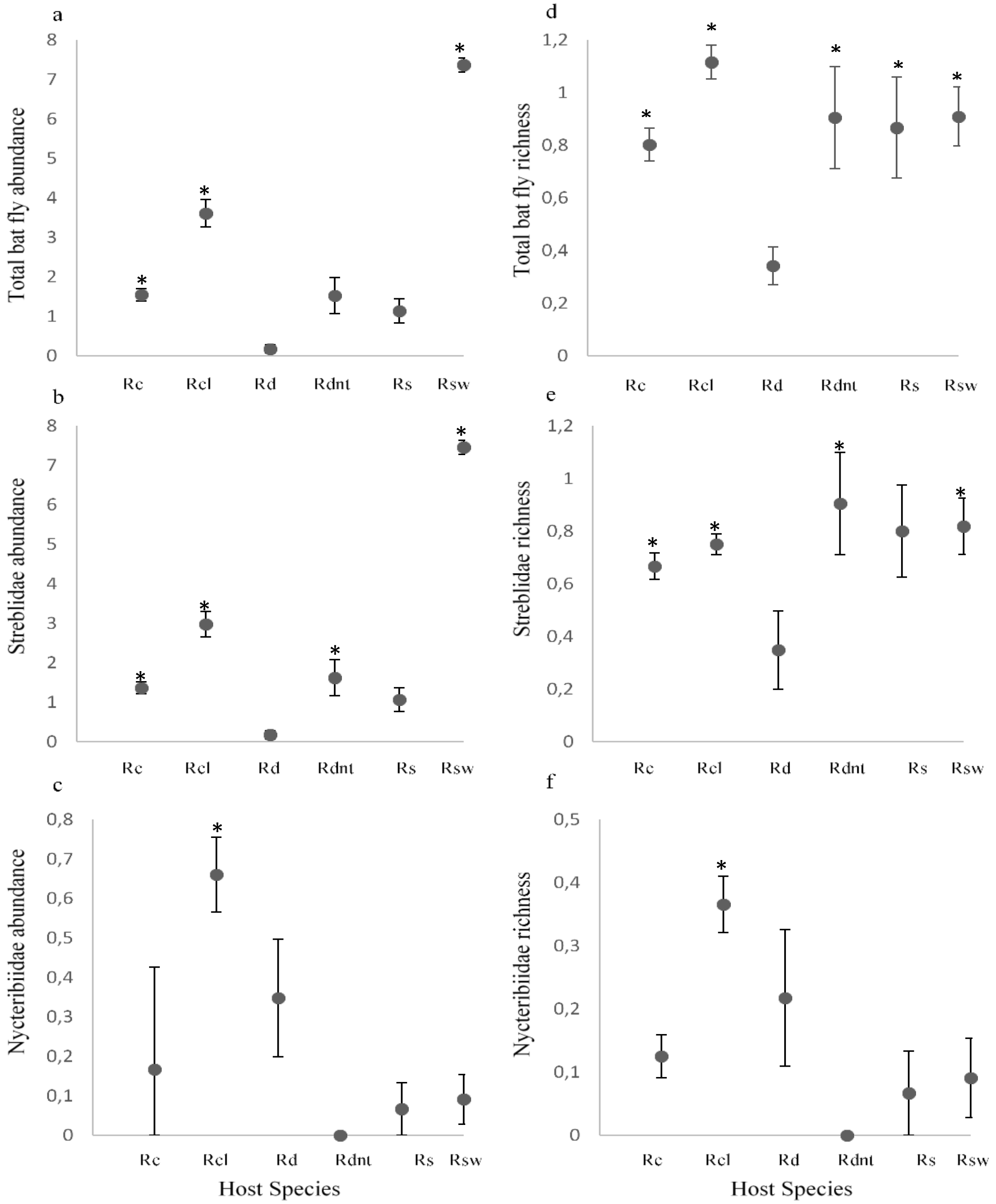

Figure 6 Mean (± SD) a. total bat fly abundance; b. total bat fly morphospecies richness; c. Streblidae abundance; d. Streblidae morphospecies richness; e. Nycteribiidae abundance; f. Nycteribiidae morphospecies richness on host species. Rc = *Rhinolophus capensis*, Rcl = *Rhinolophus clivosus*, Rd = *Rhinolophus damarensis*, Rdnt = *Rhinolophus denti*, Rs = *Rhinolophus simulator*, Rsw = *Rhinolophus swinnyi*. Significant differences among species ($p < 0.05$) are denoted by the *.

I found no significant differences in bat fly abundance or morphospecies richness between host sexes (Figure 7).

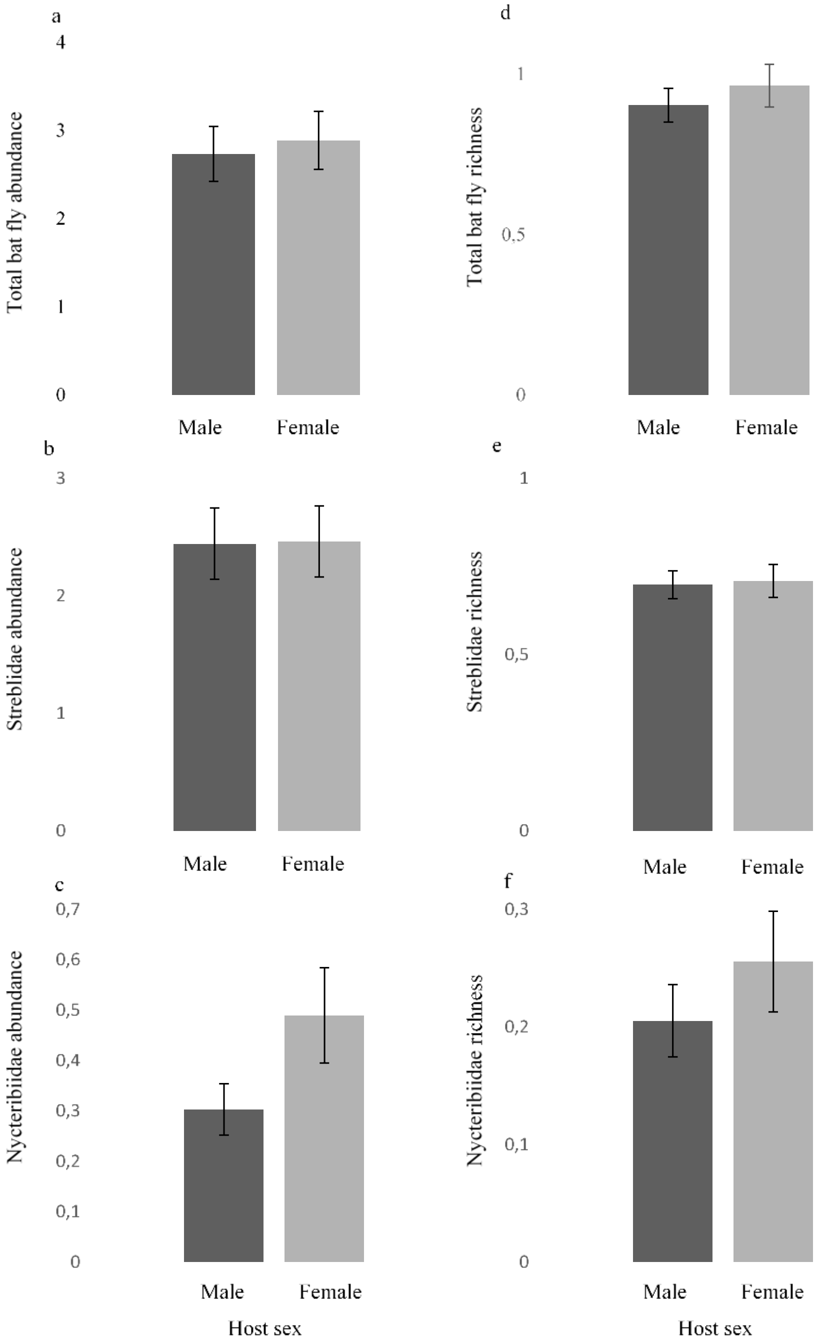

Figure 7 Mean (± SD): a. total bat fly abundance; b. total bat fly morphospecies richness; c. Streblidae abundance; d. Streblidae morphospecies richness; e. Nycteribiidae abundance; and f. Nycteribiidae morphospecies richness between male and female bats. There were no significant differences between sexes.

I found no significant differences in bat fly abundance or morphospecies richness between seasons (Figure 8).

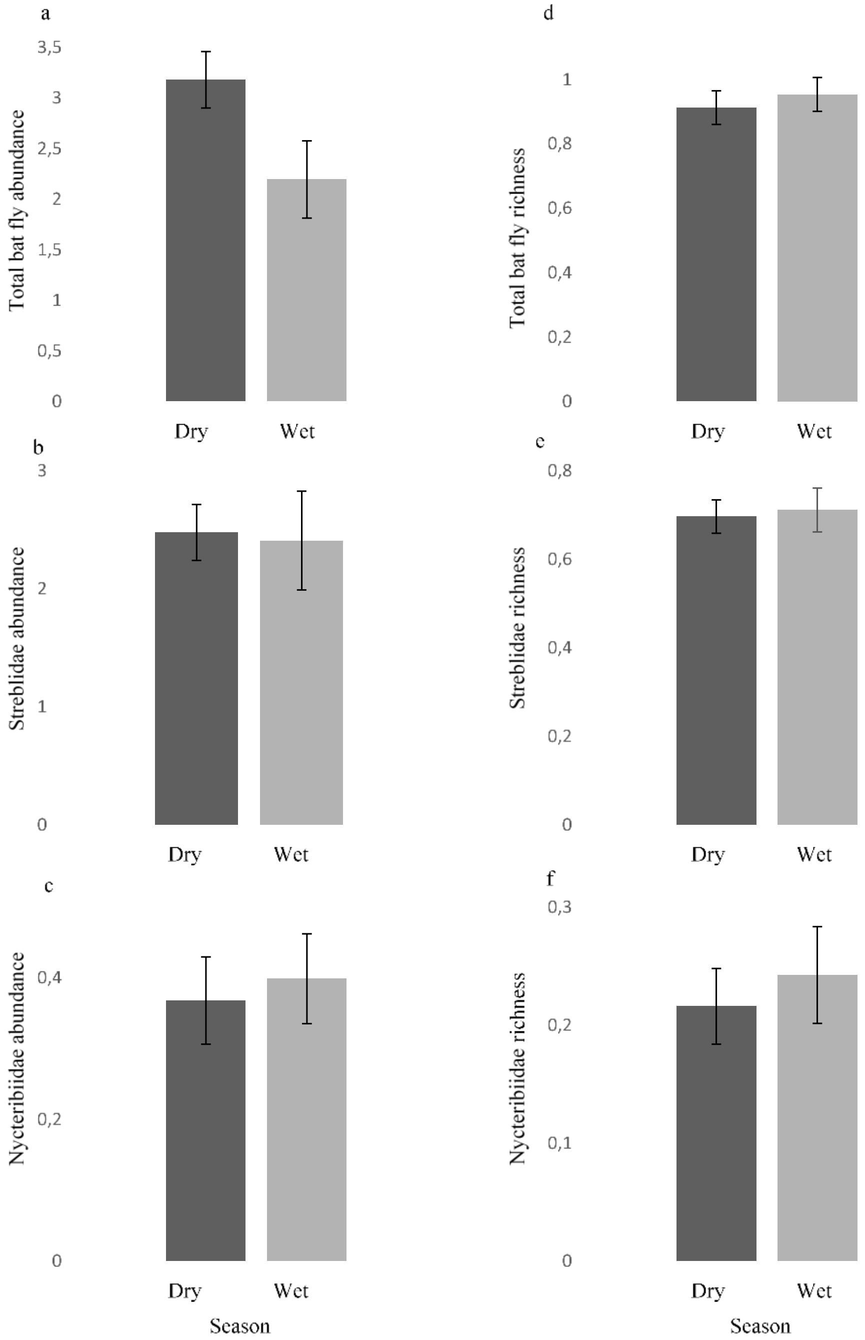

Figure 8 Mean (± SD): a. total bat fly abundance; b. total bat fly morphospecies richness; c. Streblidae abundance; d. Streblidae morphospecies richness; e. Nycteribiidae abundance; and f. Nycteribiidae morphospecies richness between dry and wet seasons. There were no significant differences between seasons.

3.1. Habitat heterogeniety

I did not include Streblidae morphospecies 2 in morphospecies analyses because they were collected from one site only.

I found strong support for the hypothesis that habitat heterogeniety influenced bat fly abundance, morphospecies richness and prevalence.

3.1.1. EVI

There were strong relationships between bat fly abundance and morphospecies richness and EVI . At bat fly family level, total bat fly abundance ($p < 0.01$; Figure 9a) and Streblidae abundance ($p < 0.01$; Figure 10a) was significantly positively associated with EVI. Conversely, total bat fly morphospecies richness ($p < 0.05$, Figure 9b), Nycteribiidae abundance ($p < 0.0001$; Figure 11a), Streblidae morphospecies richness ($p = 0.01$; Figure 9c) and Nycteribiidae morphospecies richness ($p < 0.0001$, Figure 9d) was significantly negatively associated with EVI.

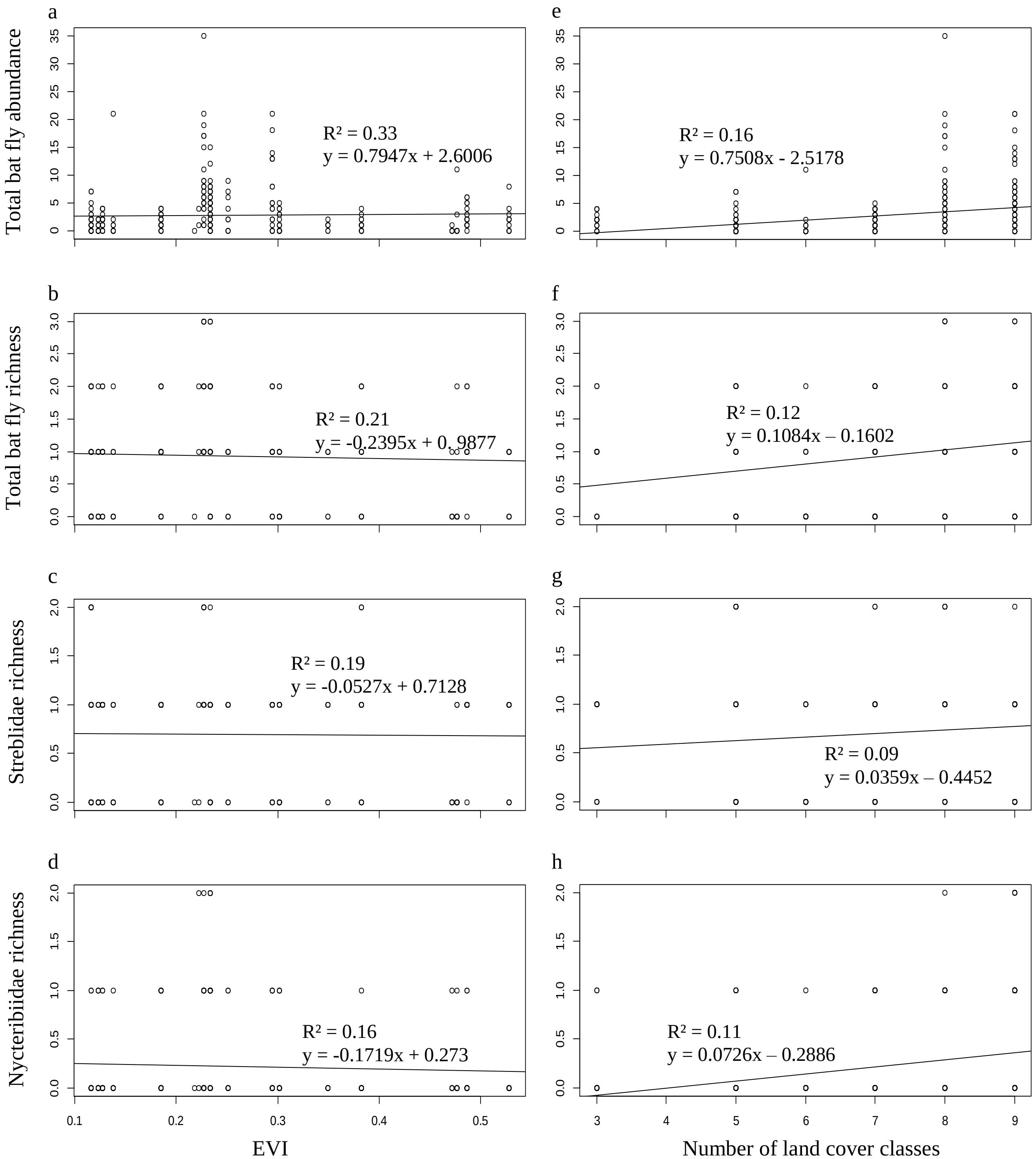

Figure 9 Relationship between EVI and a. Total bat fly abundance, b. Total bat fly morphospecies richness, c. Streblidae morphospecies richness, d. Nycteribiidae morphospecies richness. Relationship between number of land cover classes and e. Total bat fly abundance, f. Total bat fly morphospecies richness, g. Streblidae morphospecies richness, h. Nycteribiidae morphospecies richness. The solid line indicates a significant relationship ($p<0.05$).

At a bat fly morphospecies level, a significant positive association was found between Streblidae morphospecies 1 and EVI ($p < 0.0001$, Figure 10b). Conversly, a significant negative association was found between Nycteribiidae morphospecies 1 abundance and EVI ($p < 0.0001$, Figure 11b).

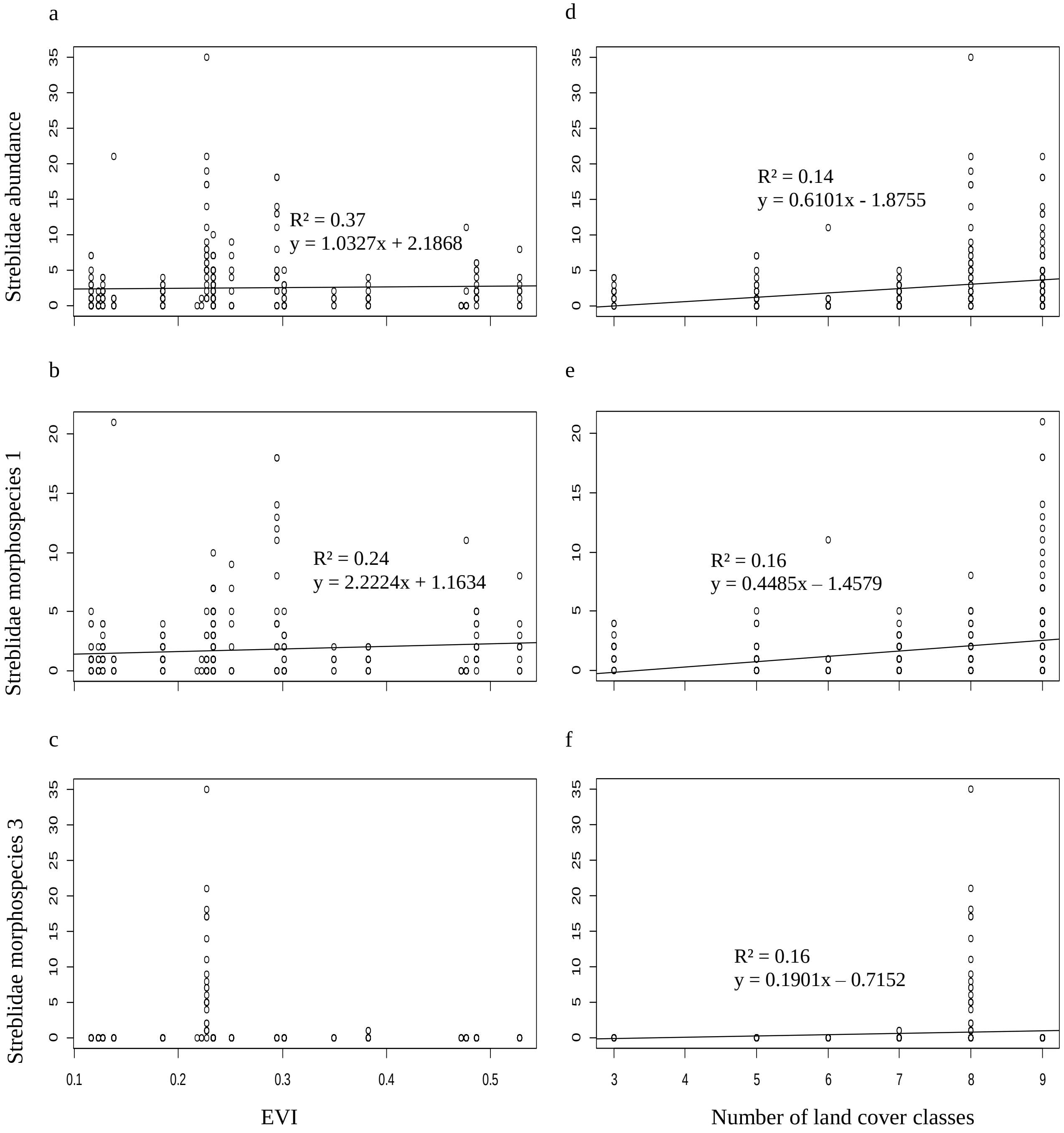

Figure 10 Relationship between EVI and a Streblidae abundance, b. Streblidae morphospecies 1 abundance, c. Streblidae morphospecies 3 abundance. Relationship between number of land cover classes and d. Streblidae abundance, e. Streblidae morphospecies 1 abundance, f. Streblidae morphospecies 3 abundance. The solid line indicates a significant relationship ($p < 0.05$).

I found no relationship between EVI and bat fly prevalence ($\chi^2_{(1)} = 0.001$, $p = 0.97$).

3.1.2. Number of land cover classes

At bat fly family level, total bat fly abundance ($p < 0.001$; Figure 9e), total bat fly morphospecies richness ($p < 0.001$, Figure 9f), Streblidae abundance ($p < 0.001$; Figure 10d), Streblidae morphospecies richness ($p < 0.001$, Figure 9g), Nycteribiidae abundance ($p < 0.001$; Figure 11e) and Nycteribiidae morphospecies richness ($p < 0.001$, Figure 9h) was significantly positively associated with number of land cover classes.

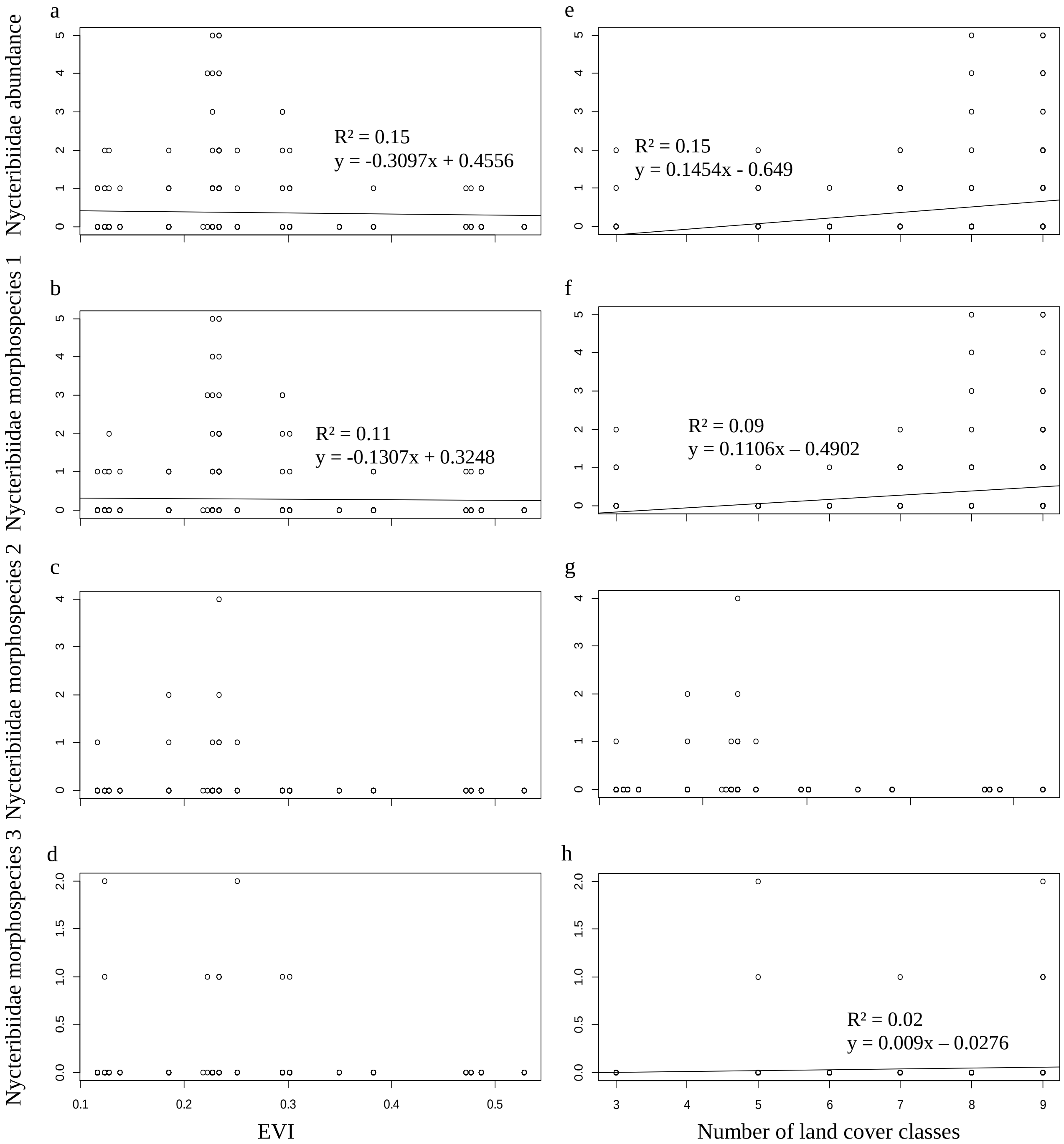

Figure 11 Relationship between EVI and a. Nycteribiidae abundance, b. Nycteribiidae morphospecies 1 abundance, c. Nycteribiidae morphospecies 2 abundance, and d. Nycteribiidae morphospecies 3 abundance. Relationship between number of land cover classes and e. Nycteribiidae abundance, f. Nycteribiidae morphospecies 1 abundance, g. Nycteribiidae morphospecies 2 abundance, and h. Nycteribiidae morphospecies 3 abundance. The solid line indicates a significant relationship ($p < 0.05$).

At bat fly morphospecies level, the abundance of Streblidae morphospecies 1 ($p < 0.001$, Figure 10e), Streblidae morphospecies 3 ($p < 0.001$, Figure 10f), Nycteribiidae morphospecies 1 ($R^2 = 0.09$, $p < 0.001$, Figure 10f) and Nycteribiidae morphospecies 3 ($R^2 = 0.02$, $p < 0.05$, Figure 11h) was significantly positively related with number of land cover classes.

At host species level, only *R. clivosus* and *R. capensis* were collected at multiple sites with different vegetation complexity values. At family level, total bat fly abundance ($p < 0.01$), Nycteribiidae abundance ($p < 0.01$), Nycteribiidae morphospecies richness ($p < 0.01$), Streblidae abundance ($p < 0.01$) and Streblidae morphospecies richness ($p < 0.05$) was significantly positively related with number of land cover classes. At a morphospecies level, significant positive correlations were found between habitat complexity and Nycteribiidae morphospecies 1 abundance ($p < 0.01$), Nycteribiidae morphospecies 3 abundance ($p < 0.05$), Streblidae morphospecies 1 abundance ($p < 0.01$) and Streblidae morphospecies 3 abundance ($p < 0.001$).

Bat fly prevalence ($\chi^2_{(1)} = 14.3$, $p < 0.001$) was significantly positively related to number of land cover classes.

3.2. Host sex

At host family level, I found no evidence that host sex predicted total bat fly morphospecies richness ($\chi^2_{(1)} = 0.183$, $p = 0.669$; Figure 13a) or abundance ($\chi^2_{(1)} = 3.252$, $p = 0.071$; Figure 13b). In contrast, sex was a significant predictor of Streblidae morphospecies 2 abundance ($\chi^2_{(1)} = 6.073$, $p < 0.05$; Figure 14c) and Streblidae morphospecies 3 abundance ($\chi^2_{(1)} = 19.253$, $p < 0.001$; Figure 14d). Streblidae morphospecies 2 abundance was higher on female hosts. Whereas, Streblidae morphospecies 3 abundance was higher on male hosts. However, there were no significant relationships between season alone or in combination with sex and bat fly abundance nor morphospecies richness when host species were combined.

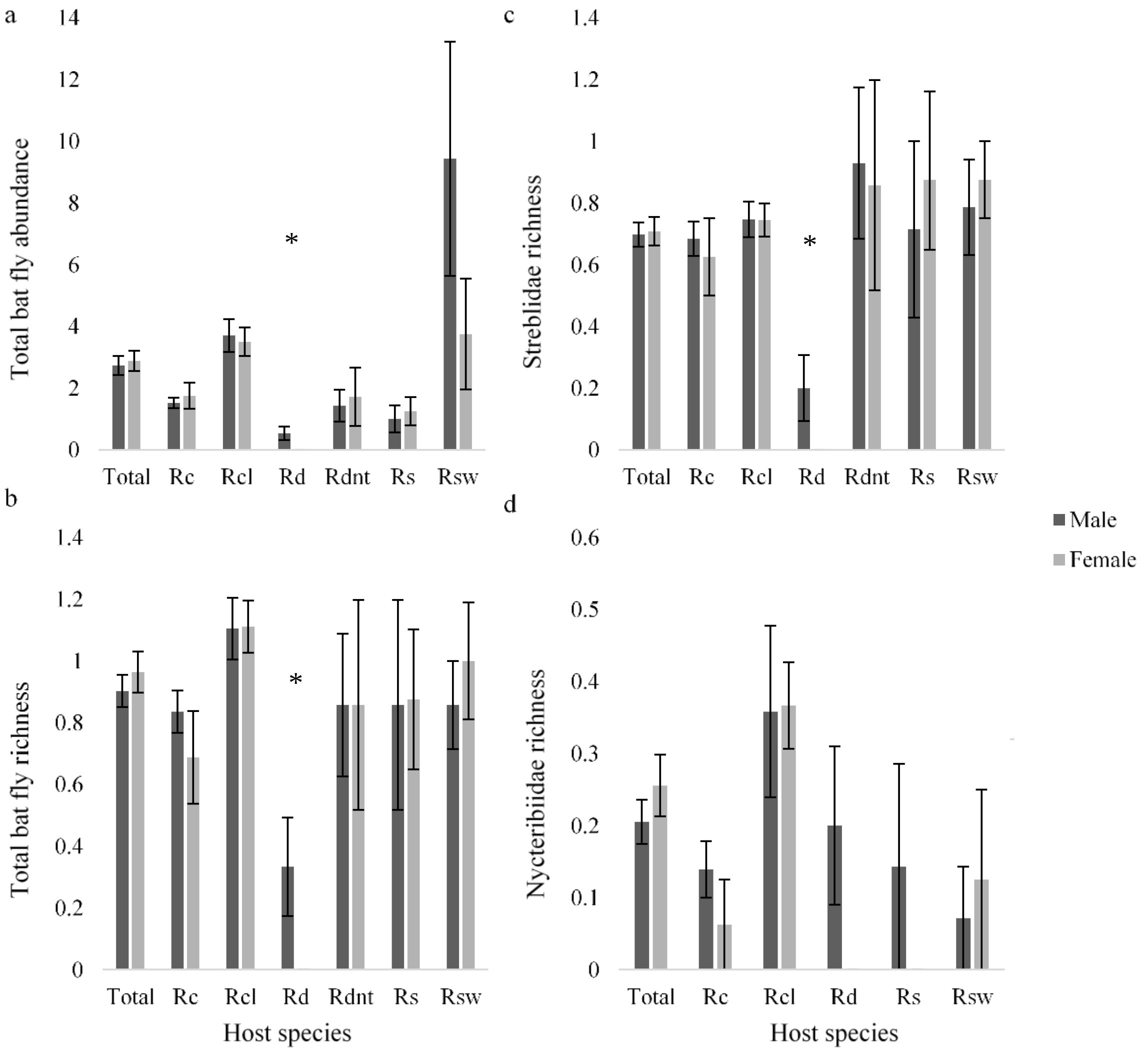

Figure 13 Mean (±SD) a. total bat fly abundance; b. total bat fly morphospecies richness; c. Streblidae morphospecies richness; and d. Nycteribiidae morphospecies richness on male and female *Rhinolophus* bat species. Rc = *Rhinolophus capensis*, Rcl = *Rhinolophus clivosus*, Rd = *Rhinolophus damarensis*, Rdnt = *Rhinolophus denti*, Rs = *Rhinolophus simulator*, Rsw = *Rhinolophus swinnyi*. Significant differences among species ($p < 0.05$) are denoted by the *.

At a host species level, *R. denti* and *R. simulator* were not included in LMM analyses, because individuals of each species were collected from single sites. Host sex was a significant predictor of bat fly abundance on *R. capensis* and *R. damarensis*., Host sex was a significant predictor of Nycteribiidae morphospecies 3 ($\chi^2_{(1)} = 9.582$, $p < 0.05$; Figure 15d) on *R. capensis*, with abundance higher on female hosts. Host sex was a significant predictor of total bat fly abundance ($\chi^2_{(1)} = 4.115$, $p < 0.05$; Figure 13a), total bat fly richness ($\chi^2_{(1)} = 6.073$, $p < 0.05$; Figure 13b) and Streblidae richness ($\chi^2_{(1)} = 4.115$, $p < 0.05$; Figure 13c) on *R. damarensis*, with all indices higher on male hosts.

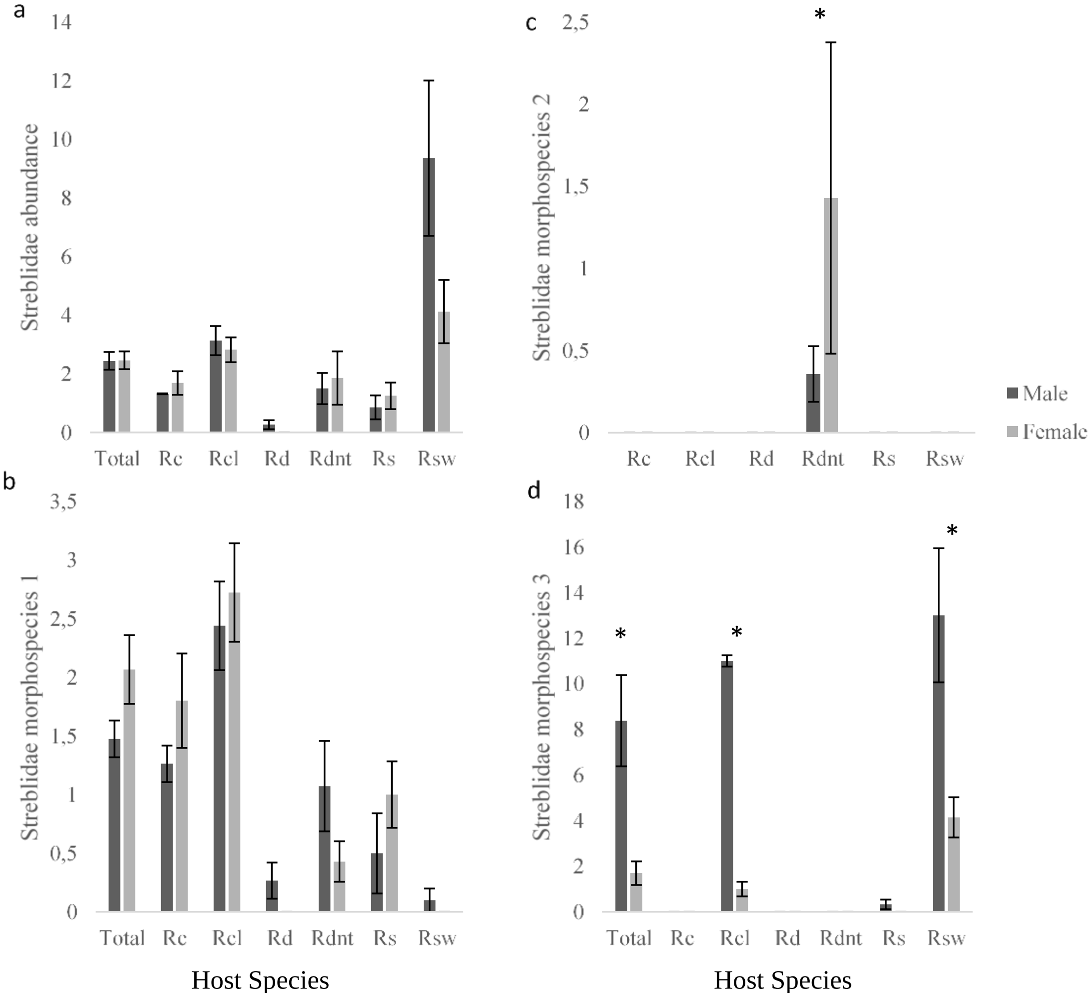

Figure 14: Mean (±SD) a. Streblidae abundance; b. Streblidae morphospecies 1 abundance; c. Streblidae morphospecies 2 abundance; d. Streblidae morphospecies 3 abundance on male and female *Rhinolophus* bats. Rc = *Rhinolophus capensis*, Rcl = *Rhinolophus clivosus*, Rd = *Rhinolophus damarensis*, Rdnt = *Rhinolophus denti*, Rs = *Rhinolophus simulator*, Rsw = *Rhinolophus swinnyi*. Significant differences among species ($p<0.05$) are denoted by the *.

There were significant interactions between host sex, host species and Streblidae morphospecies 3 abundance ($\chi^2_{(1)} = 16.187$, $p < 0.001$, Figure 14d) and Nycteribiidae morphospecies 3 abundance (χ^2

$_{(1)} = 4.475$, $p < 0.05$, Figure 15d). Streblidae morphospecies 3 abundance was significantly high on male bat hosts (i.e. *R.* clivosus and R. *swinnyi*). Conversely, Nycteribiidae morphospecies 3 abundance was significantly high on female bat hosts (i.e. *R. capensis*).

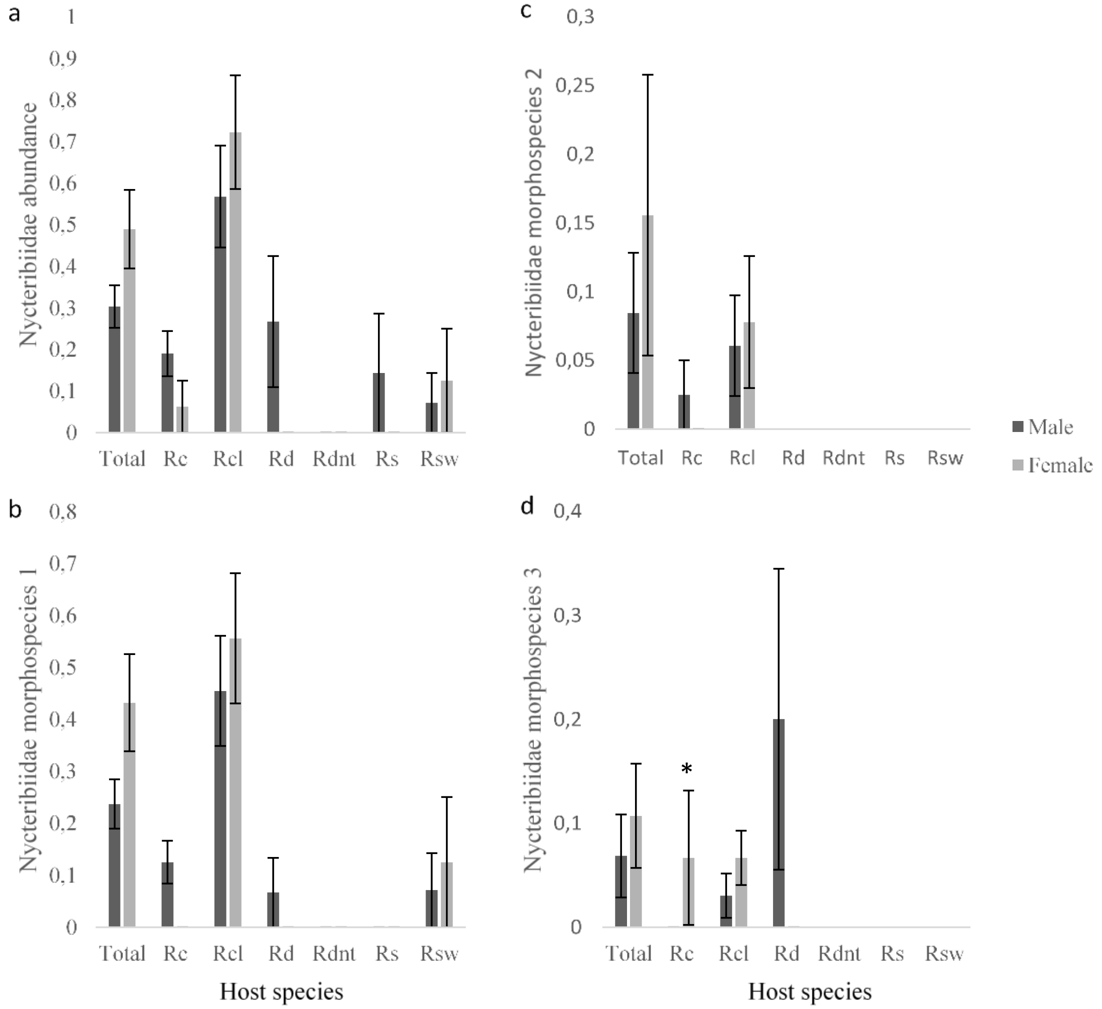

Figure 15: Mean (±SD) a. Nycteribiidae abundance; b. Nycteribiidae morphospecies 1 abundance; c. Nycteribiidae morphospecies 2 abundance; d. Nycteribiidae morphospecies 3 abundance between male and female bats. Rc = *Rhinolophus capensis*, Rcl = *Rhinolophus clivosus*, Rd = *Rhinolophus damarensis*, Rs = *Rhinolophus simulator*, Rsw = *Rhinolophus swinnyi*. Significant differences among species (p<0.05) are denoted by the *.

On *R. capensis* there were significant interactions between season, host sex and total bat fly abundance ($\chi^2_{(1)} = 6.677$, $p < 0.01$, Figure 16a), Streblidae abundance ($\chi^2_{(1)} = 6.685$, $p < 0.01$,

Figure 17a) and Streblidae morphospecies 1 abundance ($\chi^2_{(1)} = 6.508$, $p < 0.01$, Figure 17b); all indices were significantly higher on female bats in the dry season.

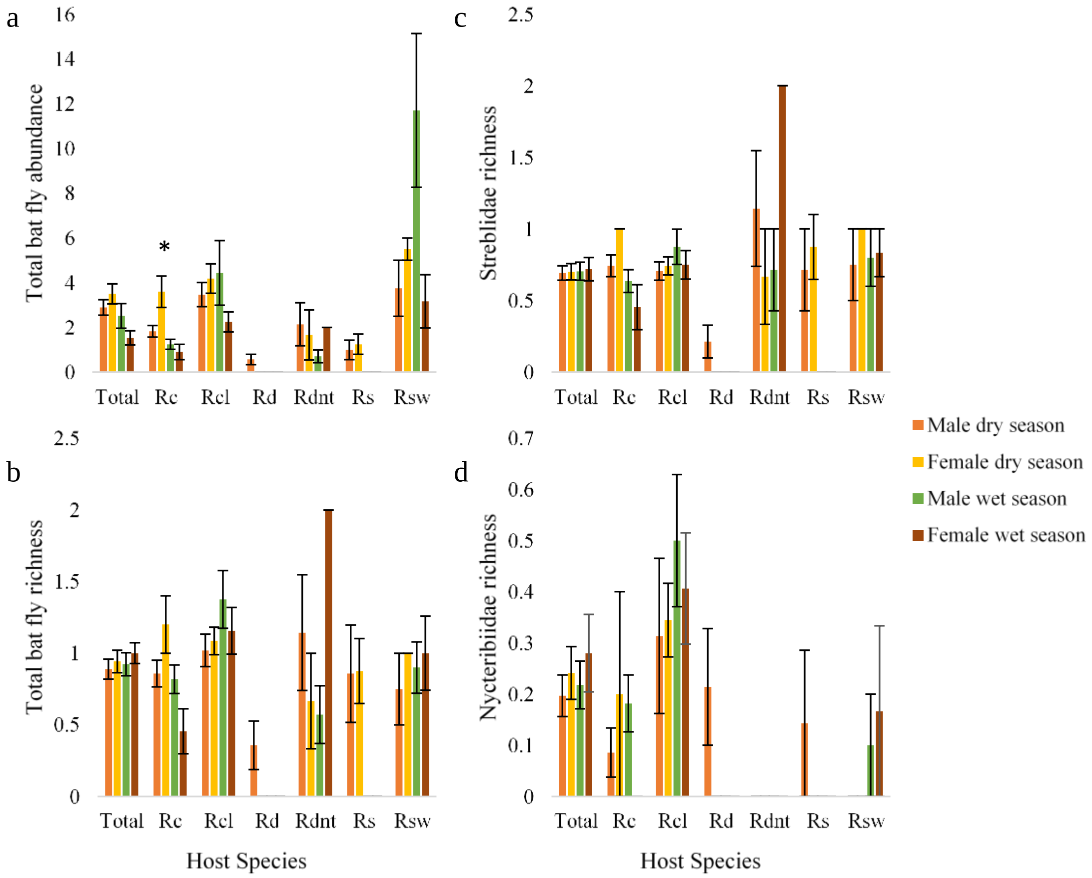

Figure 16 Mean (±SD) a. total bat fly abundance; b. total bat fly morphospecies richness; c. Streblidae morphospecies richness; d. Nycteribiidae morphospecies richness between male and female hosts in the dry and wet season. Rc = *Rhinolophus capensis*, Rcl = *Rhinolophus clivosus*, Rd = *Rhinolophus damarensis*, Rs = *Rhinolophus simulator*, Rsw = *Rhinolophus swinnyi*. Significant differences among species (p<0.05) are denoted by the *

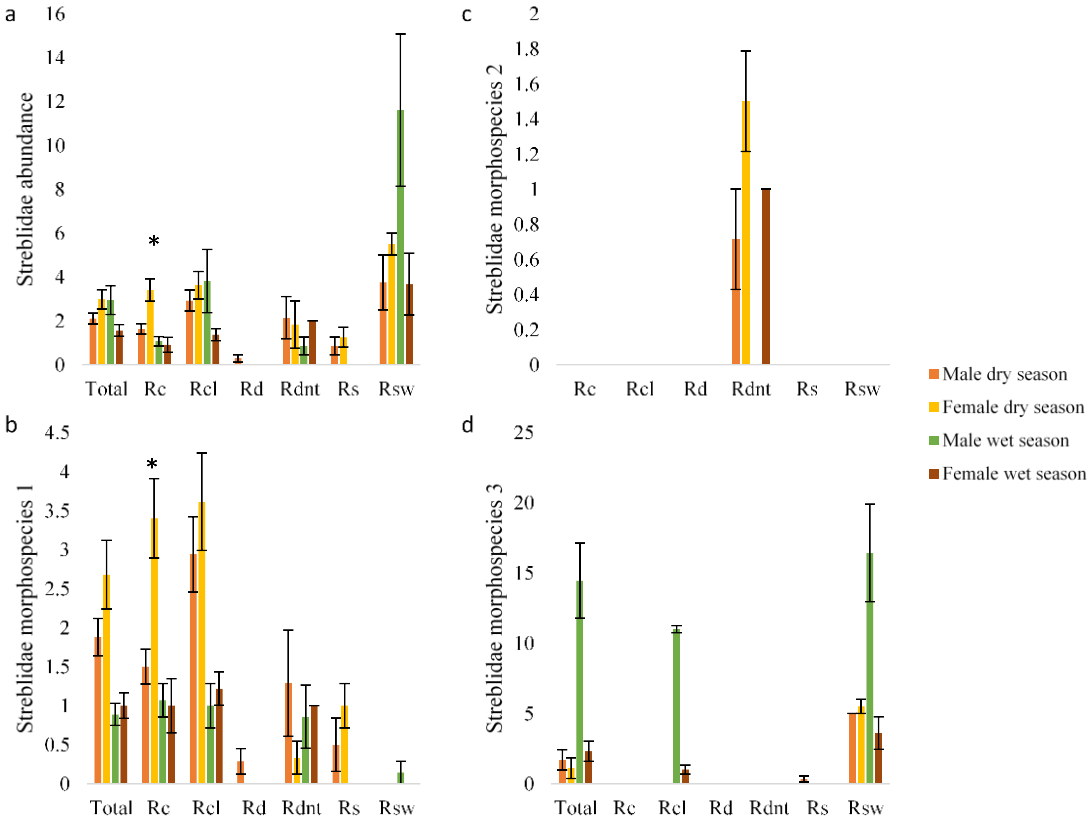

Figure 17 Mean (±SD) a. Streblidae abundance; b. Streblidae morphospecies 1 abundance; c. Streblidae morphospecies 2 abundance; d. Streblidae morphospecies 3 abundance between male and female hosts in the dry and wet season. Rc = *Rhinolophus capensis*, Rcl = *Rhinolophus clivosus*, Rd = *Rhinolophus damarensis*, Rs = *Rhinolophus simulator*, Rsw = *Rhinolophus swinnyi*. Significant differences among species (p<0.05) are denoted by the *

I found no evidence that host sex influenced the probability of bat fly prevalence ($\chi^2_{(1)} = 0.24$, p = 0.62).

3.3. Body size and condition

I did not include *R. denti* and *R. simulator* in analyses because they were each collected from single sites. I found mixed support for the hypothesis that host body size and condition influenced bat fly abundance and morphospecies richness.

3.3.1. Host forearm length

There was no evidence that host forearm length influenced bat fly abundance ($\chi^2_{(1)}$ = 1.463, p = 0.284) or morphospecies richness ($\chi^2_{(1)}$ = 0.006, p = 0.94). However, Streblidae morphospecies 2 abundance increased significantly with decreasing forearm length ($\chi^2_{(1)}$ = 6.677, $p < 0.01$; Figure 18c).

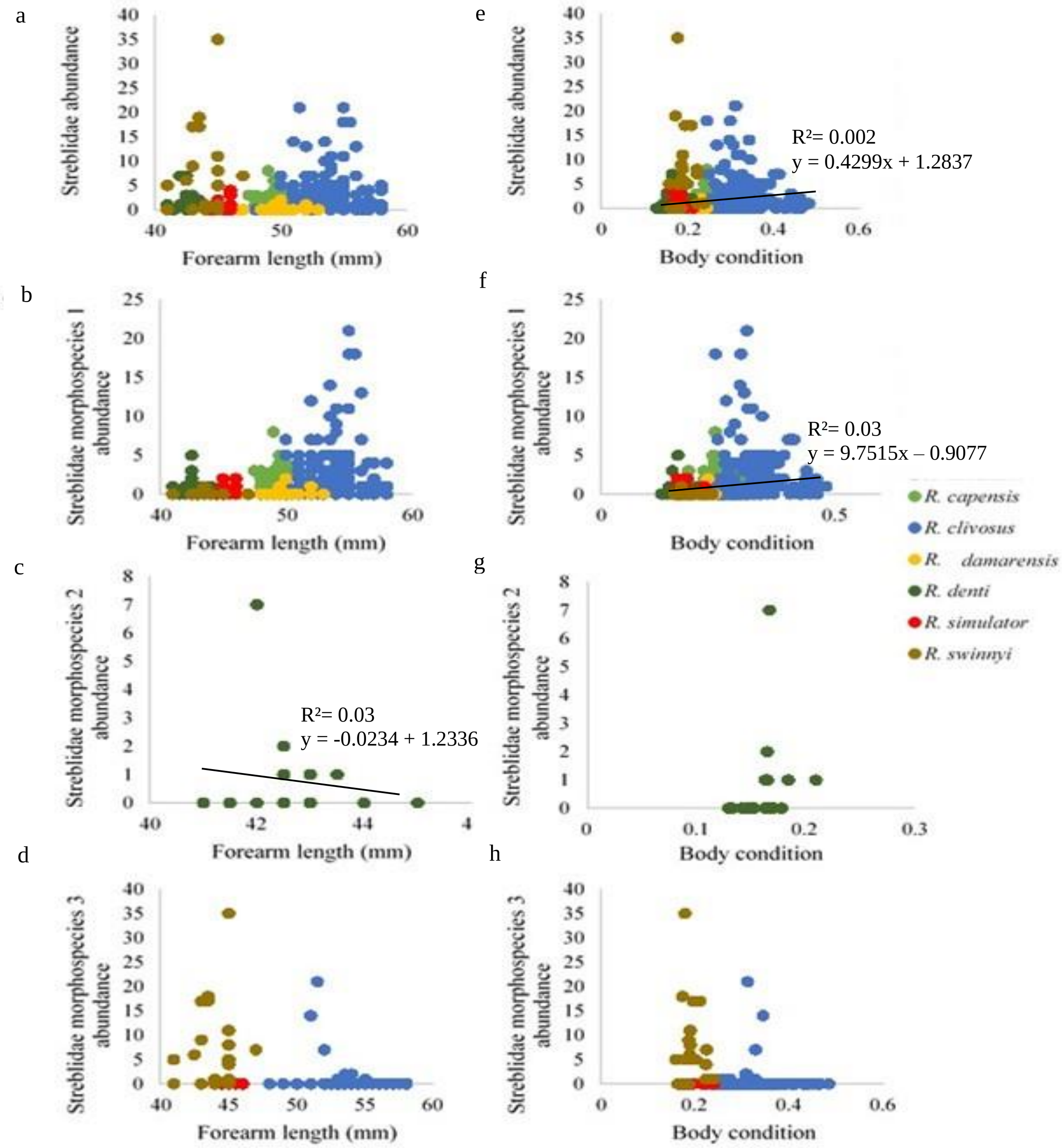

Figure 18 Relationships between host forearm length (mm) and a. total Streblidae abundance, b. Streblidae morphospecies 1 abundance, c. Streblidae morphospecies 2 abundance, and d. Streblidae morphospecies 3 abundance. Relationship between host body condition and e. total Streblidae abundance, f. Streblidae morphospecies 1 abundance, g. Streblidae morphospecies 2 abundance, and h. Streblidae morphospecies 3 abundance. R^2-value and equation of best fit line are shown only for significant relationships.

At a host species level, host body size did not significantly influence bat fly abundance on *R. capensis* ($\chi^2_{(1)} = 1.379$, $p = 0.24$), *R. clivosus* ($\chi^2_{(1)} = 0.919$, $p = 0.338$), *R. damarensis* ($\chi^2_{(1)} = 1.734$, $p = 0.188$) and *R. swinnyi* ($\chi^2_{(1)} = 0.231$, $p = 0.631$; Figure 18a) or the morphospecies richness on *R. capensis* ($\chi^2_{(1)} = 0.505$, $p = 0.477$), *R. clivosus* ($\chi^2_{(1)} = 0.886$, $p = 0.347$), *R. damarensis* ($\chi^2_{(1)} = 1.456$, $p = 0.06$) and *R. swinnyi* ($\chi^2_{(1)} = 0.068$, $p = 0.795$).

Host forearm length did not influence the probability of bat fly prevalence ($\chi^2_{(1)} = 2.2$, $p = 0.14$).

3.3.2. Body condition index

For *Rhinolophus* species combined, host body condition influenced total bat fly abundance ($\chi^2_{(1)} = 5.895$, $p < 0.05$; Figure 19e), Streblidae abundance ($\chi^2_{(1)} = 4.812$, $p < 0.05$; Figure 18e) and Streblidae morphospecies 1 ($\chi^2_{(1)} = 4.812$, $p < 0.05$; Figure 18f), with abundance significantly higher on individuals with high body condition scores.

Additionally, there were significant intractions between season and host body condition, and Nycteribidae abundance ($\chi^2_{(1)} = 7.145$, $p < 0.01$) and Nycteribiidae morphospecies 1 abundance ($\chi^2_{(1)} = 10.106$, $p < 0.01$), where abundances were significantly higher in the wet season on individuals with high body condition scores.

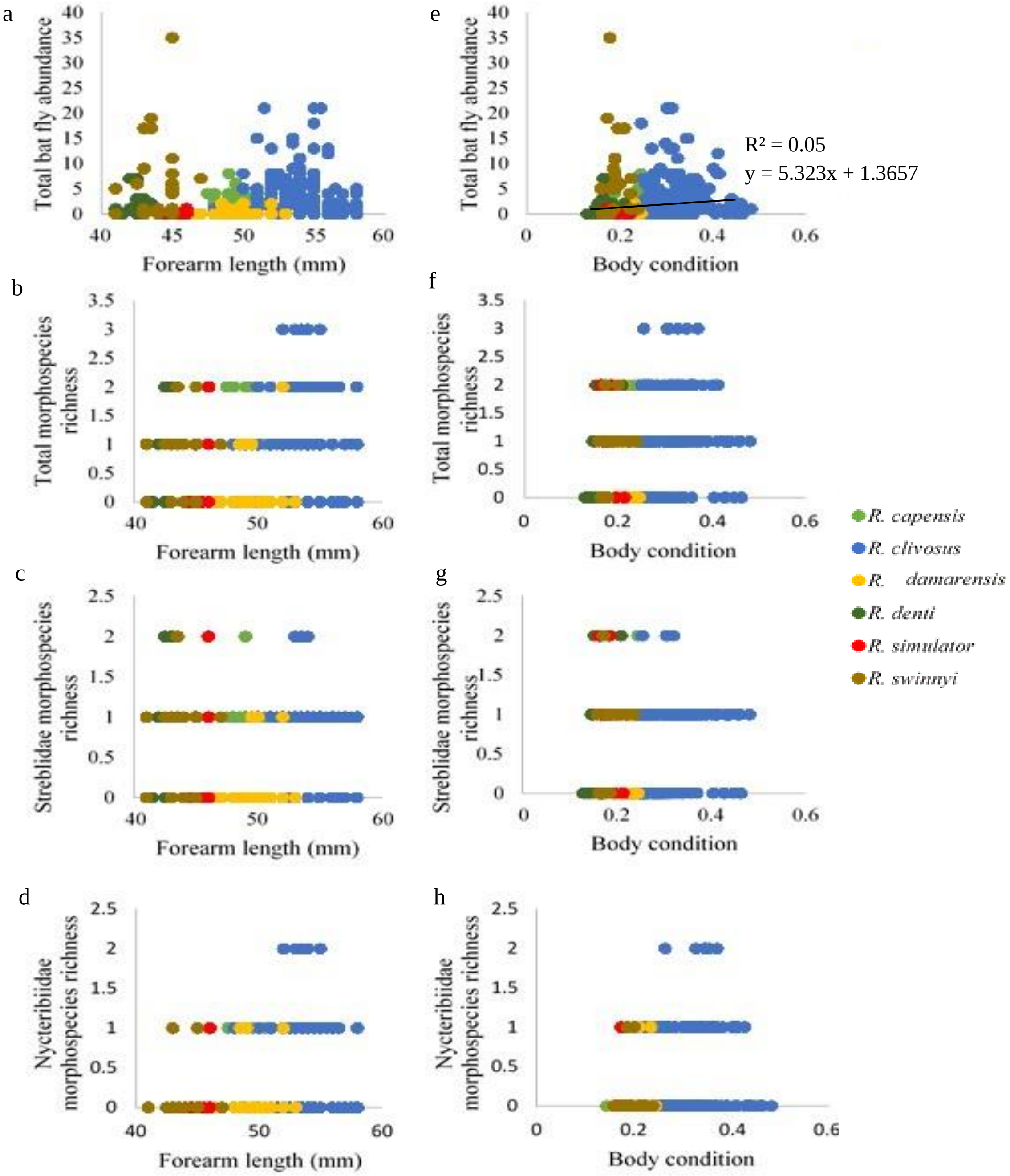

Figure 19 Relationships between host forearm length (mm) and a. total bat fly abundance; b. total bat fly morphospecies richness; c. Streblidae morphospecies richness; d. Nycteribiidae morphospecies richness; total bat fly abundance and host body condition. Relationships between host body condition and: e. total bat fly abundance; f. total bat fly morphospecies richness; g. Streblidae morphospecies richness; h. Nycteribiidae morphospecies richness. R^2-value and equation of best fit line are only shown for significant relationships.

Body condition scores of *R. clivosus* were significantly positively associated with total bat fly abundance ($\chi^2_{(1)}$ = (5.619), $p < 0.05$; Figure 19e), Streblidae abundance ($\chi^2_{(1)}$ = 4.554, $p < 0.05$; Figure 18e) and Streblidae morphospecies 1 abundance ($\chi^2_{(1)}$ = 6.186, $p < 0.05$; Figure 18f). Conversely body condition scores of *R. swinnyi*, were significantly inversely related to Streblidae morphospecies 3 abundance ($\chi^2_{(1)}$ = 4.247, $p < 0.05$; Figure 18h).

Additionally, I found significant intractions between season, host body condition and bat fly diversity in *R. clivosus* and *R. swinnyi*. Body condition scores of *R. clivosus* were positively associated with high total bat fly morphospecies richness ($\chi^2_{(1)}$ = 8.422, $p < 0.05$), Streblidae morphospecies richness ($\chi^2_{(1)}$ = 8.422, $p < 0.05$), total bat fly abunadance ($\chi^2_{(1)}$ = 5.163, $p < 0.05$), Streblidae abundance ($\chi^2_{(1)}$ = 4.035, $p < 0.05$), Nycteribiidae abundance ($\chi^2_{(1)}$ = 5.094, $p < 0.01$) and Streblidae morphospecies 1 abundance ($\chi^2_{(1)}$ = 5.178, $p < 0.05$) in the dry season. Conversely body condition scores of *R. swinnyi* were negatively significantly associated with total bat fly abundance ($\chi^2_{(1)}$ = 4.551, $p < 0.05$) and Streblidae morphospecies 3 abundance ($\chi^2_{(1)}$ = 4.896, $p < 0.05$) in the wet season.

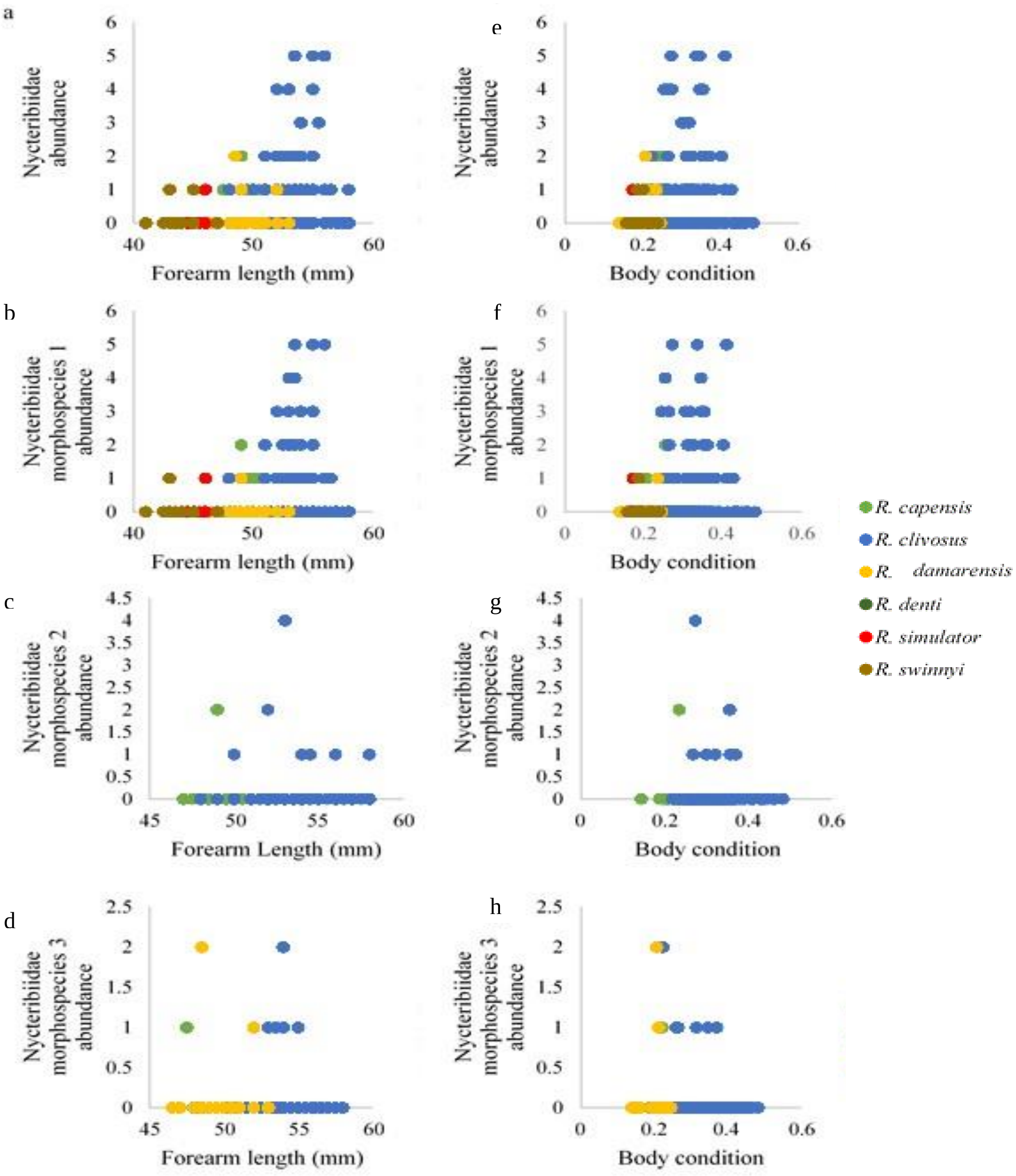

Figure 20 Relationships between host forearm length (mm) and a. total Nycteribiidae abundance, b. Nycteribiidae morphospecies 1 abundance, c. Nycteribiidae morphospecies 2 abundance, and d. Nycteribiidae morphospecies 3 abundance. Relationship between host body condition and e. total Nycteribiidae abundance, f. Nycteribiidae morphospecies 1 abundance, g. Nycteribiidae morphospecies 2 abundance, and h. Nycteribiidae morphospecies 3 abundance.

Host body condition was significantly positively related to bat fly prevalence ($\chi^2_{(1)} = 10.7$, $p < 0.05$, Figure 21).

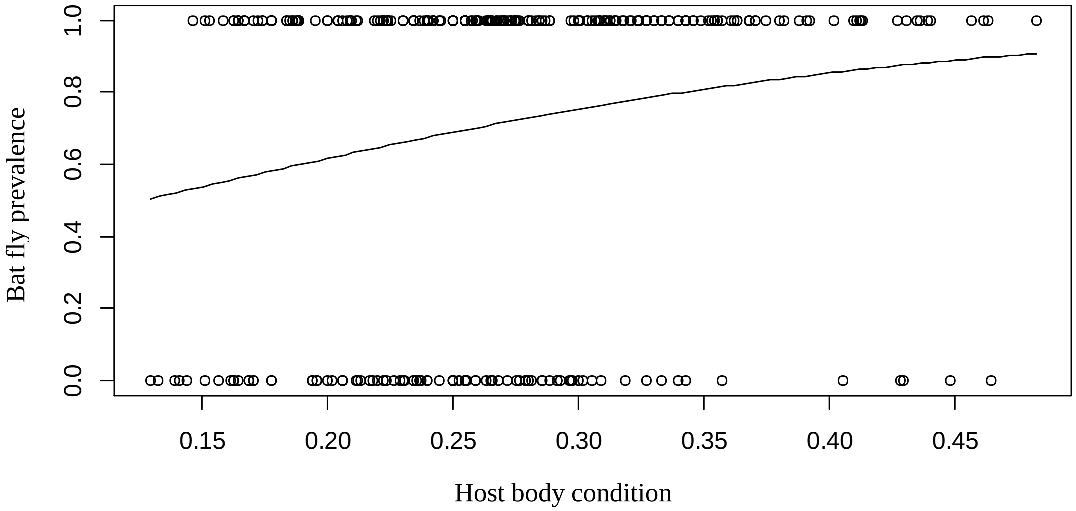

Figure 21 Relationship between probability of bat fly prevalence and host body condition of six *Rhinolophus* species in southern Africa. Line represents the line of best fit.

3.4. Relative influence of abiotic and biotic drivers

Recursive partitioning analysis showed that the most significant driver of bat fly abundance was habitat heterogeneity (Figure 22). The first split separates the abundance of bat flies based on the number of land cover classes, with high abundance in areas > 7 land cover classes (Figure 22). Abundance in habitats > 7 land cover classes was then divided based on vegetation index, with higher abundance associated with vegetation index ≤ 0.227 (Figure 22). In habitats > 7 land cover classes and vegetation index > 0.227 abundance was further split by season, with greater abundance in the dry season.

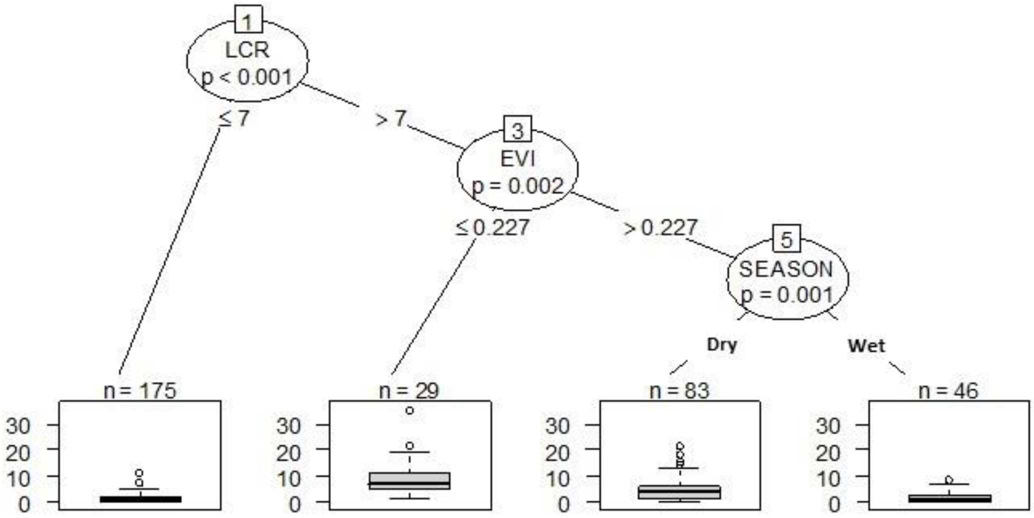

Figure 22: Recursive partitioning analysis of the three predictor variables and bat fly abundance on six *Rhinolophus* species in southern Africa.

Recursive partitioning analysis also showed that the most important predictor of bat fly morphospecies richness was habitat heterogeneity (Figure 23). The first split separates the bat fly richness based on the number of land cover classes, with high richness areas in areas > 7 land cover classes (Figure 23). Bat fly richness in areas > 7 land cover classes was then divided by the vegetation index, with higher richness in areas with vegetation index ≤ 0.234 (Figure 23). Here richness was further split by the host species, with greater richness on *R. clivosus*.

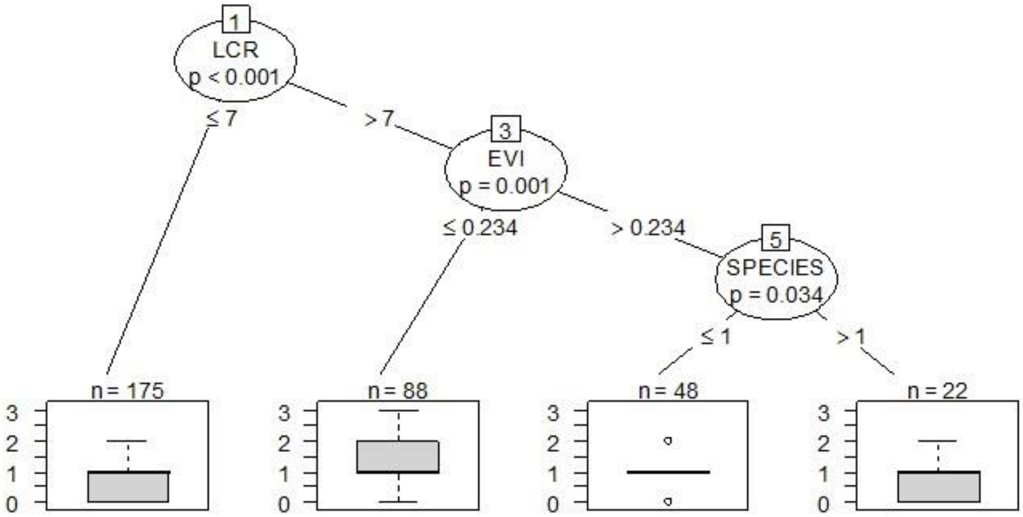

Figure 23 Recursive partitioning analysis of the three predictor variables and bat fly morphospecies richness on six *Rhinolophus* species in southern Africa.

Chapter 4. Discussion

I found mixed support for the three hypotheses, yet strongest support was for the habitat heterogeneity hypothesis. As predicted by the habitat heterogeneity hypothesis, rhinolophid species living in areas with high habitat heterogeneity had higher bat fly prevalence and intensity, probably because these areas harbour higher prey densities and increased structural complexity for the bats to utilise. I found limited support for the host sex hypothesis with most bat fly morphospecies favouring both male and female *Rhinolophus* bats. There was also little evidence that bat flies preferred bat hosts with large body size, yet I found evidence that bats with high body condition scores were more likely to be parasitised by bat flies. These results suggest that environmental and demographic factors mediate the bat fly diversity of *Rhinolophus* bat species in multilayered ways.

4.1. Patterns of parasite assemblages

4.1.1. Bat fly prevalence

Bat fly abundance was positively associated with bat abundance, probably because ectoparasites have restricted dispersal capabilities, particularly in Nycteribiidae (Patterson et al., 2008a), and spend most of their adult life stages on their host (Dick and Patterson, 2006). These results are comparable with studies in the New World where bat fly prevalence was generally high on bats that roost in permanent structures (Patterson et al., 2007). Permanent roosts provide a stable environment with respect to temperature and protection, and hence favour the formation of large and temporally stable roosting colonies (Ter Hofstede and Fenton, 2005). High host densities provide more microhabitats as well as food resources for the bat flies, attributes which promote high bat fly diversity (Patterson et al., 2007).

4.1.2. Host specificity

Six bat fly morphospecies were collected in this study: three Streblidae and three Nycteribiidae. These bat flies varied in their distribution as well as level of host specificity. The only bat fly species collected from all six bat host species was Streblidae morphospecies 1. Host specificity is driven primarily by encounter, compatibility and reproductive mechanisms (Dick and Patterson, 2007; de Vasconcelos et al., 2016). The encounter mechanism proposes that parasites need to locate and colonise hosts, therefore parasites associated with hosts that are range restricted or geographic isolated are likely to be host specific (Ter Hofstede et al., 2004; Krasnov et al., 2015). The compatibility mechanisms suggests that parasites must be able to successfully feed without succumbing to the hosts' parasite defences, such as grooming, roost switching or immune incompatibility (Kuris et al., 2007). Studies on New World bats indicate that host compatibility is an important driver of specificity, with bat flies evolving immune mimicry to avoid detection from their primary host (Dick and Patterson, 2007; de Vasconcelos et al., 2016; Obame-Nkoghe et al., 2016). The reproductive mechanism proposes that successful transfer to the new host is achieved if the parasite can encounter members of the opposite sex and successfully reproduce (Dick and Patterson, 2007). This mechanism may be important in maintaining high levels of host specificity in bat flies in the New World (Dick and Patterson, 2007).

The single monoxenous bat fly in this study may be the result of geographical isolation of the host (Seneviratne et al., 2009): *R. denti* is geographically isolated from *R. simulator* and *R. swinnyi*. This species is found only in the arid west of southern Africa. Geographic isolation limits gene flow between the bat fly populations associated with *R. denti*, *R. simulator* and *R. swinnyi*, violating both the encounter and reproductive mechanisms. This may have led to the evolution of the two morphologically distinct small Streblidae morphospecies (Seneviratne et al., 2009; de Vasconcelos et al., 2016), but this remains to be verified with genetic analyses. The relatively short evolutionary history of the southern African *Rhinolophus* species (Stoffberg et al., 2010), coupled with the co-occurrence of congeneric species within the same roosts may favour oligoxenous parasitism over monoxenous parasitism, because the three specificity mechanisms are not violated (Giorgi et al., 2004; Dick and Patterson, 2007; Dick et al., 2009; de Vasconcelos et al., 2016).

4.1.3 Host species, sites and season

Bat fly diversity differed significantly among species. For example, total bat fly abundance was significantly higher on *R. clivosus* and *R. swinnyi* hosts than all other hosts, whereas fly morphospecies richness was significantly lower on *R. damarensis* than other bat hosts. The largest host, *R. clivosus* had higher Nycteribiidae abundance and morphospecies richness than other hosts except *R. damarensis*. Conversely, total bat fly abundance and Streblidae abundance were highest in the smallest host *R. swinnyi*. Bat fly abundance may therefore not follow predictable patterns with regards to host species or body size (Presley and Willig, 2008). Hence other demographic or behavioural characteristics of hosts may drive bat fly diversity. Demographic characteristics include host colony size - large colonies provide more food and habitat for bat flies; spacing between individuals - decreased spacing between hosts increase the chances of successful vertical and horizontal parasite transfer; and longevity of the roost - newly emerged adult flies find their first blood meal within the first few hours easier in more established and permanent roosts (Reckardt and Kerth, 2006; Patterson et al., 2007). Behavioural characteristics of hosts include roost switching – for example moving from roosts with high bat fly diversity to sites with lower diversity; and grooming, which is probably the greatest cause of mortality in bat flies (Komeno and Linhares, 1999; Ter Hofstede and Fenton, 2005; Dick and Patterson, 2008; Presley, 2011).

Bat fly diversity also differed significantly among sites. Three sites (Babanango Valley Lodge Mine, Mooiplaas Mine and De Kelders Cave 2) in three biomes (Grassland, Savanna and Fynbos, respectively) had significantly higher total bat fly abundance and richness than most other sites. Further, Nycteribiidae abundance and morphospecies richness was significantly higher at Babanango Valley Lodge Mine than most sites. Ten sites had two or more bat species roosting in close proximity to *Rhinolophus* species, including *Miniopterus natalensis*, *Hipposideros caffer*, *Myotis tricolor* and *Nycteris thebaica*. However bat flies exhibited a high degree of host specificity, notably at multispecies sites. Two to three *Rhinolophus* species co-roosted at six of the twenty sites, of which two sites (Mooiplaas Mine and De Kelders cave 2) were associated with significantly higher bat fly abundance and richness. Additionally, the largest *Rhinolophus* colony (Babanango Valley Lodge Mine, ~1500 *R. clivosus*) was associated with significantly higher bat fly abundance and richness. Space of roosts were highly variable yet size of mine/cave was not correlated with bat population size. The space between individuals in large roosting systems with low bat densities may have limited bat fly abundance and richness because the parasites may be unable to successfully transfer between individuals or find mates (Patterson et al., 2008b). Moreover, differences in ectoparasite diversity between sites may be attributed to differences in abiotic conditions in roosts that were not measured in this study, such as temperature, humidity, microhabitats, and longevity (Patterson et al., 2007). Studies of host-parasite interactions on non-volant small mammals have found that abiotic conditions, such as temperature, rainfall and humidity, significantly affect survival and reproductive rates of the parasites (Krasnov et al., 2005; Krasnov et al., 2015; Morand, 2015). Low ambient temperatures in the roost and decreased metabolism and temperature on the host decrease the reproductive activity of parasites (Reckardt and Kerth, 2006; Christe et al., 2007; Lourenço and Palmeirim, 2008) For example, bats in the New World and Europe that hibernate during the winter have low parasite abundance (Lourenço and Palmeirim, 2008). However, abiotic conditions of many natural permanent structures are relatively stable even in temperate areas during winter months (McDonald et al., 1990), and probably have little effect on bat fly reproductive or pupal survivorship rates. Further, prolonged hibernation during winter is not observed in southern African *Rhinolophus* bats, with bats entering torpor for only a few days (McDonald et al., 1990), which may account for the absence of seasonal variation in bat fly abundance or morphospecies richness.

4.2. Habitat heterogeneity hypothesis

My results suggest that habitat heterogeneity was the most important predictor of bat fly abundance and morphospecies richness on *Rhinolophus* bats in southern Africa. This corroborates recent studies that show bat richness in southern Africa is significantly correlated with structural habitat heterogeneity (Qian et al., 2009; Schoeman et al., 2013). An increase in vegetation structural heterogeneity and habitat complexity leads to an increase in the number of niches available for bats as well as their insect prey to exploit.

4.2.1. EVI

EVI and bat fly diversity was significantly related. In support of the habitat heterogeneity hypothesis, significantly positive relationships were found between EVI and total bat fly abundance, Streblidae abundance and Streblidae morphospecies 1 abundance. Because bat flies are obligate parasites they are highly reliant on their associated hosts for both food as well as habitat. Therefore bat fly abundance should be highest in areas that favour high diversity of their hosts (Andrews and O'Brien, 2000; Camilotti et al., 2010; Gay et al., 2014). In support, Lepidoptera diversity, the major component of many *Rhinolophus* species' diets (Schoeman and Jacobs, 2003; Monadjem et al., 2010; Schoeman and Jacobs, 2011), is positively associated with vegetation cover (Fuentes-Montemayor et al., 2013). Further, southern African Rhinolophidae are significantly positively related to structural heterogeneity of habitat (Stoffberg et al., 2010).

Contrary to the habitat heterogeneity prediction, EVI was significantly negatively associated with total bat fly morphospecies richness, Nycteribiidae abundance and morphospecies richness, as well as Streblidae morphospecies richness. Much of southern Africa's surface area is dominated by habitats with large open areas with small shrubs and interspersed with woody areas such as grasslands, savannas and thickets (Fenton et al., 1998). Previous studies reported EVI values for savanna woodland habitats between 0.2 and 0.3 (Jin et al., 2013). Moreover, southern Africa is water scarce. Drought conditions in southern Africa over the last decade has decreased EVI values (Linderman et al., 2005). Regardless, these results suggest that the presence of the hosts in roosts within habitats rather than the structural characteristics of the surrounding habitat may influence bat fly richness (Camilotti et al., 2010; Lourenço et al., 2016).

4.2.2. Number of land cover classes

There were significantly positive relationships between the number of land cover classes and bat fly prevalence, abundance and richness, Streblidae abundance and species richness, Streblidae morphospecies 1, Streblidae morphospecies 3, Nycteribiidae abundance and species richness, Nycteribiidae morphospecies 1 and Nycteribiidae morphospecies 3. My results are consistent with studies on non-volant mammals that found strong positive relationships between parasite diversity and habitat complexity (Halos et al., 2010; Young et al., 2015). These patterns were attributed to hosts moving through many habitats whilst foraging and being in contact with the substrate that their associated parasites are found in or on. However, bats probably come into contact with their parasites mainly at their roosts or during swarming events (Dick and Dick, 2006; Presley and Willig, 2008). An alternative explanation may be that the high number of land cover classes ensure high niche availability for both bats and parasites. Stein et al. (2015) found that the number of land cover classes was positively correlated with the mammalian species richness. There is evidence from Africa that habitats with high number of land cover classes favoured bats from different foraging functional groups, and increased the local bat diversity (Fahr and Kalko, 2011; Schoeman et al., 2013). In Brazil bat fly richness was low in habitats with poor bat richness (Camilotti et al., 2010). Additionally, a large number of land classes may favour high prey diversity (Fuentes-Montemayor et al., 2013), which in turn, may result in good health of resident bat populations, thus providing the resident bat flies with large and nutritious blood meals and hence high fitness (Christe et al., 2003; Young et al., 2015).

4.3. Host sex hypothesis

Although there is evidence that parasites often prefer one host sex above the other sex (Morand, 2015), I found little support for host sex influencing bat fly richness or abundance and no support for differences in prevalence between sexes. There was some evidence that Streblidae morphospecies 2 abundance was higher on male hosts, whereas Streblidae morphospecies 3 abundance was higher on female hosts. Similarly at an individual host species level, little support was found for host sex influencing bat fly diversity. Except, abundance of Nycteribiidae morphospecies 3 was significantly higher on female *R. capensis*, whereas total bat fly abundance, total bat fly richness and Streblidae richness were higher on male *R. damarensis* hosts.

Most studies on vertebrate-parasite interactions suggest strict male-biased host preferences (Krasnov et al., 2012; Kowalski et al., 2015; Morand, 2015; Young et al., 2015). Reasons for this pattern include: males of most vertebrate species have wider dispersal (larger home ranges) than females (Kowalski et al., 2015), potentially bringing them into contact with a wider range and number of parasites (Krasnov et al., 2012); males may have larger body sizes (Kowalski et al., 2015); high testostarone levels in males reduce immunocompetence (Krasnov et al., 2012); and male bats may roost solitarily but move between multiple congregation sites within the roost bringing them into contact with more co-specifics and increasing their exposure risk to parasites (Komeno and Linhares, 1999). Sexual dimorphism in African *Rhinolophus* bats is rare, but, as in many other bat genera, usually results in larger females (Bernard and Cumming, 1997). Male southern African *Rhinolophus* bats roost both solitary and in small multi-sex groups (McDonald et al., 1990). Additionally, large multi-sex congregations are often formed at the roost entrance prior to emergence (Dick and Patterson, 2007). Thus, spatial and demographic roosting dynamics are probably not the cause of bat fly preference for males as there is equal opportunity for bat fly dispersal to individuals of either sex (Patterson et al., 2007).

There is evidence that both bat flies and mites prefer female bats (Christe et al., 2007; Patterson et al., 2008a; Sharifi et al., 2008). When sexual dimorphism was present, female *Rhinolophus* bats were larger than males. Larger size renders females a larger target for free living (host seeking) life stages of bat flies, such as newly emerged adults as well as females returning to the host after larval deposition (Patterson et al., 2008a). Further, female *Rhinolophus* bats often roost in large colonies, particularly after the gestation season. These large colonies provide the bat flies with increased food and habitat resources, reduced inter-bat spacing which facilitates ectoparasite movement between host individuals (Krasnov et al., 2012), decreased immune defences of hosts, and increased ambient temperatures (Christe et al., 2007; Krasnov et al., 2012). All of these factors are known to positively influence bat fly parasitism by increasing their population size and fitness (Zahn and Rupp, 2004).

Male and female bats have seasonal fluctuations in their immunocompetence. Immune systems are compromised in males during the mating season due to higher testosterone level and in females during the gestation period due to the stresses of rearing their pups (Patterson et al., 2008b). However, I found very little evidence for the proposed prediction that host sex influenced bat fly abundance or morphospecies richness seasonally. Except, *R. capensis* females hosted significantly higher total bat fly and Streblidae abundance in the dry season. This season corresponds to the gestation period of this species in the Fynbos biome. During the gestation period, ectoparasites may favour female hosts because much of their energetic budget is utilised rearing their young. This leaves the females vulnerable to increased ectoparasite abundance as they form large maternity colonies and have less energy reserves for parasite avoidance activities, such as grooming, roost switching and immune responses. Additionally, parasites have access to pups which are immature groomers and have naive immune systems (Dick and Patterson, 2007). Similarly, *R. mehelyi* females have higher parasite loads before and after the gestation period (Sharifi et al., 2013). Regardless, further studies are needed to elucidate seasonal preferences for host sex by bat flies.

4.4. Body size and condition hypotheses

My results indicate mixed support for the host body size and body condition hypotheses. There appeared to be partitioning of micro-habitats on bats between the two bat fly families, with Streblidae collected only from membranous surfaces, and Nycteribiidae collected only from the fur.

4.4.1. Host Forearm Length

Host body size is positively correlated with parasite abundance and richness in many species (Young et al., 2015). This is because parasites locate larger individuals more easily. Additionally, once located, large hosts provide more niches and food resources for the parasite to exploit (Froeschke et al., 2013; Morand, 2015). However, no support was found for the prediction that larger bats should have higher bat fly abundance and morphospecies richness than smaller bats. Previous studies also found no significant relationships between host body size and bat fly abundance, richness or prevalence (Patterson et al., 2008a; Presley and Willig, 2008; Kamiya et al., 2014). Host body size may not influence bat fly colonisation or diversity if bat flies are capable of moving between individuals without direct physical contact (Patterson et al., 2008a). However, Nycteribiidae are poor dispersers because they lack wings, yet they also did not exhibit preferences for larger hosts. Host body size may also not influence ectoparasite diversity if bats roost in permanent structures and form large colony sizes because bats of all sizes and their ectoparasites come into regular contact (Patterson et al., 2007). Thus the presence and numbers of bats in roosts probably play a larger role in the levels of bat fly parasitism and may result in the random distribution of the parasites.

The body size of the host may be correlated with the body size of the parasite, with smaller bat hosts having smaller parasites than larger bats. A reduction in parasite body size may reduce their risk of being groomed off by the hosts (Dick and Patterson, 2008). In this study the three smallest host species (*R. simulator*, *R. denti* and *R. swinnyi*) harboured the two smallest Streblidae morphospecies. Indeed, Streblidae morphospecies 2 abundance was highest on small *R. denti* individuals. Similarly, the two largest Nycteribiidae morphospecies were only found on the three largest host species (*R. clivosus*, *R. damarensis* and *R. capensis*).

4.4.2. Host Body Condition

I found strong support for the body condition hypothesis: for *Rhinolophus* species combined, total bat fly abundance, Streblidae abundance and Streblidae morphospecies 1 abundance were higher on bats with good body conditions. Additionally, Nycteribiidae morphospecies 1 abundance was higher on hosts with good body condition in the wet season. The wet season usually corresponds to the period of highest insect diversity, and hence good body conditions in bats (McDonald et al., 1990).

At individual host species level, however, different bat fly diversity patterns were found for two of the six host species. On *R. clivosus* a significantly positive relationship was found between body condition and total bat fly abundance, Streblidae abundance and Streblidae morphospecies 1 abundance, as well as with total bat fly morphospecies richness and abundance, Streblidae morphospecies richness and abundance, Nycteribiidae abundance and Streblidae morphospecies 1 abundance in the dry season. Conversely, on *R. swinnyi* an inverse relationship was found between body condition and Streblidae morphospecies 3 abundance and total bat fly abundance, particularly in the wet season. At Mooiplaas, these two species co-occur during the wet season. During this season the bat diversity at Mooiplaas increased from two to five species. Streblidae morphospecies 3 was recorded on both of these rhinolophid species, therefore the extra habitat and food resources available due to the presence of *R. clivosus* during the wet season may have increased the reproductive activity of the bat fly leading to an increased abundance. The extra blood meals taken by the bat flies particularly from the smaller *R. swinnyi* as well as increased energetic outputs on parasite defences may have led to a reduction in their body condition. However this was the only roost system in which a seasonal host presence/absence pattern was observed and thus needs to be studied further.

One reason that bat flies may prefer bats with good body condition is that healthy hosts are more likely to persist in populations. Preference for hosts with good body conditions has also been demonstrated in mites on bats (Christe et al., 2003; Christe et al., 2007). On the other hand, hosts in good body condition have a higher energy budget (Young et al., 2015) and therefore could spend more time on parasite avoidance (grooming and roost switching). Bats need to weigh up the costs of using extra energy in parasite avoidance with the costs imposed by the parasite. Experimental studies on grooming rates of bats exposed to different bat fly abundance found no significant relationship between grooming rates and bat fly abundance (Ter Hofstede and Fenton, 2005). This may imply that the bat flies on the host have little or no effect on the body condition of their host. By comparison, mites, which spend their entire life cycle on the host, elicit the highest grooming rates when present (Godinho et al., 2013). To date, however, studies have found no evidence that bat flies impose any significant negative effects on their associated hosts (Zahn and Rupp, 2004). High levels of host specificity of bat flies may have evolved to avoid detection by bats and reduce grooming responses through immune mimicry (Dick and Patterson, 2007), allowing bat flies to colonise hosts with good body conditions throughout the year. Competition in the form of density compensation between bat flies has been recorded, where one abundant species restricts the abundance of another (Dick and Dick, 2006; Patterson et al., 2008a; Tello et al., 2008). Additionally, bat flies may self-regulate their numbers on the host to reduce the negative costs they impose on their hosts (Dick and Dick, 2006; Patterson et al., 2008a).

4.5. Relative influence of abiotic and biotic drivers

My results from the recursive partitioning analysis showed that the most significant driver of bat fly abundance and morphospecies richness was habitat heterogeneity. This corroborates results from the analyses testing each of the three hypotheses that found strong support for the habitat heterogeneity hypothesis, yet weaker support for host sex and host body size and condition hypotheses. The recursive partitioning analyses also confirmed that bat fly diversity is influenced by the complexity of the habitat (number of land cover classes) rather than vegetation structure. However, it remains difficult to conclude whether the habitat directly influences bat fly diversity or morelikey indirectly through affecting bat diversity. To date few studies have evaluated the influence of habitat on both bat and parasite diversity. With the exception of Camilotti et al. (2010) who found bat fly richness was lower in habitats (Brazilian decideous forest) with lower bat diversity.

4.6. Caveats and future work

The main caveats of this study are as follows. First, I sampled a limited number of sites in different biomes, and some biomes were underrepresented. Future work should increase sites and biomes coverage. Second, I identified bat flies based on morphology only. Future work should include genetic identification to accurately confirm species richness and abundance. Third, sampling in this study was limited temporally. Future work should sample bat fly diversity over more than one year, and sampling of bat flies should be more sustained during seasons.

Future studies should also investigate if ectoparasites on other bat species and functional groups are also influenced mainly by habitat complexity, and if diversity of endoparasites in bats are similarly influenced by factors. For example, endoparasite richness of bats in south-east Asia was negatively associated with habitat fragmentation presumably due to the reduction in suitable habitat for intermediate hosts (Gay et al., 2014), whereas Filarial nematode infections in Malagasy bats were driven by bioclimatic conditions (Ramasindrazana et al., 2016). Moreover, the relative influence of biotic processes such as competition should be investigated. In social rodents, increased competition among ectoparasites led to competitive exclusion and lower parasite richness (Bordes et al., 2007). Although competition appears have little effect on bat fly assemblages on hosts, density compensation has been found in bat flies specialising on the same micro-habitat (Tello et al., 2008). On the other hand, facilitation between bat flies specialising on different microhabitats led to aggregations of multiple bat fly species on the hosts (Ter Hofstede et al., 2004; Dick and Patterson, 2006; Tello et al., 2008). Additionally, in this study the effects of micro-habitat conditions in roosts (e.g. moisture, temperature and humidity) on bat fly diversity and reproduction was not investigated. There is evidence that these abiotic roosting characteristics can negatively impact the reproductive activity and survival of off host life stages of bat ectoparasites (Lourenço and Palmeirim, 2008), including bat flies (Reckardt and Kerth, 2006). Finally, more data are needed on the impact of roosting behaviour of hosts on the ectoparasite assemblages and diversity in Old World bats (Patterson et al., 2007; Reckardt and Kerth, 2007).

4.8. Conclusions

To analyse factors that influence bat fly diversity on *Rhinolophus* bats, I tested predictions for three hypotheses using a battery of analytical techniques. This approach facilitated the identification of the principal predictor of bat fly prevalence and diversity, namely habitat heterogeneity. Habitat heterogeneity probably mediated bat fly diversity along many direct and indirect pathways. Given that *Rhinolophus* community and population structure are strongly related to the habitat characteristics of biomes in southern Africa (Stoffberg et al., 2010), this parasite-host model may be a good example of parasites co-evolving and mirroring the life history strategies of their hosts (Dick and Patterson, 2007; Tortosa et al., 2013; van Schaik et al., 2014). From an applied perspective, conservation and management strategies to protect both the *Rhinolophus* populations and their obligate bat fly parasites should focus on maintaining the habitat diversity that surrounds roosts and foraging grounds.

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Appendix A

Table 1: Bat fly distribution and prevalence on six *Rhinolophus* species at 20 roost sites in southern Africa.– = no capture, C = combined season, W = wet season and D = dry season.

		Bat species																				
	Biome	**All bat spp**			***R. capensis***			***R. clivosus***			***R. damarensis***			***R. denti***			***R. simulator***			***R. swinnyi***		
		Season			**Season**			**Season**			**Season**			**Season**			**Season**			**Season**		
		C	**W**	**D**	**C**	**W**	**D**	**C**	**W**	**D**	**C**	**W**	**D**	**C**	**W**	**D**	**C**	**W**	**D**	**C**	**W**	**D**
All Sites																						
Number of bats		333	129	204	96	39	57	156	48	109	23	1	22	21	13	8	15	-	15	22	16	5
Number of infested bats		233	91	142	67	31	36	122	39	83	5	0	5	12	7	5	10	-	10	18	13	5
Prevalence (%)		70	71	70	70	80	63	78	81	76	22	0	23	57	54	63	67	-	66	82	81	100
Table Farm Grahamstown	**Albany Thicket**																					
Number of bats		35	11	24	35	11	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Number of infested bats		30	10	20	30	10	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Prevalence (%)		86	91	83	86	91	83	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sleepy Hollow Maitland	**Fynbos**																					
Number of bats		19	7	12	4	1	3	15	6	9	-	-	-	-	-	-	-	-	-	-	-	-
Number of infested bats		17	7	11	3	1	2	15	6	9	-	-	-	-	-	-	-	-	-	-	-	-
Prevalence (%)		90	100	92	75	100	67	100	100	100	-	-	-	-	-	-	-	-	-	-	-	-
Phillips Tunnel Hankey	**Fynbos**																					
Number of bats		23	10	13	23	10	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Number of infested bats		10	9	1	10	9	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Prevalence (%)		44	90	8	44	90	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 1 continued

Klaassies River Cave	**Fynbos**																					
Number of bats		12	-	12	12	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Number of infested bats		8	-	8	8	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Prevalence (%)		67	-	67	67	-	67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hopefield Farm Postmasburg 1	**Nama-Karoo**																					
Number of bats		44	20	24	-	-	-	21	6	15	2	1	1	21	13	8	-	-	-	-	-	-
Number of infested bats		25	12	13	-	-	-	13	5	8	0	0	0	12	7	5	-	-	-	-	-	-
Prevalence (%)		57	60	54	-	-	-	62	83	53	0	0	0	57	54	63	-	-	-	-	-	-
Hopefield Farm Postmasburg 2	**Nama-Karoo**																					
Number of bats		15	-	15	-	-	-	1	-	1	14	-	14	-	-	-	-	-	-	-	-	-
Number of infested bats		6	-	6	-	-	-	1	-	1	5	-	5	-	-	-	-	-	-	-	-	-
Prevalence (%)		40	-	40	-	-	-	100	-	100	36	-	36	-	-	-	-	-	-	-	-	-
Blink Klip Grotte Postmasburg	**Nama-Karoo**																					
Number of bats		10	4	6	-	-	-	7	4	3	3	-	3	-	-	-	-	-	-	-	-	-
Number of infested bats		4	2	2	-	-	-	4	2	2	0	-	0	-	-	-	-	-	-	-	-	-
Prevalence (%)		40	50	33	-	-	-	57	50	67	0	-	0	-	-	-	-	-	-	-	-	-
Babanango Valley Lodge	**Grassland**																					
Number of bats		9	9	-	-	-	-	9	9	-	-	-	-	-	-	-	-	-	-	-	-	-
Number of infested bats		6	6	-	-	-	-	6	6	-	-	-	-	-	-	-	-	-	-	-	-	-
Prevalence (%)		67	67	-	-	-	-	67	67	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 1 continued

Babanango Valley Lodge Main Mine	**Grassland**																					
Number of bats		50	13	37	-	-	-	50	13	37	-	-	-	-	-	-	-	-	-	-	-	-
Number of infested bats		45	11	34	-	-	-	45	11	34	-	-	-	-	-	-	-	-	-	-	-	-
Prevalence (%)		90	85	92	-	-	-	90	85	92	-	-	-	-	-	-	-	-	-	-	-	-
Babanango Valley Lodge Exploratory Mine 1	**Grassland**																					
Number of bats		9	-	9	-	-	-	9	-	9	-	-	-	-	-	-	-	-	-	-	-	-
Number of infested bats		6	-	6	-	-	-	6	-	6	-	-	-	-	-	-	-	-	-	-	-	-
Prevalence (%)		67	-	67	-	-	-	67	-	67	-	-	-	-	-	-	-	-	-	-	-	-
Babanango Valley Lodge Exploratory Mine 2	**Grassland**																					
Number of bats		2	-	2	-	-	-	2	-	2	-	-	-	-	-	-	-	-	-	-	-	-
Number of infested bats		2	-	2	-	-	-	2	-	2	-	-	-	-	-	-	-	-	-	-	-	-
Prevalence (%)		100	-	100	-	-	-	100	-	100	-	-	-	-	-	-	-	-	-	-	-	-
Mooiplaas Gold Mine	**Savanna**																					
Number of bats		26	21	5	-	-	-	9	9	-	-	-	-	-	-	-	-	-	-	17	12	5
Number of infested bats		26	21	5	-	-	-	9	9	-	-	-	-	-	-	-	-	-	-	17	12	5
Prevalence (%)		100	100	100	-	-	-	100	100	-	-	-	-	-	-	-	-	-	-	100	100	100

Table 1 continued

Doornhoek Mine	**Savanna**																					
Number of bats		15	-	15	-	-	-	-	-	-	-	-	-	-	-	-	15	-	15	-	-	-
Number of infested bats		10	-	10	-	-	-	-	-	-	-	-	-	-	-	-	10	-	10	-	-	-
Prevalence (%)		67	-	67	-	-	-	-	-	-	-	-	-	-	-	-	67	-	67	-	-	-
De Kelder Cave 1	**Fynbos**																					
Number of bats		6	-	6	-	-	-	6	-	6	-	-	-	-	-	-	-	-	-	-	-	-
Number of infested bats		4	-	4	-	-	-	4	-	4	-	-	-	-	-	-	-	-	-	-	-	-
Prevalence (%)		67	-	67	-	-	-	67	-	67	-	-	-	-	-	-	-	-	-	-	-	-
De Kelder Cave 2	**Fynbos**																					
Number of bats		19	1	18	1	1	-	18	-	18	-	-	-	-	-	-	-	-	-	-	-	-
Number of infested bats		16	0	16	0	0	-	16	-	16	-	-	-	-	-	-	-	-	-	-	-	-
Prevalence (%)		84	0	89	0	0	-	89	-	89	-	-	-	-	-	-	-	-	-	-	-	-
De Kelder Cave 3	**Fynbos**																					
Number of bats		1	-	1	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Number of infested bats		0	-	0	-	-	-	0	-	0	-	-	-	-	-	-	-	-	-	-	-	-
Prevalence (%)		0	-	0	-	-	-	0	-	0	-	-	-	-	-	-	-	-	-	-	-	-
Sandile's Cave	**Forest**																					
Number of bats		4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	4	-
Number of infested bats		1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-
Prevalence (%)		25	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25	25	-

Table 1 continued

Forest Edge Knysna	**Forest**																					
Number of bats		10	2	8	1	1	-	9	1	8	-	-	-	-	-	-	-	-	-	-	-	-
Number of infested bats		1	0	1	0	0	-	1	0	1	-	-	-	-	-	-	-	-	-	-	-	-
Prevalence (%)		10	0	13	0	0	-	11	0	13	-	-	-	-	-	-	-	-	-	-	-	-
Suikerhoek Mine	**Desert**																					
Number of bats		4	-	4	-	-	-	-	-	-	4	-	4	-	-	-	-	-	-	-	-	-
Number of infested bats		0	-	0	-	-	-	-	-	-	0	-	0	-	-	-	-	-	-	-	-	-
Prevalence (%)		0	-	0	-	-	-	-	-	-	0	-	0	-	-	-	-	-	-	-	-	-
Steenkampskraal Mine	**Succulent -Karoo**																					
Number of bats		20	15	5	20	15	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Number of infested bats		16	11	5	16	11	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Prevalence (%)		80	73	100	80	73	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-