A comparison of two species of bandicoots (*Perameles nasuta* & *Isoodon obesulus*) influenced by urbanisation: population characteristics, genetic diversity, public perceptions, stress and parasites

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Summary

Bandicoot populations and species such as the southern brown bandicoot (*Isoodon obesulus*) are in decline throughout Australia, with the notable exception of the long-nosed bandicoot (*Perameles nasuta*). Urbanisation is a major contributor to this decline, creating habitats that are unfavourable to the long-term persistence of many native species. Knowledge of how animals cope with their environment and adapt to its changes is fundamental to the management of urban and wild populations. This study investigated issues pertaining to the management of two bandicoot species in a wild (National Park) and peri-urban (backyard) setting in northern Sydney. Specifically, the study provided an ecological snapshot, examined parasite loads (*Cryptosporidium* and ecto-parasites), investigated stress levels through faecal glucocorticoid metabolites (FGMs), defined genetic diversity and gene flow across populations and explored public perceptions of the local community towards bandicoots.

The attitudinal survey provided context behind the conflicts between bandicoots, humans and domestic pets. A direct interaction, the age of a respondent and pet ownership was pivotal in influencing the respondents' perception of a bandicoot. Live trapping and hair-tube surveys revealed a single *I. obesulus* record outside Ku-ring-gai Chase National Park. Genetic analysis identified gene flow, albeit low, between the long-nosed bandicoots of Ku-ring-gai Chase and Garigal National Park, suggesting a single interbreeding population. Through similar genetic history, ecological traits and sympatric nature in Ku-ring-gai Chase, connectivity of habitat between the two National Parks is also likely to be present for the southern brown bandicoot. FGM analysis of long-nosed bandicoots revealed that capture overnight did not represent a prolonged physiological response. Additionally, FGM analysis failed to detect a difference across habitat types and between body condition values of bandicoots. This was despite the presumed increased risks of obtaining resources in the more open suburban backyard environments. The parasite analyses observed bandicoots on the urban interface carrying the paralysis tick (*Ixodes holocyclus*) and potential zoonotic types of *Cryptosporidium*. It also highlighted the opportunity for the transmission of parasites to occur between host species in an area of elevated contact between wildlife, humans and domestic pets. The information generated from the investigations will find application with the bandicoots of northern Sydney and provide management with suitable information to employ conservation strategies conducive to the persistence of threatened and non-threatened Australian fauna, particularly *I. obesulus.*

Declaration

The work described is original and has not been submitted, in any form, for a higher degree at any other university or institution. I agree to allow a copy of my thesis to be deposited in the Macquarie University library for consultation, loan and photocopying forthwith.

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Matthew Dowle

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Date

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List of Publications

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1. Introduction

Early Aboriginals honoured the bandicoot as a Creator God. The 'dreamtime' stories, tell of 'Karora', a giant bandicoot who slept in darkness under the earth, until he awoke and gave birth to man from beneath his armpit.

1.1 General introduction

In 1845, Gould described the southern brown bandicoot (*Isoodon obesulus*) as "one of the very commonest of Australian mammals". During the 1800s the distribution of bandicoots spanned the continent and was a common source of food for Aboriginals and early settlers. Today, *I. obesulus* has suffered from the impacts of anthropogenic disturbances, resulting in large-scale declines from its former habitat. *Isoodon obesulus* is currently listed as threatened under National and State (New South Wales, South Australia and Victoria) legislation (http://www.environment.gov.au/cgi-bin/sprat/public/publicspecies.pl?taxon_id=68050).

The long-nosed bandicoot (*Perameles nasuta*) is sympatric across much of the same habitat as *I. obesulus*, particularly along the east coast of Australia. In these habitats, *P. nasuta* endures the same threats and environmental processes as *I. obesulus*, but seems to be robust to the pressures contributing to the decline of *I. obesulus*. *Perameles nasuta* is classified as common across its range with populations persisting in a wide range of habitats, including remnant bushland and suburban backyards.

Knowledge of how wildlife such as bandicoots cope with their environment and its perturbations is fundamental to the management of urban and wild populations. This thesis examines and compares the dynamics of *I. obesulus* and *P. nasuta* across the undisturbed (National Parks) and peri-urban (backyards) habitats of northern Sydney. The thesis provides conservation managers with information toward the management of Australian mammals in an urban landscape, and in particular, *I. obesulus* and *P. nasuta* of northern Sydney.

The thesis incorporates a diversity of issues pertaining to the bandicoots of northern Sydney, but also provides an integrated context for research to a surprisingly understudied group of Australian marsupials. The research interests are assimilated in the thesis through the assessment of the encroachment of the anthropogenic environments in which the bandicoots inhabit, in particular, the suburban backyards and National Parks of northern Sydney. In

addition, one of the powerful features of the thesis is the ability to compare the investigations between two bandicoot species differing in conservation status. The topics of the thesis are not only linked through the analyses of the dynamics of the species to anthropogenic environments, but also by providing an ecological snapshot and picture of overall health of the populations of the two bandicoot species.

In order to set the context for future management opportunities, the thesis initially asks the community on the urban fringe what their attitudes are towards the bandicoots and how often conflicts between bandicoots, humans and domestic pets (potential predators) occur. The thesis then looks at the dynamics of both species across the different habitats and provides an analysis of their genetic health. The genetic examination focuses on the factors potentially limiting the ability of *I. obesulus* to succeed in a peri-urban habitat in the long-term, such as restrictions to gene flow between habitats, the occurrence of a population bottleneck and inbreeding. The thesis then investigates if bandicoots in a peri-urban habitat have an adverse physiological response compared to bandicoots in National Parks, which may be explained by increased interactions with domestic cats and dogs. Lastly, the thesis tackles the issues of parasitism. It highlights the pathway for the transmission of ecto and endo-parasites to occur across host species on the urban fringe (e.g. wildlife and domestic pets) through the opportunities created by the peri-urban environment.

The dynamic in which the bandicoots inhabit the urban influenced environments is of paramount importance for their successful long-term management, especially for the threatened *I. obesulus*. This thesis not only provides a platform for further research, but also tackles a diversity of issues to provide novel research ideas, outcomes and conservation management opportunities.

1.1.1 Species descriptions

Two species of bandicoot have been used for this thesis; *Isoodon obesulus* (southern brown bandicoot) and *Perameles nasuta* (long-nosed bandicoot). Southern brown bandicoots have a shorter snout and more rounded ears than the long-nosed bandicoot. Both species have coarse fur covering the body. The southern brown bandicoot has a mostly brown coat, flecked with yellow-brown and a pale underbelly, while the long-nosed bandicoot has a grey-brown coat with a creamy white underside (Figure 1-1).

Figure 1-1: Southern brown bandicoot – *Isoodon obesulus* (above) and long-nosed Bandicoot – *Perameles nasuta* (below). Major differences between the two species can be noted in the relatively shorter snout, smaller ears and the browner (golden flecked) fur of the southern brown bandicoot.

1.2 Biology of bandicoots

The name bandicoot comes from an initial description and loose translation meaning pig-rat, referring to the Bandicoot rats (*Bandicota*) in India.

Bandicoots are small to medium sized marsupials that show sexual dimorphism. They are generally nocturnal and solitary in nature with an omnivorous diet (Tyndale-Biscoe 2005). They are the only marsupial with a placenta-like organ similar to eutherian mammals and have been credited with the shortest gestation for any mammal (11.5 to 12.5 days) (Lyne 1974; Cockburn 1990; Tyndale-Biscoe 2005). Bandicoots have a high reproductive potential, which is offset by a high mortality rate during juvenile dispersal. Bandicoots are also the only Australian marsupial with the combination of syndactyly toes and polyprodont dentition (Ashby *et al*. 1990; Tyndale-Biscoe 2005).

Despite these unique traits, bandicoots are the most ecologically under-studied group of all marsupials. This is reflected in the lack of public affection and perceptions' towards this 'rat like animal' (Ashby *et al.* 1990). Up until the 1990's and the save the 'Eastern Barred Bandicoot' campaign, there had been relatively little ecological work undertaken on any species of bandicoot. However, recent changes in public attitudes towards conservation in urban landscapes (Mankin *et al*. 1999; Savard *et al.* 2000; Manfredo *et al*. 2003) have seen a significant rise in research, focusing on the management of wildlife including bandicoots and their interactions with humans.

1.2.1 Taxonomy

Bandicoots are marsupials (Metatheria), a group of species that give birth to relatively undeveloped young. Within the Metatheria, bandicoots belong to the Peramelidae, a family within the order Peramelemorphia (Figure 1-2). The Peramelemorphia includes Australian and New Guinea species showing polyprotodontia and syndactyly of the second and third digits of the hind limbs (Figure 1-3).

Order Peramelemorphia

Peramelemorphia is made up of marsupial omnivores, identified by their distinctive bandicoot shape. Peramelemorphia is a very ancient group, co-equal with the didelphids and caenolestids in South America and not closely related to either the dasyurid or diprotodont

marsupials of Australasia (Kirsch *et al.* 1977; Springer *et al.* 1994; 1998; Burk *et al.* 1999; Tyndale-Biscoe 2005).

Peramelemorphia has historically been difficult to categorise because of their polyprotodontia and syndactyly traits. The anatomical features of syndactyly and polyprotodontia would imply a link with two major groups of Australasian taxa, the diprotodontians or dasyuromorphians (Baker *et al*. 2004). However, recent studies using modern DNA techniques have put the time of separation of bandicoots from other Australian marsupials at an estimated 60 million years (Tyndale-Biscoe 2005). This has an important consequence; it implies the complex evolution of syndactyly occurred twice throughout evolution.

Within the order Peramelemorphia, bandicoots form three distinct groups covering species from Australia and New Guinea. This thesis has followed the Tyndale –Biscoe (2005) classification to treat the three groups as two Australian families and a New Guinea family; Peramelidae, Thylacomyidae and Peroryctidae (Figure 1-4).

Linnaean Classification of Bandicoots

- **Kingdom**: Animalia

  - **Phylum**: Chordata (vertebrates)

    - **Class**: Mammalia (mammals; warm blooded vertebrates)

      - **Subclass:** Theria (live-bearing mammals)

        - **Infraclass**: Metatheria (marsupials)

          - **Order**: Peramelemorphia (bandicoots and bilbies)

            - **Family**: Peramelidae (bandicoots)

              - **Subfamily**: Peramelinae (Australian bandicoots)

Figure 1-2: Scientific classification of bandicoots. Source: adapted from Tyndale-Biscoe (2005), Bennett (2008) and Van Dyck and Strahan (2008).
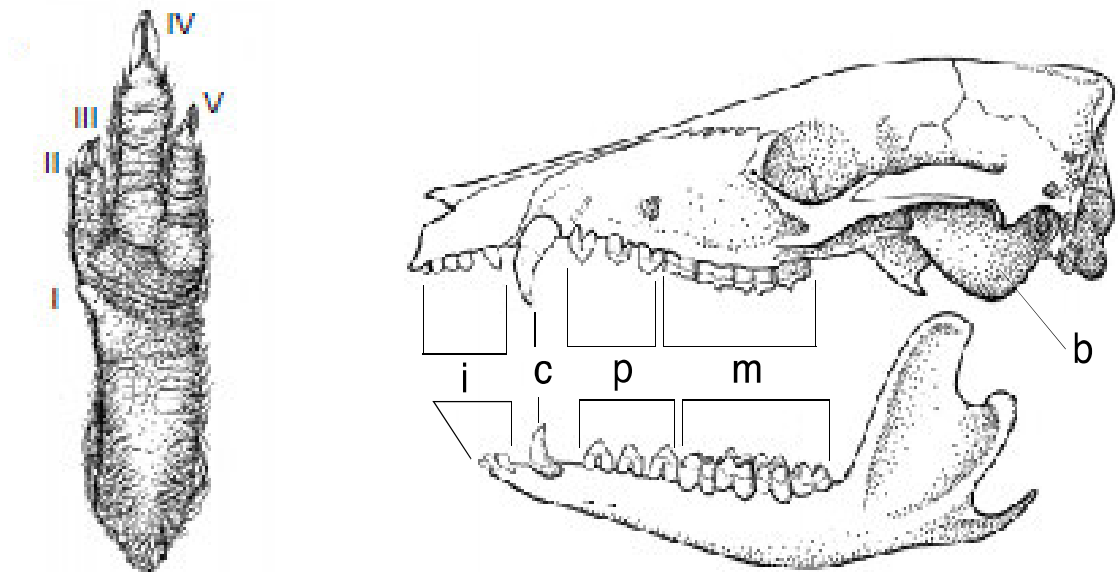
Figure 1-3: *Left* - Syndactyly of the hind limb. Note the fusion of the second and third digits. *Right* - Mandible of a northern brown bandicoot (*Isoodon macrourus*); b = bullae; c = canine; i = incisors; m = molars; p = premolars; pf = palatal foramina. Pictures extracted from Gordon and Hulbert (1989).

Figure 1-4: The phylogeny of the order Peramelemorphia. Shows the relationships of living species of Australian bandicoots and establishes their association with the New Guinea species. Source: adapted from Tyndale-Biscoe (2005) and Bennett (2008).

Family Peramelidae

As with higher order systematics, debate has occurred over the grouping of subfamilies, genera and species within the Peramelidae. Early studies used morphology and mtDNA to ascertain evolutionary position (Kirsch *et al*. 1977; Szalay 1982; Meredith *et al.* 2008). Traditionally, Peramelidae included eight genera from New Guinea and Australia. However, their specific interrelationships were the subject of ambiguity (Westerman and Krajewski 2000). Advances in technology have allowed a revision of classification, with the Peramelidae now limited to Australian bandicoots (Tyndale-Biscoe 2005). The Peramelidae comprises six extant and one extinct species in two closely related genera:

- The short-nosed bandicoots *Isoodon* (*I. auratus, I. macrourus, I. obesulus*).
- The long-nosed bandicoots *Perameles* (*P. bougainville, P. gunnii, P. nasuta*).

<u>*Isoodon obesulus*</u>

Isoodon obesulus has adapted to a range of environments from temperate to tropical and mesic to arid, resulting in distinctive regional morphologies, which have impeded attempts to develop a reliable taxonomic standing (Zenger *et al*. 2005). This led to the division of the species into sub-species based on their fragmented geographical distribution and associated morphological features (Table 1-1). Zenger *et al*. (2005) recommends the recognition of three *I. obesulus* sub-species as opposed to the previously identified five (Pope *et al*. 2001), which comprise a single morphologically diverse species that was once widespread across Australia (Zenger *et al*. 2005).

Table 1-1: A table showing *Isoodon obesulus* sub-species and their distribution.

Sub-species	Distribution
Isoodon obesulus obesulus	South Australia, Victoria, Tasmania & New South Wales
Isoodon obesulus fusciventer	South-west of Western Australia
Isoodon obesulus peninsulae	Far north-east Queensland

Throughout this thesis the sub-species classification has been removed from *Isoodon obesulus obesulus* for ease of reference. The thesis refers to the southern brown bandicoots populations in northern Sydney as just *Isoodon obesulus*.

1.2.1 Reproduction and development

The bandicoot reproductive strategy is based on high productivity and early weaning of young (Gordon 1974; Merchant 1990). They do this by responding to suitable environmental conditions with quick reproduction and a rapid colonisation of adjacent habitats (Tyndale-Biscoe 2005).

Two features of bandicoot biology contribute to the unusually fast rate of reproduction: the complex placenta and the composition of the bandicoot milk. The placenta in bandicoots is a highly efficient organ of exchange during gestation, allowing quick growth for the young bandicoot (Tyndale-Biscoe 2005). This produces a newborn bandicoot substantially larger than that of a dasyurid or didelphid of similar adult size and 20 times the size of the Tasmanian bettong newborn (Cockburn 1990; Tyndale-Biscoe 2005). The composition of bandicoot milk during lactation also allows for rapid development of the pouch young. The ability to rapidly change the quality of milk to cope with successive litters suggests an adaptive coping strategy suited to fast reproduction and juvenile development (Merchant 1990).

Gestation for *P. nasuta* and *I. obesulus* is shorter than any other mammal, lasting for only 12.5 days. Lactation lasts approximately 45 to 60 days (Heinsohn 1966; Lyne 1964; Gemmell 1982; Cockburn 1990; Merchant 1990). The female also has the ability to return to oestrus during the last 10 days of lactation to give birth to new young at the time of weaning the older litter (Merchant 1990). The number of young produced varies slightly according to species with mean litter size of 2-4 for *I. obesulus* and 2-3 for *P. nasuta,* but can be as many as six for either species (Heinsohn 1966; Gordon 1974; Stoddart and Braithwaite 1979; Gemmell 1982; Lobert and Lee 1990; Short *et al*. 1998).

The initiation of the breeding cycle is influenced by a range of environmental variables such as day length, minimum temperature and rainfall, and can be highly predictable (Heinsohn 1966; Stoddart and Braithwaite 1979; Gemmell 1982; Barnes and Gemmell 1984; Friend 1990; Lobert and Lee 1990). Heinsohn (1966) suggested the breeding season correlated with food supply and not directly to rainfall and temperature, and hence the cessation of breeding activity is correlated with a decline in the abundance of food. In later studies, the remarkable synchrony among females entering oestrus is thought to result from photoperiod (Stoddart and Braithwaite 1979; Lobert and Lee 1990). These later studies did not accept ephemeral factors

such as rainfall and prey availability due to a large variation in the start of the breeding season. However, Barnes and Gemmell (1984) in a study of captive bandicoots correlated the number of births to rates of change of variables such as day length, rainfall, maximum and minimum temperature. Despite conflicting studies, it is apparent that breeding activity of bandicoots is highly variable, but predictable, and is most likely dependent upon geographical distribution.

Although bandicoots have the ability to breed throughout the year, they show evidence of annual cycles in their reproductive activity, which is more pronounced with increasing latitudes (Gemmell 1982; Barnes and Gemmell 1984; Short *et al*. 1998). Breeding cycles are slightly later in the northern compared to southern populations. Breeding peaks occur at early spring in Tasmania and late spring in New South Wales and Queensland and early summer in the Northern Territory (Barnes and Gemmell 1984). These seasonal breeding peaks correspond to the wetter months when there is maximum food abundance (Heinsohn 1966; Gordon 1974; Short *et al*. 1998; Friend 1990). For example, with *P. gunnii* in Tasmania, Heinsohn (1966) showed the breeding peaked during winter, spring and early summer when the food is most abundant while the non-breeding period occurred through April and May in late Autumn. This is similar to Mallick *et al*. (1998b) where a peak in breeding for *I. obesulus* was observed to occur during spring and summer. *Perameles nasuta* at North Head in NSW is comparable, but with a slightly reduced length in breeding season. The females cease lactation by late autumn / early winter after a peak reproduction during late spring / early summer. This coincides with a lower thermoregulatory demand in summer, increased availability of invertebrates and the increased growth of new and more palatable vegetation (Scott *et al*. 1999).

1.2.2 Diet

Bandicoots have an omnivorous and insectivorous diet (Heinsohn 1966; Quin 1985; Mallick *et al*. 1998a; Scott *et al*. 1999; Thums *et al*. 2005) with insects accounting for the major component. Their teeth are rooted, sharp and adapted to eating insects (Heinsohn 1966). Their long slender muzzle and powerful fore claws are used for searching in crevices and digging in soil, which creates distinctive conical holes (Gordon and Hulbert 1989; Van Dyck and Strahan 2008). Bandicoots are hindgut fermenters and possess a caecum, suggesting plant material constitutes a significant part of the diet (Gordon and Hulbert 1989; Keiper and

Johnson 2004). The vegetative diet is thought to be a by-product of their insectivorous foraging, but can also be taken for its nutritional value (Mallick *et al*. 1998a).

In the habitats of northern Sydney, it is thought that *I. obesulus* and *P. nasuta* fulfil an opportunistic omnivore niche by utilizing a variety of invertebrate, plant and fungal material, through which the components shift with the seasons. Gut analyses of bandicoot diet suggests that the primary target in the dryer months are insects and small amounts of plant material, while plant material and fungal species have a much greater intake in the wetter months (Heinsohn 1966; Quin 1985; Mallick *et al*. 1998a; Scott *et al*. 1999; Thums *et al*. 2005).

In Tasmania, earthworms constitute the major diet of *P. gunnii* and *I. obesulus* during the wetter months, and insects (moths, butterflies, beetles and multiple types of larvae) form the primary diet in the summer months (Heinsohn 1966). However, Quin (1985) and Mallick *et al*. (1998a) identified a broader range of food types including plant material such as grasses, seeds, root nodules and different types of fungi. The discrepancies between studies were hypothesised to be the local availability of prey (Quin 1985). It is most likely that this availability of prey and food items in the bandicoot diet is linked strongly to habitat, season and microclimates.

1.2.3 Habitat

Bandicoots have been known to establish themselves across a wide variety of habitats, through their ability to rapidly colonise areas as a response to favourable conditions. Factors such as habitat complexity, floristic characteristics, diet and large-scale and local climatic factors are important in determining habitat preference and distribution. For example, the habitat preference for *I. macrourus* and *I. obesulus* in the northern half of the continent is partly determined by rainfall zones and rainfall gradients, indicating a seasonal shift in habitat utilisation (Keiper and Johnson 2004; Gordon *et al*. 1990; Van Dyck and Strahan 2008). *Isoodon macrourus* in particular show a greater response to fine-scale habitat partitioning than their southern counterparts (Vernes 2003). This is possibly because of the response from vegetation and insects to seasonal rains.

Gordon and Hulbert (1989) identified the *Isoodon* genus as having adaptations for use of close ground cover in semi-arid to arid areas, whereas *Perameles* show adaptations for the use

of open habitat and arid through to humid areas. *Perameles* has been associated with rainforests through wet and dry woodland to areas of little and open ground cover (Gordon *et al*. 1990; Chambers and Dickman 2002; Van Dyck and Strahan 2008). More specifically *P. nasuta* has been described as a habitat generalist with the flexibility to make widespread use of alternative habitats (Gordon and Hulbert 1989), while *I. obesulus* has been described as more of a specialist driven by habitat structure, vegetation complexity and floristics (Braithwaite and Gullan 1978; Gordon and Hulbert 1989; Dufty 1994). This is evident in a Victorian heathland where *I. obesulus* shows cause to be a food generalist and habitat specialist, with a dry vegetative community driving habitat preference (Braithwaite and Gullan 1978). However, in northern Sydney, both species are commonly found in heathland habitat (Figure 1-5 and Figure 1-6).

Figure 1-5: Heathland habitat of bandicoots in Ku-ring-gai Chase National Park.

Figure 1-6: Heathland habitat on a sandstone ridge in Ku-ring-gai Chase National Park.

Perameles nasuta and *I. obesulus* are attracted to recently burnt environments (Figure 1-7) and early seral (early to intermediate stage of ecological succession) habitats. This is supported by their high reproductive rates and ability to utilise suboptimal habitat (Heinsohn 1966; Stoddart and Braithwaite 1979; Menkhorst and Seebeck 1990; Scott *et al*. 1999; Van Dyck and Strahan 2008). *Isoodon obesulus* in heathland habitat in Victoria were found to favour regenerating habitat between 4-8 years of age after bulldozing activity, relative to other available habitats (Braithwaite and Gullan 1978; Stoddart and Braithwaite 1979). During the early stages of regeneration after fire or disturbance, the diversity of growing vegetation supports a higher productivity and abundant insect food, which is particularly advantageous for lactating females with higher energy requirements (Heinsohn 1966; Stoddart and Braithwaite 1979; Lobert 1990; Dyck and Strahan 2008). The vegetation and insect components provide resources for the omnivorous and generalist diet of bandicoots, which are often obtained by searching in crevices and digging in softer soils to leave behind the distinctive conical foraging holes (Figure 1-8).

Whilst geographically distant to mainland Australia and northern Sydney, Tasmania represents a comparable situation with sympatric bandicoot species (*Perameles gunnii* and *I. obesulus*). *Isoodon obesulus* prefers areas with low ground cover, scrubby vegetation, heath and early seral habitats (Heinsohn 1966; Gordon and Hulbert 1989; Hocking 1990; Paull 1995; Paull 1999; Dyck and Strahan 2008). *Perameles gunnii* on the other hand will inhabit and forage in these areas, as well as open paddocks and areas with less ground cover (Heinsohn 1966; Hocking 1990). The different habitat preferences for the sympatric bandicoot species was also detailed by Gordon and Hulbert (1989). It also supports the suggestion by Dueser and Porter (1986) that competition in sympatric species of ground-dwelling mammals has a relatively smaller influence on habitat use than habitat structure (Keiper and Johnson 2004). Thus, the two species may persist tolerably within a shared habitat by utilising niches on a fine scale.

Figure 1-7: Recently burnt bushland in Ku-ring-gai Chase National Park.

Figure 1-8: Distinctive conical hole dug by bandicoots whilst foraging.

1.2.4 Distribution and status

Bandicoots, including the *Perameles* and *Isoodon,* are one of the most wide-ranging marsupial groups of the Australian region (Gordon and Hulbert 1989). They occur throughout the mainland, Tasmania and numerous islands off the Australian coast. Despite their wide distribution, mainland bandicoots have suffered severe declines since European arrival with 8 of 11 species extant before arrival now classified as either extinct or threatened (**Table 1-2**). All species have shown range reductions (Tyndale-Biscoe 2005). In Tasmania, the two original species of bandicoot still prosper despite large changes to their habitat (Hocking 1990; Heinsohn 1966).

During the Plio-Pleistocene the Australian continent underwent a climatic shift from warm humid rainforest habitat to a cooler dry environment. The *Perameles* remained in wetter environments while the *Isoodon* took advantage of the newly derived drier habitats in the south of the continent (Gordon and Hulbert 1989). These different adaptive traits have ensured that they occupy a broader ecological niche than suggested by their morphology.

Table 1-2: Distribution and status of Australian bandicoots. Table modified from Seebeck *et al.* (1990), Tyndale-Biscoe (2005) and Van Dyck and Strahan (2008).

Species	Original Distribution	Declined	Status
Perameles bougainville	Southern Australia	1860-1900	2 island populations
Perameles eremiana	Central Australia	1940-1970	Extinct
Perameles gunnii	Victoria Tasmania	1930 – now -	1 relict population Abundant
Perameles nasuta	Eastern Australia	-	Secure
Isoodon auratus	North Western Australia	1940-1970	3 island, 1 relict population
Isoodon macrourus	Northern Australia	-	Reduced but secure
Isoodon obesulus	Southern Australia Tasmania	- -	Reduced, patchy distribution Secure
Macrotis lagotis	Southern & Central Australia	1930-now	Northern part of range only
Macrotis leucura	Central Australia	1920-1960	Extinct
Chaeropus ecaudatus	Southern & Central Australia	1860-1950	Extinct
Echymipera rufescens	North Queensland	-	Secure

Isoodon obesulus

Isoodon obesulus has a fragmented range extending across the Australian continent (Figure 1-9). Since European settlement, its distribution has been reduced on a regional scale particularly in New South Wales, South Australia, Victoria and southern Western Australia (Rees and Paull 2000). *Isoodon obesulus* currently has a patchy distribution along the eastern seaboard from Sydney in New South Wales down into southeast of South Australia. This species also occurs in Tasmania, on a number of islands off the coast of South Australia and as isolated patches in the far northeast of Queensland and the southwest of Western Australia. Due to its reduced distribution and known threats, *I. obesulus (obesulus)* is listed as:

- Endangered under National legislation (*Environment Protection and Biodiversity Conservation Act 1999*);
- Endangered under the State legislation of New South Wales (*Threatened Species Conservation Act 1995*); and
- Vulnerable under the State legislation of South Australia (*National Parks and Wildlife Act 1972*).

Perameles nasuta

Perameles nasuta has a broad distribution along the eastern seaboard of Australia from the far north-east of Queensland extending down along the south coast of Victoria and across the Bass Straight into the north and eastern sections of Tasmania (Figure 1-10). Along its range, *P. nasuta* occurs in many similar habitats to *I. obesulus,* such as the Gippsland in Victoria (Opie *et al.* 1990). The distribution for *P. nasuta* in New South Wales appears to be correlated to areas of shrubby open forests, vacant overgrown urban land, shrub land and coastal heath (Opie *et al.* 1990; Scott *et al.* 1999; Chambers and Dickman 2002).

Perameles nasuta is not listed as threatened under any National or State legislation. However, two populations of *P. nasuta* have an Endangered Population listing under the New South Wales *Threatened Species Conservation Act 1995*:

- Long-nosed Bandicoot, North Head; and
- Long-nosed Bandicoot population in inner western Sydney.
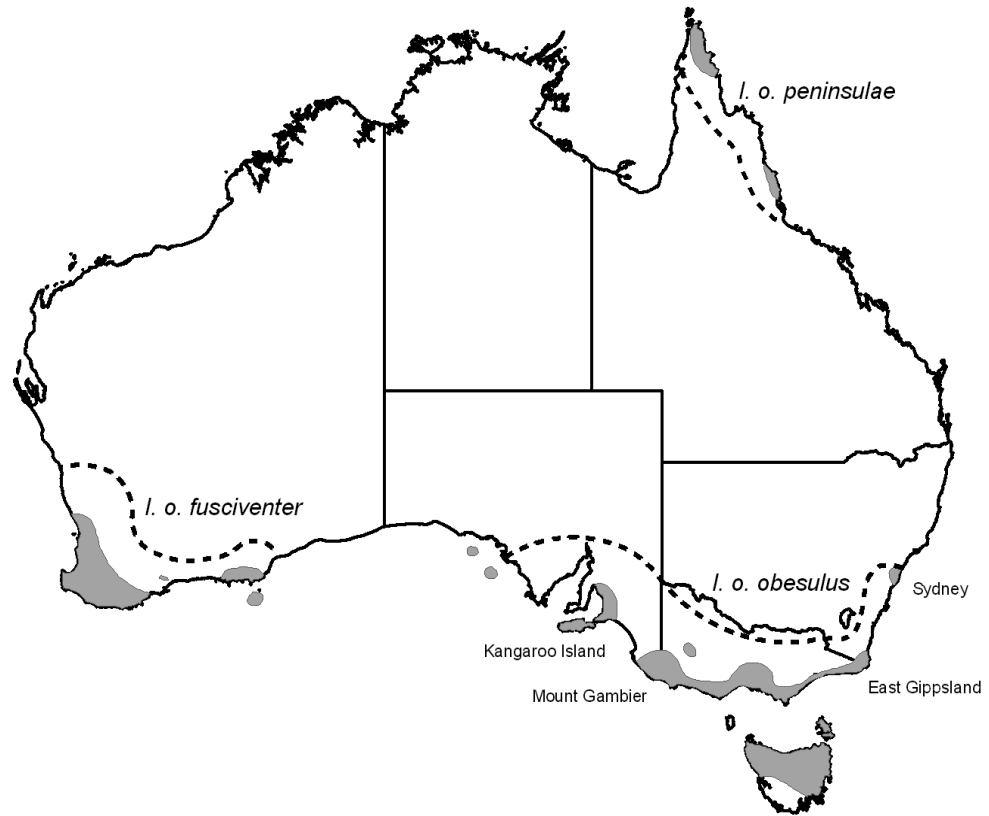
Figure 1-9: Current distribution of *Isoodon obesulus* (grey shading). Modified from Gordon and Hulbert (1989) and Zenger *et al.* (2005).
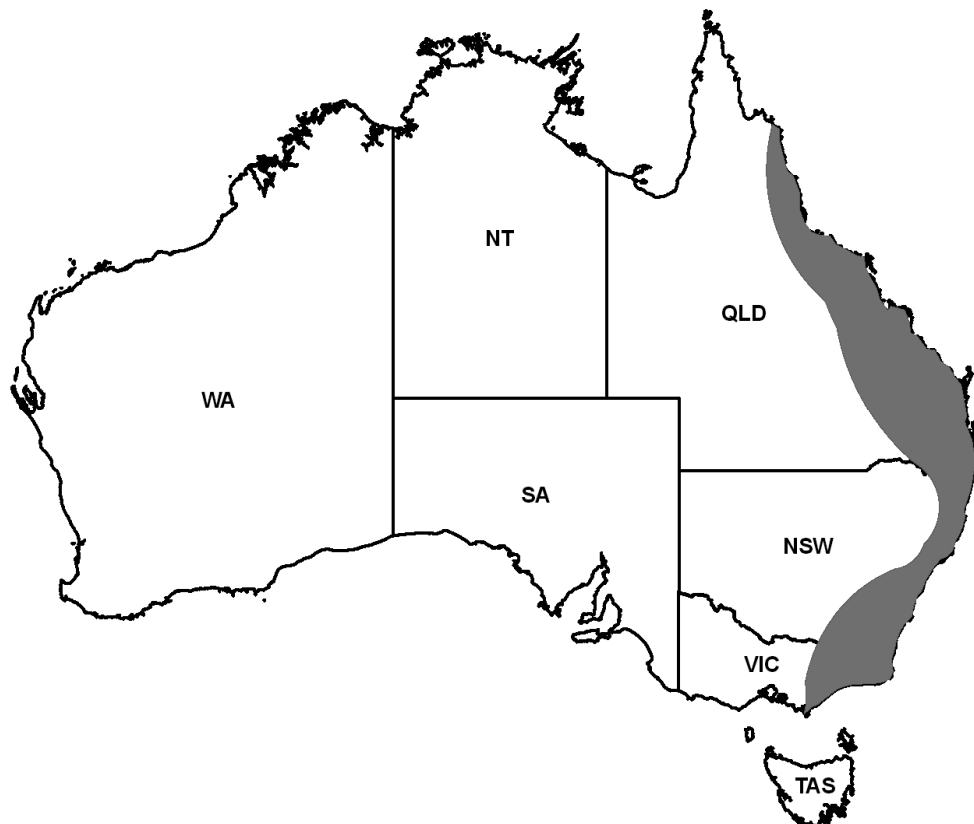
Figure 1-10: Distribution of *Perameles nasuta* (grey shading). Modified from Gordon and Hulbert (1989) and Tyndale-Biscoe (2005).

1.2.5 Threats and key threatening processes

A number of factors have contributed to the overall decline of *I. obesulus* and continue to threaten its survival, resulting in a fragmented distribution within its former range (Ashby *et al.* 1990; Zenger *et al.* 2005). Drivers for population decline and continuing threats include anthropogenic disturbances such as, clearing of vegetation for agriculture and urban sprawl, modification of remnant habitats, introduction of predatory species (foxes, cats and dogs), changed fire regimes and road mortalities (Ashby *et al.* 1990; Zenger *et al.* 2005; Harris *et al.* 2010).

The NSW State and Commonwealth Endangered listing and the subsequent NSW State Recovery Plan for the Southern Brown Bandicoot (*Isoodon obesulus*) (2006), are of immeasurable importance for the long-term survival of this species within northern Sydney and across the broader Australian landscape. A number of key threatening processes have been listed under Commonwealth legislation and within the NSW Recovery Plan as prohibiting the species long-term viability. The key threatening processes considered most important to the bandicoot populations of northern Sydney include:

- predation by the European red fox (*Vulpes vulpes*);
- predation by feral cats;
- land clearance; and
- loss and degradation of native plant and animal habitat by invasion of escaped garden plants, including aquatic plants.

The key threatening processes outlined above forms a platform to base management strategies and on-ground recovery actions for *I. obesulus*.

One example of an on-ground management action is the Fox Tap experiment. This is a commitment by the former Department of Environment and Climate Change (now Office of Environment and Heritage) to the pest management strategy in the region (Fox TAP, Sydney North Regional Pest Management Strategy 2004-2007 and 2008-2011). The Fox Tap experiment is a broad-scale baiting and monitoring program designed to reduce the number of foxes in the area. The autumn and spring live-trapping surveys presented in this research were conducted as part of the Fox Tap monitoring program.

Foxes are listed as a key 'threatening process' under multiple State and Commonwealth legislations, because they are frequently recorded as predators of multiple bandicoot species

(Coates and Wright 2003; Coates 2008) This has led to the development of fox-control programs across the country (similar to the Fox Tap experiment in northern Sydney) with the aim to aid the recovery and persistence of all native fauna. Increase in fox numbers are likely to have resulted from the encroachment of urbanisation and agriculture. Foxes are thought to limit dispersal and suppress bandicoot populations (and other species) through opportunistic predation, particularly in isolated populations (Coates 2008). Fox predation has contributed to the disappearance of bandicoots from several areas on mainland Australia; while in Tasmania the absence (or near absence) of the fox (*Vulpes vulpes*) is thought to be a factor contributing to the widespread distribution and abundance of bandicoots (Hocking 1990).

Bushfires are another threat that can have a major influence on *I. obesulus* and the persistence of bandicoots across Australia. Bushfires have the potential to alter the vegetation and floristic structure of a habitat, which bandicoots depend on for shelter. Bushfires can also change foraging habits through shifts in available invertebrate populations (Stoddart and Braithwaite 1979; Rees and Paull 2000). However, bushfires also have the potential to enhance bandicoot habitat through the regeneration of new vegetation and by providing novel resources in a recently created open and closed habitat mosaic. However, if fires burn too intensely or too frequently, bandicoots can experience direct loss of life, inadequate resources for foraging, a lack of shelter and over-exposure to predators such as foxes, cats and dogs.

The direct and indirect impacts of the urban sprawl and the clearing of land for agriculture cannot be underestimated in their capacity to influence the ability of bandicoots to persist in their natural habitats. Urbanisation has fragmented natural habitats and isolated patches of remnant vegetation (McKinney 2002; Garden *et al.* 2006). Urbanisation also increases pressure on wildlife by changing resource availability and increasing interactions with predators (McKinney 2002). This inevitably leads to significant reductions in bandicoot populations and other native fauna. For a more detailed overview of the impacts of urbanisation, refer to Chapter section 1.3 below.

1.3 Bandicoots in the urban environment

1.3.1 The urban environment

Urbanisation is a rapidly growing cause of many environmental problems and has been associated with altered ecological conditions (McKinney 2002; Drinnan 2005; Garden *et al.* 2007; Gordon *et al.* 2009). Impacts from urbanisation have been linked to a reduction in biodiversity and an increase in the number of threatened species through the removal and fragmentation of habitat, increased predation, altered resource availability and changed land management practices (Collinge 1996; Garden *et al.* 2007; Gordon *et al.* 2009). Urbanisation may ultimately result in an environmental existence of adverse physiological responses, conflicts and perturbations for native wildlife (Lindemayer 2007; Watson *et al.* 2008).

Urbanisation generally results in the reduction of native wildlife. This is particularly relevant for habitat specialists who require specific habitat features for shelter and food and connectivity between patches for dispersal (Dickman 1987; Chambers and Dickman 2002). In some cases, the novel surroundings formed by urbanisation may advantage generalist species by providing alternative food, shelter or nesting options (Chambers and Dickman 2002). These urban adapters or "edge species" are generally evident along the urban interface where stands of remnant bushland and surrounding open areas are present (McKinnery 2002).

Habitats in an urban environment are mostly homogenous towards the urban centre and increase in diversity as you move towards the edge. Environmental heterogeneity becomes evident on small scales by the mosaic of suburban backyards and the horticultural choices of homeowners (Savard *et al.* 2000; McKinney 2002). The degree of heterogeneity can influence population persistence and species richness and is favoured in conservation to habitats of a more homogeneous nature (Collinge 1996).

As urbanisation continues to replace natural landscapes, the significance of peri-urban areas, including suburban backyards becomes increasingly important for retaining native biodiversity and increasing the persistence of wildlife in urban areas (Savard *et al.* 2000; Garden *et al.* 2007; Gordon *et al.* 2009; Goddard *et al.* 2009; Hughes and Banks 2010). Many studies have described how native species richness in a remnant habitat is positively correlated to the size of that habitat (McKinney 2002; Dickman 1987), or fidelity to a distinct foraging patch (Hughes and Banks 2010). Integrating conservation ideals with urban

backyards and green spaces may be an effective and cheap management strategy to preserve as much natural habitat and connectivity as possible.

If native species are to persist within the urban interface, it is important that we first identify the habitat requirements and sensitivities of these species (Garden *et al.* 2007). For example, Chambers and Dickman (2002) determined the importance of conserving a mosaic of open and dense vegetation to ensure the continued persistence of the endangered population of long-nosed bandicoots at North Head. Hughes and Banks (2010) identified a high level of fidelity to distinct foraging patches in the same population of long-nosed bandicoots. Additionally, functional connectivity was the only factor in a study by Fitzgibbon *et al.* (2007) that successfully predicted the presence of bandicoots within fragmented vegetation on the outskirts of Brisbane.

1.3.2 Adaptability of bandicoots

The adaptability of an animal allows it to cope with unexpected changes or perturbations in the environment. Tyndale-Biscoe (2005) described features of bandicoot life history that are "clear adaptations for rapid exploitation of habitats that are temporarily favourable and give them a distinct advantage over other mammals". These features include a rapid reproduction through short gestation and weaning, quick growth, juvenile dispersal and a broad diet (Cockburn 1990; Tyndale-Biscoe 2005). Along with a high responsiveness to environmental and nutritional conditions, these qualities allow bandicoots to fill the opportunistic generalist niche among marsupials and allow movement into newly burnt areas, open paddocks or suburban backyards where spatially heterogeneous and structurally complex habitats become available.

Arguably, these same adaptive ecological traits should allow bandicoots to exploit new opportunities and resources provided by urbanisation, which has favoured the survival of species that have generalist feeding and habitat preferences (Chambers and Dickman 2002; McKinney 2002). However, the documented declines in bandicoot populations and distributions across Australia demonstrate that not all environmental changes are alike, and that some species have been more successful in responding to these changes than others.

1.3.3 Protected areas and management within urban environments

The advent of agriculture and urbanisation in Australia has altered natural vegetation, fragmented ecosystems and changed land management practices to create an existence of constant stresses and perturbations for native species (Lindemayer 2007; Watson *et al.* 2008). As a result, countless species within Australia are declining in distribution, which is increasing the demand for conservation management and the establishment of protected areas.

Protected areas are considered the first line of defence in an effort to conserve biodiversity and threatened species (Jenkins and Joppa 2009; Watson *et al.* 2011). Protected areas have grown in number and size over the last 20 years through the need to protect natural ecosystems and conserve the world's biodiversity (Jenkins and Joppa 2009). However, the success of the conservation outcome within these protected areas is reliant on a combination of the network of protected areas themselves, the ecosystems and habitats protected and the resources and management available.

Within Australia, National Parks, Nature Reserves and other protected areas (under the National Reserve System) have been set aside by the Commonwealth Government for conservation to reverse the trend in declining species numbers (Commonwealth of Australia 2009; Watson *et al.* 2011). Yet the number and size of protected areas may be insufficient for the long-term survival of many threatened species, particularly those whose habitat lies within the increasingly urbanized environment.

In New South Wales (NSW), government agencies have developed programs to enlist local communities living within the urban interface to promote conservation. These programs are based on a rationale that an enhancement of biodiversity in urban ecosystems can improve the quality of life (Mankin *et al.* 1999; Savard *et al.* 2000; Manfredo *et al.* 2003). To promote and sustain native wildlife populations such as bandicoots, communities on the outskirts of cities like those in Sydney are encouraged to adopt native backyards using local species and to maintain close control of family pets at night.

One of the challenges management face is the need to balance conservation and wildlife needs with negative connotations about wildlife-related damage (Layden *et al.* 2003). This also needs to be coupled with the challenge of incorporating the infrastructure needs of the community and the frequency of unwelcome wildlife interactions with humans. An

understanding of these factors, along with the tolerance and acceptance capacities of communities is fundamental for the conservation of wildlife within the urban interface and the expansion of the protected areas network (Manfredo and Dayer 2004; Treves *et al.* 2007).

1.3.4 Public perceptions of bandicoots on the urban interface

Bandicoots may suffer from an adverse public perception due to their habit of digging conical holes in lawns and their role in the transmission of ticks to pets and humans (Dowle and Deane 2008). In line with conserving and managing wildlife populations involving the community, these types of unwanted conflicts between people and wildlife can erode local support for conservation (Gadd 2005).

In northern Sydney, the problem for management is compounded because there are two species that are not readily distinguished by the community and one species (*Isoodon obesulus*) is classified as endangered under both State and Commonwealth legislation. However, understanding the driving factors behind the public's perception of wildlife can enable managers to develop effective and socially acceptable conservation outcomes, which can be applicable along the urban interface and more broadly across natural habitats.

Therefore, in a move to provide a management context for the bandicoots of northern Sydney, this thesis approached the local community to determine their attitudes towards bandicoots and wildlife. The community survey was conducted on the urban fringe in locations where reports of bandicoot activities occurred. This allowed the frequency of conflicts and interactions with bandicoots to be determined, as well as the factors that need to be addressed to successfully achieve community based conservation efforts. In addition, the community survey served as a resource to investigate whether urbanisation has indirectly influenced bandicoot distribution, such as through increased interactions with cats and dogs. Lastly, the survey invited local residents to participate in the research by permitting live-trapping on their properties, which would allow an analysis of the dynamics of bandicoots in a peri-urban habitat and a direct comparison to the National Park habitats.

1.4 Genetic diversity of a threatened and non-threatened marsupial

1.4.1 Genetic diversity and variation within a population

Many studies have focused on the genetic diversity of a species, which has permitted high profile animals such as the Tiger (*Panther tigris*) (Hendrickson *et al.* 2000), Giant Panda (*Ailuropoda melanoleuca*) (Hu *et al.* 2010) and Black Rhino (*Diceros bicornis*) (Karsten *et al.* 2011; Van Coeverden de Groot *et al.* 2011), amongst others to benefit. Threatened species such as these face extinction risks compounded by an inherent lack of genetic diversity within a population and across the broader landscape (Garner *et al.* 2005; Jamieson 2007).

The genetic diversity of a species or population allows it to adapt to the changing environment and hence, increase its chance of survival (Jamieson 2007). However, genetic variation of a species allows a population to evolve in response to environmental change, through the provision of raw material and genetic mutations (Frankham 1997). Genetic and ecological factors can act together within similar timescales to play a role in increasing extinction risks for threatened species, particularly in dynamic environments (Jamieson 2007). Connectivity of habitats and dispersal ability of a species is intrinsically linked to gene flow. Habitat connectivity provides an avenue to promote flow of genetic material, which is central to the dynamics and viability of populations in changing environments (Moritz 1995; Estes-Zumpf 2010; Lancaster *et al.* 2011).

1.4.2 Impact of genetic diversity on a small population

Isolation from conspecifics and decline in population size are typical demographic factors that can lead to population bottlenecks and reduce levels of diversity within populations (Gibbs 2001; Frankham *et al.* 2002). This promotes the non-random mating of individuals and increases the level of homozygotes in the population (Luikart *et al.* 1998), which can further lead to inbreeding depression (reduced fitness) (Frankham 1997; 2005; Frankham *et al.* 2001; Brook *et al.* 2002; Jamieson 2007). Inbreeding depression is predicted to have an increased impact in species and populations suffering from stressful environmental conditions, such as from gradual habitat loss, low food availability, higher predator interactions and range contraction (Reed *et al.* 2007 and Jamieson 2007). These perturbations are not uncommon in the fragmented and urbanised environments utilised by the bandicoots of northern Sydney.

Frankham (1997) revealed that the majority of extinctions of mammalian species since 1600 have resulted from insular forms and that most island populations have less genetic variation than mainland counterparts. Island populations can experience increased genetic limitations, stemming from the lack of opportunity for gene flow between related populations and resulting in reduced genetic diversity and inbreeding (Frankham 1997; 2005; Frankham *et al.* 2002; Jamieson 2007). Inbreeding, lack of gene flow and loss of genetic variation in small populations can play an important role in population decline and impact on a species ability to survive (Frankham 1997; 2005; Frankham *et al.* 2001; Brook *et al.* 2002; Jamieson 2007).

1.4.3 Genetic diversity in relation to management of taxa

Evidence indicates that the majority of threatened species, including island endemics have a lower genetic diversity than taxonomically related non-threatened species (Speilman *et al.* 2004; Jamieson 2007). Eldridge (2010) in a review of the genetic diversity of marsupials in Australia highlighted that bandicoots along with large dasyurids, wombats, and koalas generally had less diversity than the marsupial average. Additionally, populations with the lowest levels of diversity (through studies using microsatellite markers) were typically from species with restricted distributions, threatened or found on islands (Eldridge 2010). Conversely, higher levels of diversity as observed through studies using microsatellite loci, occur in widespread and abundant populations.

Adaptive management of wildlife and habitat requires information on genetic diversity, gene flow and connectivity, population size and other demographic variables (Mortiz 1995). It is also important to conserve biodiversity at all levels no matter the threatened status, as populations of both rare and common mammals are currently losing genetic diversity (Garner *et al.* 2005). Emphasis on management principles should be placed on maintaining genetic variation, encouraging gene flow and avoiding inbreeding at the population level, particularly for threatened species. The maintenance and reestablishment of gene flow is a major challenge for wildlife managers (Eldridge 2010). Conservation strategies focused below the species level at the population level or within Evolutionary Significant Units (ESU) (as described by Moritz 1999), encourages the protection of the extent of persisting genetic diversity (Garner *et al.* 2005).

1.4.4 Conducting diversity studies

Technological advancements have created a surge in population genetics relating to the ecology and conservation of populations and species (Eldridge 2010). At the forefront of these advancements is the use of hyper-variable microsatellite loci in DNA sequencing (Schuelke 2000; Sunnucks 2000). Genotyping with microsatellite loci has allowed studies to differentiate at the individual level (Sunnucks 2000). Studies include, monitoring demographic variables, determining the impact of ecological connectivity, examining the influence of urbanisation, determining the influence of disease and parasite load, and investigating the suitability for species recovery programs (Banks *et al.* 2003; Zenger *et al.* 2005; Partecke 2006a; Smith and Hughes 2008; Lachish *et al.* 2011; Lancaster *et al.* 2011; Pacioni *et al.* 2011).

To sufficiently determine genetic diversity within a species or population, multiple microsatellite loci are required. Increasing the number of microsatellite markers used in the analyses also increases the costs and time associated with analysing the loci separately. However, microsatellite loci can be multiplexed in the one PCR by using a fluorescent dye label, providing the amplified DNA fragments can be appropriately separated by size (Schuelke 2000).

Eldridge (2010) identified that 506 polymorphic microsatellite loci had been isolated from 38 marsupial species, many of which exhibit an ability to amplify loci in multiple closely related species (cross-species applicability). Microsatellite loci developed by Zenger and Johnston (2001) were shown to have cross-species applicability with the *I. macrourus, P. nasuta* and *P. gunnii.* These microsatellite loci were used to conduct a study on the phylogenetic and population structure of *I. obesulus* across Australia to inform management principles for this endangered species (Zenger *et al.* 2005). Zenger *et al.* (2005) demonstrated that the populations in Sydney have experienced significant reductions in genetic diversity and that translocations could occur between the Sydney Ku-ring-gai and Garigal populations, but not between the other populations across south-east Australia.

The research adopted developments in genetic analytical techniques to investigate the diversity and dynamics of the bandicoots of northern Sydney. Genetic analyses are an important component of determining factors that may be compromising the survival of threatened populations. In the case of the bandicoots of northern Sydney, restrictions on gene

flow as a result of urbanisation is a hurdle for the management and long-term survival of the southern brown bandicoot. A major road currently separates two habitats (populations) where bandicoots are frequently recorded in Ku-ring-gai Chase and Garigal National Park.

Multiplexed microsatellite loci were incorporated into the methodology to investigate whether genetic factors are compromising the persistence of the southern brown bandicoot in northern Sydney. Genetic factors include isolation of habitats, gene flow restrictions and inbreeding. The ability to compare information for the long-nosed bandicoot also allows hypothesises to be formed to determine if other factors are interacting with genetic variables in these habitats. For example, factors limiting genetic diversity can be combined with impacts from the urban environment, such as increased stresses due to change in resource availability and interactions with predators, to enhance the risks to the long-term survival and successful management of the southern brown bandicoot in these habitats (Jamieson 2007).

1.5 The urban environment and the stress response

1.5.1 Physiological response

Wherever there is a constraint on natural resources or increased interactions with conspecifics or with humans and predators, there is greater competition for resources and the population may experience higher stress levels or exert an adverse physiological response (Thomas 1990). Recent studies (Wasser *et al*. 1997; Wasser *et al*. 2000; Romero and Wikelski 2002; Reeder and Kramer 2005; Partecke *et al*. 2006b) have shown that a wide range of human activities (such as tourism, logging and urbanisation) can result in elevated or altered levels of the 'stress hormones' known as glucocorticoids.

When an animal perceives a stimulus as threatening or stressful (i.e. experiences a 'stressor'), the hypothalamic-pituitary-adrenal axis (HPA-axis) is activated and the end result is the production and release of glucocorticoids by the adrenal gland (Baker *et al*. 1998; Sapolsky *et al*. 2000; Reeder and Kramer 2005). An increase in an animal's stress hormones causes an alteration in metabolism and diverts energy away from non-essential activities to cope with the immediate threatening or demanding situation (Romero and Wikelski 2002; McKenzie and Deane 2005; Peel *et al*. 2005; Reeder and Kramer 2005). This occurs as an endocrine response whereby the HPA-axis is triggered to release adrenocorticotrophin (ACTH), which then activates the production and release of glucocorticoids into the blood by the adrenal gland (Baker *et al*. 1998; Sapolsky *et al*. 2000; Reeder and Kramer 2005).

High or chronic elevations of glucocorticoids can have an immunosuppressive effect causing a reduction in reproductive potential and impacting the physiological response of the individual (Thomas 1990; Sapolsky *et al*. 2000; McKenzie and Deane 2005; Peel *et al*. 2005; Partecke *et al*. 2006b). For animals living in an urban environment or on the urban fringe with a frequent exposure to stressors, an alteration in coping styles of a reduced stress response may be necessary to succeed (Partecke *et al*. 2006b; French *et al*. 2008). To effectively manage free-ranging wildlife under the influence of stressors', wildlife managers require knowledge of how animals cope with their environment and these stressors. Measurements of the levels of a physiological response can be an effective way to represent variables influencing energy allocation, physiological constraints, urban disturbances and habitat quality at an individual or population level (Romero 2004).

1.5.2 Non-invasive investigate techniques

Recently, non-invasive techniques to assess animal welfare such as measuring glucocorticoids (corticosterone and cortisol) in faeces have been favoured in ecology and conservation biology over traditional capture and blood sampling (Miller *et al*. 1991; Monfort *et al*. 1998; Goymann *et al*. 1999; Wasser *et al*. 2000; Harper and Austad 2001; Millspaugh *et al*. 2001; Dehnhard *et al*. 2003; Huber *et al*. 2003; McKenzie and Deane 2005). Timely capture, restraint and bleeding of animals pose many difficulties in the field. Circulating blood hormone levels increase rapidly in response to the stress of handling, physical restraint or capture in a trap (Harper and Austad 2000; Romero and Wikelski 2002; Romero and Reed 2005). Romero and Reed (2005) indicated a rise in hormone levels from capture occurred within 2 minutes for a house sparrow (*Passer domesticus*). If not sampled quickly enough, blood hormone levels will represent the physiological response induced by the sampling procedure itself and not homeostasis or environmental stress (Harper and Austad 2000).

Non-invasive methods, such as faecal collection, can be conducted without observing or disturbing the animals, or the need to capture and handle it (Von der Ohe and Servheen 2002; Washburn and Millspaugh 2002; Touma *et al*. 2004). Unlike blood hormone levels, faecal glucocorticoids (FGs) represent a physiological response integrated over a period of time, rather than a single point (Goymann *et al*. 1999; Von der Ohe and Servheen 2002). Thus, faecal sampling like those deposited in a trap, may be a more accurate method of assessing the animals' stressed state and the glucocorticoid levels from the current environment by measuring the integrated hormonal levels occurring hours before sample collection (Washburn and Millspaugh 2002; Huber *et al*. 2003; Touma *et al*. 2004).

The thesis intended to add to the literature that has used non-invasive techniques to measure FGs as a tool for evaluating a physiological response. However, few studies in free-ranging wildlife populations have been conducted. The use of the non-invasive method allows higher sample numbers to be collected in a shorter time than blood sampling. This method can also be used to compare a range of environmental and demographic variables between bandicoots living on the urban fringe, to bandicoots living within undisturbed habitats. Bandicoots exposed to the stresses associated with the urban environment may exhibit lower body condition or higher parasite load (Wikel 1999). This physiological response may be expressed through higher FG levels in order to compensate for the increased immunological demands. Other FG variations may be expressed through seasonal differences or reproductive demands.

1.6 Parasites, disease potential and wildlife

1.6.1 Parasites and the spread of disease

Parasites are a significant contributor to the dissemination and spread of disease in wildlife, agricultural and human populations. However, wildlife diseases have typically been considered significant only when impacting on agriculture or human health (Daszak 2000). Little attention has been given to the way diseases and parasites affect the ecology of animals in a wild environment (May 1988). Yet, disease transmission and parasite carriers are likely to have serious implications for management of wildlife on the urban fringe where interactions with domestic animals and humans are at their highest. Greater consequences are also likely for the conservation of threatened or iconic species.

Outbreaks of disease in the Tasmania devil (*Sarcophilus harrisii*) and increased costs associated with conservation efforts and recovery programs for the devil have lifted the importance of wildlife disease in Australia (McCallum 2008; Lachish *et al.* 2011). Similarly, the identification in 1994 of the Hendra virus, which have severe implications for the horseracing industry has heightened the consequences of disease outbreaks and lifted the importance of obtaining disease knowledge. The Hendra virus is a zoonotic pathogen primarily carried by flying-foxes. It can infect horses and humans, causing serious health consequences and leading to death in some cases (Field *et al.* 2007; Breed *et al.* 2010).

Urbanisation can give rise to highly stressed wildlife populations that are adversely affected by habitat fragmentation, increased predation risk and the alteration of resource availability (Collinge 1996; McKinney 2002; Drinnan 2005; Garden *et al.* 2007; Gordon *et al.* 2009). Urban environments also provide a range of additional parasite hosts compared to natural environments through increased interactions with domestic pets, livestock and humans (Pearce and O'Shea 2007; Hill 2008). As a result, urban environments are thought to be conducive to the range increase and survival of many parasites, particularly ecto-parasites and the transmission of pathogens including those with zoonotic potential (Spratt 2005; Bradley and Altizer 2007; Hill 2008).

The interactions of parasites (ecto-parasites and endo-parasites) and Australian wildlife that live at the urban interface are poorly known. Documenting these interactions, including the impacts of parasite burdens to host fitness and variances in habitat on ecto-parasite abundance

and species richness, is a step to providing management with a comprehensive tool for investigating concerns for public safety and impacts on non-endemic hosts.

1.6.2   Parasite host relationship

Parasites form a symbiotic relationship with a host animal. They become dependent on the host to provide them with food resources and a habitat to survive (Combes 2001; Shenbrot *et al*. 2007). In this way, dynamic host-parasite relationships can evolve over time, including the co-evolution of the parasite and host, or the diversification and speciation of parasites due to host switching (Poulin and Thomas 1999). However, the symbiotic relationship is usually at a cost to the host. In some cases the parasite burdens may be extensive enough to compromise the hosts' defense system. This can result in reduced host fitness, direct infection of disease and in turn making the host susceptible to other diseases, predation and perturbations within the environment (Scott 1998; Vilcins *et al.* 2005; Bradley and Altizer 2007).

Endangered host species and hosts from small, isolated or fragmented populations may be more susceptible to increased parasitic burdens and be at a heightened risk of experiencing the deleterious effects of parasites. These populations are usually associated with lower genetic diversity and inbreeding depression, which has been linked to lower fitness and a reduced reproductive performance in wild populations (Jamieson 1997; Frankham 1997; Reed *et al*. 2007). Deteriorating environmental conditions, increased predation and habitat fragmentation serve to increase population stresses and can interact with disease threats (parasitism) to increase the risk of extinction in continually declining populations (Reed *et al*. 2007; Jamieson 1997). A study by Whiteman *et al.* (2006) on island populations of Galapagos hawks (*Buteo galapagoensis*) directly linked reduced host genetic diversity from inbreeding to increased parasitic burdens. The more inbred the population of Galapagos hawks the higher parasite abundance that was recorded compared to relatively outbreed populations. The reduced fitness and weaker immune system reduces the disease resistance potential in the Galapagos hawks, increasing their susceptibility to extinction risk (Whiteman *et al.* 2006).

1.7 Ecto-parasites on the urban fringe

1.7.1 Ecto-parasites

Ticks (Acari: Ixodida) and mites (Acari: Mesostigmata) are ecto-parasites that have evolved to spend at least one part of their life-cycle on the outside of a host. They are often associated with having a detrimental effect to their host by influencing reproduction, growth and survival (May 1988; Scott 1998; Weaver and Aberton 2004; Vilcins 2005). Ecto-parasites may also play a broader ecological role by shaping host population dynamics and acting as a selective force (Geue and Partecke 2008; Hill 2008). Ecto-parasites, particularly ticks are also known to be highly efficient in transmitting pathogens including bacteria, *Rickettsia* and protozoa and vectors of disease such Lyme disease and Q fever (Vilcins *et al.* 2009). In extreme circumstances, ticks are known to cause anaphylactic reactions in humans and death through paralysis (neurotoxin) in domestic pets and livestock (Stone *et al.* 1982).

Ticks comprise two families, the Ixodidae (hard ticks) and the Argasidae (soft ticks), and along with mites, constitute the subclass Acarina. These ecto-parasites depend highly on the microhabitat features of their abiotic environment when not attached to a host (Shenbrot *et al.* 2007). They generally require a warm and humid environment, with slight changes in vegetation cover or temperature, able to drive the composition of parasites at a particular site (Lorch *et al.* 2007). Urbanised environments and habitats, such as suburban backyards often provide these warmer aseasonal conditions and locally warmer and humid microclimates (Geue and Partecke 2008). As a consequence of living within these urban habitats, wildlife such as bandicoots may be adversely impacted by ecto-parasite burdens.

Whilst on host, ecto-parasite density and ecto-parasite load are little impacted by the abiotic factors that drive abundance off-host (Lorch *et al.* 2007). Temperature, humidity and other microclimate elements give way to possible influences, such as host traits and social behaviour. For example, host body weight and lack of social grooming due to aggressive behaviour towards cons-specifics have been attributed to higher species richness and higher abundance of ecto-parasites species on bandicoots (*Isoodon obesulus*) (Lobert 1990; Thomas 1990; Weaver and Aberton 2004).

1.7.2 The paralysis tick (*Ixodes holocyclus*)

Ecto-parasites are not only responsible for impacting the health of their hosts, but they are also known to carry a number of pathogens causing disease in other animals with which they may come into contact. One such ecto-parasite is the paralysis tick (*Ixodes holocyclus*). It injects a neurotoxin into its host that may cause paralysis in domestic pets, livestock and humans (Stone *et al.* 1989). *Ixodes holocyclus* is one of approximately 90 species of ticks in Australia and is common in the outer expanses of Sydney and other similarly humid environments (Roberts 1970; Storer *et al.* 2003). *Ixodes holocyclus* is one of the most non host-specific Australian ticks having been recorded on a wide range of host species, including *P. nasuta* and *I. obesulus* (Roberts 1970). As a result, *Ixodes holocyclus* is a common cause of concern for wildlife managers and owners of domestic pets, particularly on the urban fringe where the opportunity for the transmission of the tick between host species is increased.

Anecdotal information from community reports suggests that the tick carrying capacity of bandicoots is almost solely responsible for the seasonal infestation of ticks in northern Sydney. The intention of the thesis was to confirm or deny the anecdotal information through a community survey and to document the types of ecto-parasites recorded on the bandicoots of northern Sydney. Tick species would serve as the focus for ecto-parasite investigations for the research, with a direct comparison conducted on the prevalence and abundance (load) of the paralysis tick between backyard habitats and National Parks. Ecto-parasite load would also be used to investigate the impacts on individual bandicoots through body condition values and faecal glucocorticoid measurements. Higher ecto-parasite loads are likely to lower body condition indices and lead to a increased physiological response (Scott *et al.* 1988; Vilcins *et al.* 2005), which is also likely to be exaggerated in small, isolated and genetically compromised populations (Whiteman *et al.* 2006).

1.8 Endo-parasites as a public health risk

1.8.1 Endo-parasite in bandicoots

A variety of endo-parasites have been recorded in bandicoots in a natural environment, including populations occurring along the urban interface. Some of the more commonly recorded include *Hepatozoon* (Wicks *et al.* 2006), *Toxoplasmosis* (Obendorf *et al.* 1996), *Cryptosporidium* (O'Donoghue 1995), and *Giardia* (Bettiol *et al.* 1997; Adams *et al.* 2004).

Enteric disease-causing parasites such as *Cryptosporidium* have been responsible for infections in wildlife, domestic animals and humans (O'Donoghue 1995; Bettiol *et al.* 1997). *Cryptosporidium* also has zoonotic potential because a number of species in the genus do not show strong host adaption, allowing infection to occur in animal and human hosts alike (Xiao *et al.* 2002). Public health risks to communities and individuals arise because of this zoonotic potential, particularly in areas where high interactions occur with the parasite host.

Studies on *Cryptosporidium* and other parasites have generally focused on humans and domestic animals due to the inherent risks of zoonotic transmission (Hill *et al.* 2008; Power 2010). Therefore, documenting occurrences of parasites such as *Cryptosporidium* in wildlife populations, particularly those living within the urban interface is essential for identifying possible reservoirs of parasites that may pose a health risk to local communities.

This research uses recent diagnostic and molecular tools to investigate the occurrence and phylogenetic relationships of *Cryptosporidium* from the bandicoots of northern Sydney. The presence of zoonotic species and/or genotypes is also examined, as is the pathway for transmission to occur between host species by comparing positive isolates from bandicoots inhabiting suburban backyards.

1.8.2 *Cryptosporidium* parasite

Cryptosporidium is responsible for the gastrointestinal illness Cryptosporidiosis, and is a principal disease causing parasite in humans (Waldron *et al.* 2011). Infections are typically associated with mild to severe forms of diarrhea that can be fatal in immuno-compromised individuals (O'Donoghue 1995; Rose *et al.* 2002; Fayer 2004). Infection of *Cryptosporidium* occurs through the ingestion of oocysts in contaminated food or water, or by contact with

infected persons or animals. Once ingested, oocysts excyst in the gastrointestinal tract releasing infective motile sporozoites that invade intestinal epithelial cells (O'Donoghue 1995; Fayer *et al.* 2000). All stages of the *Cryptosporidium* life cycle occur within one host (O'Donoghue 1995). However, the oocyst stage is the most important for dispersal, survival and infectivity of the parasite (Fayer *et al.* 2000). Oocysts have a thick outer shell that allows the parasite to remain viable in the environment, resisting desiccation, chemical disinfection and extreme temperatures until ingestion by the next host (Fayer 2004;). The oocyst stage is also of most importance for detection and identification of the parasite (Fayer *et al.* 2000).

1.8.3 *Cryptosporidium* in marsupials and other vertebrate hosts

Natural infections of *Cryptosporidium* have been recorded in over 200 vertebrate hosts, of which 16 species are marsupials, including one report from *I. obesulus* (O'Donoghue 1995; Fayer *et al.* 2010; Power 2010). Infections from *Cryptosporidium* across all vertebrate are the result of 23 known species with other novel genotypes yet to be genetically characterized (Power 2010; Robinson *et al.* 2011).

Species and novel genotypes of *Cryptosporidium* observed naturally in marsupials (Table 1-3) include *C. macropodum* (McCarthy *et al.* 2008), *C. fayeri* (Morgan *et al.* 1997; Power *et al.* 2003), opossum genotype II (Xiao *et al.* 2003) and possum genotype I (Hill *et al.* 2008). An occurrence of *C. muris* was observed in captive bilbies, but this was thought to have ensued through the transmission from rodent faeces, rodents being the natural host to *C. muris* (Warren *et al.* 2003). Early identifications of *Cryptosporidium* (such as in *I. obesulus*) were conducted using oocyst morphology and were often only to the genus level (Plutzer and Karanis 2009). Thus, early observations of *Cryptosporidium* in marsupial hosts, which are not included in Table 1-3, cannot be attributed to an identified species.

Cryptosporidium parvum and *C. hominis* are the primary species infecting humans and are among the most medically important and geno-typically diverse species (Xiao *et al.* 1999; Rose *et al.* 2002; Fayer 2010). *Cryptosporidium parvum,* because of its zoonotic potential is also the primary species posing a public health risk (Xiao *et al.* 1999; Rose *et al.* 2002; Xiao *et al.* 2002). *Cryptosporidium parvum* also infects cattle and other ruminants, particularly neonates, causing implications in the agricultural industry (Faubert and Litvinsky 2000). In a review of the taxonomy of *Cryptosporidium*, Fayer (2010) documented C. *parvum* infections

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in a broad range of hosts including cattle, sheep, dogs, white-tailed deer, mice, pigs, gemsboks and humans. *Cryptosporidium hominis* however, almost exclusively infects humans, and thus has a low zoonotic potential compared to *C. parvum* (Feltus *et al.* 2006).

Table 1-3: *Cryptosporidium* species and novel genotypes observed in marsupial hosts (table extracted and modified from Power 2010).

Species / Genotype	Marsupial Host	Reference
C. fayeri	Koala (*Phascolarctos cinereus*)	Morgan *et al.* (1997)
	Eastern grey kangaroo (*Macropus giganteus*)	Power *et al.* (2004)
	Red kangaroo (*Macropus rufous*)	O' Donoghue (1995)
	Yellow footed rock wallaby (*Petrogale xanthopus*)	Power *et al.* (2003; 2009)
	Western grey kangaroo (*Macropus fuliginosus*)	McCarthy *et al.* (2008)
	Western barred bandicoot (*Perameles bougainville*)	Weilinga *et al.* unpublished
C. macropodum	Eastern grey kangaroo (*Macropus giganteus*)	Power *et al.* (2004)
	Western grey kangaroo (*Macropus fuliginosus*)	McCarthy *et al.* (2008)
	Swamp Wallaby (*Wallabia bicolor*)	Ryan *et al.* unpublished
Opossum genotype II	Virginia opossum (*Didelphis virginiana*)	Xiao *et al.* (2002)
Brushtail possum genotype I	Brushtail possum (*Trichosurus vulpecula*)	Hill *et al.* (2008)

1.8.4 Techniques to identify *Cryptosporidium* species

Oocyst size and morphology have been shown to be unreliable for identification of *Cryptosporidium* species (Jex *et al.* 2007). Early studies using morphology were conducted prior to the development of the molecular methods that have led to the current understanding of the complexity of the *Cryptosporidium* genus (Xiao *et al.* 2002; Power 2010). Studies have since revealed that free-living and captive wildlife can carry both host-adapted and zoonotic strains of *Cryptosporidium* (Appelbee *et al.* 2005). The zoonotic potential of *Cryptosporidium* species has prompted the requirement for an improved understanding of the epidemiology of this parasite and its hosts using modern molecular and diagnostic tools (Jex *et al.* 2007; Jex and Gasser 2010; Xiao 2010).

Many studies have recently assessed the application of molecular techniques for the specific and genotypic identification of *Cryptosporidium* as well as assessing the epidemiology and genetic polymorphism of this parasite (Jex *et al.* 2007; 2008; Plutzer and Karanis 2009; Jex and Gasser 2010; Xiao 2010). A number of genetic markers and loci are commonly used, of which three were selected for this study (Table 1-4).

Table 1-4: Selected genetic markers commonly used for high-level classification of species and genotypes of *Cryptosporidium* for diagnostic and epidemiological studies (extracted and modified from Jex *et al.* 2008).

Genetic marker or locus	Degree of variability	Main purpose
Small subunit of nuclear ribosomal DNA at the *18S* locus	Low	Identification to species and genotype
Actin gene	Low	Identification to species and genotype
60 kDa glycoprotein (*GP60*) gene	High	Identification to genotype and subgenotype

The small subunit ribosomal RNA (*18S rRNA*) has a low intraspecific and high interspecific sequence variation making it a regularly used marker for *Cryptosporidium* identification (Jex *et al.*, 2007; 2008). The *18S rRNA* to date has been used for identification of all recognized *Cryptosporidium* species and genotypes (Jex *et al.* 2007). However, differentiation using only one genetic locus has limited applicability in phylogenetic studies. The single locus maybe under selection pressure and the rate of gene mutation may not be the same for all individuals (Sulaiman *et al.* 2002). Another phylogenetic marker commonly used to provide species-level identification and determine evolutionary relationships of *Cryptosporidium* is *Actin* (Sulaiman *et al.* 2002; Xiao et al. 2004). *Actin* has been used with *18S rRNA* to form the basis for the current classification of the *Cryptosporidium* genus (Morgan *et al.* 1999; Xiao *et al.* 2004; Jex *et al.* 2008). The *gp60* locus is the most single polymorphic marker identified so far in the *Cryptosporidium* genome (Xiao *et al.* 2010). It has a high intraspecific sequence variability and considerable variability (Strong *et al.* 2000; Jex *et al.* 2008). The *gp60* locus is the most commonly employed genetic marker for population genetic and epidemiological assessments, particularly for *C. parvum* and *C. hominis* (Jex and Gasser 2010). Subtyping of *Cryptosporidium* using the *gp60* loci has provided a means for the identification of temporal and geographic differences in *Cryptosporidium* transmission (Plutzer and Karanis 2009).

1.9 Research aims and methods

1.9.1 Research aims

The southern brown bandicoot (*Isoodon obesulus*) and long-nosed bandicoot (*Perameles nasuta*) live sympatrically in much of the available habitat in northern Sydney. Yet, *I. obesulus* is rare and listed as nationally endangered while *P. nasuta* is common and abundant. Considering morphological similarities and the analogous ecological pressures locally, an opportunity presented itself to compare the dynamics of *I. obesulus* and *P. nasuta* across different habitats. The dynamics in which the free-ranging bandicoots inhabit the urban influenced and undisturbed environments of northern Sydney and a diversity of issues pertaining to these habitats are examined in this thesis.

Chapter 2: *Biology and population characteristics of the bandicoots of northern Sydney.*
This chapter describes the study area and details the live-trapping and hair-tubing survey methodology pertaining to the collection of samples and data for chapters 3 through to chapter 8. The study area incorporates the undisturbed National Parks of northern Sydney and the anthropogenic environments of the adjacent suburban backyards.

Chapter 3: *Attitudes to native bandicoots in an urban environment.*
This research has been published in the Journal of European Wildlife Research (Dowle and Deane 2009; Appendix D). This study explores the public perceptions towards bandicoots from the local community and provides a context for future conservation management opportunities for the southern brown bandicoot. The study documents the presence of bandicoots in backyards and provides the background for interactions and conflicts that are occurring on the urban fringe between humans, domestic pets and bandicoots.

Chapter 4: *Population and habitat characteristics of captured bandicoots in northern Sydney.*
This chapter presents the live-trapping and hair-tubing survey data for the thesis and contrasts the biological characteristics of the two bandicoot species. It highlights the limited distribution of the southern brown bandicoot in the study and the ubiquitous nature of the long-nosed bandicoot. Moreover, the documentation of the frequent occurrence and data collection of bandicoots in suburban backyards is pivotal for the ability to investigate the dynamics of bandicoots on the urban fringe and for a comparison to the National Park habitats.

Chapter 5: *Genetic diversity of the free-ranging bandicoots (Perameles nasuta and Isoodon obesulus) of northern Sydney.*

This chapter examines the genetic forces present within the anthropogenic-altered habitats of northern Sydney and what might be contributing to the inability of *I. obesulus* to succeed in this area in the long-term. It examines the genetic diversity and variability of both bandicoot species between the habitats of Ku-ring-gai Chase and Garigal National Park. The study primarily focuses on the potential restriction of gene flow between habitats, the previous occurrence of a population bottleneck and inbreeding. It also uses genetic information acquired for the long-nosed bandicoot to infer a similar genetic position for the southern brown bandicoot.

Chapter 6: *Faecal glucocorticoid metabolite concentrations in the free-ranging bandicoots (Perameles nasuta and Isoodon obesulus) of northern Sydney.*

This research was published in Australian Mammalogy online in August 2012 (Dowle *et al*. 2012; Appendix E). This study examines the physiological response of bandicoots in the habitats of northern Sydney to a range of common environmental and demographic variables using a non-invasive technique. Faecal glucocorticoids are used to measure the physiological response to variations in body condition, ecto-parasite load, season and reproductive status. This chapter also determines whether bandicoots on the urban fringe have a higher physiological requirement compared to bandicoots in National Parks.

Chapter 7: *Does Cryptosporidium exist within P. nasuta and I. obesulus in a wild and urban habitat in northern Sydney?*

This chapter has been submitted for publication in Veterinary Parasitology (Dowle *et al*. September 2012; Appendix F). This study uses recent diagnostic and molecular tools to investigate the occurrence of *Cryptosporidium* in the bandicoots of northern Sydney. It uses the same techniques to examine the phylogenetic relationship of positive *Cryptosporidium* isolates. The presence of *Cryptosporidium* in the bandicoots of suburban backyards serves to highlight the potential transmission pathway for zoonotic species or genotypes to occur between bandicoots, domestic pets and humans.

Chapter 8: *A comparison of the ecto-parasites of P. nasuta and I. obesulus in a wild and peri-urban habitat.*

This chapter documents the range of ecto-parasites recorded on the bandicoots of northern Sydney, with a focus on a comparison between bandicoots inhabiting suburban backyards and National Parks. The study also highlights the opportunities for the transmission of the paralysis tick (*Ixodes holocyclus*) to occur between host species on the urban fringe. In addition, this study investigates the relationship between ecto-parasite presence, richness and load to body condition and bandicoot sex.

Chapter 9: *Discussion for the investigations into the bandicoots of northern Sydney*
This chapter concludes the thesis by discussing the dynamics in which the bandicoots inhabit the urban influenced environments of northern Sydney. This chapter presents novel research outcomes from the thesis in relation to each chapter and presents future research ideas. The information provided will assist the development of conservation strategies conducive to the long-term persistence of the bandicoots of northern Sydney, particularly *I. obesulus*.

2. Biology and population characteristics of the bandicoots of northern Sydney

2.1 Methods

This chapter describes the survey methodology for the live-trapping and hair-tubing surveys. Hypotheses and the capture data from the live-trapping surveys have been outlined in Chapter 4 and the hypotheses for the hair-tube surveys have been outlined below in section 2.1.3.

2.1.1 Study area

The study area incorporated three broad trapping locations in the greater Sydney region, Australia. Sites included Ku-ring-gai Chase National Park (33°39′3.6″S, 151°12′3.6″E), Garigal National Park (33°42′21″S 151°14′11″E) and suburban backyards adjacent to the National Parks and remnant bushland (Figure 2-1).

The National Parks of northern Sydney provide one of the last refuges for the endangered southern brown bandicoot in New South Wales and serve as a habitat for many native plants and animals. Ku-ring-gai Chase National Park is approximately 15,400 ha and is situated 25 km north-west from central Sydney. The National Park is bordered by urban developments in the south and the Hawkesbury River to the north (Figure 2-1). Garigal National Park is approximately 2,200 ha in size, is 20 km northwest from central Sydney and almost entirely bordered by urban developments. The bandicoot populations inhabiting the National Parks currently live in a free-ranging habitat and have only minimal interactions with humans and domestic pets. However, populations living in suburban backyards maybe frequently subjected to interactions with domestic pets and humans.

2.1.2 Survey methods (live-trapping)

Bandicoots from the two National Parks and surrounding backyards were trapped over 10 trapping sessions (March 2005, September 2005, November 2005, January 2006, March 2006, July 2006, September 2006, March 2007, June 2007 and September 2007), incorporating 4336 trapping nights. Three trapping designs A, B and C were used throughout the study and have been detailed in Table 2-1. There was a minimum of 3 months duration between each trapping session of the same trapping design.
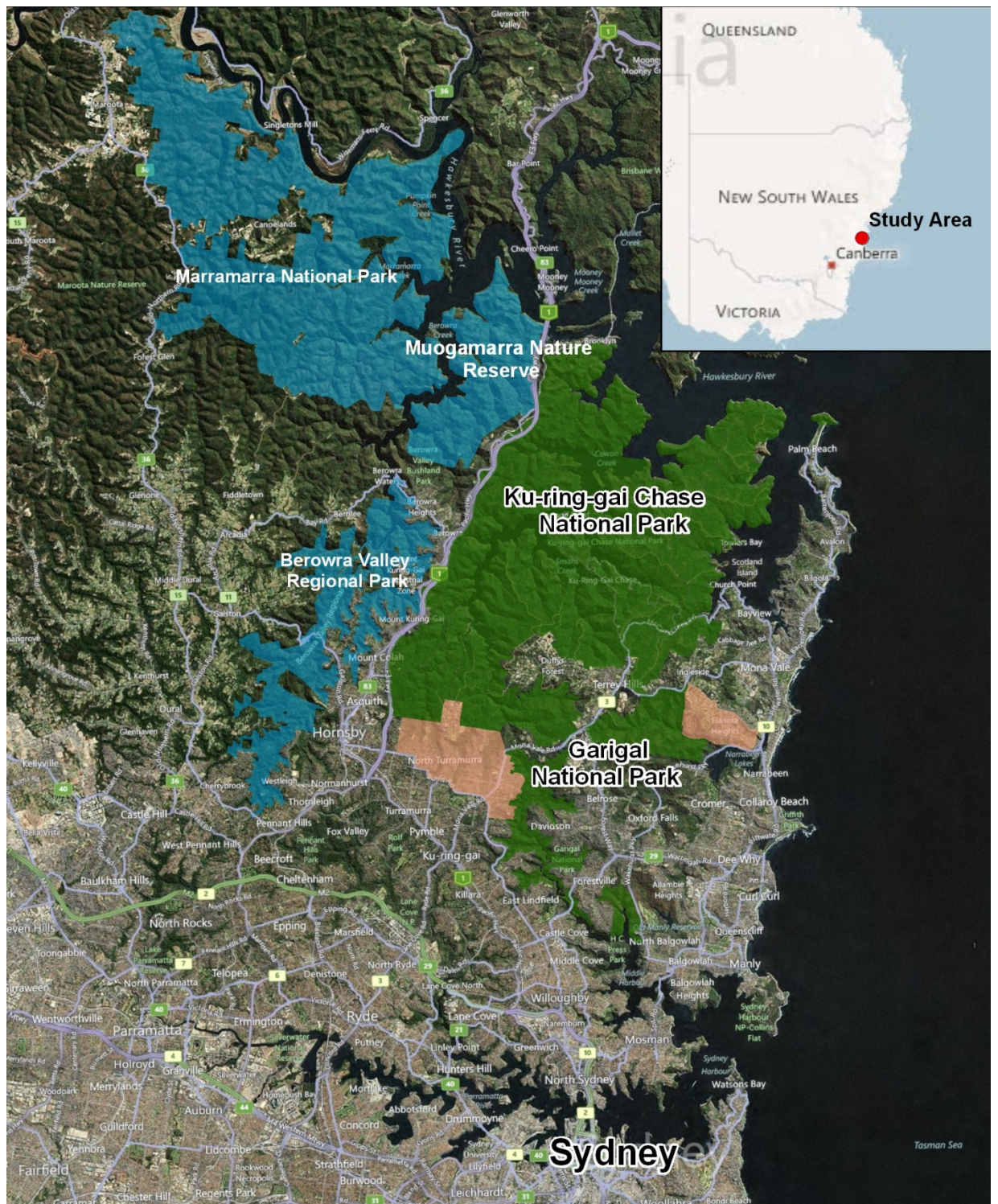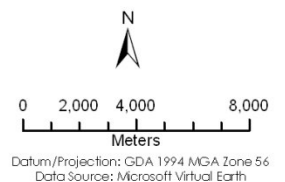
Figure 2-1: Study area showing Ku-ring-gai Chase and Garigal National Park and suburban backyards in northern Sydney.

Table 2-1: Survey live-trapping methods. KCNP = Ku-ring-gai Chase National Park. GNP = Garigal National Park.

Design	Cage traps	Elliot traps	Locations	Sites per location	Trapping duration	Description	Total cage trap nights
"A" Transect	104	208	4 (KCNP) 4 (GNP)	13	4	1 cage and 2 Elliot traps were set at 13 sites 50m apart at each of the 8 locations. Traps were open for a total of 4 nights each. The trapping session was conducted over a 2-week period with one National Park (4 locations) trapped at a time. Transect trapping was conducted in March 2005, November 2005, March 2006, July 2006 and March 2007.	2080
"B" Dispersal	80	160	1 (KCNP) 1 (GNP)	40 (KCNP) 40 (GNP)	8	1 cage and 2 Elliot traps were set at 40 sites approximately 300m* apart within both Ku-ring-gai Chase and Garigal National Parks. Traps were open for a total of 8 nights each. The trapping session was conducted over a 4-week period with one National Park (2-weeks) trapped at a time. Dispersal trapping was conducted in September 2005, 2006 & 2007.	1920
"C" Backyards	42	84	42	1	4	1 cage and 2 Elliot traps were at set at each of the 42 backyards chosen from the community attitudes survey (Chapter 3^). Traps were open for a total of 4 nights each. The trapping session was conducted over a 3-week period with 16 backyards trapped each week. This trapping design was conducted in January 2006 and July 2007.	336

* Dispersal trapping sites followed fire trails and encompassed as much southern brown bandicoot habitat as possible in each National Park. Therefore, in some cases traps were set more than 300m apart.

^ Suburban backyard trapping sites were chosen through the community attitudes survey conducted for Chapter 3. As part of the questionnaire, residents were asked if they would contribute to further research on the bandicoots of northern Sydney by allowing on-going trapping on their property.

Transect and Dispersal trapping (Design A & B) was conducted in conjunction with the National Parks and Wildlife Service (NPWS, Sydney North Region) biannual monitoring surveys. These surveys accounted for six trapping sessions and were conducted in March (Autumn) and September (Spring) each year in Ku-ring-gai Chase and Garigal National Park. Two additional trapping sessions (Design A) in these National Parks were conducted to provide data across all seasons. Two other trapping sessions (Design C) employed 42 suburban backyards along the edge of the urban matrix in northern Sydney. Backyard trapping sites were chosen from a community attitudes survey described in Chapter 3. These sites were chosen based on the frequency of regular bandicoot visits as indicated by the respondent in the community attitudes survey (Dowle and Deane 2009).

Bandicoots were captured in a wire cage trap (30 cm x 30 cm x 60 cm) overnight and samples collected at first light (Figure 2-2). Bandicoots were released near cover, as close as possible to the point of capture (Figure 2-3). Cage traps were baited with a mixture of rolled oats, peanut butter, honey and sardines with dry leaves added to the bottom of the traps for insulation. All traps were partially covered with plastic and placed under shelter where possible to provide protection from the elements. Two baited small Elliot traps (Figure 2-2) were placed adjacent to each cage trap. The use of Elliot traps was to limit the capture of non-target species in the cage traps, such as bush rats (*Rattus fuscipes*). The presence of bush rats in a cage trap would then prevent any capture of bandicoots. Upon capture, bandicoots were placed into a fleece lined pillowcase to be identified and if first capture, animals were micro-chipped and an ear biopsy taken. A total of 108 ear biopsies were collected using a 2mm Miltex Sterile Disposable Biopsy Punch and stored in ethanol at 4°C until analysis.

Measurements of the individuals' weight, sex, ecto-parasite load, reproductive status and body condition (fat stores) were recorded to collect general information from the bandicoot populations and to ensure consistency with the NPWS monitoring program. Weight was measured to the nearest 25 grams using spring scales. Sex and reproductive status was determined by examining the genitalia of each individual. The presence of pouch young was determined by inspecting the pouch on the underside of each female. Faecal samples deposited on the bottom of the cage traps overnight were collected in a plastic vial and stored at -20°C until analysis. A total of 171 faecal samples were collected over the course of the study and used in the *Cryptosporidium* and / or faecal glucocorticoid metabolite analyses as described in Chapter 6 and Chapter 8, respectively.

Figure 2-2: Cage trap and two Elliot traps showing positioning at each trapping site.

Figure 2-3: Long-nosed bandicoot being released after capture.

Ecto-parasites such as ticks (Acari: Ixodida), fleas (Acari: Siphonaptera) and mites (Acari: Mesostigmata) were collected from approximately two-thirds (n=157) of all captures (including recaptures) using fine forceps. Ecto-parasites were stored in a plastic vial filled with ethanol (70%) until further analysis. Ecto-parasites were collected by inspecting the head, ears, rump and underside of each individual for a period of 5 minutes and removing any parasites present. All ecto-parasite were identified to species level where possible, or genus level if species could not be determined. Ticks (Ixodida) and fleas (Siphonaptera) were identified under a stereo-microscope based on shared morphology, following Roberts (1970) and Dunnet and Mardon (1974), respectively. Mites (Mesostigmata) were identified under a compound microscope following Domsrow (1987; 1992). Individuals were assigned an ecto-parasite load based on the number and types of parasites collected.

The body condition of each individual was assessed with a method previously used for western barred bandicoots (*Perameles bougainville*), by calculating the cube root of weight divided by the pes (right hind foot) length (Short *et al.* 1998).

2.1.1 Survey methods (hair-tubing)

Hair tubes have been identified as an effective and non-invasive means of gathering presence/absence data for a variety of species, including *I. obesulus* (Catling *et al.* 1997; Garden *et al.* 2007). Animals are attracted to the hair-tube via the smell of the bait and leave behind shed hairs that are later identified. Hair-tube surveys were conducted across greater north-western Sydney in a report to the NPWS, Sydney North Region (Dowle 2007) and a report to the Hawkesbury-Nepean Catchment Management Authority (DECC 2008, ed. Dowle).

The hair-tube field surveys were undertaken during the period of March 2007 to July 2008. Surveys were conducted in Muogamarra Nature Reserve (MNR); Berowra Valley Regional Park (BVRP; includes Hornsby Shire Council); Marramarra National Park (MNP); Baulkham Hills Shire Council (BHSC); Ku-ring-gai Municipal Council (KMC); Ku-ring-gai Chase National Park (KCNP) and Garigal National Park (GNP) and represented over 17,632 trap nights.

Sites (lines of transects) were selected based on previous NSW Wildlife Atlas records, critical or likely habitat as identified in Visser (2004), known habitat from previous studies and suitable habitat identified from vegetation maps (see, Benson and Howell 1994). Southern brown bandicoots in northern Sydney appear to prefer vegetation that is mainly heathland or open woodland with a heath understorey and sandy soils. They also prefer habitats that are along ridgetops and upper/shallow slopes. Vegetation communities fitting these characteristics in northern Sydney include, 'coastal sandstone heath' and 'Sydney sandstone ridge-top woodland' from the Royal Botanic Gardens classification system (Chapter 4; Benson and Howell 1994). Hair-tubes were generally set close to fire trails for accessibility and along walking tracks. Some hair-tube transect lines were also set in unsurveyed areas along ridge-tops where there was no formed track.

Hair-tubes were set in transect lines of varying lengths at 100m-300m apart. Length of transects and distances between hair-tubes varied depending on ground and vegetation characteristics, and accessibility along fire trails or walking tracks. The hair-tubes were pinned to the ground and consisted of curved plastic with double sided tape attached to protrusions on the inside of the tube (designed by Andrew Murray, Department of Sustainability and Environment, Victoria, Figure 2-4 and Figure 2-5). The entrance diameter was about 75mm and in the middle of the tube was fixed bait made up of rolled oats, honey, peanut butter and sardines. Hair-tubes were left in place for approximately 14 nights (often depending on weather conditions). Upon collection, hair-tubes were inspected for the presence of hairs and if present, were sent off for further analysis. Leaving hair-tubes out for 10-14 nights is common practise (Catling *et al.* 1997; Garden *et al.* 2007) and was considered an adequate time for the bandicoot to inspect any hair-tube within its territory. Bandicoot home ranges and/or territories have been recorded to range from 0.5 ha to 9 ha (Heinsohn 1966; Mallick *et al.* 1998b).

It was hypothesised that the long-nosed bandicoot would be recorded in all Parks and Reserves included in the hair-tube surveys, due to anecdotal reports of its broad distribution. However, it was hypothesised that the endangered southern brown bandicoot would be observed less frequently and only within Ku-ring-gai Chase and Garigal National Park. It was also hypothesised that long-nosed bandicoots and southern brown bandicoots would illustrate their sympatric nature within the National Park habitats.

Figure 2-4: Bait and hook (Murray 2005).

Figure 2-5: Victorian design hair tube (Murray 2005).

2.1.2 Limitations

The timing of six live-trapping sessions was dictated by the ongoing NSW NPWS bi-annual (spring and autumn) monitoring surveys for the southern brown bandicoot and influenced the timing of other live-trapping sessions. The monitoring surveys are conducted as part of the Fox Tap experiment; a commitment by the NPWS of the Department of Environment and Climate Change (now NSW Office of Environment and Heritage) to the pest management strategy in this Region (Fox TAP, Sydney North Regional Pest Management Strategy 2004-2007 and 2008-2011) (DECC 2004; 2007) Cage trapping and hair-tubing surveys have been an on-going part of this monitoring program in Ku-ring-gai Chase and Garigal National Park.

One of the initial objectives of the trapping survey was to set a series of traps in suburban backyards containing domestic cats and/or dogs. This would allow the study to compare the frequency of bandicoot captures between backyards with and without domestic pets. The respondents to the community attitudes survey as part of Chapter 3 indicated that bandicoots were just as likely to frequent backyards (presence of distinctive conical diggings) with pets compared to backyards without pets. The physical trapping data would have been used to confirm the community survey anecdote and determine if there was a direct correlation between bandicoot presence/absence to habitats containing domestic pets. However, without control over the movements of pets with the trapped backyards, the pets would be free to inspect the traps throughout the night. As such, it was deemed too stressful to allow the trapping to occur in these backyard habitats, particularly for females weaning young. It was considered to be an undue risk to health of the individual bandicoots and it was considered to be in violation of general animal ethics principles.

3. Attitudes to native bandicoots in an urban environment

3.1 Introduction

Urbanization not only fragments natural ecosystems, it creates opportunities for increasing human and animal interactions and a potential for conflict (Decker and Chase 1997; Patterson *et al*. 2003; Casey *et al*. 2005). Within Australia, National Parks and Nature Reserves have been set aside for conservation, yet they are considered insufficient for the long-term survival of many threatened species, particularly those whose habitat lies within the increasingly urbanized coastal seaboard. In New South Wales (NSW), government agencies have developed programs to enlist local communities living close to urban bushland to promote conservation and are based on a rationale that an enhancement of biodiversity in urban ecosystems can improve the quality of life (Mankin *et al*. 1999; Savard *et al*. 2000; Manfredo *et al*. 2003). These strategies are also based on research that shows that urbanization is as a key influence in changing attitudes towards wildlife.

The challenge wildlife managers face is balancing conservation and wildlife needs with complaints about wildlife-related damage and the infrastructure needs of the community (Layden *et al*. 2003). Effective wildlife management requires consideration of ecological components along with social values and public knowledge of wildlife and the environment (Decker and Chase 1997; Riley *et al*. 2002; Miller and Jones 2005). An understanding of the tolerance and acceptance capacities of residents is vital to improve management efficiency of urban wildlife (Manfredo and Dayer 2004; Treves *et al*. 2007).

The bandicoots of northern Sydney, Australia require this approach to urban management. The southern brown bandicoot (*Isoodon obesulus*) is a medium-sized (400– 1,600 g) ground-dwelling marsupial that inhabits heathland, shrubland, dry sclerophyll forest with heathy understorey, sedgeland and woodland (Ashby *et al*. 1990; Menkhorst and Seebeck 1990). It has been listed as Endangered (Schedule 1, Part 1) on the NSW Threatened Species Conservation Act 1995 (TSC 1995) and Endangered on the Commonwealth Environmental Protection and Biodiversity Conservation Act 1999. Historical records for the southern brown bandicoot indicate major range contractions since European settlement, likely due to

fragmentation of habitats via urbanization and the introduction of predators (Ashby *et al*. 1990; Dufty 1994; Paull 1999; Rees and Paull 2000; Department of Environment and Climate Change 2006). The long-nosed bandicoot (*Perameles nasuta*) is of a similar size, occupies a similar habitat and forages in slightly more open vegetation and is exposed to the same threats as the southern brown bandicoot. This species however seems to be robust against the pressures of urbanization and is not considered either endangered or threatened. Bandicoots are generalists and opportunists (Quin 1985; Gordon and Hulbert 1989; Mallick *et al*. 1998a), which allows it to exploit urban habitats for food, shelter from predators and refuge from bushfires (Gordon and Hulbert 1989).

To incorporate public opinion into conservation strategies, managers require information regarding local attitudes and knowledge as well as timing and locations of conflicts (Casey *et al*. 2005). With respect to bandicoots, anecdotal reports suggest the greatest conflicts arise from the damage to lawns and gardens and the perception that bandicoots play a role in transmission of ticks, specifically the paralysis tick (*Ixodes holocyclus*). The problem for conservation managers is compounded because of the nationally endangered status of the southern brown bandicoot, which the community can not distinguished from the long-nosed bandicoot.

An attitudinal survey was conducted within local communities in northern Sydney adjacent to a National Park, which provides habitat for both species of bandicoots. These communities were also the location of a large number of anecdotal reports of interactions with bandicoots. It was hypothesised that most respondents would notice signs of bandicoot presence, because bandicoots regularly visit suburban backyards, due to the increase opportunities provided by the peri-urban habitat. However, it was also hypothesised that bandicoots were less likely to visit backyards with domestic pets due to the increased predation risks. Additionally, it was postulated that the majority of respondents would regard bandicoots as a pest species, because of the anecdotal reports on the local conflicts between bandicoots and humans, particularly in relation to bandicoots carrying ticks. This study was the initial step toward the development of strategies to conserve urban bandicoot populations, particularly the endangered southern brown bandicoot.

3.2 Materials and methods

3.2.1 Study area

The study site incorporated the communities surrounding Ku-ring-gai Chase and Garigal National Parks, approximately 20 km north from the centre of Sydney on the east coast of Australia (33º39´S, 151º12´E). The location of these two parks is shown in Figure 2-1. These National Parks provide one of the last habitats for the southern brown bandicoot in NSW and serve as a refuge for many native plants and animals, including the long-nosed bandicoot. Ku-ring-gai Chase and Garigal National Parks were established in 1894 and 1923 respectively, they have since been divided by a major road creating two fragmented and disjunct populations. Ku-ring-gai Chase National Park (154km²) borders approximately 6,500 properties at a medium density development level and some rural properties. It is one of the most accessible and popular parks in Australia. Garigal National Park (22km^2) is even more accessible and heavily surrounded by residential properties. Increasing urbanization has placed pressures on the parks management to provide a range of recreational activities in the parks environs from bushwalking and horse riding, to boating and picnicking as well as hazard reduction burns to provide a safety net against wildfires.

3.2.2 Survey design and questionnaires

Properties bordering the Ku-ring-gai Chase and Garigal National Parks were selected for inclusion in the survey. The detailed selection of areas to be surveyed was based on residential reports to the National Parks and Wildlife Service of bandicoot activity within the last 5 years. The National Parks and Wildlife Service at Ku-ring-gai Chase and Garigal National Parks frequently receive reports by residents of bandicoot activity. Survey areas were further defined by their close proximity (within 500m) to bushland that is connected to the bandicoot trapping sites within the National Parks (Ku-ring-gai Chase and Garigal) that are monitored by the NSW Department of Environment and Climate Change during their biannual surveys. The attitude surveys were then distributed to all houses with backyards within these pre-determined survey areas. The study was conducted towards the end of the breeding season, which is regulated by the annual peak in food abundance during November and December 2005.

A self-completion style of survey was chosen due to its perceived benefits in response rate and reduced interviewer effects along with the associated time and monetary restrictions.

Residents were requested to complete the questionnaire and place the completed form by their letterbox for collection. Accompanying the questionnaire was a covering letter introducing the study to potential respondents and emphasizing confidentiality of responses. The survey also included an invitation to residents to have traps set on their property for the capture and identification of bandicoots.

Six hundred and thirty questionnaires were distributed within the local community. The questionnaire (Appendix B) consisted of three parts:

a) Residents were asked to describe their experiences with bandicoots in their backyard through a series of 25 yes/no or multiple-choice questions. These types of questions permitted easier analysis of data than open-ended questions (Robertson and Lawes 2005). Questions asked respondents to identify frequency and level of bandicoot activity, pet interactions with bandicoots, what pets they own, perceptions as to native or pest status of bandicoots and other potential sources of conflict between humans and bandicoots. Respondent age, sex, make up of household, and distance from the National Park were also collected.

b) In a second section, residents were asked to indicate their level of agreement on a symmetric five-point Likert scale (5 being strongly agree to 1 being strongly disagree) to 23 statements in relation to bandicoots' threats, legal status, intrinsic value and aspects of living with bandicoots and other wildlife. These factors were analysed against demographic data to highlight areas of negative perceptions.

c) Residents were invited to provide personal details including name, contact details and address to allow future studies to trap bandicoots on their property.

Questionnaire responses were collated in SPSS Windows (version 14). A factor analysis using maximum likelihood extraction and varimax rotation was applied to the statement section of the questionnaire. Scaling was reversed for the unfavorable / negative statements in section two so that higher scores indicated favorable attitudes. Bartlett's test of sphericity was used to test the appropriateness of the factor model and the Kaiser–Meyer–Olkin measure of sampling adequacy is sufficiently large to suggest good factorability. The scree plot and eigenvalue scores (about equal to or greater than 1) from the maximum likelihood extraction were used to extrapolate three factors accounting for the variance. Meaningful categories were then created from these factors using variables with values over 0.40 from pure and highest loading variables (when loaded on more than one factor). An index was then created for every

respondent based on the factor scores from the maximum likelihood extraction (factor scores were weighted, e.g., a factor score $<0.5=1$, $0.5–0.7=2$, and $>0.7=3$). Regression analyses were used to compare respondent demography (age, sex, distance to Park boundary and pet ownership) with bandicoot activity (perceived presence and frequency of visits).

Cross tabulations were carried out on nominal data and were analyzed by Chi-squared tests. Only data with expected frequencies of >5 were used in analysis. These cross tabulations were applied to the experiences and interactions property owners had with bandicoots. The factor analyses and cross tabulations were only conducted using fully completed questionnaires.

3.3 Results

Six hundred and thirty questionnaires were distributed with 190 being returned, representing a response rate of 30%. Of the returned questionnaires, 177 were fully completed by the respondents.

Females accounted for almost two-thirds of the respondents (n=110), and the age of respondents ranged from 15–98 years of age with 67% between 46–75 years of age. Most (83%) residents indicated that they had experienced bandicoots in their backyard but the majority (78%) could not stipulate the species. Of the respondents who indicated bandicoot presence in their backyard, almost two-thirds reported visits at least three times a week and up to 27% were having daily visits. Bandicoot visits occurred throughout the year with a slight seasonal bias in the spring (83%) and summer (67%) and fewer visits in autumn (45%) and winter (52%).

Over a third of respondents had a property bordering the National Park and 59% were located within 50 m of a park boundary. Only 4% of houses were located greater than 500 m. Residents living closer to the park boundaries experienced bandicoot interactions more than residents who lived further away ($\chi2$ =7.174, df=2, p=0.028).

Of the 177 respondents, 59% reported pet ownership. Of these, 48% owned a dog, 33% owned a cat, and 10% owned both. Of the pet owners, 24% indicated that their pet was not locked up at night. The identified presence of bandicoots on respondents' properties and the frequency of bandicoot visits were not affected by the presence of pets (Table 3-1), nor were they affected by the pets being left out after dark (Table 3-2).

Table 3-1: The results of Chi-squared tests (n=178) for the influence of pet ownership on bandicoot presence and frequency of visits (survey question 9). Significance was taken at the p=0.05 level.

Variable	Pet ownership				
	Yes % (n=105)	No % (n=73)	χ^2	df	p
On property					
Yes	91	52	0.264	1	0.607
No	14	21			
Frequency					
7/week	48	52			
3/week	60	40			
1/week	58	42	3.250	4	0.517
1-3/month	64	36			
No visits	62	38			

Table 3-2: The results of Chi-squared tests (n=105) for bandicoot presence and frequency of visits when pets are left out after dark (survey question 10). Significance was taken at the p=0.05 level.

Variable	Pets out after dark				
	Yes % (n=81)	No % (n=24)	χ^2	df	p
On property					
Yes	66	23	2.953	1	0.086
No	15	1			
Frequency					
7/week	40	60			
3/week	40	60			
1/week	42	58	3.54	4	0.472
1-3/month	27	73			
No visits	19	81			

Almost two-thirds of respondents indicated bandicoots were a native animal and only 5% indicated that they were feral. However, 33% of residents perceived bandicoots as a pest, because they leave diggings in the garden (64%) or they carry ticks (44%). Residents who were visited by bandicoots were more likely to perceive bandicoots as a pest than residents who had no bandicoot visits (Table 3-3). However, the frequency of bandicoot visits did not influence a resident's perception of a bandicoot as a pest, nor did the sex of the respondent. However, older residents were more likely to identify bandicoots as a pest than younger residents. Conversely, respondents who owned a pet were less likely to perceive bandicoots as a pest.

Table 3-3: Position of residents to the pest and/or nuisance status of bandicoots, survey question 15a and results of Chi-square tests ($n=178$). Significance was taken at the $p=0.05$ level.

Variable	Pest and/or nuisance		χ^2	df	p
	Yes % ($n=57$)	No % ($n=120$)			
Gender					
Male	38	62	1.950	1	0.110
Female	28	72			
Age					
26-35	17	83			
36-45	20	80			
46-55	27	73	14.112	4	0.007
56-65	34	66			
66-75	55	45			
Presence					
Bandicoots	37	63	13.830	1	<0.0005
No bandicoots	0	100			
Frequency					
7/week	40	60			
3/week	40	60			
1/week	42	58	7.703	4	0.103
1-3/month	27	73			
No visits	19	81			
Pet ownership					
Pet	23	77	10.327	1	0.001
No pet	46	54			

Of the 177 fully completed questionnaires, there was a general trend to agree with the positively worded statements and disagree with the negatively worded statements (Table 3-4). The most positive responses to statements were "it's a pleasure to live with bandicoots and other wildlife" and "conservation of bandicoots is necessary" while the statement eliciting most disagreement was "bandicoots are a nuisance". The maximum likelihood extraction revealed three factors, which combined, accounted for 65% of the variation of the attitude statements and Cronbach's alpha revealed a very high strength of correlation within the factors (ά 0.891–0.942). These three factors were the basis for creating the meaningful categories (Table 3-5).

Table 3-4: Percentage of responses to attitude statements towards bandicoots. One hundred and seventy seven respondents fully completed the statement section.

Statement (abbreviated)	Strongly Agree (%)	Agree (%)	Neutral (%)	Disagree (%)	Strongly Disagree (%)
Bandicoots are cute	22	41	25	6	6
Bandicoots are nice	23	30	25	10	12
Bandicoots are a nuisance	12	16	20	28	24
They damage gardens	9	15	26	32	18
Bandicoots carry ticks	35	33	28	2	2
These ticks are dangerous	34	36	32	3	5
Have right to exist	34	40	23	1	2
Bandicoots allowed in backyards	27	25	22	15	23
Resulted in making yards less attractive	12	25	25	15	23
Conservation of bandicoots necessary	43	38	15	2	2
Survival threatened by cars	19	27	36	15	3
Survival threatened by cats	36	39	20	5	2
Survival threatened by dogs	29	38	24	6	3
Survival threatened by foxes	37	41	19	1	2
Survival threatened by habitat loss	46	45	6	1	2
Pleasure to live with bandicoots	24	24	32	10	10
Pleasure to live with other wildlife	36	48	12	1	3
Bandicoots should be protected	36	36	22	3	3
Bandicoots should be relocated	14	34	25	20	7
Important part of environment	36	44	17	1	2
People should receive more info	34	49	14	2	1
There are too many bandicoots	8	8	30	34	20
Bandicoots are welcome in my yard	28	32	16	13	11

Meaningful category one (Cronbach's alpha = 0.942) represents the residents' tolerance for

bandicoots and accounted for 48% of the variation among responses to attitude statements. The two highest loading variables were where residents indicated a positive response to the statements; "bandicoots are nice to have around" and; "bandicoots should be allowed in residents' backyards" (Table 3-5). Regression analysis for category one indicated that responses differed among age groups (Table 3-6) with a trend that older respondent were less willing to tolerate bandicoots. Pet owners were more willing to tolerate bandicoots (Table 3-6) compared to respondents who don't own a pet.

Table 3-5: Associations between attitude statements and meaningful categories identified by the factor analysis. Four factors accounted for 69% of variance. The Bartlett's test of sphericity was significant (<0.005) and the Kaiser-Meyer-Olkin measure of sampling adequacy (0.928) is sufficiently large to suggest good factorability.

Factor	Variance explained	Meaningful category	Variables (recoded questions are marked with a '-' symbol)
1	48%	"tolerance"	Q7: Bandicoots should be allowed (0.83)
			Q2: Nice to have around (0.79)
			Q22: Bandicoots are welcome (0.74)
			-Q8: Owners should not deter them (0.61)
			-Q18: Bandicoots shouldn't be relocated (0.53)
2	10%	"value of bandicoots"	Q9: Conservation of bandicoots necessary (0.64)
			Q6: Bandicoots have rights (0.63)
			Q19: Important part of natural environment (0.60)
			Q15: Pleasure to live with bandicoots (0.54)
			Q17: Bandicoots should be protected (0.54)
3	7%	"threats to bandicoots"	Q12: Dogs threaten bandicoots (0.78)
			Q11: Cats threaten bandicoots (0.77)
			Q13: Foxes threaten bandicoots (0.73)
			Q10: Cars threaten bandicoots (0.50)
			Q14: Habitat loss threatens bandicoots (0.49)

Table 3-6: Regression analyses for meaningful category one (tolerance). Significance was taken at the $p = 0.05$ level.

Regression	Coefficient	S.E.	*p*-value
constant	3.6119	0.3735	< 0.0001
Sex			0.2009
Male	0		
Female	-0.2205	0.1717	
Age group			0.0329
26-35	0		
36-45	-0.0218	0.3148	0.9449
46-55	-0.2317	0.2889	0.4238
56-65	-0.4822	0.3171	0.1302
>65	-0.7963	0.3238	0.0149
Pet ownership			0.0072
No pet	0		
Pet	0.4697	0.1727	
On property			0.7895
No	0		
Yes	-0.0689	0.2577	
Regression	**adj R-sq**	**Analysis of variance - from mean**	
	0.1041	**DF** = 9,167 **F** = 3.2720 ***p*-value** = 0.0011	

Meaningful category two (Cronbach's alpha = 0.927) represents the 'intrinsic rights and ecological values of bandicoots' and accounted for 10% of the variation among responses to attitude statements. Most positive loadings occurring on category two statements: "it's a pleasure to live with bandicoots and other wildlife" and "conservation of bandicoots is necessary". Regression analyses (Table 3-7) revealed that pet owners have a greater appreciation for the value of bandicoots than residents who don't own pets.

Meaningful category three (Cronbach's alpha=0.891) accounted for 7% of the variation among responses to attitude statements. This category represents the respondents' 'awareness of threats to bandicoots' survival. Regression analyses for category three ($F_{(9,167)} = 2.08$, $p = 0.034$, R-squared = 0.1) revealed that females were more willing to identify the threats towards bandicoots than males ($p = 0.039$).

Table 3-7: Regression analyses for meaningful category two (intrinsic rights and ecological values of bandicoots). Significance was taken at the $p = 0.05$ level.

Regression		Coefficient	S.E.	*p*-value
constant		4.1271	0.2660	< 0.0001
Sex				0.7940
Male		0		
Female		0.0320	0.1223	
Age group				0.0567
26-35		0		
36-45		-0.1843	0.2242	0.4122
46-55		-0.1438	0.2058	0.4847
56-65		-0.3620	0.2258	0.1109
>65		-0.5809	0.2306	0.0127
Pet ownership				0.0324
No pet		0		
Pet		0.2653	0.1230	
On property				0.9530
No		0		
Yes		0.0108	0.1835	
Regression	**adj R-sq**	**analysis of variance - from Mean**		
	0.0761	**DF** = 9,167	**F** = 2.6097	*p*-**value** = 0.0076

3.4 Discussion

This study has documented the attitudes of a selected group of residents to bandicoots in an urban area of Sydney, Australia adjacent to natural habitats of the animals. Our observations are consistent with those studies undertaken in other communities affected by urban wildlife including mountain lions (*Felis concolor*), black-tailed prairie dogs (*Cynomys ludovicianus*), beavers (*Castor canadensis*), giant Canada geese (*Branta canadensis maxima*) in North America (Zinn and Andelt 1999; Coluccy *et al*. 2001; Lamb and Cline 2003; Casey *et al*. 2005), wolves (*Canis lupus*) and urban foxes (*Vulpes vulpes*) in northern Europe (Heberlein and Ericsson 2005; Bisi *et al*. 2007; König 2008), wildlife outside conservation parks in Africa (Gadd 2005) and possums (*Trichosurus vulpecula*), pythons (*Morelia spilota*), koalas (*Phascolarctos cinereus*) and general vertebrate fauna in Australia (Lunney *et al*. 1997; Lunney *et al*. 2000; Fearn *et al*. 2001; FitzGibbon and Jones 2006; Hill *et al*. 2007). These studies reflect increased engagement of local communities with wildlife conservation issues as wildlife managers strive to include sociological and ecological information in their conservation management strategies (Casey *et al*. 2005).

Attitudes of people involved in human–animal interaction will likely influence the success of any conservation program for bandicoots in northern Sydney. Approximately 63% of all respondents gave permission to have traps set in their backyards and contribute in further research, regardless of whether they identified the presence of bandicoots in their yards. The respondents from this survey provided the platform for bandicoot trapping in backyards, which contributed to the broader research aims of the thesis (Chapter 2). The high positive response of residents to have traps set in their backyard to participate in research is indicative of an elevated interest in bandicoots and wildlife in general. However, the potential bias in the site selection process for the survey delivery is acknowledged. The aim of the study was to highlight the attitudes of the community members who experience interactions (and potential conflicts) with bandicoots, as these community members were more likely to be the target of educational programs and community based conservation management strategies. The attitude surveys also doubled as a request for permission to trap in residential backyards, thus conducting the survey in an area known to have visits by bandicoots was important.

Of the returned questionnaires, 83% of respondents indicated that bandicoots visited their property, which suggests respondents to the survey were influenced by exposure to bandicoots. Alternatively, it may be that bandicoots in this urban environment are highly

abundant and responding well to the local fox-baiting programs from the Department of Environment and Climate Change (DECC) and Local Councils. Bandicoots are known to be a preferred food option for foxes and baiting will cause a reduction in predation (Roberts *et al.* 2006).

The generalist and opportunistic habits of bandicoots allow them to persist in fragmented habitats of remnant bushland (Quin 1985; Gordon and Hulbert 1989; Mallick *et al.* 1998b; Garden *et al.* 2006). Refuge and foraging options of bandicoots are increased as urbanization produces spatially heterogeneous and structurally complex habitats (Dufty 1994; Scott *et al.* 1999; Chambers and Dickman 2002; Garden *et al.* 2006; Pickett and Cadenasso 2006). Residents commonly stated that bandicoots nested under their houses and used the garden vegetation for food. Dufty (1994) reported a similar finding with the eastern barred bandicoot (*P. gunnii*) preferring a range of natural and artificial structures as shelter sites.

The hypothesis that the majority of respondents would identify bandicoots as a pest was rejected by this study. Only one-third of residents perceived bandicoots as a pest. Of these respondents, almost two-thirds indicated it was because bandicoots dug holes in the garden and 44% because they carried ticks (some respondents identified both). Although no correlation was found between frequency of bandicoot activity and a negative experience, there was a correlation between the presence of bandicoots and an increase in perceived nuisance status. The study concluded that a direct interaction is pivotal in influencing positive or negative attitudes towards bandicoots. Jonker *et al.* (2006) similarly concluded that residents who had a direct experience with beavers (*Castor canadensis*) or beaver damage were more likely to perceive them as a nuisance. Jonker *et al.* (2006) also suggested that attitudes towards beavers by respondents with a direct experience might remain positive, although they were generally less favorable than attitudes of respondents who did not experience this interaction. This is reflected in the study by the generally positive nature of statements and a general appreciation of bandicoots.

The public can tolerate some nuisance aspects of living with wildlife until these activities result in a safety concern or economic loss (Wittman *et al.* 1998; Coluccy *et al.* 2001). The survey reflected anecdotal reports from National Park managers, where bandicoot diggings and ticks are cited as a major cause of conflict. Veterinary costs associated with ticks and fencing to exclude bandicoots is a financial burden few residents would willingly pay. The

fear for managers is that rather than developing a higher tolerance over time, community members may take action to reduce bandicoot populations. Of the respondents who indicated bandicoots were a nuisance, 64% believed bandicoots left holes in the lawn and 44% believed they carry ticks. However, two-thirds of residents did not identify bandicoots as a nuisance even though most (83%) experienced direct bandicoot interactions.

Older residents were more likely to indicate bandicoots were a nuisance than younger residents. Other studies have found younger individuals are more likely to value wildlife, like they value pets or people (Mankin *et al*. 1999). Younger residents may also be more educated about environmental issues. Similarly, respondents who owned a pet were less likely to call bandicoots a nuisance, perhaps reflecting more positive environmental views. Interestingly, this study rejected the hypothesis that bandicoot visits were less frequent in backyards containing pets. This study observed that bandicoots were not deterred by pets, as pet owners received just as many visits as residents with no pets. A parallel study by Hill *et al*. (2007) found that brushtail possums in another urban area of Sydney were also not deterred by pets being left out after dark.

Similar studies have demonstrated that attitudinal surveys are important for involving the community in management initiatives by increasing public awareness and support for conserving biodiversity (Lunney *et al*. 2000; Lamb and Cline 2003; Casey *et al*. 2005; Heberlein and Ericsson 2005; Raik *et al*. 2005). Community engagement also builds the goodwill necessary to access private properties for research (Pickett and Cadenasso 2006). However, to introduce an effective community-based conservation program, conservation managers of northern Sydney should address misconceptions educating the older members of the community. The education and participation of the community is a key management strategy in the conservation of wildlife and biodiversity internationally (Zinn and Andelt 1999; Scott *et al*. 1999; Lunney *et al*. 2000; Sah and Heinen 2001; Casey *et al*. 2005; Robertson and Lawes 2005).

Conflict between wildlife and people can erode local support for conservation (Gadd 2005). Educating residents about the behaviour and benefits of bandicoots in an urban environment may help reduce anxieties surrounding human–bandicoot co-existence. To reduce conflicts such as bandicoot diggings, residents may use fences or participate in bush regeneration programs to promote natural habitat in areas outside of the residential properties.

Interestingly, very few respondents were affected by the tick problem, suggesting the perception of tick infestation in bandicoots is over represented. Nevertheless, the education process should inform residents on ways to lessen exposure to ticks.

The bandicoots of northern Sydney pose a unique problem to conservation managers. The long-nosed bandicoot is a nuisance to a minority of residents, whilst the continued viability of the southern brown bandicoot may depend on increasing habitats of conservational importance, including urban backyards. However, wildlife managers have a duty to defend the interests of the minority while maintaining populations for the majority of the community to enjoy (Coluccy *et al*. 2001).

4. Population and habitat characteristics of captured bandicoots in northern Sydney

4.1 Introduction

Urban expansion is occurring at an unprecedented rate with people choosing to live in cities and their suburban environs (Goddard *et al.* 2009). Urbanisation has become a growing cause of many environmental problems and has been associated with altered ecological conditions and reduced biodiversity (McKinney 2002; Drinnan 2005; Garden *et al.* 2007; Gordon *et al.* 2009). With the growth of urbanisation, the preservation, restoration and management of environmental values in urban areas becomes increasingly important (Savard *et al.* 2000). Knowledge of how animals cope with their environment and its perturbations is fundamental to the effective management of wildlife. However, knowledge gaps of the response from wildlife to natural and disturbed environments are one of the biggest inhibitors of accurately managing and conserving biodiversity. Consequently, no outcomes or actions often result in lieu of focused conservation management, due to a lack of available information.

Researching wildlife populations inhabiting environments with diverse levels of disturbance is vital for long-term management of biodiversity in these urban areas. Measuring multiple independent indicators rather than one indicator of population health can provide a more inclusive and informative description of the response of a species to disturbance (Johnstone *et al.* 2010). Various measures of population status, including density, growth rates, body condition, parasite loads, reproductive status, juvenile dispersal and habitat types, among others have been used to collect a variety of individual and population level information. This has traditionally been conducted through mark-recapture surveys and laboratory observations, with non-invasive monitoring such as opportunistic observations, audio calls, scat counts, infra-red cameras, sand pads and hair-tubing also proving useful (Witmer 2005).

This chapter contains live-trapping data from *I. obesulus* and *P. nasuta* across the habitats of northern Sydney. The species are sympatric across much of their range and display similar life history traits, but demonstrate different responses to urbanisation. The objective of this chapter is to provide a snap shot of ecological data for both species over the trapping period. The study also aimed to compare the dynamics and biological characteristics of both species based on the hypotheses outlined below. This information will help provide an overall picture

of population characteristics for the two species, provide a backdrop to the following chapters and provide a comparison of ecologically relevant data that will guide *in situ* management.

It was predicted that *P. nasuta* would be caught more often during the live-trapping surveys than *I. obesulus*, due to the limited past records of *I. obesulus* and its national level threatened status. It was also hypothesised that *P. nasuta* would be captured in all habitats, where as *I. obesulus* would be limited to Ku-ring-gai Chase and Garigal National Park (as well as the hypothesis for Chapter 2). In addition, due to the larger home range of male bandicoots compared to females and the fact that backyards are considered fringe habitat, it was predicted that males would be more likely to be caught in backyards than females. It was hypothesised that both species of bandicoot would exhibit sexual dimorphism. Furthermore, due to the reproductive requirements of females from an almost year breeding season, male bandicoots were predicted to have a higher body condition than female bandicoots. Body condition was also hypothesised to be higher in National Park populations than suburban backyards, because of the likely increase in physiological demands required for obtaining resources in backyards.

4.2 Methods

The study area included sites in Ku-ring-gai Chase National Park, Garigal National Park and adjacent suburban backyards. Bandicoots were captured in a wire cage trap (30 cm x 30 cm x 60 cm) overnight and samples were collected at first light for analyses. For a full description of the study area and live-trapping methodology, refer to Chapter 2.

4.2.1 Analysis

Chi-squared tests were used to compare the sex ratios of captured bandicoots with the expected 1:1 (female:male) ratio. Analyses of variance (ANOVA) were used to test the effects of factors 'species' and 'sex' on the demographic variables of 'weight', 'pes length' and 'body condition'. A two-way ANOVA was used to examine whether the body condition of bandicoots differed between habitats (National Parks versus backyards). Body condition was used as the dependent variable with 'habitat' and 'sex' as the factors. Significance was accepted at the $p < 0.05$ level. Means are given with standard errors unless otherwise stated.

4.3 Results and discussion

The results and discussions for this chapter have been merged to enable a direct discussion from the outputs of the data analysis. The raw capture data is provided in Appendix A.

4.3.1 Habitat associations in northern Sydney

Isoodon obesulus were only recorded in Ku-ring-gai Chase National Park, except for a single capture in March 2005 of an individual in Garigal National Park. Prior to 2005, *I. obesulus* were relatively populous in two distinct sites in Garigal National Park. However, a hazard reduction burn reduced their prime habitat at one of these sites to about 30% of its former extent (Mel Hall pers. comm., NPWS Metro North East Pest Management Team, Senior Ranger). Bandicoots are known to use early seral habitats and favour a mosaic of habitats features created by the different stages of regeneration after fire (Heinsohn 1966; Braithwaite and Gullan 1978; Stoddart and Braithwaite 1979; Lobert 1990). Although *I. obesulus* were thought to have moved to adjacent habitat, and were expected to return after regeneration of the habitat, no individuals were recorded during the trapping period and no *I. obesulus* have been recorded in Garigal National Park since (at time of thesis submission).

Trap locations were based on historical records, potential habitat and known habitat. Potential and known habitat is strongly linked to vegetation characteristics, floristics, slope, aspect and shallow soils (Braithwaite and Gullan 1978; Gordon and Hulbert 1989; Dufty 1994). Three broad vegetation types typically represent bandicoot habitat in the northern Sydney region:

- Sydney Sandstone Ridgetop Woodland;
- Sydney Sandstone Gully Forest, and
- Coastal Sandstone Heath.

These three vegetation types have been mapped in *The Natural vegetation of the Sydney 1:100 000 map sheet* (Benson and Howell 1994) and summarised below.

<u>Sydney Sandstone Ridgetop Woodland</u> is a structurally variably dry woodland type associated with dry plateaus and ridgetops (Figure 4-1). The structural variability is influenced by time since last fire, aspect, soil and drainage conditions. Sydney Sandstone Ridgetop Woodland is typically represented in the Ku-ring-gai Chase and Garigal National Park areas by *Eucalyptus gummifera – Eucalyptus haemastoma – Eucalyptus Sparsifolia – Eucalyptus racemosa* woodland / low woodland; or *Banksia ericifolia – Hakea teretifolia* open-scrub. These vegetation communities generally have a rich sclerophyllous shrubby understorey with species of *Hakea, Banksia, Grevillia, Pultenaea, Hibbertia, Isopogon, Boronia, Leucopogon, Dillwynia,* and *Baeckea* present. Other Eucalypt species, Angophoras and *Allocasuarina* sp. may also be present in the overstorey in localised patches.

Figure 4-1: Sydney Sandstone Ridgetop Woodland

Sydney Sandstone Gully Forest has a wide distribution and is usually confined to gullies and sheltered hillsides, particularly on the southern to eastern aspects (Figure 4-2). Sydney Sandstone Gully Forest is typically represented in the Ku-ring-gai Chase and Garigal National Park areas by *Eucalyptus piperita – Angophora costata – Eucalyptus pilularis* open-forest / woodland; *Eucalyptus pilularis – Syncarpia glomulifera* tall open-forest; or *Ceratopetalum apetalum – Tristaniopsis laurina* closed-forest. These vegetation communities have an understorey dominated with a variety of shrubs 0.5-2 metres high with species of *Persoonia, Acacia, Pultenaea, Dodonaea* and *Leptospermum* present. Considerable variation occurs in this community influenced by creeks, exposure, slope and rocky substrates.

Figure 4-2: Sydney Sandstone Ridgetop Woodland leading to Sydney Sandstone Gully Forest

<u>Coastal Sandstone Heath</u> can occur in habitats along broad ridges, gently to moderately inclined slopes, wide rock benches with low broken scarps, small hanging valleys and areas of poor drainage (Figure 4-3). Coastal Sandstone Heath is typically represented in the Ku-ring-gai Chase and Garigal National Park areas by *Allocasuarina distyla – Banksia ericifolia* open-heath / closed scrub; *Baeckea diosmifolia – Baeckea brevifolia* open-heath; *Hakea teretifolia – Banksia oblongifolia* closed heath; *Banksia robur – Viminaria juncea – Gymnoschoenus sphaerocephalus* sedgeland / shrubland; or *Eucalyptus luehmanniana* shrubland (mallee). The structure of Coastal Sandstone Heath can vary from shrubland, open-heath, mallee and sedgeland. It is heavily influenced by fire frequency and time since last fire.

Figure 4-3: Coastal Sandstone Heath

4.3.2   Population biology of live-captured bandicoots in northern Sydney

A total of 258 bandicoot captures (southern brown bandicoot (SBB) = 71; long-nosed bandicoot (LNB) = 187) including recaptures over 4336 trapping nights were recorded from Ku-ring-gai Chase National Park, Garigal National Park and suburban backyards from March 2005 to September 2007 (Table 4-1). However, the southern brown bandicoot had a significantly reduced distribution in comparison and was not recorded outside of Ku-ring-gai Chase National Park, except for a single capture in Garigal National Park in 2005.

A total of 136 individuals were recorded from all captures with southern brown bandicoots accounting for 21 individuals and long-nosed bandicoots accounting for 115 individuals (Table 4-1). A total of 45 individuals (33%) were recaptured at least once over the life of the study. Recaptured bandicoots were generally within 3 to 6 months of their original capture. However, the maximum number of captures from a single individual was from a southern brown bandicoot caught 15 times between September 2005 and September 2007 (24 months).

Table 4-1: Capture numbers for the southern brown bandicoot (SBB) and long-nosed bandicoot (LNB).

	SBB	LNB	Total
Captures	71	187	258
Individuals	21	115	136
Recaptures (%)	10 (48%)	35 (30%)	45 (33%)

The 258 captures over 4336 cage trapping nights was at an overall trapping success of 6% with long-nosed bandicoots counting for the vast majority of captures. The cryptic nature of bandicoots, particularly the southern brown, is likely to have contributed significantly to the low captures in this study. Opie *et al.* (1990) considered the southern brown bandicoot in Victoria trap shy, quoting a trapping success from a broad scale regional survey as 0.01%. A study in Tasmania by Mallick *et al.* (1998b) on the demography of the southern brown bandicoot received a total of 178 captures with a trapping success of 2.6%, while another study on the western barred bandicoot by Short *et al*. (1998) reported 265 captures at a average trapping success of 14.7%. The trapping rates recorded by Short *et al.* (1998) varied from as low as 5.7% to 25.8%. Additionally, a study by Scott *et al.* (1999) on the long-nosed bandicoot at North Head in Sydney recorded a trapping success of 31%, considerably higher than the trapping success in this study. However, this population occurs in an area much

reduced compared to the bandicoots of northern Sydney, and thus the North Head bandicoots are likely to be more densely populated and account for the higher trap success.

However, in this study, it is important to consider the trap success in the context of each trapping site and environmental variables such as habitat characteristics, as not all sites recorded the presence of both species. In his Masters research, Atkins (1998) managed only one live-capture of the southern brown bandicoot and six hair-tube records over 36 transect locations, indicating the typically low capture success and cryptic nature of the southern brown bandicoot. Atkins (1998) revealed only one habitat model comprising the presence of three tree species (*Allocasuarina littoralis, Eucalyptus haemastoma* and *Eucalyptus piperita*) and three shrub species (*Platysace linearifolia, Allocasuarina distyla* and *Leptospermum trinervium*) was a reliable predictor of presence. These flora species are present within Ku-ring-gai Chase and Garigal National Park (Wilson 2004).

The accurate estimation of wildlife population abundance can often be difficult and require considerable resources (Witmer 2005). However, it is commonly one of the first sources of information used by wildlife managers in designing and focusing conservation strategies. Population estimates and population indices are often conducted on mark-recapture surveys using a range of models such as 'minimum number known alive (MKNA)' and 'population indexing' (Krebs 1966; Engeman 2005; White 2005; Southwell *et al.* 2007). These models are based on a range of basic assumptions, and the population estimates need to be interpreted within the constraints of these assumptions. Commonly, the populations are required to be closed, the survey design needs to be appropriate and the capture probability of individuals needs to be consistent throughout the study (Krebs 1966; White 1999; Engeman 2005). In addition, studies estimating population size through common models such as these are also likely to be negatively biased, unless the population exhibits high trap-ability (Dufty 1991). Population estimates on bandicoot populations have the potential to be underestimated due to their cryptic and trap-shy nature and would be influenced by low capture rates.

Population viability analysis (PVA) is another model used by conservation managers to guide management decisions of rare and threatened species (Southwell *et al.* 2007). PVA is particularly useful when management decisions require both quantitative and qualitative population information (Banks 2004). For example, for populations inhabiting unpredictable environments such as the urban fringe (Banks 2004). Banks (2004) used PVA with the

endangered population of long-nosed bandicoots at North Head in Sydney. Banks demonstrated that for this population, managing small changes in habitat availability is not as immediately important as managing the by-products of urbanisation (e.g. predation by pets or road kill). However, a short fall of PVA is that it requires detailed information on habitat characteristics and other environmental variables, which are often not part of the research design (Southwell *et al.* 2007).

Low capture success can intrinsically undermine abundance estimates on wildlife populations. McKelvey and Pearson (2001) in a five-year literature review found that 98% of small mammal studies analysed capture data using too few data points for valid mark-recapture population estimates (Engeman 2005; White 2005). McKelvey and Pearson (2001) do not suggest a minimum number required for valid population estimates, however, it is likely to be study-specific and influenced by a range of environmental and demographic variables.

Due to the inherent difficulties in providing an accurate population estimate, the unbalanced survey design, low southern brown bandicoot captures (21) and the potential for some basic assumptions to be violated, it was considered not appropriate to conduct a population estimate for the bandicoots of northern Sydney. Notwithstanding, given the total captures across a three year period and two National Parks of only 21 southern brown bandicoots, the total effective population size for this species in northern Sydney can be assumed to be low. In particular, the southern brown bandicoot population size must be low relative to that of the long-nosed bandicoot, which is sympatric in many of the habitats surveyed and was consistently observed at all trapping locations.

4.3.3 Sex and reproduction of captured individuals

Southern brown bandicoots

A total of 10 female and 11 male individual southern brown bandicoots were caught throughout all trapping sessions, representing a sex ratio (female to male) of 1:1.1 (Table 4-2). This sex ratio was not significantly different from an expected 1:1 sex ratio ($\chi^2 = 0.05$, df = 1, $p = 0.83$). However, when including recapture data, male southern brown bandicoots were preferentially caught in a cage trap compared to females. The capture ratio represented significant departure from the expected 1:1 capture ratio ($\chi^2 = 6.21$, df = 1, $p = 0.01$).

Interestingly, only four of the 10 female southern brown bandicoots were caught during the 2006 and 2007 trapping sessions and only three of the 11 male southern brown bandicoots were caught during the 2005 trapping sessions.

Of the ten female southern brown bandicoots caught over the trapping period, six individuals were caught with an average of 1.8 pouch young present (Table 4-2). This is the same as the litter size quoted by Sanderson and Kraehenbuehl (2006) for a population of southern brown bandicoots in Belair National Park and similar (2.1) to that recorded by Copley *et al.* (1990) on the Franklin Islands, South Australia. However, it was at the lower end of the litter sizes quoted by Lobert and Lee (1990) for southern brown bandicoots in Victoria and Mallick *et al.* (1998b) and Heinsohn (1966) in Tasmania. No female southern brown bandicoot was caught with pouch young in more than one trapping session, but signs of recently used teats were observed.

Breeding activity (pouch young present or used teats) for the southern brown bandicoot was observed during the months of September to March only, suggesting breeding activity occurs seasonally in northern Sydney. This is thought to coincide with the warmer months, an increase of vegetative biomass and an increase in food supply and other resources (Heinsohn 1966). Southern brown bandicoot populations have been recorded breeding year-round in South Australia (Copley *et al.* 1990; Sanderson and Kraehenbuehl 2006), seasonal in Victoria from July to December and in Tasmania from July to February (Heinsohn 1966; Lobert and Lee 1990).

Table 4-2: Sex and reproductive status of southern brown bandicoots (SBB) and long-nosed bandicoots (LNB).

	SBB	LNB
Males	11	62
Females	10	53
Sex Ratio (female:male)	1:1.1	1:1.17
Females with pouch young (average litter size)	6 (1.8)	28 (2.2)

Long-nosed bandicoots

A total of 53 female and 62 male individual long-nosed bandicoots were caught throughout all trapping sessions, representing a sex ratio (female to male) of 1:1.17 (Table 4-2). This sex ratio was not significantly different from an expected 1:1 sex ratio (χ^2 = 0.70, df = 1, p = 0.40). Additionally, when incorporating recapture data, female and male long-nosed bandicoots were equally likely (1.01:1) to be caught in a cage trap (no difference from expected 1:1 ratio; χ^2 = 0.02, df = 1, p = 0.88).

The distribution of male and female long-nosed bandicoots within National Parks and suburban backyards did not differ significantly from expected, despite more female captures recorded for suburban backyards than males (National Parks: χ^2 = 0.59, df = 1, p = 0.44; Backyards: χ^2 = 2.81, df = 1, p = 0.09). Therefore, neither male nor female long-nosed bandicoots were statistically more likely to be caught within either habitat. This was contrary to the prediction that males would be more likely to be caught in suburban backyards than females, due to the larger home range of male bandicoots and the suburban backyards considered as fringe habitat.

Of the 47 female long-nosed bandicoots of breeding age captured, 28 individuals were caught with an average of 2.2 pouch young present (Table 4-2). Only one individual was caught in multiple trapping sessions with pouch young present. The average litter size observed in this study was similar (2.3) to that recorded by Scott *et al.* (1999) for long-nosed bandicoots at North Head in Sydney. Breeding activity for the long-nosed bandicoot in this study was observed for three-quarters of the year from June to February, almost identical to the breeding period recorded by Scott *et al.* (1999). Approximately 86% of the captures of pouch young occurred during September to February, suggesting a strong seasonal bias of breeding activity towards the spring and summer months, similar to that of the southern brown bandicoot and when food resources are typically at their highest.

Female southern brown bandicoots typically have between 2 to 4 young in the pouch (Heinsohn 1966; Stoddart and Braithwaite 1979). The maximum number of pouch young recorded in this study was three in long-nosed bandicoots and two in southern brown bandicoots. Female bandicoots have eight teats, more than double the maximum number of pouch young observed at any one time in this study. Having smaller litters allows rapid re-use of the pouch and multiple litters throughout the year. The newborn young entering the pouch

have been recorded as preferring to suckle on the smaller unused teats not suckled by the previous litter (Merchant 1990). No juvenile southern brown bandicoots were caught over the trapping period compared to 14 juvenile long-nosed bandicoots. Southern brown and long-nosed bandicoots are known to become sexually mature at approximately 4 months for females and 6 months for males, however this maybe dependent on geographical boundaries (Heinsohn 1966; Lobert and Lee 1990). Heinsohn (1966) recorded the smallest female southern brown bandicoot to have young weighing at 567g, compared to 450g recorded for this study. Similarly for the long-nosed bandicoot, Scott *et al.* (1999) recorded the smallest female with pouch young at 545g, compared to 500g for this study. Thus, all bandicoots weighing less than 400g and 450g were recorded as a juvenile for the southern brown and long-nosed bandicoots, respectively. It is likely that bandicoots weighing less than this were yet to reach sexual maturity.

The number of juvenile captures was relatively low and it is thought the young bandicoots, if dispersed, moved into habitats outside of the survey area. Lobert and Lee (1990) determined that the weight of a southern brown bandicoot on completion of the weaning period is typically < 150g, whilst Heinsohn (1966) recorded a weight range of 61 to 143g (mean 82.5g) for the eastern-barred bandicoot (*Perameles gunnii*.) Upon emergence the young gains independence almost immediately and disperses into new habitat (Gordon 1974). This dispersal enables bandicoots to spatially and temporally exploit ephemeral habitats, such as those influenced by periodic fire (Cockburn 1990). High mortality of juvenile bandicoots has been associated with the dispersal period, and thus, the reproductive strategy of multiple litters throughout the year increases the chances of population survival (Copley *et al.* 1990).

4.3.4   Weight of captured bandicoots

Both species in this study showed sexual dimorphism (Table 4-3; $F_{(1,151)} = 6.74$, $p = 0.01$), supporting the research hypothesis. Long-nosed bandicoots were also significantly heavier than southern brown bandicoots (Table 4-3; $F_{(1,151)} = 4.01$, $p = 0.047$). Male southern brown bandicoot were on average 130% heavier than females, while male long-nosed bandicoots were on average 119% heavier. The mean (± s.e.) weight of adult female southern brown bandicoots at first capture was 556g ± 21g while males averaged 724 ± 29g (Table 4-3). The smallest southern brown bandicoot caught was an adult female weighing 425g whilst the largest caught was a male weighing 1200g. The mean weight of adult female long-nosed

bandicoots at first capture was 795g ± 36g while males averaged 946g ± 62g (Table 4-3). The smallest long-nosed bandicoot caught was a male juvenile weighing 75g. The smallest adult long-nosed bandicoot was a male weighing 450g, while the largest caught was also a male, but weighing in a 2050g. There were no apparent seasonal effects on body weights, although no statistical analysis was conducted.

Table 4-3: Weight and right-hind leg (pes) measurements for southern brown bandicoots (SBB) and long-nosed bandicoots (LNB). Within each column, different letters in superscript indicate significant differences between males and females.

	SBB	**LNB**
Weight (± s.e.)		
Male	724g ± 29g (475-1200g)[a]	946g ± 62g (450-2050g)[e]
Female	556g ± 21g (425-700g)[b]	795g ± 36g (500-1250g)[f]
Pes Length (± s.e.)		
Male	57mm ± 0.48mm (51-61mm)[c]	64mm ± 1mm (41-77mm)[g]
Female	53mm ± 0.42mm (50.6-55mm)[d]	61.75mm ± 0.8mm (42-70mm)[h]

Male bandicoots of both species also had a significant longer right-hind foot (pes) than female bandicoots (Table 4-3; $F_{(1,151)} = 5.99$, $p = 0.016$), while long-nosed bandicoots had a significantly longer pes length than southern brown bandicoots ($F_{(1,151)} = 40.99$, $p = <0.001$).

4.3.5 Body Condition of captured bandicoots

The body condition of an animal may reflect its energetic or physiological state and relate ultimately to population fitness. An animal in good condition will have higher energy stores than an animal in poor condition (Schulte-Hostedde *et al*. 2001) and provide an increased ability to cope with environmental pressures (Jakob *et al*. 1996). It was hypothesised that both species would have a similar body condition, whilst male bandicoots would have a higher body condition than female bandicoots due to the physiological needs related to an almost year-round breeding season. It was also hypothesised that bandicoots inhabiting backyards would have a lower body condition than those in National Parks. It was thought the increased pressures such as higher risk of predation and competition for resources in backyards would require higher physiological demands. The body condition index (BCI) of each individual was calculated by using the cube root of weight divided by pes length (Short *et al.* 1998).

There was no difference in the BCI for male and female bandicoots of both species in northern Sydney (Figure 4-4; $F_{1,151} = 0.88$, $p = 0.35$), rejecting the original hypothesis. However, southern brown bandicoots had a higher body condition than long-nosed bandicoots and were more likely to be recorded in moderate to good condition (Figure 4-4; $F_{(1,151)} = 27.63$, $p = <0.001$). Comparison of the calculated BCI to the qualitative assessment of condition upon capture (made by the experimenter), showed that animals recorded as "moderate to good" had an average BCI above 0.142, while those recorded as "low" condition tended to have an average BCI below 0.135. It is possible that rainfall or other measureable environmental factors influenced bandicoot body condition, as the availability of food resources such as vegetation and invertebrates can be linked to rain events. Although no rainfall data was captured for this study, past studies such as Short *et al.* (1998) related the body condition of local bandicoots to rainfall within the last two months.

The body condition analysis across habitats was in contrast to the original hypothesis, with long-nosed bandicoots captured in backyards recorded with a higher body condition than long-nosed bandicoots in Ku-ring-gai Chase and Garigal National Park (Figure 4-5; $F_{1,118} = 7.66$, $p = 0.007$). The higher body condition values suggests that the peri-urban habitats maybe favourable in comparison to the more undisturbed National Parks. Furthermore, they may provide bandicoots in backyards with an increased ability to cope with environmental pressures. The availability of resources in backyards and the soft moist grassy lawns that are likely to provide easy access to soil invertebrates, are appearing to compensate for the higher physiological demands in obtaining them. For example, the energy demands required to cope with the increase in vigilance behaviour for potential predators (domestic cats and dogs).
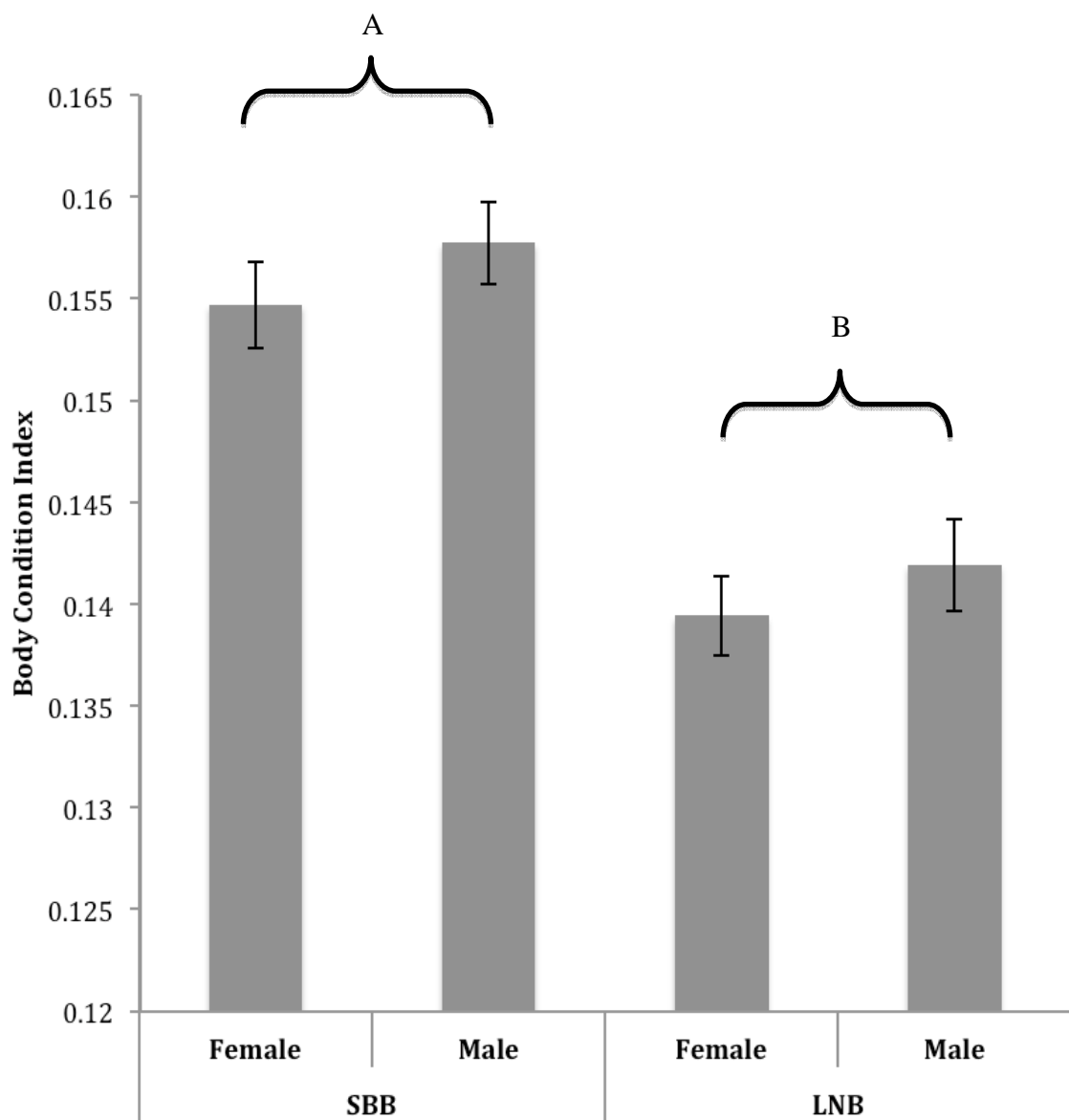
Figure 4-4: Body condition index for the southern brown bandicoots (SBB) and long-nosed bandicoots of northern Sydney. The different letters represents a significance difference ($p < 0.05$).
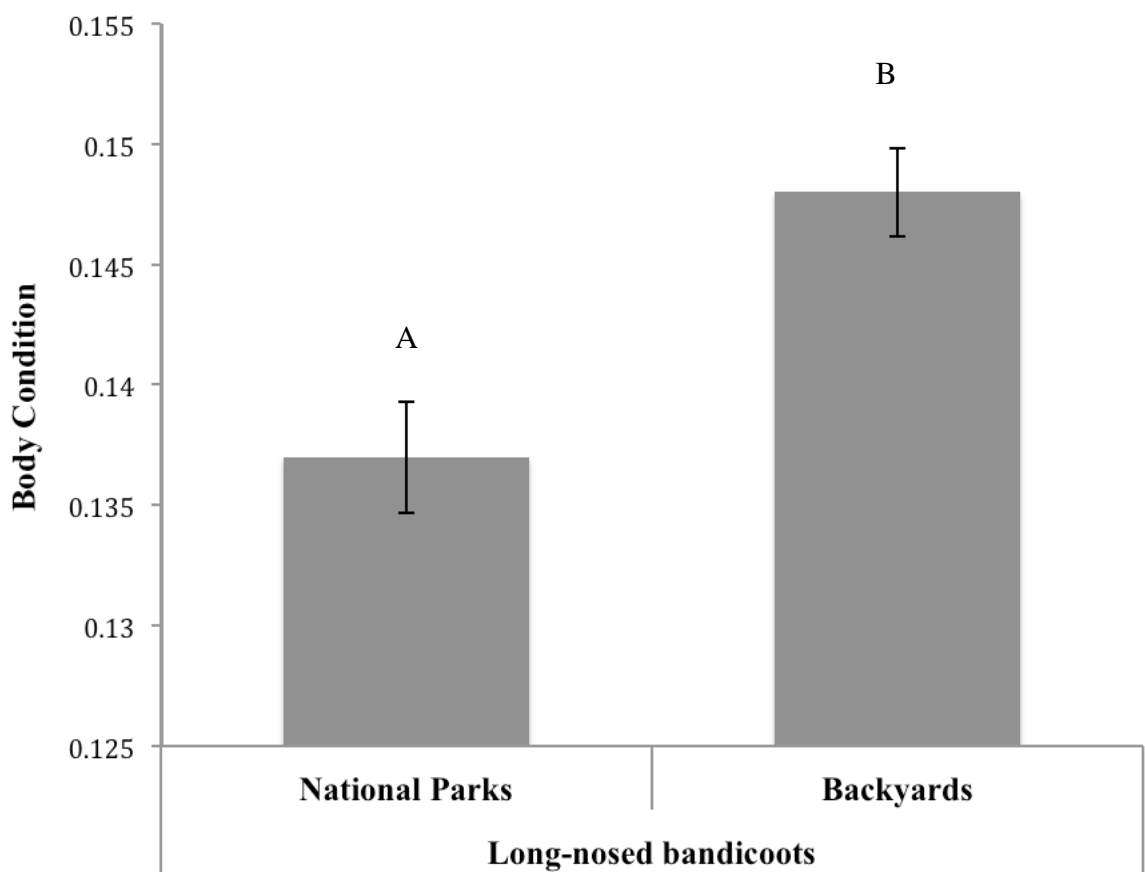
Figure 4-5: Body condition index for the long-nosed bandicoots in the different habitats types of northern Sydney. The different letters represents a significance difference ($p < 0.05$).

4.3.6 Age and Longevity of captured bandicoots

The longest period between captures for a southern brown bandicoot was 24 months; two males were caught in both the November 2005 and October 2007 trapping sessions. An estimated minimum life span for these individuals is approximately 2.5 years. The weight of these two individuals at first capture was 475g and 625g, indicating that they were already sexually mature adults. The longest period between captures for a long-nosed bandicoot was 16 months; a single female was caught in both the November 2005 and March 2007 trapping sessions. An estimated minimum life span for this individual is approximately 2 years. The weight at first capture was 550g, indicating a sexually mature adult. The average longevity of bandicoots in the wild, particularly for the southern brown bandicoot is not well known. However, Heinsohn (1966) in a mark-recapture study estimated that most bandicoots live for at least two years. Another field-based study conducted by Lobert and Lee (1990) reported individuals living up to 3.5 years of age.

4.3.7 Movement of bandicoots in northern Sydney

Male southern brown and long-nosed bandicoots are known to have a home range of 0.5-9 ha, depending on resource availability and quality (Heinsohn 1966; Lobert 1990; Mallick *et al.* 1998b; Mallick *et al.* 2000). This is compared to the female home range females of both species ranging from 0.5-5 ha (Mallick *et al.* 1998b; Scott *et al.* 1999). Bandicoots are solitary animals and are thought to establish rough territories within their home range (Heinsohn 1966). Home ranges of bandicoots have been shown to overlap, but due to their pugnacious behaviour, contact between other bandicoots, particularly outside the breeding season is rare (Copley *et al.* 1990; Lobert 1990; Mallick *et al.* 2000).

Individuals in this study were caught in a similar area in each trapping session with recaptures occurring in the same or immediately adjacent traps. However, a male southern brown bandicoot was observed dispersing between trapping sessions into adjacent habitat, approximately 2 km from the first point of capture. The new habitat in the Ku-ring-gai Chase National Park represented regenerating vegetation, approximately 4 years after the most recent fire event. This was the first capture of a southern brown bandicoot in this habitat during the trapping period and occurred in March 2007, despite previous records (prior to 2005) from the immediate area.

4.3.8 Non-target captures during live-trapping

Two Elliot traps (30cm x 10cm x 8cm) were placed within a two-metre radius of a cage trap at each trapping location to minimise the number of captures of non-target species and hence maximise the likelihood of bandicoot captures. The most common non-target captures were bush rats (*Rattus fuscipes*) which numbered 1538, followed by brown antechinus (*Antechinus stuartii*) numbering 52 individuals. Other non-target species that were opportunistically caught in Elliot traps or cage traps include, brush-tail possum (*Trichosurus vulpecula*), blue-tongued lizard (*Tiliqua scincoides*), eastern pygmy possum (*Cercartetus nanus*), Australian magpie (*Cracticus tibicen*), pied currawong (*Strepera graculina*), eastern water skink (*Eulamprus quoyii*), eastern whipbird (*Eulamprus quoyii*), Indian minor (*Acridotheres tristis*), lace monitor (*Varanus varius*), water dragon (*Physignathus lesueurii*) and peaceful dove (*Geopelia striata*).

4.3.9 Hair-tubing in greater north-west Sydney

Other monitoring methods such as the use of infra-red cameras or hair-tubing may provide additional information on habitat usage and population size on a greater scale than the live-trapping surveys. Hair tubes have been identified as an effective and non-invasive means of gathering presence/absence data for a variety of species, including *I. obesulus* (Catling *et al.* 1997 and Garden *et al.* 2007). However, hair-tubing undertaken across greater north-west Sydney during 2007 and 2008 presented a bleak forecast for habitat usage and distribution of the southern brown bandicoot. No hair-tube sites outside of those where live-trapping was conducted recorded the presence of the southern-brown bandicoot. This was in contrast to the long-nosed bandicoot that was recorded in all the National Parks, Nature Reserves and local government areas surveyed. The hair-tube records that identified the ubiquitous nature of the long-nosed bandicoot and the rare occurrence of the southern brown bandicoot, served to support live trapping data.

Additionally, previous hair-tube and cage trapping surveys conducted in Berowra Regional Valley Park have failed to locate the southern brown bandicoot outside of their current known distribution (Visser, 2004). The hair-tube surveys outlined in the methodology (Chapter 2) were used to provide an indication of presence / absence for both species across the broader locality, and did not participate in further analyses (despite being undertaken by the same researcher during the PhD candidature).

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4.4 Conclusion

The two bandicoot species of northern Sydney offer a contrasting view to the impacts on their habitat, with the long-nosed bandicoot population seemingly more prominent relative to the southern brown bandicoot. The study observed a clear difference in the relative distribution and population sizes of the bandicoot species in the habitats surveyed, using both live-trapping and hair-tubing techniques. The long-nosed bandicoot was caught in all habitats and live-trapping locations while the southern brown bandicoot was confined to Ku-ring-gai Chase National Park, supporting the hypothesis relating to the limited distribution of this species. The breeding, longevity and body condition figures of the southern brown bandicoot are consistent with the majority of other studies and comparable to those of the long-nosed bandicoot.

There were an equal abundance of females and males in the populations of long-nosed and southern brown bandicoots in northern Sydney. Sexual dimorphism was recorded for right-hind foot length and weight measurements as predicted. However, in contrast to the original hypothesis, there was no difference in body condition values between the male and female bandicoots. In addition, long-nosed bandicoots were generally larger than southern brown bandicoots, having higher weight measurements and a longer pes length. However, long-nosed bandicoots were observed to have a lower body condition index than southern brown bandicoots.

This chapter has provided an ecological snap shot over the survey period of the population demographics and habitat use for long-nosed and southern brown bandicoots of northern Sydney. The demographic and habitat variables were consistent with other studies for the long-nosed and southern brown bandicoot and will provide important information to guide management of both species in situ. The live-trapping survey also served to provide a basis for data and sample collection for the remaining chapters of the thesis.

5. Genetic diversity of the free-ranging bandicoots (*Perameles nasuta* and *Isoodon obesulus*) of northern Sydney

5.1 Introduction

Understanding the genetic diversity, variation and relationships among populations is an important component of threatened species conservation and wildlife management (Pacioni *et al.* 2011). Furthermore, understanding the interaction between environmental stressors, such as urbanisation and genetic factors, has significant repercussions for efforts aimed at conserving biodiversity in situ, and within developing environments (Reed *et al.* 2007). Populations threatened by anthropogenic forces have rarely become threatened due to one cause. Multiple factors contributing to population decline can interact with each other to enhance the decline of a species, with greater impacts occurring on isolated populations (Clark *et al.* 2011). However, there is a paucity of genetic studies demonstrating the level of impact that these multiple factors can have in the decline of isolated populations. There is an even greater lack of genetic studies from the increasing trend of urban wildlife publications (Clark *et al.* 2011; Magle *et al.* 2012).

Numerous studies on genetic diversity of island populations (in the traditional sense, i.e. surrounded by water) have been conducted (Frankham 1997; 2005; Frankham *et al.* 2002; Blackburn *et al.* 2005; Jamieson 2007; Clegg and Phillimore 2010). However, few studies apply similar theories of limited connectivity and genetic diversity to urbanised island populations (Estes-Zumpf *et al.* 2010). Urbanised island populations can be geographically and demographically challenged, and therefore may exhibit genetic signatures similar to young island populations. Urbanised island populations have been fragmented by anthropogenic environments and cut off from conspecifics by impassable roads (Gerlach and Musolf 2000; Riley *et al.* 2006), removing connectivity with adjacent habitats and limiting gene flow between populations. This can result in genetic erosion, inbreeding and loss of genetic diversity that in turn can enhance population decline (Riley *et al.* 2006). This may be further amplified in small populations, particularly under stressful environmental conditions (Frankham 1997; 2005; Frankham *et al.* 2001; Brook *et al.* 2002; Jamieson 2007; Clark *et al.* 2011).

The National Park and urban habitats of northern Sydney provide an opportunity to examine genetic diversity information of two sympatric species of bandicoot. This information may assist management in determining suitable conservation strategies for the free-ranging bandicoots of northern Sydney and across the broader landscape. This would be particularly relevant for the southern brown bandicoot, which has experienced significant population decline and range contractions on a continental scale (Ashby *et al.* 1990). This study used eight microsatellite loci with cross-species applicability designed by Zenger and Johnston (2001), combined with multiplexing fluorescently tagged loci to undertake a study on the genetic diversity of bandicoots in northern Sydney.

This study aimed to compare the genetic diversity and gene flow between the long-nosed and southern brown bandicoot populations in northern Sydney. It also aimed to determine whether inbreeding is present and if any population has undergone a bottleneck, similar to Zenger *et al.* (2005). It is hypothesised that the southern brown bandicoot, due to its reduced distribution and endangered status, has a lower genetic diversity than the long-nosed bandicoot in northern Sydney. It is also hypothesised that the southern brown bandicoot exhibits signs of inbreeding depression resulting from a population bottleneck. In addition, the lower genetic diversity of the southern brown bandicoot has been amplified by reduced gene flow between fragmented populations. In contrast, it is hypothesised that a bottleneck in the long-nosed bandicoot is unlikely due to higher population numbers. However, similar to the southern brown bandicoot, a reduced gene flow is present, due to the fragmented habitats of the long-nosed bandicoot populations.

5.2 Methods

5.2.1 Study area and sample collection.

The study area incorporated locations in greater northwest Sydney. Sites included Ku-ring-gai Chase National Park, Garigal National Park and nearby suburban backyards. For a full description of the study area and live-trapping methodology, refer to Chapter 2. Bandicoots were captured in a wire cage trap (30 cm x 30 cm x 60 cm) overnight and samples were collected at first light for analyses. Upon capture, an ear biopsy was taken from all individuals using a 2mm Miltex Sterile Disposable Biopsy Punch. The samples (ear biopsy) were collected in a plastic tube and stored in ethanol (70%) at 4°C until analysis. A total of 108 ear biopsies from two species of bandicoots were collected and used in the analysis (Table 5-1).

Table 5-1: The number of ear biopsies collected from southern brown bandicoots (SBB) and long-nosed bandicoots (LNB) in northern Sydney.

	Number of Samples		Total
	SBB	LNB	
Ku-ring-gai Chase National Park	21	48	69
Garigal National Park	3	16	19
Suburban backyards	0	20	20
Total	24	84	108
Ku-ring-gai Chase National Park*	21	59	80
Garigal National Park*	3	25	28

*Two populations; bandicoots caught in suburban backyards were assigned to their respective, Greater Ku-ring-gai or Garigal area.

5.2.2 DNA extraction from tissue samples

DNA was extracted from tissue samples using the Qiagen DNeasy tissue extraction kit (Qiagen Pty Ltd., Victoria, Australia) following the manufacturer's instructions. Briefly, 25 mg of ear tissue was placed in 180 μl ATL buffer and 20 μl of Protienase K and then incubated on a shaker overnight at 56°C. The sample was then vortexed and 200 μl of AL buffer and 200 μl of ethanol (96-100%) was added. The mix was transferred to a DNeasy spin column where it was centrifuged to bind to the DNA. To the column, 500 μl of AW1 buffer was added and centrifuged, and then 500 μl of AW2 buffer was added and centrifuged again to dry the ethanol from the spin column, discarding the flow through each time. Lastly, 50 μl

of AE buffer was added directly to the spin column to elute DNA. To ensure maximum yield, the elution step was repeated. The DNA sample was then stored at -20°C for analysis.

5.2.3   Microsatellite analysis at eight loci

Eight polymorphic microsatellite loci (*B3-2, B7-2, B15-1, B20-5, B34-1, B34-2, B35-3, B38-1*) were used to obtain information on the genetic variation of the southern brown and long-nosed bandicoots (Table 5-2). The eight microsatellite loci were developed with cross-species amplification by Zenger and Johnston (2001) for the southern brown bandicoot. The microsatellite loci were shown to be suitable for similar population studies with other bandicoot species (*Perameles nasuta, P. gunnii* and *Echymipera rufescens*) and other related marsupials (Zenger and Johnston 2001). Forward primers were tagged with a fluorescent maker to enable multiplex PCR pairings based on colour and product size (Table 5-2).

Amplification of multiplexed primer pairings were performed in 50 μl reactions. A 10 μl DNA template, 1uL in 9 μl of GeneReleaser$^{®}$ (Integrated Sciences, Sydney, Australia) was heated for 7 min at 500 W in a microwave as per manufactures instructions. The reaction mixture (50 μl) of 1 x PCR buffer, 2.5 mM MgCl$_2$, 20pM of each forward and reverse primer, and 1 U of Go Taq$^{®}$ Green Mastermix (Promega, Australia) was added to gene releaser DNA mix after microwave exposure. Conditions for the Multiplex 1 and 2 PCRs comprised an denaturation step at 94°C for 3 min, followed by a touchdown sequence of 94°C for 30 sec, 60°C for 45 sec, and 72°C for 1 min with the annealing temperature dropping by 2°C every cycle until 50°C, followed by 30 cycles at this temperature and a final cycle of 72°C for 3 min. Multiplex 3 and 4 PCR conditions compromised an altered annealing sequence of 40 cycles of 94°C for 30 sec, 52°C for 45 sec, and 72°C for 1 min. The PCR products were visualized by 2% agarose gel electrophoresis and SYBR$^{®}$ (Invitrogen, Victoria, Australia) Safe DNA gel stain (2 μl).

DNA samples were diluted at 1:30 or 1:50 depending on amplification strength and then sequenced and visualised using an Applied Biosystems 3130 sequencer (Applied Biosystems, Forster City, California) and scored alleles using GeneMapper version 3.7 (Applied Biosystems).

Table 5-2: Eight microsatellite markers extracted and adapted from Zenger and Johnston (2001). Primer sequences and characteristics of each locus are indicated.

Locus	Florescent tag	Multiplex	Repeat	Primer sequence (5' to 3')	Size (bp)
B3-2F	6-FAM^{TM} (blue)	1	$(GT)_{21}$	GGG AGT AAT GTG TTT GTG CTT G	132-148
B3-2R				TCC AGT CAT TAT CCC CTA GAA TG	
B7-2F	NED^{TM} (yellow)	1	$(GACA)_3GATA$ $(GACA)_{10}(GATA)_{15}$	AGA TTT TCC ATT TTC ACC TGA G	184-208
B7-2F				GAA AAT GTA GGT TCT ACT TTC TAA CC	
B15-1F	VIC^{TM} (green)	3	$(GT)_{21}$	GAG GCA AGT GAC AGT ATG ATG C	110-136
B15-1R				TTC TGT CTC TCT ATC TCT GTT TCT GTC	
B20-5F	NED^{TM} (yellow)	3	$(GT)_{14}$	TTC TGA CCA TTT CTC ACC TTT G	160-166
B20-5R				ACA AAT CTC CTA GGC TCT GGT G	
B34-1F	NED^{TM} (yellow)	4	$(CA)_{19}$	GAT TGG TGT CAT GGG CTA TTG	133-153
B34-1R				TCC TCA GTC TTT CCA TCC TCT C	
B34-2F	6-FAM^{TM} (blue)	3	$(CA)_{20}$	CAG GAA CTA ATC TTG TAT TTT CTC CAG	138-150
B34-2R				TGA ACT TTT CAA CAT CCA ATC ATC	
B35-3F	VIC^{TM} (green)	4	$(TC)_{11}ATATA(CA)_{17}$	ACC TCC AGT AGC CTC AAT TTC C	164-200
B35-3R				GAA ATG AGG AAA TGA TAA GGA AGG	
B38-1F	6-FAM^{TM} (blue)	2	$(CA)_3GA(CA)_{13}$	GTG ATC TTT TGC ACG TTG TCT C	130-148
B38-1R				GGG TCT TCC AGT AAA GAT TTG G	

5.2.4   Statistical analysis of microsatellite data

The statistical analyses were conducted using microsatellite data across two populations (Ku-ring-gai and Garigal) for both the long-nosed bandicoot and southern brown bandicoot. The individuals caught within the adjacent suburban backyards were included into a greater Ku-ring-gai or Garigal habitat, as functional connectivity of habitat between backyards and respective National Parks is evident. The Zenger *et al.* (2005) data were also used for a comparative analysis in the discussion.

Deviations from Hardy-Weinberg equilibrium at the eight microsatellite loci for all populations were tested using GENALEX version 6.4 (Peakall and Smouse 2006). The Chi-Squared method was used to test for significance from Hardy-Weinberg equilibrium following Hedrick *et al.* (2000). The expected heterozygosity (H_E) observed heterozygosity (H_O) and allelic richness (A) was estimated using GENALEX. Allelic richness was used to compare genetic diversity among the Sydney populations with varying sample size. The Weir and Cockerham (1984) analogues of Wright's F-statistics (F_{IS}, F_{ST}) were estimated, and significance tested by appropriate permutations in FSTAT (Goudet 1995). The F_{IS} was estimated to determine the shortage of heterozygotes for the Ku-ring-gai and Garigal population of both species, while F_{ST} was estimated to compare the differentiation between the populations of each species. The D value (genetic differentiation based on the distribution of allele frequency) was also calculated using SMOGD (Crawford 2010) for pairwise comparisons between populations for each species as an additional measure of pairwise differentiation. The F_{ST} can underestimate the degree or differentiation that exists among populations (Rousset 1996).

Evidence for population bottlenecks (reductions in effective population size (N_e)) using the Wilcoxon's heterozygosity tests and the allele frequency distribution mode shift analysis (Luikart *et al.* 1998) were performed in BOTTLENECK version 1.2 (Piry *et al.* 1999). Populations were examined under the two-phased mutation model (TPM) using 95% stepwise mutation model (SMM) to 5% infinite alleles model (IAM) with a variance among multiple steps of 12, which is considered best for microsatellite data (Piry *et al.* 1999). Significance for all analyses was accepted at the $p < 0.05$ level. Means are given with standard errors unless otherwise stated.

5.3 Results

The genetic diversity of the southern brown and long-nosed bandicoot populations was evaluated across the greater Ku-ring-gai and Garigal areas in northern Sydney. However, no captures or samples were obtained from suburban backyard habitats for the southern brown bandicoot. Samples from a total 24 southern brown bandicoots and 84 long-nosed bandicoots were genotyped at eight microsatellite loci. Locus *B38-1* was shown to be monomorphic at all the populations except for southern brown bandicoots in Ku-ring-gai.

5.3.1 Southern brown bandicoot

Of the 24 samples, 21 southern brown bandicoot samples were obtained from Ku-ring-gai and three samples from Garigal. Due to the low number of captures in Garigal, the interpretation of results for this population should be treated with caution.

Both populations of the southern brown bandicoot were in Hardy-Weinberg equilibrium for all loci except for *B3-2* and *B15-1* in Ku-ring-gai ($P < 0.01$ Chi-Squared). Additionally, loci *B20-5* and *B38-1* in Garigal were shown to be monomorphic loci for the southern brown bandicoot.

Southern brown bandicoot microsatellite variability ranged from three to six alleles in the Ku-ring-gai population and one to four alleles in the Garigal population (monomorphic alleles *B20-5* and *B38-1*). The average effective alleles (Allelic diversity = *A*) from the eight loci in Ku-ring-gai were observed as 2.47 ± 0.31 (range 1.42 - 3.90) and from the six non-monomorphic loci in Garigal as 1.92 ± 0.33 (1.00 - 3.60) (Table 5-3).

The Ku-ring-gai population had observed and expected heterozygosity for the eight loci of $H_0 = 0.49 \pm 0.09$ and $H_E = 0.55 \pm 0.06$ (ranging $H_O = 0.10$ - 0.86; $H_E = 0.30$ - 0.74) while the Garigal population, excluding the monomorphic loci, was less diverse with $H_0 = 0.42 \pm 0.14$ and $H_E = 0.38 \pm 0.10$ (ranging $H_O = 0.00 – 1.00$; $H_E = 0.28$ - 0.67). The levels of allelic richness (*A*) were similar when compared to other endangered bandicoots species, while expected heterozygosity (H_E) was relatively moderate compared with other species (Table 5-3).

A broad variation was recorded for the inbreeding coefficient (F_{IS}) among microsatellite loci ranging from -0.279 (B7-2) to 0.796 (B15-1) in the Ku-ring-gai population with an overall F_{IS} value among all loci significantly higher than zero ($F_{IS} = 0.131$, $p = 0.003$). The F_{IS} for the Garigal population was not included in the analysis.

Table 5-3: Microsatellite diversity for the four populations of bandicoots in northern Sydney from eight loci, including a comparison to other species and populations.

Population	Number (n)	Polymorphic loci	H_O	H_E	A
Southern brown bandicoot (this study)					
Ku-ring-gai	21	8	0.49 ± 0.09	0.55 ± 0.06	2.47 ± 0.31
Garigal	3	6	0.42 ± 0.14	0.38 ± 0.10	1.92 ± 0.33
Total / Mean	24 (total)		0.45 ± 0.08	0.46 ± 0.06	2.20 ± 0.23
Long-nosed bandicoot (this study)					
Ku-ring-gai	59	7	0.51 ± 0.10	0.56 ± 0.11	3.27 ± 0.65
Garigal	25	7	0.55 ± 0.10	0.60 ± 0.10	3.25 ± 0.56
Total / Mean	84 (total)		0.53 ± 0.07	0.58 ± 0.07	3.23 ± 0.44
Zenger et al. (2005); southern brown bandicoot					
Ku-ring-gai	19	8	0.54 ± 0.09	0.57 ± 0.05	3.8 ± 0.6
Garigal	11	8	0.51 ± 0.08	0.43 ± 0.06	2.1 ± 0.2
Sydney	30	8	0.53 ± 0.08	0.57 ± 0.05	3.8 ± 0.6
E Gippsland	22	8	0.80 ± 0.05	0.79 ± 0.05	7.4 ± 0.9
Mt Gambier	22	8	0.61 ± 0.05	0.69 ± 0.04	6.1 ± 1.0
Smith and Hughes (2008); western barred bandicoot					
Heirisson	29	6		0.27 ± 0.1	1.8 ± 0.3
Bernier Is.	33	6		0.27 ± 0.1	2.0 ± 0.4
Dorre Is.	26	6		0.32 ± 0.1	2.3 ± 0.4
Dryandra	38	6		0.54 ± 0.1	2.9 ± 0.3
Moritz et al. (1997); bilby					
Queensland	27	5		0.75	7.7
Taylor et al. (1994); northern hairy-nosed wombat					
	26	15		0.27	1.8
Jones et al. (2004); Tasmanian devil					
Little Swanport	36	11		0.41 ± 0.1	3.2 ± 0.3
Marawntapu	37	11		0.47 ± 0.0	3.3 ± 0.3

There were no significant deviations between observed and expected levels of heterozygosity within populations ($p > 0.05$). For an extensive comparison of diversity levels amongst marsupial populations, refer to Eldridge (2010).

5.3.2 Long-nosed bandicoot

A total of 84 long-nosed bandicoots were sampled from Ku-ring-gai Chase National Park, Garigal National Park and suburban backyards. Backyard captures were incorporated into the National Park populations, which equated to 59 long-nosed bandicoots from Ku-ring-gai and 25 from Garigal.

Both populations of the long-nosed bandicoot were in Hardy-Weinberg equilibrium for all loci except for *B20-5* and *B34-2* in Ku-ring-gai and Garigal ($p < 0.001$ Chi-Squared). Additionally, locus *B38-1* was shown to be monomorphic for the long-nosed bandicoot in both populations.

Long-nosed bandicoot microsatellite variability ranged from three to eleven alleles in the Ku-ring-gai population and one to eight alleles in the Garigal population (monomorphic allele, *B38-1*). Levels of diversity were relatively higher for the long-nosed bandicoot across both populations than the southern brown bandicoot. The average effective alleles (Allelic diversity = *A*) from the seven non-monomorphic loci were similar between the two populations at 3.27 ± 0.65 (range $1.28 - 6.34$) in Ku-ring-gai and 3.25 ± 0.56 ($1.86 - 5.59$) in Garigal (Table 5-3).

The Ku-ring-gai population of long-nosed bandicoots had observed and expected heterozygosity for the seven non-monomorphic loci of $H_0 = 0.51 \pm 0.10$ and $H_E = 0.56 \pm 0.11$ (ranging $H_O = 0.19 - 0.82$; $H_E = 0.22 - 0.84$) while the Garigal population, excluding the monomorphic loci, had observed and expected heterozygosity of $H_0 = 0.42 \pm 0.14$ and $H_E = 0.38 \pm 0.10$ (ranging $H_O = 0.36 - 0.77$; $H_E = 0.46 - 0.82$) (Table 5-3). The levels of allelic richness (*A*) were higher when compared to other bandicoot species, while expected heterozygosity (H_E) was relatively moderate to high compared with other species (Table 5-3).

A broad variation was found for the inbreeding coefficient (F_{IS}) among loci ranging from -0.112 (*B3-2*) to 0.489 (*B20-5*) in the Ku-ring-gai population and -0.105 (*B34-2*) to 0.558 (*B20-5*) in the Garigal population. The overall F_{IS} value among all loci was significant higher than zero for both populations (Ku-ring-gai $F_{IS} = 0.102$, $p = 0.003$; Garigal $F_{IS} = 0.090$ $p = 0.003$).

5.3.3 Species level comparisons of genetic diversity

The southern brown bandicoot (SBB) had a lower observed (SBB $H_O = 0.45 \pm 0.08$; LNB $H_O = 0.53 \pm 0.07$) and expected heterozygosity (SBB $H_E = 0.46 \pm 0.06$; LNB $H_E = 0.58 \pm 0.07$) than the long-nosed bandicoot (LNB). Similarly, the southern brown bandicoot displayed a lower diversity in allelic richness (SBB $A = 2.20 \pm 0.23$; LNB $A = 3.23 \pm 0.44$) than the long-nosed bandicoot (Table 5-3).

The fixation index ($F_{ST} = 0.014$) for long-nosed bandicoots was not significant ($p > 0.05$; 95% CI, 0.005 – 0.035), showing no differentiation between populations. Additionally, the fixation index ($F_{ST} = 0.158$) for the southern brown bandicoot was not significant between populations ($p > 0.05$; 95% CI, -0.029 – 0.365). The D value for the long-nosed bandicoot indicated little differentiation between populations based on seven non-monomorphic loci, ranging from 0.020 (*B3-2*) to 0.13 (*B20-5*). Conversely, larger differentiation between populations in the southern brown bandicoot was observed. The D value for the southern brown bandicoot for the eight microsatellite loci ranged from 0.032 (*B20-5*) to 0.798 (*B38-1*).

Evidence for a recent reduction in effective population N_e size was not found in any population, nor was a bottleneck found at the species level using the Wilcoxon sign-rank tests for heterozygosity, under the two-phased model in the Bottleneck analysis ($p > 0.05$) (Table 5-4). However, the mode-shift indicator highlighted a distribution away from the normal L shaped distribution for the Garigal population of southern brown bandicoots. This result should be treated with caution due to low population size (<30) and fewer than 10 loci used in the analysis (Piry *et al.* 1999).

Table 5-4: Bottleneck analyses for the bandicoots of northern Sydney.

Species / Populations	Wilcoxon's test (Heterozygosity) *p-value*			Mode-shift indicator
	H deficiency	H excess	Two-tailed	
Southern brown bandicoot	0.422	0.629	0.844	Normal L-shaped
Ku-ring-gai	0.2573	0.770	0.547	Normal L-shaped
Garigal	0.945	0.078	0.156	Shifted mode
Long-nosed bandicoot	0.055	0.961	0.109	Normal L-shaped
Ku-ring-gai	0.055	0.961	0.109	Normal L-shaped
Garigal	0.289	0.766	0.578	Normal L-shaped

5.4 Discussion

This study supported the hypothesis that southern brown bandicoots have a lower genetic diversity than long-nosed bandicoots. The observed heterozygosity (H_O) was similar between both species. However, the allelic diversity (A) values were lower in the southern brown bandicoot (Table 5-3). Additionally, the H_O was lower than the expected heterozygosity (H_E) in the populations of both species (excluding Garigal southern brown bandicoots), suggesting forces such as inbreeding may be present. Heterozygosity (inferred from the H_O and H_E) can be used as an indicator of the loss of allelic diversity and genetic variation (Allendorf 1986), and is used as a measure of contrasting genetic diversity across studies (Garner *et al.* 2005). In this regard, the higher allelic diversity of the long-nosed bandicoot in northern Sydney implies that it is more genetically diverse, and more likely to maintain genetic integrity if faced with environmental challenges and increased genetic pressures than the southern brown bandicoot (Table 5-2). Speilman *et al.* (2004) demonstrated a notable discrepancy in the levels of genetic diversity between threatened and non-threatened mammalian taxa, supporting the differences in allelic diversity observed between bandicoot species of different conservation status in this study. Speilman *et al.* (2004) observed that 84% of threatened mammals had lower diversity values than related non-threatened taxa.

The level of genetic diversity (H_O, H_E and A) detected in this study varied compared to other bandicoot species and other marsupials. The genetic diversity of all populations examined was lower than the Sydney southern brown bandicoot population reported by Zenger *et al.* (2005) ($A = 3.8$, $H_E = 0.565$), but higher than the island populations of the western barred bandicoot reported by Smith and Hughes (2008). When compared to the bilby, a closely related marsupial of the order Peramelemorphia that has undergone significant range contractions (Moritz *et al.* 1997), the bandicoot populations in this study had a much lower expected heterozygosity and allelic diversity. Moritz *et al.* (1997) determined that the viability of these bilby populations in Australia, as consequence of the favourable heterozygosity is unlikely to be limited by lack of genetic variation (Moritz *et al.* 1997).

Similarly, in a positive outcome for the management of the bandicoots in this study, it is unlikely that the long-term survival of bandicoots in Ku-ring-gai Chase and Garigal National Parks are limited by lack of genetic variation. Although the southern brown bandicoot was recorded as having a low genetic diversity, the analysis for recent reductions in effective population size (N_e), suggested that neither species have undergone a population or species

level bottleneck (Wilcoxon Significant test $P > 0.05$). This was in contrast to the hypothesis for the southern brown bandicoot and the study conducted by Zenger *et al.* (2005), but was consistent with the expectations for the long-nosed bandicoot. Zenger *et al.* (2005) observed a bottleneck in only the Garigal National Park population and not the Ku-ring-gai National Park population in his study. Bottlenecked populations show reduced evolutionary potential. The resulting inbreeding and loss of genetic variation, can in turn contribute to lower fitness of a population or species (Frankham 1997). Population bottlenecks are generally caused when there is a significant loss of the population over a short period of time, such as through stochastic environmental events (disease or wildfires). Isolation from conspecifics and decline in population size are typical demographic factors that can also lead to populations becoming bottlenecked, resulting in reduced levels of genetic diversity (Gibbs 2001; Frankham *et al*. 2002).

Additional to the N_e estimate, the mode-shift indicator test showed a normal L-distribution for all populations except southern brown bandicoots in Garigal. The assumption behind the test is that a population under mutation-drift equilibrium is expected to have a larger proportion of alleles with low frequencies (Luikart *et al.* 1998). This lends further support to the lack of a recent bottleneck in the bandicoots of northern Sydney (Shahsavarani and Rahimi-Mianji 2009). However, the result for the southern brown bandicoot in Garigal needs to be interpreted cautiously due to the low number of captures. For important management actions pertaining to the southern brown bandicoot, it may be prudent to provide a higher weighting to Zenger *et al.* (2005), than to the current study. Nevertheless, the low number of captures in this study suggests a reduced population in Garigal compared to Ku-ring-gai.

Zenger *et al.* (2005) also suggested the Ku-ring-gai Chase and Garigal National Park populations were once contiguous, but could not attribute an exact cause for the recent bottleneck observed in Garigal. Geographically and demographically challenged populations have been shown to exhibit reduced genetic diversity, typical of populations having undergone a bottleneck (Garner *et al.* 2005). Reduced genetic diversity levels and a population bottleneck were therefore expected to occur in this study if isolation of habitats is occurring. The Fixation Index (F_{ST}) and D values (Nei's D) for the long-nosed bandicoot revealed no differentiation between the Garigal and Ku-ring-gai populations, indicating that both habitats are part of one population interbreeding freely, and connectivity (although limited) still exists between the National Parks. However, the long-nosed bandicoot

population as a whole (Garigal and Ku-ring-gai) maybe suffering from inbreeding and a restriction of gene flow due to the deficiency of heterozygotes and a high number of homozygotes in the population (F_{IS} values; LNB = 0.102) (Moritz 1997). Gene flow was recorded in pygmy rabbits (*Brachylagus idahoensis*) in North America, across rural two-lane highways that were characterised by low traffic volume and a lack of human development (Estes-zumpf *et al.* 2010). Alternatively, a study on the European fallow deer (*Dama dama dama*) in Tasmania by Webley *et al.* (2007) suggested that the genetic distinction between populations of deer is due to reduced gene flow, resulting from habitat fragmentation (cleared vegetation and the Midlands Highway).

A similar analysis using the F_{ST} and Nei's D was not conducted for the southern brown bandicoot due to the limited captures within Garigal. However, population genetic statistics such as F_{ST} and Nei's D can be compared between species with differing dispersal abilities if these species are otherwise phylogenetically, geographically and demographically comparable (Bohonak 1999). Both species of bandicoot display similar adaptive ecology (Tyndale-biscoe 2005) and their sympatric nature in northern Sydney implies both species have experienced similar environmental and fragmentation pressures. This study rejected the hypothesis that gene flow for both species was restricted due to the fragmented nature of the habitats (divided by a major road) and showed that connectivity between the National Parks exists, albeit limited (absence of signs of bottlenecks (N_e) and long-nosed bandicoot Fixation Index and D values). Therefore, something in addition to limited flow of genetic material and reduced genetic diversity is contributing to the southern brown bandicoots inability to persist in both National Parks. Furthermore, this unidentified factor(s) is contributing to the concern for the species' long-term survival in northern Sydney. However, it's important to note that the presence of gene flow between southern brown bandicoot populations could not be tested directly in this study, due to the low number of samples from Garigal National Park.

This study has provided basic genetic diversity information for the sympatric species of bandicoots in northern Sydney that will guide management of the endangered southern brown bandicoot. The two species exhibited similar levels of heterozygosity, but the southern brown bandicoot displayed a lower allelic diversity. The lower diversity is not unexpected from threatened taxa when compared to non-threatened taxa. Neither species, nor any population had experienced a population bottleneck and therefore were unlikely to have experienced a significant reduction in effective population size. However, a deficiency of heterozygotes in

the northern Sydney long-nosed bandicoot population (combine Ku-ring-gai and Garigal) suggests the potential for inbreeding to be present. Additionally, gene flow between the habitats for long-nosed bandicoot does not seem to be limited by a major road. This can be inferred through analogous adaptive traits to hold for the southern brown bandicoot. A southern brown bandicoot road kill was recorded on Mona Vale Road in 2010, suggesting that bandicoots at least attempt to move between habitats (pers. comm. B. Hope, Technical Officer NPWS, 2011). However, it is likely that not all individuals share the same fate. Zenger *et al.* (2005) observed that the southern brown bandicoot in Garigal has undergone a recent genetic bottleneck. Small population size and restricted gene migration (although not totally restricted, due to possible gene flow shown in this study), resulting in non-random mating and increased homozygotes in the population, is a likely consequence of the bottleneck observed in Zenger *et al.* (2005).

6. Faecal glucocorticoid metabolite concentrations in the free-ranging bandicoots (*Perameles nasuta* and *Isoodon obesulus*) of northern Sydney

6.1 Introduction

The northern suburbs of Sydney are home to the endangered southern brown bandicoot (*Isoodon obesulus*) and the unlisted long-nosed bandicoot (*Perameles nasuta*). Both species have a similar diet, nest in dense vegetation associated with some open woodland and heath-land habitats and are sympatric in northern Sydney, southern NSW, Victoria and Queensland (Heinsohn 1966; Opie *et al.* 1990; Chambers and Dickman 2002; Keiper and Johnson 2004). Both species have the potential to exploit urbanised habitats, through increased opportunities for food, shelter from predators and refuge from bushfires (Gordon and Hulbert 1989). However, only the long-nosed bandicoot readily colonises urban habitats, such as suburban backyards. This may be due to the willingness of long-nosed bandicoots to feed in open environments (Heinsohn 1966; Cockburn 1990; Scott *et al*. 1999; Chambers and Dickman 2002) and the hypothesised need for southern brown bandicoots to shelter and feed within well-vegetated habitats (Heinsohn 1966; Paull 1995).

To effectively manage captive and free-ranging animal populations that experience stressors in their environs, managers first require knowledge of how animals cope with perturbations to their environment. Recently, non-invasive techniques to assess animal welfare such as measuring faecal glucocorticoid metabolites (FGMs) have been favoured in conservation biology over traditional capture and blood sampling (Miller *et al*. 1991; Monfort *et al*. 1998; Wasser *et al.* 2000; Millspaugh *et al*. 2001; Dehnhard *et al*. 2003). Measuring FGMs can be an effective way to represent a physiological response influencing energy allocation, physiological constraints, disturbances and habitat quality on an individual or population level (Romero 2004). Unlike blood hormone levels, FGMs are integrated over a period of time, rather than a single point and may be a more accurate method of assessing the animals' welfare in its environment (Goymann *et al*. 1999; Von der Ohe and Servheen 2002).

Faecal glucocorticoid metabolites have been measured in a variety of eutherian mammals (for an extensive list, see Keay *et al*. 2006, pp. 240) but to a much lesser extent in marsupials

(McKenzie and Deane 2005). A recent study on the Gilberts potoroo (*Potorous gilbertii*) measured faecal cortisol using a radioimmunoassay showing significantly lower levels in captive females compared to wild-caught individuals (Stead-Richardson *et al.* 2010). In the tammar wallaby (*Macropus eugenii*), enzyme immunoassays (EIAs) have been used to measure both serum cortisol (McKenzie *et al.* 2004) and FGMs, with the latter being a more reliable indicator of the stress response than blood cortisol levels (McKenzie and Deane 2005). In *M. eugenii* cortisol is predominant over corticosterone in blood, but an off-the-shelf corticosterone EIA measured FGMs as well as assays specifically tailored to individual glucocorticoid metabolites identified in its faeces (McKenzie 2005). In bandicoots, cortisol has also been recorded as the major glucocorticoid in the blood of one species (see Brownell *et al.* 1967); it is not known if this is true for all bandicoots. Our approach in the present study was to use the same EIA as McKenzie and Deane (2005) to measure the metabolites of the major glucocorticoid hormone excreted in the faeces of *Perameles nasuta* and *Isoodon obesulus*.

It is known that glucocorticoid hormone levels can be affected by sex (McDonald *et al.* 1990; Boswell *et al.* 1994; Touma *et al.* 2004), reproductive status (Touma *et al.* 2004; Gladbach *et al.* 2011), body condition (Husak and Moore 2008; Gladbach *et al.* 2011), season (Millspaugh *et al.* 2001; Washburn and Millspaugh 2002; Huber *et al.* 2003) and anthropogenic influences where physiological responses reflect differences along an urban-rural landscape (Reeder and Kramer 2005; Partecke *et al.* 2006; French *et al.* 2008). Furthermore hormone levels can also reflect fluctuations due to temperature, circadian rhythms and diet (Von der Ohe and Servheen 2002; Reeder and Kramer 2005; Keay *et al.* 2006). The presence of ecto-parasites as a potential environmental stressor and the application of measuring a physiological response from parasite load through FGMs were also considered. Heavy parasite burdens may compromise the hosts' immune system, resulting in a reduced fitness and in turn makes the host more susceptible to diseases, predation and changes in the environment (Scott 1988; Vilcins *et al.* 2005).

Faecal samples were collected from *Isoodon obesulus* and *Perameles nasuta* in northern Sydney to determine whether FGMs can be used to provide physiological information on bandicoots in free-ranging habitats on the urban fringe. It was hypothesised that bandicoots with a higher ecto-parasite load and/or lower body condition would display signs of a weaker immune system, and therefore exhibit higher FGM concentrations. It was also hypothesised

that FGM concentrations would be higher throughout winter due to the reduced food availability and change in dietary preferences to a more protein rich diet.

Male bandicoots were hypothesised to have lower FGM concentrations compared to females. It was thought that female bandicoots would require a higher physiological and immune system demand during an almost year-round breeding season. In addition, it was hypothesised that higher FGMs concentrations would be observed in bandicoots living within the urban fringe compared to the more undisturbed National Parks. The open habitats of suburban backyards were considered to have the potential to increase the risk in obtaining resources. Backyard habitats are more likely to have a general lack of vegetation cover that is required for shelter and nesting, and is also likely to lead to increased predator interactions with domestic pets. It was highlighted in Chapter 3 that bandicoots were just as likely to frequent suburban backyards with pets and forage in these habitats, leaving behind distinctive conical diggings.

6.2 Materials and Methods

6.2.1 Study area

The study area incorporated three broad trapping locations in the greater Sydney region. Sites included Ku-ring-gai Chase National Park (33°39′3.6″S, 151°12′3.6″E), Garigal National Park (33°42′21″S 151°14′11″E) and adjacent suburban backyards. The National Parks of northern Sydney provide one of the last habitats for the southern brown bandicoot in New South Wales. Ku-ring-gai Chase National Park is bordered by urban properties only in the south. However, Garigal National Park is almost entirely bordered by urban properties. National Park populations are likely to only have minimal direct interactions with humans and urban developments, while the suburban backyard populations will have frequent encounters with domestic pets (cats and dogs) and humans.

6.2.2 Sample collection

For a full description of the live-trapping methodology and sample collection, refer to Chapter 2. Bandicoots were captured in a wire cage trap (30 cm x 30 cm x 60 cm) during the period of March 2005 to September 2007, incorporating 4336 trapping nights. Upon capture, bandicoots were placed into a fleece lined pillowcase to be identified and if first capture, bandicoots were micro-chipped and an ear biopsy taken. Bandicoot pellets deposited on the bottom of the traps overnight were collected for analyses. Other measurements of the individuals' weight, right hind leg, sex, reproductive status, ecto-parasite load and body condition were recorded, to collect general information from the bandicoot populations.

Ecto-parasites were collected from each individual by inspecting the head, ears, rump and underside for up to 5 mins in total. Bandicoots that were heavily loaded with ecto-parasites did not have all parasites collected due to time constraints. This Chapter did not seek to identify ecto-parasites to species or examine their prevalence but categorised parasites into ticks, mites and fleas. Individuals were assigned an ecto-parasite load based on the amount and composition of collected parasites. The body condition of each individual was assessed with a method previously used for western barred bandicoots (*Perameles bougainville*) by calculating the cube root of weight divided by the pes (right-hind foot) length (Short *et al.* 1998). The season of bandicoot capture was attributed to each individual according to the

month of capture; autumn (March-May), winter (June-August), spring (September-October) and summer (November-February).

6.2.3 Faecal sample preparation

Faecal samples that were collected from the bottom of the cage traps were placed into plastic vials to be frozen at -20 °C until extraction and analysis. Frozen samples were thawed and then placed in a lyophilizer (Christ Alpha 1-2, Germany) for 24 hours. Dried samples were ground to a powder, weighed and 0.2-0.5 g placed in 5 ml of 80 % methanol. Samples were vortexed, shaken for an hour then centrifuged (Eppendorf 5810R, Germany) at 2500 g for 15 mins. The supernatant was transferred into a new tube and used in the assay.

6.2.4 Faecal glucocorticoid assay

All faecal extracts were diluted 1:10 before being assayed. We used the Correlate-EIA Corticosterone Enzyme Immunoassay Kit (Assay Designs, Ann Arbor, Michigan, USA) to quantify faecal glucocorticoid metabolites. Manufacturer instructions were followed and then absorbance measured at 405 nm on a Multiskan microplate reader with Genesis microplate analysis software (Labsystems / Life Sciences International (UK) Ltd; now Thermo Fisher Scientific, Waltham, Massachusetts, USA). The corticosterone EIA uses a specific sheep anti-corticosterone antibody, and as detailed by the manufacturer, has a sensitivity of 26.99 pg/ml and the following cross-reactivities: corticosterone 100%, deoxycorticosterone 28.6%, progesterone 1.7%, testosterone 0.13%, tetrahydrocorticosterone 0.28%, aldosterone 0.18%, cortisol 0.046%, pregnenolone < 0.03%, β-estradiol < 0.03%, cortisone < 0.03%, 11-dehydrocorticosterone acetate < 0.03%.

Two faecal extracts (one per species) were selected for serial dilution and compared to serially diluted standards. The slopes of the displacement curves of the diluted extracts were compared to that of the serially diluted standards, to evaluate parallelism to the standard curve. Intra-assay variations were calculated from triplicate samples in each assay, and inter-assay variations were calculated from samples assayed across three separate assay plates.

The concentrations of FGMs are expressed as nanograms (ng) per gram (g) dry weight of faecal material.

6.2.5 Data Analysis

Data was analysed using SPSS 19.0 for Windows. Students' t-tests were used to demonstrate parallelism to standard curves and validate the EIA for use with long-nosed bandicoot and southern brown bandicoot faecal material. Log transformations of FGM concentrations were performed prior to all other analyses to ensure normality. Where applicable, analyses were conducted for both species of bandicoot, however limited captures of the southern brown bandicoot precluded analyses in some cases. For analysis, bandicoot habitat was divided into Ku-ring-gai Chase National Park, Garigal National Park and suburban backyards. Reproductive status was divided into: male, females without pouch young, females with pouch young and females with used teats. Ecto-parasite load was categorized as: none present; few (one type, i.e. only ticks or mites or fleas); moderate (numerous of one type or few of two types); and many (numerous of two or more types).

The effect of recapture on FGM concentrations was investigated using paired samples t-tests between bandicoots from consecutive trapping nights. ANOVAs were used to conduct the statistical analyses on the data set for each species using independent data points (i.e. excluding data points from consecutive trapping nights). To determine whether female or male bandicoots had higher FGM levels, a one-way ANOVA was conducted for each species using the demographic variable of sex. To test whether the three types of free-ranging habitats (Ku-ring-gai Chase National Park, Garigal National Park and suburban backyards) influenced FGM levels in long-nosed bandicoots, a two-way ANOVA was conducted with the factors of sex and habitat. To test the demographic variables and the effect of season and reproductive status on long-nosed bandicoot FGM levels, a two-way ANOVA was used. To test whether high parasite load resulted in higher FGM concentrations in free-ranging southern brown and long-nosed bandicoots, a one-way ANOVAs was conducted with the factors of parasite load and sex. A simple regression analysis was run for each species to determine whether there was a relationship between body condition and FGM concentrations of southern brown and long-nosed bandicoots. Significance was accepted at the $p < 0.05$ level and means are given with standard errors (SE), including in figures, unless otherwise stated. The break down of variables and sample numbers used for analyses are shown in Table 6-1. Untransformed data are used for all figures for ease of interpretation.

Table 6-1: Break down of variables and sample numbers used for analyses.

Variable	Coded	LNB samples	SBB samples*
Samples	Recaptures and non-recaptures	88	52
Species	LNB and SBB	64	29
Sex	Female	26	17
	Male	38	12
Reproductive status	Male	26	17
	Female without pouch young	17	3
	Female with pouch young	18	5
	Female with used teats	3	4
Season	Spring	27	14
	Summer	11	0
	Autumn	14	11
	Winter	12	4
Ecto-parasite load	None present	22	9
	Few	13	10
	Moderate	20	2
	Many	6	5
Body condition	Linear regression analysis	61	26
Habitat	Ku-ring-gai Chase National	30	0
	Garigal National Park	16	0
	Suburban backyards	18	0

* Analyses for the southern brown bandicoot did not include the variables reproductive status, season and body condition, due to low sample numbers or incomplete data.

6.3 Results

In total, 140 faecal samples from 93 bandicoots were collected across northern Sydney in undisturbed (National Park) and peri-urban (backyard) habitats between March 2005 and September 2007 from the free-ranging populations in Ku-ring-gai Chase and Garigal National Parks and suburban backyards. Ku-ring-gai Chase National Park was the only habitat were southern brown bandicoots were recorded and provided almost double the faecal samples from both Garigal National Park and suburban backyards (Table 6-1). Long-nosed bandicoots accounted for 64 individuals (26 male and 38 female) and southern brown bandicoots accounted for 29 individuals (17 male and 12 female). Approximately 43% of faecal samples were collected from bandicoots caught for the first time.

The corticosterone standard curve was parallel to the displacement curve from the long-nosed bandicoot and southern brown bandicoot faecal extract serial dilutions (Figure 6-1; LNB Student's $t = 1.04$, $p = 0.353$; SBB Student's $t = -1.85$, $p = 0.471$). Intra-assay variation was 10%, while inter-assay variation was 15%.
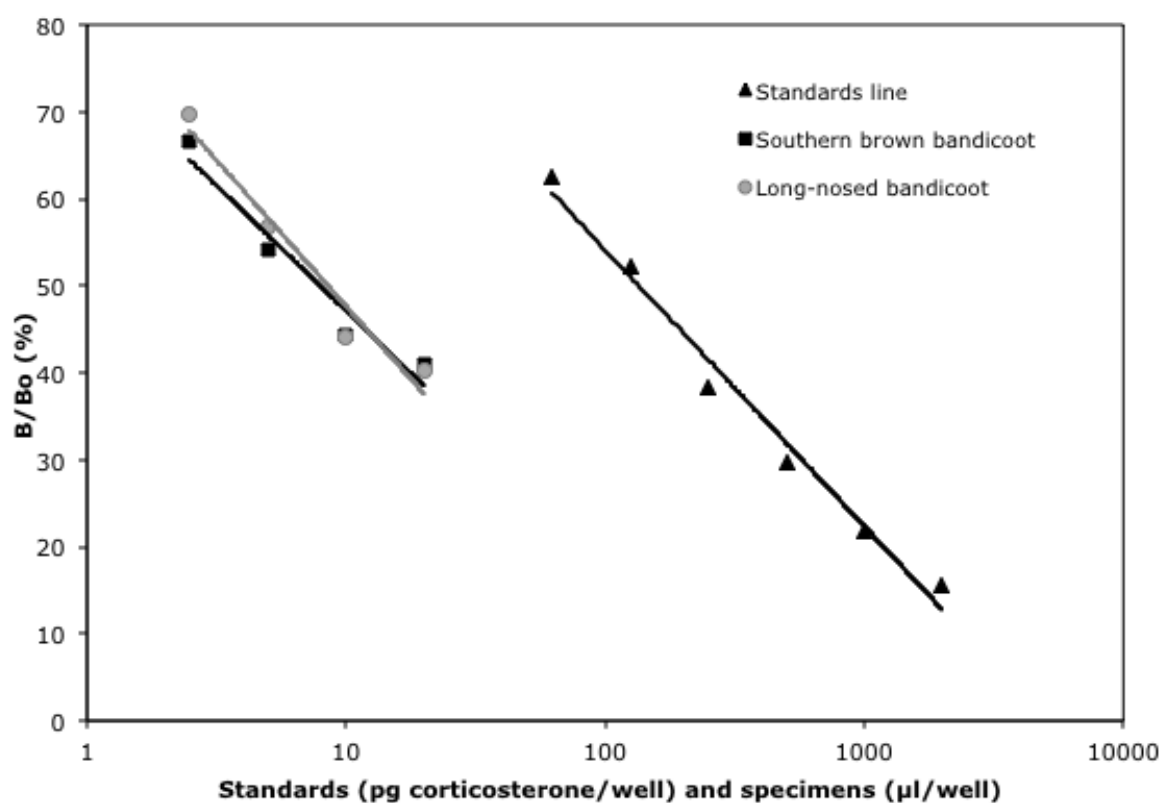
Figure 6-1: B/Bo (%) of the serial dilutions from the long-nosed bandicoot (LNB) and southern brown bandicoot (SBB) and the corticosterone standards to demonstrate parallelism (LNB: Students $t = 1.04$, $p = 0.353$; SBB: Students $t = -1.85$, $p > 0.471$). B represents the amount of binding of antibody in presence of a competitor, and Bo that bound in the absence of a competitor.

There was no significant difference in FGM concentrations detected from bandicoots caught on a second or third consecutive trapping night (t_{12} = -0.523, p = 0.610; t_6 = -0.044, p = 0.967).

FGM concentrations varied between the sexes of the southern brown bandicoot but did not differ between the sexes of the long-nosed bandicoots (Figure 6-2). Female southern brown bandicoots demonstrated higher FGM levels than male southern brown bandicoots within Ku-ring-gai Chase National Park ($F_{1,27}$ = 4.86, p = 0.036). Our analyses did not reveal any differences in FGM concentrations related to the reproductive status of the female long-nosed bandicoots ($F_{3,52}$ = 1.64, p = 0.191). Nor was there a difference in FGM concentrations detected due to the season from which long-nosed bandicoot faecal samples were collected ($F_{3,52}$ = 1.801, p = 0.158).

A comparison between the FGMs of long-nosed bandicoot populations from Ku-ring-gai Chase and Garigal National Parks (undisturbed habitat) and suburban backyards (peri-urban habitat) did not reveal any overall difference (Figure 6-3; $F_{2,58}$ = 1.35, p = 0.267). No southern brown bandicoots were captured in Garigal National Park or suburban backyards, and thus were precluded from the habitat analysis.

The body condition index of an individual was not significantly associated with FGM concentration in either species of bandicoot (LNB $F_{1,59}$ = 0.146, p = 0.70; SBB $F_{1,24}$ = 2.48, p = 0.128). The ecto-parasite load of long-nosed bandicoots did not influence FGM levels with no significant differences detected between bandicoots with none present, few, moderate or high numbers of ecto-parasites (LNB $F_{3,89}$ = 1.070, p = 0.366; SBB $F_{3,25}$ = 1.913, p = 0.153).
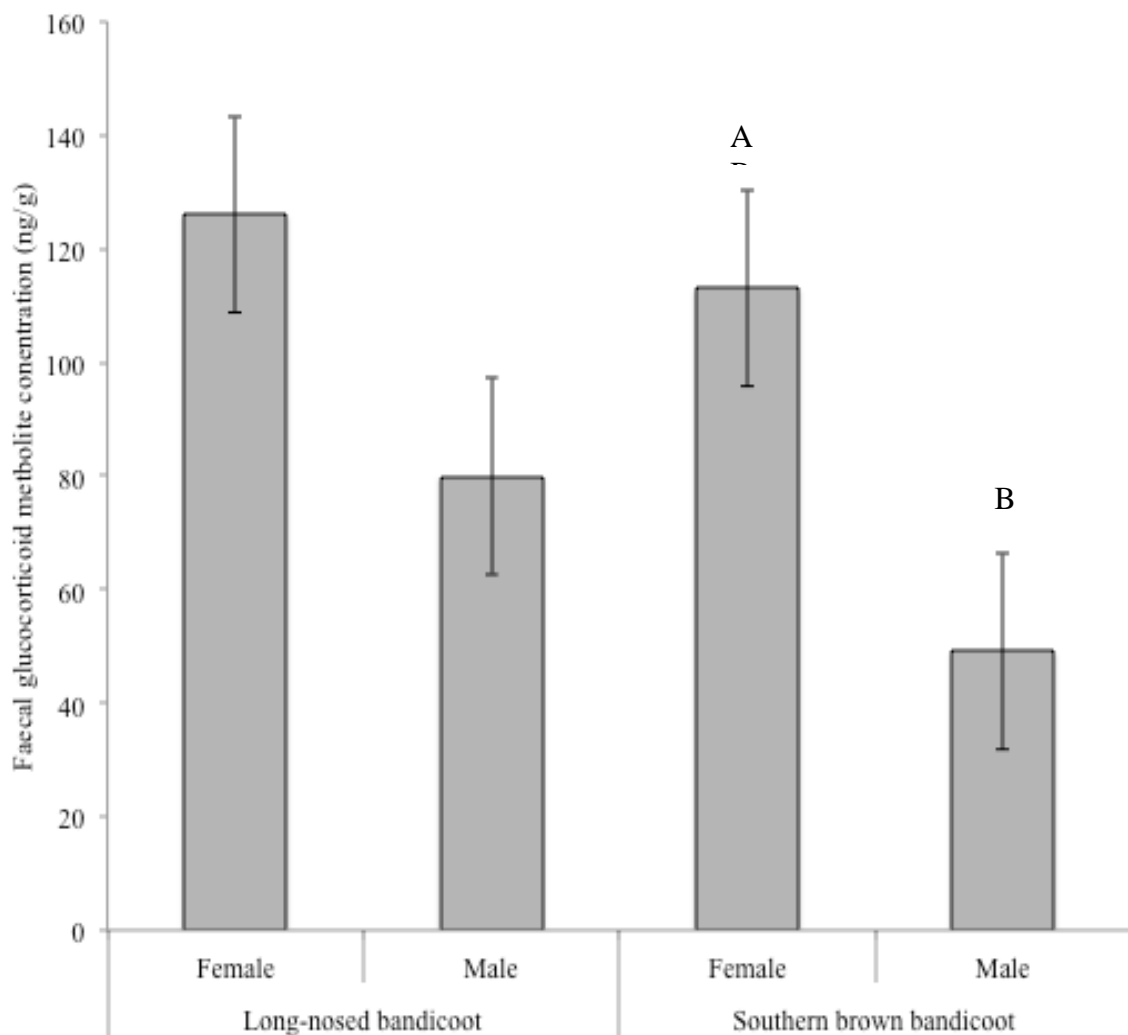
Figure 6-2: Mean (± SE) FGMs for female and male long-nosed bandicoots and southern brown bandicoots. Different letters denote significantly different results ($p = 0.036$).
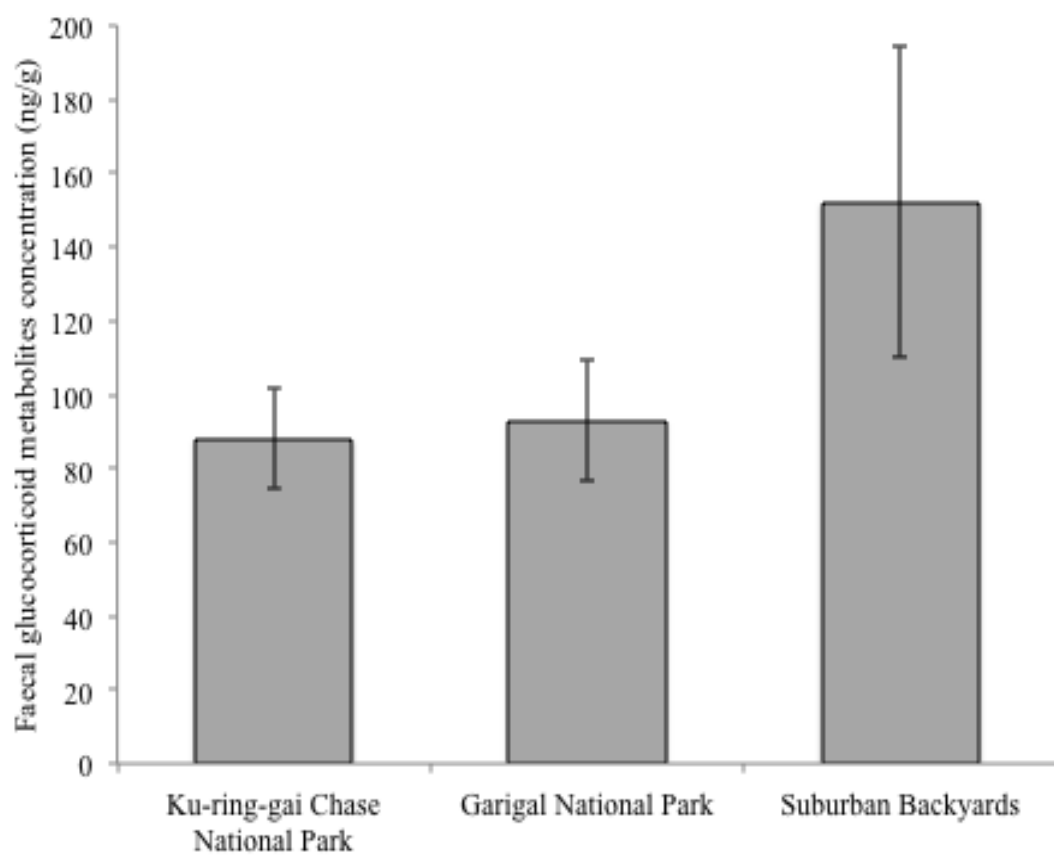
Figure 6-3: Mean (± SE) long-nosed bandicoot FGMs for the free-ranging habitats of northern Sydney. No significant differences in FGMs were detected between habitats.

6.4 Discussion

This study demonstrated that faecal glucocorticoid metabolites could be measured in two species of free-ranging bandicoots using a simple enzyme immunoassay. This is the first study to compare faecal glucocorticoid metabolites between populations of free-ranging bandicoots. It is important to note that this study aimed to conduct a full range of FGM analyses for both species. However southern brown bandicoot captures were limited to Ku-ring-gai Chase National Park preventing some analyses, such as a habitat comparison. Additionally, conclusions concerning the responses to stressors of bandicoots in northern Sydney need to be considered in the context of the absence of a biochemical (e.g. ACTH) challenge.

In a positive result for those undertaking bandicoot monitoring, the study suggests that overnight trapping of bandicoots in cage traps does not have a prolonged impact on FGM concentrations in individuals. Although capture and restraint have been recorded as elevating plasma glucocorticoid levels within several minutes in a variety of mammalian species (Harper and Austad 2001), in some species (e.g. deer mice *Peromyscus maniculatus*), the effect of capture was not enough to disguise natural adrenocortical activity (Harper and Austad 2001). If time spent in a trap on consecutive nights and the subsequent handling upon release causes a significantly increased adrenal response in bandicoots, it occurs over a shorter time period than could be recorded in our study.

In a study using red deer (*Cervus elaphus*), seasonal variations of environmental conditions and food availability in winter were shown to increase FGMs (Huber *et al*. 2003). A similar response from long-nosed bandicoots was not seen in this study, despite a known shift in dietary preference to an insect dominant diet during winter when availability of vegetation is low (Quin 1985). Romero (2002) suggested there are indications that free-living species modulate glucocorticoid levels according to season. In fact, almost all studies measuring baseline levels of glucocorticoids in free-living mammals found a seasonal variation with elevations corresponding to the species' breeding cycle (Romero 2002). This study appears to be in the minority, with little variation in FGMs across season. One possible explanation is the seasonal fluctuations in northern Sydney were not sufficient to impart a response from the adrenal cortex. Alternatively, the adreno-cortical response in Sydney bandicoots is driven strongly by the energy requirements of year-round breeding.

A sex difference in glucocorticoid concentrations is not uncommon when measuring levels in both plasma and faeces. This has been demonstrated in golden-mantled ground squirrels (*Spermophilus lateralis*; Boswell *et al*. 1994), mice (*Mus musculus*; Touma *et al*. 2004) and koalas (*Phascolarctos cinereus*; McDonald *et al*. 1990). These studies suggest that the difference reflects the seasonal reproductive cycle of females. The female southern brown bandicoots of northern Sydney showed higher FGMs than males. Bandicoots have the ability to breed almost year-round, with females weaning one litter whilst pregnant with another (Cockburn 1990; Department of Conservation and Climate Change 2006). This continuous breeding cycle may require higher energy utilisation for females throughout the year, leading to a growing need to forage, increasing competition and predation risk, which in turn increases the adrenal hormone response. However, it is important to note that where the breeding cycle is continuous and higher energy utilisation is required throughout the year, a response in glucocorticoid levels to reflect the timing of breeding may be negated, as may have occurred in female long-nosed bandicoots. Alternatively, a difference in FGMs between the sexes may be a direct result of sex-related hormones such as testosterone, oestrogen and progesterone (Von der Ohe and Servheen 2002; Reeder and Kramer 2005; Husak and Moore 2008).

Another potential influence on the adrenal response is the body condition of an animal, particularly its fat reserves. The adrenal response mediates energy utilisation, mobilisation of fat stores and the redirection of behaviour (Wingfield and Sapolsky 2003; Partecke *et al*. 2006). An animal in good condition should have higher energy stores and lower FGMs compared to an individual in low condition (Gladbach *et al.* 2011). However, our study demonstrated no FGM variation in either species of bandicoot, despite body condition being shown to influence the physiological response to stressors in other studies using American kestrels (*Falco sparverius*) (Sockman and Schwabl 2001) and wild Upland geese (*Chloephaga picta leucoptera*) (Gladbach *et al.* 2011). Body condition may also be directly related to reproductive traits, available resources, nutrition, environmental conditions and reflect an individual's ability to cope with environmental pressures (Jakob *et al*. 1996; Schulte-Hostedde *et al*. 2001). The high majority of bandicoots captured were considered to be in a moderate to good condition, suggesting the differences in body condition between individuals might not have been significant enough to see a relationship through FGM concentrations.

It was expected that high parasite loads would be linked to increased hypothalamic-pituitary-adrenal (HPA) axis activity, since heavy parasite burdens can be caused by stressors such as changed environmental conditions, increased predation or competition (Wikel 1999; Weaver and Aberton 2004; Vilcins *et al.* 2005). It is possible that the parasitic burdens of the majority of captured bandicoots may have been insufficient to activate the HPA-axis and increase FGMs, despite some individuals being heavily loaded with ticks, mites and fleas. The ecto-parasites recorded on the bandicoot of northern Sydney were demonstrating natural host-parasite relationships. This was particularly true for *I. holocyclus* (paralysis tick), which is common in the outer expanses of Sydney and accounted for the highest parasite loads observed (Chapter 8). Natural hosts to a parasite are generally unaffected by the parasite toxin, even with some individuals with heavy infestations (Campbell *et al.* 2003; Jackson *et al.* 2007). Thus, the parasite loads on the bandicoots of northern Sydney may not have be sufficient to impart a discernable FGM response.

Long-nosed bandicoots frequently venture from the constant cover favoured by southern browns to feed in open habitats such as suburban backyards, including those containing domestic pets (Cockburn 1990; Scott *et al.* 1999; Dowle and Deane 2009). This was hypothesised to result in higher FGM concentrations due to increased interactions with humans and predators. This study showed no variances in FGMs despite the increased risks of obtaining resources (such as increased vigilance for predator detection), suggesting the use of suburban backyards by long-nosed bandicoots may not be a major stressor to this species. However, more detailed studies or laboratory experiments are recommended. Changes in FGM concentrations could be used to test whether increased vigilance due to increased predation risk and a willingness to feed in open habitats leads to an increased adrenocortical response. Specifically, the faecal glucocorticoid levels and vigilance behaviour of captive bandicoots could be measured and compared between open and closed feeding environments, enclosures with differing food availability, differing density levels of conspecifics, and high or low predator threats. A similar study could also be used to test whether southern brown bandicoots behave differently to long-nosed bandicoots in an open environment, and whether these behavioural disparities (e.g. more vigilant) lead to differences in adrenocortical response and habitat use.

It is possible that a stress response in bandicoots may be detected via measurement of plasma cortisol concentrations where FGM measurement has failed to detect such a response.

However, numerous studies have indicated activation of the HPA-axis can be highly context-dependent (Wingfield and Sapolsky 2003). A study on European blackbirds (*Turdus merula*) (Partecke *et al*. 2006) showed a lower stress response for individuals born in an urban habitat (city) than their forest con-specifics, suggesting a mechanism necessary to cope with frequent human disturbances. Similarly, a study on an urban tree lizard (*Urosaurus ornatus*) (French *et al*. 2008) demonstrated a possible suppression of overall corticosterone levels as a result of frequent exposure to stressors compared to rural lizards. However, if physiological responses between undisturbed and peri-urban populations exist in other habitats, it is likely to be species specific and dependent on geographic location.

A number of animals have adapted to Australian urban and peri-urban environments including possums (*Trichosurus vulpecula*), koalas (*Phascolarctos cinereus*), pythons (*Morelia spilota*), and bandicoots (*Perameles nasuta*), increasing the potential for human-to-animal and animal-to-animal conflicts. Associated research has generally focused on managing human activities and favourable habitats to control and monitor populations without assessing the physiological response to the stressor. This study has shown that responses of bandicoots in free-ranging habitats may be assessed and monitored through FGM analysis. However, given the current paucity of data in the literature on the activation of the HPA axis in bandicoots, strong conclusions regarding the stressors of bandicoots in northern Sydney cannot as yet be drawn. Follow-up studies with increased sample numbers over a longer time-course, complete assay validations for both species, and a biochemical (e.g., ACTH) challenge should be conducted to verify study findings and further the understanding of the influence from environmental variables. In addition, a series of experiments on captive bandicoots as proposed above would significantly improve the understanding of the relationships between behavioural ecology and the adrenocortical response in these species. Nevertheless, this non-invasive method of monitoring bandicoot welfare appears to be an effective way of gathering biological information to guide the management of bandicoots across the spectra of wild and urban, endangered and successful populations.

7. Does *Cryptosporidium* exist within *P. nasuta* and *I. obesulus* in a wild and urban habitat in northern Sydney?

7.1 Introduction

Cryptosporidium, a protozoan parasite of vertebrates, occurs predominantly in the epithelial cells of the intestine (O'Donoghue 1995; Fayer 2004). Over the past decade molecular tools have identified a significant amount of biodiversity within the genus, leading to identification of a growing number of descriptions of novel and cryptic species and genotypes (Fayer *et al.* 2010; Morgan *et al.* 2001; Zhou *et al.* 2004) with variable levels of host specificity (Xiao *et al.* 2002). Infection in humans is predominantly caused by two species, the host specific *C. hominis* and the zoonotic *C. parvum*. Other zoonotic species found in humans and domestic animals include *C. meleagridis, C. felis*, *C. canis, C. cuniculus and C. ubiquitum* (Xiao and Feng 2008; Xiao 2010; Chalmers *et al.* 2011). The recent identification of the marsupial-specific *C. fayeri,* in a sporadic human infection (Waldron *et al.* 2010) demonstrates that Australian wildlife play a larger role in zoonotic transmission than previously thought.

Surveillance of *Cryptosporidium* has typically focused on animals of economic or agricultural value (Appelbee *et al.* 2005) and overlooked wildlife populations. Studies of *Cryptosporidium* in Australia indicate that wildlife populations inhabiting urban areas (Hill *et al.* 2008) and drinking water catchments (Power *et al.* 2004; Power *et al.* 2005) are hosts for *C. fayeri* (Ryan *et al.* 2008), *C. macropodum* (Power and Ryan 2008) and other marsupial-specific species and genotypes (Hill *et al.* 2008, Yang *et al.* 2011). Interactions between humans and wildlife are increasing with urban expansion, presenting increased risks of parasite emergence and transmission between humans and wildlife species (Bradley and Altizer 2007). As the human-wildlife interface expands it is critical to identify pathways of transmission between hosts that share human-altered environments. Parasites may also spread from humans into wildlife species (Nizeyi *et al.* 2002) therefore monitoring the health of wildlife, particularly threatened species, is necessary for safeguarding biodiversity.

The endangered southern brown bandicoot (*Isoodon obesulus*) and the unlisted long-nosed bandicoot (*Perameles nasuta*) are among the few marsupials that inhabit urban Australia.

Natural infections of *Cryptosporidium* species and genotypes have been documented in 16 species of marsupials (Fayer 2010; Power 2010), including the southern brown bandicoot (*Isoodon obesulus*) (cf. O'Donoghue 1995). However current descriptions of *Cryptosporidium* from bandicoots are based on oocyst morphology alone.

This study aimed to determine the prevalence and identity of *Cryptosporidium* in bandicoots in urban Sydney, and compare infection patterns between the two sympatric bandicoot species. Furthermore, the study takes advantage of molecular tools to investigate the phylogenetic relationships of *Cryptosporidium* isolated from bandicoots. It was hypothesised that the *Cryptosporidium* parasite would be identified within the long-nosed and southern brown bandicoots of northern Sydney, with a marsupial-specific species or genotype the predominant *Cryptosporidium* parasite observed. It was also hypothesised that due to interactions with domestic pets and humans on the urban interface, bandicoots in suburban backyards would be host to more than one species of the *Cryptosporidium* parasite. Species could include those of low host specificity and/or zoonotic potential, such as *C. parvum, C. hominis, C. felis* or *C. canis*.

7.2 Methods

7.2.1 Sample collection

The study area incorporated locations in Sydney including sites in Ku-ring-gai Chase National Park, Garigal National Park and nearby suburban backyards. For a full description of the study area and live-trapping methodology, refer to Chapter 2. Bandicoots were captured in a wire cage trap (30 cm x 30 cm x 60 cm) and pellets deposited on the bottom of the traps overnight were collected for analyses. Samples were also collected from captive bandicoots housed at Taronga Zoo. Pellets from Taronga Zoo were collected from enclosures of five bandicoots by Zoo staff each morning for eight days in August 2006. All faecal samples were collected into a plastic vial and stored at -20°C until analysis.

7.2.2 Purification and DNA extraction of oocysts

Cryptosporidium oocysts were concentrated from pellets using an immunomagnetic separation (IMS) technique designed for sensitive detection of *Cryptosporidium* in faecal samples (Power *et al*. 2003). In brief, a slurry containing the equivalent of 1 g of faecal material was mixed with paramagnetic beads conjugated with Cry104 coated beads (Weir *et al*. 2000) a monoclonal antibody that targets the *Cryptosporidium* oocyst wall (BTF Australia, Sydney, Australia). The resulting bead-oocyst complex was separated from the faecal debris by magnetic concentration, followed by re-suspension in sterile water (88 µl).

DNA extraction was performed on the bead-oocyst complex using DNA extraction prepGEM™ tissue kits (Zygem, Hamilton, New Zealand). Zygem Buffer 3 (10 µl) was added to the bead-oocyst complex then snap frozen at -80°C for 15 min. The samples were thawed, vortexed, spun, then 1 µl lysozyme (5 mg/ml) and 1 µl Prepgem enzyme was added (Ferrari *et al*. 2000). Samples were incubated at 37°C, 75°C and 95°C for 15 min each in a thermocycler (Perkin Elmer), then spun at 5000 rpm for 3 min. The supernatant containing DNA was transferred to a new tube and 2 µl Tris EDTA (1mM) was added. DNA samples were stored at minus 20°C until PCR screening.

7.2.3 Amplification at *18S rRNA* Locus

DNA samples were amplified at the *18S rRNA* locus using a nested PCR protocol. The primers *18SCF2* 5'-GACATATCATTCAAGTTTCTGACC-3' and *18SCR2* 5'-CTGAAGGAGTAAGGAACAACC-3' (Ryan *et al*. 2003) were used to generate ~760 bp product, which formed the template for secondary amplification. The primers *18SIF* 5'-AGTGACAAGAAATAACAA TACAGG-3' and *18SIR* 5'-CCTGCTTTAAGCACTCAATTTTC-3' (Morgan *et al*. 1997) were used to amplify ~ 310 bp product (pending species). Amplification during primary PCR was enhanced using 1 µl DNA template in 4 µl of GeneReleaser® (Integrated Sciences, Australia) heated for 7 min at 500 W in a microwave. Primary reaction mixtures (25uL) contained 1 x PCR buffer, 4 mM MgCl$_2$, 200 µM each of dNTPs, 20pM of each forward and reverse primer, 1 U of Taq Tth Plus Polymerase® (Fisher Biotech, Australia) and were added to gene releaser-DNA mix after microwaving. Secondary reactions were identical to the primary reactions, however *18SIF* & *18SIR* primers were used and 1 µl of primary reaction mixture was added as the DNA template. Conditions for both reactions comprised an initial denaturation step at 94°C for 2 min, 58°C for 60 sec, 72°C for 2 min, followed by 48 cycles of 94°C for 40 sec, 58°C for 30 sec, 72°C for 45 sec and a final extension of 72°C for 7 min. The PCR products were visualized by agarose gel electrophoresis using 2% Buffer TBE and SYBR® (Invitrogen, Australia) Safe DNA gel stain (2 µl).

All PCR's included either *C. parvum* and/or *C. fayeri* as a control sample. Samples positive at the *18S rRNA* locus were tested against the *actin* and *gp60* loci with a nested PCR protocol following Sulaiman *et al*. (2002) and Power *et al*. (2009) respectively.

7.2.4 Amplification at *actin* and *gp60* Loci

The actin primary reaction primers, *actinF1* 5'-ATG(A/G)G(A/T)GAAGAAG(A/T)A(A/G)(C/T)(A/T)CAAGC-3' and *actinR1* 5'-AGAA(G/A)CA(C/T)TTTCTGTG(T/G)ACAAT-3' were used to produce a 1,095-bp product and the *actin* reaction primers, a*ctinF2* 5'-CAAGC(A/T)TT(G/A)GTTGTTGA(T/C)AA-3' and *actinR2* 5'-TTTCTGTG(T/G)ACAAT(A/T)(G/C)(A/T)TGG-3' were used to produce a 1,066-bp product (Sulaiman *et al*. 2002). The primary *actin* PCR mixture contained 2 µl DNA volume, Platinum® PCR SuperMix (45 µl) (Invitrogen, Victoria, Australia) and 20 pM (1 µl)

of each forward and reverse primer in a final volume of 50 µl. Conditions for the primary reaction comprised an initial denaturation 94°C for 5 min followed by 35 cycles of 94°C for 45 sec, 50°C for 45 sec, 72°C for 60 sec and a final extension of 72°C for 10 min. The secondary reaction mixture was identical to the primary mixture, except primers *actinF2* and *actinR2* were used and 5 µl of the primary reaction mixture formed the DNA template in a final volume of 50 µl. Conditions for secondary reaction comprised an initial step at 94°C for 3 min followed by 35 cycles of 94°C for 45 sec, 45°C for 45 sec, 72°C for 60 sec and a final extension of 72°C for 7 min.

Amplification for *Cryptosporidium* isolates at the *gp60* locus were performed using the primary reaction primers, *out*F5'-CCACACATCTGTAGCGTCGTCA-3' and *mar4* 5'-CAGTCGTCTTAATTCCACGGT-3' and the secondary reaction primers, *atg*F5'-ATGAGATTGTCGCTCATTATCG-3' and *mar3* 5'-CGTCAGAACATTCTGGAAGCT-3' (Power *et al.* 2009). The primary *gp60* PCR mixture contained 2 µl DNA volume, 4 mM MgCl$_2$, 200 µM each of dNTPs, 20pM of each forward and reverse primer, 1 U of Red Taq$^®$ Polymerase (Integrated Sciences, Sydney, Australia) in a final volume of 25 µl. Conditions for the primary reaction comprised an initial denaturation 94°C for 3 min followed by 35 cycles of 94°C for 45 sec, 58°C for 45 sec, 72°C for 90 sec and a final extension of 72°C for 7 min. The secondary *gp60* reaction mixture used the secondary primers in an identical mixture to the primary reaction, except 1 µl of the primary reaction mixture was used as the DNA template in a final volume of 50 µl. The PCR products of the *actin* and *gp60* loci were visualised as described above.

7.2.5   Enumeration of oocysts using flow cytometry

Faecal samples positive for *Cryptosporidium* at the *18S rRNA* locus were processed using IMS and flow cytometry (IMS-FC) to determine the number of oocysts shed. IMS protocols followed Power *et al.* (2003), however the bead-oocyst complex was suspended in 100 µl sterile H$_2$0 and stored at 4°C. Prior to FC analysis, 100 µl antibody dissociation buffer (ADB – PBS pH 7.2 containing bovine serum antibody (2%) and mouse serum (10%)) was added to the suspended bead-oocyst complex. Samples were sorted using a FACSCalibur flow cytometer (Becton Dickinson Biosciences, Australia) equipped with a SortStage attachment (MRL, Australia) that facilitated cell sorting onto Isopore^{TM} membranes (13mm, 0.8 µM;

Millipore, Australia) to separate oocysts from beads. Membranes were stained with CRY104-FITC (100 µl; 10µg/ml) for 3 min and washed with 500µl monoclonal antibody buffer (MABB - 50mM tetra sodium pyrophosphate, 0.5% bovine serum albumin and 0.05% Tween-80 at pH 8.0). Oocysts were identified by their bright green fluorescence, spherical shape and size (4-6 µM) and counted using an epifluoresence Nikon BH2 microscope.

7.2.6 Sequencing and phylogenetic analysis

Amplicons were purified for sequence analyses using the QIAquick PCR purification kit (Qiagen, Victoria, Australia) according to the manufacturer's instructions. Automatic sequencing was performed in forward and reverse directions using a 3130xl DNA capillary sequencer (Applied Biosystems, Foster City California). Sequences were assembled into optimised contigs using Geneious Bioinformatics software (version 5.4.6, Biomatters Ltd, New Zealand). Contigs were compared to existing *Cryptosporidium 18S rRNA* sequences in GeneBank using Megablast search to determine likely species match (Appendix C). Bandicoot sequences were aligned with 30 described *Cryptosporidium* species and genotypes using Clustal W (Larkin et al., 2007) with default settings. A best-fit nucleotide substitution model was selected using MrModeltest 2.3 (Nylander, J.A.A. 2004. MrModeltest v2. Program distributed by author. Evolutionary Biology Centre, Uppsala University). The model with the lowest Akaike Information Criterion corrected score was the Tamura 3-parameter model with a discrete gamma distribution. Phylogenetic trees were generated with a Bayesian Markov chain Monte Carlo (MCMC) coalescent approach implemented in BEAST 1.6.2. (Drummond and Rambaut 2007). An optimal chain length of 10 million generations and sampling frequency of 1,000 was used to achieve an Effective Sample Size >200 in Tracer 1.5 (Rambaut and Drummond 2007). Consensus trees with a 10% burn-in value were generated using TreeAnnotator 1.6.2. and visualized in FigTree 1.3.1. All trees were rooted with *Plasmodium falciparum* (M19172) as the evolutionary outgroup.

7.2.7 Statistical Analysis

An ANOVA was used to conduct the statistical analyses on the data for each species. To test whether infection of *Cryptosporidium* significantly reduced the body condition of bandicoots, a two-way ANOVA was run with the dependent variable 'body condition' (values from Chapter 4), with the factors 'infection status' (positive or negative) and sex (male and female). Significance was accepted at the $p < 0.05$ level.

7.3 Results

7.3.1 Prevalence of *Cryptosporidium* in bandicoot populations.

A total of 98 faecal samples were obtained from bandicoot trappings in northern Sydney (n = 93) and at Taronga zoo (n = 5). Twelve C*ryptosporidium* positive faecal samples (Table 7-1) were identified using *18S rRNA* PCR, representing a prevalence of 10.8% (10/93) among free-ranging bandicoots (both species) of northern Sydney and 40% (2/5) among captive long-nosed bandicoots at Taronga Zoo. Of the 12 positive samples, nine were from long-nosed bandicoots (LNB) and three were from southern brown bandicoots (SBB) (Table 7-1). All SBB positives were from Ku-ring-gai Chase National Park representing a prevalence of 16.7% (3/18) for this species. For the LNB, two positives (40% prevalence) were from the Taronga Zoo population and seven (9.3% prevalence) were from the free-ranging LNB population of northern Sydney (Table 7-1).

Table 7-1: Faecal samples collected and prevalence of *Cryptosporidium* in bandicoots of northern Sydney, expressed as a percentage of individuals recorded as positive.

	# Samples (# Individuals)		Prevalence (# Positive)		Mean prevalence (# Positive)
	SBB	LNB	SBB	LNB	
Ku-ring-gai Chase National Park	51 (17)	52 (34)	17.6% (3)	14.7% (5)	15.7% (8)
Garigal National Park	1 (1)	19 (17)	0.0%	0.0%	0.0%
Suburban Backyards	0	26 (24)	0.0%	8.3% (2)	8.3% (2)
Total / Average of free-ranging populations	**52 (18)**	**97 (75)**	**16.7% (3)**	**9.3% (7)**	**10.8% (10)**
Taronga Zoo Samples	0	40 (5)	0%	40% (2)	40% (2)

Positive samples were subsequently tested at two additional loci, *actin* and *gp60*, using nested PCR protocols. However, the positive isolates identified at the *18S rRNA* locus could not be amplified at either *actin* and *gp60* loci, despite repeated attempts. Control samples, *C. parvum* and *C. fayeri*, in *actin* and gp60 PCRs amplified as expected.

7.3.2 *Cryptosporidium* species within bandicoot populations

Sequencing was attempted for all *18S rRNA* PCR positive samples but sequences were only obtained from 4 of the 12 samples (Bandicoot samples 6, 29, 206 and 211). Sequencing

results revealed that three of the four successfully sequenced isolates from bandicoots showed highest identity with *C. parvum* (range of 98.3% to 99.0%). The fourth positive isolate showed highest identity with *C. hominis* (96.1%). Inferred phylogenetic relationships based on the partial *18S rRNA* locus loosely grouped bandicoot isolates 29 and 206 into a clade with either *C. parvum* or *C. hominis* respectively. Bandicoot isolates 6 and 211 formed a clade with an unidentified common brushtail possum (*Trichosurus vulpecula*) genotype (Figure 6-1). Posterior probabilities indicated low support for these phylogenetic assignments (<0.60).

Phylogenetic analysis at the *18S rRNA* locus for the positive isolates revealed pairwise distances ranging from 0.008 to 0.035. The greatest pairwise distance occurred between isolates from a southern brown and a long-nosed bandicoot in Ku-ring-gai Chase National Park. The isolate (sample 206) showing highest identify to *C. hominis* had the same pairwise distance from all the other isolates (0.035). The pairwise comparison between the sequenced Taronga Zoo isolate and the Ku-ring-gai National Park isolates ranged from 0.017 to 0.035.
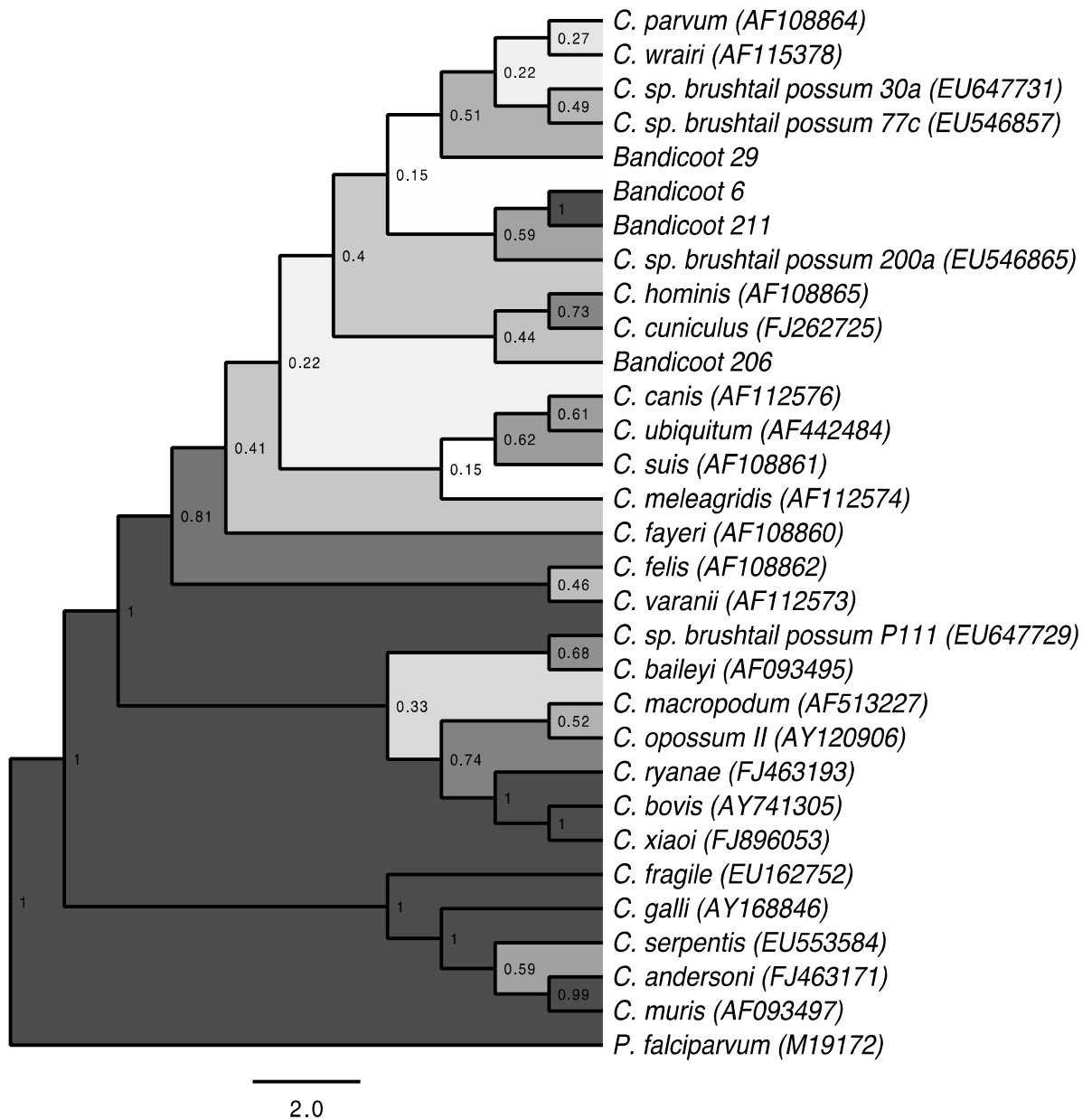
Figure 6-1: Phylogenetic relationships of the bandicoot samples (6, 29, 206, 211) and known *Cryptosporidium* species/genotypes based on a ~300-bp fragment of *18S rRNA*. Phylogenetic trees were generated with a Bayesian Markov chain Monte Carlo (MCMC) coalescent approach implemented in BEAST 1.6.2. Nodes are highlighted according to strength of posterior probability from low support (white) to high support >0.95 (dark grey). Scale bar corresponds to years.

7.3.3 *Counts of oocyst shedding in bandicoots*

Oocysts numbers in PCR positive faecal samples were determined using IMS coupled with flow cytometry. In all bandicoot samples, oocyst counts were very low (≤ 100), ranging from zero to 100-oocysts/g of faecal sample (Table 7-2). Two samples observed after flow cytometry revealed an oocyst count of zero oocysts/g of faecal sample.

Table 7-2: *Cryptosporidium* oocyst/g faeces from the 12 positive samples amplified at the *18S rRNA* locus. SBB = southern brown bandicoot; LNB = long-nosed bandicoot.

Sample ID #	Bandicoot species	Population	Sequenced at *18S rRNA*	Oocysts/g faeces
6	SBB	Ku-ring-gai	Yes	100
8	SBB	Ku-ring-gai	No	40
29	LNB	Taronga	Yes	60
37	LNB	Taronga	No	40
51	SBB	Ku-ring-gai	No	0
82	LNB	Backyards	No	80
86	LNB	Backyards	No	80
206	LNB	Ku-ring-gai	Yes	60
211	LNB	Ku-ring-gai	Yes	20
222	LNB	Ku-ring-gai	No	20
261	LNB	Ku-ring-gai	No	0
270	LNB	Ku-ring-gai	No	20

7.3.4 *Infection versus body condition*

The infection (presence of parasite) of *Cryptosporidium* in individuals did not significantly lower body condition of long-nosed bandicoots ($p > 0.05$). Body condition analysis was not conducted for the southern brown bandicoot, due to the low number of samples observed carrying the *Cryptosporidium* parasite in this species.

7.4 Discussion

This study revealed that free-ranging bandicoots of northern Sydney were shedding low levels of *Cryptosporidium* oocysts, expanding the known host range of this parasite in urban environments and supporting the study hypothesis. *Cryptosporidium* prevalence in the combined species of free-ranging bandicoots was estimated at 10.8%. Despite the small sample size, this prevalence appeared to be similar to other marsupials from greater metropolitan Sydney and other wild populations (Power *et al.* 2005; Hill *et al.* 2008; Yang *et al.* 2011). Power *et al.* (2005) recorded 6.7% prevalence in eastern grey kangaroos (*Macropus giganteus*) in Sydney's watershed; Hill et al. (2008) observed a prevalence of 5.6% and 11.3% in common brushtail possums (*Trichosurus vulpecula*) in a woodland environment and Taronga Zoo respectively. Yang *et al.* (2011) recorded an overall prevalence of 9.3% in western grey kangaroos (*Macropus fuliginosus*) from Western Australia.

Cryptosporidium infections of vertebrate hosts are the result of 26 described species, with greater than 40 novel genotypes (Power 2010; Robinson *et al.* 2011). The *Cryptosporidium 18S rRNA* sequences identified in these bandicoot species were not genetically identical to previously described species or genotypes. Phylogenetic analysis of bandicoot isolates inferred a close evolutionary relationship to species found in humans, *C. parvum* (96.1% similarity) and *C. hominis* (98.3-99.0% similarity). The identity of bandicoot isolates was not fully resolved in this study and whether the bandicoots were infected or simply passively transmitting oocysts is unknown. In addition, without a definitive identity, this study could not support the hypothesis that the bandicoots of northern Sydney were carrying a species (or genotype) that was marsupial-specific, or of zoonotic potential. Isolates failed to amplify at the *actin* and *gp60* loci despite repeated attempts. The definitive identification and resulting zoonotic potential of isolates requires confirmation by further studies with amplification at multiple loci, particularly *gp60* to alleviate subgenotype differences (Hill *et al.* 2008). Partial sequencing of isolates can create an identification bias and without a full sequence it is not possible to infer evolutionary relationships with confidence.

Inability to amplify *C. parvum* / *C. hominis* like isolates in marsupials at loci other than *18S rRNA* has been observed in possums (Hill *et al.* 2008) and kangaroos (Ng *et al.* 2011). Hill *et al.* (2008) identified *Cryptosporidium* isolates (BTP genotype 2) from common brushtail possums in an urban setting that were genetically distinct from known zoonotic species at the *18S rRNA* locus. This was attributed this to low oocyst shedding by possums preventing

amplification at the *actin* loci and morphometric analyses. Ng *et al.* (2011) identified three marsupial host species carrying *C. parvum / C. hominis* isolates based on amplification of the *18S rRNA* locus and attempts to amplify these isolates at three other loci, *hsp70*, *acetyl CoA* and *gp60* failed. Discrepancies in the ability to amplify across multiple loci were also attributed to low levels of oocysts and the polymorphic nature of the *18S rRNA* locus. Surveillance of *Cryptosporidium* in wildlife presents unique challenges owing to the low number of oocysts typically shed by reservoir species, compared to outbreak scenarios.

Primers used in routine PCR methods have been developed using sequences from described *Cryptosporidium* species and genotypes and may therefore be unsuitable for amplification of novel isolates (Power 2010). However, it is unlikely that the failure to amplify the *actin* or *gp60* gene in this study is due to incorrectly functioning PCRs, but rather a low yield of oocysts. Positive controls were amplified in PCR protocols and oocysts from the 12 positive bandicoot samples were re-extracted using IMS, flow cytometry and visualized through microscopy. Oocyst counts were less than 10^2 oocysts / g faeces, indicating chronic or asymptomatic infection. Additionally, the highly conserved multi-copy nature of the *18S rRNA* locus compared to the single copy *actin* and *gp60* loci, suggests a mechanism to impede amplification at the single copy loci (Hill *et al.* 2008; Power *et al.* 2010; Ng *et al.* 2011).

Cryptosporidium isolates from Taronga Zoo and Ku-ring-gai Chase National Park indicated pairwise distances ranging from 0.008 to 0.035. Common brushtail possum isolates (BTP2; Hill *et al.* 2008) used in the phylogenetic analysis had similar pairwise distances (0.009 to 0.022). These isolates were considered genetically distinct from other known species, including *C. parvum* and *C. hominis* (Hill *et al.* 2008). Currently recognised species of *Cryptosporidium* generally have slightly lower pairwise distances at the *18S rRNA* locus, such as *C. meleagridis* vs. *C. wrairi* (0.013) and *C. parvum* vs. *C. hominis* (0.007) (Fayer and Santin 2009). However, the pairwise comparisons at the *18S rRNA* loci in this study were conducted using partially sequenced isolates and were not fully resolved. Full-length sequences are expected to have lower pairwise distances than those currently observed. It is therefore speculated that infection of *Cryptosporidium* in bandicoots across the two habitats occurred naturally, rather than the closely inferred *C. parvum* or *C. hominis* species.

A low oocyst yield in this study suggests an outbreak of *Cryptosporidium* in the bandicoots was unlikely over the two-year study. A more feasible scenario is that bandicoots serve as a

reservoir for a marsupial-specific genotype of *Cryptosporidium*, with minimal threat of cross-infection to domestic pets and humans. Low oocyst yields in host populations are often characteristic of species that have attained immunity from coevolution with the parasite (Toft *et al*. 1993). Moreover, no infected bandicoots appeared to display physical signs associated with acute infection of the parasite. The body condition of long-nosed bandicoots was not significantly lower in infected individuals ($p > 0.05$). In fact, the body condition of all infected bandicoots were higher than the average body condition from uninfected bandicoots. However, *C. parvum* is naturally occurring in placental mammals and one of the predominant species observed in humans. Therefore, *C. parvum* is likely to have been introduced to Australia with placental mammals rather than emergence from a marsupial host. The results call for greater phylogenetic resolution of *C. parvum* in Australian fauna, as the parasite is recognized as a species 'complex' that consists of numerous species/genotypes, including many that may not yet have been identified.

A variety of pathogenic endo-parasites have been recorded in free-ranging bandicoot populations, including those occurring along the urban interface. Endo-parasites include *Hepatozoon* (Wicks *et al*. 2006), *Giardia* (Bettiol *et al*. 1997; Adams *et al*. 2004), *Salmonella* (Staff *et al*. 2012), *Toxoplasma* (Obendorf *et al*. 1996), and *Cryptosporidium* (O'Donoghue 1995). This study expands on the current knowledge and observations of *Cryptosporidium* in bandicoots. In view of the observation of parasites that are common in humans and wildlife, conservation of habitat remnants in urban Australia should be prioritized to curb contact and transmission pathways between wildlife, humans and pets. Despite the genetic similarities of *Cryptosporidium* found in marsupials to those found in humans, caution is required when interpreting the infection potential and subsequent transmission of *Cryptosporidium* between marsupials, domestic pets and humans.

8. A comparison of the ecto-parasites of *P. nasuta* and *I. obesulus* in a wild and peri-urban habitat

8.1 Introduction

Ecto-parasites are a significant cause of economic losses and contribute to the dissemination and spread of disease in wildlife, agricultural and human populations (Daszak 2000; Storer *et al.* 2003; Brossard and Wikel 2004; Jongejan and Uilenberg 2007). Ecto-parasites encompass a broad group of hard and soft-bodied parasites including, ticks (Acari: Ixodida), fleas (Acari; Siphonaptera) and mites (Acari; Mesostigmata). Of these, ticks are the leading cause of arthropod-borne disease in animals and humans, second only to mosquitoes (Estrada-Pena and Jongejan 1999; Wikel 1999). Ecto-parasites can pose a serious health risk for wildlife, particularly on the urban fringe where interactions with domestic pets and humans are at their highest (Magle *et al.* 2012). Little research has been conducted on the interactions of ecto-parasites in native Australian fauna, from their role in the transmission of diseases to disease burden (Vilcins 2008). Therefore, documenting the occurrence of ecto-parasites, including demographic and environment variables that influence abundance, is vital for effective management of wildlife in urban environments.

Ticks, above all ecto-parasites are capable of transmitting the widest range of pathogens (Estrada-Pena and Jongejan 1999; Sonenshire *et al.* 2002; Doggett 2004). Ticks are ideal vectors because of their blood feeding habits, general absence of predators, and the ability of their well-adapted bodies to shield from environmental harm (Sonenshire *et al.* 2002). Ticks also have the ability to parasitize and feed on a wide range of host species (Belan and Bull 1995). The tick life-cycle includes multiple stages (egg, larval, nymph and adult) and individuals can survive for several years (Roberts 1970). Most Australian ticks of the genus *Ixodes*, including *Ixodes holocyclus* (paralysis tick) and *I. tasmani* (marsupial tick) are three-host ticks (Roberts 1970). That is, they spend each of their three life stages on a separate host.

Ixodes holocyclus is one of approximately 90 species of Australian ticks and one of the least host-specific species. It has been recorded on a wide range of native and introduced species, including the long-nosed and southern brown bandicoot (Roberts 1970; Storer *et al.* 2003). Paralysis is usually caused by the female tick and can occur any time of the year (Doube 1979). A neurotoxin injected into the host by the female tick induces paralysis, with serious

reactions in domestic pets, livestock and humans, including respiratory and cardiac arrests in humans and death in domestic pets (Roberts 1970; Stone *et al.* 1982; 1989; Sonenshire *et al.* 2002). Marsupials, including bandicoots are a natural host to *I. holocyclus* and are unaffected by the toxin, despite records of animals with heavy infestations (Campbell *et al.* 2003; Jackson *et al.* 2007). *Ixodes holocyclus* is common in along the urban fringe of Sydney and other humid environments (Roberts 1970; Doube 1979; Storer *et al.* 2003). Interactions between native fauna and domestic pets provide a pathway for transmission of parasites across host species. The occurrence of *I. holocyclus* in urban areas and their ability to parasitise a range of animals is problematic for wildlife managers and pet owners. Chapter 3 identified a concern for managers. Community members identified the tick carrying capacity of bandicoots and the ensuing threat to humans and domestic pets, as one of the predominant reasons behind declaring bandicoots as a pest species.

With the loss of biodiversity owing to expanding urban centres, management efforts to safeguard the survival of native species becomes critical. However, urban sprawl in Australia and its major cities has reduced natural habitat and increased urban ecosystems, which has amplified the interface for conflicts to occur between wildlife, humans and pets (Magle *et al.* 2012). This has created a dilemma for managers, particularly in relation to bandicoots and the paralysis tick (*I. holocyclus*). Management requires a science-based strategy that considers the impact of ecto-parasites on the health and survival of native animals, including the documented risks of the paralysis tick to humans and pets. One of the major benefits of urban ecosystems is the ability to connect citizens with their environment for an appreciation towards wildlife (Magle *et al.* 2012). Thus management requires suitable tact to promote native wildlife as well as the priorities of the urban community.

The aim of the study was to document the diversity of ecto-parasites using bandicoots as a host at the urban fringe of northern Sydney. Ecto-parasite presence, richness and load would be examined to determine if there was a relationship with environmental and demographic variables. It was hypothesised that the paralysis tick is the most common tick on the bandicoots of northern Sydney and that a difference in parasite load occurs between male and female bandicoots. In addition, it was postulated that parasite prevalence and load is higher on bandicoots from backyards compared to National Parks, due to the increased opportunities for ecto-parasite transmission to occur in backyard habitats (created by microhabitat features).

8.2 Methods

8.2.1 Study area and data collection

The study area incorporated locations in the greater Sydney region including sites in Ku-ring-gai Chase National Park, Garigal National Park and surrounding suburban backyards. Trapping methods and details of the study areas have been described in Chapter 2. Bandicoots from the free-ranging populations were captured in cage-traps overnight and sampled prior to release the next morning. Measurements of the individuals' weight, sex, reproductive status and body condition were recorded (Chapter 2). Weight was measured to the nearest 25 grams using spring scales and sex. Reproductive status was determined by examining the genitalia and the bandicoot and females for the presence of pouch young.

The body condition of an animal may ultimately influence population fitness with an animal in good condition, having an increased ability to cope with environmental pressures (Jakob *et al*. 1996; Schulte-Hostedde *et al*. 2001). The body condition of each individual was assessed with a method previously used for western barred bandicoots (*Perameles bougainville*) by calculating the cube root of weight divided by the pes (right-hind foot) length (Short *et al*. 1998).

8.2.2 Ecto-parasite collection and identification

Ticks (order Ixodida), mites (order Mesostigmata) and fleas (order Siphonaptera) were collected from bandicoots, using fine forceps, and then stored in a plastic vial filled with ethanol (70%) until analysis. The head, ears, rump and underside of each bandicoot were inspected for a period of 5 minutes to collect parasites. Not all parasites were collected from an individual due to the time constraints associated with sampling in a natural setting and the timing limitations imposed on the trapping procedures by ethics and license approvals. However, all visible ticks were collected within the time frame and a representative sample of the fleas and mites that were present.

All ecto-parasite were identified to species level where possible, or genus level if species could not be determined. Ticks (Ixodida) and fleas (Siphonaptera) were identified under a stereo-microscope based on shared morphology, following Roberts (1970) and Dunnet and Mardon (1974), respectively. Mites (Mesostigmata) were identified under a compound

microscope following Domrow (1987; 1992). Voucher specimens of morphologically distinct mites that were collected from bandicoots in this study, were confirmed by the NSW Department of Primary Industry, Agriculture Institute in Orange. These were used as reference specimens and to document the types of species recorded on bandicoots in northern Sydney.

8.2.3 Statistical analysis

Abundance was defined as number of parasite specimens on the host (Bush *et al.* 1997). This was determined for ticks, but not fleas and mites, due to an inability to collect all individual fleas and mites from all bandicoots. Due to this potential bias, the prevalence of an ecto-parasite species was regarded as a more reliable measure of infestation, than abundance for the purposes of statistical analysis. The prevalence of parasites on bandicoots was therefore interpreted as presence/absence of an ecto-parasites species. Prevalence was calculated and analysed for all ecto-parasites, as well as just for ticks. Tick species richness was defined as the total number of tick species occurring on the host, and tick load was defined as the total number of individual ticks occurring on the host.

Where applicable, analyses were conducted for both species of bandicoot, however limited captures of the southern brown bandicoot precluded analyses in some cases. The effect of independent variables, sex and habitat type on ecto-parasite prevalence and tick prevalence was assessed using a binomial logistic model. To determine if sex and habitat type influenced tick species richness and tick load, a generalized linear model (incorporating individuals with ticks present) was used. Pairwise comparisons using a Bonferroni correction were applied where applicable to further test significant differences. A one-way ANOVA was conducted to test the impact of tick load and species richness on body condition. The effect of bandicoot species on the prevalence of parasites was assessed using a binomial logistic model, while the effect of bandicoot species on parasite load and species richness was analysed using a generalized linear model. Significance was accepted at the $p < 0.05$ level and means are given with standard errors unless otherwise stated. Data was analysed using SPSS 19.0 for Windows.

8.3 Results

8.3.1 Bandicoot and ecto-parasite populations

A total of 258 bandicoots were captured from Ku-ring-gai Chase National Park, Garigal National Park and suburban backyards of northern Sydney. New capture events involving capture of bandicoots for the first time accounted for most of the hosts sampled (n = 176: LNB = 134; SBB = 42). Of these trappings, ecto-parasites (ticks, mites and fleas) were collected from 90% (n=157) of individuals, with ticks being recorded on 102 individuals.

Eight species of ecto-parasites were recorded on the bandicoots of northern Sydney. However, it is important to note that not all mites collected were identified from this study. The ecto-parasite fauna included:

- Four species of ticks - *Ixodes holocyclus, I. tasmani, I. trichosuri* and *Haemaphysalis bancroftii*;
- Two species of fleas – *Phyiopsylla hoplia*, and *Stephanocircus dasyuri*; and
- Two species of mites – *Mesolaelaps antipodianum* and *M. australiensis*.

All ecto-parasites recorded on the southern brown and long-nosed bandicoots of northern Sydney had previously been identified on both species of bandicoot. One exception was the mite, *Mesolaelaps australiensis* on the southern brown bandicoot, which is likely to represent a new host species for *M. australiensis*. The same ecto-parasite species were recorded on both long-nosed and southern brown bandicoots across the three habitats in northern Sydney, except for the flea species *Stephanocircus dasyuri,* which was not observed on the long-nosed bandicoots captured in suburban backyards.

The paralysis tick paralysis tick (*Ixodes holocyclus*) was the most prevalent tick, accounting for 66% and 58% of the tick species recorded on the southern brown and long-nosed bandicoots respectively (Table 8-1). The paralysis tick was also the most prevalent ecto-parasite recorded in Garigal National Park and suburban backyards. The average number of paralysis ticks recorded from infected bandicoots in suburban backyards was 3.18 and ranged from 2-4 (Table 8-2).

Table 8-1: Abundance of tick species according to species of bandicoot. Means are expressed with the standard error. Percentage (%) is expressed as the percentage of the total number of ticks recorded in the population.

Ecto-parasite	Southern brown bandicoot			Long-nosed bandicoot		
	Mean (S.E.)	Range	%	Mean (S.E.)	Range	%
Ixodes holocyclus	1.37 (0.16)	1-6	66	2.13 (0.14)	0-6	58
Ixodes tasmani	0.31 (0.11)	0-3	15	1.01 (0.22)	0-10	28
Ixodes trichosuri	0.11 (0.07)	0-2	5	0.11 (0.05)	0-2	3
Haemaphysalis bancrofti	0.29 (0.15)	0-5	14	0.39 (0.09)	0-4	11

Table 8-2: Abundance of tick species according to habitat. Means are expressed with the standard error.

Ecto-parasite	Ku-ring-gai Chase N.P.		Garigal N.P.		Suburban backyards	
	Mean (S.E.)	Range	Mean (S.E.)	Range	Mean (S.E.)	Range
Ixodes holocyclus	1.26 (0.09)	0-3	2.81 (0.30)	2-6	3.18 (0.08)	2-4
Ixodes tasmani	0.63 (0.17)	0-8	0.86 (0.30)	0-3	1.18 (0.49)	0-10
Ixodes trichosuri	0.08 (0.04)	0-2	0.31 (0.18)	0-2	0.05 (0.05)	0-1
Haemaphysalis bancrofti	0.37 (0.10)	0-5	0.69 (0.28)	0-4	0.05 (0.05)	0-1

8.3.2 Ecto-parasite comparisons between bandicoot species

There were no differences observed in the prevalence of ecto-parasites between the bandicoot species of northern Sydney. However the long-nosed bandicoot was more likely to be infected with a higher abundance (load) and number of tick species (richness) than the southern brown bandicoot (load: Wald statistics = 5.182, df = 1,100, p = 0.023; richness: Wald = 4.110, df = 1,100, p = 0.043) (Figure 8-1 and Figure 8-2).
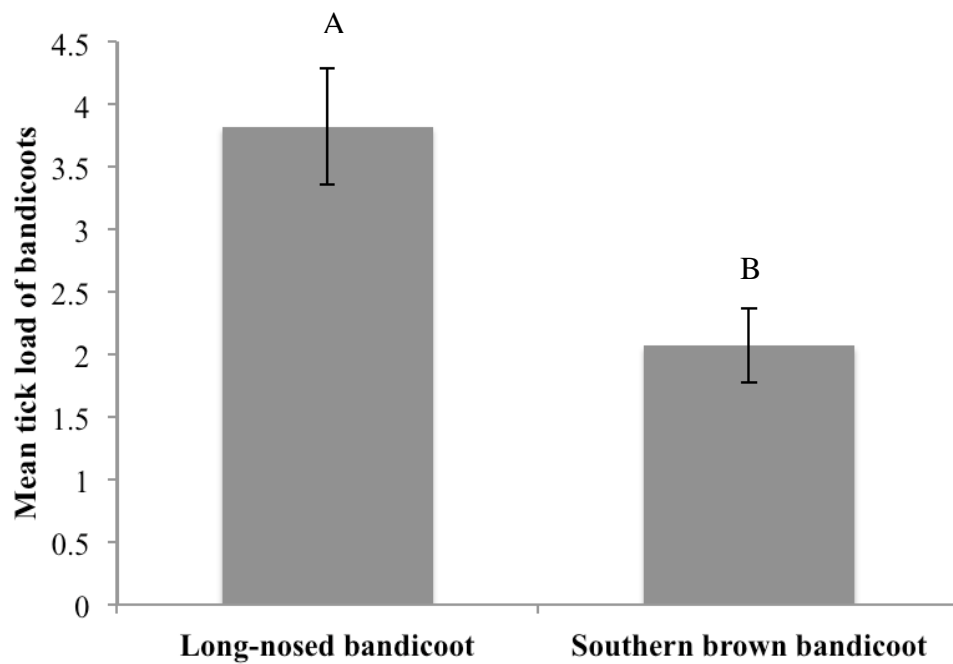
Figure 8-1: Tick load on the bandicoots of northern Sydney. Different letters denote significantly different results ($p = 0.023$).

Figure 8-2: Tick species richness of the bandicoots of northern Sydney. Different letters denote significantly different results ($p = 0.043$).

8.3.3 Influence of demographic and environmental variables on ecto-parasites

There was no significant difference between the prevalence of ecto-parasites on male and female bandicoots when analysed separately for both long-nosed and southern brown bandicoots. However, when conducting analysis on ticks alone, male long-nosed bandicoots had a significantly higher prevalence, richness and load of ticks than female long-nosed bandicoots (prevalence: Wald = 6.091, df = 1,185, $p = 0.014$; richness: Wald = 12.877, df = 1,72, $p < 0.001$; load: Wald = 12.005, df = 1,72, $p < 0.001$) (Figure 8-3 and Figure 8-4). Differences in tick load, prevalence and species richness were not detected between the sexes of the southern brown bandicoot.

The ANOVA analyses also revealed that tick species richness and tick load on both species of bandicoot in northern Sydney has no effect on the body condition of an individual ($p > 0.05$).

Analyses on the ecto-parasite fauna of bandicoots across the three habitats in northern Sydney was conducted only for the long-nosed bandicoot, due to the limited captures of the southern brown bandicoot outside Ku-ring-gai Chase National Park. The analyses detected a significant difference in the prevalence of ticks across Ku-ring-gai Chase National Park (0.32 ± 0.043), Garigal National Park (0.54 ± 0.096) and suburban backyards (0.51 ± 0.077) (Wald = 8.482, df = 2,184, $p = 0.014$). The long-nosed bandicoots of Garigal National Park had a higher prevalence of ecto-parasite fauna than Ku-ring-gai Chase National Park (Wald = 7.040, df = 2,184, $p = 0.030$) (Figure 8-5). No other differences in the prevalence of all types of ecto-parasites were detected between the habitats of northern Sydney.
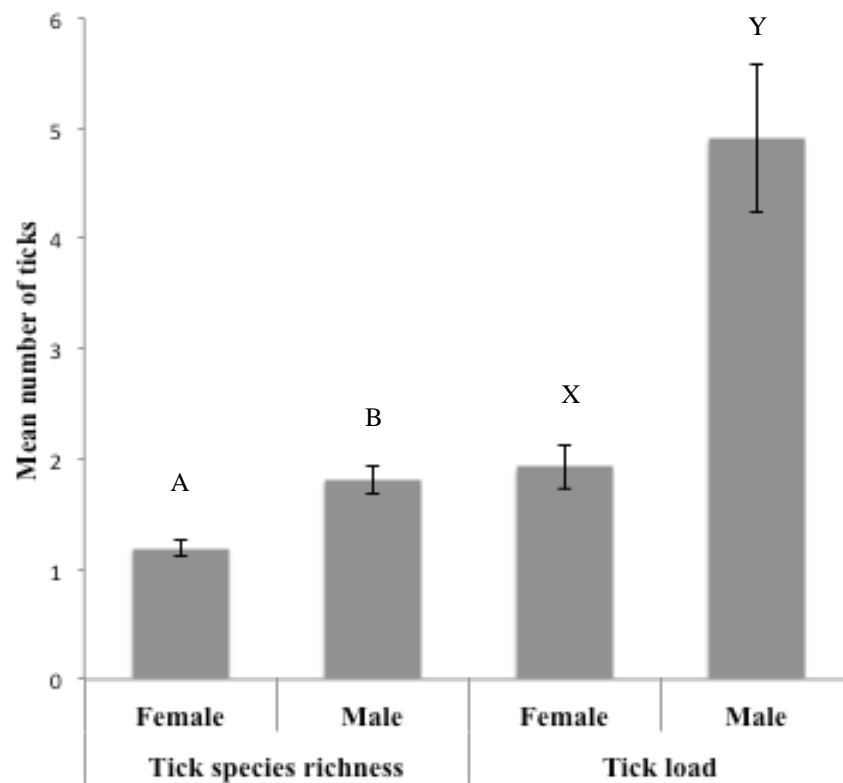
Figure 8-3: Tick richness and load of long-nosed bandicoots. Different letters denote significantly different results for richness (A/B; $p < 0.001$) and load (X/Y; $p < 0.001$).
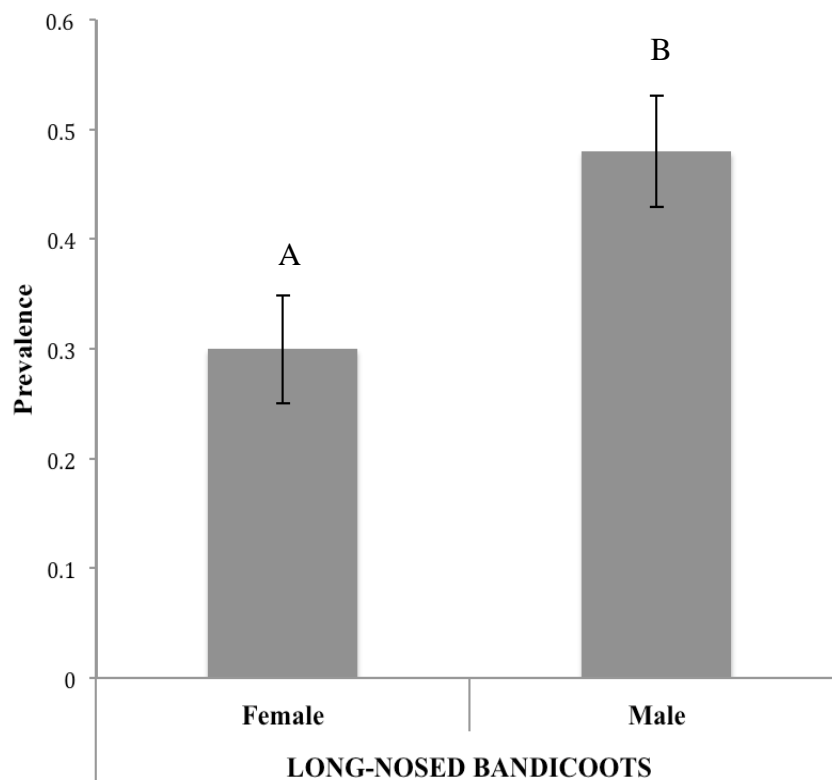
Figure 8-4: Mean prevalence of ticks for male and female long-nosed bandicoots. Different letters denote significantly different results (A/B; $p = 0.014$).
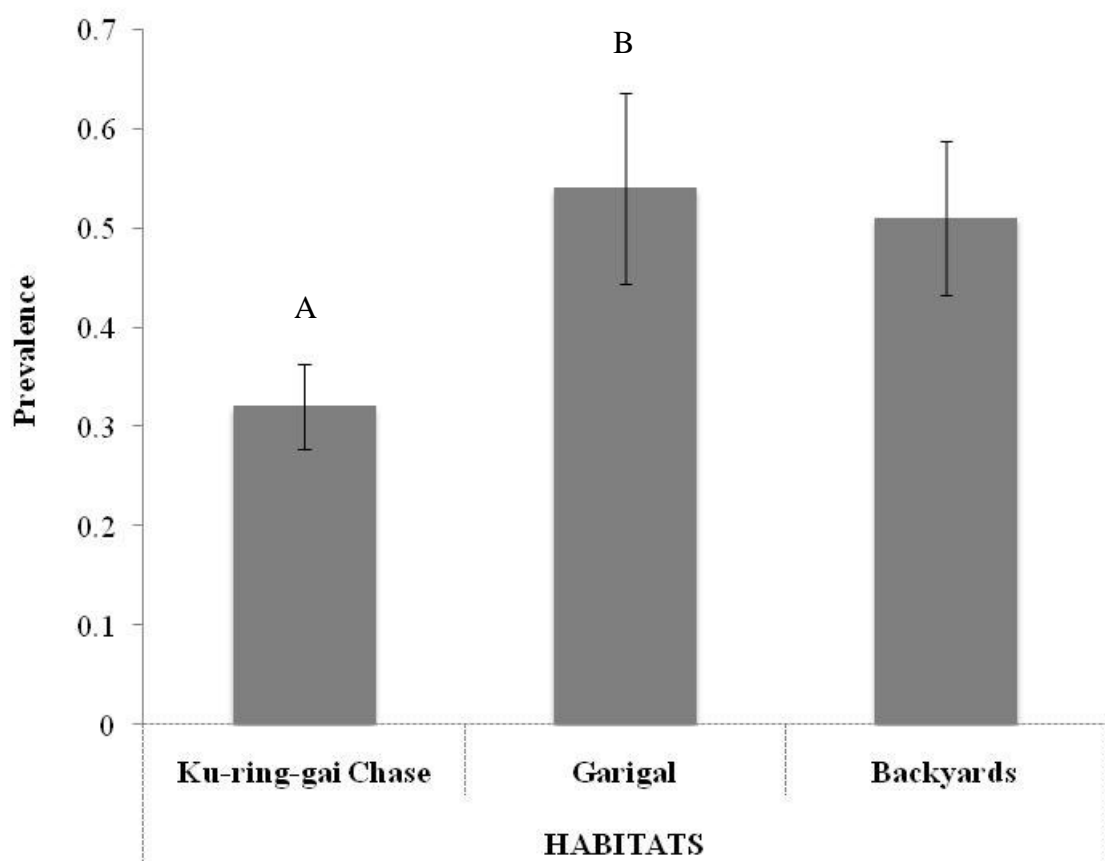
Figure 8-5: Mean prevalence of ecto-parasites across habitats for long-nosed bandicoots. Different letters denote significantly different results (A/B; $p = 0.030$).

8.4 Discussion

This study identified eight ecto-parasite species on the bandicoots of northern Sydney, with one mite species (*Mesolaelaps australiensis*; Hirst 1926) likely to indicate a new host record (Domrow 1987). The remaining species had previously been recorded on long-nosed and southern brown bandicoots and are likely to represent natural host-parasite relationships (Roberts 1970; Dunnet and Mardon 1974; Domrow 1987). *Ixodes holocyclus* and *I. tasmani* were two common species of ticks observed on bandicoots and constitute over 80% of the ticks recorded. Both tick species have been reported from most families of Australian marsupials and both have the potential to transmit pathogens to humans and domestic animals, including *Rickettsia australis* (Queensland tick typhus) (Roberts 1970; Stone *et al.* 1989; Estrada-Pena and Jongejan 1999; Murdoch and Spratt 2005).

The paralysis tick (*Ixodes holocyclus*) was the tick species most recorded on bandicoots. It accounted for 66% and 58% of ticks observed on southern brown and long-nosed bandicoots respectively, and supported the study hypothesis. *Ixodes holocyclus* is one of the least host specific and toxic species of tick in Australia (Roberts 1970; Stone *et al.* 1989). Bandicoots along with other marsupials, appear to be largely unaffected by this parasite (Campbell *et al.* 2003; Jackson *et al.* 2007). Paralysis of native marsupials by *I. holocyclus* is uncommon, but may occur in young, immuno-competent individuals and captive un-exposed individuals following release into tick infested areas (Doube 1979; Stone *et al.* 1982; Gemmell *et al.* 1991). No heavy tick infestations were recorded on the bandicoots in this study. It is unlikely that the bandicoots observed were immuno-compromised or susceptible to paralysis from *I. holocyclus*. In addition, there was no detectable difference in the body condition of individuals due to parasite abundance (load). Similar to Chapter 6, where body condition differences between individuals might not have been significant enough to impart a detectable FGM response, parasite load of captured bandicoots may not have been sufficient to impart an immune response. Therefore, would parasite load would go undetected when analysing bandicoot body condition.

Ixodes holocyclus occurs chiefly in coastal scrubland throughout its range. It is often abundant in spring and summer after rain events, indicating a preference for hot humid environments, such as the coastal expanses of Sydney (Doube 1979; Storer *et al.* 2003). A dry environment with the increased potential for water loss is a significant hurdle for ticks (Randolph 2004). As a consequence from the impact of its bite and the favourable habitats in northern Sydney,

I. holocyclus is a cause of concern for wildlife managers, veterinarians and pets owners. Chapter 3 identified that 'carrying ticks' was the second most common complaint of residents who thought bandicoots were a nuisance in northern Sydney (behind damage to manicured lawns: Dowle and Deane 2009). This study indicated that residents are justified in their view that bandicoots carry ticks, however, the potential for cross infection has not been determined.

This study did not support the hypothesis that ecto-parasite prevalence and ecto-parasite load is higher on bandicoots inhabiting suburban backyards. It was thought that the variety of microhabitats supported by the urban interface would increase the interactions occurring between wildlife, and thus increase the opportunity for parasite transmission between host species. However, higher tick prevalence was observed between long-nosed bandicoots of Garigal National Park compared to Ku-ring-gai Chase National Park. In addition, the prevalence of ecto-parasites on long-nosed bandicoots from suburban backyards was only slightly lower than the prevalence recorded in Garigal National Park. The higher prevalence of ecto-parasites in the more urbanised habitats in this study is inconsistent with other research (Bradley and Altizer 2007). Geue and Partecke (2008) found a lower risk of parasite infections (haematozoan) in populations of Eurasian blackbirds (*Turdus merula*) inhabiting urban areas compared to populations from forested habitats. Similarly, Gregoire *et al.* (2002) observed a significantly higher prevalence of *Ixodes* tick infestations on Eurasian blackbirds in a forested habitat compared to an urban habitat. Whilst there is a difference in habitat usage between a ground dwelling mammal and a bird, cross-infestation (or transmission) of parasites between host species is likely to be consistent across all habitat stratums along the urban interface. A mosaic of microhabitat types and microclimate features would be present in all stratums.

Geue and Partecke (2008) attributed the lower prevalence to decreased vector availability in treeless habitats, and extrapolated this to suggest that lower parasite prevalence creates an opportunity for invasive species to exploit urban ecosystems. However, it's important to note that backyard and Garigal National Park habitats in this study, although heavily enclosed by urban and treeless environments, are more representative of a forest habitat. The interface of the treeless and forested habitats in this study could be operating in concert to create an environment suitable for higher parasite numbers, through increased host animal diversity. This is likely to be applicable in the upper stratum of the peri-urban habitat and the environment utilised by ground-dwelling mammals. Stanko *et al.* (2002) observed that a

higher host density corresponded with an increase in species richness of fleas on small mammals. The diversity of hosts in an environment can also be crucial in determining the species richness of ecto-parasites (Krasnov *et al.* 2004).

Male bandicoots in this study recorded a higher species richness and parasite load of ticks than female bandicoots, supporting the hypothesis that a difference in parasite load between the sexes would be observed. In many wild animal populations, males tend to be bigger, disperse more regularly and have larger home ranges (Randolph 2004). Both bandicoot species in this study exhibited sexual dimorphism with males larger than females and males also likely to contain larger home ranges (Chapter 4) (Mallick *et al.* 1998b). The more active nature of males across a larger habitat than females is likely to expose them to a greater number of ticks, consistent with studies on the western fence lizard (*Sceloporus occidentalis*) (Talleklint and Eisen 1999; Randolph 2004). However, the ability of female bandicoots to breed year-round amplifies the susceptibility of females to tick infestation. The female reproductive process and the immuno-suppression that occurs during lactation, provide an opportunity to be exploited by ecto-parasites (Zuk 1990; Hill 2008). Coupled with the ability of testosterone to lower resistance to tick infestation in males (Randolph 2004), it might be expected that females would have a higher infestation than males. However, this was not observed with male bandicoots having a significantly higher parasite load than female bandicoots.

Northern Sydney exhibits trademark threats to the persistence of native species, including habitat fragmentation from urbanisation, the stress associated with increased presence of predators and increased competition from native species (Daszak *et al.* 2000; Dobson and Foufopoulus 2001). These external stressors, due to increased contact between wildlife in natural habitats with other host taxa (such as domestic pets) living in the urban matrix are known to enhance parasite transmission between individuals and reduce the ability to respond to changing environments (Dobson and Foufopolous 2001). The higher tick abundance and species richness observed on the long-nosed bandicoot, highlights the opportunities for tick infestations and the transmission of the paralysis tick to occur between bandicoots and domestic pets along the urban fringe of northern Sydney. This is enhanced by the ubiquitous nature of the long-nosed bandicoot compared to the southern brown bandicoot, and its frequent occurrence in suburban backyards. This has created a dilemma for wildlife managers in northern Sydney with the need to balance the concerns of all stakeholders. Managers are

required to balance the conservation and wildlife needs of the endangered southern brown bandicoot on one hand, with increasing complaints about wildlife related damage (bandicoot diggings) on the other (Layden *et al.* 2003; Dowle and Deane 2009). It remains a challenge for conservation efforts to recognize the role of ecto-parasites and fully consider their impacts on the host when developing management strategies (Lebarbenchon *et al.* 2007).

This study has provided an insight into the patterns of ecto-parasite infection, particularly for tick occurrence on the bandicoots of northern Sydney. The paralysis tick was the most common tick observed on the long-nosed bandicoot. The long-nosed bandicoot was also observed with higher parasite load and species richness than its endangered sympatric counterpart. The frequent occurrence of the long-nosed bandicoot in suburban backyards provides opportunities for the transmission of the paralysis tick between bandicoots, domestic pets and humans in northern Sydney. However, parasite prevalence and parasite load did not significantly differ between National Park and suburban backyard habitats.

9. Discussion for the investigations into the bandicoots of northern Sydney

9.1 Overview

Similar to other marsupials throughout Australia, bandicoots are in decline, with the notable exception of *Perameles nasuta* (long-nosed bandicoot). Urbanisation is a major contributor to this decline, creating habitats that are not favourable to the long-term persistence of many native species. The impacts of urbanisation include fragmentation of habitat, increased predation and altered resource availability. These factors coalesce to produce pressured environments that are further amplified in threatened populations (Collinge 1996; Reed *et al*. 2007; Gordon *et al.* 2009). These altered habitats create novel environments typically favoured by generalist and opportunist species, but which also increase human-wildlife interactions, leading to implications for the conservation management of a species. Bandicoots exhibit the generalist and opportunistic characteristics that should allow them to exploit such habitats, particularly on the urban fringe where increased opportunities for food and shelter may exist (Gordon and Hulbert 1989). However, urbanised habitats in comparison to natural habitats, have fewer dense refuge sites and may lack a mosaic of open foraging areas with suitable peripheral cover that bandicoots depend on for foraging and nesting (Scott *et al.* 1999).

Effective wildlife management is achieved through understanding the nature of interaction between native species and humans in urban areas. Management also relies on comprehensive studies or research in areas like the urban fringe, where the majority of interactions occur. Such investigations are integral to the development of wildlife preservation strategies, that concurrently promote human and domestic animal well-being.

The way in which the two bandicoot species inhabit urban influenced environments of northern Sydney is paramount to their successful long-term management, especially for the threatened southern brown bandicoot (*Isoodon obesulus*). In this thesis I have provided an integrated context for research on bandicoots in northern Sydney and incorporated diverse issues relating to the long-term persistence of wildlife in an urban setting. The overall investigation has examined how the bandicoots respond to the encroachment of an anthropogenic environment, and has provided an ecological snap shot of the bandicoots in

these habitats. Furthermore, the research outcomes have been strengthened through the comparison of two bandicoot species that differ significantly in conservation status.

The habitats of northern Sydney, at the eco-tone between suburban backyards and remnant bushland, provide a unique opportunity to study two sympatric bandicoot species with phylogenetic similarities. The objectives were to examine and compare the dynamics of *I. obesulus* and *P. nasuta* across the undisturbed (National Parks) and peri-urban (backyards) habitats of northern Sydney. In particular, the aim was to provide local wildlife managers with information for the conservation of Australian mammals in an urban landscape. These outcomes would apply to a broad range of species and would be pertinent for *I. obesulus* (southern brown bandicoot) in northern Sydney.

I initially investigated the frequency of interactions and causes of conflicts between bandicoots and humans (and their domestic pets) through a community attitudes survey. I then looked at the ecological characteristics of both species within the habitats of northern Sydney, and used a community survey to provide access for live-trapping in an urban environment.

After examining the context behind the conflicts and interactions between bandicoots and humans on the urban fringe, the genetic diversity of the bandicoot species was investigated in an effort to determine what might be genetically compromising the ability of *I. obesulus* to persistence in the local habitat. The genetic outcomes would provide further context for the development of management strategies crucial to the immediate and long-term survival of *I. obesulus*.

The faecal glucocorticoid concentrations of bandicoots were measured and examined against a range of demographic and environmental variables, to determine if the urban environment has an adverse physiological response on bandicoots. Finally, I determined whether the parasitic burdens of bandicoots reduces their well-being in an urban habitat and highlighted the opportunities for the transmission of ecto and endo-parasites between native host species and domestic pets and humans on the urban fringe.

9.2 Summary of chapters

9.2.1 Local community attitudes towards bandicoots

Human perception of wildlife species has become increasingly important for the conservation and management of threatened species at the urban fringe. Community surveys are important in determining the human dimensions of wildlife-human conflicts, and aid in developing management strategies to promote the peaceful existence between wildlife and people. The bandicoots of northern Sydney pose a unique challenge to conservation managers. The long-nosed bandicoot is proving to be a nuisance to a minority of residents on the urban fringe, while the survival of the southern brown bandicoot may depend on management actions that increase habitat through native designed backyards. A community attitudes survey was conducted for this thesis to identify the driving factors behind the public's perception of bandicoots. Results of this survey will enable local wildlife managers to develop effective and socially acceptable conservation strategies.

Protected areas are considered a first line of defence for conserving biodiversity (Jenkins and Joppa 2009; Watson *et al.* 2011). Yet the number and size of these areas may be insufficient for long-term survival of many threatened species, particularly those whose habitat lies within the increasingly urbanised environment. Where intensive land development has occurred, such as the outskirts of major cities, native animal biodiversity can be increased by revegetation with native plant species (McKinney 2002). In addition, understanding how remnant wildlife populations use the interface between urban and natural landscapes and implementing relatively simple management practices can boost the chance of a species' persistence within urban habitats (Hughes and Banks 2010).

To sustain native wildlife populations such as bandicoots, communities are encouraged to develop native backyards and to manage family pets at night when the most damage to native wildlife occurs. With indigenous plant species, residents can combine the habitat requirements of bandicoots while enhancing connectivity across the landscape. For example, bandicoots appear to be a matrix-sensitive species, so providing habitat that is representative of open areas for foraging, integrated with vegetation provided by remnant bush, can promote bandicoot presence (Hughes and Banks 2010). However, conservation strategies involving management and landscaping actions in backyards require the co-operation of residents. Thus,

understanding local attitudes towards bandicoots is pivotal in guiding these strategies and actions.

The objective of the community survey was to determine the underlying attitudes towards bandicoots, and use the results to promote community based conservation programs. More specifically, the survey aimed to obtain the attitudes of residents who experience direct interaction (or conflict) with bandicoots. The attitude surveys also doubled as a request for permission to trap in residential backyards in order to directly document the relationship and interactions between the urban environment and bandicoots.

The attitudes survey identified interactions between humans and bandicoots as fundamental in shaping the perception of the bandicoot as a nuisance. Age and pet ownership were also significant characteristics shaping public attitudes towards bandicoots. Therefore, educational programs should be targeted at the older demographic and pets owners, addressing ways in which interactions occur and ways to lessen exposure to ticks. This may help reduce anxieties surrounding human–bandicoot co-existence.

Interestingly, the respondents to the attitudes survey indicated that pets did not deter bandicoots. Pet owners identified that they receive as many visits by bandicoots to their backyards as residents without pets. This result was unexpected and suggests that bandicoots either do not see domestic animals as potential predators, or they have adopted mechanisms necessary to deal with increased threats of predator interactions. The concept of suburban backyards providing a lower quality habitat than the National Parks, either through increased interactions with predators or changed resource availability, is further explored in the thesis.

Despite an 83% occurrence of bandicoots on residents' properties and a heightened potential for human-bandicoot conflict, respondents were generally positive towards bandicoots. The positive attitude was further demonstrated through the response rate to the letterbox drop (30%), which is considered to be very good in the psychological literature. An expression of negative or neutral views to bandicoots would likely manifest itself in a community with low interactions and low interest in wildlife (Jonker *et al*. 2006). Therefore, identifying residents with high bandicoot interactions is supportive of an aware community and consequently encouraging for community-based conservation. Community surveys, such as the one undertaken for this thesis, have a broad application and are effective method of developing

conservation management strategies. Furthermore, surveys can provide anecdotes and supplementary background ecological information on targeted wildlife species.

9.2.2 The distribution of bandicoots within Sydney's north

The Fox Tap experiment (Fox TAP, Sydney North Regional Pest Management Strategy 2004-2007 and 2008-2011) is a fox-baiting program aimed at reducing the impact of foxes within the local environment including National Parks, Nature Reserves and remnant bushland. Bandicoots are regularly monitored by Parks Authorities as part of this fox-baiting program, through live-trapping surveys. These surveys were included in the overall investigation, and in addition to supplementary surveys, examined the regional distribution of bandicoot species and were used to collect ecological information pertinent to the research objectives.

The live-trapping surveys provided a picture of the population demographics and habitat use across the peri-urban and undisturbed habitats of northern Sydney. Backyards were initially considered fringe habitat because individuals would have to cope with the change in resource availability and the potential increase of predator interactions. The idea that bandicoots favoured National Parks rather than suburban backyards was one of the hypotheses presented in this thesis. However, comparisons of the dynamics of bandicoot species across habitats would generally reveal otherwise. The exclusion of backyards containing pets from the trapping regime, due to ethical restrictions on the survey methodology, should be considered when interpreting these results.

Body condition analysis of bandicoots across the different habitats supported the findings that backyards were not a lower quality habitat. It was thought the pressures of increased predation and competition for resources in backyards would require higher physiological demands, resulting in a lower body condition. In fact, the higher body condition observed for long-nosed bandicoots in backyards suggests these peri-urban habitats may be favourable. Suburban backyards may be providing bandicoots with the resources necessary to cope with increased pressures from the urban habitat. Additionally, the availability of resources appears to compensate for the higher physiological demands required in obtaining them.

Perhaps the most important finding from the live-trapping and hair-tube surveys, is the contrasting distribution between the two species. The long-nosed bandicoot was ubiquitous across all habitats surveyed, including hair-tubing and live-trapping locations, while the southern brown bandicoot was surprisingly confined to a few locations in Ku-ring-gai Chase National Park. The last capture of a southern brown bandicoot outside of Ku-ring-gai Chase was in Garigal National Park, March 2005 (pers. comm. B. Hope, Technical Officer NPWS 2011). A hazard reduction burn in part of Garigal National Park in 2003 unintentionally removed approximately 70-80% of prime habitat in the immediate area. Whilst the habitat is likely to recover in the long-term, the hazard reduction burn would have moved surviving bandicoots to another habitat in the short-term and/or significantly reduced the local *I. obesulus* population. However, repeated hair-tubing within Garigal National Park since the hazard reduction burn, has only recorded the presence of the southern brown bandicoot on one occasion. The hazard burn would also account for the low number of captures from the live-trapping surveys observed in this study (total of 21 *I. obesulus* over 3 years), compared to capture data for Garigal National Park collected prior to this study.

The major reduction of the northern Sydney southern brown bandicoot population documented after the hazard reduction burn could have a significant consequence for the bandicoot's long-term persistence in the area. If a major reduction of the population has occurred, it is likely to have reduced the genetic diversity of the local population and compromised its genetic integrity. This would limit the species' ability to cope with current ecological pressures and future environmental changes.

The establishment of a southern brown bandicoot translocation program has previously been considered as a management option for the conservation of this species. This program would be consistent with the NSW Government's priority for conservation, as highlighted in the NSW Recovery Plan under Section 6 (*Proposed Recovery Objectives, Actions and Performance Criteria),* Action 4.4 *Investigate the feasibility of establishing a captive breeding program.* However, the need for a translocation program is contradictory to Zenger *et al.* (2005), who suggested sufficient genetic variability existed within the species, and such a program for *I. obesulus* in northern Sydney was not currently appropriate. Nevertheless, in lieu of the recent live-trapping and hair-tubing surveys, a captive breeding program may provide an insurance against further losses for this regionally iconic species.

It is possible that southern brown bandicoots were undetected using the survey methodologies (e.g. hair-tubes). A recent survey by Paull *et al.* (2012) compared infra-red digital cameras and hair tunnels in detecting medium-sized ground-dwelling mammals. It was determined that infra-red cameras were a far more efficient way to census a broad spectrum of ground-dwelling mammals. In the Paull *et al.* (2012) study, hair-tunnels detected 8 mammals species compared to 18 species detected by infra-red cameras. It is recommended that infra-red cameras be incorporated into future surveys targeting bandicoots in northern Sydney, particularly if the aim is to expand on the current known distribution.

9.2.3   The genetic diversity of bandicoots and gene flow between habitats

The results of the live-trapping and hair-tube surveys outlined a small effective population of southern brown bandicoots that may largely be limited to Ku-ring-gai Chase National Park. This is likely to lead to inbreeding and an increased number of homozygotes in the population, which could reduce the ability of the species to respond to environmental change and increase the risk of its long-term survival in the area. The small population size highlighted the need to undertake an investigation in to the genetic diversity of the bandicoots of northern Sydney. The genetic diversity analysis would identify factors compromising the species' persistence, and be useful in informing the applicability of a translocation program.

The two bandicoot species exhibited relatively low levels of heterozygosity commonly seen in other marsupials (Eldridge 2010). The southern brown bandicoot also displayed a very low allelic diversity, typical of a threatened population and not unexpected considering the population decline and range contractions experienced by this species (Ashby *et al.* 1990). The deficiency of heterozygotes in both populations highlights the potential for inbreeding to be present. Coupled with a small population, limited gene migration and the increased potential for non-random mating, low heterozygosity and allelic diversity may have long-term consequences for the genetic health of the bandicoots. However, neither species were shown to have experienced a recent population bottleneck, suggesting that if inbreeding is present, it is currently limited.

This is one of the first studies applying the context for an island population to populations isolated by the urban environment. Urbanised island populations can be geographically and

demographically challenged, and therefore may exhibit genetic signatures similar to young island populations, such as limited connectivity and low genetic diversity. Importantly, the genetic analyses revealed a single interbreeding population of long-nosed bandicoots rather than two separate populations on either side of a major road. This major road consists of a two-lane dual carriageway, but does not seem to be limiting gene flow between long-nosed bandicoots inhabiting Garigal and Ku-ring-gai Chase National Parks. It is likely that the gene flow observations can be extrapolated from the long-nosed bandicoot to the southern brown bandicoot, because of the phylogenetic, geographic and demographic similarities of the two species (Bohonak 1999). Therefore, it can be inferred through similar adaptive traits and the sympatric nature of the bandicoot species, connectivity of habitat for the southern brown bandicoot between the National Parks, is likely to be present. Thus, something other than reduced genetic diversity and the flow of genetic material is limiting the ability of the southern brown bandicoot to persist outside of Ku-ring-gai Chase National Park.

I have demonstrated in this study that using multiplexed microsatellite loci with cross-species applicability has a broad application and can be used for research on sympatric species. The use of sympatric species in ecological studies is likely to have a high applicability for threatened taxa. It allows information to be inferred across species in situations when obtaining enough reliable data to inform management was not previously possible. Furthermore, the genetic diversity analyses can be coupled with detailed ecological, geographic and behavioural data to form a powerful tool to guide management, not only for the southern brown bandicoot but also for other threatened and non-threatened taxa.

9.2.4 The physiological response of bandicoots measured through a non-invasive technique

The pressures of urbanisation may impact the health of wildlife at the urban fringe. Stress events, such as the reduction and fragmentation of habitat, can induce the release of glucocorticoid hormones into the blood through an HPA-axis response, resulting in altered energy storage and utilisation (Reeder and Kramer 2005). A difference in HPA-axis response (or physiological response) was hypothesised to occur in the bandicoots of northern Sydney between peri-urban and natural habitats. The difference in physiological response was also

predicted to occur for bandicoots with different ecto-parasite loads and variations in body condition.

This is one of the first studies to document and compare physiological responses between free-ranging populations and the first study to compare responses between free-ranging bandicoots. Furthermore, the faecal glucocorticoid metabolites (FGMs) used to examine the physiological responses of bandicoots to a range of demographic and environmental variables, was successfully conducted with data collected using a non-invasive method. The use of non-invasive techniques has positive research outcomes, as the data collection method is not detrimental to the wellbeing of the subject. Non-invasive methods can be particularly useful when examining threatened taxa that are already dealing with environmental pressures. However, in order to test the non-invasive technique for future studies, the methods required the initial live-trapping of bandicoots. Nevertheless, a major positive outcome from this study was the identification (through non-invasive measurements of FGMs) that live-trapping does not induce a prolonged negative physiological response in captured individuals. There are obviously a number of inherent difficulties associated with undertaking studies on free-ranging endangered wildlife, such as the residual impacts associated with the stress of the trapping procedure. The data presented here suggests future studies involving live-trapping and the on-going monitoring of individual bandicoots in northern Sydney would not have a long-term negative impact.

Consistent with the analyses of body condition versus habitat type conducted for the live-trapping surveys, similar FGMs were observed in the long-nosed bandicoots of suburban backyards and National Parks. This was despite the increased risks of obtaining resources in the more open urban environments and suggests the use of suburban backyards by long-nosed bandicoots does not impart a major negative physiological response. Furthermore, no differences in the FGMs between bandicoots with high and low body condition and high or low ecto-parasite load was observed. The ecto-parasites recorded on the bandicoot of northern Sydney were demonstrating natural host-parasite relationships (Chapter 8). The parasite contributing the most to ecto-parasite load was *I. holocyclus* (paralysis tick), which is common in the outer expanses of Sydney and does not affect bandicoots with its toxin, despite records of heavy infestations (Campbell *et al.* 2003; Jackson *et al.* 2007). Considering a shared evolutionary pathway was likely between host and parasite, and that the ecto-parasite

toxin of this species does not impact bandicoots, it is unlikely that a negative physiological response would be recorded in bandicoots with a natural and/or moderate ecto-parasite load.

The study of cryptic free-ranging animals such as the southern brown bandicoot is intrinsically difficult and often presents limitations to the research. Thus, a follow-up study with increased sample numbers over a longer time-course and an ACTH challenge could further our understanding of the influence from environmental variables and increase the robustness of the analyses. Additionally, further research could be used to confirm whether a similar physiological response is likely to be observed between habitat types for future studies. A study could test whether the two species behave differently in an open environment and whether the behavioural disparities (e.g. increase in vigilance) lead to differences in physiological response and habitat use. This could directly test whether the requirements to sustain high levels of vigilance behaviour leads to an adverse physiological response. In the case of the southern brown bandicoot, the vigilance test would partially explain the reduced willingness of this species to feed in the open patches created by peri-urban habitats. Nevertheless, this non-invasive method has a broad application for scientific study and was complimentary to the other research components of the overall investigations. It should be considered an effective way of gathering biological information to guide the management of free-ranging species in a conservation context.

9.2.5 Parasites with zoonotic potential in the bandicoot populations of northern Sydney and the risks to the local inhabitants

Parasites are a significant contributor to the dissemination and spread of disease in wildlife, agricultural and human populations (Daszak *et al.* 2000). Parasites are therefore likely to have serious implications for management within the urban fringe where human-animal interactions are at their highest. Urban environments are also thought to be conducive to the range increase and survival of many parasites and the transmission of pathogens including those with zoonotic potential. From a conservation management perspective and the impact on the health of wildlife from parasites, greater consequences are also likely for threatened species, particularly populations within stressful habitats with frequent perturbations.

Recent studies of *Cryptosporidium* in Australia indicate that wildlife populations inhabiting natural or urban areas are hosts for marsupial-specific species and genotypes, as well as types with zoonotic potential (Hill *et al.* 2008; Ng *et al.* 2011; Yang *et al.* 2011). Interactions between humans and wildlife are increasing with urban expansion, presenting increased risks of parasite emergence and transmission between humans and wildlife species. As the human-wildlife interface expands, it is critical to identify pathways of transmission between hosts that share human-altered environments. Such an environment is present in northern Sydney, with frequent interactions recorded between bandicoots, humans and domestic pets. The objective of this investigation was to determine the prevalence and phylogenetic relationships of *Cryptosporidium* isolated from bandicoots in urban Sydney, and compare infection patterns between the two sympatric bandicoot species.

The information gained from the investigations has significantly contributed to wildlife disease research and to the knowledge and observations of *Cryptosporidium* in urban environments. Free-ranging bandicoots of northern Sydney were revealed to be shedding low levels of *Cryptosporidium* oocysts, with infected bandicoots having a similar body condition to uninfected individuals. Furthermore, the *Cryptosporidium* parasite was observed in bandicoots from all habitat types, highlighting the opportunity for the transmission of the parasite to occur on the urban interface between bandicoots, domestic pets and humans. This transmission pathway has management implications in an area of elevated contact between host species, particularly for any *Cryptosporidium* species with known zoonotic potential.

The *Cryptosporidium* isolates identified in this study were not genetically identical to previously described species or genotypes. Phylogenetic analysis inferred a close evolutionary relationship to *C. parvum* and *C. hominis*, two zoonotic species found in humans. However, the identity of bandicoot isolates was not fully resolved and whether the bandicoots were infected or simply passively transmitting oocysts is unknown. In addition, without a definitive identity, it could not be determined whether the bandicoots were carrying a species (or genotype) that was marsupial-specific, or of zoonotic potential. Greater phylogenetic resolution of *C. parvum* and related species in Australian fauna is required, as the parasite is recognized as a species 'complex' that consists of numerous species/genotypes, including many that may not yet have been identified.

9.2.6 Ticks and other ecto-parasites on the urban fringe; an increased opportunity for transmission between host species

Ecto-parasites are a significant cause of economic losses and contribute to the dissemination and spread of disease in wildlife, agricultural and human populations. Ticks, above all the ecto-parasites are capable of transmitting the widest range of pathogens. *Ixodes holocyclus* (paralysis tick) is one of approximately 90 species of Australian ticks and one of the least host-specific species. It can cause anaphylactic reactions in humans and death in domestic pets. Marsupials, including bandicoots are a natural host to *I. holocyclus* and are generally unaffected by its toxin. *Ixodes holocyclus* has frequently been recorded in the outer expanses of Sydney and other similar coastal and humid environments.

The encroachment of the urban environment into natural habitats has increased the interface for conflict between wildlife and humans and their pets. The urban interface also provides a pathway for the transmission of parasites across potential host species that was not historically available. However, little research has been conducted on the interactions of ecto-parasites in native Australian fauna, from their role in the transmission of pathogens to disease burden. Even fewer studies have taken both a qualitative and quantitative approach to documenting the ecto-parasites and their interactions with wildlife. I have added to the paucity of literature with this research by documenting the diversity of ecto-parasites on the bandicoots at the urban fringe of northern Sydney, and by examining the ecto-parasite relationships to a range of environmental and demographic variables.

A total of eight ecto-parasite species were documented on the bandicoots of northern Sydney, including a new host species (long-nosed bandicoot) for the mite, *Mesolaelaps australiensis*. All other ecto-parasites recorded were demonstrating natural host-parasite relationships. Considering that a shared evolutionary pathway between host and parasite was probable, it is unlikely that negative impacts from a natural ecto-parasite load would be recorded. This was confirmed by both the faecal glucocorticoid measurements conducted in Chapter 6, and the body condition analyses conducted in relation to ecto-parasite load. No difference in the body condition of individuals due to ecto-parasite load was detected, indicating that negative implications for the well-being of the southern brown bandicoot are unlikely to occur from exhibiting the parasite loads observed in this study.

The paralysis tick (*Ixodes holocyclus*) was the most common tick recorded on bandicoots, accounting for almost two-thirds of all ticks identified. Marsupials, including bandicoots are a natural host to *I. holocyclus* and are unaffected by the toxin, despite records of animals with heavy infestations. However, the high abundance of this species on bandicoots highlights the concern identified in Chapter 3 in regards to the tick carrying capacity of bandicoots. In addition, the frequent occurrence of the long-nosed bandicoot recorded in the suburban backyards of northern Sydney, provides a pathway for the transmission of the paralysis tick between host species, including bandicoots, domestic pets and humans. Of perhaps greater concern, and similar to the zoonotic potential of some *Cryptosporidium* species, is the ability of ticks to transmit other zoonosis such as Spotted Fever and Lyme's Disease (Vilcins 2008). Although there is little evidence that Lyme's Disease occurs in Australia (NSW Health 2012). The zoonosis transmission (including the paralysis tick) presents management concerns and has implications for residents, wildlife managers and the veterinary industry. Thus, management actions that are designed to promote the persistence of the southern brown bandicoot in the urban habitats of northern Sydney require consideration of the high prevalence of the paralysis tick and the ability of ticks to transmit other zoonosis.

9.3 Conclusions and further implications

The ability to study two free-ranging species with different conservation listings across predominantly the same habitat provides a great opportunity for future researchers and wildlife management to work together to deliver practical management initiatives. The management initiatives will have a wide application and be applicable to wildlife across a broad range of spectrums. The larger implications of this research are directly relevant to the long-term survival of the southern brown bandicoot and other marsupial taxa living within the urban interface. All the hallmark threats to the survival of these sympatric species exist within the outer expanses of northern Sydney. While environmental conditions present seem to be conducive to the success of the long-nosed bandicoot, the same conditions appear to limit the persistence of the southern brown bandicoot.

The current distribution of bandicoots in northern Sydney has been well established. However, continued monitoring of the populations and infra-red camera surveys would be useful in identifying the dispersal extent of both species. Direct measurement of physiological parameters was beyond the scope of this research and beyond the animal ethics and license approvals. However, detailed physiological measurements (including blood parameters) would be complimentary to this research and provide a greater understanding of the link between environmental stress and success of individuals within the current habitats.

As previously mentioned, a follow-up study of FGMs with increased sample numbers over a longer time-course and an ACTH challenge could further our understanding of the influence from environmental variables. Experiments could determine whether long-nosed bandicoots are physiologically more suited to an open environment than southern brown bandicoots. FGMs and vigilance behaviour of captive bandicoots could be measured and compared between open and closed feeding environments, enclosures with differing food availability, differing density levels of conspecifics, and high or low predator threats. Partecke *et al*. (2006b) suggests that the behavioural condition of reducing timidity and escape distance from predators is essential for species inhabiting urban environments. This supports the southern brown bandicoots' cryptic and anecdotal 'docile' nature playing a part in precluding it from occupying suburban backyards. It also suggests that southern brown bandicoots may not have mechanisms in place to cope with the increased vigilance and other requirements associated with living in open habitats.

Further investigations into the prevalence of *Cryptosporidium* would provide insight into the zoonotic potential of oocyst shedding from bandicoots in northern Sydney. This would be particularly useful on the urban interface where there is increased potential for transfer of pathogens between host species. Full sequencing from multiple genetic markers (such as *18S rRNA, actin* and *gp60* loci) to determine *Cryptosporidium* species or genotypes present and phylogenetic position would determine the zoonotic potential of *Cryptosporidium* in these habitats. Additionally, documenting a broader range of endo and ecto-parasites at the urban interface could increase our knowledge of parasites with zoonotic potential carried by bandicoots. This could be extended to include pathogens such as *Giardia, Toxoplasmosis, Hepatozoon* and tick borne pathogens such as *Rickettsia* and *Borrelia.*

The information generated from the investigations will find application with the bandicoots of northern Sydney, as well as other native marsupial species living within natural and peri-urban habitats. The research has contributed to a greater understanding of the animals in these environments and highlighted the notion that habitats on the urban fringe (suburban backyards) are in fact conducive to the survival of native species with favourable adaptive traits. Recently developed and applicable methodologies were used in the investigations to provide pertinent research that will contribute to the conservation of the southern brown bandicoot. This research will ultimately provide wildlife management with suitable information to employ conservation strategies conducive to the persistence of threatened and non-threatened Australian fauna.

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11. Appendices

Appendix A:

Capture data for the bandicoots of northern Sydney

Appendix B:

Questionnaire for the community attitudes survey

Appendix C:

GenBank Accession Numbers

Appendix D:

Publication in *European Journal of Wildlife Research* (Chapter 3)

Appendix E:

Publication in *Australian Mammalogy* (Chapter 6)

Appendix F:

Submission of publication in *Veterinary Parasitology* (Chapter 7)

Appendix G:

Final ethics approval letters

Appendix A: Capture data for the bandicoots of northern Sydney

Species	ID - Microchip	Date	Season	Reserve	Trap ID	Handler	New	Sex	Weight (g)	Pes (mm)	# of young	PY	Breeding activity
SBB	6441344	15/03/2005	3	GNP	H11		n	f					no
SBB	6442133	16/03/2005	3	GNP	H6		n	f					no
SBB	62EF643	11/03/2005	3	KCNP	V1	Peter	y	f	570	52	0	0	no
SBB	63184F8	8/03/2005	3	KCNP	BH11	Nelika	y	f	550	54	0	0	none
SBB	63184F8	11/03/2005	3	KCNP	BH11	r	r	f	550	54	0	0	no
SBB	63184F8	23/11/2005	2	KCNP	BH13	Matt	n	f	600	53	2	unfurred	pyp
SBB	63184F8	29/11/2005	2	KCNP	BH11	Matt	n	f	r	r	r	r	pyp
SBB	63184F8	1/12/2005	2	KCNP	BH11	Matt	n	f	r	r	r	r	pyp
SBB	63184F8	21/03/2006	3	KCNP	BH12	Matt	n	f	600	50.6	0	0	long teat
SBB	63184F8	22/03/2006	3	KCNP	BH12	Matt	n	f	r	r	r	r	r
SBB	63184F8	23/03/2006	3	KCNP	BH11	Alicia	n	f	r	r	0	0	lactating (3)
SBB	642D3C5	1/09/2005	1	KCNP	K35	Matt	n	f	450	55	2	unfurred	pyp
SBB	642D3C5	7/09/2005	1	KCNP	K35	Matt	n	f	475	r	r	r	r
SBB	642D3C5	8/09/2005	1	KCNP	K35	Matt	n	f	r	r	r	r	r
SBB	6442AC9	10/03/2005	3	KCNP	BH6	Matt	y	f	650	55	0	0	used teats
SBB	6442AC9	7/09/2005	1	KCNP	K4	Matt	n	f	625	54	2	unfurred	pyp
SBB	6442AC9	8/09/2005	1	KCNP	K4	Matt	n	f	r	r	r	r	r
SBB	6442AC9	9/09/2005	1	KCNP	K4	Alicia	n	f	r	r	r	r	r
SBB	6442AC9	21/03/2006	3	KCNP	BH7	Matt	n	f	600	52.8	0	0	3 large long teat
SBB	66BE792	2/10/2007	1	KCNP	K6	Matt	y	f	700	0	2	furred	pyp
SBB	66BE792	4/10/2007	1	KCNP	K6	Matt	n	f	r	r	r	r	r
SBB	66BEE17	8/09/2005	1	KCNP	K39	Matt	y	f	500	52	1	unfurred	pyp
SBB	66D4198	3/04/2008	3	KCNP	M9	Megan	y	f	425	52	0	0	none
SBB	66FF274	28/09/2007	1	KCNP	K5	Ben	y	f	500	51	0	0	long teat used
SBB	66FF274	3/10/2007	1	KCNP	K5	r	r	f	r	r	r	r	r
SBB	631D318	2/12/2005	2	KCNP	K12	Matt	y	m	800	57	m	m	m
SBB	66BDEFD	25/07/2006	4	KCNP	BH3.4	Matt	y	m	725	54.7	m	m	m
SBB	66BDEFD	16/03/2007	3	KCNP	BH1	Matt	y	m	875	58	m	m	m
SBB	66BDEFD	20/03/2007	3	KCNP	BH3.1	r	r	m	r	r	r	r	r
SBB	66BDEFD	21/03/2007	3	KCNP	BH3.5	r	r	m	r	r	r	r	r
SBB	66BDEFD	22/03/2007	3	KCNP	BH3.3.	r	r	m	r	r	r	r	r
SBB	66BE8F2	25/07/2006	4	KCNP	S5	Alicia	y	m	725	54.8	m	m	m
SBB	66CF0C4	3/04/2008	3	KCNP	V2	0	y	m	575	51	m	m	m
SBB	66FECBA	9/09/2005	1	KCNP	K4	Matt	y	m	475	55	m	m	m
SBB	66FECBA	24/11/2005	2	KCNP	BH5	Matt	n	m	575	54	m	m	m
SBB	66FECBA	29/11/2005	2	KCNP	BH4	Matt	n	m	r	r	r	r	r
SBB	66FECBA	1/12/2005	2	KCNP	BH5	Matt	n	m	r	r	r	r	r
SBB	66FECBA	21/03/2006	3	KCNP	BH10	Matt	n	m	600	61	m	m	m

Species	ID - Microchip	Condition	Ticks	Mites	Fleas	DNA	Ecto's	faeces	blood	hair	Comments - need to give measure of parasite burden
SBB	6441344	good	y	y	n	0	y	y	n	n	no weight data
SBB	6442133	good	y	n	n	0	y	y	n	n	no weight data
SBB	62EF643	good	y	y	n	1	y	y	n	n	could be sample 26 for stress - masses of poo
SBB	63184F8	good	n	n	n	1	n	y	n	n	right ear torn
SBB	63184F8	goo	n	n	n	0	n	y	n	n	recapture this session
SBB	63184F8	fair	n	n	y	0	y	y	n	n	
SBB	63184F8	fair	y	y	?	0	y	y	y	n	blood smears poor quality?
SBB	63184F8	fair	n	n	n	0	n	y	n	n	recapture this session
SBB	63184F8	good	y	y	n	0	y	n	n	n	2 holes in right ear, tick scar behind right ear
SBB	63184F8	r				0	y	n	y	y	2 slides
SBB	63184F8	good	y	y	n	0	n	n	n	n	multiple scars and scabs from tick bites
SBB	642D3C5	good	y	y	n	0	y	y	?	y	
SBB	642D3C5	fair	n	n	n	0	n	n	n	n	recapture this session
SBB	642D3C5	r	n	n	n	0	n	y	n	n	recapture (first caught 1/9/05)
SBB	6442AC9	good	y	n	n	1	y	y	n	n	
SBB	6442AC9	good	y	n	y	0	y	y	?	y	
SBB	6442AC9	good	n	n	n	0	n	y	n	n	recapture (first caught 7/9/05)
SBB	6442AC9	r	n	n	n	0	n	n	n	n	escaped before processing
SBB	6442AC9	good	y	y	y	0	y	y	n	y	tear in right ear
SBB	66BE792	fair	y	y	y	1	y	n	n	n	stumpy tail
SBB	66BE792	fair	n	n	n	0	n	y	n	n	
SBB	66BEE17	fair	y	n	y	1	y	y	n	y	
SBB	66D4198	fair	y	y	n	1	n	n	n	n	ticks and mites, might have been confused. Mites from pouch
SBB	66FF274	poor	y	n	n	1	n	y	n	n	
SBB	66FF274	r	n	n	n	0	n	y	n	n	
SBB	631D318	good	y	y	y	1	y	y	y	y	2 scars behind left ear
SBB	66BDEFD	good	y	y	y	1	y	y	n	n	150.5 - radio tracking
SBB	66BDEFD	good	y	y	n	1	y	y	n	n	very clean of parasites
SBB	66BDEFD	r	n	n	n	0	n	y	n	n	radio tracking 150.5
SBB	66BDEFD	r	n	n	n	0	n	y	n	n	
SBB	66BDEFD	r	n	n	n	0	n	y	n	n	caught during radio tracking so no data
SBB	66BE8F2	good	y	n	y	1	y	y	n	n	150.3 - radio tracking. Half a tail
SBB	66CF0C4	fair	y	y	n	1	y	n	y	y	red mites, pretty lean
SBB	66FECBA	fair	y	n	n	1	y	y	y	y	
SBB	66FECBA	fair	y	n	y	0	y	y	y	n	orange rust/fungus around skin adjacent to testes
SBB	66FECBA	r	n	n	n	0	n	y	y	n	blood smears poor quality? Recapture this session
SBB	66FECBA	r	y	n	n	0	y	y	n	n	recapture this session
SBB	66FECBA	fair	y	n	n	0	n	y	n	n	

Species	ID - Microchip	Date	Season	Reserve	Trap ID	Handler	New	Sex	Weight (g)	Pes (mm)	# of young	PY	Breeding activity
SBB	66FECBA	18/07/2006	4	KCNP	BH3	Alicia	n	m	725	58	m	m	m
SBB	66FECBA	20/07/2006	4	KCNP	BH3	r	n	m	r	r	r	r	r
SBB	66FECBA	27/07/2006	4	KCNP	BH7	r	n	m	r	r	r	r	r
SBB	66FECBA	15/09/2006	1	KCNP	DK4	Matt	n	m	700	57	0	0	0
SBB	66FECBA	20/03/2007	3	KCNP	BH6	0	n	m	700	57	m	m	m
SBB	66FECBA	3/10/2007	1	KCNP	K6	Matt	n	m	700	57	m	m	m
SBB	67006AF	27/07/2006	4	KCNP	BH10	Glen	y	m	820	53	m	m	m
SBB	67006AF	28/07/2006	4	KCNP	BH13	r	n	m	r	r	r	r	r
SBB	67006AF	13/03/2007	3	KCNP	M1	0	n	m	780	56	m	m	m
SBB	67006AF	3/10/2007	1	KCNP	M10	Matt	n	m	1050	58	m	m	m
SBB	67006AF	1/04/2008	3	KCNP	M2	Mel	n	m	1200	59.7	m	m	m
SBB	6700A94	21/03/2007	3	KCNP	JT1	RT	y	m	RT	RT	m	m	m
SBB	670155C	1/09/2005	1	KCNP	K3	Mel	y	m	625	59	m	m	m
SBB	670155C	9/09/2005	1	KCNP	K3	Alicia	n	m	625	r	r	r	r
SBB	670155C	21/03/2006	3	KCNP	BH1	Matt	n	m	675	58	m	m	m
SBB	670155C	18/07/2006	4	KCNP	BH3.2	Mel	n	m	725	59.5	m	m	m
SBB	670155C	25/07/2006	4	KCNP	BH9	r	n	m	r	r	r	r	r
SBB	670155C	7/09/2006	1	KCNP	DK3	Alicia	n	m	775	61	0	0	0
SBB	670155C	9/09/2006	1	KCNP	DK3	r	n	m	r	r	r	r	r
SBB	670155C	10/09/2006	1	KCNP	DK3	r	n	m	r	r	r	r	r
SBB	670155C	14/09/2006	1	KCNP	DK3	r	r	m	r	r	r	r	r
SBB	670155C	20/03/2007	3	KCNP	BH3.2	r	r	m	r	r	r	r	r
SBB	670155C	21/03/2007	3	KCNP	BH7	r	r	m	r	r	r	r	r
SBB	670155C	25/09/2007	1	KCNP	K1	Ben	n	m	850	58	m	m	m
SBB	670155C	26/09/2007	1	KCNP	K3	Ben	n	m	r	r	m	m	m
SBB	670155C	2/10/2007	1	KCNP	K5	0	n	m	r	r	r	r	r
SBB	670155C	3/10/2007	1	KCNP	K3	0	n	m	r	r	r	r	r
SBB	670CF36	9/10/2007	1	KCNP	K31	0	y	m	625	58	m	m	m
SBB	670F2EF	28/07/2006	4	KCNP	BH5	Matt	y	m	650	56	m	m	m
SBB	670F2EF	8/09/2006	1	KCNP	DK7	Matt	n	m	625	55	0	0	0
SBB	670F2EF	13/09/2006	1	KCNP	DK6	r	r	m	r	r	r	r	r
SBB	670F2EF	15/09/2006	1	KCNP	r	r	n	m	625	55	r	r	r
SBB	67COA23	10/10/2007	1	KCNP	K39	0	n	m	r	r	r	r	r

Species	ID - Microchip	Condition	Ticks	Mites	Fleas	DNA	Ecto's	faeces	blood	hair	Comments - need to give measure of parasite burden
SBB	66FECBA	fair	n	n	n	0	n	n	n	n	150.4 - radio tracking
SBB	66FECBA	r	n	n	n	0	n	y	n	n	recapture this session
SBB	66FECBA	fair	n	n	n	0	y	n	n	n	recapture this session
SBB	66FECBA	good	n	y	y	0	y	y	n	n	bandicoot 150.4 from radio tracking. Tail very good
SBB	66FECBA	good	n	n	n	0	n	y	n	n	radio tracking 150.8
SBB	66FECBA	poor	y	y	y	0	y	n	n	n	lots fleas, few ticks, med mites. Battle wounds on back
SBB	67006AF	good	n	n	n	1	n	n	n	n	150.1 - radio tracking
SBB	67006AF	r	n	n	n	0	n	y	n		
SBB	67006AF	good	n	y	n	0	y	n	n		
SBB	67006AF	good	y	y	n	0	y	y	n	n	few ticks, few mites - from bobbin head
SBB	67006AF	good	y	y	n	0	y	y	n	n	scat
SBB	6700A94	good	y	y	n	1	y	y	n	n	new animal, but no weights taken etc
SBB	670155C	good	y	y	n	1	y	y	n	y	1 very large engorgeed tick
SBB	670155C	r	n	n	n	0	y	y	n	n	2nd capture this session
SBB	670155C	good	n	n	n	0	n	y	n	y	couldn't get any blood
SBB	670155C	fair	n	n	n	0	n	n	n	n	150.2 - radio tracking
SBB	670155C	r	n	n	n	0	n	y	n	n	caught during radio tracking so no data
SBB	670155C	fair	y	y	y	0	y	y	n	n	inspecting tail - all good
SBB	670155C	r	n	n	n	0	n	y	n	n	recapture this session
SBB	670155C	r	n	n	n	0	y	y			recapture this session
SBB	670155C	r	n	n	n	0	y	n	n	n	
SBB	670155C	r	n	n	n	0	y	y	n	n	radio tracking 150.2
SBB	670155C	r	n	n	n	0	n	y	n	n	caught during radio tracking so no data
SBB	670155C	fair	y	n	n	0	y	n	n	n	few ticks.
SBB	670155C	fair	n	n	n	0	y	y	n	n	recapture this session
SBB	670155C	fair	n	n	n	0	n	y	n	n	
SBB	670155C	fair	n	n	n	0	n	y	n	n	
SBB	670CF36	good	y	y	n	1	y	y	n	n	
SBB	670F2EF	good	y	y	y	1	y	n	n	n	150.6 - radio tracking, short tail, lots of ecto's
SBB	670F2EF	good	y	y	n	0	y	n	n	n	scar on right hip. Tail inspected - all good
SBB	670F2EF	r	n	n	n	0	y	n	n	n	recapture this session
SBB	670F2EF	r	r	r	r	0	r	r	r	r	recapture
SBB	67COA23	r	n	n	n	0	y	y	n	n	recapture this session

Species	ID - Microchip	Date	Season	Reserve	Trap ID	Handler	New	Sex	Weight (g)	Pes (mm)	# of young	PY	Breeding activity	Condition
LNB	62E5E13	9/03/2005	3	KCNP	BH9	Glenn	y	f	500	58.8	0		none	good
LNB	62E5E13	11/03/2005	3	KCNP	BH10	r	r	f	r	r	r	r	r	r
LNB	62E5E13	23/11/2005	2	KCNP	BH8	Matt	n	f	675	57	0		used teats x2	fair
LNB	62E5E13	25/11/2005	2	KCNP	BH8	Matt	n	f	r	r	r	r	r	fair
LNB	62E5E13	29/11/2005	2	KCNP	BH9	Matt	n	f	r	r	r	r	r	r
LNB	62E5E13	30/11/2005	2	KCNP	BH10	Matt	n	f	r	r	r	r	r	r
LNB	62E5E13	1/12/2005	2	KCNP	BH13	Matt	n	f	r	r	r	r	r	r
LNB	62E5E13	22/03/2006	3	KCNP	BH13	Matt	n	f	525	59	0	0		fair
LNB	62E62D7	9/03/2005	3	KCNP	V7	Nelika	y	f	325	56	0		none	good
LNB	62E62D7	10/03/2005	3	KCNP	V8	r	r	f	r	r	r	r	r	r
LNB	62E62D7	22/03/2006	3	KCNP	V7	Matt	n	f	600	63	0	0		good
LNB	62EE42A	23/01/2006	2	Elanora	E5	Matt	y	f	1000	63	3 unfurred		pyp	good
LNB	62EE42A	24/01/2006	2	Elanora	E5	Matt	n	f	r	r	r	r	r	r
LNB	62EFF89	8/03/2005	3	KCNP	M4	Nelika	y	f	540	61	0		used teat	poor
LNB	62EFF89	29/11/2005	2	KCNP	M1	Matt	n	f	850	62	2 unfurred		pyp	good
LNB	62EFF89	1/12/2005	2	KCNP	M3	Matt	n	f	r	r	r	r	r	r
LNB	630CE75	24/01/2006	2	Elanora	E3	Matt	y	f	900	66	3 unfurred		pyp	good
LNB	6317F3B	25/11/2005	2	KCNP	BH1	Matt	n	f	600	59	1 unfurred		pyp	good
LNB	6317F3B	13/09/2006	1	KCNP	DK3	Alicia	n	f	550	62	2 unfurred		pyp	poor
LNB	631C09A	23/01/2006	2	Elanora	E12	Matt	y	f	800	67	2 furred		pyp	good
LNB	643096C	29/11/2005	2	KCNP	BH1	Matt	n	f	r	r	r	r	r	r
LNB	643096C	1/12/2005	2	KCNP	BH1	Matt	n	f	r	r	r	r	r	r
LNB	643096C	21/03/2006	3	KCNP	BH5	Matt	n	f	550	62	0		none	fair
LNB	643096C	25/07/2006	4	KCNP	BH3.2	RT	n	f	RT	RT	RT	RT	RT	RT
LNB	643096C	22/03/2007	3	KCNP	BH1.3	RT	n	f	RT	RT	RT	RT	RT	RT
LNB	6431A93	2/12/2005	2	KCNP	K8	Matt	y	f	550	59	1 unfurred		pyp	good
LNB	66BE457	14/03/2007	3	KCNP	CP5	Matt	y	f	500	60	0	0		good
LNB	66BE457	28/09/2007	1	KCNP	K29	0	n	f	300	60	0		long teat used	poor
LNB	66BE457	29/09/2007	1	KCNP	K29	0	n	f	r	r	0		long teat	poor
LNB	66BE457	1/04/2008	3	KCNP	CP5	Matt	n	f	600	64	0		none	fair
LNB	66BF214	13/09/2006	1	KCNP	DK37	Matt	y	f	650	61	2 unfurred		pyp	fair
LNB	66BF9E6	13/09/2006	1	KCNP	DK2	Alicia	y	f	685	?		0		0
LNB	66BF9E6	14/09/2006	1	KCNP	DK2	r	r	f	r	r	r	r	r	r
LNB	66BF9E6	15/09/2006	1	KCNP	DK2	Alicia	n	f	675	63	3 unfurred		pyp	fair
LNB	66C0158	27/09/2006	1	GNP	G27	Alicia	y	f	725	62	2 unfurred		pyp	good
LNB	66C94BE	22/03/2006	3	KCNP	C12	Matt	y	f	375	56	0	0		good
LNB	66C9F19	7/04/2006	3	GNP	-2A	Matt	y	f	375	68	0	0		good
LNB	66CAAB7	1/04/2008	3	KCNP	BH11	Mel	y	f	525	63.1	0		none	good
LNB	66CBB3D	8/09/2005	1	KCNP	K23	Matt	y	f	525	65			no	fair
LNB	66CBB3D	24/11/2005	2	KCNP	V13	Matt	n	f	550	66	3 unfurred		pyp	poor

Species	ID - Microchip	Ticks	Mites	Fleas	DNA	Ecto's	faeces	blood	hair	Comments - need to give measure of parasite burden
LNB	62E5E13	n	n	n	1	n	y	n	n	
LNB	62E5E13	n	n	n	0	n	y	n	n	recapture this session
LNB	62E5E13	n	y	y	0	y	y			
LNB	62E5E13	n	y	n	0	y	y			in elliot, recapture this session
LNB	62E5E13	y	y	?	0	y	y	n	n	recapture this session
LNB	62E5E13	n	n	n	0	n	y	n	n	recapture this session
LNB	62E5E13	n	n	n	0	n	n	n	n	recapture this session, no data
LNB	62E5E13	n	y	y	0	y	n	y	n	2 slides
LNB	62E62D7	n	y	n	1	n	y	n	n	
LNB	62E62D7	n	n	n	0	y	n	n	n	recapture this session
LNB	62E62D7	y	n	n	0	y	y	y	y	
LNB	62EE42A	n	n	n	1	n	y	n	y	no ecto's
LNB	62EE42A	n	n	n	0	n	y	n	n	recapture this session
LNB	62EFF89	y	y	n	1	y	n	n	n	very few ticks parasite load 2
LNB	62EFF89	?	y	y	0	y	y	n	n	
LNB	62EFF89	n	n	n	0	n	y	n	n	recapture this session
LNB	630CE75	y	n	n	1	y	y	n	n	very few ecto's
LNB	6317F3B	y	y	n	0	y	y	n	n	
LNB	6317F3B	y	n	n	0	y	y	n	n	body condition poor
LNB	631C09A	y	n	n	1	y	y	n	n	one large tick otherwise clean
LNB	643096C	y	?	?	0	y	n	n	n	recapture this session, has 2 microchips
LNB	643096C	n	n	n	0	n	y	y	n	blood smear ok
LNB	643096C	n	y	n	0	y	y	y	y	slides are poor
LNB	643096C	n	n	n	0	n	y	n	n	caught during radio tracking so no data
LNB	643096C	n	n	n	0	n	y	n	n	caught during radio tracking so no data
LNB	6431A93	y	y	n	1	y	y	y	y	
LNB	66BE457	n	y	n	1	y	y	n	n	
LNB	66BE457	n	n	y	0	n	y	n	n	caught in elliot
LNB	66BE457	n	n	n	0	y	y	n	n	
LNB	66BE457	n	y	y	0	y	n	n	n	mites, fleas
LNB	66BF214	y	y	y	1	y	y	n	n	had ear biopsy but no microchip - caught juvenile in Sept 05
LNB	66BF9E6	n	n	n	0	n	n	n	n	escaped after microchip, already had DNA - likely to be from radio tracking
LNB	66BF9E6	n	n	n	0	y	n	n	n	recapture this session
LNB	66BF9E6	n	n	n	0	n	n	n	y	animal chipped but didn't read - too stressed to re-chip, prob 66BF9E6
LNB	66C0158	n	n	y	1	y	y	n	y	
LNB	66C94BE	n	y	y	1	y	n	n	y	
LNB	66C9F19	n	y	n	1	y	y	n	n	
LNB	66CAAB7	n	y	n	1	n	y	n	n	mites in pouch, scats
LNB	66CBB3D	y	y	n	1	y	y	n	n	torn right ear, so biopsy of left ear
LNB	66CBB3D	n	y	y	0	y	y	n	n	large injury on back of neck (was open wound) and half left ear missing

Species	ID - Microchip	Date	Season	Reserve	Trap ID	Handler	New	Sex	Weight (g)	Pes (mm)	# of young	PY	Breeding activity	Condition
LNB	66CBB3D	30/11/2005	2	KCNP	V11	Matt	n	f	r	r	r	unfurred	pyp	poor
LNB	66CBB3D	24/03/2006	3	KCNP	V12	Matt	n	f	700	66	0		long teat (3)	good
LNB	66D498F	22/09/2005	1	GNP	G12	Matt	y	f	500	61	2	unfurred	pyp	good
LNB	66D5609	23/01/2006	2	Elanora	E4	Matt	y	f	1100	63	3	unfurred	pyp	good
LNB	66D5748	13/03/2007	3	KCNP	V1	0	y	f	700	66	0	0		good
LNB	66D5F11	13/09/2007	1	GNP	G26	Tash	y	f	1255	66	2	furred	pyp	good
LNB	66D6BF2	7/09/2006	1	KCNP	DK6	Matt	y	f	750	63.2	0	0		fair
LNB	66D6BF2	9/09/2006	1	KCNP	DK6	0	y	f	0	0	0	0		0
LNB	66D6BF2	14/09/2006	1	KCNP		r	r	f	r	r	r	r	r	r
LNB	66D6BF2	20/03/2007	3	KCNP	BH1.3	RT	n	f	RT	RT	RT	RT	RT	RT
LNB	66D6BF2	21/03/2007	3	KCNP	BH1.3	r	r	f	625	r	r	r	none	good
LNB	66D6D05	21/09/2005	1	GNP	G9	Alicia	y	f	700	68	3	unfurred	pyp	fair
LNB	66FE91F	22/03/2007	3	KCNP	J4	RT	y	f	RT	RT	RT	RT	RT	RT
LNB	66FF3F6	27/03/2008	3	GNP	H12	Mel	y	f	575	65.8	0		none	good
LNB	67006DC	2/04/2008	3	KCNP	CP1	Matt	y	f	600	64	0		long teat used (2)	poor
LNB	670EA70	28/09/2006	1	GNP	G28	Alicia	y	f	650	53	0		long teats	good
LNB	670EA70	12/09/2007	1	GNP	G28	Tash	n	f	1010	63.1	2	furred	pyp	good
LNB	670EA70	19/09/2007	1	GNP	G28	r	r	f	r	r	r	r	r	r
LNB	671004D	5/04/2006	3	GNP	SP05	Matt	y	f	700	?	0		long teat	good
LNB	671004D	12/04/2006	3	GNP	SP05	Matt	n	f	r	r	r	r	r	r
LNB	BH13	25/07/2006	4	KCNP	BH13	RT	y	f	RT	RT		RT	RT	RT
LNB	EH4	27/06/2007	4	Elanora	EH4	Matt	y	f	700	60	0		none	good
LNB	EH5	26/06/2007	4	Elanora	EH5	Matt	y	f	1000	65	0		none	good
LNB	EH5B	27/06/2007	4	Elanora	EH5	Matt	y	f	850	68	1	unfurred	pyp	good
LNB	juvenile - CP6	30/11/2005	2	KCNP	CP6	Matt	y	f	150	48			no	good
LNB	juvenile - K37	6/09/2005	1	KCNP	K37	Alicia	y	f	125	42			no	fair
LNB	juvenile - K37	7/09/2005	1	KCNP	K37	Matt	n	f	140	42	r	r	r	fair
LNB	juvenile - K37	8/09/2005	1	KCNP	K37	Matt	n	f	r	r	r	r	r	r
LNB	juvenile - K40	9/09/2005	1	KCNP	K40	Matt	y	f	100	44			no	fair
LNB	no chip - E9	25/01/2006	2	Elanora	E9	Matt	y	f	1050	68	3	furred	pyp	good
LNB	no chip - S1	6/02/2006	2	St.Ives	S1	Matt	y	f	850	65			used teats	good
LNB	no chip - S12	6/02/2006	2	St.Ives	S12	Matt	y	f	1000	63	3	unfurred	pyp	good
LNB	no chip - T1	30/01/2006	2	Turramurra	T1	Matt	y	f	900	64	2	furred	pyp	good
LNB	no chip - T10	30/01/2006	2	Turramurra	T10	Matt	y	f	1200	70	2	unfurred	pyp	good
LNB	no chip - T16	30/01/2006	2	Turramurra	T16	Matt	y	f	1000	65	2	unfurred	pyp	good
LNB	no chip - T16	1/02/2006	2	Turramurra	T16	Matt	n	f	r	r	r	r	r	r
LNB	no chip - T16b	1/02/2006	2	Turramurra	T16	Matt	y	f	1050	65	2	furred	pyp	good
LNB	no chip - T16b	2/02/2006	2	Turramurra	T16	Matt	y	f	r	r	r	r	r	r
LNB	no chip - T9	30/01/2006	2	Turramurra	T9	Matt	y	f	900	63	3	unfurred	pyp	good
LNB	no chip - T9	31/01/2006	2	Turramurra	T9	Matt	n	f	r	r	r	r	r	r

Species	ID - Microchip	Ticks	Mites	Fleas	DNA	Ecto's	faeces	blood	hair	Comments - need to give measure of parasite burden
LNB	66CBB3D	n	n	n	0	n	y	n	n	injuries have improved, young still present
LNB	66CBB3D	n	n	n	0	n	y	n	n	no ears to get blood
LNB	66D498F	y	y	n	1	y	y	y	n	
LNB	66D5609	y	y	n	1	y	y	n	n	very few ecto's
LNB	66D5748	n	n	n	1	n	n	n	n	
LNB	66D5F11	n	n	n	1	n	n	n	n	biopsy from left ear
LNB	66D6BF2	n	n	n	1	n	y	n	n	clean of ectos
LNB	66D6BF2	n	n	n	1	n	n	n	n	pouriing rain, no other data gathered
LNB	66D6BF2	n	n	n	0	y	n	n	n	recapture this session
LNB	66D6BF2	n	n	n	0	y	n	n	n	caught during radio tracking so no data
LNB	66D6BF2	n	n	n	0	n	y	n	n	caught during radio tracking so no data
LNB	66D6D05	y	n	y	1	y	y	n	y	
LNB	66FE91F	n	n	n	1	y	y	n	n	caught during radio tracking so no data
LNB	66FF3F6	n	y	n	1	y	n	n	n	
LNB	67006DC	n	n	n	1	n	n	n	n	no parasites
LNB	670EA70	y	n	n	1	y	n	n	y	clean pouch - weird pouch.
LNB	670EA70	y	n	n	0	y	n	n	n	
LNB	670EA70	n	n	n	0	n	y	n	n	recapture this session
LNB	671004D	y	n	n	1	n	y	n	n	escaped
LNB	671004D	r	r	r	0	y	y	n	n	recapture this session
LNB	BH13	n	n	n	1	n	y	n	n	caught during radio tracking so no data
LNB	EH4	y	n	n	1	y	y	n	n	ticks medium
LNB	EH5	n	n	n	1	n	y	n	n	
LNB	EH5B	y	y	n	0	y	y	n	n	many mites, ticks few
LNB	juvenile - CP6	y	y	y	1	y	y	n	n	too small to chip
LNB	juvenile - K37	y	y	y	1	y	n	n	n	juvenile, too small to microchip
LNB	juvenile - K37	y	n	y	0	y	n	n	n	same as 6/9/05 K37
LNB	juvenile - K37	r	r	r	0	n	n	n	n	recapture (released, nothing)
LNB	juvenile - K40	y	n	n	1	y	y	n	n	juvenile, too small to microchip
LNB	no chip - E9	y	n	n	1	y	y	n	n	very few ticks
LNB	no chip - S1	y	n	n	0	y	n	n	n	no faeces, no marks, no DNA
LNB	no chip - S12	y	n	n	1	y	y	n	n	small tare in right ear. Very clean of ecto's
LNB	no chip - T1	y	y	n	1	y	y	n	n	very ticks and mites
LNB	no chip - T10	n	y	n	1	n	y	n	n	chunk from left ear missing, no ticks
LNB	no chip - T16	y	y	n	1	y	y	n	n	left eye missing or injured, right ear split
LNB	no chip - T16	n	n	n	0	n	y	n	n	recapture this session
LNB	no chip - T16b	y	y	n	1	y	y	n	n	left ear small scar, few mites on rump, py really big
LNB	no chip - T16b	n	n	n	0	n	n	n	n	recapture this session
LNB	no chip - T9	n	y	n	1	y	y	n	n	missing tail, split left ear, no ticks
LNB	no chip - T9	n	n	n	0	n	y	n	n	recapture this session

Species	ID - Microchip	Date	Season	Reserve	Trap ID	Handler	New	Sex	Weight (g)	Pes (mm)	# of young	PY	Breeding activity	Condition
LNB	no chip - T9	2/02/2006	2	Turramurra	T9	Matt	n	f	r	r	r	r	r	r
LNB	not chipped	14/09/2006	1	KCNP	DK37	Matt	n	f	650	62	2	0	pyp	poor
LNB	NT1	27/06/2007	4	Turramurra	NT1	Matt	n	f	r	r	r	r	r	r
LNB	NT1	26/06/2007	4	Turramurra	NT1	Matt	y	f	1100	68	1	unfurred	pyp	good
LNB	NT11A	26/06/2007	4	Turramurra	NT11	Matt	y	f	950	64	2	unfurred	pyp	good
LNB	NT11A	28/06/2007	4	Turramurra	NT11	Matt	n	f	r	r	r	r	r	good
LNB	NT5	27/06/2007	4	Turramurra	NT5	Matt	y	f	650	64		0	none	fair
LNB	NT7	26/06/2007	4	Turramurra	NT7	Matt	n	f	1050	66	2	unfurred	pyp	good
LNB	NT7	28/06/2007	4	Turramurra	NT6	Matt	n	f	r	r	r	r	r	r
LNB	RTBH1	27/07/2006	4	KCNP	BH1	RT	n	f	RT	RT		RT	RT	RT
LNB	RTS4	19/07/2006	4	KCNP	S4	RT	n	f	625	RT		RT	RT	RT
LNB	6700115	19/09/2007	1	GNP	G10		0 y	m	0	0	m	m	m	good
LNB	6442E81	23/01/2006	2	Elanora	E1	Matt	y	m	650	64	m	m	m	good
LNB	6442E81	24/01/2006	2	Elanora	E1	Matt	n	m	r	r	r	r	r	good
LNB	1F124F8	6/09/2005	1	KCNP	K21	Matt	y	m	625	66	m	m	m	good
LNB	1F124F8	30/11/2005	2	KCNP	V1	Matt	n	m	850	65	m	m	m	good
LNB	1F124F8	21/03/2006	3	KCNP	V1	Matt	n	m	925	67	0	0	0	good
LNB	1F15E2D	3/04/2008	3	KCNP	V11		0 y	m	565	56	m	m	m	fair
LNB	62E3736	8/03/2005	3	KCNP	V2	Mel	y	m	1100	64	m	m	m	good
LNB	62E3736	9/03/2005	3	KCNP	V3	r	r	m	r	r	r	r	r	good
LNB	62E3736	30/08/2005	1	KCNP	K23	Matt	n	m	1150	64	m	m	m	good
LNB	62E3736	7/09/2005	1	KCNP	K22	Matt	n	m	1100	64	m	m	m	good
LNB	62E3736	8/09/2005	1	KCNP	K22	Matt	n	m	r	r	r	r	r	good
LNB	62E3736	9/09/2005	1	KCNP	K22	Matt	n	m	1100	r	r	r	r	good
LNB	630F95A	9/03/2005	3	KCNP	M5	Glenn	y	m	885	70.7	m	m	m	good
LNB	631AF47	30/08/2005	1	KCNP	K14	Mel	y	m	1100	71	m	m	m	fair
LNB	631D665	20/09/2006	1	GNP	G32	Alicia	n	m	1100	69	0	0	0	fair
LNB	631FB4F	23/09/2005	1	GNP	G13	Matt	n	m	950	70	m	m	m	good
LNB	642D095	6/04/2006	3	GNP	SP06	Matt	y	m	400	60	0	0	0	good
LNB	643278F	23/09/2005	1	GNP	G31	Matt	n	m	775	67	m	m	m	good
LNB	643278F	26/09/2006	1	GNP	G29	Matt	n	m	975	45	0	0	0	good
LNB	643F268	7/09/2005	1	KCNP	K40	Alicia	y	m	1000	67	m	m	m	good
LNB	643F9CD	1/09/2005	1	KCNP	K19	Mel	y	m	900	67	m	m	m	good
LNB	643F9CD	7/09/2005	1	KCNP	K19	Matt	n	m	850	66	m	m	m	good
LNB	66BD806	2/04/2008	3	KCNP	CP3	Matt	y	m	450	59.5	m	m	m	good
LNB	66BD9C9	4/04/2008	3	KCNP	CP3	Ben	y	m	225	0	m	m	m	fair
LNB	66BEOF5	27/07/2006	4	KCNP	BH3.5	Tash	n	m	820	67	m	m	m	good
LNB	66BFE7D	20/09/2006	1	GNP	G28	Alicia	y	m	2050	72	0	0	0	fair
LNB	66CFA75	30/11/2005	2	KCNP	V2	Matt	y	m	450	60	m	m	m	good
LNB	66CFA75	1/12/2005	2	KCNP	V1	Matt	n	m	r	r	r	r	r	r

Species	ID - Microchip	Ticks	Mites	Fleas	DNA	Ecto's	faeces	blood	hair	Comments - need to give measure of parasite burden
LNB	no chip - T9	n	n	n	0	n	n	y	n	recapture this session, blood - 2 slides - poor
LNB	not chipped	y	n	n	0	y	n	n	n	Right ear torn DNA hole, torn skin on back x 2, not chipped cause of py
LNB	NT1	n	n	n	0	n	n	n	n	no samples
LNB	NT1	y	n	n	1	y	y	n	n	few ectos
LNB	NT11A	n	y	n	1	y	y	n	n	few ectos
LNB	NT11A	n	n	n	0	n	y	n	n	recapture this session
LNB	NT5	y	y	n	0	y	y	n	n	mites heaps, ticks few
LNB	NT7	y	y	n	0	y	y	n	n	few ectos
LNB	NT7	n	n	n	0	n	y	n	n	recapture this session, no tail
LNB	RTBH1	n	n	n	0	y	n	n	n	caught during radio tracking so no data
LNB	RTS4	n	n	n	0	y	y	n	n	caught during radio tracking so no data
LNB	6700115	y	y	y	1	y	y	n	n	ticks lots, fleas med, mites low
LNB	6442E81	y	n	n	1	y	y	n	y	no ecto's
LNB	6442E81	n	n	n	0	n	y	y	n	recapture this session
LNB	1F124F8	y	n	n	1	y	y	y	y	
LNB	1F124F8	y	n	y	0	y	y			
LNB	1F124F8	y	n	n	0	y	y	y	y	slides are poor
LNB	1F15E2D	n	n	n	1	n	n	n	n	no parasites
LNB	62E3736	y	y	n	1	y	y	n	n	very few ticks parasite load 2
LNB	62E3736	n	n	n	0	n	n	n	n	recapture this session
LNB	62E3736	y	y	n	0	y	y	n	y	full tail
LNB	62E3736	y	?	?	1	y	n	n	y	
LNB	62E3736	n	n	n	0	n	n	y	n	thin smear maybe?
LNB	62E3736	n	n	n	0	n	y	n	n	recapture (first caught 7/9/05)
LNB	630F95A	n	n	n	1	n	y	n	n	
LNB	631AF47	y	n	y	1	y	y	n	y	skin fairly orange and stumpy tail (5cm)
LNB	631D665	y	n	y	0	y	y	n	y	
LNB	631FB4F	y	n	y	0	y	y	n	y	half left ear missing
LNB	642D095	n	y	n	1	y	y	n	y	
LNB	643278F	y	n	y	0	y	y	y	y	
LNB	643278F	y	n	y	0	y	y	n	n	
LNB	643F268	y	n	n	1	y	y	n	n	
LNB	643F9CD	y	y	n	1	y	y	n	y	
LNB	643F9CD	y	n	n	0	y	y	n	y	mattered hair
LNB	66BD806	n	n	y	1	y	n	n	n	not confident chip will stay in, fleas
LNB	66BD9C9	n	n	n	0	n	y	n	n	escaped while chipping
LNB	66BEOF5	n	n	n	0	n	n	n	n	radio tracking - no tail
LNB	66BFE7D	y	y	y	1	y	y	n	y	a few large ticks, stumpy tail
LNB	66CFA75	?	y	n	1	y	y			
LNB	66CFA75	y	n	n	0	y	n	n	n	recapture this session

Species	ID - Microchip	Date	Season	Reserve	Trap ID	Handler	New	Sex	Weight (g)	Pes (mm)	# of young	PY	Breeding activity	Condition
LNB	66COA23	27/09/2007	1	KCNP	K22	Megan	y	m	1100	66	m	m	m	good
LNB	66COA23	10/10/2007	1	KCNP	K22		0 n	m	r	r	r	r	r	good
LNB	66D4500	1/04/2008	3	KCNP	BH7	Mel	n	m	700	65.3	m	m	m	good
LNB	66D4B16	30/11/2005	2	KCNP	V4	Matt	y	m	450	59	m	m	m	good
LNB	66D4B16	1/12/2005	2	KCNP	V2	Matt	n	m	r	r	r	r	r	r
LNB	66D4BA9	7/09/2005	1	KCNP	K15	Matt	y	m	750	64	m	m	m	good
LNB	66D4BA9	8/09/2005	1	KCNP	K15	Matt	n	m	r	r	r	r	r	good
LNB	66D517F	21/03/2006	3	KCNP	BH11	Matt	y	m	350	58.8	0	0	0	fair
LNB	66D517F	22/03/2006	3	KCNP	BH10	Matt	n	m	r	r	r	r	r	r
LNB	66D517F	20/07/2006	4	KCNP	BH8	RT	n	m	RT	RT	RT	RT	RT	RT
LNB	66D517F	25/07/2006	4	KCNP	BH8	RT	n	m	RT	RT	RT	RT	RT	RT
LNB	66D517F	26/07/2006	4	KCNP	BH8	r	n	m	r	r	r	r	r	r
LNB	66D517F	13/03/2007	3	KCNP	BH7		0 n	m	750	67	m	m	m	good
LNB	66D517F	14/03/2007	3	KCNP	BH7	Matt	n	m	r	r	r	r	r	r
LNB	66D517F	15/03/2007	3	KCNP	BH7	r	r	m	r	r	r	r	r	r
LNB	66D517F	20/03/2007	3	KCNP	J1	r	r	m	r	r	r	r	r	r
LNB	66D6149	23/09/2005	1	GNP	G25	Matt	y	m	1200	71	m	m	m	good
LNB	66D6A07	14/09/2006	1	KCNP	DK19	Matt	y	m	600	61.5	0	0	0	good
LNB	66D6A07	27/09/2007	1	KCNP	K18	Megan	n	m	900	68	m	m	m	good
LNB	66D6D69	3/10/2007	1	KCNP	K7	Matt	y	m	1850	74	m	m	m	good
LNB	66D7356	23/09/2005	1	GNP	G9	Alicia	y	m	675	59	m	m	m	fair
LNB	66D764B	22/11/2005	2	KCNP	CP5	Matt	y	m	1300	71	m	m	m	good
LNB	66D764B	25/11/2005	2	KCNP	CP9	Matt	n	m	r	r	r	r	r	r
LNB	66D7AOF	27/03/2008	3	GNP	C10	Mel	n	m	1050	70.9	m	m	m	good
LNB	66FE829	13/09/2007	1	GNP	G9	Matt	y	m	1000	67	m	m	m	good
LNB	66FF1E8	31/03/2006	3	GNP	Cayeli 12	Matt	y	m	475	64	m	m	m	good
LNB	66FF557	22/09/2006	1	GNP	30	Matt	y	m	525	63	0	0	0	good
LNB	66FFC9C	10/10/2007	1	KCNP	K31	Ben	y	m	700	63	m	m	m	poor
LNB	66FFC9C	11/10/2007	1	KCNP	K31		0 n	m	r	r	m	m	m	r
LNB	66FFC9C	12/10/2007	1	KCNP	K31		0 n	m	r	r	m	m	m	r
LNB	6700C2C	20/09/2007	1	GNP	G9	Matt	y	m	1250	66	m	m	m	good
LNB	6700DC8	4/04/2008	3	KCNP	M6	Matt	y	m	600	62.5	m	m	m	good
LNB	670D79E	28/03/2008	3	KCNP	LC1		0 y	m	540	63	m	m	m	good
LNB	670O945	22/09/2006	1	GNP	G9	Matt	y	m	950	67	0	0	0	good
LNB	67105D5	27/03/2008	3	GNP	Cayeli 7	Mel	y	m	1250	648	m	m	m	good
LNB	EH5C	28/06/2007	4	Elanora	EH5	Matt	y	m	750	66	m	m	m	good
LNB	juvenile - E7	26/01/2006	2	Elanora	E7	Matt	y	m	150	47	m	m	m	fair
LNB	juvenile - M12	29/11/2005	2	KCNP	M12	Matt	y	m	275	53	m	m	m	good
LNB	juvenile - M12	30/11/2005	2	KCNP	M11	Matt	n	m	r	r	r	r	r	r
LNB	juvenile - M12	1/12/2005	2	KCNP	M10	Matt	n	m	r	r	r	r	r	r

Species	ID - Microchip	Ticks	Mites	Fleas	DNA	Ecto's	faeces	blood	hair	Comments - need to give measure of parasite burden
LNB	66COA23	y	n	y	1	y	y	n	n	scarring along dorsum
LNB	66COA23	n	n	n	0	n	y	n	n	
LNB	66D4500	n	n	n	0	n	n	n	n	very clean, no ecto's. Had ear hole but no chip
LNB	66D4B16	y	y	y	1	y	y			
LNB	66D4B16	y	n	n	0	y	y	n	n	recapture this session
LNB	66D4BA9	y	n	y	1	y	y	n	n	caught in elliot
LNB	66D4BA9	n	n	n	0	n	n	y	n	recapture (first caught 7/9/05)
LNB	66D517F	n	n	y	1	n	n	n	n	young animal
LNB	66D517F				0	n	n	y	n	2 slides
LNB	66D517F	n	n	n	0	n	n	n	n	radio tracking, so no data
LNB	66D517F	n	n	n	0	n	n	n	n	radio tracking, so no data
LNB	66D517F	n	n	n	0	y	n	n	n	recapture this session
LNB	66D517F	n	n	n	0	n	n	n	n	
LNB	66D517F	n	n	n	0	y	y	n	n	recapture this session
LNB	66D517F	n	n	n	0	n	y	n	n	recapture this session
LNB	66D517F	n	n	n	0	n	y	n	n	caught during radio tracking so no data
LNB	66D6149	y	n	y	1	y	y	y	y	
LNB	66D6A07	n	n	y	1	y	n	n	n	
LNB	66D6A07	n	n	n	0	n	n	n	n	
LNB	66D6D69	y	y	n	1	y	y	n	n	no tail - few old scars on back
LNB	66D7356	y	n	y	1	y	y	n	y	
LNB	66D764B	y	n	n	1	y	y	n	n	large scar on back, several smaller scars
LNB	66D764B	n	n	n	0	y	n	n	n	recapture this session
LNB	66D7AOF	n	n	y	0	y	n	n	n	
LNB	66FE829	y	y	y	1	y	y	n	n	lots of fleas
LNB	66FF1E8	n	n	n	1	n	n	n	n	no ecto's - clean - first bandicoot caught at Cayelii traps
LNB	66FF557	y	y	y	1	y	y	n	n	
LNB	66FFC9C	y	y	y	1	y	n	n	n	
LNB	66FFC9C	n	n	n	0	n	y	n	n	
LNB	66FFC9C	n	n	n	0	n	y	n	n	
LNB	6700C2C	y	y	y	1	y	y	n	n	
LNB	6700DC8	n	y	y	1	y	n	n	n	lots of mites
LNB	670D79E	n	n	y	1	y	n	n	n	2-3 of tail
LNB	670O945	y	n	y	1	y	y	n	n	white tipped tail
LNB	67105D5	y	y	n	1	y	n	n	n	stumpy tail
LNB	EH5C	y	n	y	1	y	y	n	n	ticks few, fleas few
LNB	juvenile - E7	y	y	n	1	y	n	n	n	small ticks / mites, no faeces
LNB	juvenile - M12	n	n	n	1	n	y	n	n	too small to chip
LNB	juvenile - M12	n	n	n	0	n	y	n	n	recapture this session
LNB	juvenile - M12	n	n	n	0	n	n	n	n	recapture this session

Species	ID - Microchip	Date	Season	Reserve	Trap ID	Handler	New	Sex	Weight (g)	Pes (mm)	# of young	PY	Breeding activity	Condition
LNB	juvenile BH7	14/03/2007	3	KCNP	BH7	Ben	y	m	275	50				poor
LNB	juvenile BH7	15/03/2007	3	KCNP	BH7	r	r	m	r	r	r	r	r	r
LNB	juvenile C8	8/03/2007	3	GNP	C8	Tash	y	m	310	58	m	m	m	good
LNB	juvenile K8	7/03/2007	3	GNP	K8		Y	m						
LNB	juvenile M8	11/03/2005	3	KCNP	M8	Peter	y	m	100	44	m	m	m	good
LNB	no chip - E6	24/01/2006	2	Elanora	E6	Matt	y	m	1375	77	r	r	r	good
LNB	no chip - E6	25/01/2006	2	Elanora	E6	Matt	n	m	r	r	r	r	r	r
LNB	no chip - T13	31/01/2006	2	Turramurra	T13	Matt	y	m	1000	64	m	m	m	good
LNB	no chip - T5	31/01/2006	2	Turramurra	T5	Matt	y	m	1600	74	m	m	m	good
LNB	no chip - T6	2/02/2006	2	Turramurra	T6	Matt	y	m	1050	65	m	m	m	good
LNB	no chip - T7	31/01/2006	2	Turramurra	T7	Matt	y	m	800	68	m	m	m	good
LNB	not chippedA	14/09/2006	1	KCNP	DK22	Matt	y	m	840	68.5	0	0	0	good
LNB	not chippedB	9/10/2007	1	KCNP	K31	Ben	y	m	75	42	m	m	m	poor
LNB	not chippedC	4/04/2008	3	KCNP	CP1	Ben	y	m	150	41	m	m	m	poor
LNB	NT11B	27/06/2007	4	Turramurra	NT11	Matt	y	m	1050	73	m	m	m	good
LNB	NT11B	28/06/2007	4	Turramurra	NT11	Matt	n	m	r	r	r	r	r	r
LNB	NT4	27/06/2007	4	Turramurra	NT4	Matt	y	m	950	70	m	m	m	good
LNB	NT4B	28/06/2007	4	Turramurra	NT4	Matt	y	m	1350	72	m	m	m	good
LNB	NT5B	28/06/2007	4	Turramurra	NT5	Matt	y	m	1450	73	m	m	m	good
LNB	NT8	27/06/2007	4	Turramurra	NT8	Matt	y	m	800	68	m	m	m	good
LNB	RTBH1	19/07/2006	4	KCNP	BH1	RT	n	RT	655	RT	RT	RT	RT	RT
LNB	RTBH11	28/07/2006	4	KCNP	BH11	RT	y	RT	RT	RT	RT	RT	RT	RT
LNB	RTBH5	27/07/2006	4	KCNP	BH5	RT	n	RT	RT	RT	RT	RT	RT	RT
LNB	RTJT4	26/07/2006	4	KCNP	JT4	RT	y	RT	RT	RT	RT	RT	RT	RT
LNB	RTS1	19/07/2006	4	KCNP	S1	RT	n	RT	RT	RT	RT	RT	RT	RT
LNB	RTS2	26/07/2006	4	KCNP	S2	RT	y	RT	RT	RT	RT	RT	RT	RT
LNB	RTS4	26/07/2006	4	KCNP	S4	RT	y	RT	RT	RT	RT	RT	RT	RT

Species	ID - Microchip	Ticks	Mites	Fleas	DNA	Ecto's	faeces	blood	hair	Comments - need to give measure of parasite burden
LNB	juvenile BH7	n	y	n	1	y	n	n	n	
LNB	juvenile BH7	n	n	n	0	n	y	n	n	recapture this session
LNB	juvenile C8	n	y	y	1	y	y	n	n	
LNB	juvenile K8	y								
LNB	juvenile M8	n	n	n	1	n	n	n	n	no ectos taken
LNB	no chip - E6	n	n	n	1	n	y	n	n	no ecto's
LNB	no chip - E6	y	n	n	0	y	y	n	n	recapture this session, no blood - wouldn't keep still
LNB	no chip - T13	y	n	n	1	y	n	y	n	no marks
LNB	no chip - T5	y	y	n	1	y	y	n	n	no tail, left ear split, few other scars, no poo ticks or biopsy
LNB	no chip - T6	y	y	n	1	y	y	n	n	few to no ticks
LNB	no chip - T7	y	n	n	1	y	n	n	n	no scars, younger animal, no poo or blood
LNB	not chippedA	y	?	?	1	y	?	?		no tail, biopsy right ear, nick in left
LNB	not chippedB	n	n	y	1	n	n	n	n	very very small
LNB	not chippedC	n	n	n	1	n	n	n	n	2 biopsy's - very small, no parasites visible
LNB	NT11B	y	y	n	0	y	y	n	n	heaps mites, ticks med
LNB	NT11B	n	n	n	0	n	y	n	n	recapture this session
LNB	NT4	y	y	n	0	y	y	n	n	mites med, ticks few
LNB	NT4B	y	y	y	1	y	y	n	n	ticks med, mites, few, fleas few
LNB	NT5B	y	y	y	1	y	y	n	n	ticks many, mites few, fleas few
LNB	NT8	y	y	n	0	y	y	n	n	mites heaps, ticks few
LNB	RTBH1	n	n	n	0	y	y	n	n	caught during radio tracking so no data
LNB	RTBH11	n	n	n	1	y	n	n	n	caught during radio tracking so no data
LNB	RTBH5	n	n	n	0	y	n	n	n	caught during radio tracking so no data
LNB	RTJT4	n	n	n	1	n	n	n	n	caught during radio tracking so no data
LNB	RTS1	n	n	n	0	y	y	n	n	caught during radio tracking so no data
LNB	RTS2	n	n	n	1	n	n	n	n	caught during radio tracking so no data
LNB	RTS4	n	n	n	1	n	n	n	n	caught during radio tracking so no data

Appendix B: Questionnaire for the community attitudes survey

Bandicoots in Your Backyard
Survey 2005

This survey is divided into 3 sections. The first asks you to tell me the level of bandicoot activity in your backyard. The second and third asks what you know about bandicoots through questions and a series of statements. Thank you in advance for your participation. If filling it out as a word document, please just click on the appropriate box. If you need to type anything just click in the shaded area next to the question.

<u>Bandicoot Activities</u>

1) Do you know what a bandicoot looks like?   **Yes** ☐
                                                     No ☐

2a) Have you ever seen a bandicoot before?   **Yes** ☐
                                                     No ☐

   2b) If so, where?

3a) Have you ever had any bandicoots on your property?   **Yes** ☐
                                                         No ☐ (go to Q8)

   If you answered yes, then
   3b) When was the last bandicoot activity? (e.g. last week)   **approx:**

   3c) How frequently does this occurs? (e.g. 3 times a month)   **approx:**

   3d) What season/s do they visit?   **Winter** ☐
                                             Spring ☐
                                             Summer ☐
                                             Autumn ☐

   3e) Why do you think bandicoots use your backyard?   **Shelter** ☐
                                                        Food ☐
                                                        Curiosity ☐
                                                        Playtime ☐
                                                        Other:

4a) Do you leave food out for the bandicoots?   **Yes** ☐
                                                No ☐

   4b) If so what food?

5) What evidence do you have for bandicoots on your property?   **Sightings** ☐
                                                               Diggings ☐
                                                               Pet attacks ☐
                                                               Droppings ☐
                                                               Other:

6) Do you know what species of bandicoot inhabits your property?   **Long-nosed** ☐
                                                                  Southern Brown ☐
                                                                  Don't Know ☐

7a) Do you know / are you aware of any bandicoot nests or burrows on your property?

Yes ☐

No ☐

    7b) If so where?

8) Are you aware of bandicoots on neighbouring properties?

Yes ☐

No ☐

9) Do you have pets?

Dog/s ☐

Cat/s ☐

Other

10) Are they locked up at night?

Yes ☐

No ☐

11) Can or does your pet enter the National Park during the day or night?

Yes ☐

No ☐

Unknown ☐

12) Does your property have a fence bordering the national park?

Yes ☐

No ☐

13) How far is your property to the National Park border? (e.g. next to it, 50m, 100m etc)

approx:

14a) Have you done anything to make your backyard less attractive to bandicoots?

Yes ☐

No ☐

    14b) if so what?

15a) Do you consider bandicoots as a?

   (more than one maybe appropriate)

Pest ☐

Native Animal ☐

Nuisance ☐

Feral Animal ☐

Unsure ☐

    15b) If they are a pest / nuisance, what are your reasons?

16a) Are you aware of any endangered species in Ku-ring-gai or Garigal National Parks?

Yes ☐

No ☐

    16b) If so what?

17) Are you aware that bandicoots are a protected species?

Yes ☐

No ☐

Attitudes / Feelings Towards bandicoots.

Below are a set of statements about bandicoots. Please click / tick the numbered response that most closely matches how you feel about the statement. For example, clicking a 5 in the corresponding box would indicate that you agree strongly with the statement and clicking 1 would indicate that you disagree strongly with the statement. There are no correct answers.

Statements	Strongly Agree 5	Agree 4	Neutral 3	Disagree 2	Strongly Disagree 1
1) Bandicoots are cute	☐	☐	☐	☐	☐
2) Bandicoots are/would be nice to have around	☐	☐	☐	☐	☐
3) Bandicoots are a nuisance	☐	☐	☐	☐	☐
4) Bandicoots cause too much damage to my garden	☐	☐	☐	☐	☐
5a) Bandicoots carry ticks	☐	☐	☐	☐	☐
b) These ticks are dangerous	☐	☐	☐	☐	☐
6) Bandicoots have a right to exist in their natural environment	☐	☐	☐	☐	☐
7) Bandicoots should be allowed to live in urban backyards	☐	☐	☐	☐	☐
8) Home owners should be allowed to reduce bandicoot numbers in their backyard by making gardens less attractive	☐	☐	☐	☐	☐
9) Conservation of bandicoots is necessary	☐	☐	☐	☐	☐
10) Bandicoot survival is threatened by cars	☐	☐	☐	☐	☐
11) Bandicoot survival is threatened by cats	☐	☐	☐	☐	☐
12) Bandicoot survival is threatened by dogs	☐	☐	☐	☐	☐
13) Bandicoot survival is threatened by foxes	☐	☐	☐	☐	☐
14) Bandicoot survival is threatened by the removal of natural habitat	☐	☐	☐	☐	☐
15) It is a pleasure to live with bandicoots	☐	☐	☐	☐	☐
16) It is a pleasure to live with other wildlife	☐	☐	☐	☐	☐
17) Bandicoots should be protected by law	☐	☐	☐	☐	☐
18) Bandicoots should be relocated to their natural environment (e.g. National Park)	☐	☐	☐	☐	☐
19) Bandicoots are an important part of the natural environment	☐	☐	☐	☐	☐
20) People should receive more information about bandicoots and if they have any threats to people (e.g. do they carry ticks)	☐	☐	☐	☐	☐
21) There are too many bandicoots in this area	☐	☐	☐	☐	☐
22) Bandicoots are welcome on my property	☐	☐	☐	☐	☐

<u>Other Questions</u>

Please indicate your sex   **Male**   ☐

                                             Female ☐

Please indicate your age in years

Residents at this address indicate   **0-10yrs**   ☐

                                             11-18yrs ☐

                                             19-30yrs ☐

                                             31-45yrs ☐

                                             46-65yrs ☐

                                             >65yrs   ☐

Thank you for completing this questionnaire. Can you please send this to mdowle@bio.mq.edu.au, or fax it through to 9850 9671 or post it to Matthew Dowle, Biological Sciences, Macquarie University, North Ryde 2001.

Part 2 & 3: Trapping and Video Taping Bandicoots

As part of this study I am interested in further studying any bandicoots which may come into your property. If you are willing to allow this part of the study please tick below and provide your name and address.

- Trapping   ☐

- Video Taping   ☐

Name:

Address:

Contact details

* **Phone Number**:

* **Email**:

INVESTIGATOR'S COPY

Consent to trapping and video taping

Bandicoot Research

Any information or personal details gathered in the course of the study are confidential and only Prof Elizabeth Deane and I will have access to the data. No individual will be identified in any publication of the results. If you decide to participate, you are free to withdraw from further participation in the research at any time without having to give a reason and without consequence.

Please return the investigators consent form together with your contact details (phone number and / or email address) with the questionnaire. You can return the forms via email mdowle@bio.mq.edu.au, by fax 9850 9671 or by post to Matthew Dowle, Biological Sciences, Macquarie University, North Ryde 2109.

I,   have read and understand the information above including the covering letter and any questions I have asked have been answered to my satisfaction. I agree to participate in this research, knowing that I can withdraw from further participation in the research at any time without consequence. I have been given a copy of this form to keep.

Please check box

I consent to the trapping of bandicoots in my backyard: ☐

I consent to the video taping of bandicoot activities in my backyard ☐

Participant's Name: Contact (ph) Number:
(block letters)
                                             email:

Participant's Signature: Date:

Investigator's Name:
(block letters)

Investigator's Signature: Date:

PARTICIPANT'S COPY

Consent to trapping and video taping

Bandicoot Research

Any information or personal details gathered in the course of the study are confidential and only Prof Elizabeth Deane and I will have access to the data. No individual will be identified in any publication of the results. If you decide to participate, you are free to withdraw from further participation in the research at any time without having to give a reason and without consequence.

Please return the investigators consent form together with your contact details (phone number and / or email address) with the questionnaire. You can return the forms via email mdowle@bio.mq.edu.au, by fax 9850 9671 or by post to Matthew Dowle, Biological Sciences, Macquarie University, North Ryde 2109.
.

I,   have read and understand the information above including the covering letter and any questions I have asked have been answered to my satisfaction. I agree to participate in this research, knowing that I can withdraw from further participation in the research at any time without consequence. I have been given a copy of this form to keep.

Please check box

I consent to the trapping of bandicoots in my backyard: ☐

I consent to the video taping of bandicoot activities in my backyard   ☐

Participant's Name: Contact (ph) Number:
(block letters)
                                                      email:

Participant's Signature: Date:

Investigator's Name:
(block letters)

Investigator's Signature: Date:

Appendix C: GenBank Accession Numbers

Table: *Cryptosporidium* species and GenBank accession numbers used for Megablast search. Hill *et al.* (2008) and Ng *et al.* (2011) isolates have been included.

Cryptosporidium species	Sequence length (modified)	Accession number
Cryptosporidium **species**		
C. baileyi	288	AF093495
C. muris	291	AF093497
C. fayeri	296	AF108860
C. suis	296	AF108861
C. felis	312	AF108862
C. parvum	295	AF108864
C. hominis	298	AF108865
C. varanii	292	AF112573
C. meleagridis	294	AF112574
C. canis	290	AF112576
C. wrairi	295	AF115378
C. serpentis	289	AF151376
C. ubiquitim	296	AF442484
C. macropodum	293	AF513227
C. opossum II	353	AY120906
C. galli	287	AY168846
C. bovis	285	AY741305
C. fragile	289	EU162752
C. brushtail possum	287	EU647729.1
C. cuniculus	296	FJ262725
C. andersoni	291	FJ463171
C. ryanae	285	FJ463193
C. xiaoi	285	FJ896053
Hill *et al.* (2008)		
Isolate *C.* sp. 200a	295	EU546857
Isolate *C.* sp. 200b	294	EU546859
Isolate *C.* sp. 30a	294	EU546860
Isolate *C.* sp. 30b	292	EU546865
Isolate *C.* sp. 204a	292	EU546866

Due to copyright laws, the following articles have been omitted from this thesis. Please refer to the following citations for details.

Dowle, M. & Deane, E. M. (2009). Attitudes to native bandicoots in an urban environment. European Journal of Wildlife Research 55: 45-52.

Dowle, M., Webster, K. N. & Deane, E. M. (2012). Faecal glucocorticoid metabolite concentrations in the free-ranging bandicoots (Perameles nasuta and Isoodon obesulus) of northern Sydney. Australian Mammalogy Vol. 35(1). Available at http://dx.doi.org/10.1071/AM11033.

Appendix F: Chapter 7 submitted for publication in *Veterinary Parasitology*

1

2

3

4   *Cryptosporidium* from a free-ranging marsupial host: bandicoots in

5   urban Australia

6   _____

7

8   **Matthew Dowle*, Nichola J. Hill & Michelle L. Power**

9

10

11   Department of Biological Sciences, Macquarie University, North Ryde, NSW 2109, Sydney,

12   Australia

13   * Corresponding author: matthew.dowle@students.mq.edu.au

14

16

17

18

19

20

Abstract

The southern brown bandicoot (*Isoodon obesulus*) and long-nosed bandicoot (*Perameles nasuta*) are among the few marsupials that inhabit suburban Australia, benefitting from increased opportunities for food, shelter from predators and refuge from bushfires. Expansion of human settlement has increased the interface between people and bandicoots with implications for the emergence and spread of zoonotic parasites. The host status of bandicoots inhabiting suburban areas and their potential role in *Cryptosporidium* transmission remains unresolved. Our study aimed to determine the prevalence and identity of *Cryptosporidium* and compare infection patterns between the two sympatric bandicoot species. Twelve positive isolates were identified through amplification of the *18S rRNA* from faecal DNA extraction. This study revealed that free-ranging bandicoots of northern Sydney were shedding *Cryptosporidium* oocysts at a prevalence of 10.8%, similar to marsupial species that act as reservoirs for *Cryptosporidium*. Phylogenetic inference placed the isolates in a clade with *C. parvum,* a species with a broad host range and zoonotic potential, or loosely related to *C. hominis*. However, the identity of the bandicoot isolates was not fully resolved and whether they were infected or simply passively transmitting oocysts is unknown. Our findings expand the range of hosts known to shed *Cryptosporidium* in urban areas. In view of this, conservation of habitat remnants in urban Australia should be prioritized to curb contact and transmission pathways between wildlife, humans and pets.

Keywords: bandicoot, *Cryptosporidium*, urban wildlife, emerging pathogen

Introduction

Cryptosporidium, a protozoan parasite of vertebrates, occurs predominantly in the epithelial cells of the intestine (O'Donoghue, 1995; Fayer, 2004). Over the past decade molecular tools have identified a significant amount of biodiversity within the genus, leading to identification of a growing number of descriptions of novel and cryptic species and genotypes (Fayer et al., 2010; Morgan et al., 2001; Zhou et al., 2004) with variable levels of host specificity (Xiao et al., 2002). Infection in humans is predominantly caused by two species, the host specific *C. hominis* and the zoonotic *C. parvum*. Other zoonotic species found in humans and domestic animals include *C. meleagridis, C. felis*, *C. canis, C. cuniculus and C. ubiquitum* (Xiao and Feng, 2008; Xiao, 2010; Chalmers et al., 2011). The recent identification of the marsupial specific *C. fayeri,* in a sporadic human infection (Waldron et al., 2010) demonstrates that Australian wildlife play a larger role in zoonotic transmission than previously thought.

Surveillance of *Cryptosporidium* has typically focused on animals of economic or agricultural value (Appelbee et al., 2005) and overlooked wildlife populations. Studies of *Cryptosporidium* in Australia indicate that wildlife populations inhabiting urban areas (Hill et al., 2008) and drinking water catchments (Power et al., 2004; Power et al., 2005) are hosts for *C. fayeri* (Ryan et al., 2008), *C. macropodum* (Power and Ryan, 2008) and other marsupial specific species and genotypes (Hill et al., 2008, Yang et al., 2011). Interactions between humans and wildlife are increasing with urban expansion, presenting increased risks of parasite emergence and transmission between humans and wildlife species (Bradley and Altizer, 2007). As the human-wildlife interface expands it is critical to identify pathways of transmission between hosts that share human-altered environments. Parasites may also spread from humans into wildlife species (Nizeyi et al., 2002) therefore monitoring the health of wildlife, particularly threatened species, is necessary for safeguarding biodiversity.

3

69   The endangered southern brown bandicoot (*Isoodon obesulus*) and the unlisted long-

70   nosed bandicoot (*Perameles nasuta*) are among the few marsupials that inhabit urban Australia.

71   Both species occur within the heavily populated northern suburbs of Sydney, and have the

72   potential to benefit from resources offered by urbanisation, such as increased food, shelter from

73   predators and refuge from bushfires (Gordon and Hulbert, 1989). Natural infections of

74   *Cryptosporidium* species and genotypes have been documented in 16 species of marsupials

75   (Fayer, 2010; Power, 2010), including the southern brown bandicoot (*Isoodon obesulus*) (cf.

76   O'Donoghue, 1995). However current descriptions of *Cryptosporidium* from bandicoots are

77   based on oocyst morphology alone. Our study aimed to determine the prevalence and identity of

78   *Cryptosporidium* in bandicoots in urban Sydney, and compare infection patterns between the two

79   sympatric bandicoot species. Furthermore, our study takes advantage of molecular tools to

80   investigate the phylogenetic relationships of *Cryptosporidium* isolated from bandicoots.

81

82   **Methods**

83   *Sample collection*

84   The study incorporated three trapping locations in Sydney; Ku-ring-gai Chase National Park

85   (33°39′3.6″S, 151°12′3.6″E), Garigal National Park (33°42′21″S 151°14′11″E) and in suburban

86   backyards adjacent to the National Parks. Ku-ring-gai Chase National Park is approximately

87   15,400 ha, is situated 25 km northwest from central Sydney and is bordered by urban properties

88   in the south. Garigal National Park is approximately 2,200 ha in size, 20 km northwest from

89   central Sydney and almost entirely bordered by urban properties. National Park populations have

90   minimal direct interactions with humans and urban developments, while the suburban backyard

91   populations have frequent encounters with pets (cats and dogs) and humans.

92   Bandicoots from free-ranging populations were sampled four times annually between

93   March 2005 and September 2007, resulting in a total capture effort of 4336 trapping nights.

94   Bandicoots were captured in a wire cage trap (30 cm x 30 cm x 60 cm) overnight and samples

95   collected at first light. Pellets deposited on the bottom of the traps overnight were collected for

96   analyses. Samples were also collected from captive bandicoots housed at Taronga Zoo. Pellets

97   were collected from enclosures of five bandicoots by Zoo staff each morning for eight days in

98   August 2006. All faecal samples were collected into a plastic vial and stored at -20°C until

99   analysis. All trapping and manual handling procedures were approved by the Macquarie

100   University Animal Ethics Committee (2005/010 & 2007/036), and a scientific license was

101   granted by the NSW National Parks and Wildlife Service (s11675).

102

103   *Purification and DNA extraction of oocysts*

104   *Cryptosporidium* oocysts were concentrated from pellets using an immunomagnetic separation

105   (IMS) technique designed for sensitive detection of *Cryptosporidium* in faecal samples (Power et

106   al., 2003). In brief, a slurry containing the equivalent of 1 g of faecal material was mixed with

107   paramagnetic beads conjugated with Cry104 coated beads (Weir et al., 2000) a monoclonal

108   antibody that targets the *Cryptosporidium* oocyst wall (BTF Australia, Sydney, Australia). The

109   resulting bead-oocyst complex was separated from the faecal debris by magnetic concentration,

110   followed by re-suspension in sterile water (88 µl).

111   DNA extraction was performed on the bead-oocyst complex using DNA extraction

112   prepGEM™ tissue kits (Zygem, Hamilton, New Zealand). Zygem Buffer 3 (10 µl) was added to

113   the bead-oocyst complex then snap frozen at -80°C for 15 min. The samples were thawed,

114   vortexed, spun, then 1 µl lysozyme (5 mg/ml) and 1 µl Prepgem enzyme was added (Ferrari et

115   al., 2000). Samples incubated at 37°C, 75°C and 95°C for 15 min each in a thermocycler (Perkin

116 Elmer), then spun at 5000 rpm for 3 min. The supernatant containing DNA was transferred to a

117 new tube and 2 µl Tris EDTA (1mM) was added. DNA samples were stored at minus 20°C until

118 PCR screening.

119

120 *Amplification at 18S rRNA locus*

121 DNA samples were amplified at the *18S rRNA* locus using a nested PCR protocol. The primers

122 *18SCF2* 5'-GACATATCATTCAAGTTTCTGACC-3' and *18SCR2* 5'-CTGAAGGAGTAAGG

123 AACAACC-3' (Ryan et al., 2003) were used to generate ~760 bp product, which formed the

124 template for secondary amplification. The primers *18SIF* 5'-AGTGACAAGAAATAACAA

125 TACAGG-3' and *18SIR* 5'-CCTGCTTTAAGCACTCAATTTTC-3' (Morgan et al., 1997) were

126 used to amplify ~ 310 bp product (pending species). Amplification during primary PCR was

127 enhanced using 1 µl DNA template in 4 µl of GeneReleaser$^{®}$ (Integrated Sciences, Sydney,

128 Australia) heated for 7 min at 500 W in a microwave. Primary reaction mixtures (25uL)

129 contained 1 x PCR buffer, 4 mM MgCl$_2$, 200 µM each of dNTPs, 20pM of each forward and

130 reverse primer, 1 U of Taq Tth Plus Polymerase$^{®}$ (Fisher Biotech, Australia) and were added to

131 gene releaser-DNA mix after microwaving. Secondary reaction components were identical to the

132 primary reactions however *18SIF & 18SIR* primers were used and 1 µl of primary reaction

133 mixture was added as DNA template. Conditions for both reactions comprised an initial

134 denaturation step at 94°C 2 min, 58°C 60 sec, 72°C 2 min, followed by 48 cycles of 94°C 40 sec,

135 58°C 30 sec, 72°C 45 sec and a final extension of 72°C for 7 min. The PCR products were

136 visualized by agarose gel electrophoresis using 2% Buffer TBE and SYBR$^{®}$ (Invitrogen,

137 Victoria, Australia) Safe DNA gel stain (2 µl).

138

139 All PCR's included either *C. parvum* and/or *C. fayeri* as a control sample. Samples positive at the

140 *18S rRNA* locus were tested against the *actin* and *gp60* loci with a nested PCR protocol following

141 Sulaiman et al. (2002) and Power et al. (2009) respectively.

142

143 *Amplification at actin and gp60 loci*

144 The actin primary reaction primers, *actinF1* 5'-

145 ATG(A/G)G(A/T)GAAGAAG(A/T)A(A/G)(C/T)(A/T)CAAGC-3' and *actinR1* 5'-

146 AGAA(G/A)CA(C/T)TTTCTGTG(T/G)ACAAT-3' were used to produce a 1,095-bp product

147 and the *actin* reaction primers, a*ctinF2* 5'-CAAGC(A/T)TT(G/A)GTTGTTGA(T/C)AA-3' and

148 *actinR2* 5'-TTTCTGTG(T/G)ACAAT(A/T)(G/C)(A/T)TGG-3' were used to produce a 1,066-bp

149 product (Sulaiman et al., 2002). The primary *actin* PCR mixture contained 2 µl DNA volume,

150 Platinum® PCR SuperMix (45 µl) (Invitrogen, Victoria, Australia) and 20 pM (1 µl) of each

151 forward and reverse primer in a final volume of 50 µl. Conditions for the primary reaction

152 comprised an initial denaturation 94°C for 5 min followed by 35 cycles of 94°C 45 sec, 50°C 45

153 sec, 72°C 60 sec and a final extension of 72°C 10 min. The secondary reaction mixture was

154 identical to the primary mixture, except the primers *actinF2* and *actinR2* were used and 5 µl of

155 the primary reaction mixture formed the DNA template in a final volume of 50 µl. Conditions for

156 secondary reaction comprised an initial denaturation at 94°C for 3 min followed by 35 cycles of

157 94°C 45 sec, 45°C 45 sec, 72°C 60 sec and a final extension of 72°C 7 min.

158 Amplification for *Cryptosporidium* isolates at the *gp60* locus were performed using the

159 primary reaction primers, *out*F5'-CCACACATCTGTAGCGTCGTCA-3' and *mar4* 5'-

160 CAGTCGTCTTAATTCCACGGT-3' and the secondary reaction primers, *atg*F5'-

161 ATGAGATTGTCGCTCATTATCG-3' and *mar3* 5'-CGTCAGAACATTCTGGAAGCT-3'

162 (Power et al., 2009). The primary *gp60* PCR mixture contained 2 µl DNA volume, 4 mM MgCl$_2$,

163   200 μM each of dNTPs, 20pM of each forward and reverse primer, 1 U of Red Taq® Polymerase

164   (Integrated Sciences, Sydney, Australia) in a final volume of 25 μl. Conditions for the primary

165   reaction comprised an initial denaturation 94°C for 3 min followed by 35 cycles of 94°C 45 sec,

166   58°C 45 sec, 72°C 90 sec and a final extension of 72°C 7 min. The secondary *gp60* reaction

167   mixture used the secondary primers in an identical mixture to the primary reaction, except 1 μl of

168   the primary reaction mixture was used as the DNA template in a final volume of 50 μl. The PCR

169   products of the *actin* and *gp60* loci were visualised as described above.

170

171   *Enumeration of oocysts using flow cytometry*

172   Faecal samples identified as positive for *Cryptosporidium* at the *18S rRNA* locus were processed

173   using IMS and flow cytometry (IMS-FC) to determine the number of oocysts shed. IMS

174   protocols followed Power *et al.* (2003) as described above, however the bead-oocyst complex

175   was suspended in 100 μl sterile H_2O and stored at 4°C. Prior to FC analysis, 100 μl antibody

176   dissociation buffer (ADB – PBS pH 7.2 containing bovine serum antibody (2%) and mouse

177   serum (10%)) was added to the suspended bead-oocyst complex. Samples were sorted using a

178   FACSCalibur flow cytometer (Becton Dickinson Biosciences, Sydney, Australia) equipped with

179   a SortStage attachment (MRL, Sydney Australia) that facilitated cell sorting onto Isopore™

180   membranes (13mm, 0.8 μM; Millipore, Sydney Australia) to separate oocysts from beads.

181   Membranes were stained with CRY104-FITC (100 μl; 10μg/ml) for 3 min and washed with

182   500μl monoclonal antibody buffer (MABB - 50mM tetra sodium pyrophosphate, 0.5% bovine

183   serum albumin and 0.05% Tween-80 at pH 8.0). Oocysts were identified by their bright green

184   fluorescence, spherical shape and size (4-6 μM) and counted using an epifluoresence Nikon BH2

185   microscope.

186

187 *Sequencing and phylogenetic analysis*

188 Amplicons were purified for sequence analyses using the QIAquick PCR purification kit

189 (Qiagen, Victoria, Australia) according to the manufacturer's instructions. Automatic sequencing

190 was performed in forward and reverse directions using a 3130xl DNA capillary sequencer

191 (Applied Biosystems, Foster City California). Sequences were assembled into optimised contigs

192 using Geneious Bioinformatics software (version 5.4.6, Biomatters Ltd, New Zealand). Contigs

193 were compared to existing *Cryptosporidium 18S rRNA* sequences in GeneBank using Megablast

194 search to determine likely species match. Bandicoot sequences were aligned with 30 described

195 *Cryptosporidium* species and genotypes using Clustal W (Larkin et al., 2007) with default

196 settings. A best-fit nucleotide substitution model was selected using MrModeltest 2.3 (Nylander,

197 J.A.A. 2004. MrModeltest v2. Program distributed by author. Evolutionary Biology Centre,

198 Uppsala University). The model with the lowest Akaike Information Criterion corrected score

199 was the Tamura 3-parameter model with a discrete gamma distribution. Phylogenetic trees were

200 generated with a Bayesian Markov chain Monte Carlo (MCMC) coalescent approach

201 implemented in BEAST 1.6.2. (Drummond and Rambaut 2007). An optimal chain length of 10

202 million generations and sampling frequency of 1,000 was used to achieve an Effective Sample

203 Size >200 in Tracer 1.5 (Rambaut and Drummond 2007). Consensus trees with a 10% burn-in

204 value were generated using TreeAnnotator 1.6.2. and visualized in FigTree 1.3.1. All trees were

205 rooted with *Plasmodium falciparum* (M19172) as the evolutionary outgroup.

206

207 **Results**

208 *Prevalence of Cryptosporidium in bandicoot populations*

209 A total of 98 faecal samples were obtained from bandicoot trappings in northern Sydney (n = 93)

210 and at Taronga Zoo (n = 5). Twelve C*ryptosporidium* positive faecal samples (Table 1) were

211 identified using *18S rRNA* PCR, representing a prevalence of 10.8% (10/93) among free-ranging

212 bandicoots (both species) of northern Sydney and 40% (2/5) among captive bandicoots at

213 Taronga Zoo. Of the 12 positive samples, nine were from long-nosed bandicoots (LNB) and three

214 were from southern brown bandicoots (SBB) (Table 1). All SBB positives were from Ku-ring-gai

215 Chase National Park representing a prevalence of 16.7% (3/18) for this species. For the LNB, two

216 positives (40% prevalence) were from the Taronga Zoo population and seven (9.3% prevalence)

217 were from the free-ranging LNB population of northern Sydney (Table 1).

218 Positive samples were subsequently tested at two additional loci, *actin* and *gp60*, using

219 nested PCR protocols. However, the positive isolates identified at the *18S rRNA* locus could not

220 be amplified at either *actin* and *gp60* loci, despite repeated attempts. Control samples, *C. parvum*

221 and *C. fayeri*, in *actin* and gp60 PCRs amplified as expected.

222

223 *Cryptosporidium species within bandicoot populations*

224 Sequencing was attempted for all *18S rRNA* PCR positive samples but sequences were only

225 obtained from 4 of the 12 samples (Bandicoot sample 6, 29, 206 and 211). Sequencing results

226 revealed that three of the four successfully sequenced isolates from bandicoots showed highest

227 identity with *C. parvum* (range of 98.3% to 99.0%). The fourth positive isolate showed highest

228 identity with *C. hominis* (96.1%). Inferred phylogenetic relationships based on the partial *18S*

229 *rRNA* locus loosely grouped bandicoot isolates 29 and 206 into a clade with either *C. parvum* or

230 *C. hominis* respectively. Bandicoot isolates 6 and 211 formed a clade with an unidentified

10

231   common brushtail possum (*Trichosurus vulpecula*) genotype (Figure 1). Posterior probabilities

232   indicated low support for these phylogenetic assignments (<0.60).

233   Phylogenetic analysis at the *18S rRNA* locus for the positive isolates revealed pairwise

234   distances ranging from 0.008 to 0.035. The greatest pairwise distance occurred between isolates

235   from a southern brown and a long-nosed bandicoot in Ku-ring-gai Chase National Park. The

236   isolate (sample 206) showing highest identify to *C. hominis* had the same pairwise distance from

237   all the other isolates (0.035). The pairwise comparison between the sequenced Taronga Zoo

238   isolate and the Ku-ring-gai National Park isolates ranged from 0.017 to 0.035.

239

240   *Counts of oocyst shedding in bandicoots*

241   Oocysts numbers in PCR positive faecal samples were determined using IMS coupled with flow

242   cytometry. In all bandicoot samples, oocyst counts were very low (≤ 100), ranging from zero to

243   100-oocysts/g of faecal sample (Table 2). Two samples observed after flow cytometry revealed

244   an oocyst count of zero oocysts/g of faecal sample.

245

246   **Discussion**

247   This study revealed that free-ranging bandicoots of northern Sydney were shedding low levels of

248   *Cryptosporidium* oocysts, expanding the known host range of this parasite in urban

249   environments. *Cryptosporidium* prevalence in the combined species of free-ranging bandicoots

250   was estimated at 10.8%. Despite the small sample size, this prevalence appeared to be similar to

251   other marsupials from greater metropolitan Sydney and other wild populations (Power et al.,

252   2005; Hill et al., 2008; Yang et al., 2011). Power et al. (2005) recorded 6.7% prevalence in

253   eastern grey kangaroos (*Macropus giganteus*) in Sydney's watershed; Hill et al. (2008) observed

254   a prevalence of 5.6% and 11.3% in common brushtail possums (*Trichosurus vulpecula*) in a

255 woodland environment and Taronga Zoo respectively. Yang et al. (2011) recorded an overall

256 prevalence of 9.3% in western grey kangaroos (*Macropus fuliginosus*) from Western Australia.

257   *Cryptosporidium* infections of vertebrate hosts are the result of 26 described species, with

258 greater than 40 novel genotypes (Power, 2010; Robinson et al., 2011). The *Cryptosporidium 18S*

259 *rRNA* sequences identified in these bandicoot species were not genetically identical to previously

260 described species or genotypes. Phylogenetic analysis of bandicoot isolates inferred a close

261 evolutionary relationship to species found in humans, *C. parvum* (96.1% similarity) and *C.*

262 *hominis* (98.3-99.0% similarity). The identity of bandicoot isolates was not fully resolved in this

263 study and whether the bandicoots were infected or simply passively transmitting oocysts is

264 unknown. Isolates failed to amplify at the *actin* and *gp60* loci despite significant attempts. The

265 definitive identification and resulting zoonotic potential of isolates requires confirmation by

266 further studies with amplification at multiple loci, particularly *gp60* to alleviate subgenotype

267 differences (Hill et al., 2008). Partial sequencing of isolates can create an identification bias and

268 without a full sequence it is not possible to infer evolutionary relationships with confidence.

269   Inability to amplify *C. parvum* / *C. hominis* like isolates in marsupials at loci other than

270 *18S rRNA* has been observed in possums (Hill et al., 2008) and kangaroos (Ng et al., 2011). Hill

271 et al. (2008) identified *Cryptosporidium* isolates (BTP genotype 2) from common brushtail

272 possums in an urban setting that were genetically distinct from known zoonotic species at the *18S*

273 *rRNA* locus. This was attributed to low oocyst shedding by possums preventing amplification at

274 the *actin* loci and morphometric analyses. Ng et al. (2011) identified two marsupial host species

275 carrying *C. parvum* / *C. hominis* isolates based on amplification of the *18S rRNA* locus but

276 attempts to amplify these isolates at three other loci, *hsp70*, *acetyl CoA* and *gp60* failed.

277 Discrepancies in the ability to amplify across multiple loci were also attributed to low levels of

278 oocysts and the polymorphic nature of the *18S rRNA* locus. Surveillance of *Cryptosporidium* in

279 wildlife presents unique challenges owing to the low number of oocysts typically shed by

280 reservoir species, compared to outbreak scenarios.

281   Primers used in routine PCR methods have been developed using sequences from

282 described *Cryptosporidium* species and genotypes and may therefore be unsuitable for

283 amplification of novel isolates (Power, 2010). However, it is unlikely that the failure to amplify

284 the *actin* or *gp60* gene in this study is due to incorrectly functioning PCRs, but rather a low yield

285 of oocysts. Positive controls were amplified in PCR protocols and oocysts from the 12 positive

286 bandicoot samples were re-extracted using IMS, flow cytometry and visualized through

287 microscopy. Oocyst counts were less than 10^2 oocysts / g faeces, indicating chronic or

288 asymptomatic infection. Additionally, the highly conserved multi-copy nature of the *18S rRNA*

289 locus compared to the single copy *actin* and *gp60* loci, suggests a mechanism to impede

290 amplification at the single copy loci (Hill et al., 2008; Power et al., 2009; Ng et al., 2011).

291   *Cryptosporidium* isolates from Taronga Zoo and Ku-ring-gai Chase National Park

292 indicated pairwise distances ranging from 0.008 to 0.035. Common brushtail possum isolates

293 (BTP2; Hill *et al.,* 2008) used in the phylogenetic analysis had similar pairwise distances (0.009

294 to 0.022). These isolates were considered genetically distinct from other known species,

295 including *C. parvum* and *C. hominis* (Hill *et al.,* 2008). Currently recognised species of

296 *Cryptosporidium* generally have slightly lower pairwise distances at the *18S rRNA* locus, such as

297 *C. meleagridis* vs. *C. wrairi* (0.013) and *C. parvum* vs. *C. hominis* (0.007) (Fayer and Santin,

298 2009). However, the pairwise comparisons at the *18S rRNA* loci in this study were conducted

299 using partially sequenced isolates. Full-length sequences are expected to have lower pairwise

300 distances than those currently observed. We therefore speculate that infection of *Cryptosporidium*

301 in bandicoots across the two habitats occurred naturally, rather than the closely inferred *C.*

302 *parvum* or *C. hominis* species.

303   A low oocyst yield in this study suggests an outbreak of *Cryptosporidium* in the

304   bandicoots was unlikely over the two-year study. A more feasible scenario is that bandicoots

305   serve as a reservoir for a marsupial-specific genotype of *Cryptosporidium*, with minimal threat of

306   cross-infection to domestic pets and humans. Low oocyst yields in host populations are often

307   characteristic of species that have attained immunity from coevolution with the parasite (Toft et

308   al., 1993). Moreover, no clinical signs associated with acute infection were observed from

309   bandicoots in the urban or zoo setting. However, *C. parvum* is naturally occurring in placental

310   mammals and one of the predominant species observed in humans. Therefore, *C. parvum* is likely

311   to have been introduced to Australia with placental mammals rather than emergence from a

312   marsupial host. Our results call for greater phylogenetic resolution of *C. parvum* in Australian

313   fauna, as the parasite is recognized as a species 'complex' that consists of numerous

314   species/genotypes including many that may not yet have been identified.

315   A variety of pathogenic endo-parasites have been recorded in free-ranging bandicoot

316   populations, including those occurring along the urban interface. Endo-parasites include

317   *Hepatozoon* (Wicks et al., 2006), *Giardia* (Bettiol et al., 1997; Adams et al., 2004), *Toxoplasma*

318   (Obendorf et al., 1996), and *Cryptosporidium* (O'Donoghue, 1995). This study expands on the

319   current knowledge and observations of *Cryptosporidium* in bandicoots. In view of the

320   observation of parasites that are common in humans and wildlife, conservation of habitat

321   remnants in urban Australia should be prioritized to curb contact and transmission pathways

322   between wildlife, humans and pets. Despite the genetic similarities of *Cryptosporidium* found in

323   marsupials to those found in humans, caution is required when interpreting the infection potential

324   and subsequent transmission of *Cryptosporidium* between marsupials, domestic pets and humans.

325

326   **Acknowledgements**

333

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437

438
439 **Tables**

440 **Table 1:** Faecal samples collected and prevalence of *Cryptosporidium* in bandicoots of northern

441 Sydney, expressed as a percentage of individuals recorded as positive.

	# Samples (# Individuals)		Prevalence (# Positive)		Mean prevalence (# Positive)
	SBB	LNB	SBB	LNB	
Ku-ring-gai Chase National Park	51 (17)	52 (34)	17.6% (3)	14.7% (5)	15.7% (8)
Garigal National Park	1 (1)	19 (17)	0.0%	0.0%	0.0%
Suburban Backyards	0	26 (24)	0.0%	8.3% (2)	8.3% (2)
Total / Average of free-ranging populations	**52 (18)**	**97 (75)**	**16.7% (3)**	**9.3% (7)**	**10.8% (10)**
Taronga Zoo Samples	0	40 (5)	0%	40% (2)	40% (2)

442

443

444

Table 2: *Cryptosporidium* oocyst/g faeces from the 12 positive samples amplified at the 18S

rRNA locus. SBB = southern brown bandicoot; LNB = long-nosed bandicoot.

| Sample ID #	Bandicoot species	Sample Location	Sequenced at *18S rRNA*	Oocysts/g faeces
6	SBB	Ku-ring-gai	Yes	100
8	SBB	Ku-ring-gai	No	40
29	LNB	Taronga Zoo	Yes	60
37	LNB	Taronga Zoo	No	40
51	SBB	Ku-ring-gai	No	0
82	LNB	Backyards	No	80
86	LNB	Backyards	No	80
206	LNB	Ku-ring-gai	Yes	60
211	LNB	Ku-ring-gai	Yes	20
222	LNB	Ku-ring-gai	No	20
261	LNB	Ku-ring-gai	No	0
270	LNB	Ku-ring-gai	No	20

447

Figures

450
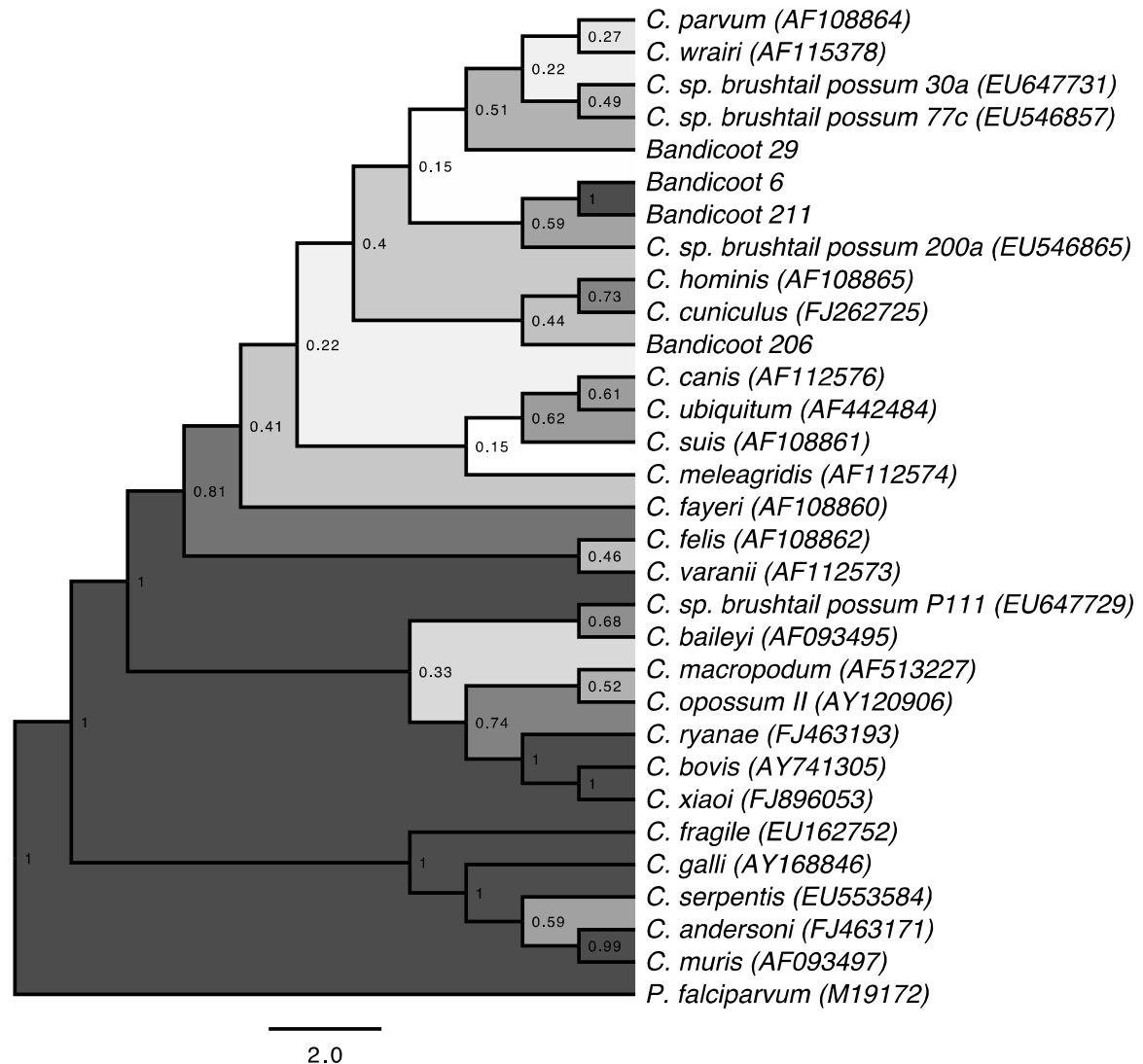
Figure 1: Phylogenetic relationships of the bandicoot samples (6, 29, 206, 211) and known

Cryptosporidium species/genotypes based on a ~300-bp fragment of *18S rRNA*. Phylogenetic

trees were generated with a Bayesian Markov chain Monte Carlo (MCMC) coalescent approach

implemented in BEAST 1.6.2. Nodes are highlighted according to strength of posterior

probability from low support (white) to high support >0.95 (dark grey). Scale bar corresponds to

years.

Appendix G: Final ethics approval letters

MACQUARIE
UNIVERSITY

10 February 2009

Mr Matthew Dowle
42 Darnley Street
GORDON NSW 2072
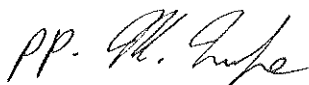
Reference: HE23SEP2005-D04289

Dear Mr Dowle,
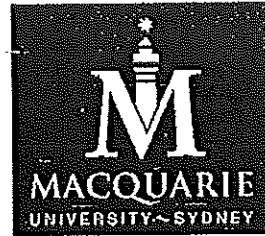
FINAL REPORT APPROVED

Title of project: "Community perceptions of the bandicoots of Northern Sydney (Ku-ring-gai and Garigal National Park)"

Your final report has been received and approved, effective 10 February 2009. The Committee is grateful for your cooperation and would like to wish you success in future research endeavours.

Yours sincerely

PP. *[signature]*

Dr Shirley Wyver
Acting Chair, Ethics Review Committee (Human Research)

ETHICS REVIEW COMMITTEE (HUMAN RESEARCH)
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http://www.research.mq.edu.au/researchers/ethics/human ethics

9 December 2008

Mr Matthew Dowle
42 Darnley Street
Gordon
NSW 2072

Dear Mr Dowle

FINAL REPORT

Title of project: Health, disease and status of bandicoots in the National Parks of Northern Sydney (ARA 2007/036)

Your Final Report for the above project was considered and accepted at the Animal Ethics Committee meeting of 4 December 2008. The Committee wishes to thank you for this report and advises that no further action is required.

Yours sincerely

Dr Darren Burke
Acting Chair, Animal Ethics Committee