DEMOGRAPHIC AND GENETIC VARIABILITY IN CAPE DWARF CHAMELEONS, *Bradypodion pumilum*, WITHIN A FRAGMENTED, URBAN HABITAT

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ABSTRACT

Habitat fragmentation is recognized as a primary cause of biodiversity loss. To maximize biodiversity maintenance, researchers in the field of conservation biology often investigate population demography and genetic variability for species inhabiting fragmented landscapes. Findings from such work enable effective conservation management, maximizing viability for potentially imperiled populations. Previous research has relied predominately on spatial analysis when investigating population demography and genetic variability; however, temporal analysis is also important to species conservation.

As of 2006, reptiles and amphibians had the highest threat status among small, terrestrial vertebrates, warranting continual investigation of herpetofaunal species inhabiting fragmented landscapes. Of the two, reptiles are the more poorly studied, though are suggested to be equally or more threatened than amphibians. The Cape Dwarf Chameleon, *Bradypodion pumilum*, exemplifies one potentially threatened reptile species which has suffered habitat loss, due to urbanization, inducing fragmentation and transformation among much of its habitat. As a result, many *B. pumilum* populations currently exist as a collection of isolated groups inhabiting critically endangered ecosystems. Continued habitat alteration may prove detrimental to *B. pumilum's* continued existence.

This study investigated temporal dynamics in the local demographic and genetic characteristics of a group of Cape Dwarf Chameleons; the population inhabits a patch of transformed, fragmented habitat within the Noordhoek Wetlands Nature Reserve, Cape Town, South Africa. One year of capture-mark-recapture data indicated the study site typically supports a fluctuating abundance of adult individuals, ranging from 25-100 adult chameleons. Chameleons experienced annual survival similar to other small-bodied lizards as well as size-dependent survival, where larger individuals showed higher survival rates. Sex and season, however, did not appear to have significant effects on chameleon survival within the study site as both sexes had similar thirty-day survival rates which remained constant throughout the study period . Results corroborate previous ten-day survival estimates for *B. pumilum* inhabiting the Noordhoek site, and suggest *B. pumilum* experiences similar short-term and extended survival. Demographic assessment also revealed that *B. pumilum* engaged in minimal movement between the core site and adjacent patch fragments during thirty-day periods, independent of time and/or sex, executed exclusively through the narrow corridors of continuous vegetation linking the core site to adjacent fragments.

A three-year genetic assessment using eight microsatellite molecular markers was conducted to investigate temporal stability in allelic variation for *B. pumilum* inhabiting the field site. Specific focus was directed towards uncovering a potential relationship between allelic variation and stochastic events (e.g. temporary site-vacancy of adult chameleons). Quantitative genetic analysis (overall levels of polymorphism including number of alleles and observed heterozygosity) of *B. pumilum* samples indicated typical levels of genetic diversity within the Noordhoek population compared with other small-bodied lizards inhabiting more natural landscapes. Additionally, small, but detectable, differences in allele frequencies (R_{ST} = 0.017, 0.015, 0.015; *P*-value = 0.019, 0.046, 0.045) were observed between pre- versus post-vacancy periods for *B. pumilum* inhabiting the field site. Interestingly, despite these observed shifts, there are nonsignificant differences between pre- and post-vacancy periods for the F_{ST} statistics (as opposed to R_{ST} statistics) provided by the analysis of molecular variance (AMOVA). Cumulatively, results indicated a degree of genetic stability for *B. pumilum* inhabiting the Noordhoek site across the three-year period, particularly between years 2 and 3. Results support the initial hypotheses that *B. pumilum* inhabiting the Noordhoek site experience predominately stable allelic structure except following stochastic events such as the disappearance of the adults between years 1 and 2.

This investigation of demographic and genetic variability provides important temporal data on local population dynamics and habitat use for a potentially vulnerable species inhabiting primarily fragmented landscapes. Findings from this study benefit *B. pumilum's* ongoing evaluation for IUCN Redlist status and provide empirical data useful to identifying and mitigating threats to chameleon viability through the implementation of effective conservation management strategy. Results suggest *B. pumilum* is capable of short-term occupation within fragmented landscapes with the potential for long-term viability, though further study is required. Management strategy may be required to maintain quality habitat for chameleons inhabiting this fragmented landscape. This study exemplifies both the necessity and benefits of conducting species-specific investigations for small-vertebrate populations inhabiting fragmented landscapes. The combination of demographic and genetic approaches offers insight into local population dynamics likely unobtainable through their individual application. Results will likely aid in effective species conservation, maximizing the continual existence of potentially imperiled species inhabiting fragmented landscapes.

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CHAPTER ONE

AN INTRODUCTION TO *(1)* CHAMELEONS: WITH FOCUS ON DWARF CHAMELEONS (*Bradypodion* sp.) AND *(2)* THE EFFECTS OF HABITAT FRAGMENTATION ON SPECIES WITH ADDITIONAL FOCUS ON HERPETOFAUNA

CHAMELEONS

Chameleons (family: Chamaeleonidae) likely originated within the last ninety million years on the island of Madagascar, following the break-up of the supercontinent Gondwana (Africa, Madagascar, Seychelles, India, Antarctica and Australia). The family has since undergone multiple dispersal events between Madagascar, the Seychelles Islands, mainland Africa and its surrounding islands, southern coastal regions of the Mediterranean from southern Portugal and Spain (Greece and Turkey and various Mediterranean islands), the Arabian peninsula and India (Raxworthy *et al*. 2002; Tolley and Burger 2007; Townsend *et al*. 2011). Today, Chamaeleonidae includes approximately 180 species spanning eleven genera; nearly half of which are thought to be endemic to Madagascar. Genus *Chameleo* exhibits one of the more extensive distributions, spanning much of Africa and extending into Europe, the Middle East, Arabia and the Indian sub-continent (Tilbury and Tolley 2009 *[A]*). The oldest known chameleon fossil, *Chameleo andrusovi*, is estimated to be 18 million years old (*type locality*: Dolnice, Czech Republic; *type stratum*: Lower Miocene, Ottnangian, zone MN 4; Čerňanský 2010).

Basic chameleon morphology includes a skull characterized by a bony casque, prominent crests, and eyes encased within sockets capable of independent movement (Alexander and Marais 2007; Tolley and Burger 2007). Acrodont dentition prevents effective sawing or chewing; however, chameleons are primarily insectivorous and crush prey between their jaws swallowing it whole. Insects are caught using a long, club-shaped projectile tongue controlled by specialized bones and muscles (Herrel *et al*. 2000). The moistened tip of the tongue is shaped like a suction cup, enabling the high force of adhesion (Herrel *et al*. 2000). Though basic morphology is widespread amongst chameleons, significant range does exist for select traits. Chameleon species inhabiting Madagascar exemplify the immense range observed between body-size when comparing large species (ex. *Calumma parsonii* and *Furcifer oustaleti*) measuring up to nearly seventy centimeters in total length with small species (*Brookesia minima* and *Brookesia tuberculata*) barely measuring three centimeters in total length. Chameleon bodies are laterally compressed and covered by skin composed of modified scales called granules. Color changing ability is generated within sub-epidermal cells called chromatophores and melanophores; rearrangement of pigments beneath the skin controlled by nerves initiates the color change. Color change in chameleons is a physiological reaction to alterations in their physical and

social environment (e.g. thermoregulation or response to behavioral stimuli). At night when sleeping, chameleons lose their bright coloration and display a muted, pale coloration (Alexander and Marais 2007; Tolley and Burger 2007; E.M. Katz, personal observation). Most species are arboreal, reliant upon long, developed limbs with fused, opposing digits and a prehensile tail optimized for grasping vegetation. Unlike most other lizards, chameleons cannot shed their tails or regenerate them when damaged. Terrestrial species exhibit special adaptations, such as dull coloration, stubby tails, and comparatively small body size, disguising them as leaves amongst ground foliage (Alexander and Marais 2007; Tolley and Burger 2007).

Sex is distinguished by a hemipenal bulge present in males (absent in females) on the underside of the tail posterior to the cloaca. Males have two hemipenes, used for reproductive purposes. Most chameleon species are oviparous, though viviparous species do exist (Burrage 1973; Blackburn 1999; Necas 2004; Jackson 2007; Tolley and Burger 2007). Following birth, most chameleons probably take about a year to reach maturity, during which time parents provide no care for their offspring. Chameleons' exhibit indeterminate growth, though adults grow at an ever-slowing rate, and like all reptiles, chameleons periodically shed their skin as they grow (Tolley and Burger 2007).

Life expectancy for most species is probably less than five years in the wild. Common predators include birds and snakes, while domestic cats are also a likely predator within urban areas (Tolley and Burger 2007). Wild-chameleon populations are also threatened by the international pet-trade. Global chameleon trade from 1977 to 2001 totaled 845,000 recorded exports with 96% coming from African countries. Though national and international legislation has diffused export from the previously dominant nations, continually rising demand has initiated an increase in wild-caught chameleon export from other countries, in particular, Uganda, Benin, Mozambique, Yemen and Comoros (Carpenter *et al*. 2004).

Chameleons of Southern Africa

Seven genera within the family Chamaeleonidae occur across the continent of Africa; *Bradypodion*, *Chameleo*, *Rhampholeon*, *Rieppelon*, *Kinyongia*, *Trioceros*, and *Nadzikambia* (Tolley and Burger 2007; Tilbury and Tolley 2009 *[A]*; Tilbury 2010). *Bradypodion*, *Chameleo*, and *Rhampholeon* all occur within southern Africa, accounting for the twenty-one currently recognized species within the region (Tolley and Burger 2007; Raw and Brothers 2008; Tilbury and Tolley 2009

[A]; Tilbury 2010). Species distributions within the region span nine different biomes; the Forest biome currently contains the greatest species richness (Tolley and Burger 2007; Tolley *et al*. 2008).

Dwarf Chameleons

Seventeen of the twenty-one species currently recognized within the southern African region are part of the genus *Bradypodion* (Figure 1). Fifteen of these species are endemic to South Africa, with the earliest species likely originating ten to fifteen million years ago (Tolley and Burger 2007). *Bradypodion* species often have highly restricted distributions, predominately within the Forest and Fynbos biomes of southern Africa, where no two species inhabit the same locality; however, some species exhibit parapatric distribution. Changes in *Bradypodion* species distributions appear linked to changes in the extent and fragmentation of natural forests, typically resulting in fragmented/isolated

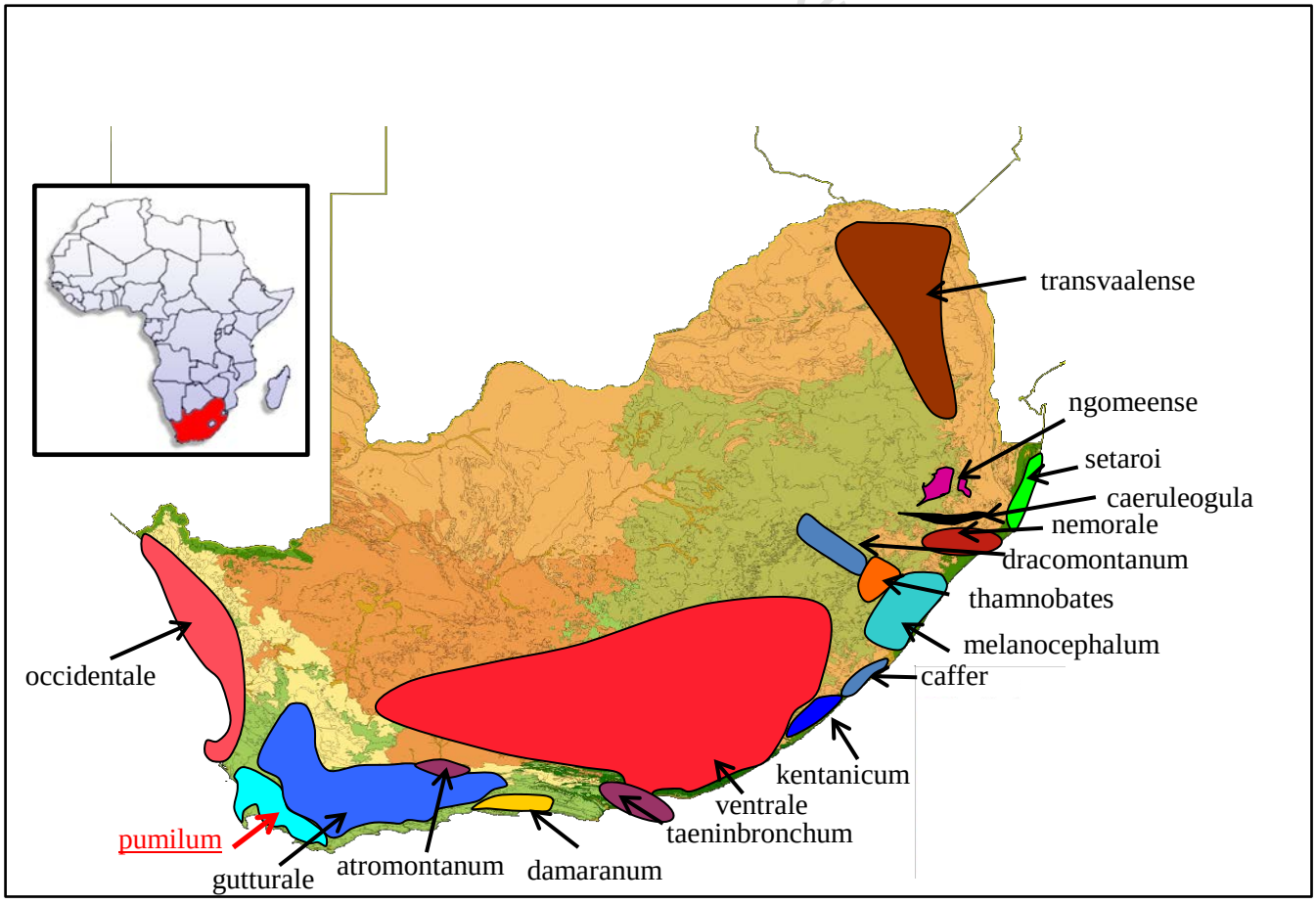


Figure 1 Distribution of *Bradypodion* species inhabiting South Africa (Tilbury and Tolley 2009*[B]*; K.A. Tolley, personal communication)

populations inhabiting small remnant patches (Figure 1; Tolley *et al*. 2006; Alexander and Marais 2007; Tolley and Burger 2007; Tolley *et al*. 2008, 2010).

Most *Bradypodion* species are morphologically distinct from other chameleons, characterized by the presence of both gular and dorsal crests. Snout-vent length for *Bradypodion* species typically ranges between 45.0-90.0 mm and tail length is typically less than 100.0 mm (Tilbury *et al*. 2006; Alexander and Marais 2007; Tolley and Burger 2007).

All *Bradypodion* are arboreal and can usually be found year-round on exposed perches, though some species are harder to locate during the winter season; it has been suggested that they enter a state of torpor, perching deep within vegetation (K.A. Tolley, personal communication; E.M. Katz, personal observation). Dwarf chameleons are both diurnal and heliothermic often climbing into exposed positions following sunrise in order to bask. Throughout the day they traverse in-habitat vegetation to forage, moving slowly with a swaying gait presumably to mimic the movement of leaves. Towards dusk chameleons suspend movement, usually perching among the upper segments of branches until the following sunrise (Alexander and Marais 2007; Tolley and Burger 2007; E.M. Katz, personal observation).

As with most chameleon species, male-male and male-female encounters amongst *Bradypodions* generally result in aggressive behavior. When face-offs occur participants will fight, threaten, and/or chase each other until one individual retreats. Females are particularly aggressive towards males, often to prevent unwanted copulation, and usually succeed in intimidating males into retreat. During such encounters *Bradypodion* females may display contrasting shades of light and dark body coloration, successively open and close their mouth, and continually rock back-forth upon perch vegetation. Receptive females suspend male-oriented aggression allowing select male approach and the commencement of copulation. Males attempt to entice potential female mates through vibrant color display and rapid head wagging (Burrage 1973; Stuart-Fox and Whiting 2005; Alexander and Marais 2007; E.M. Katz, personal observation). Receptive females can mate with and store sperm from multiple males (Burrage 1973; Jackson 2007). Other chameleon species (e.g. *Chamaeleo chamaeleon*) exhibit mate guarding behavior by males (Caudrado 2001), and it is speculated that *Bradypodion* males also engage in post-copulatory mate-guarding (E.M. Katz and K.A. Tolley, personal observation), following a specific female until she is no longer receptive. *Bradypodion* species are viviparous and following an approximate three month gestation period females give birth to five to fifteen babies

(Jackson 2007). Reproduction is aseasonal and females are capable of producing multiple clutches per year (Burrage 1973; Blackburn 1999; Jackson 2007).

THE EFFECTS OF HABITAT FRAGMENTATION ON SPECIES

Habitat destruction, a form of landscape modification, is characterized by all processes, particularly anthropogenic, leading to the elimination of an ecosystem and the loss of its former biological function (Bender *et al*. 1998; Dodd and Smith 2003). Anthropogenic habitat destruction has eliminated approximately half of the fourteen to eighteen million km^2 of species-rich tropical forests once existent on our planet (Skole and Tucker 1993). Habitat destruction is the primary cause of species extinction (Pimm and Raven 2000) and is most likely responsible for the increased extinction rates and biodiversity loss observed within previous decades (Korneck and Sukopp 1988; Henle and Streit 1990; Seabloom *et al*. 2002; McKee *et al*. 2003; IPCC 2007). One conservative estimate quantifies species extinction induced by anthropogenic change at a rate of approximately one thousand species per million per decade (Pimm *et al*. 1995). To further contextualize this statistic, approximately seven million species of eukaryote exist on our planet; therefore approximately seven thousand species are becoming extinct per decade. Therefore, one direct implication of habitat destruction on biodiversity is local extinctions induced by the loss of physical habitat (Ehrlich and Ehrlich 1970; Soule 1983; Groombridge 1992; Burkey 1995; Bender *et al*. 1998). Additionally however, habitat destruction also induces the process of habitat fragmentation; the transformation of large expanses of habitat into a number of smaller patches of smaller total area isolated from each other by a matrix of habitats unlike the original (Wilcox and Murphy 1985; Wilcove *et al*. 1986; Burkey 1995; Bender *et al*. 1998). Like habitat destruction, habitat fragmentation is also recognized as a primary cause of biodiversity loss (Ehrlich and Ehrlich 1970; Soule 1983; Vitousek *et al*. 1997), incorporating declines in floral and faunal species richness and genetic variation within affected areas (Connor and McCoy 1979; Saunders *et al*. 1991; Andren 1994; Haden and Westbrooke 1996; Lynch 1996; Brown 2001; McGarigal and Cushman 2002; Woinarski and Ash 2002; Dodd and Smith 2003).

Two very different approaches are applied to study the effects of habitat fragmentation on species and populations:

Table 1 Approaches to study the effects of habitat fragmentation on species (MacArthur and Wilson 1967; Haila 2002; Fischer and Lindenmayer 2006; Fischer and Lindenmayer 2007).

Species-Oriented Approaches	Pattern-Oriented Approaches
Concept: Each species responds individualistically to a range of processes related to its requirement for food, shelter, space and suitable climatic conditions, as well as interspecific processes like competition, predation, and mutualism.	**Concept**: Typically involves human-perceived landscape patterns and their correlation with measures of species occurrence (e.g. species richness). Originates from island biogeography and composes the bulk of existent fragmentation related research.

Species-oriented approaches typically focus on the range of processes that threaten individual species/populations and can be broadly classified as deterministic or stochastic. Deterministic threats predictably lead to population declines, whereas stochastic threats are driven by chance events (Fischer and Lindenmayer 2007). Species decline caused by habitat fragmentation is usually affected by both deterministic and stochastic threats (Clark *et al*. 1990). In contrast, pattern-oriented approaches are more concerned with key physical attributes of landscape pattern and structure.

Species- and patch-oriented approaches both have unique strengths and weaknesses in studying the effects of habitat fragmentation on species/populations. The former has the advantage of being based on well-established ecological knowledge, but is often limited by the impracticality of studying every single species inhabiting a given landscape (Fischer and Lindenmayer 2007). The latter offers an opportunity to gain broadly applicable general insights; however, pattern-oriented approaches frequently aggregate across individual species and ecological processes. Aggregation may lead to an under-appreciation of the complexity of ecological processes and differences between individual species (Fischer and Lindenmayer 2007). Because of this, key insights regarding the effects of habitat fragmentation on species/populations often require the complementary contributions of both approaches.

Species vary in their tolerance to the threatening processes associated with habitat fragmentation (Fischer and Lindenmayer 2007; Table 2). Two of the biological attributes listed in Table 2, ‘ability to move through the matrix’ and ‘dispersal ability’, could potentially be combined and

relabeled as 'species vagility'. Table 2 and other supporting studies suggest species' vagility effects extinction proneness (Hanski 1994; With and Crist 1995; Lindenmayer and Possingham 1996; Fahrig 1998; Gibbs 1998; Casagrandi and Gatto 1999; Carr and Fahrig 2001). It is important to note that although increased vagility can help species to move between habitat patches, it may also lead to an increased number of individuals dispersing into unsuitable habitat, thereby threating population persistence (Gibbs 1998; Casagrandi and Gatto 1999). Studies reliant upon models not directly incorporating the effects of dispersal mortality on population dynamics suggest that more vagile species have a higher tolerance to habitat fragmentation (e.g. Hanski 1994; With and Crist 1995; Lindenmayer and Possingham 1996) while those reliant upon models which do incorporate the effect of dispersal mortality on population dynamics suggest the opposite due to an increased susceptibility to dispersal mortality (e.g. Fahrig 1998; Gibbs 1998; Casagrandi and Gatto 1999; Carr and Fahrig 2001). Whether species vagility is advantageous or detrimental to extinction proneness appears linked to the level of mortality experienced by dispersing individuals within the matrix portion of the landscape (Carr and Fahrig 2001). For example, species with increased vagility inhabiting patch fragments where roads represent a large mortality factor in the adjacent matrix may be at greater risk of population decline than less vagile species (Carr and Fahrig 2001).

Table 2 Proposed relationships between key threatening processes associated with landscape modification and biological attributes of species which contribute towards or ameliorate extinction proneness (*taken from* Fischer and Lindenmayer 2007).

Threatening process	Ameliorating biological attribute	Explanation
Habitat loss and habitat degradation	Low habitat specialization	Specialized species are more likely to lose their habitats as a result of landscape change
	Disturbance tolerance	Disturbance-tolerant species are more likely to find suitable habitat in modified landscapes
	Ability to live in the matrix	Species that can live in the matrix experience no habitat loss as a result of landscape modification
Habitat isolation and sub-division	Ability to move through the matrix	Species that can move through the matrix are less likely to suffer the negative consequences of habitat isolation
	Dispersal ability	Strong dispersers may be more likely to maintain viable metapopulations (but note this is contentious — see text)
Disrupted species interactions	Limited dependence on particular prey or mutualist species Competitive ability	Species that can switch prey or mutualists are more likely to withstand landscape change Species that are strong competitors are less likely to be outcompeted by species whose habitat expands as a result of landscape change
Disrupted biology	Low biological and behavioral complexity	Species with a complex biology (e.g. social or breeding systems) are more likely to have their biological processes disrupted as a result of landscape change than species with simpler biological systems
Stochastic events	Population density	High density populations contain many individuals even in a small area, and hence are more resilient to stochastic threats

The Effects of Habitat Fragmentation on Herpetofauna

There is a wealth of previous research investigating the effects habitat fragmentation on herpetofauna (amphibians and reptiles; Appendix 1). As of 2006, reptiles and amphibians had the highest threat status of all terrestrial vertebrates, with significantly more species at risk than either birds or mammals (IUCN 2006; Gardner *et al*. 2007). Of the two, reptiles are the more poorly studied, though are suggested to be equally or more threatened than amphibians (IUCN 2006). It is therefore critical to continue investigating reptile species inhabiting fragmented landscapes aimed towards maintaining their continual existence. Doing such will increase our understanding of reptile life histories and population dynamics for populations inhabiting fragmented landscapes, enabling more effective conservation management and maximizing viability potential for potentially imperiled species. Compared with similar-sized homeotherms, many reptiles species exhibit lower energetic and moisture requirements, smaller home ranges, and higher densities (Pough 1980; Jellinek *et al*. 2004), potentially increasing their tolerance to fragmentation (Dickman 1987; Burkey 1995; Smith *et al*. 1996; Gascon *et al*. 1999) and corroborating the suggested increased ability of generalists to persist in smaller degraded remnants (Table 2; Kitchener 1982; Humphreys and Kitchener 1982; Kitchener and How 1982; Fischer and Lindenmayer 2007). Despite these biological attributes, other studies suggest reptile species and communities are quite sensitive to the effects of habitat fragmentation (Sarre 1995; Smith *et al*. 1996; Cosson *et al*. 1999; MacNally and Brown 2001; Templeton *et al*. 2001; Driscoll 2004). In contrast, though rare, examples of increased reptile species richness accompanying fragmentation do exist (e.g. Tocher *et al*. 1997). However, the apparent local successes of reptile species within fragmented agricultural landscapes within such studies may be misleading; a need to incorporate a more broad-scale method of assessment may be required. Over time, the continuous decrease in the range of, and connectivity between, remnant patches created by fragmentation could result in small, isolated populations facing high risks of stochastic variation leading to extinction with decreased probability of recolonization (Appendix 1; Soule *et al*. 1992; Stacey and Taper 1992; Hanski 1998; Lande 1998; Fischer and Lindenmayer 2007). Further support towards reptile sensitivity to fragmentation is apparent within studies offering mounting evidence for the widespread decline of reptile species from fragmented agricultural areas (Brown and Bennett 1995; Smith *et al*. 1996; Covacevich *et al*. 1998; Sarre 1998; Dorrough and Ash 1999; Diaz *et al*. 2000; MacNally and Brown 2001). The contrasting results described here suggest the difficulty of applying universal theory to

explain the reptilian response to the effects habitat fragmentation. In-part this may be because reptiles represent too large of a biological study group. Studies focused on more specific taxa (e.g. family: Chamaeleonidae and/or genus: *Bradypodion*) may better serve to elucidate the effects of habitat fragmentation.

The Cape Dwarf Chameleon, *Bradypodion pumilum*, is one reptile species in which landscape modification, due to urbanization, has induced fragmentation and transformation of much of its habitat resulting in critically endangered ecosystems (Driver *et al*. 2005). Distributed within the Western Cape, extending from Cape Town to Cape Agulhas, *B. pumilum* utilizes a variety of habitats including: fynbos, renosterveld, thicket, exotic and native trees and riparian vegetation, planted garden vegetation within urban environments, and isolated patches of land within the Cape Town metropolitan area (Tolley and Burger 2007; Tolley *et al*. 2010). Habitat transformation is continuing, with at least 6.5 km^2 of undeveloped land lost to urbanization annually within the Cape Town metropolis region (Rebelo *et al*. 2011). Habitat alteration within this area has already resulted in a collection of *at least* semi-isolated populations/groups of *B. pumilum* likely experiencing the negative effects of habitat fragmentation. *Bradypodion pumilum* is not currently listed as an IUCN Redlist species; however, is currently under evaluation (K.A. Tolley, personal communication). Conservation policy does not currently exist for *B. pumilum*; however, management plans would be recommended should it be included within the IUCN Redlist of Threatened species.

Study Aims

This study uses temporal monitoring to investigate local population stability for a group of *B. pumilum* inhabiting a patch of transformed, fragmented habitat at the Noordhoek Wetlands Nature Reserve, Cape Town, South Africa. The study site has previously hosted a short-term demographic assessment investigating survival and movement for *B. pumilum*, providing preliminary insight into local population dynamics and conservation management strategy for chameleons inhabiting highly fragmented, urban habitats. However, more expansive and extended demographic study is required to elucidate how isolated chameleon populations persist within fragmented landscapes. Additionally, because the scientific community recognizes that habitat fragmentation affects both demographic and genetic components of population structure and species' vulnerability to extinction, a combination of demographic and genetic approaches are applied here to offer insight into local population dynamics

unobtainable through their individual application. Data collection and analyses rely on a combination of capture-mark-recapture-, radio-tracking-, and vegetation survey, as well as microsatellite molecular markers. The major aims of this study are to:

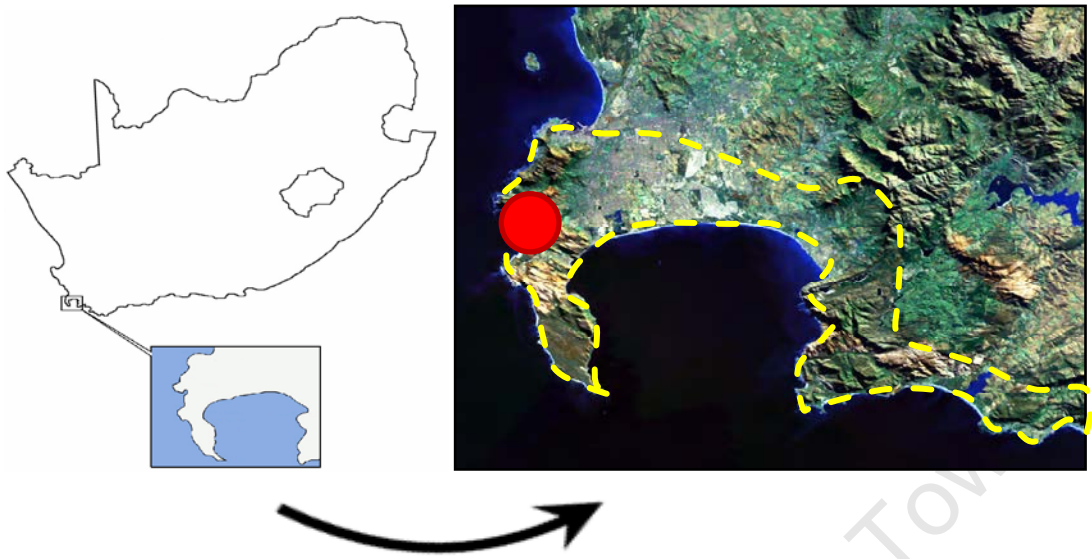
1.) *Estimate demographic parameters (survival, movement, and abundance) to uncover local chameleon population dynamics for a group of Bradypodion pumilum inhabiting a patch of transformed fragmented habitat.*
2.) *Use microsatellite markers to estimate temporal genetic variation and stability (through shifts in allele frequencies) to uncover local chameleon population variability for a group of Bradypodion pumilum inhabiting a patch of transformed fragmented habitat.*
3.) *Use a combination of demographic and genetic approaches to uncover local habitat use and suggest basic conservation management strategy required by chameleons inhabiting fragmented landscapes.*

This investigation of demographic and genetic variability provides important temporal data on local population dynamics and habitat use for a potentially vulnerable species inhabiting primarily fragmented landscapes. Such knowledge of species' life-history allows for more effective conservation management thereby facilitating maintenance of viable populations within potentially imperiled species. Findings from this study will benefit *B. pumilum's* ongoing evaluation for IUCN Redlist status and provide empirical data useful to identifying and mitigating threats to chameleon viability through the implementation of effective conservation management strategy. Additionally, results from this study will provide empirical data (e.g. parameter estimates) for long-term monitoring and/or comparison in future studies focused on spatial and temporal population structure and stability for *B. pumilum* and possibly other small-vertebrate populations. Lastly, this study exemplifies both the necessity and benefits of conducting species-specific investigations for small-vertebrate populations inhabiting fragmented landscapes.

DESCRIPTION OF THE STUDY AREA

This study was conducted within a patch of transformed, fragmented habitat at the Noordhoek Wetlands Nature Reserve (34°11'S, 18°38'E), Cape Town, South Africa. Bordering anthropogenic impacts include residential and commercial real-estate, roads, and recreational parkland. The study area is also bordered by fragmented patches of mixed native and exotic vegetation (specific species listed below). Human impact on the study area includes pedestrian use as a thoroughfare and recreational trail use for walking and horseback riding. The study area was divided into two nested 'sites': an interior 'core site' approximately 5659.0 m^2 completely encompassed within a surrounding 'outer site' approximately 18520.0 m^2 (excluding the area of the core site, Figure 2). A short-term demographic assessment investigating survival and movement for *B. pumilum* was previously conducted on the core site to investigate potential conservation management for chameleons inhabiting highly fragmented, urban habitats (Tolley *et al*. 2010). More detailed demographic parameter estimates and the introduction of genetic parameter estimates are still needed to identify and mitigate potential viability threats to chameleons in these areas; therefore the site was reselected for the current study. The outer site was determined so as to include areas of what appeared to be highly unsuitable vegetation bordering the core site as well as the narrow corridors of suitable vegetation linking the core and outer sites (Figure 2) to test hypotheses regarding *B. pumilum* habitat use within a highly fragmented habitat. The outer site was also shaped by what was judged to be the maximum size within which a manageable survey of the area could be conducted. At its interior, the core site supports a dense, homogenous mix of vegetation, including native shrubs species *Osteospermum* sp. and *Senecio* sp. (Asteracae), and exotic restios (Restionaceae) and reeds (Typhaceae), Port Jacksons (*Acacia saligna*), and papyrus (Cyperaceae). Exterior to the core site supported a patchier distribution of vegetation, including all species found within the interior of the site as well as lawn grasses and a large patch of sand. Outer site vegetation appears predominately split between *(1)* short lawn grasses to the east with a few small patches of sparse vegetation and sand, and *(2)* to the west a less dense, though near identical mix of vegetation found within the interior core site. Connections between the core and outer site existed through narrow corridors of continuous vegetation (Figure 2). Sporadic removal and management of *Acacia saligna* by the City of Cape Town resulted in its discontinuous presence within the study area throughout the study period.

(A)



(B)

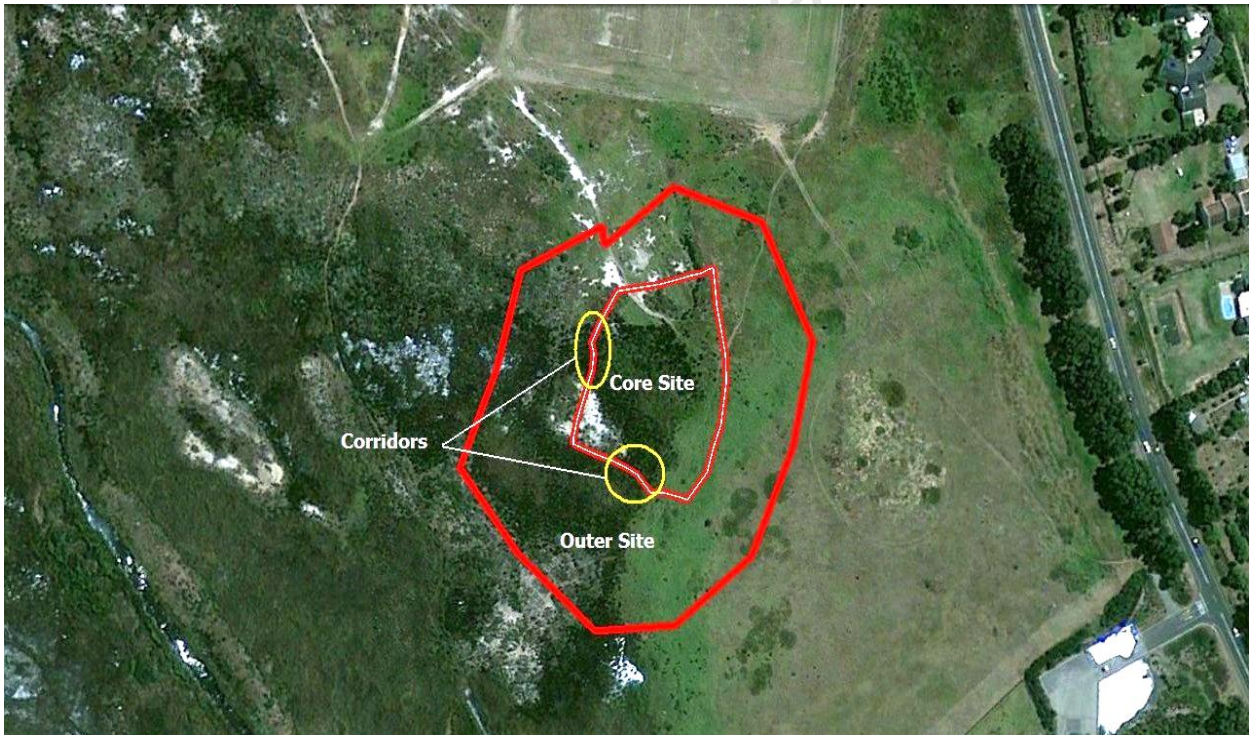


Figure 2 *(A)* Location of the Noordhoek Wetlands Nature Reserve, Western Cape, South Africa (red dot) and species distribution for *Bradypodion pumilum* (yellow dotted lines). *(B)* The perimeters of the 'core' and 'outer' sites superimposed over a Google Earth image.

Appendix 1 Previous studies investigating the effects of habitat fragmentation on herpetofauna.

Study Species (scientific/ common)	**Geographic Location**	**Main Focus**	**Conclusions/Implications**
Amphibian species inhabiting the geographic study area (*n*=10) (Lehtinen *et al*. 1999)	Central and south-western Minnesota (USA)	Impacts of habitat loss and fragmentation on amphibian assemblages in wetland environments.	1.) Species richness was lower in areas with increased isolation and road density, therefore decreases in landscape connectivity via fragmentation and habitat loss can affect amphibian assemblages 2.) Protection and restoration aimed at reversing habitat loss and fragmentation will likely be most effective conservation strategy for wetland complexes. 3.) Additional long-term studies are needed to elucidate patch dynamics and to determine the level of fragmentation at which populations become vulnerable to extinction. 4.) Documenting dispersal patterns, extinctions, and recolonizations through mark-recapture studies or other methods is essential to improving our understanding of the assemblage dynamics.
Anuran species inhabiting the geographic study area (*n*=28) (Vallan 2000)	Ambohintantely Nature Reserve (Madagascar)	The effects of rainforest fragmentation on amphibian diversity.	1.) Species' richness positively correlated with fragment area. 2.) The relative individual density was negatively correlated with the fragment size. 3.) Compared to other taxa, amphibians generally seem to react less sensitively to fragmentation.
Herpetofauna inhabiting the geographic study area (*n*=46) (Alcala *et al*. 2004)	Negros Occidental Province (south-western Negros Island, Philippines)	Determine the amphibian and reptile species inhabiting forest fragments and assess their population densities.	1.) Local extinctions within this area over the last 50 years (16.4-24.6%) are attributed to the removal of large trees and the resulting fragmentation and degradation of the original forest. 2.) Herpetofaunal species exhibited low population densities; has important implications for herpetofaunal species inhabiting the tropical rainforest as it continues to contract in size, become fragmented and be degraded. 3.) Continued related research is critical because 13–42% of animal species in South-east Asia may become extinct over the next century as a result of habitat loss (Brook *et al*. 2003).
Reptile species captured within the geographic study area	South-central New South Wales (Australia)	1.) The effects of habitat loss, patch-size, and vegetation structure on reptile distributions 2.)The level of dispersal existent in reptile	1.) All but one species declined from up to 90% of their former range due to land clearing. 2.) Linear strips do not provide adequate connectivity for many reptile species, either because they are too narrow or because the habitat within them is altered. 3.) Reptile species inhabiting this area have already become locally extinct, and it seems likely that additional species will face extinction without major intervention. Intervention options include habitat restoration (Saunders *et al*. 1993) and properly planned translocations (Burke

(*n*=19) (Driscoll 2004)		populations inhabiting agricultural areas 3.) The effects of land clearing on the reptile community	1991; Dodd and Seigel 1991; Krauss *et al*. 2002). 4.) Supports the urgency of habitat restoration in fragmented areas (Sinclair *et al*. 1995).
Lizard species captured within the geographic study area (*n*=10) (Jellinek *et al*. 2004)	Hobart, Tasmania (Australia)	1.) Lizard species richness, composition, overall abundance and abundance at the species level in urban fragments versus continuous bushland areas 2.) The effects of edge environments on lizard species richness, composition, overall abundance and abundance at the species level 3.) The effects of abiotic and biotic influences on lizard species richness and abundances	1.) Lizard species richness and abundance were not significantly influenced by habitat fragmentation or fragment size. 2.) Vegetation type and structure as well as environmental variables influenced the structure of reptile communities. 3.) Species that were able to use a number of different habitat types were found to persist at most sites, irrespective of fragment size. 4.) Edge environment did not significantly influence lizard species richness or abundance in remnant areas. 5.) Lizard species richness was significantly lower in sites that had a high ratio of exotic to native plant species. Therefore, if remnants continue to be invaded by exotic plants, lizard species that require native plant communities will become increasingly vulnerable to local extinction. 6.) Lizard species requiring specialized habitats may persist in large urban remnants rather than small urban remnants because large reserves are more likely to encompass rare habitats. Habitat heterogeneity, rather than size, may be the key to their persistence.

CHAPTER TWO

MOVEMENT, ABUNDANCE, AND SURVIVAL OF *Bradypodion pumilum* WITHIN A FRAGMENTED, URBAN HABITAT

INTRODUCTION

Species inhabiting fragmented landscapes are often at increased risk of population decline and/or local extinction through deterministic and/or stochastic threats (Soule 1987; Lande 1988; Saunders *et al*. 1991; Vitousek *et al*. 1997; Bender *et al*. 1998; Holsinger 2000; Brown 2001; Dodd and Smith 2003; Driscoll 2004; Bell and Donnelly 2006). Population decline and/or extinction may be particularly evident in small-vertebrate species with limited vagility inhabiting fragmented landscapes (*see* Table 2 and Chapter One; Hanski 1994; With and Crist 1995; Lindenmayer and Possingham 1996; Fahrig 1998; Gibbs 1998; Casagrandi and Gatto 1999; Carr and Fahrig 2001; Fischer and Lindenmayer 2007). For example, the combination of biotic and abiotic factors in such populations is likely to restrict dispersal/migration, hindering the ability to maintain abundance and/or recolonize other imperiled local populations within the overall fragmented landscape (Cooper and Walters 2002; Soule *et al*. 2004). As a result, such populations will likely require conservation management to ensure their continual existence. Investigations uncovering local demography within potentially imperiled populations inhabiting fragmented landscapes will help determine the necessity- and type- of management strategy required for effective species conservation.

Estimating demographic parameters is central to investigations of wildlife management, conservation, and evolutionary ecology (Sandercock 2006). Demographic and statistical models are commonly used to identify threats to local viability and/or monitor the effectiveness of conservation management strategy within potentially imperiled populations (Lande 1993; Lindenmayer *et al*. 1993; Lindenmayer and Possingham 1995; Beissinger and Westphal 1998). Traditionally, demographic models are applied in population viability analysis (PVA): the probability that a population will go extinct in a given number of years (Caughley 1994; Hedrick *et al*. 1996). Alternatively, statistical models are applied outside of PVA, often aimed toward estimating demographic parameters uncovering local population dynamics. In either case, the use of models provides increased knowledge of local demography facilitating effective conservation management (e.g. Hokit and Branch 2003; Tolley *et al*. 2010). Therefore, population assessments to create models estimating local demographic parameters and stability for species inhabiting fragmented landscapes may prove critical to species conservation (Table 3; Caughley 1994; Holsinger 2000).

Demographic parameter estimates (e.g. abundance, survival, and movement) often guide management and conservation decisions for wildlife populations (Sandercock 2006). Population size, as measured by abundance, is the most basic variable of demography (Holsinger 2000). Abundance can be used to determine population growth rate and the combination of the two can then be used to assess potential demographic threats to persistence, i.e. those that arise from changes in the number of individuals present from one generation to the next without respect to any associated genetic changes (Holsinger 2000). In all taxa, the demographic parameters affecting the local abundance of small populations often include survival and movement (e.g. dispersal and migration). Therefore, estimating survival and movement is critical to uncovering local demographic structure (Holsinger 2000, Sandercock 2006).

Estimates of survival are valuable for understanding population dynamics and life-history evolution in wild populations (Stearns 1992; Sandercock 2006). Analyses based on projection matrices frequently identify survival as the demographic parameter with the greatest potential impact on the finite rate of population change (Crone 2001; Sandercock 2006). Survival rates may vary based upon characteristics such as age, sex, mass, genotype, or phenotype, and also as a function of biotic and abiotic environmental variables (Lebreton *et al*. 1992). For example, in declining populations, the rate of population change is often most sensitive to changes in survival rates of the oldest age class or the largest state class (Heppell 1998; Saether and Bakke 2000; Sandercock 2006). High sensitivity implies that management actions affecting survival rates will have the greatest potential to modify rates of population change (Sandercock 2006). This type of knowledge is often beneficial to conservation management strategy.

Uncovering a species' ability to disperse and move through the matrix between patches within the overall fragmented landscape is also critical to the management and conservation of potentially imperiled populations (King and With 2002; Vuilleumier and Metzger 2006; Fischer and Lindenmayer 2007). Modeling these types of movement aid in determining a species'/population's sensitivity to habitat fragmentation (Table 2, Ewers and Didham 2006; Fischer and Lindenmayer 2007). In general, species' with limited dispersal capability are more sensitive to habitat fragmentation (Table 2; Hanski 1994; With and Crist 1995; Lindenmayer and Possingham 1996; Fahrig 1998; Gibbs 1998; Casagrandi and Gatto 1999; Carr and Fahrig 2001; Fischer and Lindenmayer 2007). In-part, this is because one of the more immediate consequences of fragmentation is often the disruption of dispersal or movement

through the matrix between habitat patches, which may lower colonization success and lead to local extinction (King and With 2002; Fischer and Lindenmayer 2007). However, it is important to note that whether or not the level of species-specific vagility is truly advantageous or detrimental to populations inhabiting fragmented landscapes appears dependent on the level of mortality experienced by dispersing individuals within the matrix portion of the landscape (*see* Chapter One; Hanski 1994; With and Crist 1995; Lindenmayer and Possingham 1996; Fahrig 1998; Gibbs 1998; Casagrandi and Gatto 1999; Carr and Fahrig 2001). Additionally, estimating movement also provides useful information for investigating the complex interaction between movement behavior and landscape pattern and use (King and With 2002; Vuilleumier and Metzger 2006). Whether or not dispersal success is affected by landscape pattern depends on the scale of movement relative to the scale of fragmentation (Doak *et al*. 1992; With and King 1999). Examining movement of populations inhabiting fragmented landscapes will uncover aspects of local demography and landscape ecology and should therefore prove an essential conceptual tool for landscape conservation planning (Kareiva and Wennergren 1995; King and With 2002; Kramer-Schadt *et al*. 2004; Vuilleumier and Metzger 2006).

Previous study supports the necessity/benefit of conducting population assessments towards the creation of statistical models to estimate demographic parameters such as survival, abundance, and/or movement within herpetofauna and other small-vertebrate species (e.g. Coffman *et al*. 2001; Tolley *et al*. 2010; Table 3).

Table 3 Studies incorporating statistical models to estimate life-history parameters.

Study Species (scientific/ common)	**Geographic Location**	**Duration of Study**	**Field Methods**	**Number of Samples**	**Main Focus**	**Conclusions/Implications**
Bradypodion pumilum (Cape Dwarf Chameleon) (Tolley *et al*. 2010)	Noordhoek Wetlands Nature Reserve Cape Town, South Africa	March-May, 2009	Capture-mark-recapture survey	97 Individuals (multiple captures for some individuals)	Estimate survival and migration of chameleons inhabiting a fragmented, urban habitat	1.) Smaller chameleons have a substantially lower survival per ten-day period than larger chameleons. 2.) Male chameleons were more prone to temporary emigration than females, and smaller chameleons more readily moved between sites than larger chameleons.
Microtus pennsylanicus (Meadow Vole) (Coffman *et al*. 2001)	Patuxent Wildlife Research Center, Laurel, Maryland, USA	April, 1993-September, 1994	Capture-mark-recapture survey	5,700	The effects of corridors on local population dynamics.	1.) Movement rates increased to a greater extent on constructed corridor-linked grids than on the non-fragmented or non-linked fragmented grids. 2.) Significant differences in local survival were found on the corridor-linked grids compared to the fragmented and non-fragmented grids. 3.) Non-fragmented grids were found to be more stable than the fragmented grids based on lower temporal variability in population size. 4.) Demonstrates that corridors constructed between existing fragmented populations can indeed cause increases in movement and associated changes in demography, supporting the use of constructed corridors in conservation biology.

Findings from additional studies investigating abundance, survival, movement, and/or other demographic parameters within lizard populations further support the necessity of investigating potentially imperiled herpetofauna inhabiting fragmented landscapes. Though overall abundance often declines in areas following urban development, some lizard species may be capable of persisting in localized remnants (Endriss *et al*. 2007). Assessing adult and juvenile survival rates and adult reproductive output for populations inhabiting such areas can be used to determine their viability (Endriss *et al*. 2007). For example, the Texas horned lizard, *Phrynosoma cornutum*, is characterized by high survival and moderate reproductive output and is capable of persisting within small urban remnants (Endriss *et al*. 2007). Other research suggests the extent of connectivity through the matrix between remnants patches can directly modify demographic parameters for lizards inhabiting fragmented habitats (Boudjemadi *et al*. 1999). For example, dispersal patterns, survival rates, and reproduction are three demographic parameters sensitive to patch connectivity for the common lizard, *Lacerta vivipara* (Boudjemadi *et al*. 1999). Therefore, conservation management strategy for the common lizard and similar species (e.g. installation of dispersal corridors), should take both landscape characteristics and behavioral features into account (Boudjemadi *et al*. 1999).

Although previous demographic assessments investigating abundance, survival, and/or movement already exist for lizard species inhabiting fragmented environments (e.g. Boudjemadi *et al*. 1999; Hokit *et al*. 1999; MacNally and Brown 2001; Driscoll 2004; Jellinek *et al*. 2004; McCoy *et al*. 2004; Bell and Donnelly 2006; Endriss *et al*. 2007; Tolley *et al*. 2010; Rubio and Simonetti 2011), chameleons are still understudied in this respect (Tolley *et al*. 2010). While lizards do show common demographic trends and patterns (i.e. typically low survival and high fecundity), potential exists for family/species-specific responses to the effects of fragmentation due to differing biological attributes and habitat requirements (Hokit *et al*. 1999). Because of this, demographic assessments for chameleon populations inhabiting fragmented landscapes are needed to uncover local demography and aid in conservation management strategy. Additionally, as restoration and maintenance of urban fragments often incurs great cost and effort, knowledge of long term viability potential and effective management strategy for chameleon populations is essential (Tolley *et al*. 2010).

The Cape Dwarf Chameleon (*Bradypodion pumilum*) inhabits fragmented, urban habitats within the city of Cape Town, South Africa. Much of its distribution lies within the metropolitan area where severe habitat loss (Driver *et al*. 2005; Rebelo *et al*. 2011) is likely inducing negative effects on

the inhabiting populations (K.A. Tolley, personal communication). A short-term demographic assessment investigating survival and movement for *B. pumilum* has provided some insight into potential conservation management for chameleons inhabiting highly fragmented, urban habitats (Tolley *et al*. 2010). However, more detailed demographic parameter estimates are needed to identify and mitigate potential viability threats to chameleons in these areas.

This portion of my study focuses on temporal monitoring of population demography and habitat use for a group of Cape Dwarf Chameleons inhabiting a patch of transformed, fragmented habitat at the Noordhoek Wetlands Nature Reserve, Cape Town, South Africa. Demographic and habitat use data was collected from a combination of capture-mark-recapture-, radio-tracking-, and vegetation survey conducted within a one year period from *B. pumilum* inhabiting the Noordhoek site. Demographic data was used to estimate *B. pumilum* population demographic parameters and their fluctuations. Habitat use data was analyzed to investigate *B. pumilum* vegetation use and the potential benefit of corridors of continuous vegetation connecting fragmented patches in a patch of transformed fragmented habitat. The main aims of this portion of my study are:

1.) *Estimate survival, abundance, and movement for a group of* Bradypodion pumilum.
2.) *Examine variation in thirty-day and/or annual survival, abundance, and movement rates for a group of* Bradypodion pumilum.
3.) *Investigate* Bradypodion pumilum *use of native and exotic vegetation.*
4.) *Investigate* Bradypodion pumilum's *use of corridors of continuous vegetation to move between otherwise fragmented patches.*

Hypotheses (for *Bradypodion pumilum* inhabiting the Noordhoek site):

1.) *Annual survival similar to other small-bodied lizards. Thirty-day survival rates are anticipated to be (1) similar amongst males and females, (2) higher for larger chameleons versus smaller individuals, and (3) variable with season.*

A short-term demographic study monitoring survival ($\boldsymbol{\Phi}$) for *B. pumilum* estimated ten-day rates ranging from 0.49 to 0.98 dependent on- and positively correlating with- body-size and showing no differences between males and females (Tolley *et al*. 2010). From these values, an approximate range estimation for annual survival rate ($\boldsymbol{\Phi_A}$) can be extrapolated ($\boldsymbol{\Phi_A} = \boldsymbol{\Phi^{36}} =$ *7.03E-12* to *0.48*). The range of these values is large therefore $\boldsymbol{\Phi_A}$ from additional studies focused on other small-bodied lizards were referenced to aid in generating predictions ($\boldsymbol{\Phi_A} <$ 0.30, Schoener and Schoener 1982; Wright *et al*. 1984; Andrews and Nichols 1990). Though no previous work has investigated seasonal variation in *B. pumilum* survival, other studies indicate seasonal abiotic factors (e.g. temperature and moisture) often exert proximate influences on lizard life histories, including survival (Tinkle 1972; Ballinger 1977, 83; Dunham 1978, 81; Abts 1987; Jones and Ballinger 1987; Jones *et al*. 1987; Sinervo and Adolph 1989; Sinervo 1990).

2.) *Low levels of chameleon movement between the core site and adjacent vegetation fragments (separated by the matrix). Movement is anticipated to be higher amongst males than females. All movement between fragments is anticipated to be reliant upon linking corridors of suitable vegetation rather than movement across the ground.*

A short-term demographic study monitoring movement for *B. pumilum* found equal bi-directional ten-day migration rates (γ'' and γ') indicative of male-bias dispersal ($\gamma'' = \gamma' = 0.20$ in males; $\gamma'' = \gamma' = 0.05$ in females, Tolley *et al*. 2010). *Bradypodion* species are primarily arboreal; therefore in areas containing fragmented vegetation (e.g. the study area) they often experience restricted distributions, separated by gaps of unsuitable habitat. Movement between

these gaps may be restricted by the availability of connecting corridors of continuous vegetation.

3.) Bradypodion pumilum *use both native and exotic vegetation for perching, movement, and all other routine activities.* Bradypodion pumilum *are anticipated to abstain from intentional movement off of suitable vegetation to actively engage short lawn grass or bare ground (other than to escape predators).*

Bradypodion pumilum's species distribution spans a range of habitats including fynbos, renosterveld, thicket, exotic and native trees and riparian vegetation (Tolley and Burger 2007). *Bradypodion* species are primarily arboreal and *B. pumilum* microhabitat choice is based on perch size rather than vegetation species (Herrel *et al*. 2011). Previous study indicates that dwarf chameleons of the genus *Bradypodion* may drop to the ground from vegetation as an anti-predator escape tactic (Stuart-Fox *et al*. 2006); however, no other use of bare ground or short ground vegetation (lawn grasses) for routine daily activity has been documented.

Results from this study will further our understanding of population demography and habitat use for chameleons inhabiting fragmented landscapes and possibly other small-vertebrate populations, enabling effective conservation management strategy. Additionally, results will provide empirical data (e.g. parameter estimates) for comparison in future studies focused on population demography and stability for *B. pumilum* and other small-vertebrate populations. Lastly, results will help determine the need- and type- of preliminary management recommendations required for *B. pumilum* inhabiting the patch of transformed, fragmented habitat at the Noordhoek Wetlands Nature Reserve.

MATERIALS, METHODS, AND ANALYSIS

Chameleon Field Survey

This capture-mark-recapture (CMR) study followed a Robust Design (RD) where primary sessions consisted of multiple secondary sampling occasions during which the system was assumed closed to migration, death, and recruitment (Kendall *et al*. 1997). Closure was not assumed between primary sessions creating a combination of open and closed designs that enabled estimation of survival, abundance, and temporary emigration.

Primary sessions (n =11) were conducted monthly, 24-33 days apart[1], over a one year period (30 November 2009- 4 November 2010). Within each primary session, five secondary sampling occasions[2] were conducted over five consecutive nights, for two hours[3], after dark. There were four secondary occasions[4] inclusive to the core site (Figure 2) and a final sampling occasion devoted to surveying the outer site[5] (Figure 2). During survey, a team of two field workers located and captured adult chameleons[6] ($\geq$ 40 mm snout-vent length) with the aid of torch light. Nocturnal surveys were conducted because *B. pumilum* is readily visible at night when asleep, as they tend to perch higher up vegetation enabling their pale coloration to be distinguished against darker vegetation via torchlight (Tolley and Burger 2007). Secondary sampling occasions were initiated from alternating start points, progressing in a circular direction, switching nightly between clockwise and counter-clockwise directions. Prior to commencing nightly sampling, local weather observations (e.g. wind-strength and precipitation) were recorded.

[1] A 45 day gap separated primary sessions #8 and #9

[2] Four secondary sampling occasions were conducted within primary sessions #1 and #11; three were conducted within primary session #8

[3] One secondary sampling occasion was terminated early due to weather within primary sessions #6 and #11; two secondary sampling occasions were terminated early due to weather within primary session #7

[4] Three core site sampling occasions were conducted within primary session #8

[5] No outer site sampling occasions were conducted within primary sessions #1, #8, or #11

[6] Minimum male and female adult sizes were determined using male chameleons. In adult males, hemipenes were exposed, indicating sexual maturity. Results from previous study indicated a similar adult size (> 41.5 mm, Jackson 2007).

Upon encounter, point coordinate locations (latitude/longitude) were recorded for captured chameleons (Garmin GPS Map 60Cx). Chameleons were then removed from their roost and perch vegetation data was recorded. First-time captures received two marks, a unique identification number written on the ventral surface (using indelible ink) and a small (< 3 mm) tail clipping was collected to serve as a batch mark and provide a tissue sample for future DNA analysis (*see* Chapter Three; Tolley *et al*. 2010). Photographs (Cannon Power Shot SX100 IS) were taken of the right body side, gular region, and top of the head for all encountered chameleons and used to identify individuals that had lost their ink number after shedding, but who were identified as recaptures through the batch mark (Figure 3; Tolley *et al*. 2010). Sex was distinguished by the presence or absence of a hemipenal bulge (present in males, absent in females) and in unclear cases, males were identified by everting the hemipenes. Snout-vent length (SVL) and tail length (TL) (nearest 0.1 mm) and mass (nearest 0.25 g) were recorded for all chameleons using a set of calipers ($\pm$ 0.1 mm accuracy) and a spring-scale (PESOLA Micro-Line 20030). Chameleons were handled for less than five minutes (usually ca. two minutes) upon each capture to minimize disturbance to the animal. After processing, chameleons were returned to the exact perch where they were found. The duration of total search time was recorded at the conclusion of each night of sampling.

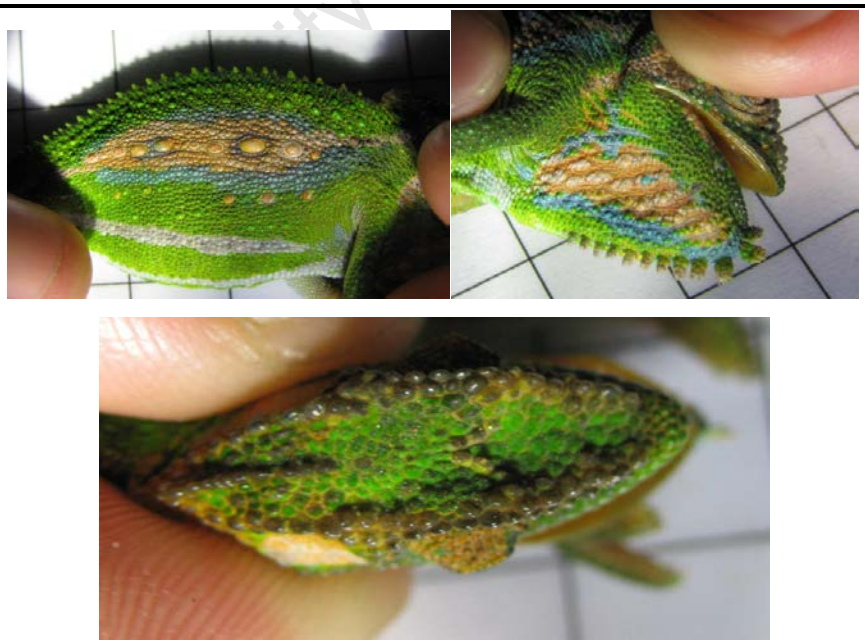


Figure 3 Typical photographs of the right body-side (upper-left), gular region (upper-right), and top of the head (bottom) taken during capture-mark-recapture study.

Survival, Abundance, and Movement

Standard capture-mark-recapture (CMR) models implemented in MARK 5.1 (White and Burnham 1999) were used to estimate survival, abundance, and site movement (temporary emigration; inter-site movement) (Lebreton *et al*. 1992; White and Burnham 1999). Survival, abundance, and temporary emigration estimates were generated solely using data from the core site while inter-site movement was estimated incorporating encounter data from the outer site as well. Underlying assumptions of the CMR models are equal probability of survival and recapture for all individuals, marks are not lost or missed, and all samples are instantaneous, relative to the interval between (i) and (i + 1).

Cormack-Jolly-Seber Models

The Cormack-Jolly-Seber (CJS) approach was selected for preliminary survival analysis, chosen for its basic structure and availability of well-established goodness of fit (GoF) tests (Burnham *et al.* 1987). CJS models are composed of two sets of parameters: $\boldsymbol{\Phi}$ (survival rate) and $\boldsymbol{P}$ (capture probability). Individual encounter histories created for CJS analysis were pooled across secondary sampling occasions (Tolley *et al*. 2010). Time intervals between primary sessions were standardized around a basal time unit of thirty days (Tolley *et al*. 2010). Testing the general model for GoF utilized program RELEASE ('tests 2 and 3', Burnham *et al.* 1987) and the median-$\hat{c}$ approach in program MARK (White and Burnham 1999). The general model was fit incorporating the effects of time and sex upon survival rate and capture probability, following which subsequent models were fit using simplified versions of the general model. Standard procedures in model fitting and notation were used, and model selection was based on Akaike's Information Criterion (Lebreton *et al*. 1992). Additional covariates including: *(1)* the effect of season[7] and/or search time upon capture probability and *(2)* the effect of season upon survival rate were added to the most parsimonious models. Models were then reanalyzed to determine the most parsimonious models. Annual survival rate ($\boldsymbol{\Phi_A}$) was derived from the thirty day survival estimates ($\boldsymbol{\Phi_A} = \boldsymbol{\Phi^{12}}$).

[7] Seasons: *(1)* December-March, *(2)* April-May, *(3)* June-August, and *(4)* September-November

Robust Design Models

The Robust Design (RD) with Huggins closed captures approach was selected for final analysis, estimating survival, abundance, and temporary emigration (Tolley *et al*. 2010). RD models allow for heightened flexibility when estimating population demographics by introducing parameters for temporary unavailability (interpreted as temporary emigration from the core site; Kendall *et al*. 1997; Cooch and White 2009). RD models are composed of five sets of parameters: $\boldsymbol{S}$ (survival rate), γ'' (emigration rate), γ' (immigration rate), $\boldsymbol{P}$ (capture probability), and $\boldsymbol{C}$ (recapture probability), with site abundance estimated as a derived value (Kendall *et al*. 1997). The RD approach has additional underlying assumptions to those previously mentioned: the population is closed to additions and deletions across secondary sampling occasions within a primary session; temporary emigration is either completely random, Markovian, or non-existent; and survival rate is the same for all animals in the population, regardless of availability for capture (Kendall *et al*. 1997). The assumption of closure across secondary sampling occasions may be violated here, especially with respect to emigration (Tolley *et al*. 2010); however, as long as emigration is either random or non-existent; the parameter estimates should remain unbiased (Kendall 1999). 'No-emigration' and random movement models combined to accumulate approximately 76% of the overall model support during primary periods (Table 5), it is therefore reasonable to assume that movement between secondary occasions was also either close to random or non-existent (Tolley *et al*. 2010). There is no specific GoF analysis for the RD with Huggins closed captures approach; however, RD models share assumptions with the CJS approach and therefore GoF for RD models can be satisfied by previous GoF testing conducted within CJS analysis (following, e.g. Tolley *et al*. 2010). Model building, fitting, notation, selection, and annual survival estimation followed procedures applied within CJS analysis, though incorporating additional appropriate parameters. Additional covariates were used: *(1)* the effects of season, wind, or search time upon (re)capture probability and *(2)* the effect of season upon survival rate.

Multi-Strata Models

The Multi-strata (MS) approach was selected to estimate *(1)* the effect of chameleon body-size upon survival and *(2)* inter-site movement between the core and outer sites (Cooch and White 2009; Tolley *et al*. 2010).

Body size was anticipated to affect chameleon survival within the study site (Tolley *et al*. 2010). Incorporating individual covariates that stay constant for the duration of the study is straight-forward in the CMR modeling framework, however, notable growth of individual chameleons was observed during the study and body-size could therefore not be treated as constant. To examine the effect of size upon survival while allowing for growth, a multi-strata approach was used for $\boldsymbol{\Phi}$ where the strata were size classes based upon SVL[8] with transitions between the strata, $\boldsymbol{\psi}$, to account for growth. Secondary occasions were pooled, and because chameleons do not shrink, transition rates to smaller size classes were set to zero. GoF analysis relied upon the median-$\hat{c}$ approach in program MARK. Model building, fitting, notation, and selection followed procedures applied within CJS analysis. In a different application of the multi-strata approach, physical movement was estimated between two geographic areas, treating the core site and the outer site as two different strata (Figure 2). The parameters within this model were $\boldsymbol{\Phi}$ (survival rate), $\boldsymbol{P}$ (capture probability), and inter-strata movement ($\boldsymbol{\psi_{co}}$ {core site to outer site} and $\boldsymbol{\psi_{oc}}$ {outer site to core site). The general model incorporated the effects of time, sex, and site upon survival rate and capture probability and the effects of time and sex upon movement. Subsequent models were fit using simplified versions of the general model. Time intervals, GoF analysis, model fitting procedures, notation, and selection all followed procedures used within previous MS analysis.

[8]Size Classes (SVL): *(1)* < 50.0 mm, *(2)* 50.0-60.0 mm, *(3)* 60.0-70.0 mm, and *(4)* > 70.0 mm

Radio-Tracking Survey

The radio-tracking (RDT) study incorporated repetitive chameleon location coupled with short-term observation to provide site movement and habitat use data for resident chameleons. Six previously encountered chameleons (CMR recaptures) were selected (12-15 March 2010) from different locations spanning the core site: three males and three females weighing at least 6.5g, with no physical deformities. GPS point coordinates were recorded at the capture site and these individuals were placed in cloth bags for transport to a field station for formal measurement, transmitter attachment, and post-attachment observation. Prior to transmitter attachment, measurements and data collection included: CMR unique identification number, mass (nearest 0.25g) SVL and TL measurements (nearest 0.1mm), and photo documentation.

RDT equipment included transmitter Model BD-2 (Holohil Systems Ltd.) and radio receiver Model TRX-1000WR (Wildlife Materials Inc.). *Bradypodion* have not been the subject of previous RDT study, but other species of chameleon have been successfully radio tracked using a similar set up: *Chameleo chameleon* (Cuadrado 2001) and *Furcifer labordi* (Karsten *et al*. 2008; K. Karsten, personal communication). Custom-designed transmitters with a modified arch shape for dorsal attachment (Cuadrado 2001), and mass of 0.65g were attached to chameleons with tissue glue (1.0x0.5 mL Histoacryl, AESCULAP). Transmitters were only attached to individuals where total mass of the transmitter accounted for less than 10% of body mass, as recommended by the tag manufacturer. After attachment, each chameleon was observed for a period of several hours to ensure that the attachment was not snagging or loose, and that transmitter placement minimized the risks of discomfort, immobility, or becoming stuck on vegetation. Individuals were then returned to the study area and released at their encounter location.

RDT study was conducted across a thirteen day period (16-28 March 2010), initiating daily survey prior to sunrise (06:30-08:00) and continuing through to and commencing at dusk (18:30-19:30). Start and end times were based upon a predicted ‘activity period’ outside of which chameleons were believed to remain immobile upon their nighttime perch (E.M. Katz, personal observation). Daily surveys were separated into three to five ‘observation sessions’ as time permitted, during which tagged chameleons were located and observed in rounds. The order of chameleon location remained consistent across observation sessions throughout a single day, however, daily orders were alternated to minimize bias in data collection.

Tagged chameleons were located via radio receiver by triangulating an approximate location and subsequently adjusting the receiver signal strength to home in on a more precise location leading to visual confirmation. Upon location, GPS point coordinate location and perch vegetation data were recorded.

During the RDT study, two of the tagged chameleons lost their transmitters and were replaced by new individuals. On 17 March 2010 chameleon (ID: N684) was replaced by chameleon (ID: N670) and on 23 March 2010 chameleon (ID: N522) was replaced by chameleon (ID: N690). Replacement chameleons were slightly larger than the original chameleons, and thus the transmitters had a better fit.

At the end of the thirteen day study period, all tagged chameleons were recaptured and transported to a field station where the transmitters were removed through the light application of acetone to the glue, as recommended by a certified veterinarian (T. Townsend, personal communication). Chameleons were then observed for a day for any adverse effects from transmitter removal before returning them to the study area to be released.

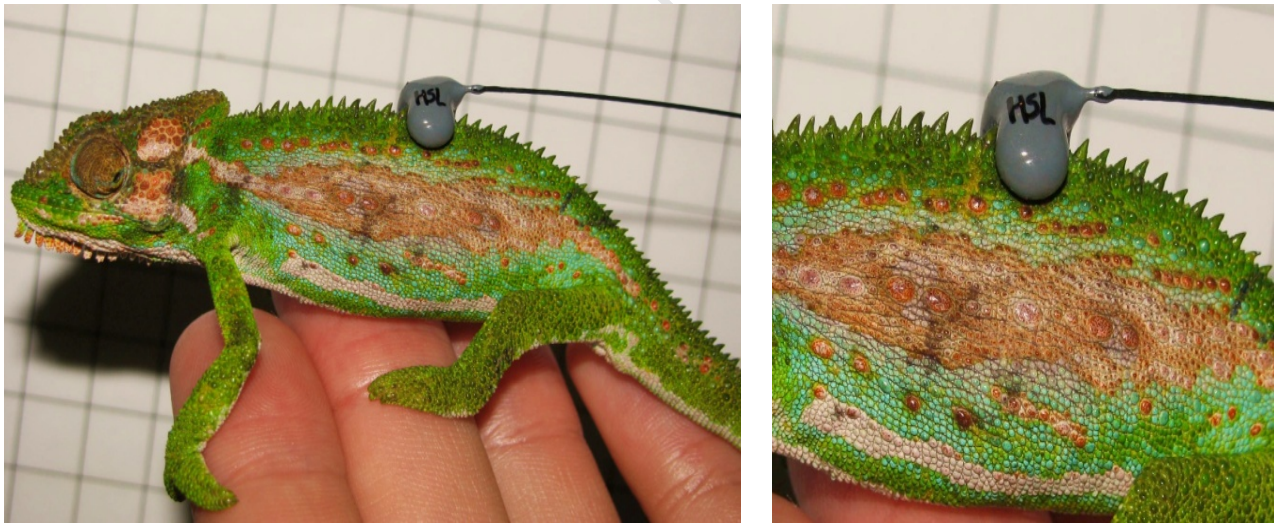


Figure 4 Chameleon with radio-transmitter attached.

Site Movement and Habitat Use

GPS point coordinate capture locations collected during CMR and RDT surveys were uploaded into Google Earth and superimposed over satellite images of the study area containing outlines of the core and outer sites (Figure 2). Point-coordinate maps served as a secondary source of temporary emigration and inter-site movement analysis and were combined with personal observation to suggest broad scope habitat use.

RESULTS

Throughout capture-mark-recapture (CMR) survey 728 chameleons (re)captures were conducted in which 118 individual males and 101 individual females were encountered (Appendix 2). Two-sample *t*-tests indicated a significantly greater snout-vent-length (t = 3.4537, $d.f.$ = 720, P-value = 0.0006) and mass (t = 5.7706, df = 681, P-value < 0.0001) in females, and a greater tail-length (t = 8.0125, $d.f.$ = 7230, P-value < 0.0001) in males (Appendix 2).

Cormack-Jolly-Seber (CJS) Models

Goodness of Fit

Goodness of fit tests (program RELEASE, 'tests 2 and 3'; median $\hat{c}$ approach in program MARK) showed that the general model (Table 4) provided a good fit of the data, with slight under dispersion (χ^2 = 23.59, $d.f.$ = 38, P-value = 0.968; ĉ = 0.62 {RELEASE, tests 2 and 3}; ĉ = 0.99, S.E. = 0.020 {median ĉ}). The data did not therefore violate the model assumptions significantly.

Model Selection

The most parsimonious model suggested a thirty day survival rate of 0.78 (95% confidence interval 0.74-0.82) independent of both time and sex, accumulating approximately 59% of the model support (Table 4). Model 2 allowed for sex-dependent survival independent of time, but its deviance was almost identical with model 1, which shows that adding a parameter for the sex effect did not improve the fit of the model (Table 4). Time-dependent survival constrained by season improved model support more than four-fold compared to full time dependence (Model 3 vs. 4), but still had over three times less model support than the most parsimonious time-independent survival model (Table 4). Additional models allowing for sex- and/or time-dependent survival rates were all less well supported by the data (Table 4). Annual survival rate was estimated at 0.05 ($\boldsymbol{\Phi_A} = 0.7812265^{12} \rightarrow \boldsymbol{\Phi_A} =$ 0.0516). The four most parsimonious models suggested an exclusively time-dependent capture probability ranging from 0.21 (0.10-0.38) to 0.95 (0.71-0.99), accumulating approximately 99% of the model support (Table 4). Season and search time covariates applied to capture probability reduced model parsimony (Table 4).

Table 4 Summary of Cormack-Jolly-Seber model selection for *Bradypodion pumilum*. The models consist of two sets of parameters: ***Φ*** (survival rate) and ***P*** (capture probability). Model selection was based on the sample-size adjusted Akaike's Information Criterion (**AICc**). ***K*** is the number of estimated parameters.

Rank	Model	AICc	ΔAICc	AICc Weight	*K*	Deviance
1	{***Φ***() ***P***(time)}	804.276	0.000	0.587	11	329.108
2	{***Φ***(sex) ***P***(time)}	806.350	2.074	0.208	12	329.044
3	{***Φ***(season) ***P***(time)}	806.797	2.522	0.166	14	325.178
4	{***Φ***(time) ***P***(time)}	809.932	5.657	0.035	19	317.310
5	{***Φ***() ***P***(sex*time)}	814.735	10.460	0.003	21	317.622
6	{***Φ***(sex) ***P***(sex*time)}	816.852	12.576	0.001	22	317.473
7	{***Φ***() ***P***(search time)}	829.728	25.453	0.000	3	371.242
8	{***Φ***() ***P***(season)}	832.629	28.353	0.000	5	370.042
General (GoF)	{***Φ***(sex*time) ***P***(sex*time)}	835.900	31.624	0.000	38	298.388

Robust Design Models

Goodness of Fit

Goodness of fit for Robust Design (RD) models was satisfied using GoF tests from CJS analysis (*see above*).

Model Selection

RD analysis confirmed results obtained within pooled secondary analysis (Table 4). The most parsimonious models suggested thirty day survival rates of 0.78-0.79 (95% confidence interval 0.73-0.83) independent of both time and sex, accumulating approximately 47% of the model support (Models 1-3, Table 5). Model 4 allowed for sex dependent survival independent of time, but its deviance was almost identical with model 1, which shows that adding a parameter for the sex effect did not improve the fit of the model (Table 5). Time-dependent survival constrained by season improved model support more than five-fold compared to full time dependence (Model 5 vs. 18), but still had over three times less model support than the most parsimonious time-independent survival model (Table 5). Additional models using one of the parameter formats included within one of the top five

models were all less well supported by the data (Table 5). Within the most parsimonious model annual survival rate was estimated at 0.05 ($\boldsymbol{S_A} = 0.7776875^{12} \rightarrow \boldsymbol{S_A} = 0.0489$).

The top five models suggested temporary emigration ($\boldsymbol{\gamma''}$) and immigration ($\boldsymbol{\gamma'}$) were independent of both time and sex including: models suggesting no temporary emigration or immigration ($\boldsymbol{\gamma''=\gamma'=0}$) (Models 1, 4, and 5), a model suggesting emigration equals immigration at a rate of 0.04 (0.01-0.23) under random movement (Model 2), and a model suggesting an emigration rate of 0.04 (0.01-0.18) and an immigration rate of 0.67 (0.03-0.99) under Markovian movement (Model 3) (Table 5). All models carrying an AICc weight $>1.0 \times 10^{-5}$, suggested time-dependent (re)capture rates were equal and independent of sex, with top model estimates ranging from 0.90×10^{-15} ($0.00\text{-}0.14 \times 10^{-7}$) to 0.70 (0.53-0.83) (Model 1, Table 5). Season, wind-strength, and/or search time covariates applied to (re)capture probability reduced model parsimony (Model(s) 19-21, Table 5). Monthly abundance estimates ranged from 11.4 (6.9-26.2) to 50.4 (38.6-75.2) for males and from 13.7 (10.5-24.4) to 41.4 (31.4-63.3) for females within the most parsimonious model (Table 5, Figure 5).

Table 5 Summary of Robust Design model selection, using Huggins closed captures, for *Bradypodion pumilum*. The models consist of five sets of parameters: ***S*** (survival), γ'' (emigration), γ' (immigration), ***C*** (capture), and ***P*** (recapture) rates. Model selection was based on the sample-size adjusted Akaike's Information Criterion (**AICc**). ***K*** is the number of estimated parameters.

Rank	Model	AICc	ΔAICc	AICc Weight	*K*	Deviance
1	{***S***() γ''=γ'(Fixed at 0) ***P***=***C***(time)}	2711.858	0.000	0.236	44	3061.366
2	{***S***() γ''=γ'() ***P***=***C***(time)}	2712.660	0.802	0.158	45	3059.843
3	{***S***() γ''() γ'() ***P***=***C***(time)}	2714.089	2.231	0.077	46	3058.938
4	{***S***(sex) γ''=γ'(Fixed at 0) ***P***=***C***(time)}	2714.126	2.268	0.076	45	3061.308
5	{***S***(season) γ''=γ'(Fixed at 0) ***P***=***C***(time)}	2714.492	2.633	0.063	47	3056.999
6	{***S***() γ''=γ'(sex) ***P***=***C***(time)}	2714.514	2.656	0.063	46	3059.363
7	{***S***(sex) γ''=γ'() ***P***=***C***(time)}	2714.936	3.078	0.051	46	3059.785
8	{***S***(season) γ''=γ'() ***P***=***C***(time)}	2715.363	3.505	0.041	48	3055.520
9	{***S***() γ''(sex) γ'() ***P***=***C***(time)}	2715.500	3.642	0.038	47	3058.007
10	{***S***() γ''() γ'(sex) ***P***=***C***(time)}	2715.650	3.792	0.035	47	3058.157
11	{***S***(sex) γ''() γ'() ***P***=***C***(time)}	2716.373	4.514	0.025	47	3058.880
12	{***S***(sex) γ''=γ'(sex) ***P***=***C***(time)}	2716.729	4.871	0.021	47	3059.236
13	{***S***() γ''=γ'(time) ***P***=***C***(time)}	2717.002	5.144	0.018	54	3042.883
14	{***S***(sex) γ''(sex) γ'() ***P***=***C***(time)}	2717.154	5.296	0.017	48	3057.311
15	{***S***(season) γ''() γ'() ***P***=***C***(time)}	2717.269	5.411	0.016	49	3055.068
16	{***S***() γ''=γ'(season) ***P***=***C***(time)}	2717.405	5.547	0.015	48	3057.562
17	{***S***() γ''(sex) γ'(sex) ***P***=***C***(time)}	2717.669	5.811	0.013	48	3057.826
18	{***S***(time) γ''=γ'(Fixed at 0) ***P***=***C***(time)}	2717.792	5.934	0.012	53	3046.074
19	{***S***() γ''=γ'(Fixed at 0) ***P***=***C***(search time)}	2726.596	14.738	0.000	3	3164.952
20	{***S***() γ''=γ'(Fixed at 0) ***P***=***C***(season)}	2766.462	54.604	0.000	17	3175.840
21	{***S***() γ''=γ'(Fixed at 0) ***P***=***C***(wind strength)}	2801.760	89.902	0.000	5	3236.057
General (GoF)	{***S***(sex*time) γ''(sex*time) γ'(sex*time) ***P***(sex*time) ***C***(sex*time)}	3041.428	329.570	0.000	198	2902.640

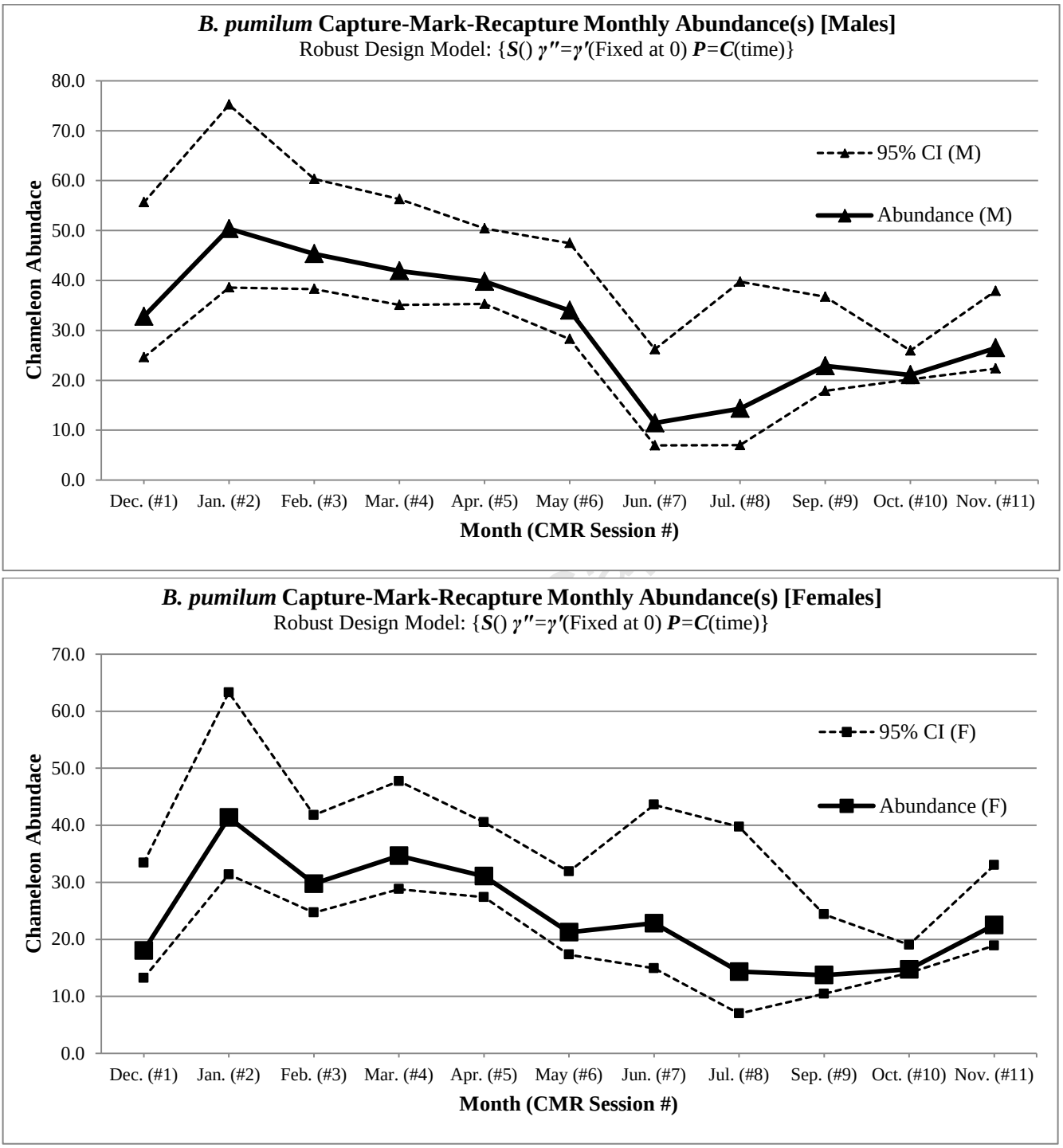


Figure 5 Male and female abundance estimates (separate) obtained from the most parsimonious Robust Design model (Table 5) for *Bradypodion pumilum* in a habitat patch near Noordhoek, South Africa during 2009/2010. The solid lines represent Robust Desgin abundance estimates with accompanying dotted lines representing 95% confidence intervals.

Multi-Strata Models

Multi-Strata Size-Survival Models

Goodness of Fit

The goodness of fit test (median ĉ approach in program MARK) showed that the model allowing for the effects of body size and sex upon survival and size-category movement and the effects of time upon capture rates (Model 7, Table 6) provided a good fit of the data (ĉ = 0.99, S.E = 0.039). The data did not therefore violate the model assumptions significantly.

Model Selection

Three of the four most parsimonious models suggested size-dependent survival rates, accumulating approximately 90% of the model support (Table 6). The top model, accumulating approximately 72% of the model support, suggested size-dependent thirty day survival rates independent of sex or time of: 0.56 (95% confidence interval 0.39-0.72 for chameleons <50.0 mm), 0.78 (0.65-0.87 for chameleons 50.0-60.0 mm), 0.76 (0.67-0.83 for chameleons 60.0-70.0 mm), and 0.84 (0.78-0.89 for chameleons >70.0 mm) (Table 6, Figure 6). All models carrying an AICc weight $>1.0 \times 10^{-5}$, suggest time-dependent capture probabilities independent of sex and size, with top model estimates ranging from 0.21 (0.10-0.39) to 0.95 (0.70-0.99) (Table 6). Chameleon growth was almost exclusively size class dependent, sex- and time-independent accumulating approximately 97% of the model support within appropriate models (Table 6). During thirty day periods, chameleons most commonly grew into the next largest size class, however, growth across two size classes did occur (Table 7). Smaller chameleons were more likely to grow into the next size class than larger individuals.

Table 6 Summary of Multi-Strata size-survival models for *Bradypodion pumilum*. The models consist of three sets of parameters: $\boldsymbol{\Phi}$ (survival rate), $\boldsymbol{P}$ (capture probability), $\boldsymbol{\psi}$ (transitions among size categories). Model selection was based on the sample-size adjusted Akaike's Information Criterion (**AICc**). $\boldsymbol{K}$ is the number of estimated parameters.

Rank	Model	AICc	ΔAICc	AICc Weight	*K*	Deviance
1	{$\boldsymbol{\Phi}$(size) $\boldsymbol{P}$(time) $\boldsymbol{\psi}$(size}	1004.984	0.000	0.724	20	662.356
2	{$\boldsymbol{\Phi}$(sex*size) $\boldsymbol{P}$(time) $\boldsymbol{\psi}$(size)}	1008.168	3.184	0.147	24	656.453
3	{$\boldsymbol{\Phi}$() $\boldsymbol{P}$(time) $\boldsymbol{\psi}$(size)}	1009.593	4.610	0.072	17	673.644
4	{$\boldsymbol{\Phi}$(size) $\boldsymbol{P}$(time) $\boldsymbol{\psi}$(sex*size)}	1011.418	6.434	0.029	26	655.078
5	{$\boldsymbol{\Phi}$(sex) $\boldsymbol{P}$(time) $\boldsymbol{\psi}$(size}	1011.742	6.759	0.025	18	673.580
6	{$\boldsymbol{\Phi}$() $\boldsymbol{P}$(time) $\boldsymbol{\psi}$(sex*size)}	1015.802	10.818	0.003	23	666.379
7 (GoF)	{$\boldsymbol{\Phi}$(sex*size) $\boldsymbol{P}$(time) $\boldsymbol{\psi}$(sex*size)}	1044.222	39.238	0.000	42	648.819
General	{$\boldsymbol{\Phi}$(sex*time*size) $\boldsymbol{P}$(sex*time*size) $\boldsymbol{\psi}$(sex*size)}	1297.146	292.163	0.000	140	540.711

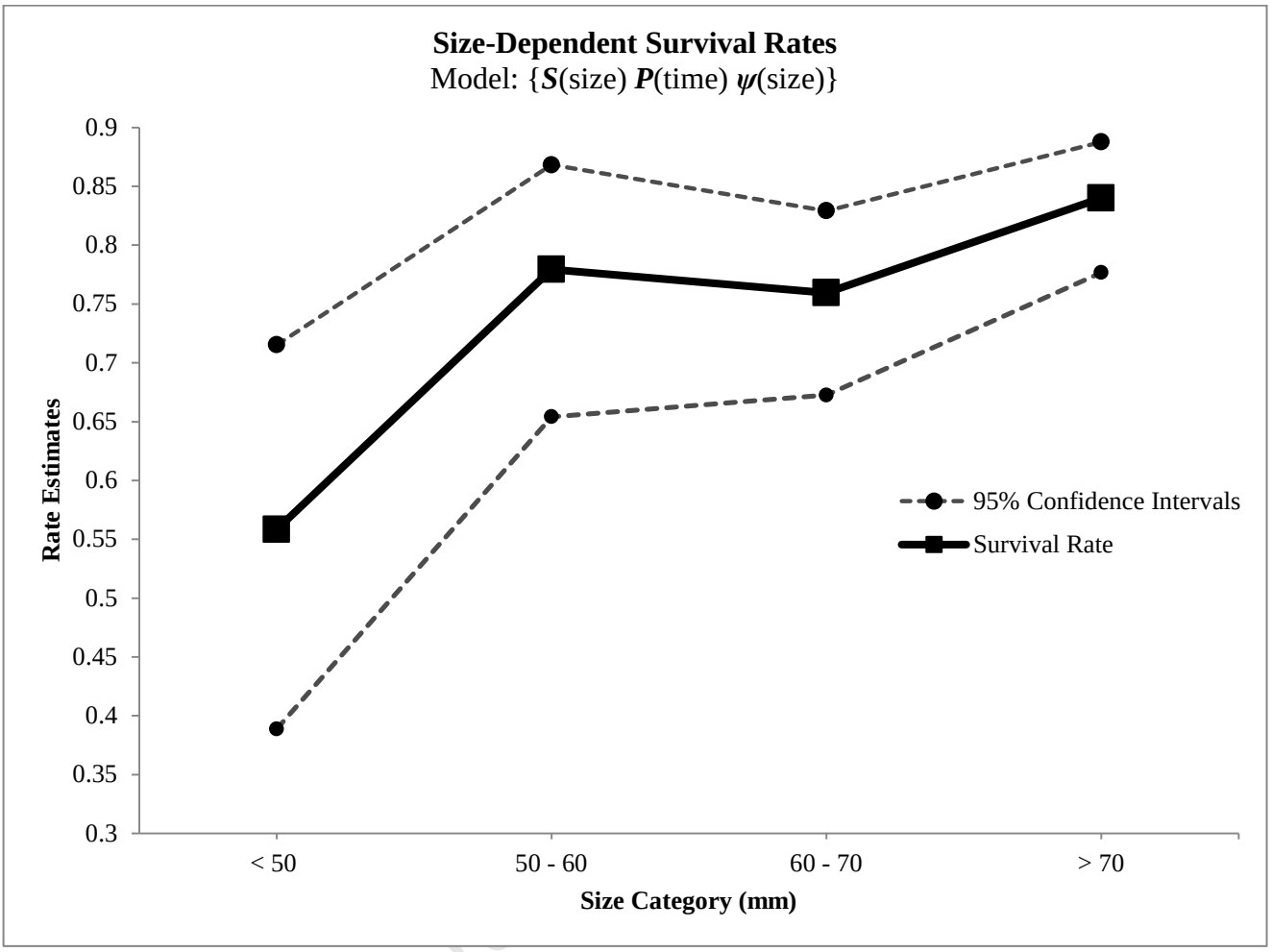


Figure 6 Summary of size-dependent thirty day survival rates for *Bradypodion pumilum*, within most parsimonious Multi-Strata size-survival model (Table 6). The heavy solid line represents thirty day survival rate estimates with accompanying dotted lines representing 95% confidence intervals.

Table 7 Upper triangular symmetric matrix summarizing the probability of chameleon size-class transition(s) per thirty day period (growth rates) for *Bradypodion pumilum* within the most parsimonious Multi-Strata size survival model (Table 6).

Size Classes (mm)	**< 50**	**50-60**	**60-70**	**> 70**
< 50	0.177	0.737	0.086	0.00
50-60	---	0.510	0.490	0.00
60-70	---	---	0.788	0.212
> 70	---	---	---	1.00

Multi-Strata (Inter-Strata) Movement Models

Goodness of Fit

The goodness of fit test (median ĉ approach in program MARK) showed that the general model (Table 8) provided a good fit of the data (ĉ = 1.09, S.E = 0.010), with slight over dispersion. The data did not therefore violate the model assumptions significantly.

Model Selection

Four of the five most parsimonious models suggested bi-directional equal movement between the core and outer sites, accumulating approximately 59% of the model support (Table 8). The top two models suggested sex- and time-independent site movement at a rate of 0.05 (95% confidence interval 0.03-0.08), accumulating approximately 39% of the model support, while models 3 and 5 suggested sex-dependent, time-independent site movement (Table 8). All models suggesting equal movement between the core and outer sites combine to accumulate approximately 71% of the model support (Table 8). The most parsimonious model suggesting differing inter-site movement rates does so independent of both time and sex at rates of 0.05 (0.03-0.11) and 0.04 (0.02-0.10), accumulating approximately 9% of the model support (Table 8). Many of the top models suggested site-dependent, time- and sex- independent chameleon survival; however, results may have been heavily biased by the study design leaving a large uncertainty as to whether survival differed between sites (Table 8).

Table 8 Summary of Multi-Strata movement models for *Bradypodion pumilum*. The models consist of four sets of parameters: $\boldsymbol{\Phi}$ (survival rate), $\boldsymbol{P}$ (capture probability), $\boldsymbol{\psi_{co}}$ (core site to outer site movement), and $\boldsymbol{\psi_{oc}}$ (outer site to core site movement). Model selection was based on the sample-size adjusted Akaike's Information Criterion (**AICc**). $\boldsymbol{K}$ is the number of estimated parameters.

Rank	Model	AICc	ΔAICc	AICc Weight	*K*	Deviance
1	{$\boldsymbol{\Phi}$(site) $\boldsymbol{P}$(site*time) $\boldsymbol{\psi_{co}}$=$\boldsymbol{\psi_{oc}}$()}	1124.426	0.000	0.233	23	515.394
2	{$\boldsymbol{\Phi}$() $\boldsymbol{P}$(site*time) $\boldsymbol{\psi_{co}}$=$\boldsymbol{\psi_{oc}}$()}	1125.216	0.791	0.157	22	518.397
3	{$\boldsymbol{\Phi}$(site) $\boldsymbol{P}$(site*time) $\boldsymbol{\psi_{co}}$=$\boldsymbol{\psi_{oc}}$(sex)}	1125.654	1.229	0.126	24	514.401
4	{$\boldsymbol{\Phi}$(site) $\boldsymbol{P}$(site*time) $\boldsymbol{\psi_{co}}$() $\boldsymbol{\psi_{oc}}$()}	1126.384	1.958	0.088	24	515.131
5	{$\boldsymbol{\Phi}$() $\boldsymbol{P}$(site*time) $\boldsymbol{\psi_{co}}$=$\boldsymbol{\psi_{oc}}$(sex)}	1126.676	2.251	0.076	23	517.645
6	{$\boldsymbol{\Phi}$() $\boldsymbol{P}$(site*time) $\boldsymbol{\psi_{co}}$() $\boldsymbol{\psi_{oc}}$()}	1126.893	2.467	0.068	23	517.861
7	{$\boldsymbol{\Phi}$(sex) $\boldsymbol{P}$(site*time) $\boldsymbol{\psi_{co}}$=$\boldsymbol{\psi_{oc}}$()}	1127.226	2.800	0.057	23	518.194
8	{$\boldsymbol{\Phi}$(site) $\boldsymbol{P}$(site*time) $\boldsymbol{\psi_{co}}$(sex) $\boldsymbol{\psi_{oc}}$()}	1127.577	3.152	0.048	25	514.092
9	{$\boldsymbol{\Phi}$(site) $\boldsymbol{P}$(site*time) $\boldsymbol{\psi_{co}}$=$\boldsymbol{\psi_{oc}}$(time)}	1128.466	4.041	0.031	31	501.376
10	{$\boldsymbol{\Phi}$(site) $\boldsymbol{P}$(site*time) $\boldsymbol{\psi_{co}}$() $\boldsymbol{\psi_{oc}}$(sex)}	1128.599	4.173	0.029	25	515.114
11	{$\boldsymbol{\Phi}$(sex*site) $\boldsymbol{P}$(site*time) $\boldsymbol{\psi_{co}}$=$\boldsymbol{\psi_{oc}}$()}	1128.646	4.221	0.028	25	515.161
12	{$\boldsymbol{\Phi}$(sex) $\boldsymbol{P}$(site*time) $\boldsymbol{\psi_{co}}$() $\boldsymbol{\psi_{oc}}$()}	1128.918	4.492	0.025	24	517.664
13	{$\boldsymbol{\Phi}$(site) $\boldsymbol{P}$(site*time) $\boldsymbol{\psi_{co}}$(sex) $\boldsymbol{\psi_{oc}}$(sex)}	1129.756	5.331	0.016	26	514.029
14	{$\boldsymbol{\Phi}$(sex*site) $\boldsymbol{P}$(site*time) $\boldsymbol{\psi_{co}}$() $\boldsymbol{\psi_{oc}}$()}	1130.634	6.209	0.010	26	514.907
General (GoF)	{$\boldsymbol{\Phi}$(sex*time*site) $\boldsymbol{P}$(sex*time*site) $\boldsymbol{\psi_{co}}$(sex*time) $\boldsymbol{\psi_{oc}}$(sex*time)}	1265.165	140.740	0.000	105	433.293

Site Movement and Habitat Use

Throughout CMR and radio-tracking (RDT) survey, chameleons were encountered exclusively on suitable vegetation (*see 'Description of Study Area'* in Chapter One), never directly on the ground, grass, or sand (Figure(s) 7-9). Chameleons were regularly encountered within and proximal to the narrow corridor(s) of continuous vegetation linking the core and outer sites (Figure(s) 7-9). Additionally, chameleons were recaptured in a different site than their initial capture and in most cases this was within or proximal to the vegetation corridors that linked the core and outer sites (Figure(s) 8 and 9).

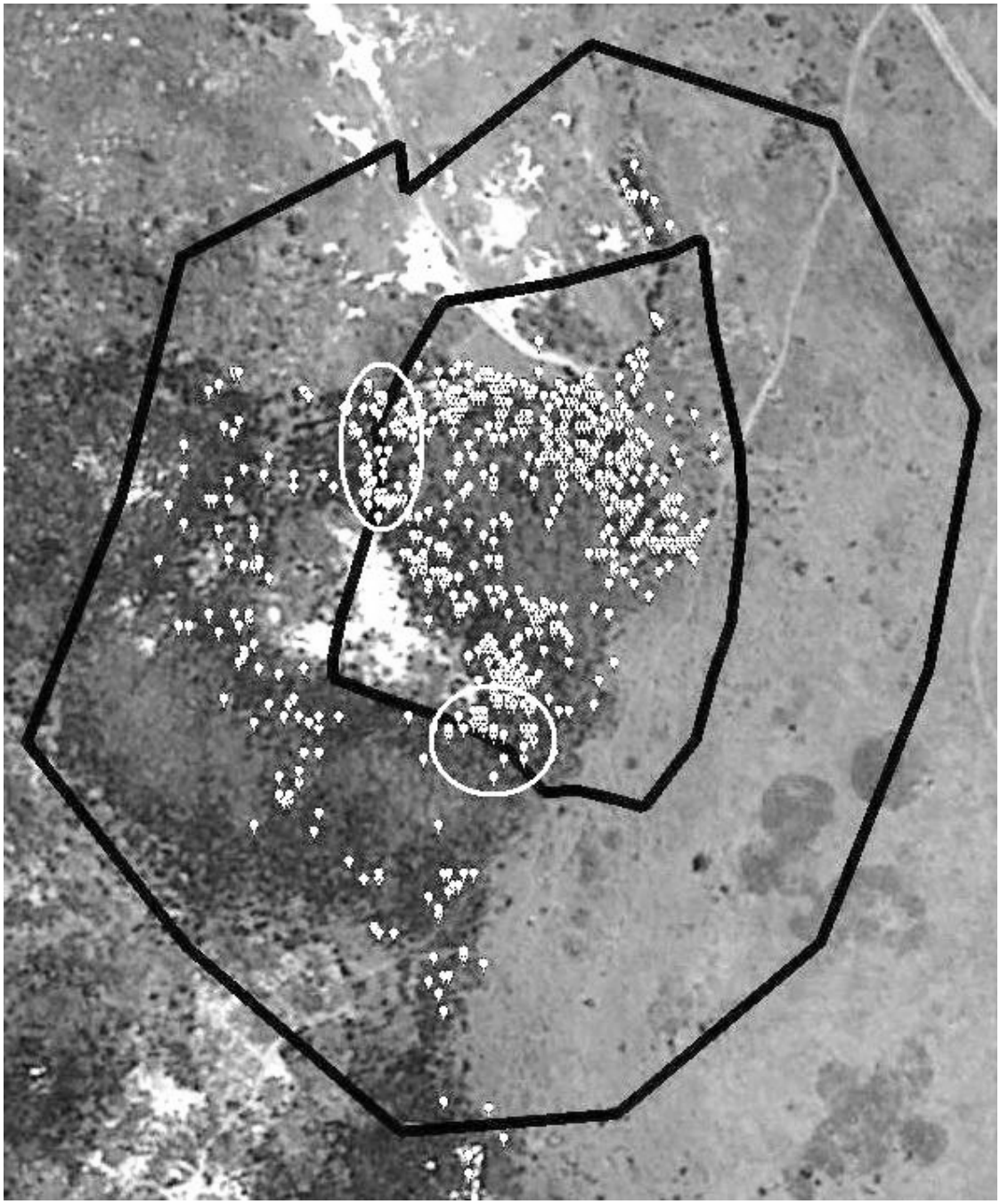


Figure 7 Summary of the encounter locations for all *Bradypodion pumilum* individuals collected during capture-mark-recapture survey superimposed over a satellite image of the study area (Google Earth) accompanied by outlines of the core and outer sites (black lined polygons) and corridors (white circles).

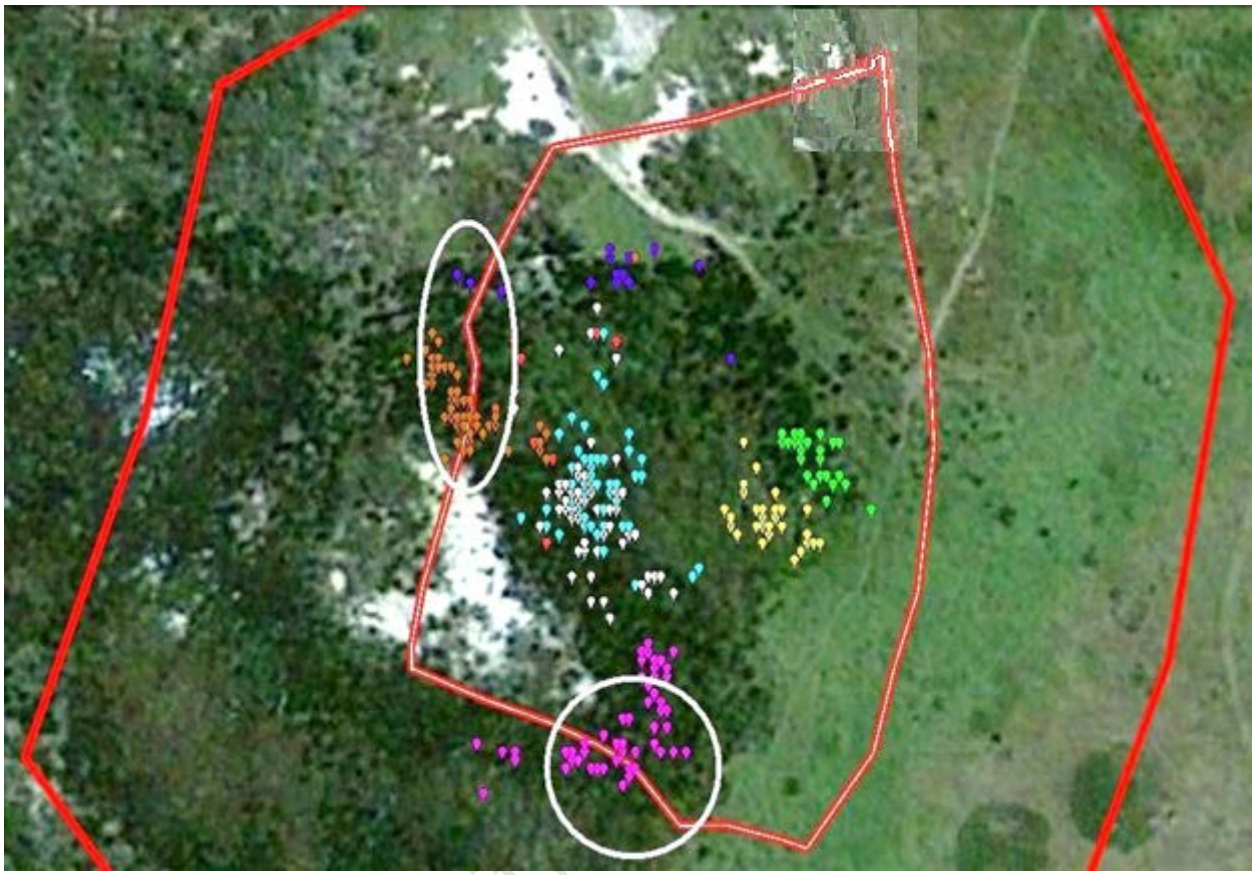


Figure 8 Summary of encounter locations for the eight *Bradypodion pumilum* individuals studied during radio-tracking survey superimposed over a satellite image of the study area (Google Earth) accompanied by outlines of the core and outer sites (red lined polygons) and corridors (white circles). Each color marker represents all point-coordinate encounter locations for a specific individual chameleon during radio-tracking survey.

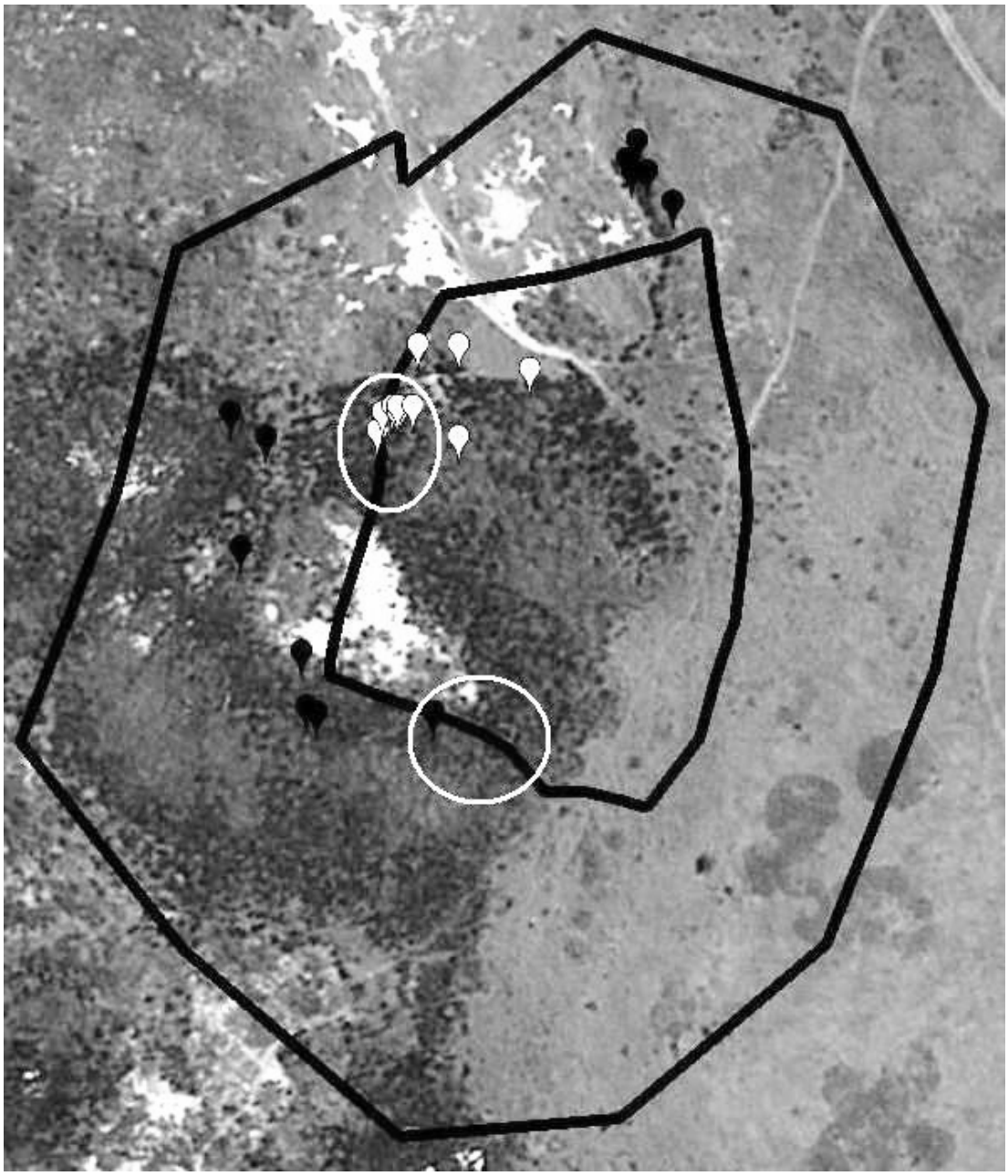


Figure 9 Summary of site fidelity for *Bradypodion pumilum* during capture-mark-recapture survey superimposed over a satellite image of the study area (Google Earth) accompanied by outlines of the core and outer sites (black lined polygons) and corridors (white circles). Markers (black) represent chameleons encountered within the outer site that were initially encountered within the core site and (white) represent chameleons encountered within the core site that were initially encountered within the outer site.

DISCUSSION

Bradypodion pumilum Survival

Over the one year period, chameleons showed size-dependent survival, where larger individuals had higher survival rates (Tables 5 and 6). In contrast, sex and season did not appear to have significant effects on survival as Cormack-Jolly-Seber (CJS), Robust Design (RD), and Multi-Strata (MS) approaches indicated males and females had similar thirty-day survival rates which remained constant throughout the study period (Tables 4-6, and 8). Size-dependence was identified as important and influential in the MS survival models. Less parsimonious models suggesting chameleons experienced size-independent, sex- and/or time-dependent survival rates were dismissed based on their AICc, ΔAICc, AICc weight, and/or model deviance values (Tables 4-6, and 8). Results are supported by previous ten-day estimates for *B. pumilum* survival at the Noordhoek site (Tolley *et al*. 2010), and suggest chameleons inhabiting fragmented landscapes experience similar short-term and extended survival.

No evidence was found suggesting differential male and female survival among chameleons inhabiting the Noordhoek site. Among lizard species, general theory suggests survival rates are often similar for both sexes (Turner 1977); however, previous case studies both support and reject sex-dependent survival (Alcala and Brown 1967; Tinkle 1967; Tuner *et al*. 1969; Shine 1980; Schoener and Schoener 1980, 82; Turner *et al*. 1982; Andrews and Nichols 1990; Watkins 1996; McCoy *et al*. 2004; Rutherford 2004; Endriss *et al*. 2007; Bock *et al*. 2010; Tolley *et al*. 2010). Supporting study suggests that the underlying mechanisms are linked to reproductive 'costs' (trade-offs between fecundity and survival), conspicuous social behavior often associated with mating in highly polygynous species, and/or sexual size dimorphism (SSD). All of these behaviors/attributes increase the risk of predation within one of the sexes (Shine 1980; Schoener and Schoener 1982; Watkins 1996; McCoy *et al*. 2004; Bock *et al*. 2010). Studies rejecting sex-dependent survival in lizards, document specific species experiencing known SSD and/or conspicuous social behavior (e.g. *A. distichus, A. angusticeps*, and *A. carolinensis*) *and* similar male and female survival (Schoener and Schoener 1982). The notable difference appears to be the intensity with which these behaviors/attributes occur within each species. Further support for the rejection of sex-dependent survival in lizards comes from studies in which specific species experience low levels of- or no reproduction costs, conspicuous social

behavior, and/or SSD *and* similar male and female survival (Schoener and Schoener 1980, 82; Turner *et al*. 1982; Andrews and Nichols 1990; Rutherford 2004; Endriss *et al.* 2007). In most *Bradypodion* species, including *B. pumilum*, females are larger than males (Stuart-Fox and Whiting 2005; Stuart-Fox and Moussalli 2007; Hopkins and Tolley 2011; E.M. Katz, personal observation). *Bradypodion* females exhibit an aseasonal reproductive cycle and are capable of producing multiple clutches per year probably as a result of sperm storage (Jackson 2007). Although *Bradypodion's* reproduction costs and mating system are not well-studied, other chameleon species (e.g. *Chameleo chameleon*, *Furcifer labordi*, and *F. verrucosus*) exhibit conspicuous social behavior (mate-guarding and territoriality) and polygynous mating systems (Cuadrado 2001; Karsten *et al*. 2009). Therefore, it appears plausible that *Bradypodion* exhibit some of the same traits. If *Bradypodion* do exhibit some/all of these traits it could explain the similar male and female survival rates observed for the group inhabiting the Noordhoek site. An alternative explanation is that *B. pumilum* is a unique example of a lizard species that does not display these traits, however, experiences similar male and female survival. Long-term field study is often required in order to clarify demographic patterns (Ferguson *et al*. 2004), therefore it is also possible that the current study was too short to find sex differences in survival.

Smaller adult chameleons showed lower survival than larger individuals inhabiting the Noordhoek site. In lizards, general theory suggests adult survival is either unrelated or indirectly related to body-size (Trivers 1976; Schoener and Schoener 1982; Pike *et al*. 2008). For example, in Bahamian *Anolis* lizards, only one of four species experienced a significant effect of body-size upon survival rate, and this relationship only existed on islands experiencing low levels of overall predation (Schoener and Schoener 1982). Size-dependent dispersal is another phenomenon experienced within a number of lizard species, including chameleons, in which juveniles and/or smaller adults move into and colonize less favorable habitats (M'Closkey *et al.* 1998; Keren-Rotem *et al.* 2006; Warner and Shine 2008; Tolley *et al*. 2010). Size-dependent dispersal in lizards is likely induced by habitat instability, intraspecific competition, and/or inbreeding depression (Johnson and Gaines 1990; MacDonald and Smith 1990). Because dispersal may reduce food intake and increase the risk of predation, a relationship may exist between the size-dependent survival and increased dispersal among smaller adult chameleons (Lidlicker 1975; Christian and Tracy 1981; Shields 1987; Snell *et al*. 1988). In *B. pumilum*, smaller adults showed substantially lower thirty-day survival rates than larger individuals, ranging from 0.56 to 0.84 over the created size classes (Figure 6). Gravid *Bradypodion*

pumilum females typically deposit their clutches in dense clusters on a single bush (Jackson 2007; K.A. Tolley, personal communication), necessitating eventual dispersal (to reduce intraspecific competition and/or inbreeding depression). If decreased food intake and/or increased predation are experienced by smaller, dispersal-prone chameleons it could partially explain the lower survival rates found within smaller adults inhabiting the Noordhoek site. Additionally, prior study and personal observation indicate smaller chameleons may be driven off favorable vegetation by larger more aggressive individuals (Tolley *et al*. 2010; E.M. Katz, personal observation). Typically, smaller chameleons are restricted to inhabiting marginal areas with decreased access to food sources and/or increased predation risks. This pattern is likely amplified in disturbed and fragmented habitats such as the study site, possibly explaining their lower survival rates.

Annual survival estimates for *B. pumilum* inhabiting the Noordhoek site are comparable with those for other small-bodied lizards (< 100.0 mm SVL; $\boldsymbol{\Phi}_A < 0.30$; Schoener and Schoener 1982; Wright *et al*. 1984; Andrews and Nichols 1990). Species-specific survival among other things appears to be function of predation intensity; species inhabiting areas experiencing an increased risk of predation often exhibit lower annual survival (Schoener and Schoener 1982; Andrews and Nichols 1990). For example, Bahamian anoles inhabiting mainland areas have significantly lower annual survival rates compared to those residing in island habitats (< 0.06 vs. 0.21-0.28). Mainland habitats contain large numbers and high diversities of species that prey upon anoles while island habitats have depauperate predator communities (Schoener and Schoener 1982; Wright *et al*. 1984; Andrews and Nichols 1990). Within the current study, CJS and RD analysis (omitting the effect of body-size) indicated a constant thirty-day survival rate ($\boldsymbol{S}$=0.78), extrapolated to yield an estimated annual survival rate of approximately 0.05 for chameleons inhabiting the Noordhoek site. *Bradypodion pumilum's* annual survival rate compares with those of *Anolis* species (lizards of similar body-size) inhabiting areas experiencing higher levels of predation (Wright *et al*. 1984; Andrews and Nichols 1990). Therefore, it is plausible that *B. pumilum's* annual survival could be explained by high levels of predation occurring within the Noordhoek site.

Results from this study offer thirty-day survival rates for chameleons inhabiting the Noordhoek site for a full year. Interestingly, thirty-day survival estimates remained fairly constant throughout the year. Seasonal abiotic factors (e.g. temperature and moisture) often exert proximate influences on lizard life-history parameters, including survival (Tinkle 1972; Ballinger 1977, 83; Dunham 1978, 81;

Abts 1987; Jones and Ballinger 1987; Jones *et al*. 1987; Sinervo and Adolph 1989; Sinervo 1990). Temperature is often linked to daily activity; many lizards thermoregulate causing daily activity periods to fluctuate throughout the year based on seasonal temperature changes. Periods of behavioral thermoregulation are typically short or non-existent during the winter months, and long during the summer with intermediary durations during spring and fall (Porter *et al*. 1973; Porter and Tracy 1983; Adolph and Porter 1993). Populations experiencing extended potential activity seasons/daily activity periods yield lower annual survival rates, suggesting increased mortality in active (vs. inactive) lizards (Adolph and Porter 1993). Moisture (precipitation) affects insect/prey abundance (i.e. increased moisture yields increased insect/prey abundance; Bates 1945; Dobzhansky and Pavan 1950; Owen 1969; Frith 1975). Long-term climate data for the city of Cape Town indicates minimal temperature change (0^{o}-9^{o}C) between seasons per annum, though drastic differences in average monthly precipitation (14.0-93.0 mm) (data from 1961-1990, South African Weather Service). The result is warm, dry summers and mild, wet winters with intermediary conditions during spring and fall. Current results suggest seasonality and the associated biotic/abiotic changes do not significantly affect *B. pumilum* survival for individuals inhabiting the Noordhoek site (Tables 4 and 5). Minimal temperature fluctuation between seasons may allow for the relative maintenance of daily activity periods for *B. pumilum* throughout the year. This could partially explain the non-season-dependent survival experienced by these chameleons. Furthermore, the *minimal* inverse relationship between temperature and moisture may allow for *slight* increases in daily activity periods during seasons with reduced prey abundance enabling maintenance of adequate foraging throughout the year, contributing towards *B. pumilum's* non-season-dependent survival rate.

Results support hypotheses of *(1)* annual survival for chameleons, similar to other small-bodied lizards, *(2)* similar male and female chameleon survival, and *(3)* that larger chameleons experience higher survival than smaller individuals. However, results reject the hypothesis of significant seasonal variation in survival for chameleons inhabiting the Noordhoek site. These results are further supported by previous ten-day estimates for *B. pumilum* survival (Tolley *et al*. 2010).

Bradypodion pumilum Site Movement and Use of Corridors

Bradypodion pumilum engaged in minimal movement between the core and outer sites during thirty-day periods, independent of time and/or sex. Inter-site movement was executed exclusively through narrow corridors, containing suitable native and exotic vegetation, linking the core and outer sites (Tables 5 and 8, Figures 7-9). Capture-mark-recapture (CMR) model estimates suggest chameleons engage in low levels of temporary emigration and inter-site movement (γ'' and ψ ~0.05). It is important to note that though these parameter estimates are quite low, they do still hold biological meaning. Results suggest chameleons inhabiting the Noordhoek site are capable of dispersal/migration between patches via available connecting corridors (Tables 5 and 8). Inter-site movement estimates within MS analysis relied on chameleon encounter data, reaffirming actual chameleon movement into and out of the core site. Results suggest that chameleons from the small patch studied, are not completely isolated from other patches. This may hold important implications regarding continued maintenance of connecting corridors and other conservation management strategy.

Bradypodion species are primarily arboreal. Because of this, *Bradypodion* populations inhabiting fragmented landscapes often exhibit a heterogeneous distribution, restricted to areas containing suitable vegetation (E.M. Katz, personal observation). Patches of suitable vegetation are often separated by areas of unsuitable habitat (e.g. the matrix). Movement between patches (e.g. dispersal/migration) may be restricted by the availability of connecting corridors containing suitable vegetation. Throughout the study period, chameleons moved into and out of the core site exclusively through narrow corridors, containing native and exotic vegetation, linking the core and outer sites (Figures 7-9). Additionally, throughout CMR and radio-tracking (RDT) survey, chameleons were never observed in direct interaction with bare ground, short grasses, or sandy areas (E.M. Katz, personal observation). *Bradypodion pumilum's* arboreal behavior supports the necessity of corridors containing suitable vegetation to maintain movement between patch fragments within the overall landscape (Figures 7-9).

Male and female chameleons experienced similar rates of temporary emigration and bi-directional inter-site movement ($\gamma''=\psi_{co}=\psi_{oc}$ ~0.05) per thirty-day period (Tables 5 and 8). Results contrast with previous short term study estimating ten-day movement patterns for *B. pumilum* in which males showed greater temporary emigration rates (males = 0.20; females = 0.05, Tolley *et al*. 2010). Sex-dependent temporary emigration within previous work was likely the result of limited data

collection or some short term movement trend that either was not repeated during the present study, or was undetected over a larger time scale.

Results support hypotheses of *(1)* low levels of adult chameleon inter-site movement, *(2)* the necessity of corridors containing suitable vegetation to maintain inter-site movement, and *(3)* chameleon use of both native and exotic vegetation. However, results reject hypotheses of increased movement among male chameleons versus females (Tables 5 and 8).

Bradypodion pumilum Site Abundance

Derived abundance estimates suggest the core site typically supports 25-100 adult chameleons throughout the year (Figure 5). Short-term chameleon abundance appeared to fluctuate, with upwards of seventy individuals inhabiting the core site during warmer, dryer periods (January-April), dropping to less than forty individuals during colder, wetter periods (June-October) (Figure 5). Additionally, adult males almost always appeared to inhabit the core site in greater numbers than adult females, even though the confidence intervals widely overlapped (Figure 5). It is important to note that abundance estimates were generated as derived values within the most parsimonious RD model, rather than through actual counting methods. These estimates stand to quantify a realistic range of short term site abundance(s) more so than to compare/deduce abundance variation throughout the year-long study period. Credible estimates of abundance variation would require incorporating measurement uncertainty under a random effects model (R. Altwegg, personal communication). The required model assumptions combined with the difficulty of teasing apart noise from true process variance deterred attempts to quantify true abundance variation (R. Altwegg, personal communication).

There was a drastic increase in chameleon site abundance from December to January (Figure 5). As December included the first set of CMR surveys, low numbers were likely the result of inefficient chameleon location and data processing rather than actual decreased abundance (E.M. Katz, personal observation). More realistically, December site abundance was likely similar (>70 individuals) to that of the other warmer, dryer months (January-April). Decreased abundance values during colder, wetter months may be the result of chameleon site vacancy through permanent/temporary emigration. Alternative explanations include *(1) B. pumilum* site recruitment predominately occurs during summer months or *(2)* that local climatic conditions affected abundance estimates. Chameleons are ectotherms, therefore their body temperature and rate of metabolism

decreases during colder months. Low body temperatures and decreased metabolism might cause chameleons to move deep down in vegetation and remain there for up to a few days (protecting them from predators and reducing body temperature loss; K.A. Tolley, personal communication). This behavior would make chameleon encounter during CMR survey increasingly difficult and could explain the reduced abundance estimates observed during winter months.

Individuals not part of the trappable population would not have been part of these abundance estimates.

Conclusion

This portion of the study focused on the use demographic data collected from the Noordhoek chameleon population to estimate local demographic parameters. Findings suggest the core site (Figure 2) is typically inhabited by 25-100 adult chameleons (established using combined male and female monthly chameleon abundance estimates, Figure 5) continuously throughout the calendar year. Additional results suggest that chameleons inhabiting the Noordhoek site *(1)* experience annual survival comparable to other small-bodied lizards, *(2)* experience variable survival rates related to biological attributes, *(3)* are primarily arboreal avoiding movement over the ground, and *(4)* engage in minimal movement between patch fragments and to do so, rely on linking corridors containing 'suitable' native and exotic vegetation. Results support many of the initial hypotheses (discussed in detail below) as well as *(1)* further our understanding of population demography and habitat use for small-vertebrates inhabiting fragmented landscapes, *(2)* enable effective conservation management strategy aimed towards maintaining viable demographic parameters within potentially imperiled populations, *(3)* provide empirical data (e.g. parameter estimates) for comparison in future study focused on population demography and stability for *B. pumilum* and other small-vertebrate populations, and *(4)* help determine the need- and type- of preliminary management recommendations required for *B. pumilum* inhabiting the patch of transformed, fragmented habitat at the Noordhoek Wetlands Nature Reserve.

Demographic parameter estimates suggest Cape Dwarf Chameleons are capable of short-term occupation within a fragmented landscape. However, because *B. pumilum's* natural survival is low compared to larger vertebrates (normal compared to other lizard species), they may be more sensitive to perturbations in the system and/or stochastic events. Therefore, continual monitoring of local

chameleon populations would prove beneficial to ensure these parameters remain at viable levels. Additionally, it appears important to maintain the availability of corridors of suitable vegetation linking patch fragments within the overall landscape. The wide array of deterministic and stochastic threats to persistence often induced by the effects of habitat fragmentation prevent conclusions regarding *B. pumilum's* capability of long-term inhabitance within fragmented landscapes and warrants the need for continual long-term demographic study.

Results offer empirical evidence that small-vertebrate populations, specifically herpetofauna with limited vagility, are capable of short-term inhabitance within fragmented landscapes.

Appendix 2 Summary of biometric data from capture-mark-recapture (CMR) survey from November, 2009-November, 2010 for *Bradypodion pumilum* inhabiting the Noordhoek site.

Site(s)	**Total Captures**	**Unique Individuals**		**Mean, {Std. Dev.} Snout-Vent-Length (mm)**		**Mean, {Std. Dev.} Tail-Length (mm)**		**Mean, {Range} Mass (g)**	
		Males	*Females*	*Males*	*Females*	*Males*	*Females*	*Males*	*Females*
Core + Outer Sites*	728	118	101	63.31 {10.48}	66.25 {12.40}	67.95 {13.52}	60.40 {11.15}	5.59 {2.59}	7.10 {4.19}
Core Site Only	620	86	68	63.53 {11.95}	67.19 {15.43}	67.88 {14.77}	61.16 {15.66}	5.65 {2.73}	7.48 {4.61}
Outer Site Only	108	41	36	61.42 {10.43}	61.52 {10.40}	67.69 {13.88}	58.43 {8.56}	5.16 {2.42}	5.33 {2.43}

* *'Core + Outer' CMR data was combined and then edited to correct for individuals found in both sites. As a result the 'core + outer unique individuals' count does not equal the sum of 'core only' + 'outer only' unique individuals counts.*

CHAPTER THREE

EXPLORING POPULATION GENETIC VARIABILITY IN *Bradypodion pumilum* WITHIN A FRAGMENTED, URBAN HABITAT

INTRODUCTION

The *combined* interaction of environmental factors and demographic- and genetic-responses to habitat fragmentation contribute to population viability and effective species conservation (Lande 1988; Newman and Pilson 1997; Groom 1998; Haig 1998; Saccheri *et al*. 1998). Molecular tools are valuable for ensuring the maintenance of long-term genetic diversity and for clarifying demographic and ecological issues early in species recovery (Haig 1998; Frankham *et al*. 2002; Kuo and Janzen 2004; Epps *et al*. 2005; Keyghobadi *et al*. 2005; Xu *et al*. 2005; Martinez-Cruz *et al*. 2007; Segelbacher *et al*. 2008). Understanding relative levels of within- and among- population genetic variation can help focus efforts of specific populations in need of recovery (Templeton *et al*. 1990; Lacy 1997; Haig 1998; Epps *et al*. 2005; Keyghobodi *et al*. 2005; Martinez-Cruz *et al*. 2007). Additionally, defining the structure of a population (e.g. founder relationships, rates of effective dispersal among groups of individuals, and effective population size [*N*e]) leads to more accurate population management from the beginning of recovery efforts when options may be the most flexible (Haig 1998; Kuo and Janzen 2004; Vignieri 2005; Riley *et al*. 2006). Therefore, investigating population genetics within small-vertebrate populations inhabiting fragmented landscapes will benefit species conservation.

Habitat fragmentation can create dispersal barriers which divide a population into smaller disjunct groups with limited or no connectivity (Sarre *et al*. 1990; Rolstad 1991). Loss of genetic variation is among the genetic changes in population structure which can be caused by fragmentation of a species' habitat (Templeton *et al*. 1990; Sherwin and Moritz 2000). Increased isolation amongst fragmented groups can lead to and/or amplify a number of effects which erode genetic variation: demographic fluctuations (decreased abundance), reduced gene flow, genetic drift, and/or inbreeding depression (Wright 1978; Templeton *et al*. 1990; Hitchings and Beebee 1997; Gerlach and Musolf 2000; Sherwin and Mortiz 2000; Andersen *et al*. 2004). These effects are often interconnected and build upon and/or initiate one another. Fragmented groups may experience temporary or sustained decreases in abundance resulting from population bottlenecks and/or decreased immigration/dispersal between groups. Population bottlenecks reduce genetic variation through random events in the transmission of small numbers of alleles (Wright 1931; Wright 1978; Franklin 1980; Frankel and Soule 1981; Lande and Barrowclough 1987; Frankham 1995) while decreased immigration/dispersal may

reduce gene flow between fragmented groups within the overall population (Wright 1978). Over time, groups experiencing reduced gene flow may suffer decreased access to genetically diverse mates (Hitchings and Beebee 1997; Gerlach and Musolf 2000) resulting in a loss in genetic variation. Decreased immigration/dispersal may be particularly evident in small-vertebrate populations with limited vagility inhabiting fragmented landscapes. The combination of biotic and abiotic factors in such populations is likely to decrease movement capability between fragmented groups (*see* Chapter One). In either case, groups experiencing decreased abundance are at increased risk for inbreeding depression (increased homozygosity caused by breeding of related individuals) which may result in reduced fitness (Lande 1988; Templeton *et al*. 1990; Andersen *et al*. 2004). Small populations are also more susceptible to the negative effects of genetic drift (fluctuation in gene frequencies due to random sampling), further eroding genetic variation (Lande 1988; Templeton *et al*. 1990; Andersen *et al*. 2004). When habitat fragmentation results in a loss of genetic variation, adverse implications for species conservation may arise including a reduced ability to adapt to the changing environment, reduced survival and reproduction, and an increase in the probability of extinction (Fisher 1930; Ayala 1965, 69; Frankham 1980; Mitton and Grant 1984; O'Brien *et al*. 1985; Allendorf and Leary 1986; Palmer and Strobeck 1986; Goodman 1987; Templeton *et al*. 1990; Burger and Lynch 1995; Frankham 1995; Madsen *et al*. 1996; Lacy 1997; Saccheri *et al*. 1998; Westemeier *et al*. 1998; Sherwin and Mortiz 2000; Ebert *et al*. 2002; Reed and Frankham 2003). Differential responses of vertebrate species to habitat fragmentation are based upon the interaction of species specific attributes with abiotic landscape attributes (*see* Chapter One). Because of this, quantitative genetic analysis has become an important tool for estimating the level of genetic diversity within potentially imperiled populations inhabiting fragmented landscapes (O'Brian 1994; Haig 1998) to recognize and mitigate species-specific loss of genetic variation (Sherwin and Mortiz 2000).

Because genetic variation is both a trait of individuals and of populations, it can be quantified in a number of ways including estimates of gene diversity, allelic diversity, and/or by the percentage of polymorphic loci (Frankel and Soule 1981; Lacy 1997; O'Connell and Wright 1997; Nielsen *et al*. 1999; Sherwin and Mortiz 2000; Stow and Briscoe 2005; Xu *et al*. 2005). Most experimental investigations of genetic variation rely on quantitative analysis using variable number tandem repeats (VNTR) regions within neutral loci because of their utility for the investigation of gene flow and their relative ease of analysis (Sherwin and Mortiz 2000). Changes in genetic variation can be characterized

qualitatively by collecting temporal measures of allelic diversity (Sherwin and Moritz 2000). Because allelic diversity may decrease more rapidly than local heterozygosity, this relationship can be used as an early indicator for species at risk for long-term problems associated with population fragmentation (Allendorf 1986; Gravitol *et al*. 2000; Stow and Briscoe 2005). Microsatellites are a highly variable class of molecular markers used to assess allelic diversity within the VNTR regions of neutral loci (e.g. O'Connell and Wright 1997; Nielsen *et al*. 1999; Stow and Briscoe 2005; Xu *et al*. 2005). Also known as simple sequence repeats (SSRs) or tandem repeats, microsatellites are repeating sequences of one to five base pairs of non-coding, eukaryotic DNA (Bruford and Wayne 1993; Queller *et al*. 1993; Page and Holmes 1998; Ellegren 2004; Turnpenny and Ellard 2005). Typically neutral or co-dominant, microsatellites most commonly consist of di-, tri-, and tetra-nucleotides repeated three to one hundred times (Ellegren 1992, 2004; Whittaker *et al*. 2003). Using the unique sequences within flanking regions as primers, microsatellites can be amplified for identification by the polymerase chain reaction (PCR) (Ellegren 1992): DNA is repeatedly denatured at a high temperature to separate the double strand, then cooled to allow annealing of primers and the extension of nucleotide sequences through the microsatellite, creating and exponential increase in the replicated segment (Griffiths *et al*. 1996). PCR products are then genotyped, from which DNA profiles are created. Using DNA profiles, changes in genetic diversity can be estimated through comparison against empirical values obtained from undisturbed populations of the same or closely related species (Akst *et al*. 2002; Gautschi *et al*. 2002). Microsatellite DNA profile analysis can also provide estimates for spatial and temporal shifts in genetic variation among- and within- populations (Waples and Teel 1990; Whitlock 1992; Richards and Leberg 1996; White *et al*. 1998; Ciofi *et al*. 1999; Lundy *et al*. 2000; Rossiter *et al*. 2000; Pertoldi *et al*. 2001; Stow *et al*. 2001; Melgaard *et al*. 2003; Williams *et al*. 2003; Johnson *et al*. 2004; Small *et al*. 2005; Noel *et al*. 2007; Chevolot 2008; Echodu *et al*. 2011). The high variability among- and within-population allele compositions make microsatellites optimal genetic markers for studying population processes over ecological timescales. Furthermore, microsatellites can be surveyed from small amounts of DNA collected non-lethally via small tissue samples, making microsatellites especially useful in small-bodied and/or rare taxa (Roberts *et al*. 2009).

There is an enormous quantity of research implementing quantitative genetic analysis to investigate genetic variation within small-vertebrate species (e.g. Waples and Teel 1990; Whitlock 1992; Richards and Leberg 1996; White *et al*. 1998; Ciofi *et al*. 1999; Rossiter *et al*. 2000; Pertoldi *et*

al. 2001; Stow *et al*. 2001; Melgaard *et al*. 2003; Williams *et al*. 2003; Johnson *et al*. 2004; Noel *et al*. 2007; Chevolot 2008). Because of the quantity and varied focuses of available research in this subject, I refrain from attempting explanation at such a broad scale within the scope of this thesis. Rather, this chapter investigates a subset of quantitative genetic analysis; specifically temporal genetic variation within a small-vertebrate population. Temporal genetic monitoring (quantifying genetic changes in population genetic metrics using molecular markers, Schwartz *et al*. 2007) can be separated into two categories: (*1*) diagnostic molecular markers for traditional population monitoring (e.g. abundance, survival, hybridization, geographic range) and (*2*) monitoring population genetic parameters (e.g. genetic variation, effective population size, population structure and migration) (Schwartz *et al*. 2007). This chapter will focus on the latter with specific regards to the genetic variability and temporal stability of allele frequencies within a small-vertebrate population, *Bradypodion pumilum*, inhabiting a fragmented landscape. Previous studies investigating temporal genetic variability within- and among-small-vertebrate populations offer valuable life history information and aid in species conservation (Appendix 3). Temporal monitoring of genetic variation provides the opportunity to track fragmented populations over time and to evaluate when populations reach critical thresholds that demand management action (Schwartz *et al*. 2007). Despite these benefits, temporal genetic variation and stability in small-vertebrate populations inhabiting fragmented landscapes remains understudied, especially in reptiles (E.M. Katz, personal observation).

The Cape Dwarf Chameleon, *Bradypodion pumilum*, exemplifies a small terrestrial vertebrate species with limited vagility in which habitat loss, due to urbanization, has led to the fragmentation and transformation of much of its habitat (Driver *et al*. 2005; K.A. Tolley and E.M. Katz, personal observation). *Bradypodion pumilum's* distribution is primarily concentrated within the Cape Town metropolitan area; an area suffering severe habitat transformation (Driver *et al*. 2005; Rebelo *et al*. 2011). Continued habitat alteration within this area has resulted in a collection of isolated local populations/groups of *B. pumilum* possibly vulnerable to genetic diversity loss resulting from stochastic events (e.g. demographic fluctuations), decreased gene flow, and/or increased genetic drift and inbreeding depression.

Chapter two provided demographic information for *B. pumilum* inhabiting a patch of transformed, fragmented habitat at the Noordhoek Wetlands Nature Reserve, Cape Town, South Africa. Chameleons within this area are believed to have undergone a recent demographic bottleneck;

an approximate three month vacancy of adults from the site. Capture-mark-recapture (CMR) surveys indicated an absence of adult chameleons (juveniles were observed within the core site during this period) from the study site in late October and November, 2008 (K.A. Tolley, personal communication; Appendix 4). The data suggested a temporary site vacancy continuing through as late as February, 2009 when re-occupancy was confirmed (K.A. Tolley, personal communication). Vacancy occurred during spring/summer when adult chameleons should have been observable in higher numbers than previous months (K.A. Tolley, personal communication; *see* Chapter Two). A comparison of chameleon recaptures between years 2 and 3 further supports a temporary site vacancy (Appendix 5). CMR data prior to site-vacancy (year 1) is here after referred to as 'pre-vacancy' and CMR data following re-occupancy (years 2 and 3) as 'post-vacancy' and these groupings are used in subsequent analyses. Possible explanations for adult vacancy include an extreme predation event, or alternatively adult dispersal for some unknown reason. Following adult re-occupancy, questions arose as to whether new adults were the remnant juveniles within the core site who had since grown to maturity, or rather new immigrant chameleons from adjacent vegetation fragments. None of the new chameleons were recaptures from the previous year (K.A. Tolley, personal communication), allowing for either scenario (since juveniles were not marked during previous mark-recapture study).

This portion of my study focuses on temporal monitoring of genetic variation and stability in a group of Cape Dwarf Chameleons inhabiting a patch of transformed, fragmented habitat at the Noordhoek Wetlands Nature Reserve, Cape Town, South Africa. Microsatellite DNA marker analysis was carried out using tissue samples collected across a three year period to monitor changes in *B. pumilum* genetic variation. The main aims of this portion of my study are:

1.) *Investigate pre-vacancy (2008) versus post-vacancy (2009-2010) genetic differentiation and allelic variation.*
2.) *Estimate genetic variability and temporal stability (shifts in allele frequency) across a three year period.*

Hypothesis:

Bradypodion pumilum *experience minimal genetic variation across the three year period indicating predominate genetic stability, except following stochastic events (e.g. demographic fluctuations associated with pre- versus post-vacancy), which are hypothesized to induce significant shifts in allelic variation through genetic drift, and/or colonization*

Low levels of connectivity are predicted to exist between the core site and adjacent vegetation patches through distinct corridors of suitable vegetation (*see* Chapter Two). In spite of fragmentation and *B. pumilum's* low vagility, core site adult immigration/dispersal may proceed through these corridors providing adequate gene flow to maintain genetic variation. However, following temporary adult site vacancy, chameleon recolonization of the core site through these corridors may have caused an allele frequency shift due to founder effects. Alternatively, the site may have rather been re-populated by the remnant juveniles who had grown to maturity. Similar allele frequencies would then be expected when compared to the previous group of individuals inhabiting the core site, assuming the juvenile population was a reflection of the previous adult population.

Results from this study will further understanding of temporal genetic variation and stability within small-vertebrate populations, specifically chameleon populations inhabiting fragmented landscapes. Such knowledge enables effective conservation management strategy aimed towards maintaining genetic diversity within potentially imperiled populations. Additionally, results will provide empirical data for comparison in future studies focused on temporal genetic variation and stability within *B. pumilum* and other small-vertebrate populations. Lastly, results will enable specific preliminary management recommendations for *B. pumilum* inhabiting the patch of transformed, fragmented habitat at the Noordhoek Wetlands Nature Reserve.

MATERIALS, METHODS, AND ANALYSIS

Study Design and Data Collection

Tissue-Sample Collection

Chameleon tissue samples (*n* =119) were collected within the core site during capture-mark-recapture (CMR) surveys[9]. Forty samples were included from year 1, 40 from year 2, and 39 from year 3[10]. A team of two field workers located and captured adult chameleons[11] (≥ 40 mm snout-vent length) with the aid of torch light. Nocturnal surveys were conducted because *B. pumilum* is readily visible at night when asleep, as they tend to perch higher up vegetation enabling their pale coloration to be distinguished against darker vegetation via torchlight (Tolley and Burger 2007). Upon encounter chameleons were removed from their roost and tissue samples were collected by removing the last 1.0-3.0 mm of the chameleon's tail with sterilized scissors and stored in 99% ethanol. Chameleons were handled for less than five minutes (usually ca. two minutes) upon each capture to minimize disturbance to the animal. After processing, chameleons were returned to the exact perch where they were found.

Microsatellite Amplification and Screening

DNA extraction followed standard salt-extraction techniques (following Aljanabi and Martinez 1997, Appendix 6) to produce extracts of total genomic DNA template for polymerase chain reaction (PCR). DNA template concentrations were determined (NanoDrop spectrophotometer, Wilmington, Delaware) and standardized to a range of 5.0-75.0 ng/µL. Microsatellite primers were selected from libraries developed and optimized for *Bradypodion* (Feldheim *et al*. 2010; Feldheim *et al.* 2011). PCR trials were conducted using ten different primer sets, from which eight polymorphic loci were selected: Dinucleotide(s) Bpu507 and Bpu557; Trinucleotide Bpu94; Tetranucleotide(s) Bpu26, Bpu28, Bpu115, Bpu132, and Bpu238 (Appendices 9-11).

[9] See '*Materials, Methods, and Analysis* (Chapter One) for detailed mark-recapture procedures

[10] Year 1 (June-September, 2008), year 2 (February-May, 2009), and year 3 (January, 2010-May, 2010).

[11] Minimum male and female adult sizes were determined using male chameleons. In adult males, hemipenes were exposed, indicating sexual maturity. Results from previous study indicated a similar adult size (> 41.5 mm, Jackson 2007).

Ten µL PCR reactions were conducted using one of two PCR protocols (Appendix 8). Optimization required adjustments to the component volumes and thermal cycling conditions (Appendices 9-11). Universal PCR recipes and thermal cycling conditions were not achieved within or across loci; rather a range was required to generate successful PCR products across all loci from all tissue samples (Appendices 9-11).

PCR products were electrophoresed through 2% agarose gels (pre-stained with Goldview Nucleic Acid Stain) at 100-105 V (400mA) for approximately 25 minutes. Products were run against a 100bp DNA ladder to determine fragment size, and following electrophoresis, viewed and photographed for preliminary size and amplification success. Products yielding no banding, inappropriate banding, or inappropriate fragment size(s) were rerun. PCR products were sent to Stellenbosch Central Analytical Facility for microsatellite genotyping (ABI3130xl or ABI3730xl sequencer using a 50 cm capillary array and POP7 and ROX 500 size standard; Applied Biosystems). Products sent for genotyping ranged from undiluted to 1:20 dilutions with ddH_2O.

Data Analysis

Microsatellite Genotyping and Screening

Microsatellite DNA profiles were scored against a ROX 500 size standard in GeneMapper 4.0 (Applied Biosystems). Profiles were screened for peaks and stutters of appropriate pattern and size, and alleles were identified using the repeat motif(s) and allelic size range(s) generated in associated study (Feldheim *et al.* 2010; Feldheim *et al.* 2011). The screening process was continuously honed and amended throughout to reduce error. Questionable DNA profiles (e.g. undefined peaks, to many peaks, small peaks, ambiguous peaks, incorrect peak or stutter patterning, incorrect fragment size, too much background noise) were discarded and samples re-run. Allele fragment sizes and frequencies were recorded in Microsoft Excel (Appendix 7) and repeatedly checked to make sure fragment size(s) correlated with repeat motif(s) and previously scored DNA profiles. In a few cases successful PCR products and/or DNA profiles were unattainable within a specific loci for a specific tissue sample in which case data was left as 'missing' within the excel file.

Pre- Versus Post-Vacancy Microsatellite Differentiation

Determining microsatellite allelic variation along with expected and observed heterozygosity (H_e and H_o) are widely used to characterize genetic differentiation among- and within- populations (Excoffier *et al*. 2005; Peakall and Smouse 2005). Allele frequencies generated from pre- and post-vacancy microsatellite datasets were used to characterize polymorphism and heterozygosity within loci (GenAlEx V.6.4 software, Peakall and Smouse 2006). First however, a combined dataset including all samples from years 1-3 (n =119) was created and tested for linkage disequilibrium (the non-random association of alleles) between loci. Any evidence of linkage disequilibrium prevents use of the suspected loci as they may alter the allele frequency results. Linkage disequilibrium testing relied on the log likelihood ratio statistic (G-test, Goudet *et al*. 1996) under Markov chain parameters of 1000 dememorizations over 100 batches at 1000 iterations per batch (GENEPOP on the Web V.4.0.10, Raymond and Rousset 1995; Rousset 2008, Appendix 12). The combined dataset was subsequently partitioned into pre- (n =39, 40) and post- (n =80, 82) vacancy datasets (*see 'Introduction'*) for frequency based microsatellite analysis including: number of alleles, allelic size range (base pairs), and H_e and H_o (with accompanying P-value) (GeneAlEx V.6.4 software, Peakall and Smouse 2006, Table 9; GENEPOP on the Web V.4.0.10, Raymond and Rousset 1995; Rousset 2008).

Frequency based microsatellite analysis served as secondary analysis (primary = the analysis of molecular variance {AMOVA}) for temporal genetic variation and stability for *B. pumilum* across a three year period using pre- versus post-vacancy comparison. Polymorphism was calculated as a percentage of polymorphic loci across all loci with a potential range from 0% to 100%. H_e and H_o were estimated for each locus as well as arithmetic means across all loci (GeneAlEx V.6.4 software, Peakall and Smouse 2005, 2006). H_e was calculated by subtracting the summation of the allele frequency(s) squared from one ($H_e = 1-\sum p_i^2$) and H_o was calculated by dividing the direct count of heterozygotes by the total number of samples (H_o = # Heterozygotes/ # of Samples) (GeneAlEx V.6.4 software, Peakall and Smouse 2005, 2006).

Additional analyses included tests for deviation from Hardy-Weinberg Equilibrium (HWE), genotyping errors, and statistical evidence of a population bottleneck. The Hardy-Weinberg Law states: in a random-mating population in which two alleles A and A' occur in frequencies p and q (= 1 - p) the three types AA, AA', and $A'A'$ are expected to remain in equilibrium from generation to generation at frequencies of p^2, $2pq$, and q^2, in the absence of mutation or selection (Stern 1943). In

other words, both the allele and genotype frequencies in a population remain constant from generation to generation unless specific disturbing influences are introduced (i.e. non-random mating, mutations, selection, limited population size, random genetic drift, gene flow). HWE never truly exists in nature, but is rather used as a baseline against which to measure genetic change. Tests for deviations from global HWE (across all loci) relied upon score {U} tests for heterozygote deficit/excess. HWE testing applied Markov chain parameters of 1000 dememorizations over 100 batches at 1000 iterations per batch (GENEPOP on the Web V.4.0.10, Raymond and Rousset 1995; Rousset 2008). Individual loci deviating from HWE were determined using *P*-values obtained from Chi-square tests (GenAlEx software V.6.4, Peakall and Smouse 2006).

Although microsatellites can be useful, errors can be introduced, so careful screening is necessary to ensure that estimates of HW are accurate. Low template DNA can result in amplification failure (Miller and Waits 2003; Wandeler *et al*. 2003) and mutations at priming sites can result in false homozygotes (null alleles; Shaw *et al*. 1999). Other errors include preferential amplification of small alleles (i.e. large allele dropout or short allele dominance, Wattier *et al.* 1998) and allele slippage during PCR amplification (additional stutter products which differ from the original template, Shinde *et al*. 2003). Loci were tested for genotyping errors using program MICRO-CHECKER (Van Oosterhout *et al*. 2004). Datasets were compared against a null distribution generated by 10,000 randomizations. Although null alleles were detected, the loci involved differed depending upon the dataset being analyzed (pre- versus post-vacancy, Appendix 13*(A)*) suggesting the results were random and possibly caused by other factors. Regardless, MICRO-CHECKER was used to generate adjusted genotypes (Brookfield 1 Method) for these loci. Homozygote frequencies were adjusted to appropriate Hardy-Weinberg proportions by scoring the second allele as missing data, however, increasing the number of missing values within analyses decreases the statistical power. The adjusted and original datasets were both run using the analysis of molecular variance (AMOVA) tests, and results did not substantially differ (Appendix 13*(B)*), therefore the original datasets were used in order to maintain statistical power.

BOTTLENECK software V.1.2.02 was used to test for signatures of recent population decline (Cornuet and Luikart 1996). Because population bottlenecks induce a transient excess of heterozygosity, finding an observed heterozygosity that is higher than the expected (equilibrium) heterozygosity for a large majority of loci in a population suggests that this population may have

recently experienced a genetic bottleneck (Cornuet and Luikart 1996). A sign rank test is one statistical test for excess heterozygosity that uses the difference (observed - expected) in heterozygosity across loci in a population sample (Cornuet and Luikart 1996).Using *n* individuals that have been scored for *L* polymorphic loci: if mutation drift equilibrium is assumed (i.e. no bottleneck), there is approximately an equal probability of getting a positive or a negative difference between the observed and the expected heterozygosities. In contrast, if there has been a recent bottleneck in the population, a positive difference (heterozygosity excess) should be observed more often than a negative difference (Cornuet and Luikart 1996). More simply, test the number of loci for which there is a heterozygosity excess and determine whether it is significantly larger than *L*/2, assuming an a priori binomial distribution of parameters *L* and ½ (Cornuet and Luikart 1996). The Wilcoxon sign rank test was conducted using the two phase model of mutation (TPM) to test for signatures of recent population decline. Analysis of frequency distributions of allele size can be used to understand mutational changes in simple sequence repeats (SSR) (i.e. microsatellites, Di Rienzo 1994). The TPM is an intermediate between the infinite allele model (IAM) and the stepwise mutation model (SMM). The TPM assumes that most mutational changes result in an increase or decrease of one SSR unit, but mutations of larger magnitude may also occur (Di Rienzo 1994). Comprehensive explanation of the IAM and SMM require detailed complexity, however, in short, the IAM provides formulae for determining the relationship between heterozygosity and the number of alleles at loci in a bottlenecked population (Watterson 1984) while the SMM provides formulae describing the change of heterozygosity for loci in a population following a bottleneck (Chakraborty and Nei 1977). The TPM is shown to best fit most microsatellite datasets and was selected for its relative tolerance to small datasets and small numbers of loci (Di Rienzo *et al*. 1994; Luikart unpublished data). TPM models were run with 95% single-step mutations and 5% multi-step mutations, with a variance of twelve among multiple steps, at 1000 iterations as recommended by Piry *et al. (1999)*.

Where applicable, throughout the analysis, corrections for multiple simultaneous comparisons, using traditional or sequential Bonferroni corrections were applied using a global significance level of 0.05 (Holm 1979; Rice 1989).

Microsatellite Variation: Detection of Temporal Shifts in Allele Frequency

The analysis of molecular variance (AMOVA) is a highly informative statistical test that allows the hierarchical partitioning of genetic variation among populations and the estimation of *F*-statistics and/or their analogues (Peakall and Smouse 2005). Hierarchical AMOVA was performed to investigate temporal shifts in allele frequencies among- and within- pre- and post-vacancy and annual periods. AMOVA analysis included estimating F_{ST} (Wright 1951) and analogue R_{ST} (Slatkin 1995) along with accompanying *P*-values (GenAlEx V.6.4 software, Peakall and Smouse 2006), obtained through random permutation (9,999 permutations).

RESULTS

Pre- Versus Post-Vacancy Microsatellite Differentiation

Pre- and post-vacancy *B. pumilum* were both genetically variable, with multiple alleles at each locus and expected heterozygosities within the range for small-vertebrates (except for Bpu557, Table 9). Pre- and post-vacancy samples for *B. pumilum* were polymorphic for all loci, containing 4-19 unique alleles (Table 9). The pre- and post-vacancy samples met Hardy-Weinberg expectations after Bonferroni correction for all loci except Bpu94 (both periods) and Bpu28 (only post-vacancy period, Table 9). Heterozygote deficit could be evidence for null alleles due to mutations in the priming sequence (Lundy *et al*. 2000), particularly for Bpu94, which showed significant heterozygote deficit in both pre- and post-vacancy analyses and was suggested to contain possible null alleles (MICRO-CHECKER, Table 9, Appendix 13*(A)*). MICRO-CHECKER also suggested possible null alleles at Bpu26 (Appendix 13*(A)*); however, as mentioned earlier, the original datasets were kept intact in order to maintain statistical power (Appendix 13*(B)*). No other genotyping errors were detected amongst the scored microsatellite datasets. Following an applied sequential Bonferroni correction, no evidence of linkage disequilibrium was detected in 28 pairwise comparisons (Appendix 12). No evidence of a recent population bottleneck was detected within pre- and post-vacancy datasets.

Microsatellite Variation: Detection of Temporal Shifts in Allele Frequency

Hierarchical analysis of molecular variance (AMOVA) indicated there were no significant differences in F_{ST} between pre- and post-vacancy periods. In contrast, R_{ST} did show significant differences between pre- and post-vacancy datasets (Table 10). R_{ST} results inspired further partitioning datasets for pairwise examination by year (1 vs. 2, 1 vs. 3, and 2 vs. 3) to assess annual differences. There were no significant differences in F_{ST} between years 1, 2, and 3. In contrast, significant differences in R_{ST} were detected between years 1 versus 2 and years 1 versus 3 (Table 10).

Table 9 Summary of allele frequency based statistics for *Bradypodion pumilum* characterized from pre- and post-vacancy microsatellite dataset(s).

CMR Period	Locus	N	Na	S	He	Ho	*P*-Value
Pre-Site Vacancy	*Bpu94*	39	7.000	161-212	0.756	0.436	0.001
	Bpu28	39	16.000	161-257	0.903	0.821	0.788
	Bpu26	39	14.000	175-267	0.908	0.795	0.369
	Bpu115	40	15.000	125-209	0.888	0.875	0.637
	Bpu132	40	12.000	158-262	0.864	0.900	0.772
	Bpu238	40	12.000	178-254	0.886	0.850	0.894
	Bpu507	40	11.000	211-263	0.866	0.850	0.201
	Bpu557	40	4.000	104-114	0.353	0.375	0.090
	Global			104-267	0.803	0.738	<0.001
Post-Site Vacancy	*Bpu94*	82	9.000	161-212	0.757	0.549	<0.001
	Bpu28	82	17.000	161-257	0.911	0.976	<0.001
	Bpu26	80	14.000	175-267	0.883	0.763	0.026
	Bpu115	80	19.000	125-213	0.908	0.888	0.098
	Bpu132	82	15.000	158-262	0.862	0.817	0.888
	Bpu238	82	12.000	178-254	0.871	0.817	0.374
	Bpu507	82	12.000	203-261	0.874	0.829	0.024
	Bpu557	82	4.000	104-114	0.330	0.354	0.908
	Global			104-267	0.799	0.749	<0.001

N = Sample Size; Na = No. Alleles; S = Allelic size range (bp); He = Unbiased Expected Heterozygosity; Ho = Observed Heterozygosity

Table 10 Analysis of molecular variance (AMOVA) results comparing *Bradypodion pumilum* microsatellite variation during the study periods

Statistic	Compared Periods/Years	F_{ST}/R_{ST} Value	*p*-Value	Source	% Variation
F_{ST}	*Pre- vs. Post-Vacancy*	0.001	0.245	Among Periods	0.117
				Within Periods	99.883
				Total	100
	Year 1 vs. 2 *Year 1 vs. 3* *Year 2 vs. 3*	<0.000 0.004 <0.000	0.446 0.067 0.450		
R_{ST}	*Pre- vs. Post-Vacancy*	0.017	0.019	Among Periods	1.741
				Within Periods	98.259
				Total	100
	Year 1 vs. 2 *Year 1 vs. 3* *Year 2 vs. 3*	0.015 0.015 <0.000	0.046 0.045 0.392		

DISCUSSION

This is the first study to monitor temporal genetic variation and stability in Cape Dwarf Chameleons and is among a small collection of research investigating these topics within small-vertebrate populations inhabiting fragmented landscapes. Quantitative genetic analysis of pre- and post-vacancy and annual temporal periods indicates typical levels (compared with other small-bodied lizards) of genetic diversity (polymorphism, number of alleles per loci, heterozygosity) for *B. pumilum* inhabiting the Noordhoek site (Table 9). Additionally, small, but detectable, differences in allele frequencies were observed between pre- and post-vacancy periods for *B. pumilum* inhabiting the Noordhoek site (Table 9, Appendix 7). Interestingly, despite these observed shifts, there are nonsignificant differences between pre- and post-vacancy periods for the F_{ST} statistics (as opposed to R_{ST} statistics) provided by the analysis of molecular variance (AMOVA) (Table 10). Cumulatively, results indicate a degree of genetic stability for *B. pumilum* inhabiting the Noordhoek site across the three-year period, particularly between years 2 and 3. Results support the initial hypotheses that *B. pumilum* inhabiting the field site experience predominately stable allelic structure except following stochastic events such as the disappearance of the adult chameleons between years 1 and 2. However, because allelic variation was predominately observed within pre- and post- vacancy periods rather than between them (Table 9) we are unable to determine whether or not demographic fluctuations were the primary catalyst for the observed shifts.

Pre- and Post- Vacancy Genetic Diversity

Comparison with prior study of *B. pumilum* from natural habitats shows similar levels of genetic diversity and heterozygosity (Feldheim *et al*. 2010; Table 9; Appendix 14), although allelic richness and number of alleles are lower for chameleons from Noordhoek (richness = 5.0-26.0, Feldheim *et al*. 2010, Appendix 14). It is important to note that Feldheim *et al*. (*2010*) incorporated chameleons from a much larger range (~100 km) as well as both transformed and natural landscapes, suggesting allelic richness is positively correlated with the geographic range of the included samples and that fragmentation can result in altered levels of genetic diversity (Wright 1931; Templeton *et al*. 1990; Falconer and Mackay 1996; Sherwin and Mortiz 2000; Andersen *et al*. 2004). Studies of other small-bodied lizards (e.g. Stow *et al*. 2001; Sumner *et al*. 2004) also reveal similar or slightly

decreased allelic diversities (range of allelic richness across- and/or within- loci; observed heterozygosity, Stow *et al*. 2001; Sumner *et al*. 2004; Table 9; Appendix 15*(A)* and *(B)*) compared to the current study (Table 9). In the present study, levels of diversity are comparable to small-vertebrate species with limited vagility inhabiting fragmented landscapes, suggesting that these populations can experience normal levels of genetic diversity.

Microsatellite Variation: Detection of Temporal Shifts in Allele Frequency

The AMOVA (R_{ST} statistics) indicate small but detectable ($R_{ST} < 0.020$, P-value <0.05) differences in allele frequencies when comparing pre- versus post-vacancy periods (Table 9, Appendix 7). The low values of R_{ST} could indicate the observed differences are not biologically meaningful (Waples 1998; Hendrick 1999), and possibly due to sampling error. In contrast, core site reoccupancy resulting from either *(1)* an influx of new adults into the core site from surrounding patch fragments (Appendices 5 and 6) or *(2)* remnant juveniles who had grown to maturity offer biological explanations for the observed shifts in allelic frequencies when comparing pre- versus post-vacancy periods.

Populations suffering recent population declines (e.g. bottlenecks) may experience a quick increase in the amount of genetic distance between groups (Hendrick 1999). As a result, the genetic distance observed between groups may not accurately reflect divergence, but may be more a function of the reduction in population size (Hedrick 1999). In the current study, however, no evidence was found indicating recent population declines (or bottlenecks). The high allelic diversity within loci and the absence of evidence indicating recent bottlenecks suggests that the differentiation indicated by the AMOVA is biologically meaningful.

Incorporating an appropriate sample size representative of the population in question is necessary to ensure accurate and precise results (Nei 1978; Baverstock and Mortiz 1996; Ruzzante 1998; Kalinowski 2005). Sampling error has previously been determined to bias estimates of genetic distance (standard genetic distance of Nei, chord distance, and/or F_{ST} and its analogues, Nei 1978; Baverstock and Mortiz 1996; Ruzzante 1998). The general theory is that larger sample sizes are required for analysis when populations are experiencing high levels of heterozygosity compared to when they are low. This is because polymorphic loci have high sampling variances when the sample size is small (Nei 1978; Baverstock and Mortiz 1996; Ruzzante 1998). However, it is important to note here that most of these studies examined the relationship between sample sizes and sampling variance.

More recent study suggests that this focus on sampling variance may be misleading because *(1)* genetic distances are derived measures of genetic differentiation (i.e. they do not necessarily have high sampling variances when estimates of allele frequencies are imprecise) or *(2)* sampling variances are not always an appropriate measure of precision to compare study design strategies (Kalinowski 2005). Computer simulations show that loci with high mutation rates produce estimates of genetic distance with lower coefficients of variation than loci with lower mutation rates without requiring larger sample sizes from each population (Kalinowski 2005). Additionally, the rate at which increasing sample sizes decreases the coefficient of variation of estimates of genetic distances has been shown to be approximately determined by the value of F_{ST} between the populations being sampled: When F_{ST} is greater than 0.05, sampling fewer than 20 individuals (per population) should be sufficient; however, when F_{ST} is less than 0.01, sampling 100 individuals (per population) is more appropriate (Kalinowski 2005). The AMOVA results (F_{ST}, R_{ST}) of the current study ranged from less than 0.000 to 0.017, with the three statistically significant R_{ST} statistics ranging from 0.015 to 0.017. Therefore, the appropriate sample sizes for the analysis of genetic distance should be approximately 100 chameleons per population (i.e. per temporal period). In the current study, the sample sizes for pre-and post-vacancy periods were n = 40 and 82 individuals respectively and n = 39 or 40 for annual periods, suggesting that sample sizes may have been too low. Therefore, the nonsignificant differences observed in the comparison of allele frequencies between pre- versus post-vacancy periods for *B. pumilum* inhabiting the Noordhoek site may not be meaningful. Had more appropriate sample size been included, these results would likely show significant shifts in allele frequencies.

Core site reoccupancy resulting from either *(1)* an influx of new adults into the core site from the surrounding patch fragments (Appendices 4 and 5) or *(2)* remnant juveniles who had grown to maturity could explain the observed shifts in allelic frequencies. Anecdotal information and observations suggest *B. pumilum* is prone to population crashes with quick recoveries via migration and/or increased birth rate[12] (K.A. Tolley, personal communication; E.M. Katz, personal observation; Chapter Two). Temporal variation in demographic parameters such as abundance, turnover- and/or migration rates have previously been shown to affect the partitioning of genetic variance (Wright 1978;

[12] There is no data regarding chameleon birth rate(s) to suggest its role in reoccupancy or its candidacy as an influential factor in the observed temporal genetic variation; however, limited personal observation suggested no unusual increase in birth rate(s) throughout the three year study.

Whitlock 1992). When local populations become extinct and are recolonized, new populations may have different variance properties than older groups (Whitlock 1992). At the Noordhoek site, chameleons enter/exit the core site to and from the surrounding patch fragments through linking vegetation corridors (E.M. Katz, personal observation, Chapter Two). Core site reoccupancy presumably resulted from an influx of new adults into the core site from the surrounding fragments (Appendices 5 and 6) rather than from remnant juveniles who had grown to maturity. Capture data from 2009 indicates new adults were *(1)* not recaptures (therefore were either remnant juveniles grown to maturity or immigrants from proximal patch fragments) and *(2)* snout-vent lengths ranged from 48.0-86.0 mm. During previous mark-recapture study within the core site, juveniles less than 40.0 mm had not been marked; although these juveniles could have grown to adults from October to February, only four of the 81 new captures were less than 50.0 mm, suggesting new adults were more likely immigrants. Increased allelic diversity among reoccupying (immigrant) chameleons compared to that of the pre-vacancy inhabitants could explain the observed shifts in allele frequencies for *B. pumilum* observed within the core site following reoccupancy.

The assumptions and limitations associated with the analysis of molecular variance, aided by results from frequency based statistics (Table 9), support the observed shifts in allelic frequencies between pre-and post- vacancy periods despite contrasting results. F_{ST} relies upon the infinite allele model, which assumes low mutation rates and is not particularly suited for the higher mutation rates often found in microsatellite loci (Weber and Wong 1993; Slatkin 1995). This model also assumes a mutation process independent of the prior allelic state, however within microsatellite loci there is abundant evidence that the size of a new mutant allele depends directly upon the size of the original allele that mutated (Weber and Wong 1993; Valdes *et al*. 1993; Goldstein *et al*. 1995). In contrast, R_{ST} relies upon a stepwise mutation model better suited for the high mutation rates and memory dependent allele mutations found within microsatellite loci (Di Rienzo *et al*. 1994; Slatkin 1995). As a result of these differences, many recent studies have incorporated R_{ST} statistics when investigating genetic variation using microsatellite loci (e.g. Hoelzel *et al*. 1998; Reusch *et al*. 2000; Taylor and McPhail 2000; Stefenon *et al*. 2007; Gileva *et al*. 2008; Ando *et al*. 2011).

Conclusion

Shifts in allele frequencies observed when comparing pre- versus post-vacancy periods for *B. pumilum* inhabiting the Noordhoek site warrant additional study incorporating larger sample sizes and if possible, extended study periods to further elucidate local chameleon population genetic structure. However, if the results of the current study are accurate, they suggest*(1)* chameleon populations inhabiting fragmented landscapes can maintain typical levels of genetic diversity (compared with other small-bodied lizards) and predominately stable allelic structure across temporal periods, *(2)* that stochastic events possibly affect the allele frequencies, and *(3)* studies investigating population genetic variation within small-vertebrate populations experiencing demographic fluctuations may be biased/skewed by a temporal effect (i.e. different population signatures may arise at the same exact geographic locality depending on the recent demographic history). The latter suggests that conclusions regarding population genetic structure for similar systems could also be biased. Such limitations are not often considered in study design and may prove problematic.

This study is novel in that it is the first to conduct temporal monitoring of genetic variation in chameleon populations. Furthermore, to my knowledge, it is the first to do such in any lizard species population. However, prior work investigating other small-vertebrate populations corroborates the above conclusions (Appendix 3). Additionally, an investigation of *Raja clavata* populations showed maintained genetic diversity despite decreasing abundance(s), largely attributed to adequate gene flow (Chevolot *et al*. 2008). It appears *Raja clavata* populations were reliant upon gene flow to maintain diversity (Chevolot *et al*. 2008). These results support the potential necessity of linking corridors containing suitable vegetation between patch fragments enabling gene flow and aiding in stable chameleon genetic diversity.

Results of the current study *(1)* provide valuable information furthering our knowledge towards management of small-vertebrate populations inhabiting fragmented landscapes and *(2)* suggest the importance of temporal genetic investigations of small-vertebrate populations inhabiting fragmented landscapes. If small-vertebrate populations regularly experience demographic shifts and/or other stochastic events, founder effects may be prominent, relegating population structure to a function of time rather than space. Genetic assessments would therefore become less useful on a spatial scale when attempting to generate conservation strategy regarding protected areas. Findings will also help

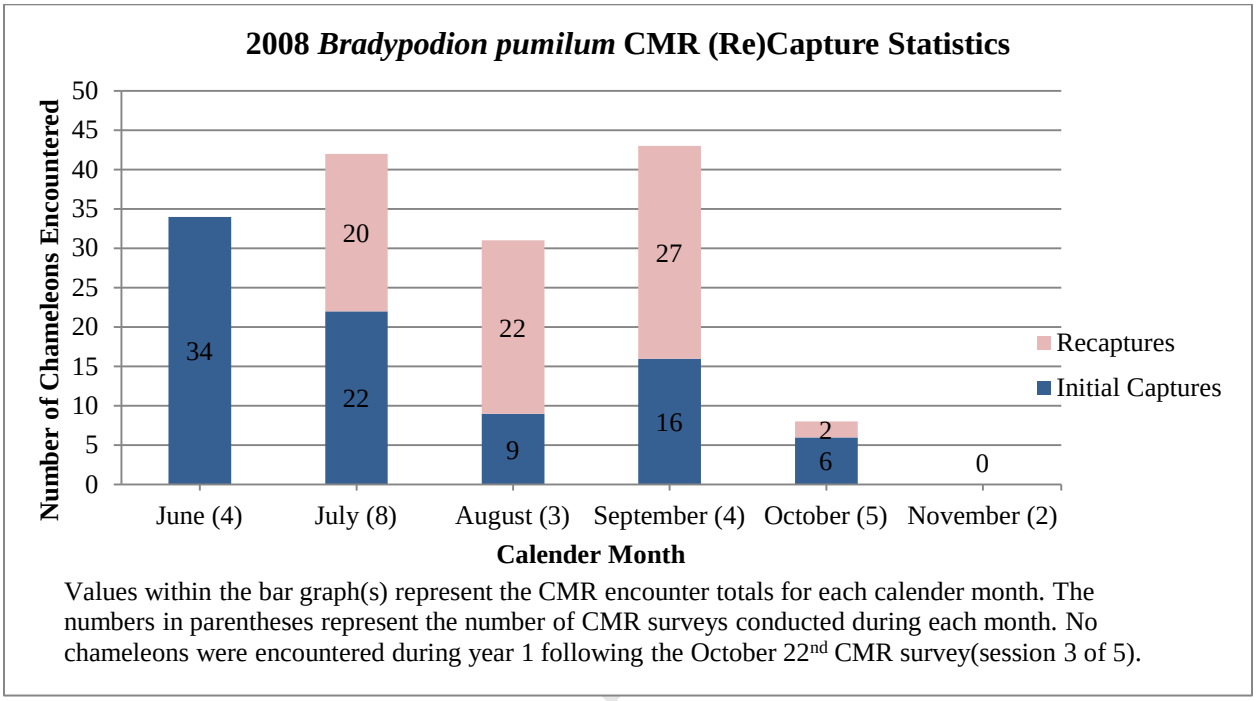
determine the long-term viability potential and necessary management strategy required to mitigate threats to chameleons and other small-bodied lizards inhabiting fragmented habitats.

Appendix 3 Previous studies focused on genetic variability and temporal stability within- and among- small vertebrate populations.

Species	**Main Focus**	**Geographic Location**	**Duration of Study**	**Type (Number) of Molecular Markers**	**Number of Samples**	**Conclusions/Implications**
Merluccius merluccius (Lundy *et al*. 2000)	Temporal genetic variation and structure in European hake	Bay of Biscay (coast of France and Spain)	1997-1999	Microsatellite (5)	600	1.) Temporal changes in allele frequencies distribution and allele size variation were more important than spatial changes. 2.) No significant differentiation observed between populations and among temporal samples within the region. 3.) Suggests no barriers to migration within the bay.
Clupea pallasii (Small *et al*. 2005)	Temporal and regional population structure in Pacific herring	1.) Puget Sound (Washington) 2.) the southern Strait of Georgia (between Vancouver island and the coast of British Columbia)	1999-2003	Microsatellite (12)	1,165	1.) Low but significant genetic structure among and within herring collections. 2.) Temporally stable genetic divergence among some Pacific herring populations.
Vulpes lagopus (Noren *et al*. 2011)	1.) Impact of immigration on genetic structure of fox populations 2.) Genetic differentiation between fox populations	Svalbard (Norway)	1997-2001	Microsatellite (7)	46	1.) Low but significant genetic differentiation between the Svalbard, North America and Siberia fox populations. 2.) Temporal differences in genetic composition of the Svalbard population between summer and winter seasons. 3.) The Arctic fox population structure varies with time and is influenced by immigration.

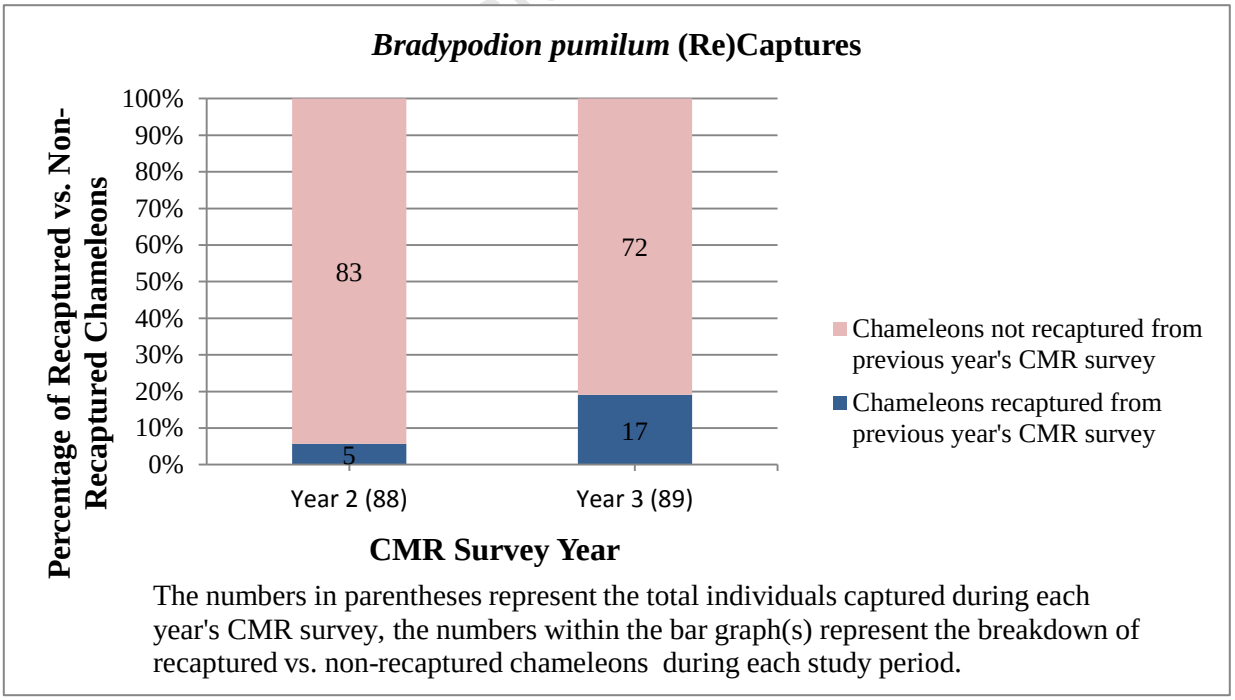
Tscherskia triton (Xie and Zhang 2006)	Temporal genetic changes over in a natural population of the greater long-tailed hamster	Hebei Province (China)	1998-2003	Mitochondrial D-loop sequence	108	1.) High levels of genetic diversity. 2.) Temporal genetic changes in haplotype frequencies likely caused by random genetic drift and migration. 3.) Positive correlation between population density and the level of genetic diversity in hamster populations. 4.) Inbreeding and genetic drift caused by reproduction, dispersal and population size may affect genetic structure and diversity.
Lutra lutra (Pertoldi *et al*. 2001)	The genetic consequences of population decline in the European otter	Islands of Funen, Sealand, and the Jutland Peninsula; the Limfiord catchment area (Denmark)	Historical samples: 1883-1963 Contemporary samples: 1989-1993	Microsatellite (11)	67 (Historical) 58 (Contemporary)	1.) Minimal evidence suggesting a recent bottleneck or loss of variability. 2.) Low but significant genetic differentiation between the extant population and historical samples of otters from other regions in Denmark. 3.) Maintained temporal stability in allele frequency and/or genetic variability within otter populations suffering population decline.
Procyon lotor (White *et al*.1998)	Long-term temporal genetic variability in a population of raccoons	Reelfoot National Wildlife Refuge (Tennessee and Kentucky, USA)	1984-1992	Type not specified (41 loci)	1,056	1.) Significant temporal genetic differentiation. 2.) Hunting appears to have had little influence on the genetic composition of raccoons in the study area. 3.) Low-level temporal variation probably caused by stochastic events.

Appendix 4 Summary of *Bradypodion pumilum* capture-mark-recapture survey data from year 1 (June-September, 2008).



Values within the bar graph(s) represent the CMR encounter totals for each calender month. The numbers in parentheses represent the number of CMR surveys conducted during each month. No chameleons were encountered during year 1 following the October 22nd CMR survey(session 3 of 5).

Appendix 5 Summary of *Bradypodion pumilum* capture-mark-recapture data survey data from years 2 vs. 3 (February-May, 2009 versus January, 2010-May, 2010)



The numbers in parentheses represent the total individuals captured during each year's CMR survey, the numbers within the bar graph(s) represent the breakdown of recaptured vs. non-recaptured chameleons during each study period.

CHAPTER FOUR

GENERAL CONCLUSIONS

This investigation of demographic and genetic variability provides important temporal data on local population dynamics and habitat use for a potentially vulnerable species inhabiting primarily fragmented landscapes. Findings from this study benefit *B. pumilum's* ongoing evaluation for IUCN Redlist status and provide empirical data useful to identifying and mitigating threats to chameleon viability through the implementation of effective conservation management strategy. Results suggest *B. pumilum* is capable of short-term occupation within fragmented landscapes with the potential for long-term viability, though further study is required. Despite the potential for stochastic events, and the effects of habitat fragmentation, chameleons inhabiting the Noordhoek site maintained predominately stable short-term survival and genetic diversity indicative of viable population structure. However, the Noordhoek chameleon population likely experiences source-sink dynamics (or some similar mechanism) in which within-fragment demographic and genetic parameter maintenance is reliant on available opportunities to refresh/recoup local populations via dispersal and migration between patch fragments within larger fragmented landscape. Therefore, continued chameleon viability may be heavily dependent on adequate connectivity with other patch fragments of suitable quality within the overall landscape. Corridors of quality habitat greatly increase the connectivity between fragments (Debinski and Holt 2000), promoting gene flow, and reducing founder effects, population bottlenecks, and local extinctions (Tolley *et al.* 2010). Corridors of suitable habitat connecting patch fragments are available to chameleons at the Noordhoek site, and their availability is likely imperative to ensure that chameleon populations remain viable in this fragmented landscape. Additionally, *B. pumilum's* arboreal behavior necessitates the continued availability of suitable vegetation (*see* Chapter One) within patch fragments. Management strategy may be required to maintain quality habitat for chameleons inhabiting this fragmented landscape. Lastly, *B. pumilum's* limited dispersal capability suggests their continued ability to inhabit fragmented landscapes requires the proximal geographic location of adjacent patch fragments. Additional study investigating chameleons inhabiting fragmented landscapes is recommended to confirm the above conclusions. Additional study will also clarify *B. pumilum's* potential for long-term survival within fragmented landscapes. The suggested focus for future study includes chameleon vegetation preferences based on body-size and/or sex as well as long-term demographic and genetic assessments using methods applied within the current study.

Habitat fragmentation has already negatively impacted a wide array of small-vertebrates populations (e.g. Robinson *et al.* 1995; Davies and Margules 1998; Templeton *et al.* 2001) and is continuing to include more and more species. The scientific community has only begun to uncover,

understand, and mitigate the effects of habitat fragmentation on biodiversity. The continued existence of imperiled species inhabiting fragmented landscapes is reliant on continued population-level investigations aimed towards species conservation and viability maintenance. Results from this study: *(1)* increase our understanding of life-history, population structure, and habitat use for herpetofauna and other small-vertebrates inhabiting fragmented landscapes, *(2)* provide insight into potential conservation management strategy required by herpetofauna and other small-vertebrates with limited vagility inhabiting fragmented landscapes, and *(3)* provide empirical demographic and genetic data for comparison in future study investigating spatial and/or temporal population structure and stability for herpetofauna and other small-vertebrates inhabiting fragmented landscapes.

This study exemplifies both the necessity and benefits of conducting species-specific investigations for small-vertebrate populations inhabiting fragmented landscapes. The combination of demographic and genetic approaches offers insight into local population structure likely unobtainable through their individual application. Results will likely aid in effective species conservation, maximizing the continual existence of potentially imperiled species inhabiting fragmented landscapes.

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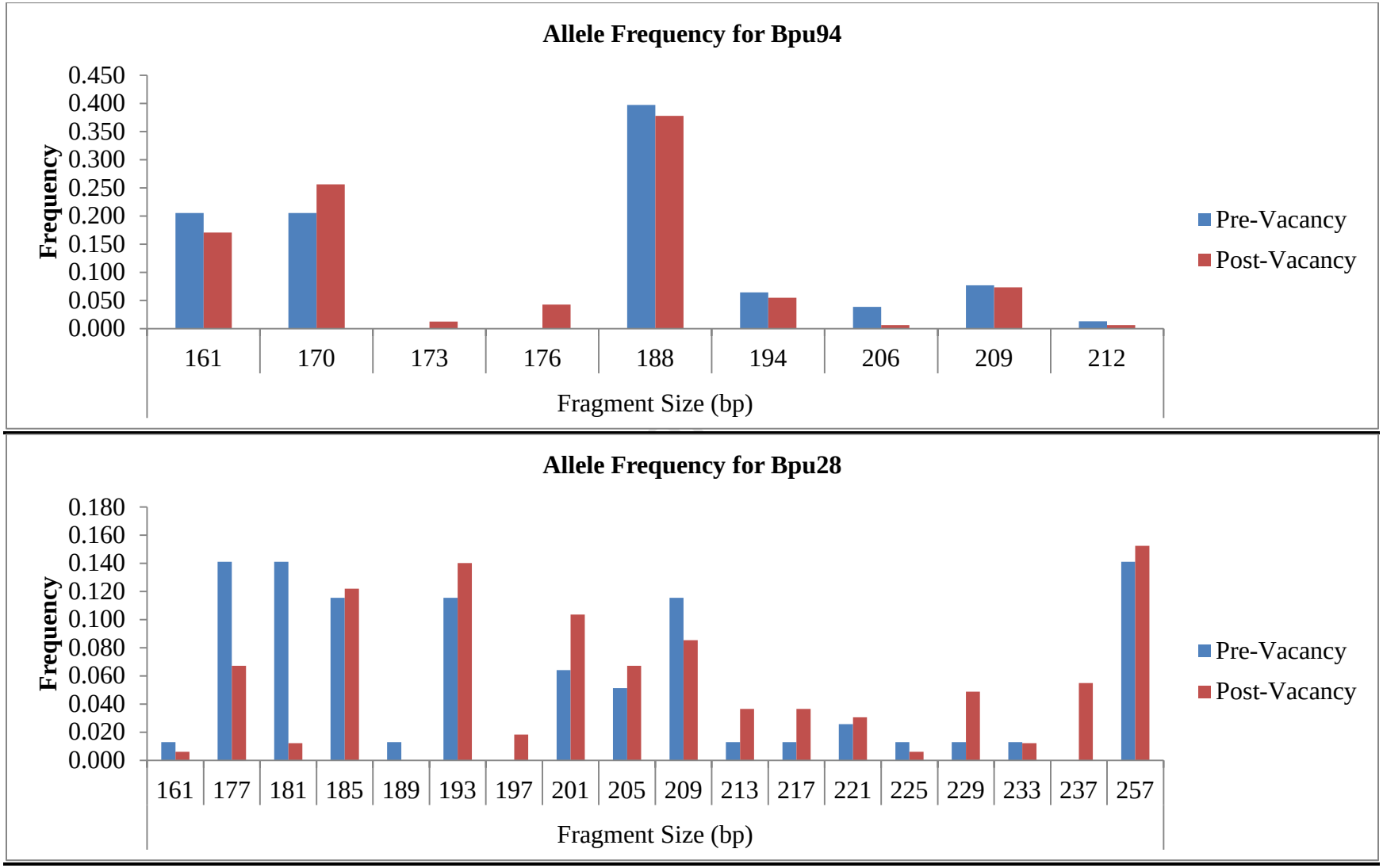
ADDITIONAL APPENDICES

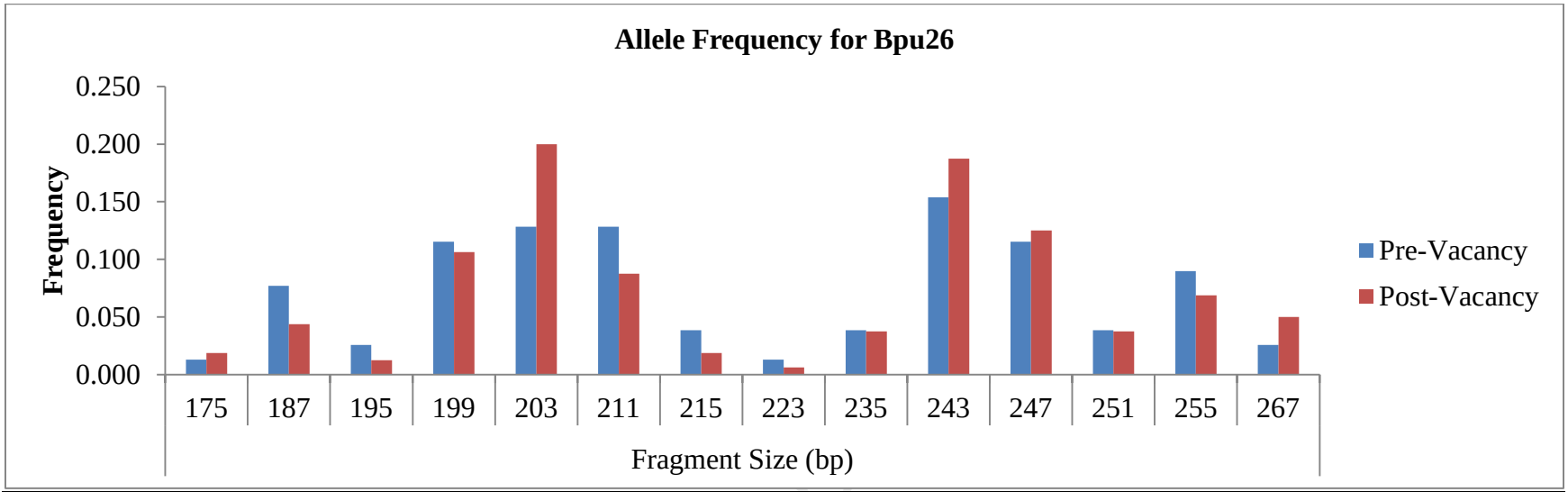
Appendix 6 DNA Salt Extraction Protocol (following Aljanabi and Martinez 1997)

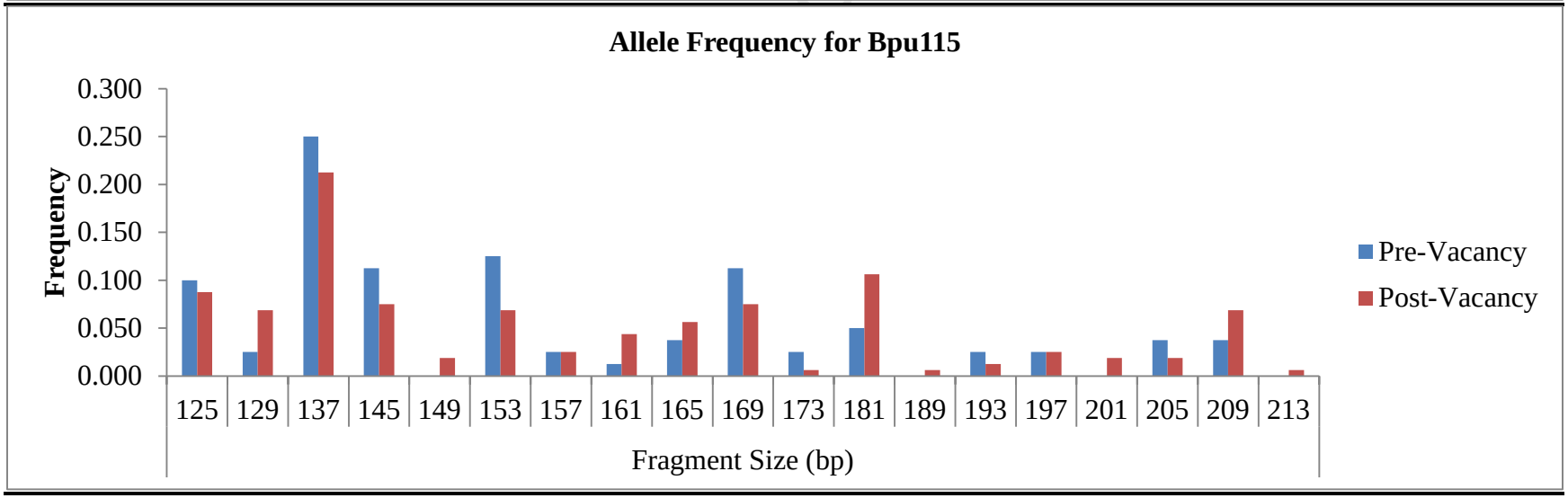
1. Tissue + 410 µl extraction buffer + 2% SDS (80 µl 10%SDS) + 10 µl Proteinase K (10mg/ml)
2. incubate 55ºC or 37ºC over night
3. 5 minutes 13000 rpm centrifuge, transfer supernatant in a new vessel + 180 µl NaCl. Mix it (turn Eppi ca. 50 times or vortex it 30 seconds)
4. 5 minutes 13000 rpm centrifuge, pipette transfer supernatant quickly in a new vessel + 420 µl cooled Isopropanol (mix it gently)
5. Leave samples in freezer for a few hours (2-4 hours)
6. 5 minutes 13000 rpm centrifuge, discard supernatant. Add 250 µl 80% Ethanol for washing (turn Eppi ca. 50 times or vortex it 30 seconds)
7. do the last washing step again
8. remove alcohol completely, dry pellet 10 to 20 minutes in the vacuum centrifuge
9. dilute DNA in 100-200 µl TE Buffer [preferably H_2O]and keep it at room temperature over night
10. freeze –20ºC or use extracts immediately

Appendix 7 Summary of *Bradypodion pumilum* microsatellite allele sizes (bp) and frequencies from pre- and post-vacancy datasets (pre-vacancy n = 40; post-vacancy n = 82).

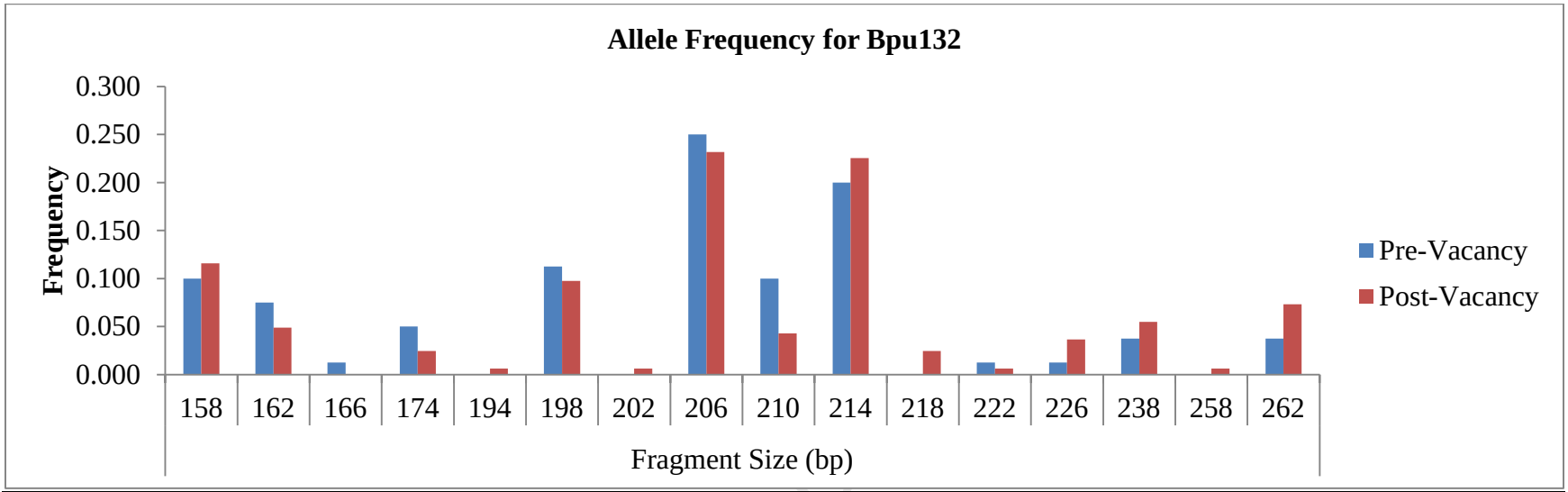


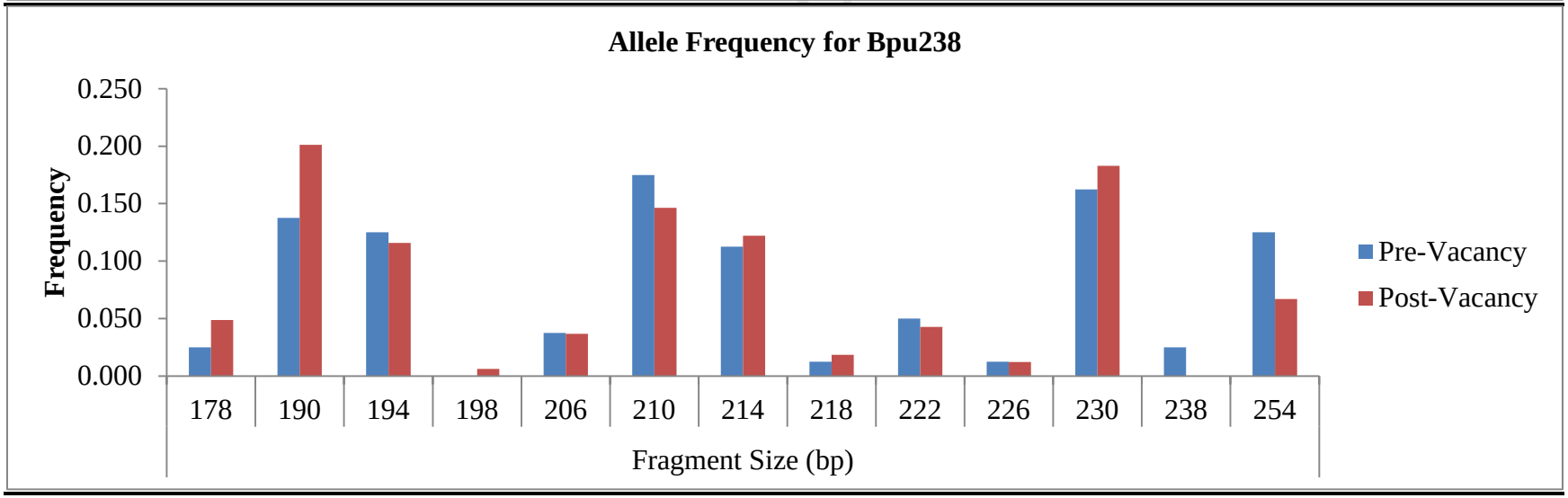
Appendix 7 continued



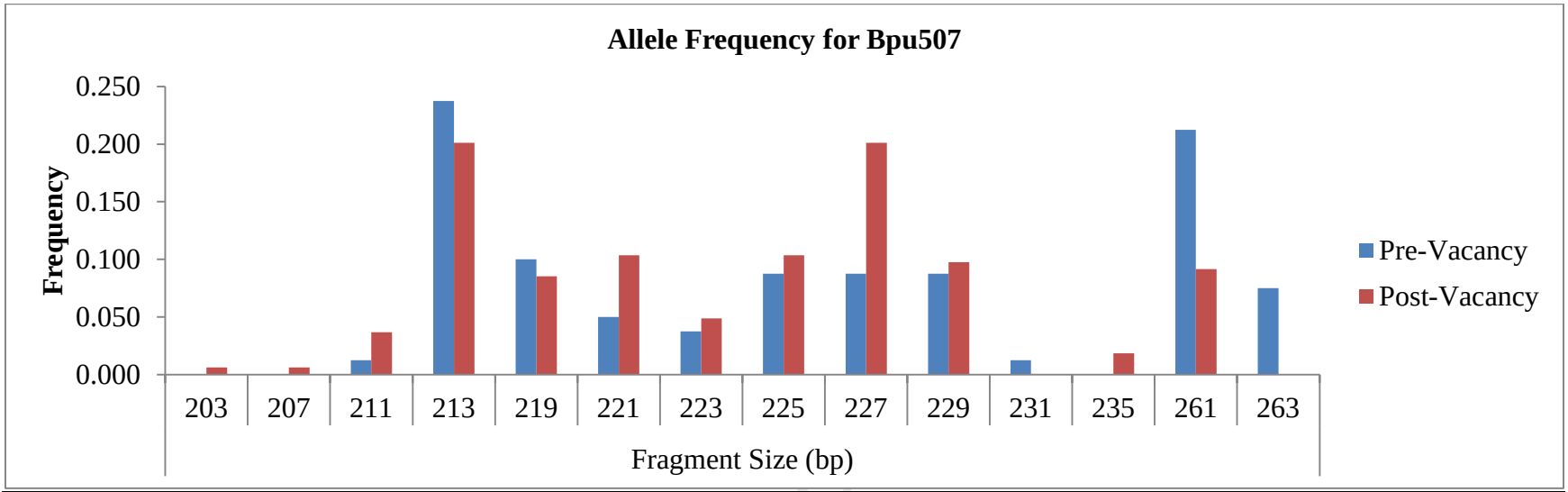


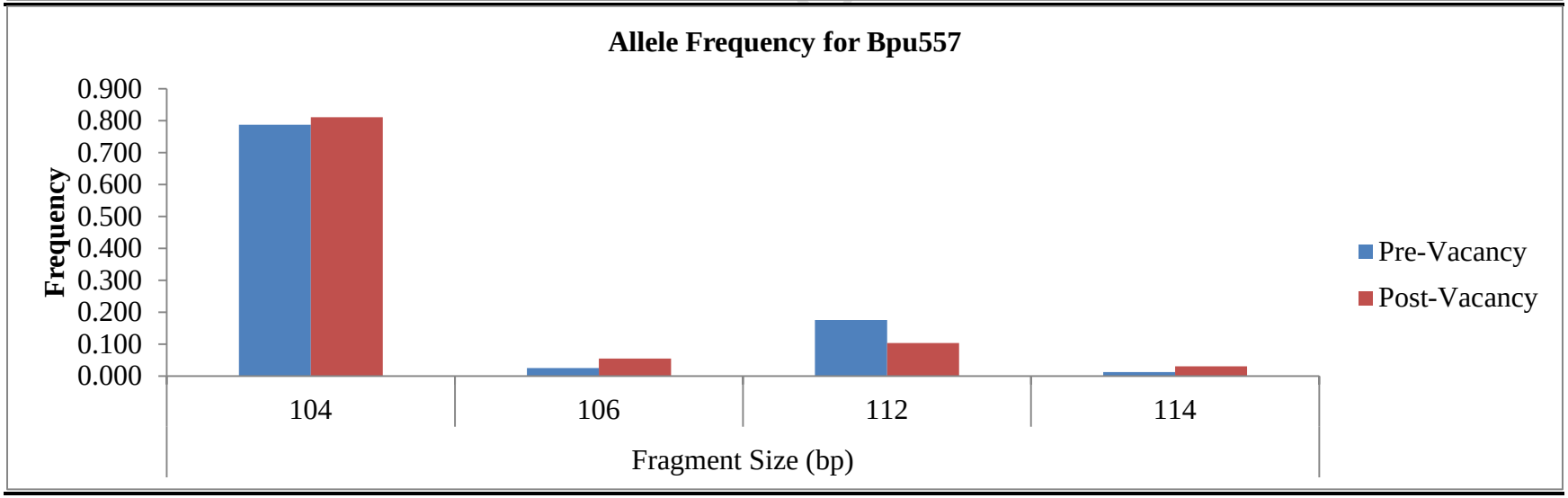
Appendix 7 continued





Appendix 7 continued





Appendix 8 Polymerase chain reaction details for *Bradypodion pumilum* microsatellite loci using different *Taq* polymerase

***GoTaq* PCR Recipe (10 µL)**	***Super-Therm* PCR Recipe (10 µL)**
Storage Buffer (10X containing 10.0 mM Tris-HCl), 25.0 mM $MgCl_2$, 10.0 mM dNTPs, 5.0 U/µL *GoTaq* polymerase (Promega Corp.), forward and reverse primers, ddH_2O, and DNA template	Storage Buffer (20.0 mM Tris-HCl, 100.0 mM NaCl, 0.1 mM EDTA, 1.0 mM DTT, stabilizers and 50% glycerol), 25.0 mM $MgCl_2$, 10.0 mM dNTPs, 5.0 U/µL *Super-Therm* polymerase (Southern Cross Biotechnology), forward and reverse primers, ddH_2O, and DNA template

Appendix 9 Component volumes (µL) within polymerase chain reactions for *Bradypodion pumilum* microsatellite loci using *GoTaq* Polymerase

Locus	**Label**	**Storage Buffer**	**$MgCl_2$**	**dNTPs**	***GoTaq* Polymerase**	**Primers (F and R)**	**ddH_20**	**DNA Template**
Bpu26 $(TTAC)_{26}$	*HEX*	3.125	0.70	0.08	0.10	0.20	3.095	2.5
Bpu28 $(TATC)_{30}$	*HEX*	3.125	1.00	0.08	0.10	0.065-0.150	2.565-4.565	1.0-3.0
Bpu94 $(GTT)_{17}$	*HEX*	1.25-3.125	0.40-2.00	0.08	0.10	0.20	3.295-6.770	1.0-4.0
Bpu115 $(TAGA)_{14}$	*HEX*	3.125	0.50-1.50	0.08	0.10	0.20	1.695-3.295	2.5-4.0
Bpu132 $(TATG)_{17}$	*6FAM*	3.125	1.00	0.08	0.10	0.10	4.495	1.0
Bpu238 $(TATC)_{26}$	*6FAM*	3.125	1.00	0.08	0.10	0.15-0.20	0.895-4.395	1.0-4.5
Bpu557 $(GT)_8$	*HEX*	3.125	0.50-1.50	0.08	0.10	0.20	2.295-3.295	2.5

Appendix 10 Component volumes (µL) within polymerase chain reactions for *Bradypodion pumilum* microsatellite loci using *Super-Therm* Polymerase

Locus	Label	Storage Buffer	$MgCl_2$	dNTPs	*Super-Therm* Polymerase	Primers (F and R)	ddH_20	DNA Template
Bpu26 $(TTAC)_{26}$	*HEX*	1.25	0.60	0.08	0.10	0.16-0.18	4.13-6.65	1.0-3.5
Bpu28 $(TATC)_{30}$	*HEX*	1.25	0.40-0.50	0.08	0.10	0.17-0.20	3.73-6.77	1.0-4.0
Bpu94 $(GTT)_{17}$	*HEX*	1.25	0.40-0.60	0.08	0.10	0.20	3.57-6.77	1.0-4.0
Bpu115 $(TAGA)_{14}$	*HEX*	1.25	0.50	0.08	0.10	0.20	6.17-6.67	1.0-2.5
Bpu132 $(TATG)_{17}$	*6FAM*	1.25	0.40-0.50	0.08	0.10	0.18-0.20	5.71-6.71	1.0-2.0
Bpu238 $(TATC)_{26}$	*6FAM*	1.25	0.40-0.60	0.08	0.10	0.18-0.20	5.71-6.77	1.0-3.0
Bpu507 $(TG)_{23}$	*6FAM*	1.25	0.40-0.50	0.08	0.10	0.16-0.20	4.25-6.81	1.0-3.5
Bpu557 $(GT)_8$	*HEX*	1.25	0.50	0.08	0.10	0.18-0.20	3.71-6.67	1.0-4.0

Appendix 11 Polymerase chain reaction thermal cycling conditions for *Bradypodion pumilum* microsatellite loci

Locus	Label	Annealing Temperature ^oC/Number of Cycles
Bpu26	*HEX*	63.0-65.0^oC/35-38 Cycles
Bpu28	*HEX*	56.1-60.5^oC/30-35 Cycles
Bpu94	*HEX*	48.0-58.0^oC/32-35 Cycles
Bpu115	*HEX*	52.4-57.9^oC/35 Cycles
Bpu132	*6FAM*	48.0-58.0^oC/35 Cycles
Bpu238	*6FAM*	60.5-63.0^oC/30-35 Cycles
Bpu507	*6FAM*	54.0^oC/35 Cycles
Bpu557	*HEX*	48.0-52.0^oC/35 Cycles

Appendix 12 Summary of linkage disequilibrium test results for *Bradypodion pumilum* using the combined dataset (GENEPOP on the Web V.4.0.10, Raymond and Rousset 1995; Rousset 2008).

Number of populations detected: 1
Number of loci detected: 8
Markov chain parameters
Dememorisation: 1000
Batches: 100
Iterations per batch: 1000

Locus #1	Locus #2	*P*-Value	S.E.	Switches
BPU94	BPU28	0.22871	0.040284	532
BPU94	BPU26	0.592480	0.045555	568
BPU28	BPU26	1.000000	0.000000	136
BPU94	BPU115	0.166240	0.033670	560
BPU28	BPU115	0.009940	0.009940	107
BPU26	BPU115	0.003710	0.003710	120
BPU94	BPU132	0.009400	0.008327	580
BPU28	BPU132	0.774310	0.041137	141
BPU26	BPU132	0.081270	0.026216	159
BPU115	BPU132	0.273220	0.043063	137
BPU94	BPU238	0.081540	0.024908	789
BPU28	BPU238	0.009030	0.009030	160
BPU26	BPU238	0.005940	0.005940	99
BPU115	BPU238	0.013230	0.010478	167
BPU132	BPU238	0.013770	0.010651	189
BPU94	BPU507	0.040710	0.015325	936
BPU28	BPU507	1.000000	0.000000	191
BPU26	BPU507	0.086950	0.027938	243
BPU115	BPU507	0.287100	0.044523	190
BPU132	BPU507	0.037630	0.018642	246
BPU238	BPU507	0.739730	0.042080	294
BPU94	BPU557	0.116970	0.019170	5332
BPU28	BPU557	0.404670	0.039018	3001
BPU26	BPU557	0.011390	0.008440	3024
BPU115	BPU557	0.118640	0.024084	3009
BPU132	BPU557	0.026160	0.011991	2959
BPU238	BPU557	0.009720	0.003991	3577
BPU507	BPU557	0.003680	0.002618	3970

Appendix 13 Summary of null allele test results for *Bradypodion pumilum* using pre- and post-vacancy datasets (Micro-Checker, van Oosterhout *et al*. 2004). *(A)* Null alleles table and *(B)* Analysis of molecular variance (AMOVA) results for adjusted and original datasets

(A)

LOCUS	***Pre-Vacancy***				***Post-Vacancy***			
BPU94	161,161	170,170	188,188	209,209	161,161	170,170	188,188	
BPU28	None				None			
BPU26	Yes*				199,199	203,203	243,243	247,247
BPU115	None				None			
BPU132	None				None			
BPU238	None				None			
BPU507	None				None			
BPU557	None				None			

** Locus was suggested to contain null alleles; however, Brookfield 1 Method contained no genotype adjustments.*

(B)

Null-Adjusted AMOVA	**Value**	***p*-Value**
F_{ST}	0.002	0.146
R_{ST}	0.019	0.018

Original AMOVA	**Value**	***p*-Value**
F_{ST}	0.001	0.245
R_{ST}	0.017	0.019

Appendix 14 Characteristics of microsatellite loci and primers developed for *Bradypodion pumilum* (Feldheim *et al*. 2010)

Locus	Repeat motif	Primer sequence (5'–3')	Label	T_a(°C)	Mgcl2 (mM)	Number of alleles (size range)	Ho	He	Genbank Access #
Bpu557	(GT)8	F: GACTTGCTGAGGGATATTAC R: GGCACTGGCATCCCTAAATA	Hex	48	1.25	5 (100–116)	0.279	0.293	GU066303
Bpu571	(GA)11	F: CAATATGCCACCTAACCATC R:CCATGACAAATTACACAAACCTC	6Fam	57	6	7 (126–139)	0.465	0.558	GU066304
Bpu94	(GTT)17	F: CAGCTTTGGCGTCTTACACA R: GCCTTAAAGGAAGGAAAGTGG	Hex	48	1.25	13 (161–206)	0.75	0.854	GU066305
Bpu115	(TAGA)14	F: GCTGTGATATGTAAATTCAGGG R: CACTTTGTTTTGGTCTCCCACT	Hex	55	1	22 (108–216)	0.786	0.939	GU066306
Bpu238	(TATC)26	F: CCCCAATCTCGTTGTTCTGT R: CTCATTTCCTCCTCCCCATT	6Fam	58	1	18 (155–239)	0.929	0.93	GU066307
Bpu26	(TTAC)26	F: TGAAATCTCGCTATCCTTGT R: CTTTCGAGTAAGGGAGACCT	Hex	63	6	26 (172–288)	0.881	0.941	GU066308
Bpu132	(TATG)27	F: CGCTATTTCCCCTCAAAATC R: TGGCTCCATATAGCAACACG	6Fam	48	0.75	22 (162–282)	0.93	0.939	GU066309
Bpu28	(TATC)30	F: CTGGAAACCTCCCTGCCTAT R: TGGACTTATAGTCCGCCTTCC	Hex	58	1	21 (162–262)	0.881	0.941	GU066310

Appendix 15 *(A)* Per loci number of alleles, size range, and heterozygosity estimates in each habitat for *Egernia cunninghami* (Stow *et al*. 2001)

Locus	Number of Alleles	Allelic Size Range	Ho	He
Cleared				
Est13	16	(164–244)	0.947	0.912
Tr3.2	20	(169–269)	0.891	0.906
Tr5.21	18	(79–145)	0.86	0.888
Tr5.20	3	(146–152)	0.266	0.291
Est9	8	(219–279)	0.419	0.446
Est1	20	(209–337)	0.957	0.913
Reserve				
Est13	17	(180–252)	0.868	0.926
Tr3.2	20	(161–261)	0.961	0.928
Tr5.21	15	(79–145)	0.887	0.875
Tr5.20	3	(146–152)	0.26	0.268
Est9	12	(215–267)	0.66	0.646
Est1	20	(209–329)	0.94	0.883

Appendix 15 continued

(B) The number of alleles (No.), the proportion of heterozygous individuals (*H*O) and the gene diversity (*H*E) for each locus at each site, and the significance levels for any deviations from Hardy–Weinberg proportions (heterozygote deficit; **P* <0.05, ***P* <0.01; ****P* <0.001). (Sumner *et al*. 2004)

Locus	Site	F1	F2	F3	F4	F5	F6	F7	C1	C2	C3	C4	C5	Average
GQ20/21	No.	6	8	5	5	9	8	5	8	8	11	7	9	7.42
	HO	0.72	0.86	0.63	0.67	0.76	0.7	0.74	0.71	0.67	0.72	0.71	0.75	0.72
	HE	0.73	0.77	0.66	0.65	0.76	0.73	0.57	0.73	0.71	0.65	0.59	0.74	0.69
GQ10/11	No.	8	11	10	7	10	8	8	8	5	9	8	12	8.67
	HO	0.72	0.76*	0.68	0.72	0.61**	0.40***	0.70*	0.67	0.67	0.88	0.64	0.65	0.68
	HE	0.68	0.73	0.75	0.77	0.72	0.67	0.78	0.66	0.67	0.84	0.57	0.66	0.71
GQ24/25	No.	9	10	10	12	14	10	13	9	11	10	11	17	11.33
	HO	0.68*	0.83	0.91	0.83	0.92	0.67	0.75*	0.79	0.8	0.66	0.85	0.82	0.79
	HE	0.78	0.83	0.86	0.87	0.89	0.8	0.83	0.84	0.8	0.69	0.81	0.82	0.82
GQ38/39	No.	3	3	4	2	4	5	3	4	3	5	4	4	3.67
	HO	0.25	0.12	0.56	0.11	0.3	0.33	0.07	0.15	0.23	0.28	0.21	0.15	0.23
	HE	0.32	0.12	0.43	0.11	0.27	0.3	0.07	0.18	0.24	0.31	0.31	0.17	0.24
GQ18/19	No.	6	6	11	7	11	10	6	9	8	7	16	10	8.92
	HO	0.63	0.48	0.64	0.61	0.71	0.79	0.58	0.82	0.7	0.65	0.89	0.53	0.67
	HE	0.7	0.46	0.71	0.6	0.7	0.73	0.66	0.73	0.71	0.66	0.84	0.46	0.66
GQ16/17	No.	9	10	3	5	8	8	6	6	8	10	9	9	7.58
	HO	0.82	0.52	0.36**	0.63	0.45*	0.75	0.81	0.59	0.8	0.77	0.68	0.56*	0.65
	HE	0.83	0.5	0.47	0.53	0.58	0.68	0.71	0.61	0.78	0.81	0.58	0.6	0.64
GQ36/37	No.	11	10	9	10	14	14	12	15	13	11	11	14	12
	HO	0.8	0.83	0.75	0.72	0.82	0.9	0.82	0.89	0.93	0.81	0.71	0.79	0.82
	HE	0.87	0.82	0.78	0.77	0.85	0.89	0.84	0.9	0.87	0.84	0.73	0.8	0.83
EA1/2	No.	7	11	13	6	8	12	11	10	12	12	11	15	10.67
	HO	0.72	0.86	0.86	0.78	0.79	0.97	0.78*	0.96	0.8	1	0.82	0.79	0.84
	HE	0.77	0.83	0.83	0.74	0.8	0.86	0.87	0.86	0.82	0.84	0.83	0.83	0.82
GQ42/43	No.	4	4	3	4	5	4	5	4	4	4	4	4	4.08
	HO	0.76	0.64	0.63	0.61	0.76	0.73	0.56	0.52*	0.67	0.68	0.82	0.48	0.65
	HE	0.66	0.59	0.65	0.64	0.76	0.74	0.65	0.73	0.59	0.58	0.68	0.53	0.65
All loci	Average no.	7	8.11	7.56	6.44	9.22	8.78	7.67	8.11	8	8.78	9	10.44	
	Average HO	0.68	0.66	0.67	0.63	0.68	0.69	0.65	0.68	0.7	0.72	0.71	0.62	
	Average HE	0.7	0.63	0.68	0.63	0.7	0.71	0.66	0.69	0.69	0.69	0.66	0.62	