Geographic variation in the echolocation calls of the endemic Cape horseshoe bat, *Rhinolophus capensis* (Chiroptera: Rhinolophidae)

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Declaration

I, Lizelle Janine Odendaal, know the meaning of Plagiarism and declare that all of the work in the document, save for that which is properly acknowledged, is my own.

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Abstract

Several intrinsic (body size) and extrinsic (foraging ecology and communication) factors are suggested to influence call frequency divergence in high duty-cycle bats. Investigating these factors within the framework of established hypotheses would contribute to understanding evolutionary changes leading to speciation in bats. Here, acoustic divergence between populations of the endemic Cape horseshoe bat, *Rhinolophus capensis* was investigated at both inter- and intraspecifc levels. No previous study has investigated geographic variation in echolocation calls of *R. capensis*. Body size, wing morphology and skull parameters associated with diet and echolocation call production and reception, were compared between populations. Adult *R. capensis* were sampled at three sites: De Hoop situated in the centre of the species distribution in the Fynbos biome; Steenkampskraal and Table Farm were ecotone populations situated in the western and eastern limits of the distribution, respectively. Interspecific analysis revealed that the two ecotone populations deviated slightly from the allometric relationship between body size and peak frequency for the African clade. In fact, the expected inverse relationship between body size and peak frequency was not evident across populations. Ecotone populations had significantly larger mean body sizes than the population at De Hoop (10.28 ± 1.08 g; 84.60 ± 0.82 kHz). However, one population in the ecotone had the highest frequency (Table Farm: 13.88 ± 0.87 g; 85.84 ± 0.73 kHz) while the other had the lowest (Steenkampskraal: 13.15 ± 0.95 g; 80.66 ± 0.50 kHz). Several hypotheses were considered to explain the patterns of echolocation and morphological variation observed. The larger body size of the ecotone populations may be explained by James' Rule or it may be an adaptation to the intrinsic habitat heterogeneity of ecotones as it affords these bats a greater niche width and possibly larger home ranges to access spatially separated resources. On the other hand, neither climatic (humidity hypothesis), habitat (foraging habitat hypothesis) nor dietary

differences (prey detection hypothesis between populations were responsible for the observed peak frequency differences between populations. Nasal chamber area was the best predictor of peak frequency and there was no relationship between the area of the nasal chamber and body size. Thus, selection may have acted directly on peak frequency altering skull parameters directly involved in echolocation independently of body size. Within each population, females were larger and used higher frequencies than males, which implies a potential social role of peak frequency for *R. capensis*. Observed differences in peak frequency may be because *R. capensis* interacts with separate rhinolophid species at either end of its distribution (Steenkampskraal: *R. swinnyi*; Table Farm: *R. darlingi*) in addition to *R. clivosus*, which results in the evolution of local dialects to facilitate intraspecific communication. These local dialects, possibly brought about by differences in local ambient noise characteristics (e.g. chorusing insects), could be maintained via cultural transmission. However, the role of gene flow for the evolution of these local dialects between populations cannot be discounted without adequate genetic analyses.

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Chapter 1

INTRODUCTION

The extraordinary ecological diversity displayed by bats (Order: Chiroptera) is evident in the fact that they represent over 25% of all known living mammals and are found on all continents except the polar regions (Gunnell & Simmons 2005). Their success is due to numerous morphological, behavioural, physiological and sensory adaptations that allow the exploitation of a wide range of nocturnal ecological niches (Neuweiler 1984; Schnitzler & Kalko 2001). Bats feed on a variety of foods including fruit, nectar, pollen, small vertebrates, blood and insects. With the exception of most bats in the Pteropodidae, all bats use echolocation, a biological acoustic imaging system, for orientation and/or prey detection (Simmons & Stein 1980). However, pteropodids in the genus *Rousettus*, do produce brief, broadband tongue clicks which are different from the echolocation calls of all other echolocating bats that produce tonal signals in their larynx (Möhres & Kulzer 1956). Echolocating bats emit calls either nasally or orally and analyse the difference between the emitted pulse and the returning echo, to gather information about their surroundings.

Echolocation is very variable both within and between species (Thomas *et al.* 1987; Heller & Von Helversen 1989; Francis & Habersetzer 1998; Guillén *et al.* 2000; Kazial *et al.* 2001; Hiryu *et al.* 2006; Armstrong & Coles 2007; Jacobs *et al.* 2007) and echolocating bats can be divided into two broad categories. Most echolocating bats produce calls at low duty cycle (ratio of call duration to interonset interval) dominated by frequency modulated (FM) signals and they separate pulse and echo in time to avoid self-deafening. High duty cycle bats viz. those in the families Rhinolophidae (horseshoe

bats) and Hipposideridae (leaf-nosed bats) as well as the mustached bat, *Pteronotus parnellii* (Mormoopidae) (Vater *et al.* 2003; Jones & Teeling 2006); avoid self-deafening by separating pulse and echo in frequency. They are thus able to transmit and receive echoes simultaneously unlike low duty cycle bats (Fenton *et al.* 1998; Ulanovsky *et al.* 2004). High duty cycle bats have evolved a unique echolocation system which serves in both resource acquisition and intra-specific communication (Kingston *et al.* 2001; Jones & Teeling 2006). They broadcast calls of long duration with a prominent constant frequency (CF) component followed by a brief frequency modulated (FM) component with most energy contained in the second harmonic (Neuweiler 1984). Whilst flying, these bats compensate for Doppler shifts generated by its flight speed relative to that of an object of interest by lowering the frequency of the emitted pulse. This ensures that the returning echo falls within the narrow frequency range of the sharply tuned neurons of the "acoustic fovea" (Schuller & Pollak 1979). The acoustic fovea is a region of the cochlea with an over-representation of neurons sensitive to a unique frequency called the reference frequency (Schuller & Pollak 1979). The returning echo is thus very similar to the reference frequency of the acoustic fovea. In turn, the reference frequency is only 100 Hz to 300 Hz higher than the frequency these bats emit when stationary, often referred to as the 'resting frequency' (RF) (Schuller & Pollak 1979; Siemers *et al.* 2005).

High duty cycle bats are ideal for studying factors affecting geographic variation in echolocation calls because the resting frequency recorded from handheld individuals can be used. Thus, recording methods for different populations or different species of high duty cycle bats can be standardized and measurement error due to variations in flight speed and the direction of the bat relative to the microphone can be reduced. Most low

duty cycle bats cannot echolocate when stationary, and because bats may change the features of the echolocation calls according to the situations in which they fly, recording situations are often hard to standardize. Thus, this study will focus on patterns of frequency variation in high duty cycle bats, particularly rhinolophids.

Within high duty cycle bats there are several intrinsic and extrinsic factors that can influence the variability of echolocation calls. Intrinsic factors include body size, sex, age and genetic drift (Heller & Von Helverson 1989; Jones 1996; Jones & Barlow 2004; Jacobs *et al.* 2007). Extrinsic factors include aspects of the foraging ecology (habitat and diet) and other bats (Kingston *et al.* 2001; Jones & Barlow 2004; Jacobs *et al.* 2007; Russo *et al.* 2007). Extrinsic factors are likely to result in geographic variation of echolocation frequency between populations of the same species occupying different habitats. Such variation can however also be brought about through non-adaptive processes such as random genetic drift.

Body size is an intrinsic factor that has been shown to scale negatively with call frequency in at least five bat families i.e. Rhinolophidae, Hipposideridae, Emballonuridae, Vespertilionidae, and Molossidae (Jones 1996); with larger bats producing echolocation calls of lower frequencies than smaller bats. The relationship between body size and frequency of acoustic signals is also found in other taxa including anurans (McClelland *et al.* 1996; Castellano *et al.* 2002), birds (Genevois & Bretagnolle 1994), insects (Brown *et al.* 1996) and other mammals (e.g. baboons: Pfefferle & Fischer 2006; mole-rats: Credner *et al.* 1997). This relationship is also found within species e.g. in some species of bats such as *Hipposideros larvatus*: (Thabah *et al.* 2006) and *Rhinolophus philippinensis* (Kingston & Rossiter 2004).

However, many other species of insectivorous bats deviate from the general allometry between body size and call frequency. For example, although *Rhinolophus clivosus* is larger than a number of other African rhinolophids, it produces calls of higher frequencies (Jacobs *et al.* 2007). Jones *et al.* (1994) found that although females were the larger sex in *Hipposideros fulvus*, no sexual dimorphism in call frequency was apparent. Similarly, although there were no significant differences in body size, there were differences in the peak frequencies of *R. euryale* and *R. hipposideros* (Russo *et al.* 2007).

Thus there are some exceptions to the allometric relationship between body size and peak frequency, which suggests that body size does not strongly constrain RF in horseshoe bats (Guillén *et al.* 2000). This is probably due to the fact that an organisms' body size is influenced by a range of factors including the physical environment (Dietz *et al.* 2007; Bro-Jørgensen 2008) as well as ecological interactions with conspecifics and competitors (LaBarbera 1989; Kingston & Rossiter 2004).

Echolocation frequency may also vary with other intrinsic factors such as age and gender. Juvenile rhinolophids produce lower frequency calls than adults in *R. euryale*, *R. mehelyi* (Russo *et al.* 2001), *R. blasii* (Siemers *et al.* 2005) and *R. ferrumequinum* (Matsumura 1979; Jones & Ransome 1993). In growing rhinolophids, the dimensions of the vocal tract (larynx, pharynx and rostrum) are constantly changing until adult proportions are reached (Pedersen 1996). This results in the shift from multi-harmonic, noisy, low frequency calls of juveniles to higher, pure tone frequencies of adults (Matsumura 1979; Rübsamen 1987). Thus it would appear that juvenile rhinolophids "grow into" their frequencies post partum (Pedersen 1996).

Call frequency differences may also be related to gender. Many species show sexual dimorphism in call frequency but not body size (Neuweiler *et al.* 1987; Jones *et al.* 1992, 1994; Russo *et al.* 2001, 2007; Siemers *et al.* 2005). For example, females emit higher frequency calls than males in *R. rouxi* (Neuweiler *et al.* 1987), *R. hipposideros* (Jones *et al.* 1992; Russo *et al.* 2007) and *R. blasii* (Siemers *et al.* 2005). Males produce higher frequency calls in *Hipposideros speoris* but no sexual dimorphism in body size occurs (Jones *et al.* 1994).

Frequency variation in high duty cycle bats may also be influenced by genetic drift (i.e. it may be non adaptive; Jones & Barlow 2004). During isolation, populations may show acoustic divergence in frequency simply as a result of random genetic drift. These differences in frequency could be maintained even after secondary contact between previously isolated populations (Jones & Barlow 2004; Kingston *et al.* 2001). Populations which are geographically isolated from one another show greater genetic and trait variation (e.g. morphological characters) than those separated by smaller geographic distances (e.g. Maharadatunkamsi *et al.* 2000, 2003; Chen *et al.* 2006; Solick & Barclay 2006). Thus frequency differences in rhinolophid bats are more likely to develop between geographically isolated populations (Russo *et al.* 2007) and differences in call frequency may be correlated to the geographic distance between populations.

The extrinsic factors that affect echolocation include aspects of the foraging habitat and diet, as well as other bats. Echolocation frequency differences between bats occupying different habitats may be reflected in differences in wing morphology because of the proposed "adaptive complex" between wing morphology and echolocation frequency

(the foraging habitat hypothesis: Jones & Barlow 2004; Jacobs *et al.* 2007). Evidence for this would be found in a correlation between wing morphology and echolocation. Aldridge and Rautenbach (1987) proposed that low frequency calls allow for long-range detection of prey and are therefore associated with bats foraging in open areas. Higher frequency calls are more directional and provide greater resolution of targets and are thus best suited for short-range detection of prey (Neuweiler 1984). Thus high frequency, calls are ideal for foraging within clutter (where clutter is defined as the number of obstacles the bat has to detect and avoid; Fenton 1990). Intraspecific variation in wingspan and individual flexibility in echolocation calls allow *Miniopterus natalensis* to forage in both open and cluttered habitats (Jacobs 1999). Bats foraging in clutter had shorter wingspans and thus lower aspect ratios (wingspan^2/ wing area) and they used higher frequency calls than bats foraging in open spaces (Jacobs 1999). Furthermore, Kalcounis and Brigham (1995) found significant correlations between body mass and wing loading (body mass/wing area) and between individual wing loading and habitat use in *Myotis lucifugus*. Heavier bats with greater wing loadings foraged in less cluttered habitats.

Geographic variation in echolocation frequency within species may also be influenced by differences in the humidity in different localities (the humidity hypothesis). Rainfall (a measure of relative humidity) may strongly influence differences in peak frequency between populations because high frequency calls are heavily attenuated in humid conditions (Lawrence & Simmons 1982; Hartley 1989). Populations found in drier, less humid environments should have higher call frequencies than populations occurring in wetter environments. A significant inverse relationship between call frequency and mean annual rainfall was found in *Hipposideros ruber* (Guillén *et al.* 2000).

Echolocation variation may also be related to diet (prey detection hypothesis: Jones & Barlow 2004, Jacobs *et al.* 2007). Echoes from insect prey are strongest when the wavelength of the emitted call is equal to or shorter than the wing length of the target insect (Houston *et al.* 2004). Thus echo strength is greatly reduced when wavelength exceeds target circumference (Pye 1983). Higher frequencies reflect stronger echoes from smaller targets (Jones 1997). Thus, bats using low frequency calls may only be able to detect relatively large prey whereas high frequency echolocators may be able to detect a wider range of insect prey sizes which would be reflected in their diet (Kingston & Rossiter 2004; Jacobs *et al.* 2007). Jones (1997) proposed that frequency differences in the CF portion of calls in rhinolophid bats could have important implications for resource partitioning in these species. For example, three size morphs of *Rhinolophus philippinensis* echolocate at different harmonics (harmonic hopping) of the same low fundamental frequency which allows them to exploit differently sized prey, thus promoting ecological divergence (Kingston & Rossiter 2004).

Although echolocation is largely used for prey detection and orientation, it may also play a role in communication, a fact that has received increased attention from biologists (e.g. Pearl & Fenton 1996; Barlow & Jones 1997; Kingston *et al.* 2001; Russo *et al.* 2001, Andrews & Andrews 2003; Kazial & Masters 2004; Siemers *et al.* 2005; Ma *et al.* 2006; Jones 2008; Kazial *et al.* 2008). Echolocation calls may convey information about the presence and location of conspecifics, feeding areas and roosts to other "eavesdropping" bats (Fenton & Bell 1981; Barclay 1982; Leonard & Fenton 1984; Fenton 1985; Jones 2008). Communication via echolocation may also be important for species that live in year-round social groups. For example, Hiryo *et al.* (2006) found that under laboratory conditions newly introduced colony members of *Hipposideros*

terasensis either shifted their original individual frequencies up or down to match the frequency of other colony members within a short period of time (8-28 days).

The need for an effective communication channel for the recognition of conspecifics is often cited as an explanation for the occurrence of morphologically cryptic but acoustically divergent bat species as it allows for effective intraspecific communication (Heller & Von Helverson 1989; Kingston *et al.* 2001; Thabah *et al.* 2006; Russo *et al.* 2007) (acoustic communication hypothesis: Jones & Barlow 2004; Jacobs *et al.* 2007, Russo *et al.* 2007). Call frequencies in rhinolophid bats may therefore be largely influenced by the frequencies used by sympatric species (Thabah *et al.* 2006; Russo *et al.* 2007). For example, *Rhinolophus clivosus* has a higher call frequency than expected for its body size and Jacobs *et al.* (2007) propose that character displacement for effective communication may be the cause for this apparent deviation.

Acoustic signals used in communication can differ within and between different populations over both small and large geographic distances. This is evident in a variety of bird vocalizations (Zann 1993; Warren 2002; Shieh 2004), anurans (Wilczynski & Ryan 1999) and mammals (e.g. primates: Mitani *et al.* 1999, Gunnistons prairie dogs: Slobodchikoff *et al.* 1998) including bats (Barclay *et al.* 1999; Guillén *et al.* 2000; Law *et al.* 2002, Gillam & McCracken 2007). Thus presence of ecologically similar species (acoustic communication hypothesis), aspects of the species foraging habitat or diet (foraging habitat and prey detection hypothesis, respectively), as well as drift (non-adaptive hypothesis), may explain inter-population differences in call frequency. All of these factors may contribute to geographic variation in echolocation call frequency.

Geographic variation in echolocation calls may also be due to geographic variation in body size because of the inverse relationship between the two variables. Body size and the size of various morphological features (e.g. skull morphology) have been found to vary geographically in response to environmental factors such as temperature and rainfall (which are determined by latitude and altitude) for a variety of taxa (e.g. anurans: Schäuble 2004; polish water shrews: Rychlik *et al.* 2006; common shrews: White & Searle 2007; vervet monkeys: Cardini *et al.* 2007; bobcats: Wigginton & Dobson 1999) including bats (e.g. *Eptesicus fuscus*: Burnett 1983; *Rhinolophus affinis*: Maharadatunkamsi *et al.* 2000; *Rousettus aegyptius* and *Pipistrellus kuhli*: Yom-Tov & Geffin 2006). Furthermore, James (1970) argued that body size variation is related to a combination of climatic factors- smaller body size is associated with hot and humid conditions, while a large body size is associated with cool and dry conditions.

It is possible that selection could bring about variation in echolocation frequency by acting on echolocation independently of body size. If so, one would expect no correlation between body size and echolocation frequency. Instead, there should be a stronger correlation between echolocation frequency and the morphological features associated with the production and emission of calls as well as the processing of echoes from those calls (Francis & Habersetzer 1998; Armstrong & Coles 2007; Stoffberg 2007). Whether a bat emits calls directly through their open mouths (oral-emitting bats e.g. Vespertilionidae) or through the nasal passages (nasal-emitting bats e.g. Rhinolophidae and Hipposideridae), the adult skull must act as an efficient acoustical horn during echolocation (Pedersen 1998, 2000). Features of the skull directly involved in signal production and reception include the dorsal nasal chambers which has been shown to act as resonance chambers (Hartley & Suthers 1988; Suthers *et al.* 1988) and,

the cochlea, the sound-processing organ in vertebrates (cochlear anatomy reviewed in Vater 2004). A few studies have provided support for the decoupling of echolocation from body size. Nasal chamber size was the only morphological feature showing significant geographic variation in the otherwise morphologically conserved *Rhinonicteris aurantia* and these differences were linked to differences in echolocation call frequency (Armstrong & Coles 2007). Similarly, Stoffberg (2007) reported that the size of the rostrum (which houses the nasal chambers) was the best predictor of echolocation call frequency in rhinolophid bats. A significant negative correlation between call frequency and cochlear diameter for 15 rhinolophid and 10 hipposiderid species has also been reported (Francis & Habersetzer 1998).

External morphological features involved in signal emission may also reflect selection on echolocation. For example, the nostrils of horseshoe bats are surrounded by elaborate folds of skin called "noseleaves" which are thought to play a significant role in controlling signal direction and improve signal resonance (Zhuang & Müller 2007). A significant negative correlation exists between noseleaf width and peak frequency in horseshoe bats (Robinson 1996). Furthermore, noseleaf width was the external morphological feature that best predicted echolocation call frequency in horseshoe bats (Stoffberg 2007).

Bats belonging to the family Rhinolophidae are ideal for testing the influence of both intrinsic (e.g. body size, sex) and extrinsic (e.g. foraging ecology, communication) factors in maintaining variability in echolocation calls because some species have wide geographic ranges and the use of hand held bats allow the collection of echolocation data in a standardized way (Houston *et al.* 2004). Call parameters are also easier to

measure accurately from calls dominated by a pure CF component than from bats using FM calls because the power spectra of the latter often fail to show clear peaks which are easy to measure (Jones 1995).

The Cape horseshoe bat, *Rhinolophus capensis*, is a medium sized rhinolophid (forearm length (FA): 46-51.8 mm, Jacobs *et al.* 2007) endemic to the Cape Floristic Region in the south western cape of South Africa. They are found in a variety of habitats along the coast of the Western and Eastern Cape Provinces and some populations experience distinctly different habitat and climatic conditions. For example, populations in the southern and eastern regions of the species distribution experience more rainfall and cooler conditions than populations in the west. Populations in the west also experience more open habitats than other areas of the species distribution. No previous study has investigated geographic variation in the echolocation calls of this *R. capensis*. In this study I investigated whether there is in fact geographic variation in echolocation call frequencies between populations of *R. capensis* as has been reported for other horseshoe bats (e.g. *R. pumilus*: Yoshino *et al.* 2006; *R. hipposideros*, *R. euryale* and *R. mehelyi*: Russo *et al.* 2007; *R. clivosus*: Jacobs *et al.* 2007), and, if so, which factors contribute to these differences.

If differences between populations of *R. capensis* result from genetic drift (non-adaptive hypothesis: Jones & Barlow 2007), echolocation parameters between the populations should vary consistently with body size (Armstrong & Coles 2007) i.e. the negative correlation between body size and echolocation frequency should be maintained in each population despite inter-population differences in echolocation frequency. Thus, the population with the higher average call frequency should have the smaller average body

size. Furthermore, the variation between populations should also be more pronounced than the variation within populations if interpopulation differences are due to drift.

On the other hand, if variation in echolocation frequency between populations of *R. capensis* is due to differences in foraging habitat (foraging habitat hypothesis), populations of *R. capensis* occupying drier and therefore potentially more open habitats, should use lower frequency calls than bats foraging within clutter. Similarly, if differences in humidity between populations drives frequency variation (humidity hypothesis), populations found in drier regions (western parts of the species distribution) should have a higher frequency than populations found in wetter regions (eastern and southern regions). Lastly, if peak frequency co-varies with body size in each population, climatic or habitat differences between populations are the likely explanation for peak frequency differences as a consequence of their influence on body size. Populations experiencing more open habitats should have a larger body size and corresponding lower frequency than populations experiencing cluttered habitats.

However, if selection is acting on echolocation frequency independently of body size (e.g. for improved communication; Kingston *et al.* 2001, Jones & Barlow 2004, Jacobs *et al.* 2007, Russo *et al.* 2007), call frequency should not be correlated with body size but strongly correlated with morphological features directly involved in call production (rostrum and nasal chamber), emission (nose leaves) and reception (cochlea).

A difference in peak frequency between populations of *R. capensis* is unlikely to be due to resource (prey detection hypothesis) or sonar partitioning. This is because *R. capensis* overlaps with only one other rhinolophid species, *R. clivosus* (Taylor 2000) across its

entire range. *Rhinolophus clivosus* echolocates at a higher frequency than *R. capensis* (CF= 90-100 kHz and CF= 85-90 kHz respectively, Taylor 2000). Similarly, differences in wavelengths at the high frequencies used by these species are not large enough to result in differences in detectable prey size (Jacobs *et al.* 2007). Indeed, Jacobs *et al.* (2007) found complete overlap in the range of insect prey taken by the two species as well as no difference in their flight patterns and habitat use in sympatry. Since differences in call frequency between *R. capensis* populations (same species) is unlikely to be larger than the difference between *R. capensis* and *R. clivosus*, differences in echolocation frequency between populations of *R. capensis* is unlikely to be related to differences in prey size. If so, different populations of *R. capensis* should take similar sized prey and this should be reflected in similarity of skull components associated with processing prey.

The aims of this study are thus to investigate geographic variation in the echolocation calls of populations of *R. capensis* and to evaluate the effect of body size, foraging habitat, communication and morphological correlates on call frequency variation in *R. capensis* .

Chapter 2

MATERIALS & METHODS

Study sites

I sampled adult *R. capensis* individuals from three populations across the species' distribution range during spring and summer 2007 to 2008: 1) De Hoop Nature Reserve (34°26′ S, 20°25′ E) near Bredasdorp in the Western Cape Province; 2) Table Farm (33°17′ S, 26°25′ E) near Grahamstown in the Eastern Cape Province; and 3) Steenkampskraal (30°58′ S, 18°37′ E) outside Vanrhynsdorp situated in the Namaqualand region of the Western Cape Province of South Africa (Figure 2.1).

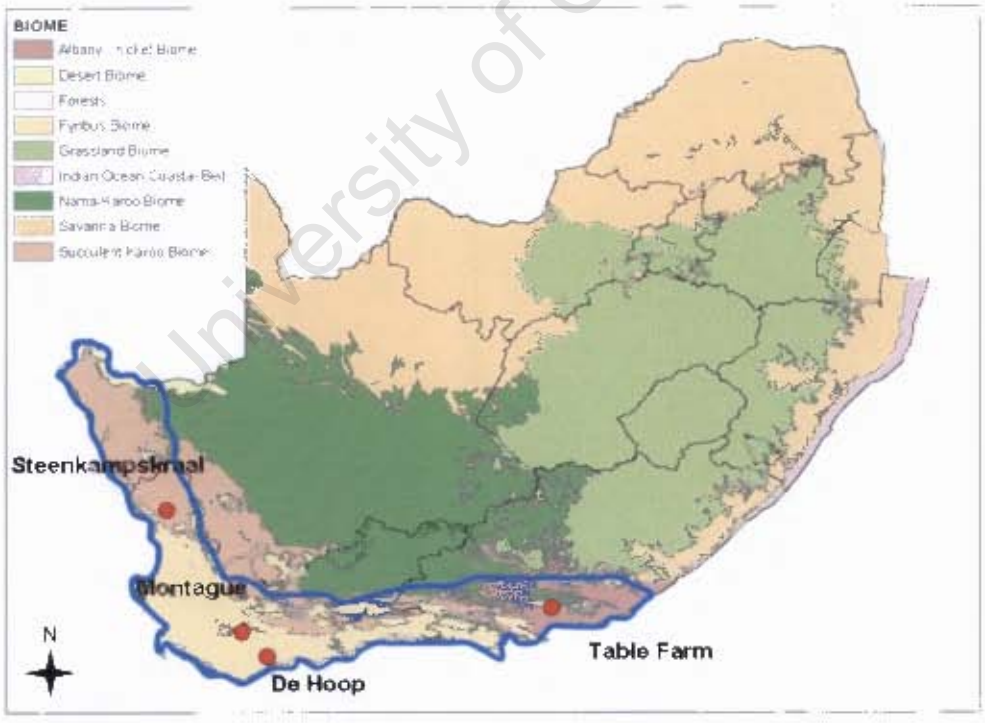

Figure 2.1: Map showing the species distribution of *R. capensis* (adapted from Taylor 2000) together with the different biomes of South Africa and the position of the three populations of sampled in this study. Museum specimens from Montague were obtained from the Iziko Museum. Biome map is derived from Mucina & Rutherford 2006)

De Hoop Nature Reserve is situated in the winter rainfall region of the Western Cape Province of South Africa. There are two main roosting colonies of *R. capensis* within De Hoop Nature Reserve: De Hoop Guano Cave and Hot Hole. The former is situated in a limestone cliff at the northern part of the land-locked De Hoop Vlei (shallow body of water that is periodically dry), while the latter is a sink hole about 10 km from the Guano Cave (Jacobs *et al.* 2007). The dominant vegetation surrounding both colonies is coastal fynbos, characterised by evergreen, sclerophyllous shrubs, and dominated by restios. *Rhinolophus capensis* shares both these roosts with other bat species: *Myotis tricolor*, *Miniopterus natalensis*, *Rhinolophus clivosus* and *Nycteris thebaica*, but the latter only roosts in the Guano Cave (Jacobs *et al.* 2007).

The Eastern Cape Province is unique in that six of the seven biomes in South Africa (Fynbos, Savanna, Nama-Karoo, Grassland, Thicket and Forest; Figure 2.1) extend into this region from adjacent areas, yet none of the biomes are actually confined to the Eastern Cape (Lubke *et al.* 1986). Thus, the Eastern Cape is recognized as the boundary between many major vegetation types, and therefore, spatial and temporal heterogeneity in the vegetation of the region is extensive (Palmer 1990). The greatest diversity of biomes and their associated vegetation types is found within a 150 km radius of Grahamstown (Lubke *et al.* 1986). Table Farm is situated on the ecotone (area of environmental transition: Kark *et al.* 2002) between Thicket in the east and Nama-Karoo in the west (Dold 2003). This region receives rain throughout the year but most rainfall occurs from spring to summer (Stone *et al.* 1998). On Table Farm, *R. capensis* roosts in an abandoned tunnel excavated for a water pipeline. *Rhinolophus capensis* shares this roost with *M. natalensis* which is the more abundant species.

Steenkampskraal farm is situated in the Succulent Karoo biome in the western part of South Africa (Figure 2.1), often referred to as Namaqualand. Although within a winter rainfall region, this area is arid and is characterised by < 150 mm of rain per annum (Cowling *et al.* 1999). The major vegetation type of Namaqualand is Lowland Succulent Karoo characterised by sparse, dwarf (*ca* 30 cm in height) succulents (Cowling *et al.* 1999). Near Vanrhynsdorp at least three major vegetation types occur, viz. Lowland Succulent Karoo, Strandveld Succulent Karoo and Sandplain Fynbos. Thus Steenkampskraal is also found within an ecotone between these vegetation types. At this study site, bats roost in an abandoned monazite mine. Here, as with the other two sites, *R. capensis* shares the roost with the more abundant *M. natalensis*.

Sampling protocols

Bats were captured using mist nets and/ or a harp trap (Austbat Harp Trap, Faunatech; Mount Taylor, Victoria, Australia) placed near the entrance of the roosts. Mist nets were checked regularly throughout trapping periods to ensure that bats were not injured by being in the net for too long. Hand-nets were also used to capture roosting bats during the day. Our capture, handling and voucher collection methods complied with the guidelines recommended by the American Society of Mammologists (Gannon *et al.* 2007) and were approved by the Science Faculty Animal Ethics Committee (approval number: 2008/V18/LO) at the University of Cape Town.

Morphology

The age (adult or juvenile) and sex of each bat was recorded. Juveniles were distinguished from adults by the presence of cartilaginous epiphyseal plates in their

finger bones which can be detected by transilluminating the bats wings (Anthony 1988). Only adults were used in subsequent analyses. For each bat I measured body mass (to the nearest 0.01 g) using a portable electronic balance (Ohaus Corporation, Pine Brook, New Jersey, USA), and forearm length and noseleaf width (to the nearest 0.01 mm) using dial callipers. To control for seasonal variation in body mass and echolocation call frequency, all sampling took place during the months of spring and summer from 2007 to 2008. Body mass may be influenced by various factors such as reproductive status or whether the bat had been foraging (Stoffberg 2007). In an attempt to eliminate variation in body mass due to foraging, bats were only measured after ensuring their gut was emptied (e.g. keeping them overnight in a soft cotton bag). To control for changes in body mass that may be due to pregnancy, only non-pregnant females were used. The reproductive condition of females was determined following Solick and Barclay (2006). However, sampling in 2008 occurred outside the reproductive period of *R. capensis* (Stoffberg 2008)

A photograph of each bat, right wing and tail extended, was taken with a Canon Powershot S60 PC1088 digital camera (Canon Inc, Japan) positioned directly 90° above the wing and parallel to the flat table top on which the wing was extended, to prevent angular distortion (Jacobs *et al.* 2007). The wing was extended (after Saunders & Barclay 1992) on graph paper to provide a reference for the calibration of SigmaScan Pro 5 software (version 3.20, SPSS Inc.), used to calculate various wing measurements. From the photographs, I measured wing parameters such as wingspan, wing area, and calculated wing indices including wing loading (weight divided by wing area) and aspect ratio (wingspan squared divided by wing area) following Norberg (1981) and Norberg & Rayner (1987).

Echolocation

Echolocation calls were recorded from hand held bats positioned 10 cm in front of an Avisoft Ultrasound Gate 416 (Avisoft Bioacoustics, Berlin, Germany) microphone. To measure the resting peak frequency (where the constant-frequency (CF) component is stable and inter-pulse variation is low; Armstrong & Coles 2007), hand-held recordings were used because they eliminate differences in peak frequency that may be due to Doppler shift compensation during flight. Initial calls emitted by rhinolophids may have lower frequency values before reaching the final RF (Siemers *et al.* 2005). Thus bats were sufficiently warmed up prior to echolocation recordings, and only calls emitted after ten seconds were used in analyses.

Echolocation calls were recorded directly onto a HP Compaq nx7010 notebook computer with Avisoft SasLab Pro software. Recordings were slowed down by ten and were analyzed using BatSound Pro software (Version 3.20, Pettersson Elektronik AB, Uppsala, Sweden). In BatSound Pro I measured the following parameters: 1) Peak frequency (kHz) – the frequency of maximum intensity determined from the Fast Fourier Transformation (FFT) power spectrum (size 1024); 2) Duration (ms) – the time from beginning to end of call, determined from the oscillogram; 3) Minimum frequency of the frequency modulated tail (kHz) - determined from the spectrogram; and 4) Inter-pulse interval (ms) – time between adjacent calls. Bandwidth (kHz) was calculated by subtracting the minimum frequency from the peak frequency. Due to the random occurrence of background noise, a Hanning window was used.

The mean values of the above-mention echolocation parameters, were calculated from ten, randomly selected, high-quality calls (calls with a high signal to noise ratio) for

each bat. The amplitude of the signal was at least three times higher than that of the background noise as displayed on the oscillogram. Because using the means from each parameter results in an echolocation call that is "constructed", and therefore not "real", the parameters of an original call that most closely resembled the calculated mean parameters, were chosen for all subsequent analyses.

Skull morphology

Skulls of *R. capensis* (n=22) were obtained from the Northern Flagship Institute and Iziko Museum in South Africa (list of museum codes provided in Appendix 1). These included skulls from an additional population; Montague, (33°47′ S, 20°7′ E). In addition I collected voucher specimens of ten bats (five males and five females) from both Table Farm and Steenkampskraal, after they were measured and their echolocation calls recorded. Skulls were also obtained from four bats (voucher specimens previously collected from De Hoop) and echolocation data for three of these were also available. Thus I had post-cranial morphological measurements and echolocation calls for 23 out of the 46 skulls.

Due to the small size of the *R. capensis* skulls (average skull length 20.62 mm), certain skull parameters were more difficult to measure than others, thus increasing the probability of measurement error. To achieve greater accuracy in the measurement of skull parameters associated with emission and reception of echolocation, x-ray radiographs were taken of the same skulls from above (transverse view) and from the side (sagittal view) (Figure 2.2) following Armstrong and Coles (2007). Radiographs were taken with a custom-made Mamex prototype high-resolution scanner at 28

kilovolts and 100 milliamps. A scale was included with the radiographs for later calibration of measurements using Sigma Scan Pro 5 software.

The following skull parameters were measured from the radiographs (Figure 2.2) following Armstrong and Coles (2007):

Transverse view: CW - cochlear width: greatest width across the cochlea; CA – cochlear area; NW - nasal width: measured between outer crowns of M^3; TA - transverse area of the nasal capsule.

Sagittal view: X-Y – nasal capsule length; Z-W - nasal capsule height; SA – sagittal area of nasal capsule.

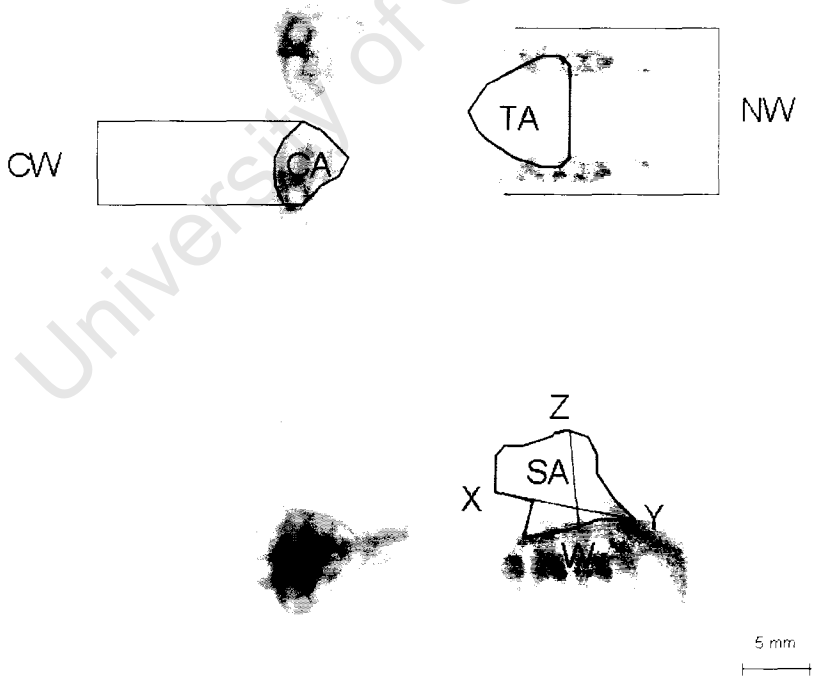

Figure 2.2: Skull radiographs of a female *R. capensis* specimen showing the skull parameters measured from the transverse and sagittal views (See text above for explanation of abbreviations).

Skull parameters associated with diet (Figure 2.3) were also measured following Jacobs (1996). The following skull parameters were measured, to the nearest 0.01 mm, using digital callipers, and under Leica dissecting microscope (x 12):

Lateral view: *a* – distance from the anterior surface of the mandibular fossa to the origin of the masseter muscle (indicated by the fusion line of the zygomatic arch with the maxilla); *b* – distance from the top of the left condyle to the bottom of the left angular process; *c* – length of the masseter muscle scar; *d* – condyle height: top of the left condyle to the plane of the alveoli of the left first and second molar; *e* – height of the coronoid process: top of the left coronoid process to the plane of the alveoli of the left first and second molar; *f* – anterior skull length: maximum length from the most posterior point of the occipital to where the palatal meets the premaxilla; *g* – dentary length: from the back of the left condyle to the epiphysis of the dentary; *h* – maxillary tooth row: from the front of left premolar to the back of left third molar; *i* – dentary thickness: from the plane of the alveoli of the left first and second molars to the bottom of the left dentary.

For all SigmaScan Pro measurements from the radiographs and digital calliper measurements, the mean value of three measurements for each skull parameter was calculated and used in subsequent analyses. Measurement error was determined by taking repeated measures (n=10) of those characters which were difficult to measure. If the mean difference between populations in any of the above measured parameters was less than the calculated measurement error, the parameter was excluded from analyses.

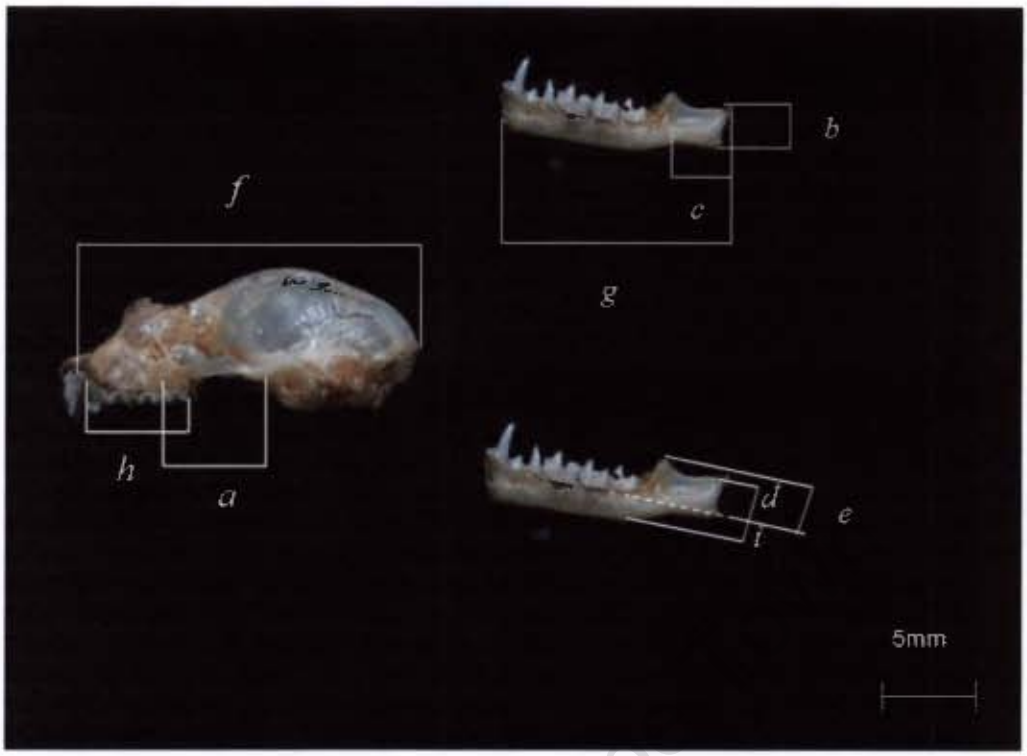

Figure 2.3: Lateral view of a skull and mandible of an adult female *Rhinolophus capensis* showing measurements taken (See materials and methods for explanations of abbreviations)

Statistical analyses

Principle Components Analysis (PCA) was used to determine the degree of variation between the three populations with respect to body mass, noseleaf width, wing morphology, echolocation call parameters and skull morphology. PCA defines new, uncorrelated variables called principle components (PC's) which are weighted linear combinations of the original variables. Thus, PCA gives an indication of the relative importance of variables responsible for the separation of populations in multivariate space.

Many studies have found significant variations in body size between populations of rhinolophid and hipposiderid bats (Jones *et al.* 1994; Francis & Habersetzer 1998; Guillén *et al.* 2000; Thabah *et al.* 2006; Yoshino *et al.* 2006; Russo *et al.* 2007). However, these studies often used forearm length as an indicator of body size instead of body mass as the latter may be influenced diurnally by a wide range of factors (e.g. reproductive state, whether the animal has just fed or not prior to capture) and therefore may be an unreliable measurement for comparative purposes. In so doing, intraspecific variation in body mass for rhinolophid bats has largely been ignored. However, in this study, regression analysis revealed that body mass and forearm length were very strongly correlated in *R. capensis* ($r^2 = 0.3462$, $F_{(1, 102)} = 54.01$, $P < 0.00001$) and variation in body mass between populations due to differences in sampling method was controlled for as much as possible (see above). Thus, body mass instead of forearm length was used in all analyses. Furthermore, because there were no significant differences in wing morphology, body size or peak frequency between bats caught at Hot Hole and the Guano Cave at De Hoop Nature Reserve (Tukey P's > 0.05); there were no colony effects on these variables. Thus, data for the two colonies were pooled and used in all analyses as representing the entire De Hoop population.

I used Analysis of Variance (ANOVA) to determine whether peak frequency differed between populations of *R. capensis*. Multivariate Analysis of Variance (MANOVA) and post-hoc Tukey tests were used to determine inter-population and sex-linked differences in those morphological (body size, noseleaf width, wing morphology, skull morphology) and echolocation call (peak frequency and minimum frequency) variables which the PCA identified as contributing most to differences between populations. 'Sex' and 'population' were used as categorical predictors. A Kolmogorov-Smirnov test for

normality and Levene's test for homogeneity of variances were used to ensure that data met the assumptions of PCA and ANOVA/MANOVA (Zar 1999). Means were regressed against standard deviations to ensure that the additional assumption of uncorrelated means and variances, required for MANOVA, was met.

To determine among species differences in the relationship between body size and peak frequency for South African rhinolophids belonging to the African clade (Stoffberg 2007), body mass was regressed against peak frequency for eleven rhinolophid species for which data were available. The mean peak frequencies of each *R. capensis* population was included in the analyses to determine whether different populations diverged from the allometry between body size and peak frequency. Data for African rhinolophids were obtained from Jacobs *et al.* (2007). To ensure that variances were equal and data were normally distributed, mean peak frequency and mean body mass were log transformed. Peak frequency was regressed against body mass, noseleaf width and wing loading for all individuals from each of the three populations.

Forward stepwise multiple regressions were used to determine which skull parameter measured from radiographs of the 23 voucher specimens for whom I had echolocation data, is the best predictor of peak frequency. Peak frequency was the dependent variable and those skull parameters which PCA revealed as contributing most to differences between populations were independent variables. All variables were log transformed to ensure linearity. To test the normality of the residuals of all regressions, residuals were plotted against their normal scores. I used Statistica (version 8.0, StatSoft Inc., Tulsa, OK, USA) for all analyses.

Chapter 3

RESULTS

Means and standard deviations of wing parameters, echolocation call parameters and body mass are provided in Table 3.1. I randomly chose equal numbers of males and females to control for the effects of potential differences between males and females (see below). Furthermore, the data used in all analyses did not depart from normality. Spectrograms of typical echolocation calls of *R. capensis* from each population is shown in Figure 3.1

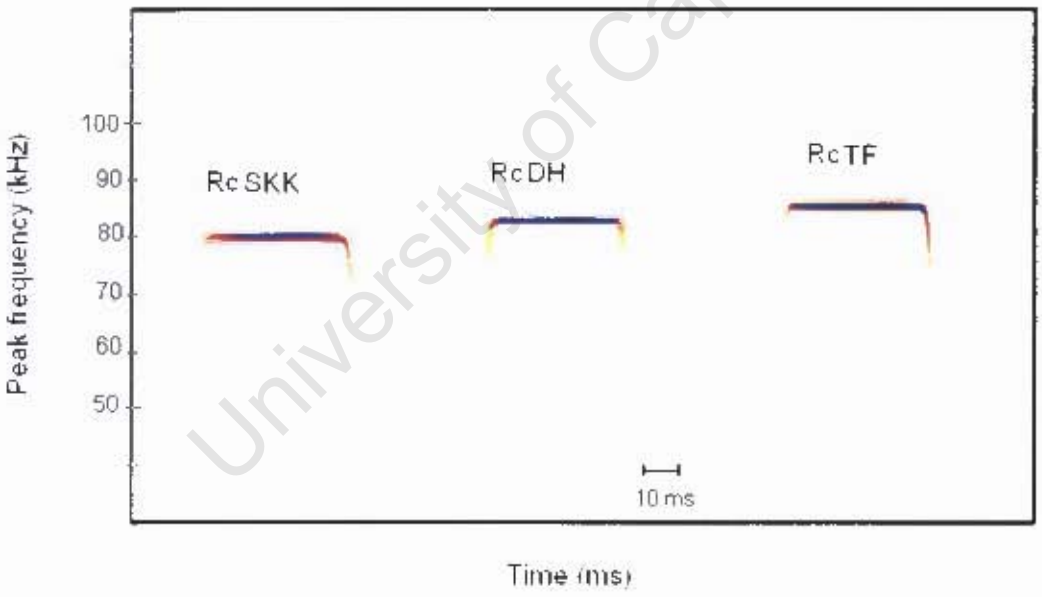

Figure 3.1: Spectrograms of typical echolocation calls of *R. capensis* from Steenkampskraal (RcSKK), De Hoop Nature Reserve (RcDH) and Table Farm (RcTF). FFT size = 1024 samples.

Among species comparison and divergence of R. capensis populations

There was an inverse correlation between body mass and peak frequency ($r^2 = 0.592$, $F_{(1, 10)} = 14.51$, $P < 0.05$; Figure 3.2) for the African clade of the rhinolophids (as per Stoffberg 2007). Furthermore, the peak frequency of *R. capensis* for all three populations sampled in my study fell within the 95% confidence limits of the relationship for the African clade (Figure 3.2). Most of the variance in echolocation frequencies is thus a consequence of differences in body size. The mean peak frequency of bats from De Hoop is consistent with the mean body mass of this population. However, Table Farm and Steenkampskraal bats show some deviation from the relationship (displaced from the line of allometry; Figure 3.2) for the African clade, suggesting that not all the variation in peak frequency is explained by body mass for these two populations of *R. capensis* (Figure 3.2).

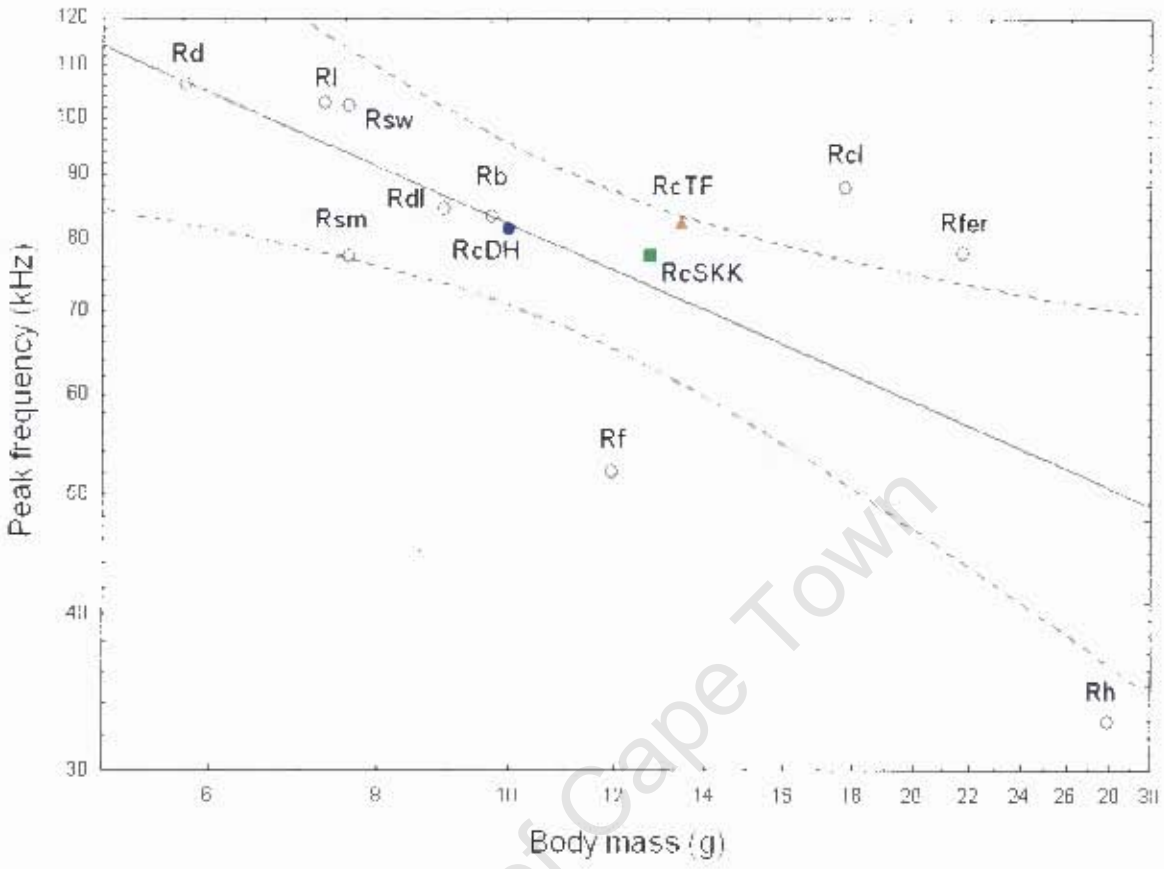

Figure 3.2: The regression of log mass (g) and log peak frequency (kHz) for rhinolophids belonging to the African clade together with three populations of *R. capensis*: Steenkampskraal ((RcSKK (■)); Table Farm ((RcTF (▲) and De Hoop ((RcDH (●)); *R. denti* (Rd); *R. landeri* (Rl); *R. swinnyi* (Rsw); *R. darlingi* (Rdl); *R. ferrumequinum* (Rfer); *R. blasii* (Rb); *R. simulator* (Rsm); *R. clivosus* (Rcl); *R. fumigatus* (Rf) and *R. hilderbrandti* (Rh). Data for South African rhinolophids were obtained from Jacobs *et al.* (2007). Dashed lines represent the 95% confidence limits. Solid line represents the best fit where Log Peak Frequency = 2.4122 – 0.4806*Log Mass.

ble 3.1: Morphological and echolocation call parameters (Mean ± SD) for three *R. capensis* populations sampled in this study. Ranges provided in entheses.

ariable	De Hoop			Table Farm			Steenkampskraal		
	Male (n=23)	Female (n=23)	All individuals (n=46)	Male (n= 18)	Female (n=18)	All individuals (n=36)	Male (n=11)	Female (n=11)	All individuals (n=22)
mass (g)	9.71 ± 0.60 (8.6-10.5)	10.85 ± 1.16 (9.0-12.9)	10.28 ± 1.08 (8.6-12.9)	13.58 ± 0.73 (12.5-14.7)	14.18 ± 0.91 (12.6-15.8)	13.88 ± 0.87 (12.5-15.8)	12.60 ± 0.72 (11.2-13.8)	13.69 ± 0.86 (12.5-15.1)	13.15 ± 0.95 (11.26-15.1)
rm length (mm)	48.33 ± 0.84 (47.1-50.6)	49.29 ± 0.86 (47.9-50.9)	48.84 ± 0.97 (47.1-50.9)	49.56 ± 0.91 (47.6-50.8)	50.67 ± 1.14 (48.6-52.7)	50.11 ± 1.16 (47.6-52.7)	50.75 ± 1.10 (48.5-53.0)	51.45 ± 0.99 (50.0-53.2)	51.45 ± 0.99 (48.5-53.2)
span (cm)	30.60 ± 0.89 (29.1-32.7)	30.42 ± 1.23 (28.0-32.4)	30.52 ± 1.07 (28.0-32.7)	29.06 ± 0.95 (27.5-30.3)	30.64 ± 1.26 (28.8-34.4)	29.85 ± 1.36 (27.5-34.1)	29.63 ± 0.79 (28.5-31.1)	29.95 ± 0.81 (28.3-30.7)	29.79 ± 0.805 (28.3-31.1)
area (cm^2)	154.11 ± 9.62 (137.9-181.2)	156.41 ± 11.33 (134.9-175.6)	155.26 ± 10.46 (134.9-181.2)	153.93 ± 9.67 (137.5-170.6)	167.15 ± 14.56 (142.6-212.5)	160.54 ± 13.90 (137.5-212.5)	162.37 ± 8.41 (149.8-172.7)	161.45 ± 6.58 (147.8-169.3)	161.91 ± 7.38 (147.8-172.7)
ading (Nm^2)	6.32 ± 0.51 (5.6-7.2)	6.97 ± 0.89 (5.7-8.7)	6.64 ± 0.79 (5.6-8.7)	8.85 ± 0.61 (7.9-9.9)	8.52 ± 0.70 (6.7-9.4)	8.68 ± 0.67 (6.7-9.5)	7.78 ± 0.60 (7.0-9.0)	8.49 ± 0.64 (7.4-9.6)	8.14 ± 0.71 (6.9-9.6)
ect ratio	6.08 ± 0.19 (5.8-6.4)	5.92 ± 0.22 (5.5-6.2)	6.00 ± 0.21 (5.5-6.4)	5.49 ± 0.22 (5.0-5.8)	5.63 ± 0.20 (5.2-5.9)	5.56 ± 0.22 (5.0-5.9)	5.41 ± 0.16 (5.1-5.7)	5.56 ± 0.22 (5.1-6.0)	5.49 ± 0.20 (5.1-6.0)
leaf width (mm)	8.05 ± 0.38 (7.5-9.1)	7.87 ± 0.35 (7.1-8.5)	7.96 ± 0.37 (7.1-9.1)	8.50 ± 0.16 (8.1-8.8)	8.37 ± 0.23 (8.0-9.0)	8.44 ± 0.21 (8.0-8.9)	8.22 ± 0.16 (7.9-8.4)	8.22 ± 0.28 (7.8-8.7)	8.22 ± 0.23 (7.8-8.7)
frequency (kHz)	84.11 ± 0.74 (82.7-85.4)	85.08 ± 0.59 (84.1-86.3)	84.60 ± 0.82 (82.7-86.3)	85.44 ± 0.65 (83.7-86.3)	86.26 ± 0.54 (85.5-87.1)	85.84 ± 0.73 (83.7-87.1)	80.47 ± 0.57 (79.3-81.4)	80.85 ± 0.35 (80.4-81.4)	80.66 ± 0.50 (79.3-81.4)
tion (ms)	40.56 ± 7.85 (29.0-58.0)	42.39 ± 6.23 (32.0-55.0)	41.48 ± 7.07 (29.0-58.0)	43.05 ± 5.53 (33.0-57.0)	44.33 ± 6.20 (34.0-54.0)	43.69 ± 5.83 (33.0-57.0)	45.54 ± 6.12 (38.0-58.0)	45.81 ± 7.73 (35.0-56.0)	45.68 ± 6.80 (35.0-58.0)
t frequency omponent (kHz)	72.35 ± 2.27 (67.9-76.7)	72.19 ± 2.76 (67.0-76.7)	72.27 ± 2.50 (67.0-76.7)	74.59 ± 2.60 (70.4-76.8)	74.59 ± 2.62 (70.7-81.10)	73.97 ± 2.28 (70.4-81.1)	70.18 ± 2.31 (66.0-74.0)	69.45 ± 1.91 (66.0-73.0)	69.45 ± 2.10 (66.0-74.0)
width (kHz)	11.76 ± 2.52 (7.1-15.7)	12.89 ± 2.73 (9.1-17.6)	12.32 ± 2.66 (7.1-17.6)	12.06 ± 1.97 (8.7-15.9)	11.67 ± 2.69 (4.7-15.1)	11.87 ± 2.33 (4.7-15.9)	10.29 ± 2.03 (7.4-14.4)	11.40 ± 1.74 (8.4-14.4)	10.84 ± 1.93 (7.4-14.4)

Patterns of interpopulation variation in body mass, wing morphology and echolocation

Principle Components Analysis (PCA) on body mass, wingspan, wing area, peak frequency and lowest frequency (Table 3.1) revealed substantial differences between the three populations of *R. capensis*. The first three principle components (eigenvalues > 1) extracted from the analysis accounted for 87.22% of the variation between populations (Table 3.2). Principle component 1 (PC 1) accounted for 33.86 % of the total variation and was associated with wingspan and wing area (Table 3.2) which suggests that wing loading and aspect ratio may differ between populations. Wingspan and wing area loaded high on PC 1 while echolocation call parameters and body mass loaded low (Table 3.2). Bats on the negative end of PC 1 had relatively larger wingspans and greater wing areas than bats on the positive end, but there was no clear separation between populations (Table 3.1; Figure 3.3 A).

A clear separation between populations was evident along PC 2 and PC 3 which accounted for 30.21 % and 23.14 % of the total variation, respectively (Table 3.2; Figure 3.3 B). PC 2 was associated with peak frequency and the lowest frequency of the frequency modulated (FM) component of the echolocation call, while body mass was responsible for the separation of the De Hoop population from the other two populations along PC 3.

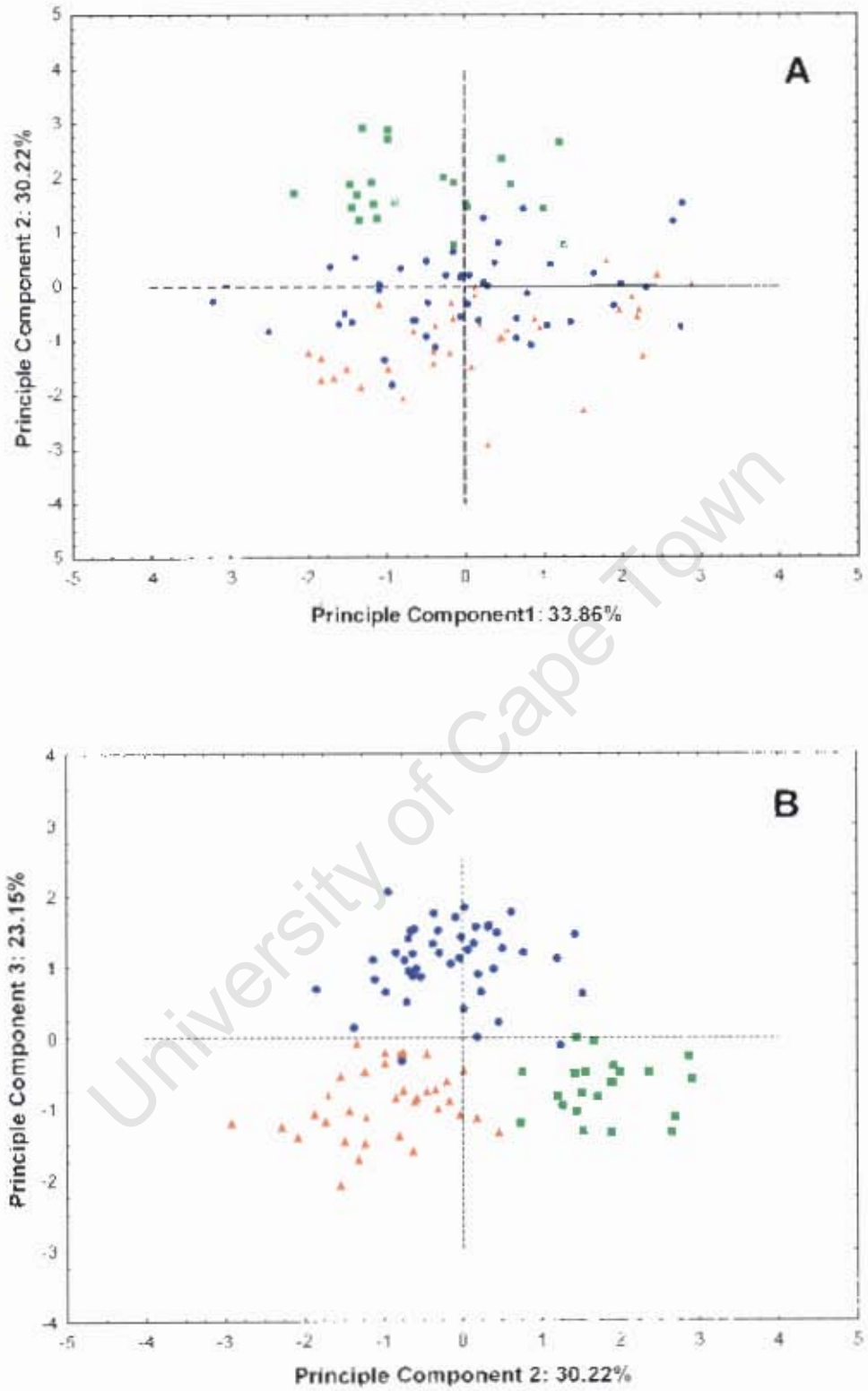

Figure 3.3: Plot of component scores for *R. capensis* at Steenkampskraal (■). Table Farm (▲) and De Hoop (●)

Table 3.2: Factor loadings, eigenvalues and percent variation obtained from PCA analysis of body size, wing morphology and echolocation call parameters.

Variables	PC 1	PC 2	PC 3
Wingspan	-0.858	-0.209	-0.393
Wing area	-0.937	-0.098	-0.210
Body mass	-0.134	-0.067	-0.970
Peak frequency	0.211	-0.846	0.097
Lowest frequency	0.120	-0.858	-0.092
Eigenvalue	1.693	1.510	1.157
% Total variation explained	33.86	30.21	23.14
% Cumulative variation	33.86	64.08	87.22

There were significant differences between the three populations (MANOVA, $F_{(10, 188)} = 146.8$, $P < 0.005$) in peak frequency, lowest frequency of the FM component of the echolocation call, body mass and wingspan. Bats from Table Farm had higher peak frequencies than both De Hoop and Steenkampskraal (Tukey HSD test, $P's < 0.005$; Table 3.1) with the latter population echolocating at the lowest frequencies (Tukey HSD test, $P < 0.005$; Table 3.1; Figure 3.1). The significant difference in the lowest frequency of the FM component of the echolocation call between populations (Tukey HSD test, $P < 0.005$) was a consequence of differences in peak frequency because there was no difference in bandwidth between populations (ANOVA, $F_{(6,198)} = 36.47$, $P < 0.005$; Tukey HSD test, $P > 0.05$; Table 3.1.) The echolocation frequencies of bats from De Hoop are more dispersed along PC 2 (Figure 3.1B). Additionally, Table Farm bats were significantly heavier than

both Steenkampskraal and De Hoop, with bats from the latter population having the lowest average body mass (Tukey HSD test, $P's < 0.005$; Table 3.1.). De Hoop individuals had significantly shorter wingspans than both Table Farm and Steenkampskraal bats (Tukey HSD test, $P < 0.005$; Table 3.1). Furthermore, bats from De Hoop also had significantly smaller wing areas than Steenkampskraal individuals (Tukey HSD test, $P < 0.005$; Table 3.1.). There was no significant difference in wingspan or wing area between Table Farm and Steenkampskraal populations (Tukey HSD test, $P's > 0.05$; Table 3.1). However, wing loading and aspect ratio differed significantly between all three populations (ANOVA, $F_{(6,198)} = 36.47$, $P < 0.005$; Tukey HSD test, $P's < 0.005$; Table 3.1.)

Sexual differences in body size, wing morphology and echolocation

There were also significant differences in peak frequency between sexes (MANOVA, $F_{(5, 94)} = 16.6$, $P < 0.005$). Females echolocated at significantly higher frequencies than males at De Hoop and Table Farm (Tukey HSD test, P's < 0.001; Table 3.1) but not at Steenkampskraal (Tukey HSD test, $P > 0.05$; Table 3.1). Body mass also differed significantly between sexes at De Hoop and Steenkampskraal where females were heavier than males (Tukey HSD test, P's < 0.001; Table 3.1). Although females were slightly larger than males at Table Farm, the difference in body mass was not significant (Tukey HSD test, $P > 0.05$; Table 3.1). The interactive term population*sex was significant (MANOVA, $F_{(10, 188)} = 3.1$, $P < 0.005$) suggesting that the magnitude of difference in peak frequency and body mass between sexes are significantly different between populations.

Influence of climate on body size and echolocation (humidity hypothesis)

Mean peak frequency and body mass of each population was plotted against mean annual rainfall, altitude and mean minimum temperature to explore the influence of these environmental variables on body mass and peak frequency (Figure 3.4). Burnett (1983) found that minimum temperatures explained geographic variation in body size of *Eptesicus fuscus* more so than maximum temperatures and thus proposed that cold stress may be more important than heat stress for body size adjustments in bats.

Although Steenkampskraal receives considerably less rainfall per annum than both De Hoop and Table Farm, bats from Steenkampskraal echolocate at significantly lower frequencies than bats from the other populations (Table 3.1; Figure 3.4). There was a striking relationship between body size and altitude. The *R. capensis* population at De Hoop, which is situated close to sea level, has the smallest average body mass, whereas the two populations at higher altitudes (Steenkampskraal and Table Farm) are larger (Table 3.1; Figure 3.4). Furthermore, Table Farm and Steenkampskraal also experience lower minimum temperatures than De Hoop.

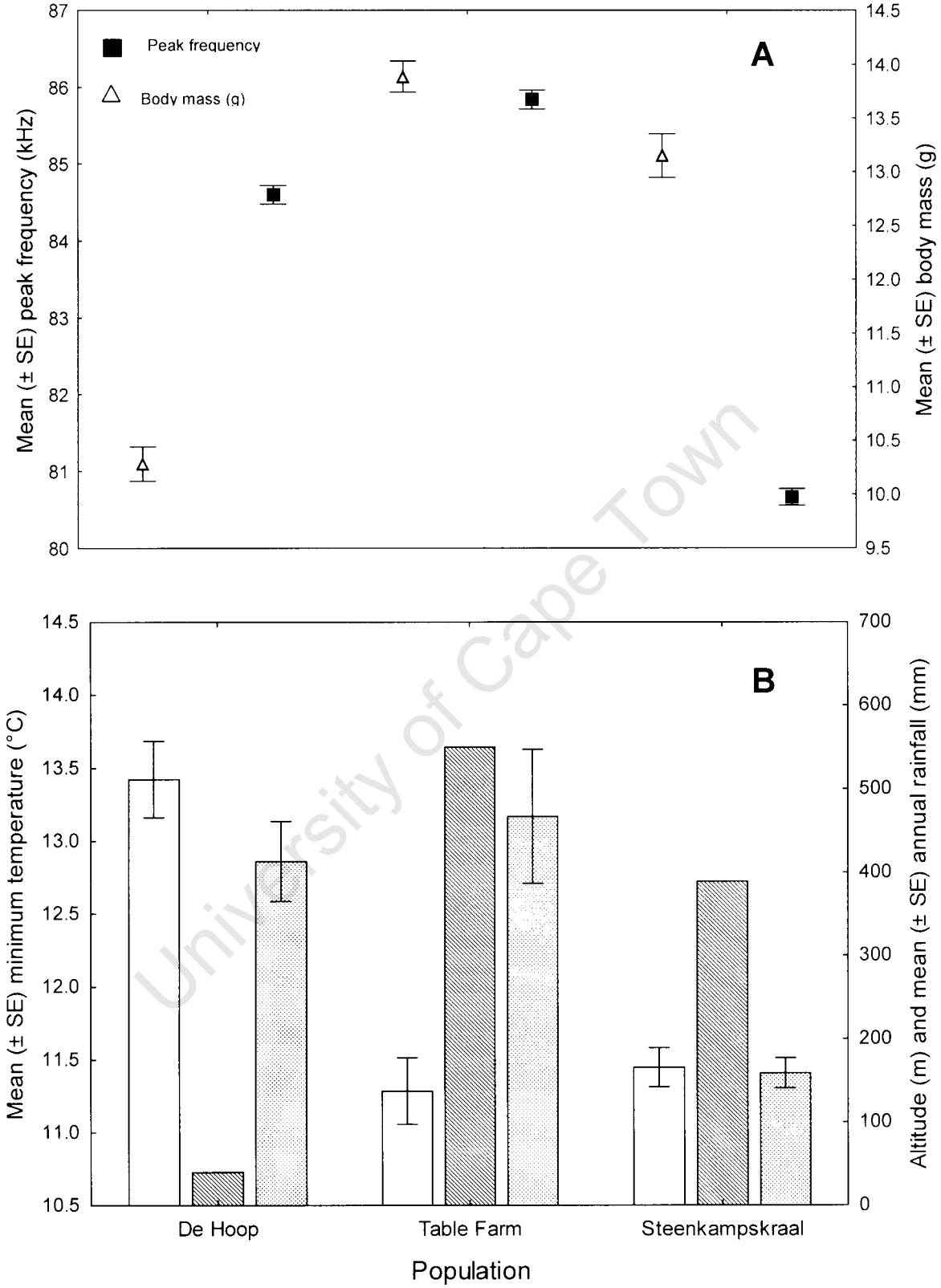

Figure 3.4: (A) Mean (± SE) body mass (g) and peak frequency (kHz) of *R. capensis* at each population. (B) Climatic (Mean minimum temperature (☐), Mean annual rainfall (⬚) and environmental (Altitude (▨)) data for De Hoop, Table Farm and Steenkampskraal.

Foraging habitat hypothesis

Intraspecific analysis revealed no significant relationship between wing loading and peak frequency across populations ($r^2 = 0.001$, $F_{(1, 102)} = 0.113$, $P > 0.05$; Figure 3.7) or within populations (De Hoop: $r^2 = 0.015$, $F_{(1, 44)} = 0.6745$, $P > 0.05$; Table Farm: $r^2 = 0.007$, $F_{(1, 34)} = 0.2466$, $P > 0.05$; Steenkampskraal: $r^2 = 0.025$, $F_{(1, 26)} = 0.5131$, $P > 0.05$).

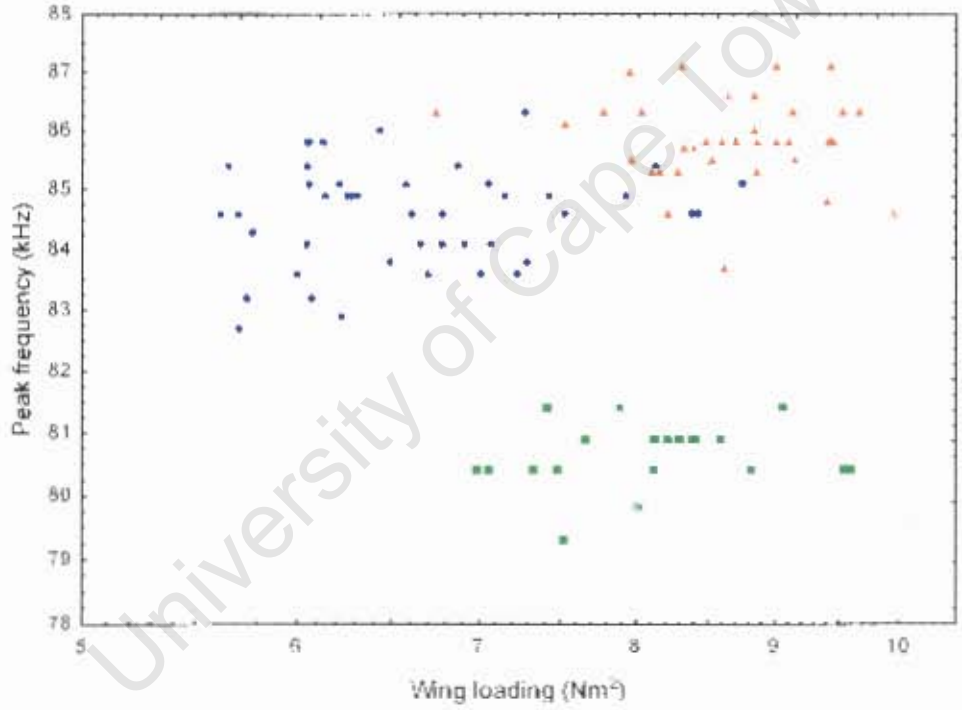

Figure 3.5: The regression of log wing loading (Nm^2) and log peak frequency (kHz) for *R. capensis* from three populations (Steenkampskraal (■), Table Farm (▲) and De Hoop (●)).

Relationship between peak frequency and body size and the echolocation apparatus (decoupling hypothesis)

There was no significant relationship between body mass and peak frequency for *R. capensis* in general ($r^2 = 0.0002$, $F_{(1, 102)}$ 0.0023, $P > 0.05$; Figure 3.6). This was also true within each geographic population of *R. capensis* (De Hoop: $r^2 = 0.270$, $F_{(1, 44)} = 1.222$, $P > 0.05$; Table Farm: $r^2 = 0.0025$, $F_{(1, 34)} = 0.0885$, $P > 0.05$; Steenkampskraal: $r^2 = 0.0014$, $F_{(1, 22)} = 0.0290$, $P > 0.05$).

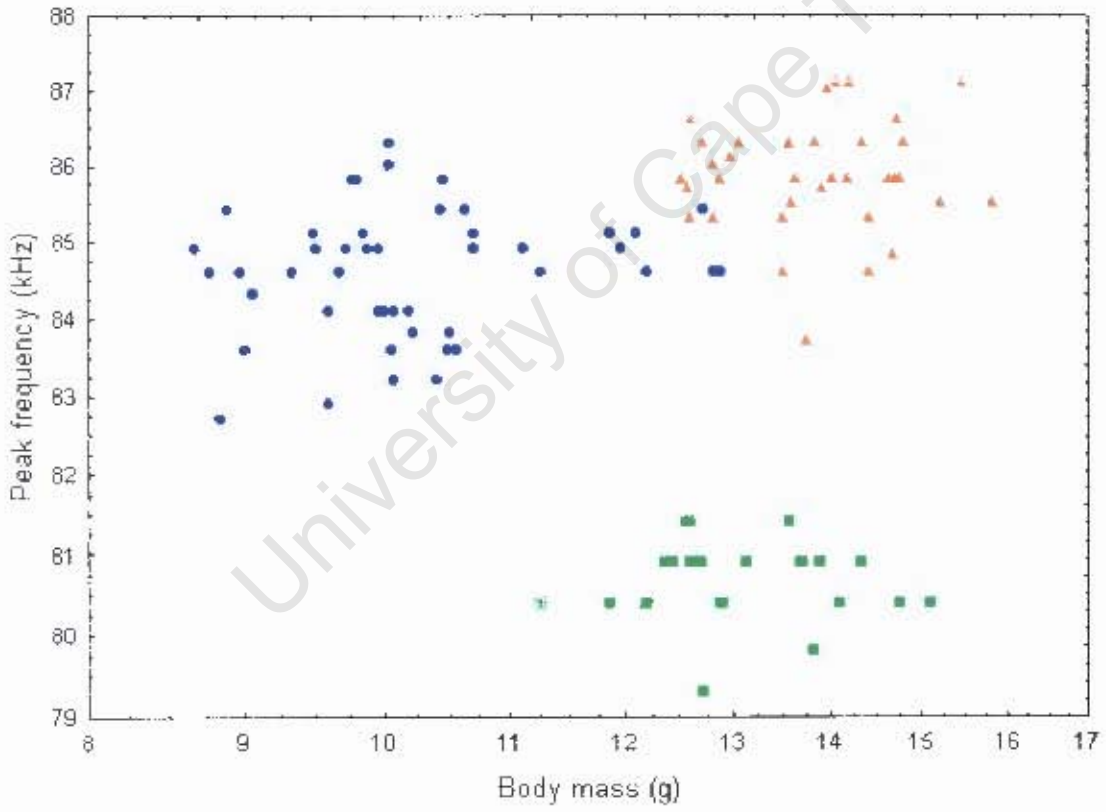

Figure 3.6: The regression of log mass (g) and log peak frequency (kHz) for *R. capensis* from three populations (Steenkampskraal (■), Table Farm (▲) and De Hoop (●)).

I ran PCA on skull radiograph measurements (Table 3.3) to determine which skull parameters differed amongst the *R. capensis* populations. The first two principle components (eigenvalues > 1) extracted from the PCA accounted for 76% of the variation between populations (Table 3.4). Rostrum width, nasal capsule area and height, as well as skull length, were responsible for the separation of populations along PC 1 (Figure 3.7). Bats from Steenkampskraal and Table Farm fell on the negative end of PC 1 and were characterised by larger skulls, greater nasal capsule areas and wider rostrums than bats from De Hoop and Montague, which fell on the positive end of PC 1 (Table 3.3; Figure 3.7). There were significant differences between populations with respect to these parameters (ANOVA, $F_{(9, 43)} = 34719$, $P < 0.005$). Bats from De Hoop and Montagu had significantly smaller skulls, rostrum widths and nasal capsule areas than bats from Table Farm and Steenkampskraal (Tukey HSD test, P's < 0.05; Table 3.3). However, there was no difference between De hoop and Montague with respect to the above skull parameters (Tukey HSD test, P's > 0.05; Table 3.3).

PC 2 only accounted for 14.9% of the total variation and was associated with cochlear size and area (Table 3.4). However, there were no significant differences between populations with respect to cochlear area (Tukey HSD test, $P > 0.05$). Bats from De Hoop had significantly narrower cochleae than bats from Steenkampskraal (Tukey HSD test, $P < 0.05$). Montagu bats had significantly narrower cochleae than both Table Farm and Steenkampskraal bats (Tukey HSD test, $P < 0.05$). However, no clear separation between populations was evident along either of the principal components, with some individuals from different populations overlapping (Figure 3.7).

Table 3.3: Mean ± SD of skull parameters (mm) taken from radiographs of *Rhinolophus capensis*. Ranges are provided in parentheses; n=sample size. Missing values were excluded when calculating the mean

Skull Parameter	De Hoop	Steenkampskraal	Table Farm	Montague
Sagittal skull length	19.97 ± 0.49 (19.34 - 21.04) n=15	20.73 ± 0.22 (20.33 - 20.99) n= 10	20.32 ± 0.34 (19.88 - 20.79) n= 10	20.07 ± 0.19 (19.78 - 20.43) n= 10
Nasal capsule length (X-Y)	4.94 ± 0.24 (4.52 - 5.32) n=15	5.57 ± 0.31 (5.08 - 6.23) n=10	5.07 ± 0.18 (4.72 – 5.30) n= 10	5.29 ± 0.09 (5.11 - 5.42) n= 10
Nasal capsule height (Z-W)	3.66 ± 0.09 (3.46 - 3.81) n=15	3.80 ± 0.11 (3.61 - 3.94) n=10	3.65 ± 0.09 (3.55 - 3.78) n= 10	3.49 ± 0.08 (3.46 - 3.71) n= 10
Sagittal nasal capsule area (SA)	12.58 ± 0.52 (11.93 - 13.55) n=15	13.93 ± 0.62 (13.04 - 14.88) n=10	13.15 ± 0.45 (12.23 - 13.93) n= 10	12.70 ± 0.45 (12.13 - 13.46) n= 10
Transverse skull length	19.92 ± 0.49 (18.92 - 20.82) n=14	20.43 ± 0.20 (20.12 - 20.77) n=10	20.48 ± 0.39 (20.03 - 21.03) n= 10	20.33 ± 0.43 (19.68 - 20.97) n= 10
Nasal width across rostrum (NW)	7.37 ± 0.12 (7.11 - 7.52) n=14	7.74 ± 0.14 (7.47 - 7.88) n=10	7.58 ± 0.18 (7.35 - 7.86) n= 10	7.39 ± 0.24 (7.01 - 7.84) n= 10
Transverse nasal capsule area (TA)	17.25 ± 0.63 (16.36 - 18.73) n=14	18.21 ± 0.62 (17.50 - 19.21) n=10	17.51 ±0.77 (16.45 - 18.58) n= 10	17.51 ± 0.70 (16.19 - 18.71) n= 10
Cochlear area (CA)	8.12 ± 0.27 (7.62 - 8.53) n=13	8.38 ± 0.36 (7.66 - 8.84) n=10	8.17 ± 0.46 (7.57 - 8.86) n= 10	7.99 ± 0.33 (7.25 - 8.30) n= 8
Cochlear width (CW)	3.76 ± 0.08 (3.61 - 3.88) n=13	3.85 ± 0.06 (3.74 - 3.93) n=10	3.82 ± 0.08 (3.72 - 3.96) n= 10	3.68 ± 0.11 (3.48 - 3.81) n= 8

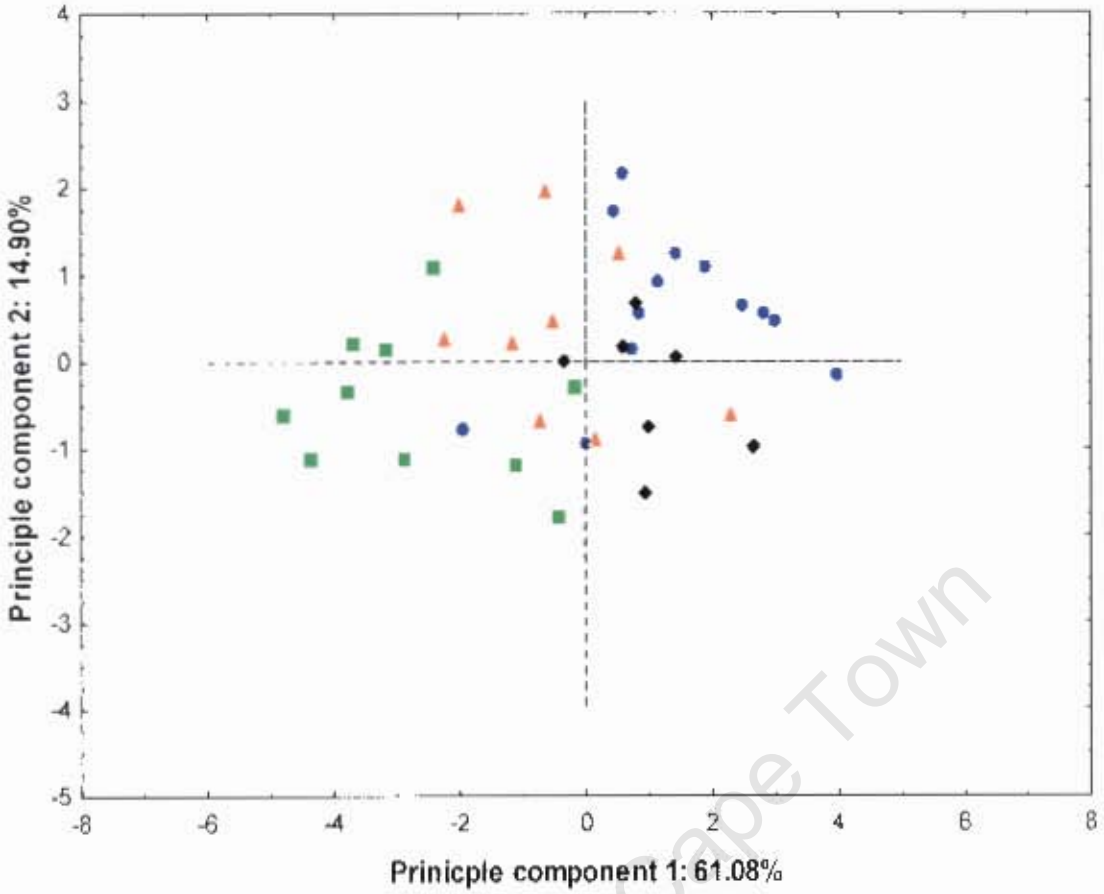

Figure 3.7: Plot of component scores based on skull radiograph measurements for *R. capensis* at Steenkampskraal (■), Table Farm (▲), De Hoop (●) and Montague (♦)

Table 3.4: Factor loadings, eigenvalues and percent variation obtained from PCA analysis of skull parameters measured from skull radiographs

Variables	PC 1	PC 2
Greater skull length	-0.736	-0.367
Nasal capsule length	-0.656	-0.497
Nasal capsule height	-0.847	-0.108
Sagittal nasal capsule area	-0.844	-0.413
Nasal width across rostrum	-0.867	0.211
Transverse nasal capsule area	-0.826	0.098
Cochlear area	-0.719	0.540
Cochlear width	-0.727	0.528
Eigenvalue	4.88	1.19
% Total variation	61.08	14.89
% Cumulative variation	61.08	75.98

There was no significant difference in cochlear width between De Hoop and Montague bats (Tukey HSD test, $P > 0.05$). Furthermore, there were no significant correlations between peak frequency and cochlear width ($r^2 = 0.095$, $F_{(1, 21)} = 2.208$, $P > 0.05$; Figure 3.8) and cochlear area ($r^2 = 0.0749$, $F_{(1, 21)} = 1.701$, $P > 0.05$; Figure 3.9) for *R. capensis*.

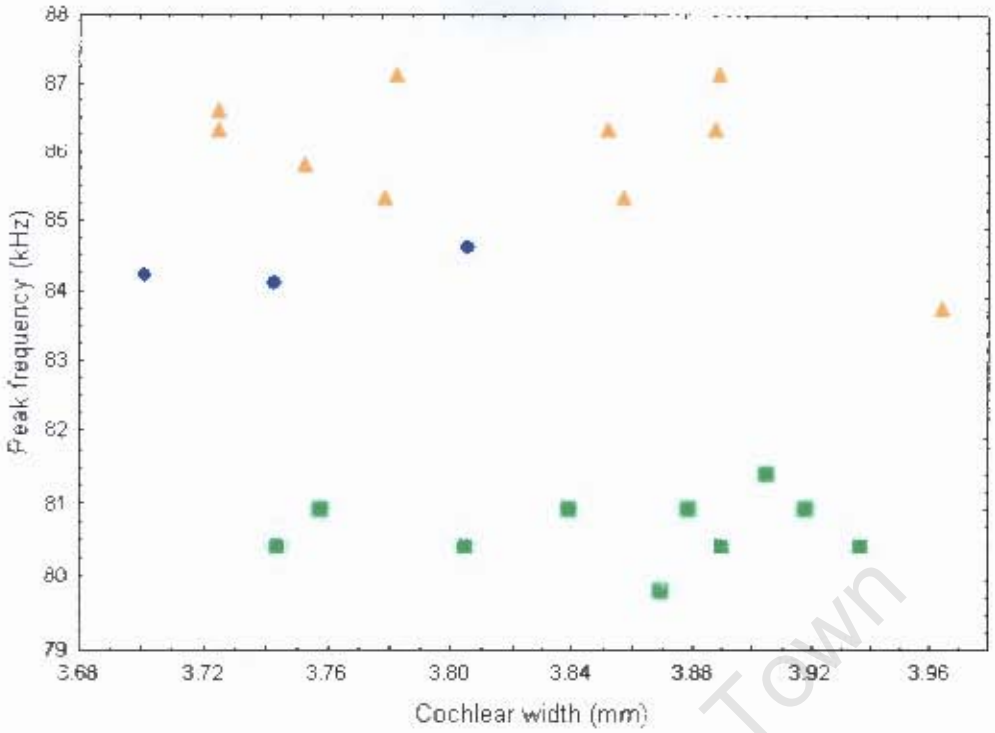

Figure 3.8: The regression of log cochlear width (mm) and log peak frequency (kHz) for *R. capensis* from three populations (Steenkampskraal (■), Table Farm (▲) and De Hoop (●)).

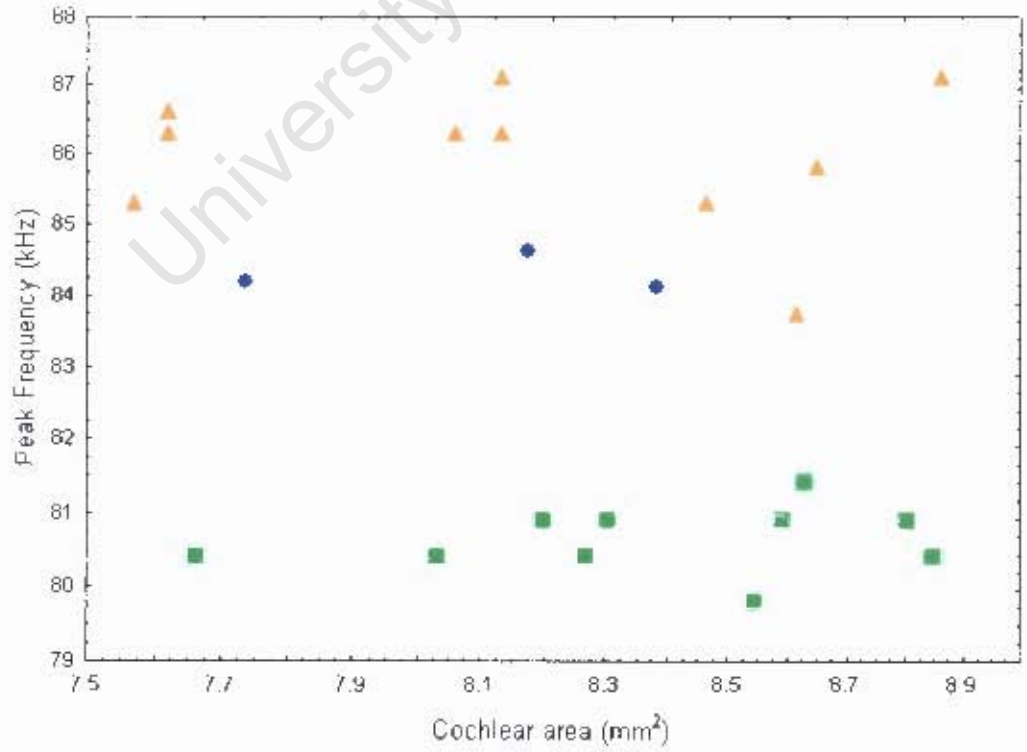

Figure 3.9: The regression of log cochlear area (mm) and log peak frequency (kHz) for *R. capensis* from three populations (Steenkampskraal (■), Table Farm (▲) and De Hoop (●)).

Forward stepwise regression of peak frequency against skull length, nasal capsule area, nasal capsule height and rostrum width, yielded a model ($r = 0.7626$, $F(2,20) = 13.902$, $P < 0.001$) which only contained nasal capsule area (t-test: $t = -2.254$, $n = 23$, $P < 0.05$) and greater skull length, the latter of which did not contribute significantly to the model (t-test: $t = -1.514$, $n = 23$, $P > 0.05$). Thus, nasal capsule area was the best predictor of peak echolocation frequency. In addition, nasal capsule area was not correlated with body mass ($r^2 = 0.137$, $F_{(1, 20)} = 3.20$, $P > 0.05$).

There was also no relationship between peak frequency and nose leaf width across populations of *R. capensis* ($r^2 = 0.002$, $F_{(1, 102)} = 0.251$, $P > 0.05$; Figure 3.10)

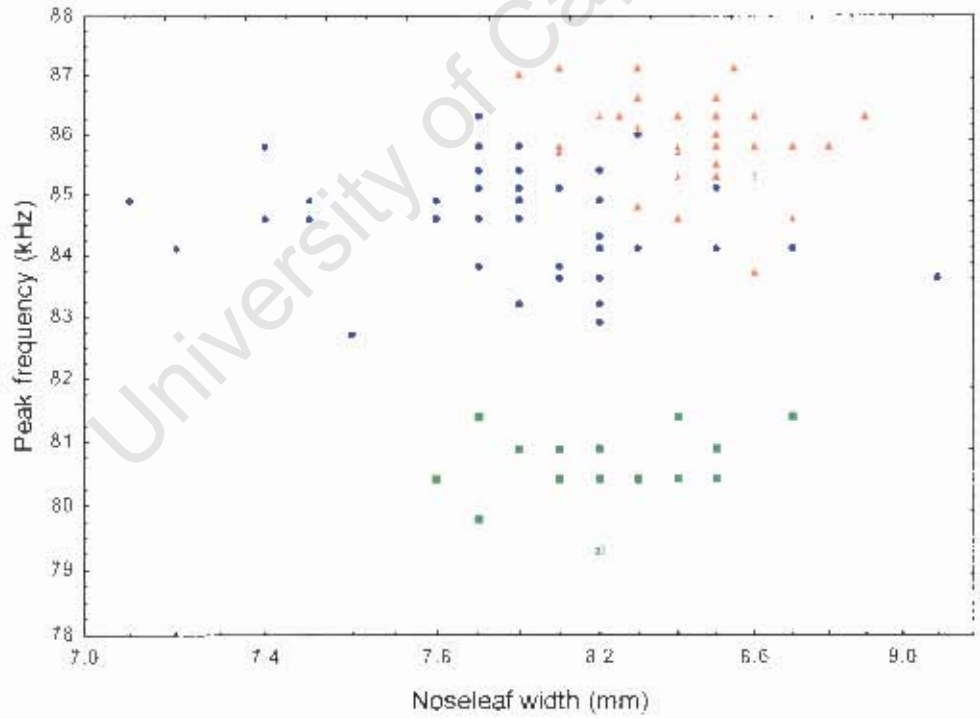

Figure 3.10: The regression of log noseleaf width (mm) and log peak frequency (kHz) for *R. capensis* from three populations (Steenkampskraal (■), Table Farm (▲) and De Hoop (●)).

Skull morphology- prey detection hypothesis

Condyle height was excluded from the PCA based on skull parameters associated with diet (Table 3.5) because the mean between populations did not differ more than the variation due to measurement error (measurement error = 0.019 mm). The first three principle components (eigenvalues > 1) extracted from this PCA accounted for 57.45% of the variation between populations (Table 3.6). PC 1 accounted for 24.54 % of the total variation and was associated with skull length, whereas PC 2 accounted for 17 % and was associated with the length of the maxillary tooth row (Table 3.6). However, no clear separation between populations was evident along either PC, with individuals from different populations overlapping considerably (Figure 3.11). There was a significant difference in dietary skull parameters between populations (ANOVA, $F_{(21, 83)} = 2.66$, $P < 0.005$). However, only the length of the maxillary tooth row was significantly different between Steenkampskraal bats and both De Hoop and Montague (Tukey HSD test, $P < 0.05$). Bats from the two ecotone populations had similar tooth row lengths (Tukey HSD test, $P > 0.05$). Furthermore, maxillary tooth row was significantly positively correlated with skull length ($r^2 = 0.009$, $F_{(1, 39)} = 0.3669$, $P < 0.005$; Figure 3.12). Thus, the difference in tooth row length is simply a consequence of differences in body size. Therefore, peak frequency differences between populations may not be due to differences in diet.

Table 3.5: Mean ± SD (mm) of skull parameters associated with diet and measured with digital callipers from four *Rhinolophus capensis* populations. Ranges are given in parentheses.

Skull parameter	De Hoop	Steenkampskraal	Table Farm	Montague
Skull length	19.90 ± 0.38 (19.36 - 20.63) n=13	20.64 ± 0.26 (20.12 - 20.98) n=10	20.35 ± 0.23 (20.02 - 20.71) n=10	20.12 ± 0.19 (19.80 - 20.44) n=8
Distance from the mandibular fossa to the origin of the masseter muscle	4.90 ± 0.24 (4.44 - 5.33) n=13	5.08 ± 0.30 (4.47 - 5.48) n=10	5.01 ± 0.05 (4.92 - 5.12) n=10	4.84 ± 0.41 (3.95 - 5.12) n=7
Distance between left condyle and the insertion of the masseter muscle	2.83 ± 0.14 (2.64 - 3.15) n=13	2.96 ± 0.10 (2.82 - 3.16) n=10	2.75 ± 0.18 (2.56 - 3.07) n=10	2.64 ± 0.14 (2.35 – 2.85) n=8
Masseter muscle scar	3.29 ± 0.17 (3.07- 3.59) n=13	3.23 ± 0.12 (3.02 - 3.40) n=10	3.27 ± 0.09 (3.08- 3.38) n=10	3.33 ± 0.15 (3.16 - 3.54) n=8
Condyle height	1.49 ± 0.29 (0.93 - 1.85) n=13	1.49 ± 0.09 (1.41 - 1.72) n=10	1.52 ± 0.11 (1.32 - 1.69) n=10	1.51 ± 0.13 (1.34 - 1.75) n=8
Coronoid process height	1.99 ± 0.18 (1.53 - 2.22) n=13	1.92 ± 0.17 (1.58 - 2.13) n=10	1.96 ± 0.12 (1.81 - 2.22) n=10	2.00 ± 0.13 (1.84 - 2.20) n=8
Dentary length	11.92 ± 0.57 (11.06 - 13.11) n=13	12.11 ± 0.80 (11.10 - 13.55) n=10	12.03 ± 0.26 (11.69 – 12.41) n=10	12.33 ± 0.45 (11.68 – 12.80) n=8
Length of left maxillary tooth row	5.78 ± 0.21 (5.39 - 6.17) n=13	6.08 ± 0.09 (5.94 - 6.23) n=10	5.95 ± 0.09 (5.83 - 6.13) n=10	5.84 ± 0.07 (5.77 - 5.96) n=8
Dentary thickness	1.58 ± 0.09 (1.43- 1.76) n=13	1.60 ± 0.10 (1.51- 1.85) n=10	1.65 ± 0.106 (1.49- 1.83) n=13	1.54 ± 0.08 (1.43- 1.65) n=8

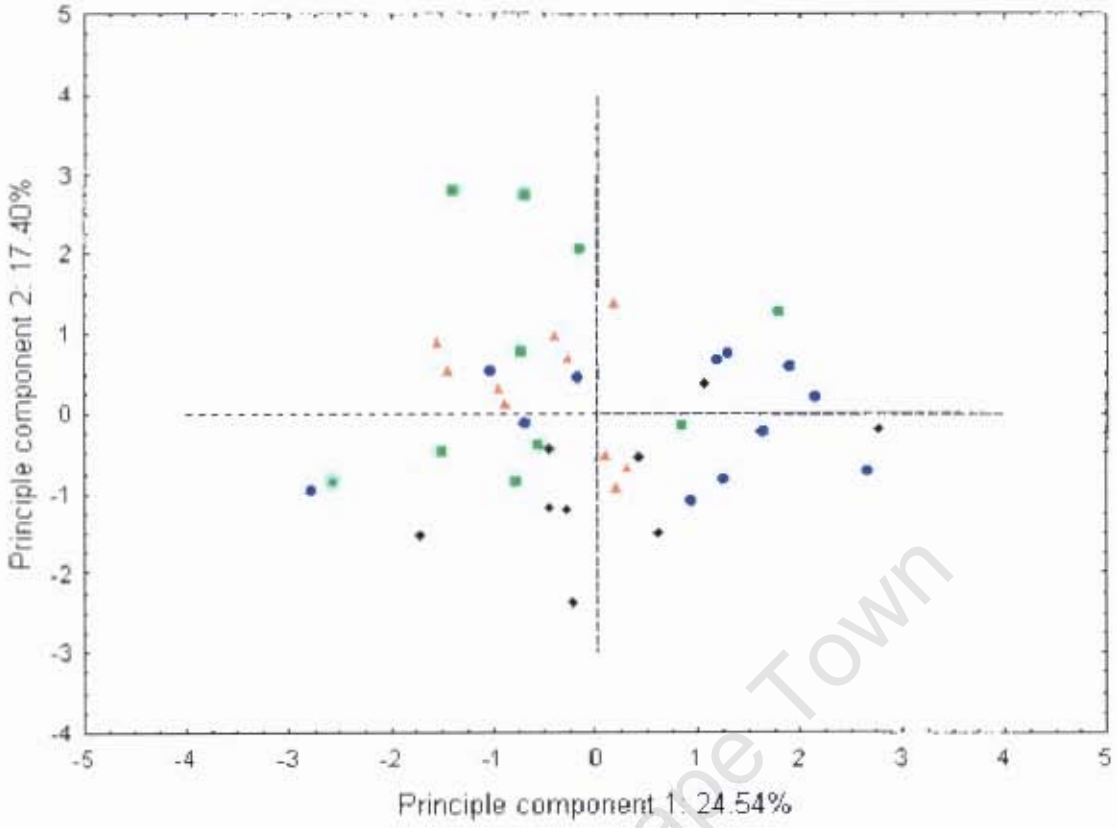

Figure 3.11: Plot of component scores based on skull parameters associated with diet for *R. capensis* at Steenkampskraal (■), Table Farm (▲), De Hoop (●) and Montague (♦)

Table 3.6: Factor loadings, eigenvalues and percent variation obtained from PCA analysis of skull parameters associated with diet.

Variable	PC 1	PC 2	PC 3
Skull length	0.843	-0.383	0.034
a:b*	0.182	0.403	-0.551
Length of masseter muscle scar	0.270	0.554	-0.321
Height of coronoid process	-0.342	-0.362	-0.577
Dentary length	0.601	0.520	-0.210
Maxillary tooth row length	0.595	-0.674	-0.260
Dentary thickness	0.335	0.191	0.722
Eigenvalue	1.71	1.21	1.08
% Total variation	24.54	17.40	15.50
% Cumulative variation	24.54	41.94	57.45

* a:b is the ratio between the joint to origin of the masseter muscle (a) and the joint to insertion of the masseter (b).

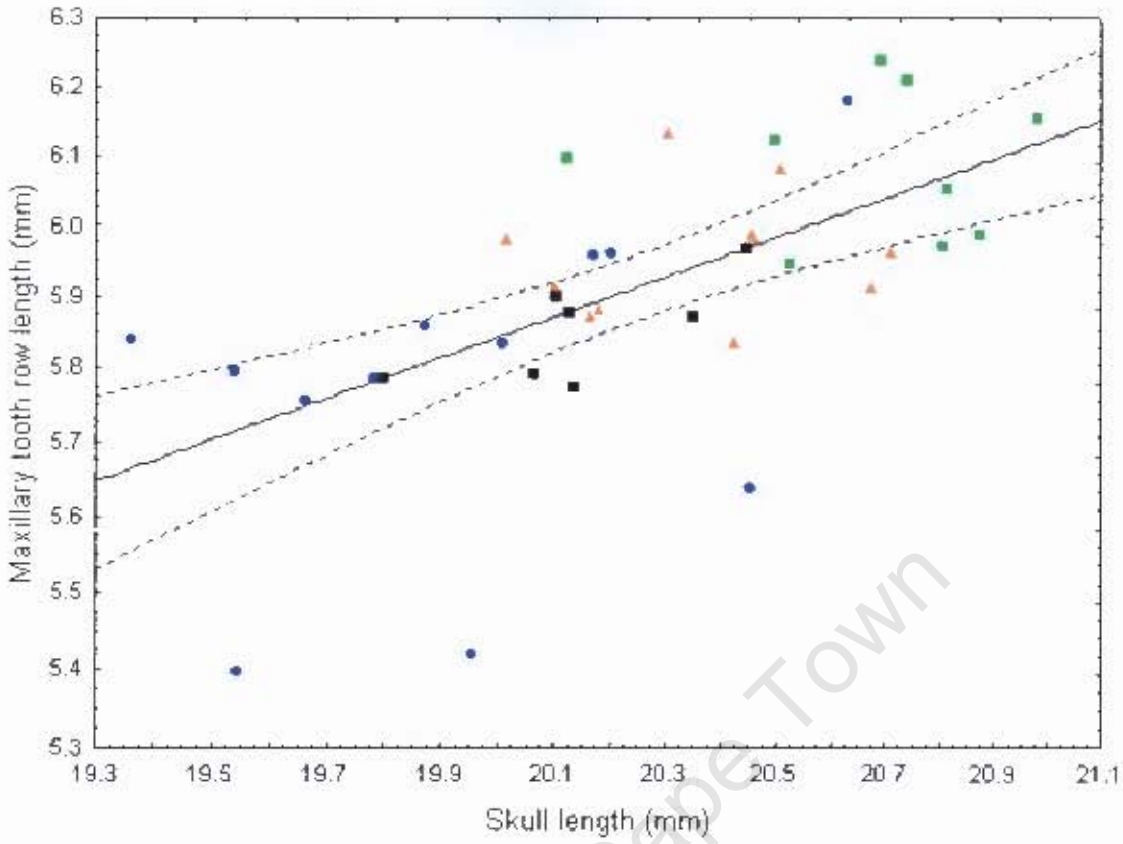

Figure 3.12: The regression of log skull length (mm) and log maxillary tooth row (mm) for *R. capensis* from four populations (Steenkampskraal (■), Table Farm (▲), De Hoop (●)and Montague (♦)).Dashed line represents the 95% confidence limits and the solid line represents the best fit where Log Maxillary Tooth Row Length = 0.2405 + 0.2801*Log Skull Length.

Chapter 4

DISCUSSION

The analysis focusing on interspecific patterns in echolocation variation in African rhinolophids confirm the significant inverse relationship between body size and peak frequency for rhinolophids (Figure 3.2) (Heller & Von Helversen 1989; Jones 1996, 1999; Jacobs *et al.* 2007; Stoffberg 2007). The three populations of *Rhinolophus capensis* studied here were located within the 95% confidence limits of the allometric relationship suggesting that body size explained most of the variation in peak echolocation frequency (Figure 3.2). However, the populations at Steenkampskraal and Table Farm deviated slightly from the allometric relationship for the genus (Figure 3.2). In fact, the expected inverse relationship between body size and peak frequency was not evident across the three populations (Figure 3.6) even though body size differed considerably between populations and between sexes. Populations situated in the two ecotones (Steenkampskraal and Table Farm) had larger mean body sizes than the population at De Hoop and females were generally larger than males within each population. The echolocation frequencies used by bats in these ecotone populations did not follow the body size/frequency relationship: the population in one ecotone had the highest frequency (Table Farm) while the population in the other ecotone had the lowest (Steenkampskraal). Furthermore, although females were larger, they used higher echolocation frequencies within each population (Table 3.1). These results suggest that while broad scale differences in peak frequency between populations may simply be a consequence of differences in body size, other factors may be responsible for the observed intraspecific divergence in peak frequency of *R. capensis* populations. Here I

will evaluate various hypotheses that have been suggested to influence acoustic divergence between populations of high duty cycle bats as explanations of the observed difference in peak frequency between populations of *R. capensis*.

Differences in body size

Bats from Table Farm and Steenkampskraal (ecotone populations) were significantly heavier and had greater skull dimensions than bats at De Hoop (Table 3.1; Table 3.3). Body size and morphological traits are known to vary with environmental conditions and ecotone populations in a variety of taxa such as birds (James 1970; Kark *et al.* 2002, Carbonell *et al.* 2003) anurans (Schäuble 2004), and mammals (Monteiro *et al.* 2003; Cardini *et al.* 2007) including bats (Burnett 1983; Storz *et al.* 2001; Yom-Tov & Geffen 2006). According to Bergmann's Rule, body sizes of individuals in populations of homeothermic vertebrates from cooler regions, tend to be larger than those from populations found in warmer climates (Wigginton & Dobson 1999). The classical explanation for this pattern is that the larger body surface area to volume ratio of small animals facilitates thermoregulation in warmer regions (Jacobs 1996). However, bats are unusual among mammals because many species are able to abandon homeothermy in favour of heterothermy in response to varying thermoregulatory requirements (Hock 1951; Lewis 1993; Solick & Barclay 2007). This lack of homeothermy may explain why evidence for size-climate relationships in the context of Bergmann's Rule is generally not convincing for bats (Finlay & Wilson 1982). Instead, intraspecific variations in size may be influenced by a combination of climatic variables and not just temperature (Jame's Rule; James 1970). Individuals in populations found in hot, humid conditions should be smaller than individuals found in cool or drier regions to facilitate

conservation of metabolic water (James 1970). This could explain the differences in body size found in my study. The De Hoop population which has a smaller average body size than the other two populations experiences a higher annual rainfall and higher average minimum temperature (low altitude) than both Table Farm and Steenkampskraal (high altitude) (Figure 3.4). In support of this, the body size of the cave fruit bat, *Eonycteris spelaea*, also increased from west to east along its distribution across the Banda Arc which may be due to the gradual increase in aridity from west to east (Maharadatunkamsi *et al*. 2003). However, other studies have found correlations between body size and climate that differs from James' Rule for other chiropteran species. For example, Burnett (1983) found a significant negative correlation between body size and minimum environmental temperature for *Eptesicus fuscus* in accordance with Bergmann's Rule. However, he also found that moisture had a statistically more significant influence on body size than did temperature but body size increased with humidity, contrary to James' Rule. Similarly, Storz *et al*. (2001) reported an increase in body size with a decrease in minimum temperature and an increase in humidity along a latitudinal transect across the distribution of the Indian fruit bat, *Cynopterus sphinx*. In contrast, Solick and Barclay (2006) found no difference in body size between populations of *Myotis evotis* which experience distinctly different climatic conditions and thus proposed that *M. evotis* possesses a body type that can cope with a range of environmental conditions. The above studies all deal with intraspecific differences in body size but support for both Bergmann's and James' Rules are inconsistent. It thus appears that some factor/s other than the level of analyses, as suggested by Blackburn *et al.* (1999), affects the validity of Bergmann's and Jame's Rules as an explanation for body size variation within a species.

The larger body size of bats at Steenkampskraal and Table Farm may instead be an adaptation to the intrinsic habitat heterogeneity of ecological transition zones as it allows these bats to have a greater niche width and possibly larger home ranges. Populations situated at the edge of a species distribution range are thought to occupy sub-optimal habitats in comparison to populations situated in the centre of the species distribution. This is based on the idea that suitable environments to which species are adapted decrease towards the species boundaries (Brown 1984). However, recent studies have argued that range edges and ecotones instead provide novel environments to which species can become locally adapted which results in phenotypic divergence of edge and ecotone populations from central populations (Smith *et al.* 1997; Kark *et al.* 1999, 2002; Carbonell *et al.* 2003). Reis *et al.* (2004) argues that populations at habitat edges have access to spatially separated resources. If resources (e.g. insect prey) are spatially separated in ecotones, then Table Farm and Steenkampskraal bats may have to cover larger distances to find preferred insect prey. The significantly larger body size and corresponding greater wing loading of ecotone populations would allow efficient commuting flight over long distances (Norberg & Rayner 1987). A more detailed comparison of home range size and resource distribution between these populations is required to adequately test whether larger bats at Table Farm and Steenkampskraal have larger home ranges than bats at De Hoop because they need to access spatially separated resources. Home range use of each population can be investigated using radio telemetry methods to determine home range size (Meyer *et al.* 2005) and foraging distance in relation to insect prey distribution (Goiti *et al.* 2008).

Influence of climate and habitat on echolocation

Higher frequencies suffer more from atmospheric attenuation than lower frequencies and this effect increases rapidly for frequencies above 90 kHz (Lawrence & Simmons 1982). Thus, we would expect Steenkampskraal bats to echolocate at higher frequencies than bats from De Hoop and Table Farm. Steenkampskraal is situated in an arid region, where attenuation would be low and the disadvantages i.e. shorter detection distances, associated with higher frequencies would not be as pronounced as in the more humid habitats of De Hoop and Table Farm. However, bats at Steenkampskraal echolocated at lower frequencies than bats from De Hoop and Table Farm (Table 3.1; Figure 3.1, 3.4), despite the large difference in mean annual rainfall between the habitats of these populations. Thus, observed differences in peak frequency between populations of *R. capensis* sampled in this study may not be due to differences in environmental humidity (humidity hypothesis) as measured by mean annual rainfall. Stoffberg (2007) also found no clear relationship between the distribution of South African rhinolophids using high and low frequency echolocation and rainfall patterns of South Africa. Thus, there is no suggestion that atmospheric conditions affect peak frequency divergence between populations of *R. capensis.*

However, even habitat structure may not adequately explain the divergence in echolocation call frequency. The lower echolocation frequencies (increased detection distance) used by Steenkampskraal bats, combined with their higher wing loading (more rapid flight; Table 3.1), may be an adaptation for faster flight in the relatively open habitat at Steenkampskraal than at De Hoop or Table Farm. Steenkampskraal is characterised by sparse, dwarf (*ca* 30 cm in height) vegetation, whereas the vegetation

in the other two habits is dense and can reach 2 m and more. However, at De Hoop *R. capensis* foraged close to the ground (Jacobs *et al.* 2007) and if *R. capensis* at Steenkampskraal does the same, they may nevertheless experience the habitat there as cluttered. More importantly, peak frequency was not significantly correlated with wing loading across the three populations (Figure 3.5) which is what one would expect if echolocation and wing morphology formed an adaptive complex in response to the physical structure of the habitat (Aldridge & Rautenbach 1987; Jacobs *et al.* 2007). Thus, peak frequencies in populations of *R. capensis* appear to have evolved independently of wing morphology and for reasons other than the exploitation of different habitats. Similarly, Russo *et al.* (2007) found no relationship between wing morphology and echolocation call frequency differences within R. *hipposideros* and *R. euryale* and thus also concluded that differences in peak frequency may not be due to the exploitation of different foraging habitats for both species. In addition, Jacobs *et al.* (2007) found no evidence for the foraging habitat hypothesis as an explanation for the use of higher than expected peak frequencies of *R. clivosus*.

Influence of diet on echolocation

We predicted that peak frequency differences between populations of *R. capensis* may not be due to differences in diet because differences in wavelengths at the high frequencies used by *R capensis* are not large enough to result in differences in detectable prey size (Jacobs *et al.* 2007). In support of this prediction, we found no differences between populations of *R. capensis* with respect to skull parameters associated with diet (Figure 3.11). The length of the maxillary tooth row was the only skull morphological feature associated with diet that differed between populations but it was also

significantly positively correlated with skull size (Figure 3.12). Therefore, differences in maxillary tooth row length are probably a consequence of differences in body size between populations rather than the result of differences in diet. A detailed analysis of dietary differences in conjunction with geometric morphometrics may shed more light on this.

Decoupling of echolocation from body size

The absence of the relationship between peak frequency and body mass within *R. capensis*, despite the significant differences in these variables between populations, implies that selection may have acted on peak frequency independently of body size. Previous within species comparisons have also found no relationship between body size and peak frequencies in rhinolophid and hipposiderid bats but reasons for the apparent decoupling were not always clear or investigated. For example, Jones *et al.* (1992) and Jones and Ransome (1993) only reported that peak frequency was not related to body size in *R. ferrumequinum* and *R. hipposideros*. However, sonar partitioning to facilitate intraspecific communication (acoustic communication hypothesis) was proposed by Russo *et al.* (2007) to explain why peak frequency but not body size differed between peninsular and Sardinian populations of *R. euryale* and *R. hipposideros*.

Similarly, the higher than expected call frequency used by *R. clivosus* may be to facilitate intraspecific communication (Jacobs *et al.* 2007)

Armstrong and Coles (2007) found no relationship between body size and peak frequency between isolated populations of *Rhinonicteris aurantia* but significant differences in skull morphological features directly involved in echolocation production.

They therefore suggested that a decoupling of the echolocation apparatus from body size may have resulted in the observed peak frequency differences between populations of *R. aurantia*. However, they recognized that a decoupling of peak frequency and the echolocation apparatus from body size can only really be confirmed in situations where both body size and peak frequency differs, such as is the case in this study.

Skull parameters measured from radiographs of *R. capensis* populations showed considerable differentiation (Table 3.4; Figure 3.7). Bats from Steenkampskraal had larger dimensions and greater areas for all skull parameters measured from radiographs (Table 3.4) which correspond to their use of lower frequency calls (Armstrong & Coles 2007; Table 3.1). Similarly, the higher frequencies used by bats at De Hoop correspond to the smaller dimensions of their skull parameters (Armstrong & Coles 2007; Table 3.4). Furthermore, skulls from De Hoop and Montague were very similar in size. This may be because De Hoop is geographically much closer to Montague (Chapter 2; Figure 2.1) than to Table Farm and Steenkampskraal, and Montague and De Hoop may form a single population. In addition, nasal chamber area was the best predictor of peak frequency for *R. capensis* and there was no relationship between the area of the nasal chamber and body size. This, coupled with the absence of a relationship between peak frequency and body size, implies that selection may have acted directly on peak frequency altering skull parameters directly involved in echolocation independently of body size which supports the decoupling of echolocation from body size hypothesis. These results are in agreement with previous studies which investigated the relationship between skull morphology and peak frequency in rhinolophids (Stoffberg 2007) and hipposiderids (Armstrong & Coles 2007), despite these studies not using calls and skull parameters from the same individuals as was done in my study.

Despite interspecific correlations between peak frequency and other phenotypic characters associated with echolocation (e.g. noseleaf width: Stoffberg 2007; and cochlea: Francis & Habersetzer 1998) I found no such correlations within *R. capensis* (cochlear width and area: Figure 3.8 and 3.9 respectively; noseleaf: Figure 3.10). Similarly, Armstrong and Coles (2007) only found a moderate relationship between noseleaf width and peak frequency in *Rhinonicteris aurantia* and Francis and Habersetzer (1998) reported overlap in cochlear width despite divergence in peak frequency between populations of *H. cervinus*. Cochlear size also did not differ greatly between geographic isolates of *Rhinonicteris aurantia* (Armstrong & Coles 2007). Thus, results from this study are in agreement with the argument that the relationship between peak frequency and cochlear size is moderately plastic (Francis & Habersetzer 1998). The overall dimensions for the cochlear may not be influenced by small changes in foveal frequency (Armstrong & Coles 2007) which simply involves fine tuning of the frequency sensitivity of the basilar membrane.

Role of communication (acoustic communication hypothesis)

Sexual dimorphism in peak frequency and body size was evident in all populations. Females were larger and echolocated at higher frequencies than males. Although average differences in peak frequency between sexes were less than 1 kHz (De Hoop: 0.97 kHz; Table Farm: 0.82 kHz; Steenkampskraal: 0.38 kHz), these differences were significant in each population. Previous studies have also found differences in peak frequency related to sex (Neuweiler *et al.* 1987; Jones 1994; Russo *et al.* 2001; 2007) but differences between sexes were often larger than those reported here for *R. capensis*. For example, females echolocate *c.* 4 kHz and 3 kHz higher than males in *R.*

hipposideros (Russo *et al.* 2007) and *H. speoris* (Jones *et al.* 1994) respectively. Nonetheless, the observed sexual differences in peak frequency may have important implications for intraspecific communication as individuals should be able to recognize the sex of echolocating conspecifics. The finding that peak frequency is more strongly associated with morphological features directly involved in echolocation production than with either body size or wing morphology, further supports the potential social role of echolocation frequency in *R. capensis*.

Acoustic communication has been cited as an explanation for the occurrence of morphologically cryptic but acoustically divergent high duty cycle bat species as it allows for effective intraspecific communication (Kingston *et al.* 2001, Thabah *et al.* 2006, Jacobs *et al.* 2007; Russo *et al.* 2007). For example, Russo *et al.* (2007) found that when *Rhinolophus mehelyi* roosted together with *R. euryale*, they used significantly higher frequencies than when roosting alone.

The western limit of the distribution of *R. capensis* is close to the Orange River at the border of South Africa and Namibia (Figure 2.1). However genetic analyses on rhinolophids sampled from this area and identified in the field as *R. capensis* (based on morphological characteristics), revealed that these bats were indeed a different species (Rowen van Eeden, pers comm.). Thus, Steenkampskraal may be situated close to the "real" edge of the species distribution. Nonetheless, the distribution of *R. capensis* overlaps with only one other rhinolophid- *R. clivosus*. However, populations situated in ecotones may also come into contact with different rhinolophid species. De Hoop is situated in the centre of the species distribution and it also shows the least divergence from the general allometry between body size and peak frequency for the clade. Thus it

is more likely that the two ecotone populations diverged from the ancestral frequency of *R. capensis*. If so, the observed peak frequency divergence of the two ecotone populations of *R. capensis* may be a consequence of *R. capensis* interacting with *R. clivosus* in addition to separate rhinolophid species at either end of its known distribution. This may result in the evolution of local dialects or accents to facilitate intraspecific communication. The lower frequency used by *R. capensis* at Steenkampskraal (80.66 kHz) may be a response to an interaction with *R. darlingi* (86 kHz) (Taylor 2000; Stoffberg 2007) because the presence of *R. clivosus* would have prevented an upward shift in peak frequency away from *R. darlingi*. *R. capensis* at Table Farm (85.8 kHz) on the other hand, may also come into contact with its sister species *R. swinnyi* (107.8 kHz) (Kelly 2008). However, because the peak frequency difference between these species is substantial (> 20 kHz), it is unlikely that *R. swinnyi* has influenced the peak frequency divergence of *R. capensis* at Table Farm. This may be because *R. capensis* may have already shifted its frequency in response to *R. clivosus* (92 kHz; Jacobs *et al*. 2007). However, beyond this, there is currently little direct evidence to support sonar partitioning as an explanation for intraspecific differences in peak frequency. Sonar partitioning may be a better explanation for interspecific rather than intraspecific peak frequency divergence, because, at the level of populations, other factors such as gene flow may mediate differences in peak frequency. There may be sufficient gene flow between De Hoop and Table Farm to result in the small, albeit significant difference in peak frequency between these populations (1.24 kHz; Table 3.1). On the other hand, the much larger geographical distance between De Hoop and Table Farm may decrease gene flow resulting in the bigger differences between Steenkampskraal and the other two populations. Thus, it is feasible that genetic drift acting on its own may explain the small but significant differences in peak frequency

observed between the three populations of *R. capensis*. The relative roles of selection and drift can only really be teased apart through the analyses of the genetic variation within and between populations. This would require samples from more populations across the species distribution, not just representatives from the central, eastern and western limits.

Results from this study provide no clear-cut support for any one of the hypotheses proposed to drive peak frequency divergence between populations of *R. capensis*. The 1-5 kHz difference between *R. capensis* populations sampled in this study is not strongly related to differences in body size, foraging habitat use, diet or mean annual rainfall. Thus, some other environmental factor may have caused the initial divergence between *R. capensis* populations. One such environmental factor could be differences in the local ambient noise characteristics between populations. Slabbekoorn (2004) argues that if ambient noise conditions (for example, noise from chorusing insects or running water) differ consistently between populations, it may cause acoustic divergence between populations of the same species. Thus, differences in ambient noise characteristics of the different populations of *R. capensis* may have initially influenced peak frequency divergence.

Once acoustic divergence between populations occurs, the differences in echolocation frequency between populations of the same species may be maintained by cultural transmission. While species-specific echolocation call structure is genetically determined, cultural transmission may also influence call frequency divergence between geographically separated populations of horseshoe bats (Russo *et al.* 2007). This is because the peak frequency of growing rhinolophids is also partly influenced by that of

its mother (Matsumura 1979; Jones & Ransome 1993). Furthermore, Hiryu *et al.* (2006) demonstrated that individual *Hipposideros terasensis* bats are able to shift their call frequency to match that of different colony members, and therefore, individuals have some control over their emitted peak frequency. Yoshino *et al.* (2008) investigated the possible role of maternal cultural transmission in shaping the bimodal distribution of regional mean peak frequency differences within an island population of *Rhinolophus cornutus pumilus*. They found that the acoustic difference of 5-8 kHz between the north and south regions of the island are maintained despite sufficient nuclear gene flow. Furthermore, using maternally inherited genes, they also found evidence for female philopatry. The 5-8 kHz difference in peak frequency between regions does not result in any difference in habitat use or prey detection between regions. Therefore, Yoshino *et al.* (2008) argued that the divergence in peak frequency may result from random cultural drift and maintained by mother-offspring transmission because of the limited dispersal of females. Cultural transmission of vocal dialects is well documented in cetaceans (e.g. Deecke *et al.* 2000; Rendell & Whitehead 2002; Yurk *et al.* 2002) as well as birds (e.g. Leader *et al.* 2008; MacDougall-Shackleton & MacDougall-Shackleton 2001; Wright *et al.* 2005, 2008). Previous studies have also documented the occurrence of vocal dialects or colony specific calls in bats (e.g. Masters *et al.* 1995; Boughman & Wilkinson 1998; Esser & Schubert 1998). Thus, it is possible that the observed peak frequency differences between populations of *R. capensis* may be maintained through cultural transmission of local dialects.

To determine whether peak frequency differences between populations of *R. capensis* are maintained via cultural or maternal transmission, similar molecular techniques as those described by Yoshino *et al.* (2008) could be applied to *R. capensis* populations to

determine the degree of gene flow and female philopatry. Recently, Li *et al.* (2008) tested the involvement of the newly discovered "hearing" gene, *Prestin*, in the evolution of echolocation. They argue that the adaptive changes of *Prestin* may have resulted in the evolution of the extraordinary frequency selectivity of the acoustic fovea of high duty cycle bats. Thus, it would be interesting to determine whether sequence differences in *Prestin* correspond to intraspecific variation in call frequency of *R. capensis*.

Implications of this study

This study would have benefited with the inclusion of more *R. capensis* populations sampled along a climatic or habitat gradient across its distribution. More robust predictive models such as Generalized Linear Mixed Models (GLMM) could then have been used to determine which factors best predict peak frequency and body size for *R. capensis* because there would have been more variation in categorical predictors. However, this may be limited because the distribution of *R. capensis* is restricted to the western and southern Cape coasts of South Africa. Also, a larger sample size of skulls with corresponding echolocation data would have been ideal but this was not possible, owing to the fact that *R. capensis* is endemic. Despite these caveats, this study has some important implications. For example, preliminary genetic analyses by Conrad Matthee (unpublished data) and my own identification using morphology (forearm length; anterior premolar in the tooth row) to distinguish them from all other known rhinolophid species, indicate that these three populations are all *R. capensis*. If this is confirmed by more detailed genetic analyses then it advises caution when using size and/or echolocation differences on their own to identify bat species. This study also provides evidence for the decoupling of the echolocation apparatus from body size and wing

morphology for rhinolophid bats which implies that the "adaptive complex" between echolocation and wing morphology may only apply at the level of the species and not at the level of the populations. This emphasizes the need for more detailed life history data which would provide greater insights into factors affecting trait variation in species. This study also highlights the importance of undertaking analyses at different levels. Although analyses at the species level indicated very little divergence, analyses at the population level indicated substantial divergence in body size and echolocation. This divergence is probably connected to resource distribution and cultural transmission, respectively, which present further avenues for future research.

Chapter 5

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APPENDIX 1

Museum codes for *Rhinolophus capensis* skulls used in this study:

Northern Flagship Institute.—TM 27066, TM 29081, TM 29064, TM 29067, TM 29080, TM 29070, TM 29077, TM 29065, TM 29063.

Iziko Museum.— ZM 14560H, ZM 14559, ZM 14560I, ZM 14561, ZM 35665, ZM 14560E, ZM 14560C, ZM 14560F, ZM 14560B, ZM 14560, ZM 14560 G.