Persistence Under Pressure: Understanding Connectivity, Detection, and Conservation of the Eastern Pygmy Possum in the Urban Matrix

Cassandra Jane Thompson

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School of Life and Environmental Sciences, Faculty of Science

THE UNIVERSITY OF SYDNEY

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Declaration

I declare that the content of this thesis is my own work. This thesis has not been submitted for any other degree or diploma. I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

Cassandra J. Thompson

29th June 2025

Preface

My thesis is written as a series of manuscripts that are either published, under review or submitted to a scientific journal. As such, formatting among chapters may vary to suit the target journal and there is some redundancy and overlap in the introduction, methods, and discussion sections among chapters. In these data chapters I also use the term 'we' to reflect the contributions of my supervisors and other co-authors.

Chapter 2 of this thesis is in the final stages of publishing: Thompson C., van der Ree E., Soanes K., Jones D., Stokes J., Bannock C. (in press). *Road Ecology in Oceania. In: Road Ecology: synthesis and perspectives* (D'Amico M., Barrientos R., Ascensão F., Eds.). Springer International Publishing AG, Cham (Switzerland). I formulated and wrote the drafts of the book chapter manuscript, with the co-authors refining and editing the drafts. All authors contributed to the final manuscript and read and approved the final manuscript.

Chapter 3 of this thesis has been submitted to Ecological Management and Restoration: Thompson C., van der Ree, R., Stokes, J. and Banks, P.B. (2025) *Success or failure? Insights form 25 years of monitoring wildlife crossings*. Manuscript submitted for publication, Ecological Management and Restoration. I collated and reviewed the grey literature, analysed the data, created figures and illustrations and wrote the draft manuscript. Concept design was contributed to by all authors. All authors contributed to the final manuscript and read and approved the final manuscript.

Chapter 4 of this thesis has been published in Wildlife Research: Thompson, C., Law, B., Gonsalves, L. and Banks, P.B. (2025) *Occupancy of an urban-sensitive specialist: the role of habitat availability and fire on the urban edge*. Wildlife Research 52(11). I co-designed the study with field data collected BL, myself and Hornsby, Pittwater and Ku-ring-gai Council staff. I compiled and analysed the field data and completed modelling with the assistance of LG. I completed the analysis, created figures and illustrations and wrote the draft manuscript. All authors contributed to the final manuscript and read and approved the final manuscript.

Chapter 5 of this thesis has been published by Australian Mammalogy: Thompson, C., Law, B., Gonsalves, L., Banks, P.B. (2025) *Assessing the detectability of a cryptic arboreal marsupial using a novel survey approach.* Australia Mammalogy 47(2). I prepared the survey, collated and analysed the data with input from LG, wrote the draft manuscript and created figures and illustrations. All authors contributed to the final manuscript and read and approved the final manuscript.

Chapter 6 of this thesis has been submitted to Australian Journal of Zoology: Thompson, C., Lott, M., Campbell, C.D., Banks, P.B, and Frankham, G. (2025) *Assessing genetic connectivity of eastern pygmy possums across a peri-urban landscape: Insights for conservation planning*. Manuscript submitted for publication, Australian Zoologist. I developed the study conception and design with the assistance of GF. I completed the data collection, with additional samples collected by EMM Consulting. DNA extraction was carried out by GF and the SNP genotyping completed by Diversity Arrays Technology (Canberra, Australia). I performed the data analysis with the assistance of ML and CC. All authors contributed to the study conception and design. The first draft of the manuscript was written by CT and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

All research was conducted under the Department of Primary Industries Secretary's Animal Care & Ethics Committee (project number 16/16207) and NSW National Parks and Wildlife Services (NPWS) Scientific Licence SL101873.

Artificial intelligence statement

During the preparation of the thesis the author used Copilot for the purposes of text enhancement and copyediting. The use of this generative AI tool includes spelling corrections, minor sentence restructuring, and clarity enhancement. The author confirms that where text was modified by generative AI, the content was reviewed for possible errors, inaccuracies, and bias. The author takes full responsibility for the submitted thesis, confirms the work is their own, and has used generative AI in accordance with the University of Sydney guidelines and policies.

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Authorship attribution statement

This is to certify that the content of this thesis is my own work. This thesis has not been submitted for any other degree or purpose.

I certify that the intellectual content of this thesis is the product of my own work, and that all assistance received in preparing this thesis and all sources have been acknowledged.

Cassandra J. Thompson

29th June 2025

Attesting author attribution statement

As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statement above is correct.

Peter B. Banks

29th June 2025

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Firstly, I would like to pay my respects to the traditional owners of the lands where my research activities took place: the Gadigal, Dharug and Garigal people. I pay my respects to Elders past, present, and future and recognise their strong connections with the land. Always was, always will be.

I would not have been able to complete this PhD without the support, enthusiasm, and kindness of many people. Firstly, my supervisors Peter Banks, Brad Law and Rodney van de Ree for providing guidance and advice. You have always believed in me, despite this taking 8.5 years! Thank you for sharing your wisdom and knowledge with me, your sage advice and laughs along the way.

The co-authors of the papers which have been submitted, thank you for the many chats, suggestions and quick turnaround on reviews. Your insights and revisions have been invaluable to the papers we produced. In particular, I would like to give a specific shout-out to Leroy Gonsalves, who was my guiding light for occupancy modelling and R code wrangling, and helped keep me sane using R! I hope that one day I can repay your kindness and patience.

I'd like to also thank the many other mentors or people that helped me through the tribulations of PhD life, chatting at conferences and offering advice. Of note are the members of the Banks Lab at the University of Sydney, the many honours and PhD students that have come and gone through my time there, as well as the research staff who have always been such a help!

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Abstract

Urbanisation continues to fragment native habitats and place increasing pressure on species that rely on continuous vegetation cover for movement and survival. Conservation of such species requires a deep understanding of how they use urban landscapes, and the efficacy of initiatives aimed at reducing urban impacts; an understanding that is lacking for many taxa. This thesis investigates the impact of urban-edge fragmentation on a small, cryptic, and urban-sensitive arboreal marsupial, the eastern pygmy possum (*Cercartetus nanus*), to identify the factors shaping persistence and connectivity in the highly modified landscapes of Australia's oldest and largest city, Sydney. Using a multidisciplinary approach that integrates SNP-based genetic analysis, dynamic occupancy modelling, species detectability comparisons, and a synthesis of long-term wildlife crossing data, this research aims to advance our knowledge of how fragmentation affects small native mammals surviving in peri-urban landscapes.

Using an extensive network of nest boxes, I found that the eastern pygmy possum occurs in fragmented bushland remnants in northern Sydney and suggest their detection can be improved by surveying their use of feed trees. But their patchiness makes long term persistence vulnerable and heavily dependent on the extent and connectivity of fragmented native vegetation. Occupancy models identified that native vegetation extent and fire history were key predictors of site use. Genetic analyses revealed subtle but emerging population structuring among remnant habitat patches, but surprisingly limited differentiation across a heavily trafficked arterial road. This suggests that roads (at least smaller ones) are not absolute barriers but will likely contribute to long-term isolation in the study area. My reviews of road ecology in Australia and beyond (Oceania) as well as the review of 25 years of wildlife crossing structure monitoring data, identified single species-focused mitigation with a lack of population and ecosystem level analysis of crossing efficacy. The reviews also show that my study species is rarely recorded using existing wildlife crossing structures and is largely absent from the literature. Least-cost path modelling and landscape resistance analysis pinpointed priority corridors for restoration and informed the design of two species-specific crossing structures now installed in the study area. My results highlight a need for mitigation strategies and long-term monitoring frameworks to integrate genetics, occupancy, and connectivity. They also reveal an

untapped potential for compliance monitoring and related grey literature to improve our understanding of structure use. Finally, I propose a replicable methodology for assessing and managing fragmentation and barrier effects in urban landscapes. By combining applied field data with broader synthesis, this work provides new insight into the challenges and solutions for conserving cryptic, arboreal fauna in a rapidly urbanising world.

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Chapter 1: Introduction

Overview

Urbanisation is a driver of biodiversity loss, producing some of the greatest local extinction rates of native species (McKinney 2009). This is particularly evident in peri-urban environments, where natural habitats are fragmented by expanding infrastructure and development associated with urban sprawl (Garden *et al.* 2006). Furthermore, conservation opportunities within built environments and small habitat remnants are underexploited and poorly understood (Soanes and Lentini 2019). Worldwide, the proliferation of roads and other linear developments has created barriers that can impede wildlife movement, especially for those sensitive to urban disturbances (Forman *et al.* 2003; Newport *et al.* 2014; Borda-de-Água *et al.* 2017). Road barriers may restrict physical movement and disrupt genetic connectivity, leading to isolated populations with reduced genetic diversity and subsequently, an increased risk of local extinction (Frankham *et al.* 2017). Despite the recognition of these issues, there remains a paucity of research examining the specific impacts of road-induced fragmentation on small mammal populations in peri-urban Australian landscapes. Consequently, we have an insufficient understanding of both the short- and long-term effects of fragmentation, which hinders the development of evidence-based management practices to ensure the survival of urban-sensitive species. This thesis aims to address this gap by investigating the effects of habitat fragmentation and road barriers on the eastern pygmy possum (*Cercatetus nanus*), a cryptic and urban-sensitive species listed as threatened, on the urban fringe of northern Sydney.

In this introduction chapter, I provide a background for the current understanding of the impacts of habitat fragmentation on small mammal populations, outlining the significance of urbanisation as a driver of fragmentation, with a particular focus on linear infrastructure such as roads. This will create the foundational rationale for understanding the ecological challenges faced by species like eastern pygmy possum in urban landscapes, and the need to understand the efficacy conservation strategies to mitigate these effects. In doing so this chapter identifies the major themes and key aims of this thesis and concludes with a short summary of the intent of each of the main chapters and their logical connections that build understanding. Note that this chapter is followed by a focussed review of road ecology in the Australian region (Chapter 2) so this topic isn't covered in detail here.

My thesis has a key focus on Australian species and environments as Australia has one of the most highly urbanised human populations in the world, and its cities are increased in size and density. I also highlight the eastern pygmy possum as a useful species for studying the impacts of urbanisation because of its needs to find food and urban sensitive habitat requirements. By examining the role of roads as barriers to movement and genetic connectivity, I aim to identify effective strategies for mitigating the effects of urbanisation, with a particular focus on wildlife crossings. Additionally, I aim to develop improved monitoring methods for cryptic species that are often overlooked in traditional monitoring, to ensure they are included in future planning and management on the urban edge.

Fragmentation on the urban edge

Habitat fragmentation occurs when large, continuous habitats are divided into smaller, isolated patches, often resulting from anthropogenic activities such as urban development and agriculture (Fahrig 1997). Urbanisation is a key driver of habitat fragmentation, leading to substantial biodiversity loss, not only from the loss of vegetation cover and reduced connectivity, but also from edge effects (Li *et al.* 2022). As urban areas expand, the isolation of remnant vegetation patches becomes more pronounced, creating reduced population sizes that are more susceptible to stochastic events and predation risk, and fragmented wildlife populations that are at increased risk of inbreeding, genetic drift, and local extinction (Frankham *et al.* 2017). As a result, habitat fragmentation is a major concern for wildlife in Australia and globally, with the clearing of native vegetation listed as a key threatening process. Fragmentation is particularly evident in urban-edge environments, where remnant bushland patches are increasingly isolated by roads, buildings, and other infrastructure (Garden *et al.* 2006; McKinney 2009). However, much remains unknown about how the unique configuration of urban landscapes, such as the proximity of roads and other infrastructure, influences the movement patterns and genetic diversity of wildlife in these environments (Tischendorf and Fahrig 2000; van der Ree *et al.* 2015).

Small mammals, particularly those with specific habitat requirements and limited dispersal capabilities, are particularly vulnerable to effects of fragmentation (Gaines *et al.* 1997), exhibiting varied responses, with some being more adaptable to urban environments and others being highly sensitive to changes (Garden *et al.* 2006). Generalist species, such as the common brushtail possum (*Trichosurus vulpecula*), tend to persist in Australian urban landscapes because of their broad ecological niches and ability to exploit diverse food sources (Tischendorf and Fahrig 2000). Similarly, mobile species such as microbats can move large distances to access foraging habitats, making them less sensitive to the impacts of urbanisation (Jung and Threlfall 2016). Urban-sensitive species often become confined to larger remnant patches of native vegetation, which are becoming increasingly fragmented as urban development intensifies (Garden *et al.* 2006), and the specific impacts of these fragmented landscapes on species persistence are still poorly understood.

Previous studies on the urban edge focus on the ability of generalist species to exploit urban areas (Garden *et al.* 2007), but modelling also identified that small, ground-dwelling mammals were most negatively affected by urbanisation (van der Ree and McCarthy 2005). Few studies have focused on urban-sensitive species, particularly arboreal mammals (Brearley *et al.* 2010; Marks and Goldingay 2023) that may be more susceptible to urban edge impacts and barriers to movement due to their need for canopy connectivity. Thus, there remains a largely unanswered question of how urban-sensitive small mammals are surviving in these patches, and are they gradually losing the genetic diversity necessary for long-term survival, particularly when considering the role of roads as barriers to movement?

Urban-sensitive species often have narrow habitat requirements and limited ability to adapt to altered environmental conditions associated with urban landscapes (Dickman and Doncaster 1987; Garden *et al.* 2006). The loss of habitat and food resources increases the vulnerability of urban-sensitive small mammals to extinction, especially when populations become disconnected from larger, more sustainable habitat networks (da Rosa *et al.* 2018). Road mortality can also pose a significant threat in urban areas, as these species attempt to cross roads to access new resources or migrate between patches (Forman *et al.* 2003). A study conducted in Tasmania found that road mortality accounted for approximately 3.8–5.7% of the annual population of the Tasmanian devil (*Sarcophilus harrisii*), a species of conservation concern (Hobday and Minstrell 2008). However, this was in forested areas and the specific impact of road infrastructure on genetic exchange and long-term survival of populations in urban fragmented landscapes remains understudied in Australia. Further research is needed to identify the scale of fragmentation and the types of barriers that are most detrimental to species survival and movement in these areas (van der Ree *et al.* 2015). This lack of understanding limits our ability to design effective conservation strategies that mitigate the negative effects of road-induced fragmentation, particularly interrupting movement for foraging and breeding and reducing genetic connectivity (Smith *et al.* 2019). Without such knowledge, the development of wildlife corridors and crossing structures are less targeted, resulting in inefficient conservation efforts and potentially further population decline.

Despite facing significant anthropogenic pressures, remnant bushland patches in urban areas can play a crucial role in preserving biodiversity, at least in the short term. These patches may provide vital refugia for small mammal populations, supporting both resident species and transient individuals from other areas (Wintle *et al.* 2019). For example, the persistence of species such as the common brushtail possum, bandicoots, and native rodents in urban areas of Australia depends on the availability of remnant habitat patches (Harper 2005; Rowland 2015). Remnant urban patches offer shelter, food, and breeding sites that may otherwise be unavailable in heavily modified landscapes. However, even though species may persist in fragmented habitats for a time, the concept of “extinction debt” suggests that they may still face long-term declines due to the delayed effects of habitat loss (Kuussaari *et al.* 2009). The ability of a habitat to sustain species in the long term is likely dependent on its size, quality, and connectivity to other natural areas. As habitat patches become smaller and more isolated, their ability to support viable populations diminishes. The fragmentation of these patches can result in populations becoming too small and isolated, limiting the possibility of genetic exchange or recolonisation from other areas (Taylor *et al.* 2011). However, it remains unclear how connectivity, particularly through green corridors and wildlife crossing structures, might mitigate this effect and restore the ecological flow between fragmented patches (Ricketts 2001; Bennett 2003).

Studies on urban-sensitive species in fragmented landscapes have shown that the connectivity between habitat patches is crucial for maintaining genetic diversity and ensuring long-term population viability (Bennett 2003; Delaney *et al.* 2010). Therefore, conservation efforts must prioritise maintaining or restoring connectivity between these patches to reduce local species extinctions. Without adequate corridors, isolated populations may suffer from inbreeding depression, which can further exacerbate the negative effects of habitat fragmentation (Frankham, 2005). However, the specific mechanisms that allow small Australian mammals to navigate fragmented landscapes and utilise these corridors remain unclear. Isolated populations may experience different rates of genetic drift depending on their access to movement pathways, and understanding the extent and drivers of this drift is critical for effective conservation planning (van der Ree *et al.* 2015).

The ability of species to move between habitat patches, or functional connectivity, is increasingly being identified as the key to enhancing connectivity for small mammals (Fahrig 2003). Functional connectivity ensures that small mammal populations can access necessary resources, such as food, mates, and shelter, and recolonise patches when necessary, facilitating gene flow and supporting the long-term survival of populations (Ricketts 2001). How functional connectivity can be effectively restored and maintained in highly fragmented, urban-edge environments remains a critical research gap. There is a pressing need for metrics as proxies for functional connectivity in fragmented landscapes particularly for urban-sensitive small mammals. The results will guide the restoration of functional connectivity for such species by identifying movement pathways, creating and maintaining green corridors, improving existing patches, and minimising barriers to movement, which are fundamental for the long-term survival of small mammals in urban environments (Ricketts 2001; FitzGibbon *et al.* 2007).

The impacts of roads in fragmented environments

Roads, particularly in urban and peri-urban environments, can be a significant barrier to wildlife movement and may sever the functional connectivity between habitat patches (Forman and Alexander 1998; van der Ree *et al.* 2011). Roads have the potential to impose barriers that affect wildlife, especially small and arboreal mammals (Goosem 2001; Taylor and Goldingay 2010). They not only fragment habitats but also directly impact species through mortality (road kills), habitat loss, and impeded movement (Forman *et al.* 2003). The effects of roads on wildlife populations have been well documented, with many studies emphasising the role of roads in the decline of small mammal populations (van der Ree *et al.* 2015). Arboreal mammals are particularly vulnerable to these impacts because they often rely on interconnected canopies for movement (van der Ree *et al.* 2010; Soanes and van der Ree 2015).

The physical presence of roads creates a clear barrier to the movement of small mammals, preventing them from accessing essential resources such as food, mates, and new territories (van der Ree *et al.* 2015). Edge effects, such as increased light levels, noise, higher daytime temperatures, higher wind speeds, and lower humidity, can significantly affect the vitality and composition of species in fragmented habitats (Saunders *et al.* 1991; van der Ree *et al.* 2015). These behavioural disruptions and impacts on habitat quality are particularly concerning for species that are already at risk because of habitat loss and fragmentation. For species such as the mosaic-tailed rat (*Melomys cervinipes*) and the brown antechinus (*Antechinus stuartii*), even relatively small, low-usage roads can become effectively impassable (Goosem 2001). These species often avoid crossing roads, particularly when they pass through critical habitats, which further isolates populations and reduces genetic exchange. Additionally, roads with higher traffic volumes not only increase the likelihood of roadkill but also create stressful environments for small mammals, leading to altered foraging behaviour and lower reproductive success (Forman and Alexander 1998). Squirrel gliders (*Petaurus norfolcensis*) near roads suffer increased stress and altered movement behaviours, further compounding the challenges faced by these arboreal species in urbanised landscapes (Brearley *et al.* 2011). These behavioural disruptions are particularly concerning for species that are already at risk due to habitat loss and fragmentation and have rarely been studied in the urban context.

Reducing the barrier effects of roads

As road networks continue to expand globally, their detrimental impacts on wildlife populations have become increasingly apparent (Laurance *et al.* 2009). Given that urban areas continue to expand, addressing the fragmentation caused by roads has become an increasingly urgent task for ecologists and urban planners. In Australia, concerns about the barrier effect of roads emerged in the early 1980s, leading to management efforts aimed at mitigating these impacts, particularly for species of conservation concern (Chapter 2). But the outcome of these efforts is not well documented and often remains hidden in unpublished 'grey' literature.

One widely implemented strategy has been the installation of wildlife crossing structures, which aim to increase the permeability of roads by facilitating safe passage over or under the roadway while reducing collision risk (van der Ree *et al.* 2007). Worldwide, wildlife crossing structures such as underpasses, overpasses, and canopy bridges, have been shown to reduce road mortality and enhance connectivity between habitat patches (Clevenger and Waltho 2005; van der Ree *et al.* 2015). In Australia, crossing structures have become a mandatory requirement for many roads, though evidence for use and efficacy has been largely based on single species studies. For example, canopy bridges have been shown to facilitate movement across roads that would otherwise create insurmountable barriers, at least for some arboreal species such as the common brushtail possum, green ringtail possum (*Pseudochirops archeri*) (Goosem 2001), and squirrel glider (Taylor and Goldingay 2012; Soanes *et al.* 2013). However, their efficacy for smaller mammals remains largely unknown, and few studies have been completed on the urban edge where roads are common.

The study of wildlife crossing structures and their effectiveness has thus emerged as a pivotal area of research within conservation biology, particularly in relation to habitat fragmentation and road ecology. A growing body of literature has emerged aimed at understanding how crossing structures, such as road underpasses and overpasses, might mitigate road impacts, especially for arboreal mammals (Rytwinski *et al.* 2016; Soanes *et al.* 2024) (see Chapter 2). But despite the widespread adoption of wildlife crossing structures in Australia, there is little literature evaluating their effectiveness (Soanes *et al.* 2024). Some studies have demonstrated that various species use these structures (e.g. (Goldingay *et al.* 2013; Rytwinski *et al.* 2016)), but many have focused on single species and confirmed crossing, limiting our ability to generalise findings across species, landscape contexts, and structure designs (Soanes *et al.* 2018; Taylor and Rohweder 2020). This may be a result of monitoring for crossing structures being completed by ecological consultants on behalf of road agencies, meaning that most of this work resides in unpublished ‘grey’ literature. This limitation restricts the applicability of findings across multiple projects and species to draw conclusions and provide a basis for future best-practice mitigation. Such practitioner-generated research needs to be included to minimise publication bias in conservation reviews (Haddaway and Bayliss 2015), but access is often due to limited access and the inability to compare results across studies (Lesbarrères and Fahrig 2012). However, this has been identified as a broader problem in other areas of scientific research (Hartling *et al.* 2017). As wildlife crossing structures become integral components of wildlife conservation strategies, unpublished data needs to be review and analysed, to guide their design and implementation across various species and ecosystems (Fischer and Lindenmayer 2007).

A model species for investigating ways to reduce the impacts of fragmentation on the urban edge

My thesis focuses on the eastern pygmy possum (*Cercartetus nanus*) as a model species for studying the impacts of fragmentation and road risk in peri-urban environments. This small, nocturnal, and cryptic marsupial inhabits heathlands, woodlands, and rainforests throughout south-eastern Australia, but is particularly associated with banksia-dominated habitats, which provide both food and shelter (Bowen and Goldingay 2000; Harris 2008; Law *et al.* 2018). The species is predominantly nectivorous, feeding on nectar and pollen from native plants such as *Banksia, Eucalyptus*, and *Callistemon* (Harris 2008; Law *et al.* 2018), with most activity occurring between 0.5 m and 2 m off the ground (Tulloch and Dickman 2006; Law *et al.* 2013; Law *et al.* 2018). As a key pollinator of certain native flora (e.g., banksia), the eastern pygmy possum plays a significant role in maintaining ecosystem function. Pollinators are vital for plant reproduction, and the eastern pygmy possum's nocturnal foraging behaviour facilitates cross-pollination that supports the resilience of local plant community (Cunningham 1991; Carthew 1993; Harris *et al.* 2007a; O'Rourke *et al.* 2020). Despite the species' critical ecological role, its persistence in urban landscapes remains under-explored, and a better understanding of the impacts of urban fragmentation and connectivity on the population is needed.

The species' breeding cycle is influenced by the availability of floral resources, particularly banksia species (Goldingay and Keohan 2017; Goldingay and Rueegger 2018). This connection means that the presence of eastern pygmy possums may be closely tied to the timing and availability of food resources, which can fluctuate with seasonal changes in vegetation (Turner 1984; Bladon *et al.* 2002) and being able to access it. The availability and quality of foraging resources could be impacted by the effects of habitat fragmentation where it persists on the urban edge as could movement between areas of such resources.

Due to its cryptic nature and the species occurring in low-density populations; the eastern pygmy possum often goes undetected in traditional surveys (Bowen and Goldingay 2000). This has hampered our understanding of its population size, persistence and movements. Where it is recorded, capture rates are very low using traditional survey methods (e.g. Elliot

Trapping and spotlighting) (Bowen and Goldingay 2000; Harris and Goldingay 2005; Harris *et al.* 2014), with the most suitable methods identified to date including: pitfall trapping and the use of nest boxes (Bowen and Goldingay 2000; Tasker and Dickman 2002; Tulloch and Dickman 2006; Goldingay 2023). However, capture rates are still low using such methods in comparison to other small mammals and like conventional techniques, nest boxes and pitfall traps can represent a labour-intensive survey method due to the ongoing need for physical checks at a site and ongoing maintenance. Reliable, cost-effective methods that increase detectability are required to ensure that all populations are identified during wildlife surveys, particularly in urban-edge areas where they are most at threat.

Past research on the eastern pygmy possum has focused on large forested or protected areas such as Royal National Park (Tulloch and Dickman 2006, 2007; Harris *et al.* 2014; Goldingay 2023) and state forests (Law *et al.* 2013; Law *et al.* 2018; Chew *et al.* 2024), but there is a notable gap in studies examining its persistence in urban remnants or at the interface between urban development and natural habitat (Harris *et al.* 2007b). This gap is especially important for understanding how the species interacts and moves within urban edges. The eastern pygmy possum has been found to persist in some urban-edge areas, potentially because suitable habitat is available and connectivity has been maintained (Harris *et al.* 2007b). The species was only identified in urban bushland patches in northern Sydney, outside large conservation areas, in 2010 (B. Law *pers comm*.). Radio-tracking in larger forested areas has revealed that home ranges can comprise a mosaic of disturbed and undisturbed areas and that logged habitat is not avoided within home ranges, including for denning (Law *et al.* 2013). Unlike forested areas investigated in this study, as urbanisation increases, these bushland remnants face the impacts of fragmentation, reducing overall habitat quality and connectivity. One of the central challenges then for the species in urban-edge habitats, is its reliance on specific food resources, such as nectar-rich banksia species, which are sensitive to fire regimes and urban development (Bradstock and Myerscough 1981). As bushland in peri-urban areas is heavily managed by prescribed fire for asset protection, the impacts of fire on the quality and quantity of habitat resources for the species needs to be better understood.

Habitat fragmentation and disturbance may create a "sink" effect, where populations persist temporarily but are unsustainable in the long term (Kuussaari *et al.* 2009). This is the idea of extinction debt, where species may survive in fragmented patches for a period but ultimately face population decline as negative demographic processes accumulate over time. In fragmented landscapes, demographic stochasticity becomes amplified due to small population sizes and isolation, accelerating the risk of extinction (Fischer and Lindenmayer 2007). Despite its vulnerability, the potential for the eastern pygmy possum to adapt to urban habitats remains unstudied. Perhaps it is not as sensitive to urbanisation as once thought? Either way, there is a gap in our understanding of how much urban fragmentation or barriers to movement these populations can withstand, and how long-term persistence in fragmented habitats can be maintained. By investigating the influence of habitat and landscape factors on occupancy for the species where it persists on the urban edge, we can better manage remaining habitat for the species survival long-term.

The eastern pygmy possum's reliance on tree hollows for shelter and its arboreal nature (Law *et al.* 2018) make the species particularly vulnerable to the sorts of disruptions in both canopy and understorey connectivity that roads create. It also generally occupies small home ranges, with males up to 20 ha and females 2.6 ha (Law *et al.* 2013). Within the home range, between 3–5 dens were used each week (Law *et al.* 2013). The species' ability to disperse has been recorded over distances of up to 500 meters in a single night (Bladon *et al.* 2002; Law *et al.* 2013), with some longer-term movements ranging from 800 to 5,500 meters by males (Harris 2010). However, their mobility may be hindered by roads, cleared areas and other barriers in fragmented landscapes due to their arboreal nature impeding the ability to access necessary resources such as food and mates (Harris *et al.* 2007b; Goldingay and Keohan 2017).(Harris *et al.* 2007b; Goldingay and Keohan 2017). Only one study has recorded a successful road crossing in the Royal National Park (1,300m between capture sites) by an independent male nestling over a period of five months (Harris 2010), and two individuals detecting crossing a small road within a National Park in another study (Goldingay 2023).. Further, Harris et al. (2007) documented several cases of roadkill, raising concerns about the vulnerability of eastern pygmy possums to road mortality, particularly in urbanising landscapes. Thus, the road may not only represent a physical break in connectivity for movement but could act as a functional barrier if road mortality is high. Studies are limited on the impact of roads of different sizes and traffic use for many small mammals persisting on the peri-urban edge in Australia (Rytwinski *et al.* 2016; Soanes *et al.* 2024).

We do not know if the eastern pygmy possum will use wildlife crossing structures or what specific measures and crossing types are needed to maintain movement for the species. Without this knowledge, the barrier effect of roads may ultimately limit access to critical resources and increasing the risk of isolation, which can lead to reduced genetic diversity and inbreeding for the local population (McKinney 2009; Frankham *et al.* 2017). Without focusing on maintaining genetic diversity and functional connectivity, the full scale of such impacts on population persistence cannot be understood. Genetic methods have not yet been used to quantify the genetic diversity of the eastern pygmy possum population either within peri-urban areas or in larger intact forest. To understand future impacts and changes to the local population, a genetic baseline is needed, as is ongoing monitoring to identify and quantify barriers to movement and fragmentation impacts. The effects of isolation may take years or even decades to manifest as a loss in genetic diversity, but the immediate risk comes from the inability of small, isolated populations to recover from random events such as mortality or poor breeding years (Fischer and Lindenmayer 2007; Kuussaari *et al.* 2009).

Aims of the thesis

The overall aim of this thesis is to investigate the effects of habitat fragmentation and road barriers on the eastern pygmy possum, an urban-sensitive species found on the urban fringe of northern Sydney. In doing so, this research explores the role of road infrastructure in creating barriers to species movement and genetic connectivity as potential causes of long-term declines in genetic diversity and population viability for the species. The study also aims to identify methods that could mitigate the impacts of urbanisation on small mammal populations.

The research is structured around several key broad aims:

- Investigating the effects of fragmentation on species persistence, with a focus on how habitat quality and landscape connectivity influence the eastern pygmy possum's occupancy and movement. This includes exploring the long-term consequences of fragmentation, beyond short-term persistence, and how these effects influence genetic structure.

- Assessing the role of roads as barriers to movement and genetic flow, and how these barriers can be mitigated through measures like wildlife crossing structures. Despite recognition of the road barrier effect, the specific impacts on gene flow and long-term population health for small mammals in urban areas are not well understood, which this study seeks to address for eastern pygmy possums.
- Improving detection methods for the eastern pygmy possum, a cryptic species that is difficult to monitor using traditional methods. Novel detection methods are necessary to improve monitoring efficiency, particularly in urban environments where the species is most vulnerable to fragmentation effects.
- Evaluating the effectiveness of current management practices and suggesting future conservation strategies for maintaining genetic diversity and population viability in an urban-sensitive species. A critical gap exists in how current habitat management approaches can be applied effectively in fragmented urban landscapes, ensuring not just survival, but long-term security for small mammal populations.

This thesis is organised as a series of manuscripts that address these themes, using a combination of field surveys, genetic analysis, literature reviews and occupancy modelling to inform conservation strategies for the eastern pygmy possum and other urban-sensitive species facing similar challenges.

Chapter structure and summary

This thesis contains chapters which are based on papers in varying stages of publication. Chapters 4, 5 and 6 present data chapters which are based on experimental studies, while Chapter 2 provides a literature review and synthesis for a book chapter, and Chapter 3 presents a quantitative review of grey literature.

In Chapter 2, I provide an overview of road ecology research in Oceania, with a particular emphasis on Australia and New Zealand. I argue that while significant progress has been made in understanding the effects of roads on wildlife, there remains a gap in landscape-scale research that evaluates the long-term ecological impacts of road infrastructure. I review mitigation efforts, such as wildlife crossing structures, and highlight the need for broader ecosystem-based solutions that go beyond single-species measures. This chapter contextualises the research conducted in this thesis within the broader field of road ecology, demonstrating the importance of integrating landscape-scale connectivity into conservation planning.

Chapter 3 investigates the effectiveness of wildlife crossing structures in Australia, using a 25-year review of monitoring data from various road projects in New South Wales, much of which is unpublished in the peer-reviewed literature. Using data from a variety of arboreal mammals, I explore the species-specific preferences for different types of crossing structures and identify critical gaps in current research, to inform recommendations for the eastern pygmy possum. This chapter contributes to the growing body of work on wildlife crossing structures by providing an evidence base for improving their design and implementation in fragmented landscapes.

In Chapter 4, I investigate the occupancy of the eastern pygmy possum in fragmented peri-urban landscapes, using occupancy modelling to assess the relationship between species presence and habitat and landscape factors. Additionally, I examine the role of fire history and the management of prescribed burning for occupancy. This chapter emphasises the importance of landscape-scale habitat management for the long-term survival of the eastern pygmy possum in fragmented urban environments.

In Chapter 5, I focus on the challenges of detecting cryptic species, such as the eastern pygmy possum, which is difficult to monitor using traditional methods like trapping and spotlighting. I introduce camera trapping as a novel survey method, demonstrating that camera traps placed on flowering banksia trees significantly improve the detectability of the species. This chapter highlights the importance of innovative survey methods for urban-sensitive species and discusses the potential applications of these methods for other cryptic species in fragmented habitats.

Chapter 6 focuses on genetic connectivity in the eastern pygmy possum across fragmented habitats in northern Sydney, particularly the impact of an arterial road as a potential barrier to gene flow. Using genetic data from 119 individuals across nine habitat patches, I assess genetic differentiation and the role of landscape features in facilitating or hindering genetic exchange. Least-cost path analysis identified critical connectivity corridors, to guide future management actions. This chapter provides insights into the effectiveness of genetic analysis in informing conservation management and the placement of wildlife corridors for small mammals in fragmented areas.

In the final chapter (Chapter 7), I synthesise the findings from the preceding chapters to discuss the implications for conservation management in urban-sensitive species. I highlight the importance of maintaining habitat connectivity in peri-urban areas, with a particular focus on the eastern pygmy possum. I discuss the role of wildlife crossing structures and survey methods in improving species detection and mitigating road impacts. I also provide recommendations for future research to fill gaps in our understanding of road ecology and genetic connectivity and propose management strategies for enhancing the long-term survival of species like the eastern pygmy possum in fragmented urban environments.

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Chapter 2: Road ecology in Oceania

Road Ecology in Oceania

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Cassie Thompson[1], Rodney van der Ree[2,3], Kylie Soanes[3], Darryl Jones[4], Josie Stokes[2], Carol Bannock[5]

1 Behavioural Ecology & Conservation Lab, The University of Sydney, 2000, Australia; and Transport for New South Wales, Australia **(Corresponding Author)** 2 WSP Australia Pty Ltd, Melbourne, 3000, Australia 3 School of Agriculture, Food and Ecosystem Sciences, The University of Melbourne, Parkville, Victoria, 3010, Australia 4 School of Environment & Science, Griffith University, Brisbane, 4111, Australia 5 Waka Kotahi New Zealand Transport Agency, New Zealand

Abstract

The study of road ecology in Oceania is commonly associated with Australia; however, this chapter shows that the identification and mitigation of environmental effects caused by roads have been developing all over the region, most notably in New Zealand. Overwhelmingly, research and management decisions on road impacts on rare or threatened species are driven by conservation concerns. Thus, mitigation efforts usually centre on legislative mandates or single-species-based solutions, with few multispecies, ecosystem-level approaches like forested overpasses being adopted. This has generated research that delves into the assessment of the effectiveness of wildlife crossing structures, while ignoring the wider implications on the population and ecosystem, information that could be useful for management. Another consequence has seen mitigation coinciding with new infrastructure developments, rather than being completed in locations of greatest environmental or conservation concern.

Lessons learnt from early research will be important for those areas under development pressure in the more remote parts of the region, including Papua New Guinea and some of the Pacific Islands. Road ecology in Oceania needs a more coordinated approach to look beyond assessing the use of wildlife crossing structures, to foster research and programs that operate beyond road project timeframes. Using the knowledge gained, we can ensure that future road projects in the region are as ecologically sustainable as possible. Research

leadership, emerging technology and creating a coordinated approach for knowledge-sharing, will be vital to road ecology in the region into the future.

Synthesis

Early research

Road ecology research in Oceania began in Australia in the early 1980s, focusing on road vegetation as wildlife habitat in heavily cleared agricultural landscapes (e.g. Bennett 1991). Roadside and unused road reserves across Australia provide habitat for a wide range of species, including rare and threatened plants and animals, and often represent an assemblage of pre-European plant communities (Spooner 2004; Van de Ree 2001). In Western Australia, roadside roads are popular tourist destinations for their seasonal native wildflowers. As a result, several road protection and advisory committees have been established to assist road authorities in managing roadsides to enhance biodiversity values (Chiles In prep). Therefore, early research in Australia focused on protecting and improving critical roadside areas. Similar research was conducted in New Zealand in the early 2000s, focusing on improving roadside biodiversity.

Aligning with global trends (Engert in prep), recording the number and location of wildlife-vehicle collisions through road crash surveys was a common feature of early Australian transport ecology research. These studies are often local and relatively short-term (e.g. Coulson 1982; Driessen 1996); there are no systematic investigations at the state or national level. Research on the road barrier effect also appeared very early (e.g. Burnett 1992.). Similar studies later emerged in New Zealand, with specific research into road fatalities not starting until the late 2000s.

At the time, there were relatively few efforts to mitigate the environmental impact of roads, although notable exceptions laid the foundation for future research and action. Perhaps the most famous example was the 'Tunnel of Love' constructed in 1985. These were small box-culverts installed to rectify population declines of the endangered Mountain Pygmy Possum (*Burramys parvus*) following the construction of an alpine road which disrupted dispersal and population dynamics (Mansergh 1989) (Figure 1A). Other early wildlife crossing structures in Australia include pipe culverts for small mammals such as kangaroos, bandicoots, native rats and wombats (Scotts 1989).

Most studies have focused on temperate regions of Australia, with little evidence of mitigation within the wider region or tropical ecosystems. However, a crossing structure (Fig. 1C) was installed for a canopy-dependent species on a narrow dirt road in a northern Australian tropical forest. Such "rope tunnels" are used by a variety of arboreal species, including Lemuroid Ringtail Possum (*Hemibelideus lemuroides)*, Striped Possum (*Dactylopsila trivirgata*) and Green Ringtail Possum (*Pseudochirops archeri*) (Weston *et al.* 2011).

Figure 1: Historical road mitigation highlights in Oceania. *(A) The tunnel of love mimics a boulder field environment which provides habitat for the critically endangered Mountain Pygmy Possum (Mansergh 1989). (B) The first roadkill-related population study on quolls and Tasmanian Devil's results in fauna fencing in Tasmania - image ©David Hamilton (Jones 2000). (C) Canopy bridges installed for arboreal rainforest possums (Weston et al. 2011). (D) First fauna land bridge constructed at Yelgun targeting Koalas. (E) Compton Road land bridge and associated glide poles and rope crossings facilitating movement for a range of species (McGregor et al. 2017). (F) Fencing and crab bridge protect hundreds of thousands of land crabs during the annual migration from the oceans to the forest on Christmas Island (Muller and Misso 2015). (G) Penguin fencing installed to mitigate road strike in New Zealand. (H) Frog-friendly crossing with a natural ground surface installed to promote movement by endangered Growling Grass Frogs, which otherwise won't use underpass structures (Koehler 2014). (I) Genetic study reveals the installation of canopy bridges and glide poles re-instated connectivity for the Squirrel Glider in habitat fragmented by the Hume Highway (Soanes et al. 2018). (J) A major focus in New Zealand is the restoration of fish movements and the Fish Passage Guidelines (National Institute of Water & Atmospheric Research Ltd) provide mitigation measures to reduce water speed, rock ramp designs and methods to retrofit existing structures. (K) Threatened microbat roosting and breeding habitat is provided by installing artificial roost structures in culverts and under bridges (Goreki 2019). (L) The newly constructed land bridge over the Tonkin Highway, the first in Western Australia, records a successful Emu crossing.*

A boom in mitigation research and practice

Road ecology in Oceania gained momentum in the 2000s, driven by increased investment in infrastructure projects, paired with heightened attention on the environmental impacts of roads. Consequently, research groups devoted to road ecology formed and a broader community of research and practice began to develop. The Australian Federal Government hosted the first 'road ecology' workshop in 2006, with approximately 30 participants from Australia and New Zealand. Subsequent road ecology conferences were hosted around Australia, leading to the establishment of the Australasian Network for Ecology and Transportation (ANET – www.ecologyandtransport.com) and global participation and interest in road ecology in the region.

Overwhelmingly, research and mitigation in the region has been driven by the need to mitigate the impact of major road construction projects on rare or threatened species. This undoubtedly created opportunities to advance the field of road ecology in the region. Partnerships between ecologists and road agencies have facilitated some long-term research, robust experimental evaluations, and raised the profile of the environmental impacts of roads throughout local, state and federal governments (e.g. Chambers and Bencini 2015; Soanes *et al.* 2018; Goldingay *et al.* 2019). As a result, there is much greater consideration of the impacts of roads in environmental planning and approvals, increased requirements for mitigation, and the development of connectivity plans and best-practice guidelines for fauna-sensitive road design.

However, a reliance on construction projects to drive the research and funding agenda has left road ecology in Australia largely reactive and opportunistic rather than strategic, with several important consequences:

- Mitigation focuses on legislative requirements and single-species measures, while larger, multispecies or ecosystem-level solutions such as forested overpasses or 'land-bridges' are scarce.
- Research focuses largely on evaluating the use of wildlife crossing structures, often in a compliance framework, rather than understanding the broader population and ecological impacts and guiding mitigation needs.

- Mitigation efforts typically coincide with the location of recent infrastructure developments and upgrades, rather than in locations of greatest environmental or conservation concerns.
- After construction, the funding and associated positions to support researchers have declined, as reflected by a downturn in research in recent years, and the closure of research hubs.

Wildlife mitigation

The construction and widening of roads led to a proliferation of mitigation measures for wildlife, primarily crossing structures and fencing. Underpasses and funnel fencing were the most common approach, with box-culverts and tunnels specifically installed for iconic mammals such as kangaroos and the Koala (*Phascolarctos cinereus*) (Jones 2012), and endangered small mammals such as the Brush-tailed Phascogale (*Phascogale tapoatafa*) and Southern Brown Bandicoot (*Isoodon obesulus*) (Chambers and Bencini 2015; Goldingay *et al.* 2019).

Forested overpasses are rare in Australia, and while fewer than ten have been constructed across the country, there is strong evidence supporting their effectiveness at facilitating movement of a range of species of wildlife (Pell and Jones 2015; McGregor *et al.* 2017) (Figure 1D&E). The slow adoption of land-bridges is likely due to road agencies tending to prefer species-specific measures that are less expensive, yet still ensure compliance with environmental regulations.

A particular focus in Australia has been mitigating the impacts of roads on arboreal mammals, such as possums and gliders. These species are often gap-limited, highly susceptible to the barrier effects of roads, and usually of high conservation concern. As a result, canopy bridges and glider poles have been widely installed across the country and their use has been documented by more than 10 species of arboreal mammal (Weston *et al.* 2011; Soanes *et al.* 2013; Taylor and Goldingay 2013).

Evaluating mitigation effectiveness

Research partnerships between road agencies and ecologists facilitated the evaluation of many mitigation measures, generating world-leading research. While many research projects established the use of these new mitigation measures by wildlife, the focus quickly turned to evaluating effectiveness. Experimental designs that incorporated before data or control sites were used to determine whether mitigation measures reduced (or prevented) the negative impacts of roads on wildlife (Soanes *et al.* 2013; Taylor and Goldingay 2014; Pell and Jones 2015).

Research then began taking a population-level approach, testing whether mitigation led to improved population survival rates, reproductive outputs, or population viability (chapter 12). The negative impacts of roads at the population level were demonstrated through:

- mark-recapture studies were used to assess survival rates of a range of species, including the Mountain Pygmy Possum (one of the first in the world to quantify a population-level effect of restored movement) (van der Ree *et al.* 2009) and the Squirrel Glider (*Petaurus norfolcensis*) (McCall *et al.* 2010), as well as population size of the Eastern Quoll (*Dasyurus viverrinus)* and Tasmanian Devil (*Sarcophilus harrisii)* (Jones 2000) (Figure 1B);
- population viability analyses for Swamp Wallaby (*Macropus bicolor)* (Ramp and Ben-Ami 2006) and Common Wombat (*Vombatus ursinus)* (Roger 2011); and
- other work investigated the presence and causes of the 'road effect zone' for bats (Bhardwaj 2021), insects (Bhardwaj 2018), microclimatic conditions (Pohlman 2007) and small mammals (Burnett 1992.).

Genetic approaches have also proved useful both to identify and prioritise locations for mitigation measures and to evaluate their success. Genetic analyses of a roadside population of Eastern Pygmy Possums (*Cercartetus nanus*) conducted prior to a major road widening project guided the design and location of wildlife crossing structures and set evaluation targets (C. Thompson, unpub. data). Genetic analyses were incorporated into a before-after-control-impact design to show that canopy bridges and glider poles restored gene flow across a pre-existing road barrier for Squirrel Gliders (Soanes *et al.* 2018).

Beyond Australia

Fewer road ecology studies have been conducted in New Zealand, but as with Australia, the approach has been largely ad-hoc and short-term. New Zealand is unique in terms of ecosystems and indigenous flora and fauna and the only native land mammals are bats. The impact of roads on ecology is a new and evolving field, with national protocols only recently established to guide the assessment of road project impacts on biodiversity. However, broader research into the impacts of roads is increasing, such as the research on Long-tailed Bats (*Chalinolobus tuberculatus*) which identified decreased bat occupancy with increasing night-time traffic volume (Borkin *et al.* 2020), research into edge effects from roads (Simcock et al., 2022) and current research into bird mortality from vehicles and roads. There has also been significant focus on the restoration of fish movements through road structures (Figure 1J) in New Zealand.

In Papua New Guinea and other islands in the Pacific and Indian Oceans, road mitigation activity has been largely non-existent. One key exception are efforts on Christmas Island to protect migrating land crabs (Figure 1F). Hundreds of thousands of the iconic Red Crabs (*Gecarcoidea natalis*) were being killed annually by vehicles. To mitigate this, numerous underpasses and a crab-specific overpass and fencing was installed in the early 2010s and has significantly reduced mortality rates (Muller and Misso 2015). Most recent road ecology studies in Papua New Guinea and the islands have focussed on the impact of creating roads in tropical forests and subsequent deforestation and poaching (Laurance *et al.* 2009; Alamgir *et al.* 2019).

Perspectives

Road ecology in Oceania needs a more coordinated approach to look beyond assessing the use of wildlife crossing structures and foster research and programs that operate beyond road project timeframes. Using the knowledge gained, we can ensure that future road projects in the region are as ecologically sustainable as possible. We propose four key needs moving forward:

Research leadership and strategic coordination

There is a clear need for a coordinated approach to mitigation, monitoring and evaluation priorities within Oceania. Continuing the ad-hoc, ‘road-project by road-project’ approach will lead to wasted resources, poor biodiversity outcomes, and will stunt the advancement of the field. The current approach can also result in several small projects independently addressing the same question, each with limited inferential strength. Inevitably, this will result in poor outcomes and limited learning, thereby minimising the knowledge base needed for decision-making. Worse still, if these lessons are not shared broadly, new projects are likely to repeat past mistakes.

A coordinated approach could involve road ecologists, agencies and government working together to prioritise and strategically assign research, based on ecological need and current knowledge gaps. Such an approach would be more cost-effective, allowing the study of a larger number of structures simultaneously, using standard and consistent methods. It would also allow for cross-jurisdictional datasets on road impacts, mitigation efforts, and evaluations of success through a collaborative funding model.

New Zealand has taken steps towards a more strategic vision for road ecology through the recently established Biodiversity Knowledge Hub within the New Zealand Ministry of Transport, which aims to identify research needs and opportunities. Similar hubs or centres of practice are needed throughout the region, particularly where the ecological impacts of new or improved roads threaten areas of high ecological value, such as Papua New Guinea and other Pacific Islands.

A strategic research agenda would ensure that research is forward thinking and responsive to ecological needs, rather than simply reactive to construction agendas. For example, the high levels of roadkill in Tasmania have been known for some time yet receives little attention or research funding because of the small state population and revenue-base. Similarly, future challenges of climate change, urbanisation, and the cumulative impacts of roads could be better considered under a coordinated approach. A strategic and coordinated approach will also support the development of a strong, networked scientific community that can respond to the coming challenges with a strong evidence-base to inform decision-making.

Future research areas

Oceania has many unique species which present challenges to reduce the negative impacts of roads. However, new approaches are being developed, and methods used elsewhere in the world are being tested, to cater to our unique biodiversity. Key research in Oceania includes:

- *Citizen science and roadkill*: A recently developed smartphone application (Roadkill Reporter App) aims to address the lack of accessible roadkill data in Australia, with the potential to quantify roadkill and identify 'hotspots' at a scale and rate not seen before.

- *Impacts on habitat values of roads during upgrades*: Designing and manufacturing permanent microbat roosting and breeding habitat for new and retrofitted structures, with successful uptake and breeding events recorded (Goreki 2019).
- *Retrofits and modifications to existing road infrastructure*: Modification to existing roads and drainage structures, providing a cost-effective method to reduce the barrier effects, including the addition of fauna fencing (to reduce mortality and funnel animals to structures), ledges (to allow the structure to be used even when inundated) and cover or furniture (to encourage use by wildlife). Rapid uptake of retrofitted ledges has been recorded by Koalas (*Phascolarctos cinereus*) (Jones 2012).
- *Evaluating the effects of roads on New Zealand's endemic bat populations*: Guidelines are being established to provide a framework to manage the effects of road projects on bats.

More broadly, habitat loss is the biggest threat to biodiversity in the region. It's noteworthy that road construction is currently the main driver of this. New or enhanced roads offer the chance to exploit new regions of tropical forest, which results in logging, mining, and poaching—often referred to as the "first cut" in pristine forests (Campbell *et al.* 2017). Due to their biological specialisation and tendency to avoid even small clearings and forest edges (less than 30 metres wide), many tropical wildlife species are particularly vulnerable to linear infrastructure. They also face increased risk of roadkill, human predation, and hunting near highways (Laurance *et al.* 2009).

To stimulate economic growth, national road networks in Papua New Guinea will double over the coming years (Alamgir *et al.* 2019), representing a substantial threat to an area of exceptionally high species endemism. In a similar vein, new roads for logging have been and are still being built in the Solomon Islands. Many developing nations in Oceania have recently signed the Belt and Road Initiative, which will lead to further investment in the region's extractive industries and new linear infrastructure.

In order to minimise encroachment and the exploitation of tropical forests, future road networks should be built using a strategic and regional strategy that avoids protected and high-value conservation areas (chapter 58). (Laurance *et al.* 2009). For instance, using best-practice road planning and design will help reduce the ecological impact of large-scale road projects, like the Highland Highway that is being considered for Papua New Guinea (Alamgir *et al.* 2019). Nevertheless, little is known about how roads affect the rare species assemblages found in these places. Research is needed in this area to develop ways to regulate and minimise the wider impacts of frontier roads, including ways to mitigate the impacts for edge-sensitive forest species.

Moving beyond compliance to long-term ecological research

The historical focus on the 'compliance performance' of wildlife mitigation measures leaves other important questions unanswered.

There are still many knowledge gaps that limit our ability to design and construct roads with minimal environmental impacts. For example, the population-level impacts of roads on wildlife remain poorly understood, as does the capacity of wildlife crossing structures to redress those impacts (Taylor and Goldingay 2010). Overwhelmingly, research projects are short-term and focus on measures of individual response (such as roadkill or movement) rather than population responses such as changes in population size, population vital rates, or viability (Van der Ree 2008).

Similarly, the environmental impacts of roads encompass much more than wildlife mortality and reduced movement. Roadsides are sometimes the only habitat remaining in agricultural areas (chapter 18), while in other areas, roads can represent an avenue for incursion by introduced species or future anthropogenic impacts. Other important issues for wildlife, such as traffic noise, artificial light at night, disturbance and stress responses, pollution and changes in community structure are poorly researched, and we have little evidence to guide and test appropriate mitigation.

Road ecology research in Oceania must move beyond short-term monitoring, to creating a deeper understanding of the biodiversity values of roadside areas, how roadsides are used by species for habitat in different regions, the extent to which mitigation structures benefit broader wildlife populations, and other measures needed to improve wildlife populations and their habitats where these are impacted by roads.

Broadening scope to species and spaces

Road ecology research in Oceania has predominantly focused on mammals, especially macropods, and in Australia often within already disturbed urban and rural landscapes. While this work has been incredibly valuable, the limited contexts in which road impacts and mitigation have been studied make broader generalisations difficult. Future work should focus on understanding the impacts of roads across a wider range of species and landscape types, and across countries which have different ecosystems, unique species and different transport needs.

For example, effective mitigation measures for amphibians remains a major knowledge gap in Australia. The few studies that have occurred suggest that current underpass designs are ineffective (e.g. Hamer *et al.* 2014) or require specific design features to be effective for amphibians (Koehler 2014). Studies of mitigation for reptiles and birds are also scarce despite these species groups often being more susceptible to road effects such as noise, artificial light at night, pollution and vehicle disturbance – factors that are not well addressed by current mitigation efforts in Australia. Research on fish movement and mitigation effectiveness are increasing in Australia and New Zealand (Keep *et al.* 2021) and resulting in the development of detailed guidelines for implementation (Franklin 2018).

The sharing of knowledge and resources among jurisdictions in the wider region and targeted training will be critical to reducing future impacts in these less-studied regions. However, New Zealand, Papua New Guinea and the Pacific Islands also contain highly specialized species and ecosystems, such as the ground-dwelling birds of New Zealand, making generalisations from existing research more difficult. This highlights the need for new research, and partnerships with local scientists, government, road agencies and the broader research community in these areas and targeted to their unique wildlife and road-ecology issues.

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Chapter 2: Summary

In Chapter 2, I provide a review of the road ecology literature across Oceania, focusing on the documented impacts of roads on wildlife, the extent of mitigation efforts, and the major research and implementation gaps. It synthesises the state of road ecology research across Oceania, revealing a strong focus on large, mobile, and charismatic species, with limited consideration for small, cryptic mammals. There is a growing recognition of roads as partial barriers that reduce functional connectivity, yet empirical evidence on mitigation efficacy remains scarce, particularly for urban-sensitive species persisting in peri-urban landscapes.

The gaps highlight the need for more targeted assessments of how wildlife crossing structures perform for lesser-studied species. The next chapter (Chapter 3) addresses this by reviewing 25 years of data on wildlife crossing structures in Australia, focusing specifically on arboreal mammals and the effectiveness of design elements in facilitating safe road crossings.

Chapter 3: Success or failure? Insights form 25 years of monitoring wildlife crossings

Success or failure? Insights from 25 years of monitoring wildlife crossings

Cassie Thompson[1], Josie Stokes[2] and Peter B. Banks[1] and Rodney van der Ree[3]

1 School of Life and Environmental Sciences, University of Sydney, 2006, Australia.

2 City of Gold Coast Council, Bundall QLD 4217, Australia.

3 WSP Australia Pty Ltd, Melbourne 3000, Australia

Short summary: Roads fragment habitats and threaten wildlife by disrupting movement and increasing rates of mortality. This study reviews 25 years of monitoring data from internal transport department-commission reports revealing species-specific use patterns which can be used to guide future mitigation in road-affected landscapes. Despite this, the effectiveness of crossing structures for maintaining wildlife populations remains largely unknown.

Abstract

Linear infrastructure, such as roads and railways, can significantly fragment vegetation and impede wildlife movement, particularly for arboreal mammals that rely on continuous canopy connectivity. In Australia, the installation of wildlife crossing structures is a common mitigation strategy to address these challenges; however, their effectiveness in facilitating movement and maintaining population viability remains poorly understood. This study synthesises 25 years of largely unpublished monitoring data from New South Wales, Australia, focusing on species-specific patterns of use of various wildlife crossing structures. Our findings reveal that wildlife crossing structures are successfully used, with distinct preferences among arboreal mammals for different crossing structure types. Underpasses with fauna furniture are being used typically by non-gliding species, glider poles are used by most gliding mammals, canopy rope bridges are used for movement by a range of arboreal species though larger gliders rarely use them, and vegetated overpasses have rarely been evaluated in monitoring reports. Despite the observed use of these structures by 16 arboreal mammal species, including species of conservation concern, the overall effectiveness in enhancing connectivity and supporting population stability remains uncertain. This review highlights critical data gaps and emphasises the need for comprehensive and consistent monitoring that assesses broader ecological outcomes, such

as genetic exchange and population viability, to ensure that mitigation strategies deliver meaningful conservation benefits on a landscape scale.

Introduction

The impact of linear infrastructure, such as roads and railways, on wildlife populations is a pressing concern globally (van der Ree *et al.* 2015), and particularly in regions like Australia, where unique arboreal mammals face significant threats from habitat fragmentation (Taylor and Goldingay 2012; van der Ree *et al.* 2015). Roads and railways disrupt movement patterns and increase mortality rates due to vehicle collisions, leading to declines in wildlife populations (Forman *et al.* 2003; Rytwinski *et al.* 2015; Barrientos *et al.* 2019). Since the early 1980s, various mitigation strategies have been implemented to address these issues, with wildlife crossing structures emerging as a widely adopted solution (van der Ree *et al.* 2007; Soanes *et al.* 2018; Thompson C. in press). These structures aim to enhance habitat connectivity by facilitating safe passage over or under roadways, thereby reducing the barrier effects posed by roads (Goldingay *et al.* 2013; Soanes *et al.* 2024).

Despite the widespread installation of wildlife crossing structures, their effectiveness in facilitating movement and maintaining population viability remains poorly understood (van der Grift *et al.* 2013; Thompson C. in press). Much of the existing research has focused on single species, limiting the ability to generalise findings across species, landscape contexts, and structure designs (Soanes *et al.* 2013). Moreover, the effectiveness of wildlife crossing structures has typically been measured by direct observations of use by target species (Taylor and Goldingay 2013; Soanes *et al.* 2024).

A significant body of monitoring data on wildlife crossing structures has been commissioned and is held by transport departments from many years of monitoring. These studies have the potential to inform a deeper understanding of the value of different types of structures, but the studies are difficult to find and access, which is particularly concerning given the substantial financial investments made in constructing wildlife crossing structures. Further, many individual reports exist in the form of grey literature, which while informative to the local system, may lack the scientific rigor needed to identify a structures' general benefits to wildlife populations. Understanding the effectiveness of wildlife crossing structures is crucial not only for the conservation of wildlife populations but also to inform future infrastructure planning and wildlife management strategies.

Thus, this study aims to address gaps in our understanding of wildlife crossing structures, by synthesising 25 years of unpublished data in monitoring reports commissioned by the New South Wales (NSW) transport agency in Australia. We focus on arboreal mammals which are particularly vulnerable to the impacts of roads because their movements are largely made via connected canopy cover, and road corridors often result in abrupt gaps in habitat that are difficult or dangerous to cross, increasing the risk of mortality and genetic isolation (Goosem 2001; van der Ree *et al.* 2007; Soanes *et al.* 2018). Specifically, we (1) Collate and summarise evidence for the use of wildlife crossing structures by arboreal mammals in NSW; (2) Provide an evidence-base for species-specific preferences regarding different crossing structure types and designs; and (3) Assess whether existing monitoring methods can reliably evaluate the efficacy of these crossing structures in mitigating road impacts and maintaining connectivity for arboreal mammals. By providing a comprehensive analysis of the use patterns of arboreal mammals across various crossing structures, this review seeks to inform ecologists, policy-makers, and road designers about mitigation strategies that can best enhance habitat connectivity and support the conservation of Australia's arboreal fauna.

Methods

Literature sources

This review focuses on data collected for monitoring programs for road projects completed throughout NSW, Australia. NSW contains the largest number of monitored wildlife crossing structures in Australia, with work commissioned by the state transport agency. Internal transport department archives were searched and all available monitoring reports completed before December 2023 were reviewed to investigate the use of wildlife crossing structures installed across the state. Our review focused only on structures that were specifically designed as wildlife crossing structures and which were intentionally monitored for use by arboreal mammals. Arboreal mammals included in the review are those that strictly (gliding and non-gliding arboreal) and generally (semi-arboreal) use trees for foraging, shelter and movement throughout the landscape.

The impetus behind the crossing structure reports were State and Federal project approval regulatory conditions to determine the effectiveness of mitigation measures, which included wildlife crossing structures. All reviewed reports were prepared by ecological consultants, except for the Hume Highway upgrades and the Oxley Highway upgrade at Port Macquarie, which were completed by academic institutions acting in a consultancy context. The effectiveness of crossing structures was measured by recording 'use' of the crossing structure by the target species, usually one that was of conservation concern under State and/or Federal legislation (i.e. species of conservation concern).

To complement the grey literature review, a search of peer-reviewed literature on wildlife crossing structures for arboreal mammals in Australia was conducted, to compare our results against published wildlife crossing structure use. This search was conducted in the ISI Web of Science database (January 2025), using the following keyword string: (road*, highway*, connect*, habitat*, barrier*, OR traffic*) AND (rope*, ladder*, passage*, overpass*, underpass*, pole*, crossing structure*, measure* OR mitigat*) AND (wildlife*, fauna*, animal*, marsupial*, mammal*, OR arboreal*). We also searched Google Scholar (January 2025) using the same combinations of these keywords. Results were filtered to papers specific to Australia.

Data extraction and analysis

All reports where arboreal mammals were recorded using wildlife crossing structures are included in this review (see Supplementary material Table 2 for a list of species included in the review). Wildlife crossing structures included underpasses (dedicated fauna and combined culverts, pipe culverts, bridge underpasses), vegetated overpasses, canopy rope bridges, glide poles and vegetated medians (Table 1). A wildlife crossing structure was considered 'used' when an arboreal mammal was detected on the structure via photographs, collection of hair samples or via observation of tracks. The following data were recorded when a species was recorded using a wildlife crossing structure: species, target species, location, type and design of crossing structure, survey method, and survey effort.

Due to the lack of consistency in the survey methods used, different survey durations, survey timing and locations across studies, we first used descriptive statistics to detect patterns in the data. We examined differences in monitoring methods, number and type of species recorded using each wildlife crossing structure type, investigated the physical attributes of the wildlife crossing structures to identify if it influenced use, and the location of the structures that were monitored. Survey effort varied widely across the monitoring reports, and the data was standardised to enable consistent summarising and comparing across projects and species. Few reports provided evidence of full crossings, that is, when an arboreal animal was recorded on both ends of a crossing structure (generally within 10 minutes of the first record), or on the median glider pole (in between traffic lanes), or heading in the opposite direction to infer complete crossing. This may have been due to the limitations of the cameras used to detect animals successfully as has been shown in other studies (Seidlitz *et al.* 2020), and differences in camera models used across studies makes comparison difficult.

The rate of use was calculated where sufficient data was available (i.e. total survey effort and number of detections) by dividing the number of detections of each species per wildlife crossing structure method (e.g. camera, sand pad etc) by the total survey duration during that monitoring period (e.g. total number of camera nights, sand pads, hair tubes, trapping or spotlighting nights at each structure). So, where detection was recorded on three nights out of 100, the rate of use was 0.03. Of note, this review didn't include structures where no use was recorded (ie presence only data was included). Heatmaps were generated using the ggplot2 package (Wickham 2016) in R (RCoreTeam 2024) based on rates of use by species, by wildlife crossing structure type to identify preferences of use. Differences in the rate of detection across species and types of wildlife crossing structures were analysed statistically using ANOVA in R (RCoreTeam 2024).

We were unable to compare rates of use of crossing structures, including structures where use by an arboreal mammal was not detected, against local population size. However less than 30% of studies had some measure of the local population size within the crossing structures reports and where they did, it focused on single species and was generally not reported in the structure use reports. Further, the vast majority of reports failed to assess or record whether arboreal species were present in the immediate adjacent area, with the exception of those that specifically targeted species of conservation concern, so we were unable to look at variation in rates of use based on movements and density in habitat surrounding crossing structures.

Results

Road projects reviewed

We reviewed 20 project's monitoring reports prepared between 1997 and 2023 by the state road department from road projects in eastern NSW, encompassing the Pacific Highway, Oxley Highway, Olympic Highway, northern section of the Princes Highway and southern section of the Hume Highway (Figure 1). Of note, several other crossing structures throughout the state have been monitored and results published in peer-reviewed literature by academics but were not included in our analysis. As the reports came from the state transport agency, all projects were large in scale, incorporating the duplication of 2-lane highways to dual carriageway 4-lane highways, or new dual-carriageway highways at greenfield sites.

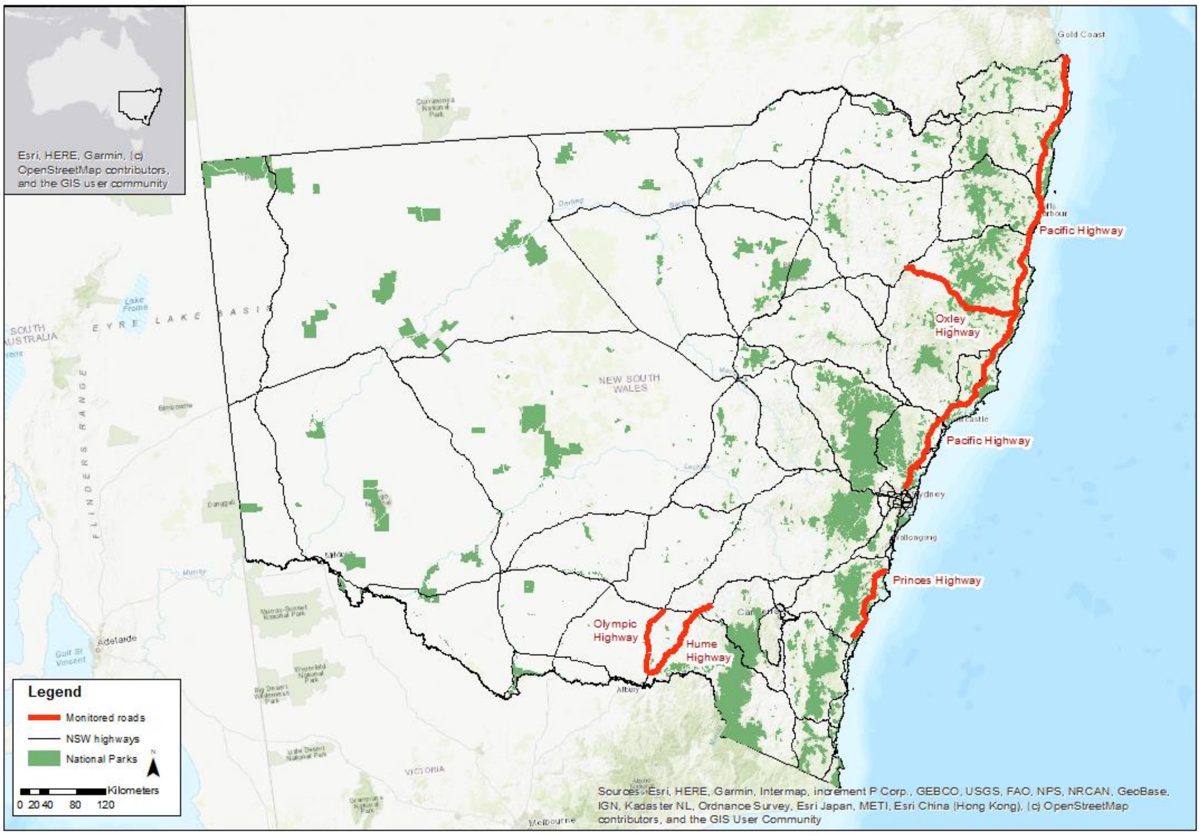

Figure 1: Location of the monitored highways in New South Wales, Australia included in this review.

Crossing structure types

Ten different wildlife crossing structure types recorded use by arboreal mammals (Table 1).

Table 1: Overview of crossing structure types included in the review.

Structure Type/ Variant		Description	Wildlife crossing structure photos	Photo source and credit
Underpass	Dedicated fauna culvert	Concrete or box culvert designed exclusively for wildlife; dry substrate year-round; often fitted with fauna furniture.		Woolgoolga to Ballina Pacific Highway Upgrade (Source: Sandpiper Ecological)
	Pipe culvert	Large-diameter pipe culvert primarily for drainage but can function as fauna passage; may carry low flow water.	 	Glenugie Pacific Highway Upgrade (Source: Sandpiper Ecological)
	Combined underpass	Multi-purpose culvert or bridge shared with drainage, stock or pedestrian use, modified to suit wildlife passage.		(Source: Josie Stokes)
	Bridge underpass	Elevated road bridge providing wide, open under-road space with natural light and substrate for multiple species.		Pacific Highway Upgrades (Source: NSW Roads and Traffic Authority)
	Bebo arch	Pre-cast concrete arch creating a spacious underpass with natural substrate and headroom for larger fauna.	 	Glenugie Pacific Highway Upgrade (Source: Snadpiper Ecological)
Vegetated overpass		Land-bridge or tunnel roof planted with native vegetation, forming continuous habitat over the road.		Bonville Pacific Highway Upgrade (Source: Josie Stokes)
Glider pole array		Vertical poles (often with glide beams that are parallel or perpendicular to the road) in median/verge offering launch & landing points for gliding mammals.		Woolgoolga to Ballina Pacific Highway Upgrade (Source: Sandpiper Ecological)

Structure Type/ Variant		Description	Wildlife crossing structure photos	Photo source and credit
Canopy rope bridge	Rope ladder bridge	Flexible rope ladder suspended between poles/trees above carriageway, facilitating arboreal crossings.		Coopernook to Heron's Creek Pacific Highway Upgrade (Source: Sandpiper Ecological)
	Rope tunnel bridge	Cylindrical or enclosed rope tunnel offering protected arboreal pathway across the road.		Karuah Bypass Pacific Highway Upgrade (Source: Theiss)
Vegetated median		Retention or planting of mature trees within dual-carriageway median enabling species to glide carriageway-to-carriageway.		(Source: Josie Stokes)

Report survey methods and study design

Of the 151 wildlife crossing structures monitored with recorded arboreal use, only 63 (42%) included surveys to investigate the presence of arboreal mammals in the habitat surrounding the crossing structure, though this was mainly restricted to methods to record the target species of conservation concern. Of these, only 34% monitored species abundance in habitat surrounding the wildlife crossing structures through trapping (live, camera and remote (eg call playback)), however few (<10%) studies included the results or discussed the outcomes of this in the structure monitoring reports. When completed, population surveys surrounding the crossing structures focused on species of conservation concern, with one exception where the non-threatened sugar glider (*Petaurus breviceps*) was radio-tracked in proximity to wildlife crossing structures for the Pacific Highway upgrades (Bonville upgrade). Of note, species of conservation concern represent only half of the arboreal mammals that have been recorded on wildlife crossing structures in this review (see Supplementary material). Thus, the full suite of arboreal species was not targeted by the survey methods employed, nor where they reported on.

Only the Hume Highway Upgrade used a Before-After-Control-Impact (BACI) study, individually tagging animals (and included radio-tracking), with population studies including genetic analysis and controls to investigate the efficacy of the crossing structures on the squirrel glider (*Petaurus norfolcensis*). Interestingly, the results of this study were published in the peer-reviewed literature as it was completed by an academic institution (four papers) as was work completed by academic institutions for projects on the Oxley and Pacific Highway upgrades (eight papers) (see Supplementary material Table 1). None of the studies completed by ecological consultants were published in peer-reviewed journals.

Detecting and quantifying use of wildlife crossing structures was completed using a range of methods (Figure 2). Most commonly this comprised wildlife detection cameras (95%), with spotlighting and hair tubes being the least used methods. Very few studies used radio-tracking (9%). Survey duration at a structure varied in length from 18 nights (a bridge underpass) to 1,420 nights (almost 4 years at a bebo arch), however the average length of time surveyed across all structures was close to one year.

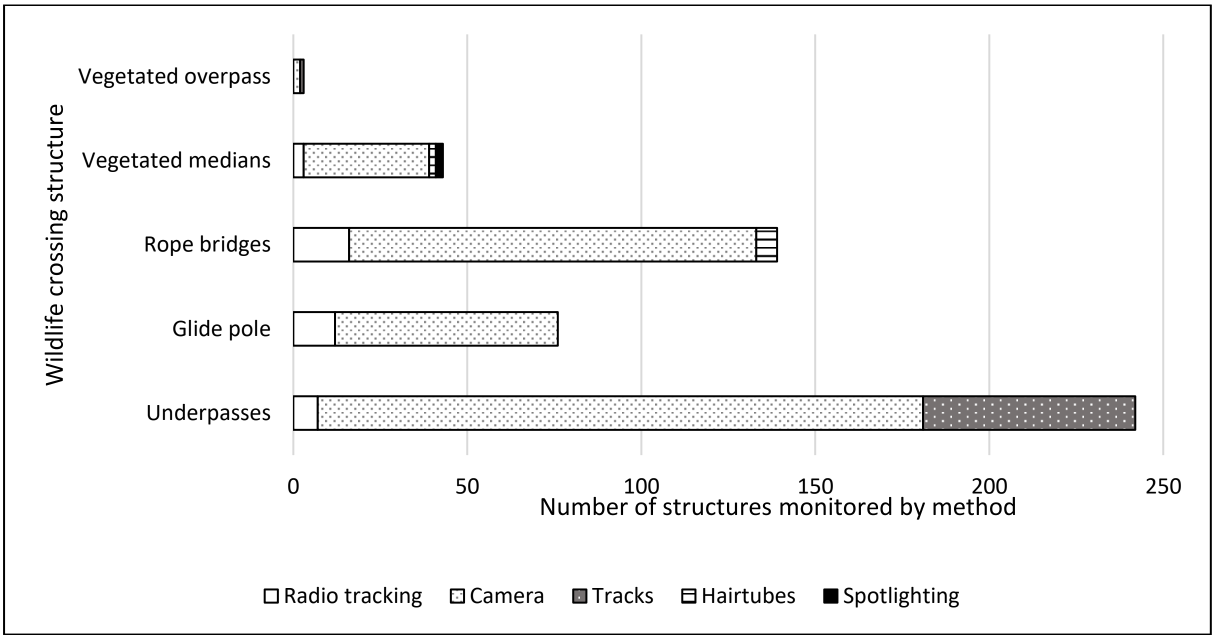

Figure 2: Survey methods used to detect wildlife crossing structure use, where arboreal species were detected (note some structures used multiple methods).

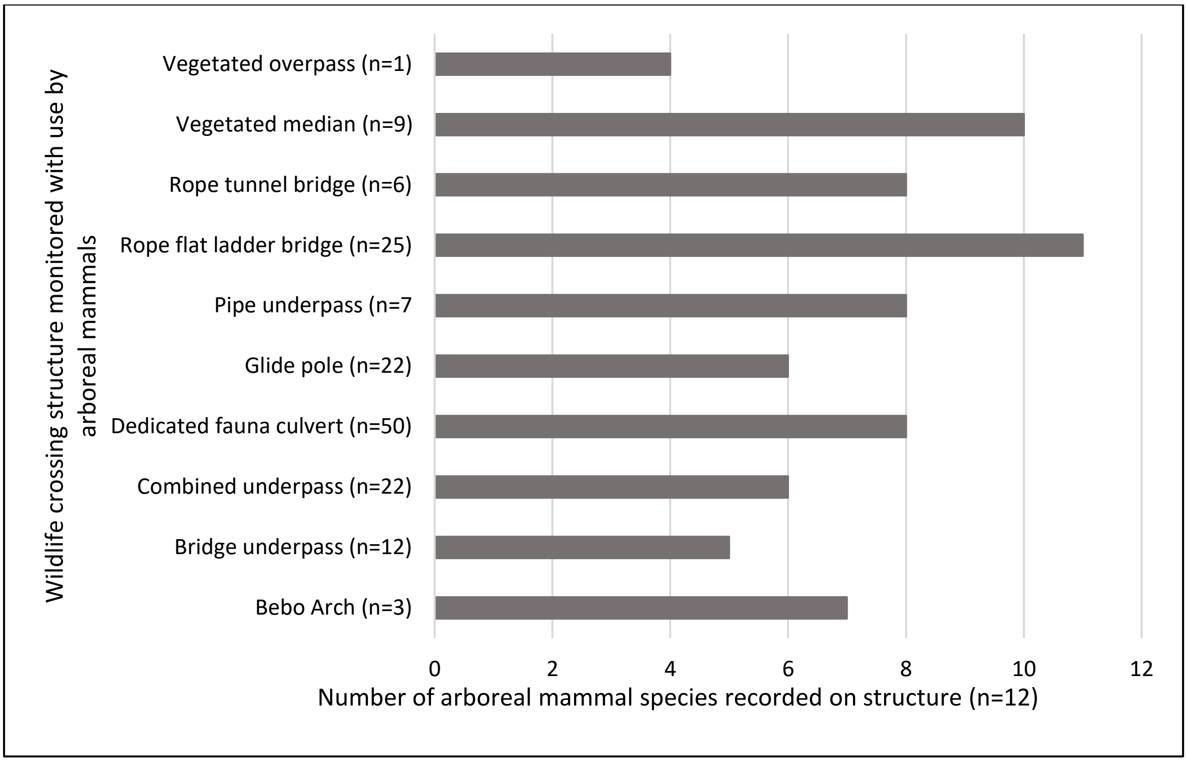

Figure 3: Number of arboreal species detected using each type of wildlife crossing structure (n=151). Data come from 20 road upgrade projects across New South Wales, Australia, reported between 1997-2023.

Crossing structure use

Sixteen arboreal mammals were detected on wildlife crossing structures in this review (see Supplementary material Table 2). No single structure recorded use by all arboreal species. Flat rope ladders were used by the most species (12), followed by a vegetated median coupled with a dedicated fauna underpass (nine species) and then a rope tunnel ladder (eight species) (Figure 3). The structure with the least species diversity detected was the vegetated overpass (three species – koala (*Phascolarctos cinereus*), short-eared brushtail possum (*Trichosurus caninus*)and spotted-tail quoll (*Dasyurus maculatus*)).

Few reports (<10%) detailed the type and condition of habitat surrounding the wildlife crossing structures. A key absence was the quantification of the condition of the approaches to wildlife crossing structures, which can have a significant influence on use, particularly for species that are dependent on canopy and shrub cover for movement and predator avoidance. Fauna furniture, defined as structural elements such as logs, poles, ledges, or ropes added to crossing structures to facilitate safe movement, provide shelter, or mimic natural habitat features for wildlife, was installed in most of the underpasses investigated, with 76% reported to contain log rails and refuge poles and 1% with concrete ledges. Interestingly, only some (<8%) of the studies included methods to recorded use of fauna furniture. Importantly, species of conservation concern such as the brush-tailed phascogale (*Phascogale tapoatafa*) and koala were detected using fauna furniture to navigate underpasses (koala recorded once (Nambucca Heads to Urunga) and brush-tailed phascogale recorded frequently (Frederickton to Eungai, Oxley Highway to Kempsey and Woologoolga to Ballina)). However, some species were more frequently detected moving along the ground in underpass structures without using fauna furniture, including the koala, and the common brushtail possum (*Trichosurus vulpecula*), bush rat (*Rattus fuscipes*) and *Antechinus spp*..

Rates of use varied across species and crossing structure type, with the highest rates (0.13) recorded for all arboreal mammals combined on rope tunnel bridges (n = 6 structures monitored) followed by both glider poles (0.12, n = 22) and dedicated culverts (0.12, n = 50), then vegetated medians (0.11, n = 9)) (Figure 4). The lowest rates of use were recorded on vegetated overpasses (0.02, n = 1) and bebo arches (0.03, (n = 3)), but it's worth noting the low sample sizes for these structures and the large surface area which may prove difficult to monitor with cameras (the only method used on these structures). There was no significant difference in the rate of use of the different types of crossing structures by combined arboreal mammal use (P=0.247).

Of the 151 monitored structures used by arboreal mammals, 80% recorded use by the target species of conservation concern that the structure was specifically built for. Despite this, common species such as *Antechinus spp*., short-eared brushtail possum and sugar gliders were among the most frequent arboreal users of wildlife crossing structures, while species of conservation concern like the yellow-bellied glider (*Petaurus australis*) and eastern pygmy-possum (*Cercartetus nanus*) used wildlife crossing structures less frequently. There was variability in the combined rates of use by different arboreal mammals and this relationship was significant (P=0.046) (Figure 5). Some species known to occur in the area surrounding the wildlife crossing structures were not recorded at all, such as the greater glider (*Petauroides volans*) (a species of conservation concern). This suggests that while some individuals or specific monitoring events may show high rates of use (possibly due to low survey effort but detection), these are outliers and the overall crossing rates remain low in comparison for most arboreal mammals (Figure 5).

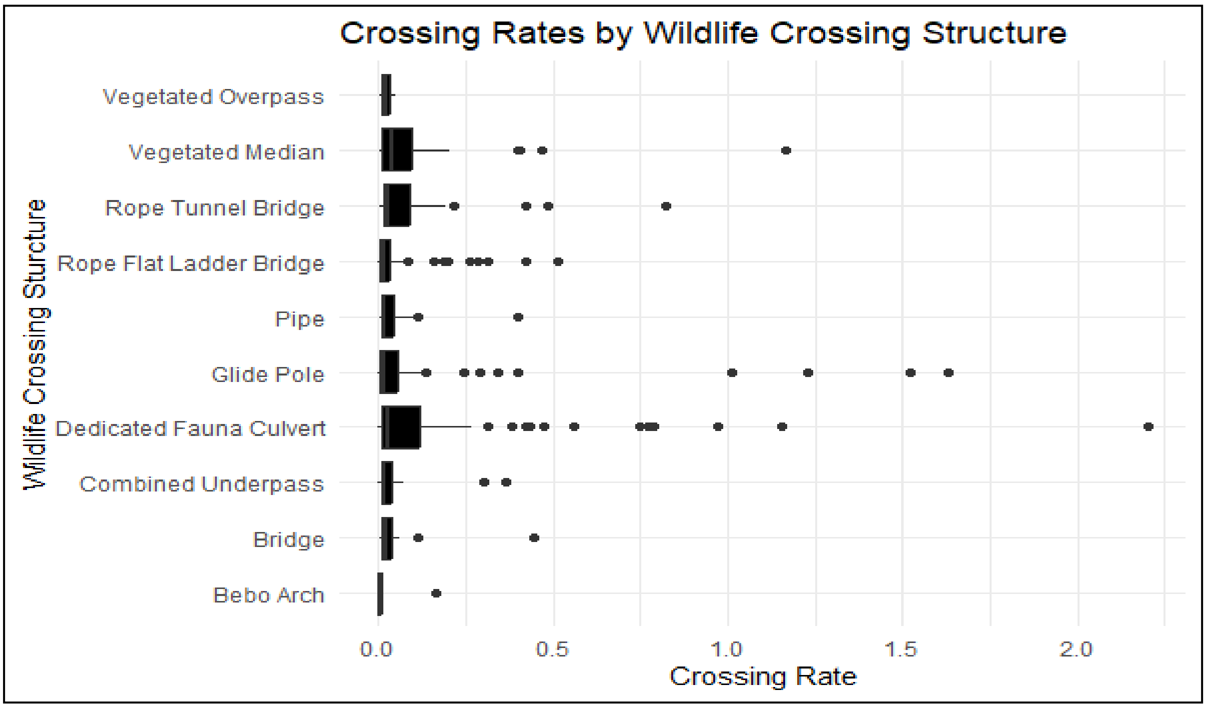

Figure 4: Average crossing rate among arboreal species by type of wildlife crossing structure, based on monitoring across New South Wales, Australia from 1997-2023 (n=151 crossing structures). Crossing rates are the number of detections of arboreal mammals divided by the total survey effort.

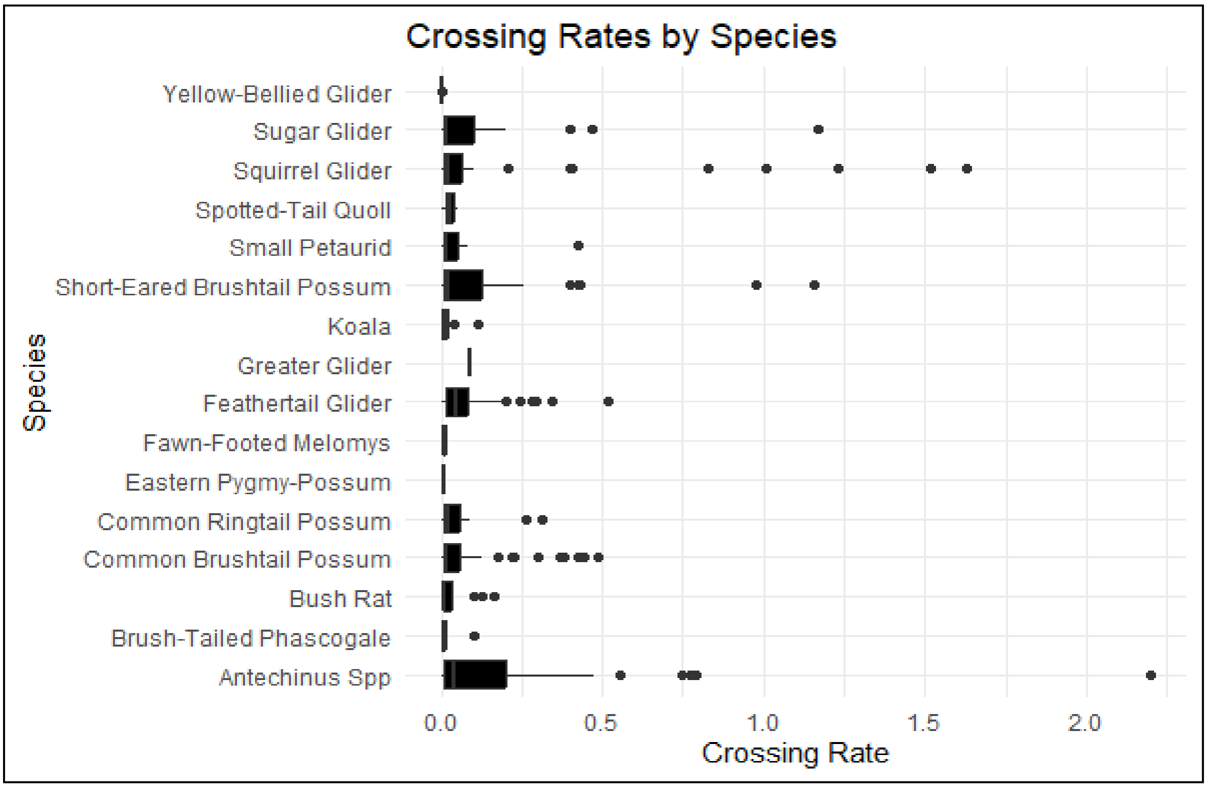

Figure 5: Crossing rates by arboreal species for all crossing structure types from monitoring across New South Wales, Australia from 1997-2023 (n=151 crossing structures). Crossing rates are the number of detections divided by the total days surveyed.

Species rates of use

Associations between crossing structure type and rates of use by arboreal mammals were identified using a heatmap, with strictly arboreal mammals (gliding and non-gliding) showing higher rates of use for canopy bridges and glide poles, while semi-arboreal mammals used a wider range of structure types (Table 2). The rates of use may identify potential species preferences for wildlife crossing structures, however these are only representative of the survey methods employed to recorded use and do not include where a species occurred in an area but did not use a structure. It is difficult to provide broad generalisations about taxonomic groups or guilds of arboreal mammals, but the results may indicate preference for:

- Underpasses by semi-arboreal mammals such as the bush rat, bridge underpasses by common brushtail possum and koala, dedicated fauna underpasses with fauna furniture by *Antechinus spp*., pipe culverts by short-eared brushtail possum, and combined underpass by common brushtail possum;

- Glider poles by squirrel glider;
- Canopy bridges by canopy-dependent mammals; for example rope tunnel bridges by sugar glider and rope ladder bridges by feathertail glider (*Acrobates pygmaeus*) and common ringtail possum (*Pseudocheirus peregrinus*);
- Vegetated overpass by spotted-tail quoll; and
- Vegetated median by sugar glider, greater glider and squirrel glider, but also used by a range of semi-arboreal mammals where these were paired with underpass structures (Table 2).

Table 2: Heatmap displaying species potential preferences for different structures by arboreal mammals based on average recorded rates of use by species for each wildlife crossing structure type (n=151), using data collected by monitoring wildlife crossing structures throughout NSW, Australia from 1997 to 2023. Darker shading indicates higher rates of use.

Arboreal species	Underpasses					Rope bridges		Vegetated median	Glider pole	Vegetated overpass
	Bebo arch	Bridge underpass	Dedicated fauna culvert	Combined underpass	Pipe culvert	Rope flat ladder bridge	Rope tunnel bridge			
Strictly arboreal - Gliding mammals										
Feathertail glider (*Acrobates pygmaeus*)	NR	NR	NR	NR	NR	0.09	0.07	0.04	0.07	P
Greater glider (*Petauroides volans)*	NR	NR	NR	NR	NR	NR	NR	0.09	NR	P
Small petaurid (*Petaurus spp*.)	0.001	NR	NR	NR	NR	0.01	0.25	0.03	0.03	P
Squirrel glider (*Petaurus norfolkensis*)	NR	NR	NR	NR	NR	0.02	0.13	0.11	0.28	P
Sugar glider (*Petaurus breviceps*)	NR	NR	NR	NR	NR	0.02	0.06	0.24	0.04	P
Yellow-bellied glider (*Petaurus australis*)	NR	NR	NR	NR	NR	0.00*	NR	P	0.00	P
Strictly arboreal - Non-gliding mammals										
Brush-tailed phascogale (*Phascogale tapoatafa*)	0.00	NR	0.01	0.01	0.10	0.01	0.00	NR	NR	P
Common ringtail possum (*Pseudocheirus peregrinus*)	NR	NR	NR	0.004	0.024	0.09	0.09	0.01	NR	P
Eastern pygmy-possum (*Cercartetus nanus*)	0.01	NR	NR	NR	NR	NR	NR	NR	NR	P
Koala (*Phascolarctos cinereus*)	NR	0.06	0.005	0.006	0.016	NR	NR	NR	NR	0.02
Short-eared brushtail possum (*Trichosurus caninus*)	NR	NR	0.155	0.029	0.20	0.005	NR	NR	NR	0.00
Semi-arboreal mammals										
Antechinus spp.	0.01	0.02	0.29	NR	0.20	0.01	0.01	0.05	NR	P
Bush rat (*Rattus fuscipes*)	0.16	0.00	0.04	NR	0.01	NR	NR	0.004	NR	P
Common brushtail possum (*Trichosurus vulpecula*)	NR	0.13	0.06	0.06	0.01	0.06	0.08	NR	NR	P
Fawn-footed melomys (*Melomys cervinipes*)	NR	NR	0.01	NR	NR	NR	NR	0.00	NR	P
Spotted-tail quoll (*Dasyurus maculatus*)	0.00	NR	NR	NR	NR	NR	NR	NR	NR	0.05

*NR = not recorded, P = Possible structure use based on what we know of their ecology/biology, however insufficient survey to confirm, *this species was recorded on the structure but did not use/cross*

Target species use

Almost 90% of the crossing structures that recorded use were specifically designed for use by threatened arboreal species. The percentage of wildlife crossing structures with use by threatened species, shows that different structure types have different success rates for the species targeted (Table 3). Koalas recorded use at 100% of the bridge underpass structures which targeted the species, as did vegetated overpasses. High use by target species was also recorded on the vegetated overpass for the spotted-tail quoll (100%), glide poles (88%) and vegetated medians (57%) for squirrel gliders, and rope flat ladder bridges for squirrel gliders (53%) and brush-tailed phascogale (50%). Additional records of threatened species also exist where they had not been targeted by the wildlife crossing design and installation, such as the brush-tailed phascogale, eastern pygmy possum and spotted-tail quoll using bebo arches, and the koala using a pipe culvert.

Table 3: Percent of crossing structures recording use by threatened arboreal species when that species was identified as a target, using data collected by monitoring wildlife crossing structures throughout NSW, Australia from 1997 to 2023.

Structure type	Brush-tailed Phascogale	Eastern Pygmy Possum	Greater Glider	Koala	Spotted-tail Quoll	Squirrel Glider	Yellow-bellied Glider
Bebo Arch (n=3)	R	R	-	-	R	-	-
Bridge (n=10)	0%	-	-	100%	0%	-	-
Combined underpass (n=20)	8%	-	-	20%	0%	-	-
Dedicated fauna culvert (n=44)	33%	0%	-	14%	25%	-	-
Glide pole (n=20)	-	-	-	-	-	88%	23%
Pipe (n=5)	25%	-	-	R	0%	-	-
Rope flat ladder bridge (n=20)	50%		0%			53%	7%
Rope tunnel bridge (n=4)	0%					25%	0%
Vegetated median (n=8)			33%			57%	0%
Vegetated overpass (n=1)	0%			100%	100%		0%

Note: R – Record of species though not specifically targeted for the structure, - not targeted by any wildlife crossing structures

Underpass design

The koala, short-eared possum, common brushtail possum, bush rat and *Antechinus spp.* used underpasses with a wide range of dimensions. Of the 94 underpass structures monitored with recorded use by arboreal mammals, dedicated fauna culverts with fauna furniture (log rails and posts) were the most numerous (53%). More than half of the dedicated fauna culverts measured 3 m by 3 m, while combined underpasses ranged in size though dimensions were only reported in 64% of cases. Some species were only recorded in one or a few underpass sizes, such as the eastern pygmy-possum in a 10 m bebo arch, or the fawn-footed melomys (*Melomys cervinipes*) in a 2.4 m high dedicated culvert (Table 4). The koala was recorded across a range of underpass structures, including pipe culverts that were 1.5 m and 1.05 m diameter (Woolgoolga to Ballina) as was the brush-tailed phascogale, with the smallest structure recording use being a pipe 0.75 m diameter (Woolgoolga to Ballina). Generally, most species appeared to use a range of underpass structures with varying heights, widths and lengths (Table 4).

Table 4: Dimension ranges of underpasses used by arboreal species in New South Wales, Australia from 1997-2023 by type. na = not available.

Structure and species	Length (L)	Width (m)	Height (m)
Bebo arch (n = 3)			
Antechinus spp	na	10	10
Brush-tailed phascogale	33	9	3
Bush rat	na	10	10
Eastern Pygmy-possum	na	10	10
Small Petaurid	41	6	3
Spotted-tail quoll	na	10	10
Bridge underpass (n = 12)			
Antechinus spp	21-82	na	na
Bush rat	na	na	na
Common brushtail possum	na	na	na
Koala	na	na	na
Combined underpass (n=22)			
Brush-tailed phascogale	10	2.7	2.4
Common brushtail possum	18	1.5-3	1.5-3
Common Ringtail possum	59.03	2.4	2.4
Koala	50	2.4	1.8
Short-eared Brushtail Possum	14.75-41	1.5-3	1.5-2.4
Dedicated fauna culvert (n=50)			
Antechinus spp	15-60	2.4-3.6	1.2-3.6
Brush-tailed phascogale	20-60	1.2-3.6	1.2-3.6
Bush rat	18-70	2.4-3.6	2.4-3
Common brushtail possum	15-70	2.1-3.6	1.2-3.6
Fawn-footed Melomys	70	3.6	2.4
Koala	20-50	2.1-3	1.8-3
Short-eared Brushtail Possum	16-70	2.4-3.6	2.4-3.6
Pipe culvert (n=7)			
Antechinus spp	31-82	0.75-1.05	0.75-1.05
Brush-tailed phascogale	31-30	0.75	0.75

Structure and species	Length (L)	Width (m)	Height (m)
Bush rat	15-82	1.05	0.9-1.05
Common brushtail possum	56-102	1.05-3	1.05-2.4
Common Ringtail possum	15-55	1.05	0.9-1.05
Koala	15	na	900
Short-eared Brushtail Possum	15	na	900

Canopy bridge and glider pole design

Arboreal mammals were detected using canopy bridges that ranged in length from 41 – 118 m. The length of canopy bridges did not appear to affect rates of use, with crossing rates of 0.04 for canopy bridges 55 m in length, compared to 0.05 at a canopy bridge of 118 m length.

The set-up of the cross-arms on glider poles did not appear to affect use by most gliders, except perhaps yellow-bellied gliders who were only documented using perpendicular cross-arms (Figure 6).

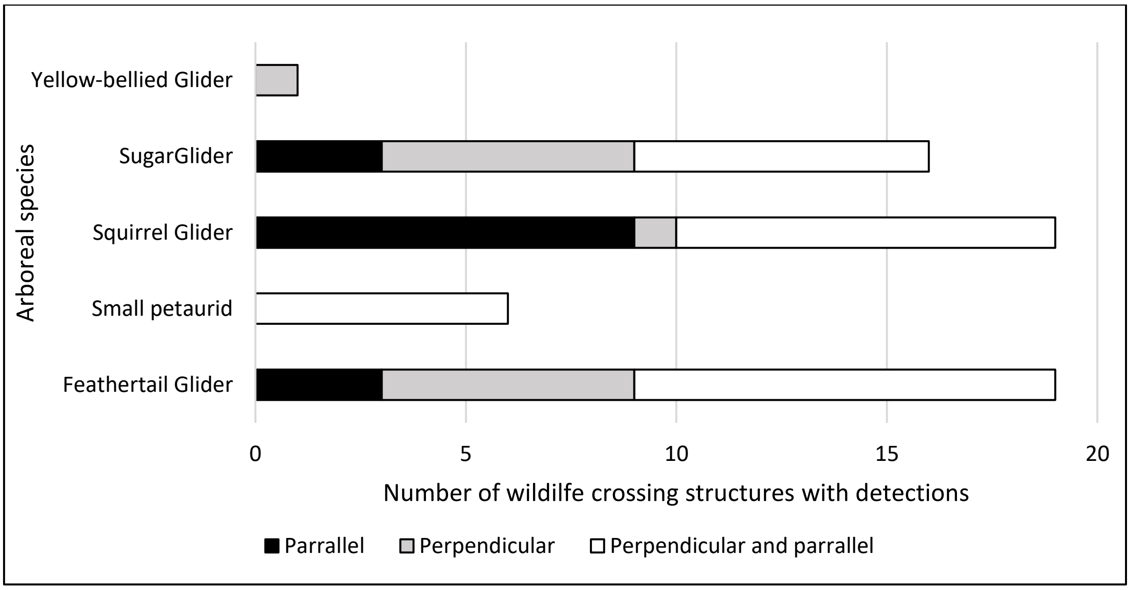

Figure 6: Documented use of glider pole arms by species according to cross-arm orientation where reported, from monitoring reports in New South Wales, Australia from 1997-2023. Small petaurids are where the species could not be distinguished between the sugar glider and squirrel glider.

Discussion

Arboreal mammals of conservation concern have been recorded using wildlife crossing structures in the literature, however this study confirms this across a range of wildlife crossing structure types and locations throughout New South Wales, Australia. Thus, this review provides the evidence that use has been demonstrated for such species and shows that the design-specific requirements and locations specifically chosen for such species are adequate in most cases. However, structures often appeared to be used at higher rates by more common species, and no structure recorded use by all arboreal mammals. This indicates that there is not a one-size-fits-all solution for wildlife crossing structures for arboreal mammals.

Insights on wildlife crossing structures

Canopy bridge and glide pole use

Our review found that canopy bridges and glider poles appear to be most used for road crossing movement by strictly arboreal mammals. Previous work on individual species has also shown these structured to be used by arboreal mammals (Weston *et al.* 2011; Taylor and Goldingay 2012, 2013; Yokochi and Bencini 2015; Goldingay and Taylor 2017; Gregory *et al.* 2022; Baker *et al.* 2023). Glide poles in particular, have been shown to have equivalent rates of use to trees in fragmented areas (Goldingay *et al.* 2018), and in our review recorded use by all gliding arboreal mammals investigated except for the greater glider. Squirrel gliders were reported as the most consistent glide pole users, with confirmed crossing events across multiple projects, whereas yellow-bellied gliders exhibited limited use. This may be a result of the lower densities of yellow-bellied gliders in the landscape given their large home-range size (Goldingay 2025). Glide pole arrays typically consist of two to three poles, with monitoring largely reliant on cameras mounted near the top of the poles, on glide beams, or at the exit to a metal collar that the animal needs to climb through to get up the pole. Thus, detections may be missed if gliders launch from the pole without triggering the camera at the top of the pole (Goldingay *et al.* 2018) and our review may under report use.

Canopy bridges were used by a diverse range of species, including the threatened brush-tailed phascogale, corroborating findings from Weston et al. (2011) and Baker et al. (2023). Rope tunnel bridges were mostly used by squirrel gliders and sugar gliders, whereas brush-tailed phascogales and short-eared possums appear to favour flat rope ladder bridges. Species-specific preferences were also identified in the literature, with sugar gliders favouring single ropes, while squirrel gliders used multiple bridge types (Goldingay & Taylor, 2017). As with glide poles, a limitation of detection methods has resulted in fewer full crossings recorded in the reviewed reports, as more mobile species may bypass canopy bridge ends entirely. For example, the yellow-bellied glider was recorded by a camera on one end of a rope flat ladder bridge (Devil's Pulpit), though was considered unlikely to have crossed the road due to the lack of evidence on the other side, and was only ever recorded once over five years. Further, despite high rates of use by a range of species, tagging studies show few individuals may using canopy bridges (Hume Highway), though it is generally accepted that even low-frequency use may enhance genetic connectivity (Soanes *et al.* 2015; Soanes *et al.* 2018).

The greater glider was not recorded using glide poles or canopy bridges in the review, likely due to their small home ranges (Hofman *et al.* 2023) and potential sensitivity to highway disturbance (Kavanagh, 1988; Ridley et al., 2024). Greater gliders have been recorded a dozen times using pole arrays across a cleared 30 m – 60 m pipeline easement (GHD 2017). A recent record of the use of a rope bridge (single rope not a ladder design) by the greater glider in the Central Highlands of Victoria (Slapp 2023), indicates that this may also be a wildlife connectivity structure worthwhile investigating for the species.

Vegetated medians are an understudied crossing structure in the published literature (van der Ree *et al.* 2010; Soanes *et al.* 2013) but we found records of many gliding arboreal mammals using vegetated medians. The squirrel glider was observed frequently gliding to vegetated medians spanning gaps of 38 – 42 m by radiotracking, suggesting that these connectivity measures may support home range connectivity. The greater glider was also detected in a vegetated median shortly after construction of the road (Devil's Pulpit), though its movement across the road remains unconfirmed and it may have been in the median when the road was built. We had 7 studies (35%) that monitored and recorded use in vegetated medians, which is higher than a recent published global review, where the efficacy of vegetated medians as a wildlife crossing structure for arboreal mammals was only assessed in 2 out of 313 studies reviewed (0.6%) (Soanes *et al.* 2024). Non-gliding and semi-arboreal mammals were only recorded in the vegetated median where underpasses that opened into the median were present, which enabled safe access via underpasses to habitat in the median (Martinig and McLaren 2019). The results suggest vegetated medians may reduce road impacts for gliding species (as was reported for the Hume Highway projects in Soanes et al., 2013) but highlight the need for further investigation into their role of supporting home range connectivity for other semi- and strictly arboreal mammals.

Underpass and overpass use

Underpasses were highly used by non-gliding and semi-arboreal mammals, with some of the highest rates of use recorded by *Antechinus spp*. in dedicated fauna culverts. This is interesting as underpass structures have been thought to provide a prey trap for introduced and native predators, and aligns with recent findings that this is not backed up by the literature (Little *et al.* 2002; Goldingay *et al.* 2022). The addition of fauna furniture to an underpass appeared to increase rates of use. Furniture may assist arboreal mammals to navigate these structures, by promoting movement and providing an escape mechanism from ground-based predators, consistent with findings that increased structural complexity improves passage use (Goldingay et al., 2018a). While dedicated fauna culverts with fauna furniture represent a third of the crossing structures recording arboreal mammal use, few specifically recorded use of furniture (8%). Of interest, reports often lacked data on underpass approaches, a key factor influencing use and crossing success (Goldingay et al., 2022). This was identified as a deficiency in study designs early on in structure use monitoring (Taylor and Goldingay 2003), but was not incorporated into later monitoring programs. Despite this limitation, our review suggests that underpasses are effectively used by many non-gliding and semi-arboreal mammals.

The apparent limited data on vegetated overpasses for arboreal mammals reflects global literature challenges in monitoring these structures (Gužvica et al., 2014). The first challenge is the very small number of vegetated overpasses in Australia, with just 7 in Australia (2 in Queensland, 1 in Western Australia and 4 in New South Wales), compared to at an estimated 54 globally from a recent assessment (Soanes *et al.* 2024), with only one overpass surveyed in the reviewed reports. The second challenge is the difficulty in surveying arboreal mammals with standard methods (camera traps) when they may be passing through a 50 m wide area cluttered with vegetation. Some studies suggest overpasses may contribute to connectivity when paired with glider poles and canopy bridges until vegetation becomes established for canopy movements (Taylor & Goldingay, 2012), but their standalone effectiveness remains undetermined, as not enough structures have been constructed and adequately studied, particularly in NSW. As suggested by van der Ree et al. (2010) and Soanes et al. (2024), further investigation into the role of vegetated overpasses in connectivity, particularly for arboreal mammals, and methods to monitor these is warranted.

Lessons and looking to the future

Rates of use can be used to identify potential preferences for specific crossing structure types among arboreal mammals, which is important for effective design (Soanes et al. 2018). Targeted designs for threatened species have largely proven successful in terms of 'use' by such species, and while designs often target one to a few threatened arboreal species, wildlife crossing structures can provide a mechanism to reduce the barrier effects of a road for a range of both common and threatened species. If structures are targeting arboreal fauna more broadly, the review indicates a need for inclusive designs that cater to a broader range of species, as highlighted by van der Grift et al. (2013). Variation in rates of use of the same structure type and design by different species, and even species with similar movement methods (e.g. gliders), suggests that one (structure) style does not fit all.

Most studies only report 'use' of crossing structures and their actual 'effectiveness' in mitigating road barrier effects remain unknown. It is important to note that many other structures have been monitored over the past 25 years without detecting arboreal mammal use, but have not been included here. Furthermore, numerous structures have been constructed for wildlife use on roads owned by the transport agency, but never monitored, and many more structures that potentially provide connectivity exist, but were not built for this purpose. For example, only one of the four fauna overpasses built in NSW has been monitored and was available for the review. Among the structures where arboreal mammal use was detected, 59% recorded presence only at a structure, without confirmation or more often inferred use of the structure to successfully cross the road safely.

While16 species were documented using various structures, presence alone on the structure neither confirms successful movement or restoration of habitat connectivity. Usage is still important, and in some cases is still unknown for some species for example the greater glider indicating that our current suite of structures may not adequately support their movement needs, but determining ‘use’ is only a first step in identifying the potential effectiveness of wildlife crossing structures. The assumption that ‘use’ alone ensures connectivity overlooks key ecological factors such as dispersal success, demographic stability, and genetic exchange (van der Grift *et al.* 2013).

What was clear from the review, monitoring hasn’t been measuring important co-variates that might influence crossing rates and the efficacy of crossing structures. The condition and rehabilitation of vegetation on crossing structure approaches following construction was often not reported. Some species are more sensitive to open areas, un-natural substrates and a lack of dense vegetative cover and thus may be deterred from using crossing structures. Additional data on surrounding habitat features need to be collected at the existing wildlife crossing structures to fill such gaps to further analyse our findings to inform future mitigation design. Further, the detection methods used in some studies, for example the use of sand pads in underpasses and attaching cameras 2-3 m above an underpass floor, creates sampling bias and makes identifications, particularly for smaller species that are difficult to distinguish, under reported as they were often not the target of monitoring. Monitoring methods and reporting practices should be standardised using the grey literature results, not only for arboreal mammals but to capture the full suite of species using wildlife crossing structures, to create best practice guidelines for future monitoring to look beyond ‘use’.

An important element of this would be to relate the rates of use to background density and rates of use in surrounding areas for arboreal mammals. While this was completed for a few projects that were part of this review, the results were not incorporated into crossing structure monitoring analysis. Often this was only completed for key species (eg call playback and spotlighting for the yellow-bellied glider at Devil’s Pulpit), using methods that were different than those used for crossing structure monitoring, making comparisons and density estimates unrelatable. The shortness of most monitoring periods (up to 5 years) following construction may be inadequate to detect changes in population densities and distinguish these from natural variation, particularly when control sites were not used. Ongoing monitoring replicating the methods used in the more comprehensive studies reviewed (eg Oxley Highway to Kempsey and Devil’s Pulpit) may provide further answers to the effectiveness of crossing structures in minimising the barrier effects of the road.

Measuring effectiveness needs to be tailored to specific goals, such as whether wildlife crossing structures prevent road barrier effects, restore movement to pre-construction conditions, or even improve movement relative to unmitigated roads (Soanes *et al.* 2024). Although crossing structures may enable some individuals to move, this may not translate into broader population-level benefits (Rytwinski *et al.* 2015). A further challenge in monitoring efficacy is that species of conservation significance usually occur at low density, so it is expected they would be detected infrequently using wildlife crossing structures (Goldingay *et al.* 2013; Goldingay *et al.* 2022). Future monitoring efforts should adopt comprehensive approaches, such as Before-After-Control-Impact (BACI) designs, to assess pre- and post-construction changes in population density (van der Grift *et al.* 2013), rather than relying solely on recorded use data (Goldingay *et al.* 2013; Soanes *et al.* 2024). Long-term genetic studies and population viability assessments may be required to allow inferences about population connectivity (Soanes *et al.* 2013). This will ensure success is not simply measured by rates of use, but rather in meaningful ways, as advocated by Goldingay et al. (2013). To increase the value of monitoring, regulatory frameworks should move beyond only requiring use data and adopt standardised evaluation methods that ensure mitigation strategies achieve their intended conservation outcomes (van der Ree *et al.* 2015; Soanes *et al.* 2024). As such, specific goals are needed to assess connectivity outcomes.

In conclusion, by synthesising grey literature and aligning our findings with existing research on the use of wildlife crossing structures, we can better inform conservation strategies aimed at mitigating the impacts of habitat fragmentation caused by linear infrastructure on Australia's unique arboreal mammals. However, while our review provides valuable insights into the use of wildlife crossing structures by arboreal mammals, it also highlights significant gaps in understanding their effectiveness. If success is measured by recording use, then wildlife crossing structures have been successful for most threatened arboreal species targeted. However, if we are trying to understand their ability to mitigate the barrier effects of the road on local populations and their viability long-term, we need to move toward monitoring that can suitably detect such changes. Addressing such gaps will be essential for enhancing habitat connectivity and supporting the conservation of arboreal mammals subjected to the barrier effects of roads into the future.

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Supplementary material

Table 1 Number and types of wildlife crossing structures monitored and recording use by arboreal species by location, from monitoring reports (grey literature) for road projects in New South Wales.

Location/ Project	Road upgraded	Bebo arch	Bridge underpass	Combined underpass	Dedicated fauna culvert	Glider pole	Pipe culvert	Rope flat ladder bridge	Rope tunnel bridge	Vegetated median	Vegetated overpass	Total	Monitored by (and published papers)
Bonville Upgrade	Pacific Highway		2					1		1		4	Australia Museum Business Services (AMBS), Sandpiper Ecological Surveys
Brunswick Heads Bypass	Pacific Highway		1									1	Australia Museum Business Services (AMBS), (Taylor and Goldingay 2003; Taylor and Goldingay 2014)
Bulahdelah Bypass	Pacific Highway											0	EcoPro
Coopernook to Herons Creek	Pacific Highway				2			2				4	Australia Museum Business Services (AMBS)
Devil's Pulpit (Grafton to Ballina)	Pacific Highway		3	5				2		2		11	GeoLink Environmental Management and Design
Frederickton to Eungai	Pacific Highway		1	3	1	3		3				10	Niche Environment and Heritage
Glenugie Upgrade	Pacific Highway	2		2	2			3				9	Sandpiper Ecological (Goldingay *et al.* 2022)
Hume Highway Upgrades	Hume Highway					7		2				8	Australian Research Centre for Urban Ecology (ARCUE) Melbourne University (Soanes *et al.* 2013; Soanes *et al.* 2015; Soanes *et al.* 2017; Soanes *et al.* 2018)
Karuah Bypass	Pacific Highway							1	3			4	ERM (Goldingay *et al.* 2013)
Karuah to Bulahdelah	Pacific Highway							1				1	Sandpiper Ecological
Kempsey Bypass	Pacific Highway					2						2	Niche Environment and Heritage
Nambucca Heads to	Pacific Highway			1	5					2		6	Sandpiper Ecological

Location/ Project	Road upgraded	Bebo arch	Bridge underpass	Combined underpass	Dedicated fauna culvert	Glider pole	Pipe culvert	Rope flat ladder bridge	Rope tunnel bridge	Vegetated median	Vegetated overpass	Total	Monitored by (and published papers)
Urunga													
Olympic Highway Kapooka	Olympic Highway							3				3	SMEC
Oxley Highway to Kempsey	Pacific Highway			4	8	2			3	1		18	Niche Environment and Heritage
Port Macquarie Upgrade	Oxley Highway			1	2	1		1				5	Southern Cross University (Taylor and Goldingay 2012; Goldingay *et al.* 2018a; Goldingay *et al.* 2018b; Goldingay *et al.* 2019)
Raleigh (part of Bonville Upgrade)	Pacific Highway				1							1	Australia Museum Business Services (AMBS)
Sapphire to Woolgoolga	Pacific Highway					1	3	1		1		6	Sandpiper Ecological
Sydney to Newcastle	Pacific Highway	1										1	Australia Museum Business Services (AMBS)
Termeil Creek	Princes Highway											0	SMEC
Warrell Creek to Nambucca Heads	Pacific Highway									1		1	Sandpiper Ecological
Woolgoolga to Ballina	Pacific Highway		5	6	29	6	4	6		1		56	Sandpiper Ecological (Taylor and Rohweder 2020)
Yelgun to Chinderah	Pacific Highway										1	1	Tweed Shire Council (Hayes and Goldingay 2009)
Total		**3**	**12**	**22**	**50**	**22**	**7**	**25**	**6**	**9**	**1**	**151**	

Note – Reports for the Termeil Creek project on the Princes Highway and Bulahdelah Bypass on the Pacific Highway were reviewed, however no detections were made by arboreal species and thus these were not included in the analysis.

Table 2: Number of wildlife crossing structures recording use by arboreal mammal and average crossing rates by species from monitoring reports (grey literature) for road projects in New South Wales.

Species (classification)	**Number of wildlife crossing structures recording use (all wildlife crossing types n=151)**	**Mean crossing rate (all structure types)**	**Standard deviation**
Strictly arboreal – Gliding mammals			
Feathertail glider	54	0.075	0.101
Greater glider*^	1	0.091	0.000
Small petaurid	16	0.049	0.102
Squirrel glider*	48	0.172	0.391
Sugar glider	45	0.087	0.192
Yellow-bellied glider*^	6	0.002	0.000
Strictly arboreal – Non-gliding mammals			
Brush-tailed phascogale*	23	0.013	0.021
Common ringtail possum *	14	0.062	0.100
Eastern pygmy-possum*	1	0.007	0.000
Koala*^	12	0.021	0.031
Short-eared brushtail possum	40	0.128	0.248
Semi-arboreal mammals			
Antechinus spp.	56	0.177	0.343
Bush rat	14	0.037	0.053
Common brushtail possum	95	0.064	0.106
Fawn-footed melomys	2	0.009	0.007
Spotted-tail quoll*^	2	0.027	0.033

*Note: *Denotes a species listed as threatened under the NSW Biodiversity Conservation Act 2016, ^ denotes a species listed as threatened under the Federal Environment Protection and Biodiversity Conservation Act 1999.*

Chapter 3: Summary

In Chapter 3, I review 25 years of grey literature and monitoring reports from New South Wales (NSW) to assess the extent to which these structures are being used by arboreal mammals, how structure design influences their use, and the major limitations in monitoring and reporting that constrain adaptive management. It highlights that despite widespread installation of structures, monitoring rarely assesses outcomes such as restored connectivity or genetic exchange. Of note, the eastern pygmy possum has only been identified on one of the over 150 wildlife crossing structures across the state, despite it's widespread distribution.

To build on these findings, the next chapter (Chapter 4) uses dynamic occupancy modelling to evaluate the environmental and landscape factors that influence the persistence of the eastern pygmy possum in fragmented peri-urban bushland. This analysis provides a critical link between structure placement, habitat configuration, and species viability.

Chapter 4: Occupancy of an urban-sensitive specialist: the role of habitat availability and fire on the urban edge

Occupancy of an urban-sensitive specialist: the role of habitat availability and fire on the urban edge

Cassie J. THOMPSON [a], Brad LAW [b], Leroy GONSALVES [b] and Peter B. BANKS [a]

a School of Life and Environmental Sciences, University of Sydney, Sydney, 2006, Australia

b NSW Department of Primary Industries (Forest Science), Sydney, 2150, Australia

Abstract

Context: Urban-sensitive species face a heightened risk of extinction in bushland remnants on the urban edge. Understanding the habitat factors that influence their occupancy is essential for effective conservation management in peri-urban landscapes. *Aims.* This study aimed to identify the key landscape and habitat factors influencing the occupancy of the eastern pygmy possum (*Cercartetus nanus*), as an urban-sensitive species, in peri-urban northern Sydney. *Methods.* We used occupancy modelling to examine the relationships between occupancy and environmental covariates, on the locality (home range) and landscape scales. Surveys were conducted in peri urban bushland remnants using nest boxes over a 7-year period. *Key results.* Detection probability peaked in autumn at 0.49 (s.e. 0.05) per visit, coinciding with the flowering of key foraging resources, and was lowest in summer at 0.27 (s.e. 0.06). The occupancy estimate under median conditions was 50%, indicating widespread occupancy by this threatened species in the peri-urban study area. Occupancy was positively associated with the extent of remnant native vegetation in the landscape (potentially a surrogate for connectivity) and an increasing proportion of the landscape that had burned. When native vegetation comprised at least 50% of a 500 m buffer (~40 ha), occupancy probability was ≥70%. Occupancy was ≥70% when at least 50% of the native vegetation (500 m buffer) had been burnt in the 30 years prior to survey. *Conclusions.* These findings highlight the need for management actions that enhance both the extent and quality of native vegetation around known populations, particularly with key foraging resources. Fire management, particularly for prescribed burns, will be important for the species; however, further research is required to determine optimal fire intervals and intensities. *Implications.* Effective habitat management, including increasing native vegetation cover and appropriate fire management practices, is crucial for the long-term survival of the

eastern pygmy possum in peri-urban areas. Further research is needed to determine optimal fire intervals and intensities for conservation efforts.

Introduction

Urban expansion is a major driver of habitat loss, with growth in peri-urban areas (on the outskirts of cities and large towns) exerting direct pressure on the environment (Bohnet and Pert 2010) and biodiversity (McKinney 2006). As cities expand, habitat on the urban edge becomes increasingly fragmented, encroached upon, and degraded. However, remnant urban bushland and peri-urban patches can be highly biodiverse, providing critical refuges for threatened species (Soanes and Lentini 2019). These areas are particularly important for conservation management (Ives *et al.* 2016; Soanes and Lentini 2019), but maintaining habitat quality is essential for the long-term survival of native species (Holland and Bennett 2007). Various anthropogenic factors influence habitat quality (Garden *et al.* 2006), including prescribed fire, which can be used to improve habitat condition in fire-prone environments. However, fire management aimed at asset protection can also alter habitat composition and degrade foraging and denning habitat for specialist species (Bradstock *et al.* 2005; Flanagan-Moodie *et al.* 2018).

Research in peri-urban environments focuses on species that are easily observed, readily identified, or elicit an emotive public response (Garden *et al.* 2006). Nocturnal arboreal mammals, which are often unseen and are more vulnerable to urbanisation as they tend to avoid or are unable to traverse the urban matrix, are comparatively understudied (van der Ree and McCarthy 2005; Garden *et al.* 2006; Goldingay *et al.* 2006). Our study investigates the persistence of one such understudied urban-sensitive species, the eastern pygmy possum (*Cercartetus nanus*), on the northern peri-urban edge of Sydney, Australia. This nectar-feeding marsupial relies on flowering resources year-round and is closely associated with habitats dominated by Proteaceae and Myrtaceae plants (Ward 1990; Tulloch and Dickman 2007). The species is considered a food specialist (Tulloch and Dickman 2006, 2007) but also consumes insects during periods of minimal flowering (van Tets 1998). Banksia-dominated habitats are strongly favoured (Ward 1990; Bladon *et al.* 2002; Harris and Goldingay 2005b; Law *et al.* 2018), with spool tracking studies confirming that banksia inflorescences are an important foraging resource (Tulloch and Dickman 2006; Law *et al.* 2018). Key threats to the species include habitat loss, fragmentation, predation by exotic species, and inappropriate fire regimes (NSW Scientific Committee 2001).

Despite its cryptic nature, the eastern pygmy possum has been studied primarily in large, forested areas (Bladon *et al.* 2002; Harris and Goldingay 2005a; Tulloch and Dickman 2006, 2007; Law *et al.* 2013; Law *et al.* 2018; Goldingay 2019). The species was rediscovered in urban remnants outside protected areas in northern Sydney in 2010 (B. Law, *pers. obs*.), yet only one study has examined it in an area under urban development pressure (Harris *et al.* 2007). Interestingly, most records from that study were obtained from animals rescued by wildlife carers, suggesting a high level of interaction with the urban environment. Although eastern pygmy possums exhibit a patchy distribution on Sydney's urban edge, no studies have explored their persistence within urban and peri-urban bushland. A deeper understanding of the factors influencing their occupancy in these areas is essential for effective management and for guiding conservation strategies for similar urban-sensitive species.

Here we examine occupancy patterns of eastern pygmy possums in remnant and peri-urban bushland to identify key habitat attributes influencing this. Given that the species occupies fire-prone habitats, we investigate the role of prescribed fire and fire history in shaping occupancy patterns (Chew *et al.* 2024). Specifically, we address three key questions: (1) What factors influence detectability? (2) Which local and landscape-level attributes correlate with eastern pygmy possum occupancy in the peri-urban matrix? (3) How does fire history influence occupancy in the peri-urban matrix? Based on our findings, we provide management recommendations to support the persistence of urban-sensitive species in bushland remnants on the urban edge.

Methods

Study area and occupancy surveys

The study area encompasses the Hornsby and Northern Beaches (formerly Warringah and Pittwater) local government areas (LGAs) in northern peri-urban Sydney, in temperate south-eastern Australia (Figure 1). In these areas, remnant native vegetation exists across several council-managed reserves (approximately 10% of the area) with some connectivity to areas of national park but generally surrounded by suburban housing.

Native vegetation is dominated by Hawkesbury sandstone woodland with a mixture of open forest, open woodland and heath dominated by myrtaceous flora species. Sites were targeted in peri-urban bushland remnants that contained the most suitable eastern pygmy possum habitat, particularly the presence of banksia species (with *Banksia ericifolia* being the dominant species), a known key food resource (Turner 1984; Huang *et al.* 1987; van Tets 1998; Tulloch and Dickman 2006; Harris *et al.* 2007; Goldingay and Keohan 2017).

We installed nest boxes in clusters of five at 41 bushland remnants at various times since 2011 to survey the normally cryptic and trap shy eastern pygmy possum (Ward 1990; Bladon *et al.* 2002; Beyer and Goldingay 2006; Law *et al.* 2013). At each bushland remnant site, five nest boxes made from salvaged hollows and PVC pipe nest boxes with small openings (2-5 cm diameter) were installed in a cluster along a 100 m transect (generally 20-50m apart). Boxes were installed 1-1.8 m above the ground for ease of checking. Nest boxes were surveyed for eastern pygmy possum presence approximately each month at the sites between May 2011 to July 2017 (representing a 7-year dataset), with between 15 and 130 checks of nest boxes at each bushland remnant completed (Table 1). Individuals were not removed from boxes during inspections.

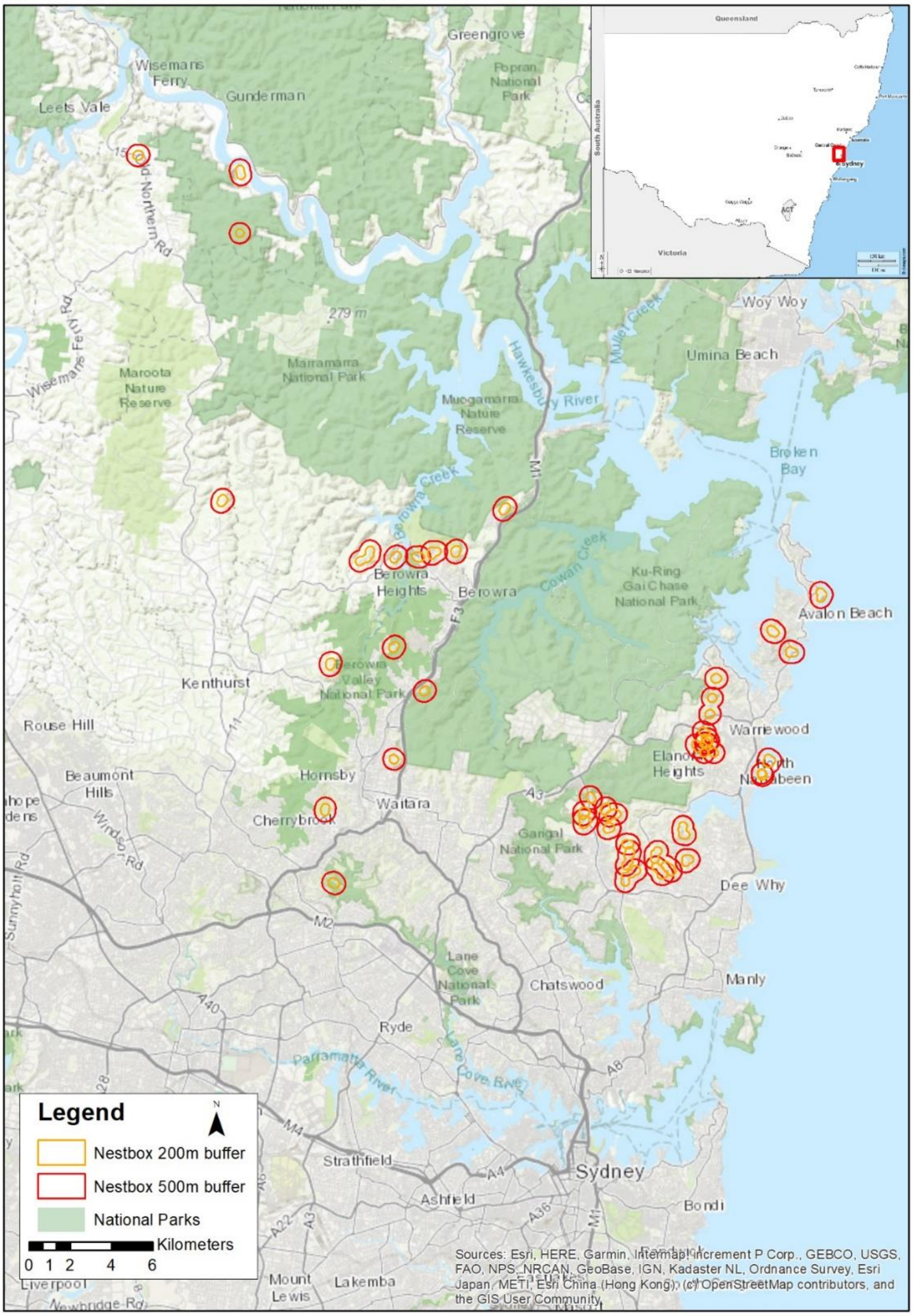

Figure 1: Study area and sites containing nest boxes as shown by the 200m and 500m nestbox buffers for the eastern pygmy possum (*Cercartetus nanus*) in peri-urban northern Sydney.

Table 1: Sampling effort and naïve occupancy per site from nest box surveys for the eastern pygmy possum in peri-urban Sydney. Note that a subset of sites was surveyed each year and the actual sites surveyed varied across each year.

Year	No. of sites surveyed (from a total 41 across the study area)	No. survey events	Naïve Occupancy
2011	11	32	0.31
2012	20	57	0.47
2013	27	76	0.40
2014	13	63	0.13
2015	17	77	0.21
2016	16	118	0.31
2017	6	24	0.63

Covariates

We assumed that occupancy was influenced by local (home range) and landscape-level variables, and modelled site occupancy as a function of a range of covariates calculated using GIS software. Aggregated buffer areas of 200 m radius (local) from each nest box in a cluster were used for spatial analysis at the scale of the home range, as the average home range for a possum is 3-4 ha (equivalent to 100m radius)(Law *et al.* 2013). A 500m (landscape) radius was used around nest box locations at a site (combined buffer around each of the five nest boxes) (Law *et al.* 2013). Covariates relating to the site's habitat availability and condition, level of connectivity and fire history were investigated (Table 2). These local and landscape factors are considered ecologically important for the eastern pygmy possum (Tulloch and Dickman 2006, 2007; Goldingay 2023) and could be manipulated through management for future conservation of the species.

In the peri-urban environment, the size of bushland remnants and connectivity can influence the abundance and diversity of small mammals (Garden *et al.* 2006) and is known to influence occupancy for many other species (Andr 1994). Thus, patch size was used to investigate the influence of fragmentation of the bushland remnants on eastern pygmy possum occupancy (Table 2). The percent of native vegetation within the buffer areas was assessed as a surrogate for connectivity (Bender *et al.* 2003), habitat condition and level of disturbance (roads, weeds and urban development). Road lengths within buffers were also investigated and a permeability matrix calculated to further analyse potential influence of connectivity and barriers to movement (Table 2).

Given the high density of houses on the urban edge, most of the remnant bushland is subjected to prescribed, often frequent, burning regimes. As such, several fire management attributes were investigated for this study, particularly as a range of post-fire responses have been shown by the eastern pygmy possum in previous studies (Dickman and Happold 1988; Thompson *et al.* 1989; Wilson *et al.* 1990; Whelan *et al.* 1996; Tulloch and Dickman 2006, 2007; Law *et al.* 2013; Chew *et al.* 2024). Fire extent within the buffer over different times since fire included: recent (area burnt less than 5 years prior), intermediate (area burnt 5-15 years prior) and long unburnt (area burnt 15-30 years prior). A pyrodiversity matrix was calculated to assess the influence of historical burn patterns and mosaics. The total area of native vegetation left unburnt within each of the buffers (1980-2017) was also calculated and included as a covariate, as was the number of fires and years since last fire (>50% of native vegetation burnt) within the 500m buffer (Table 2). Assessment of historical fire intensity or severity was not possible from the available data.

Occupancy modelling

An occupancy modelling framework was used to investigate detection and occupancy probabilities against local and landscape variables for each of the bushland remnants using nest box survey results. Site occupancy modelling accounts for imperfect detection (i.e. false absences that result when the species is present but is not detected), by using information from repeated observations at each site to estimate detection probabilities (Mackenzie 2005). Data analyses were run in the R statistical package 'R Presence' (MacKenzie 2022) using a dynamic occupancy model. We fitted a reduced dynamic occupancy model (MacKenzie *et al*. 2003) without colonisation (γ) or extinction (ε) parameters, as sampling of sites was staggered and did not take place for each site in every monitoring period. This formulation of the dynamic occupancy model assumes that occupancy changes randomly between monitoring seasons and colonisation and extinction are not estimated separately. This reduced formulation can be used to model occupancy in each season independently while still allowing for seasonal variation in occupancy and detection probabilities. The occupancy parameter (ψ) represents the mean probability of site occupancy across all surveyed seasons. Missing site–year combinations were coded as NA in the detection histories and were accommodated in the likelihood calculation without biasing ψ estimates (MacKenzie *et al*. 2003). Each 'season' for our dynamic model consisted of a year with up to 12 survey occasions with sampling completed in each month of the year. A limitation with this approach is that dynamic occupancy models lack a fully developed goodness of fit test in R Presence (MacKenzie and Hines 2022).

Detection histories were prepared for each bushland remnant for input into the model. A 'site' for the model consisted of pooled data from five nest boxes in a remnant bushland patch, resulting in one record (presence/absence or NA) per month for each site.

An eastern pygmy possum detected in at least one of the five boxes indicated presence. Using a hierarchical approach, detection probability was first modelled with occupancy held constant. As nest boxes were checked during the day for possum presence, weather covariates were not investigated. Instead, several variables which were considered likely to influence the probability of detecting a possum in a nestbox were investigated included sampling year and survey season (3 months per season, total of four seasons per year), and annual rainfall for the year prior to survey (to account for site productivity and subsequent likely increased flowering response). Detection variables were investigated against the null model which assumed constant detection across all visits to a site. The top detection covariate was subsequently used for all occupancy models.

All continuous covariates were standardised for input into the model. Covariates were modelled individually in the model set against a null model. Some of the 200m and 500m buffer values for the same variables were correlated (Pearson $r>0.7$), so only the highest performing covariates from the single variable occupancy models were taken forward in the model set (e.g. UnBurnt500 was the highest performing when compared to UnBurnt200, so this was taken forward). Similarly, PMI200 and Native200 were correlated, however both the 500 m buffers of these covariates outperformed the 200 m buffers and thus these were not taken forward in the analysis.

As with detection, an additive approach was used in the model, where the top performing occupancy covariate was taken forward and combined in subsequent models with all other covariates, until there was no improvement in model performance. Occupancy models were ranked from the lowest to highest based on Akaike Information Criterion corrected for small sample size (AICc), with the difference in AICc (ΔAICc) calculated between each model and the top-ranked model. Models with ΔAICc < 2 were considered plausible to explain the data. AICc was chosen instead of AIC to account for the relatively small sample size (n = 41) in relation to the number of model parameters (K), which can bias AIC values (Burnham and Anderson 2004). Occupancy estimates generated while holding all other supported covariates at the median value were used to determine the direction and magnitude of influence of a supported covariate.

Table 1: Summary statistics of occupancy (psi) covariates modelled for occupancy of the eastern pygmy possum (*Cercartetus nanus*) in peri-urban Sydney, NSW.

Category	Covariate	Variable	Mean	SD	Range
Native vegetation	Native200	Extent native vegetation (% buffer)	66.9	22.6	24.9-100.0
	Native500		57.4	24.2	8.7-97.2
Heath	Heath200	Extent of heath within native vegetation (% buffer)	13.1	13.8	0-55.3
	Heath500		8.9	9.0	0-37.4
Road length	Roads200	Roads (km within buffer)	0.8	0.6	0.0-2.3
	Roads500		4.0	2.2	0.1-11.7
Fire history	RecentFire200	Extent recent fire (0-5 years prior) (% native vegetation	7.0	22.0	0.0-100.0
	RecentFire500	burnt in buffer)	4.2	12.8	0.0-74.2
	IntermediateFire200	Extent intermediate fire (5-15 years prior) (% native	44.0	42.0	0.0-100.0
	IntermediateFire500	vegetation burnt in buffer)	33.8	37.2	0.0-100.0
	LongUnburnt200	Extent long unburnt (15-30 years prior) (% native	44.0	44.0	0.0-100.0
	LongUnburnt500	vegetation burnt in buffer)	37.7	36.4	0.0-100.0
	NotBurnt200	Extent unburnt (30 years prior to survey (1980-2017)) (%	29.2	39.3	0.0-100.0
	NotBurnt500	native vegetation burnt in buffer)	37.7	38.7	0.0-100.0
Pyrodiversity index	PYRO200	Pyrodiversity in the 200m buffer was calculated based on	0.5	0.3	0.0-1.1
	PYRO500	three fire intervals covariates investigated: 0-5 years (recent fire), 5-15 years (intermediate fire), 15-30 years (long unburnt). Shannon's Diversity Index (Shannon 1948) was used to calculate pyrodiversity scores for each site, using: $H = -\Sigma p_i * \ln(p_i)$ where: Σ: Sum; ln: Natural log; pi: The proportion of the buffer (% native vegetation) made up of a fire interval i	0.6	0.3	0.0-1.1

Category	Covariate	Variable	Mean	SD	Range
Permeability index	PMI200 PMI500	Permeability Matrix Indices (PMI) in the 200m buffer (as per(da Silva *et al.* 2015)) were calculated using: $PMI_a = \frac{\sum A_a \times P_a}{Area_a}$ where: PMI_a = Permeability Matrix Index at site A_a = area of a specific land-use type within the buffer P_a = permeability value assigned to that land-use type $Area_a$ = total area of the buffer zone Within the buffer, areas were derived for edge, core and urban. 'Edge' area type was calculated in GIS from the amount of native vegetation within a 50m of the edge and was assigned a permeability type of 75%. 'Core' area type was calculated in GIS from the area of native vegetation from the 'edge' area (or 50m from the edge of mapped native vegetation) and was assigned a permeability type of 100%. 'Urban' area type was calculated in GIS from the edge of native vegetation for an area of 50m and was assigned a permeability type of 25%.	68.0 64.0	16.9 17.8	37.0-99.0 29.0-95.0
Patch size	Patch	Patch size (ha)	3,032.4	7,123.5	2.5-41,758.0
Years since last fire	YSL	Years from last survey since the last fire (500m buffer)	11.3	9.2	0-36.0
Number of fires	No.Fires	Number of fire years (30 years prior to survey (1980-2017)) (500m buffer)	4.0	3.1	0-12.0

200 covariates were calculated within the 200m buffer around nestboxes, while 500 covariates were calculated within the 500m buffer.

Results

A total of 2,415 nest box checks was completed over the seven years of survey in the Hornsby and Northern Beaches LGAs, with 3-26 monthly surveys completed within each site. Average naïve occupancy of eastern pygmy possum for a site (five nest boxes) with monthly nest box checks during the study was 29 %, with the lowest occupancy year being 2014 (13 %) (Table 2). Of the 41 sites surveyed, eastern pygmy possums were never detected at 13.

Detection probability

The most supported detection probability model included the autumn survey covariate (Table 3). The other detection covariates investigated; individual seasons, year, and annual rainfall the previous year, as well as combinations of these, did not improve detection probability (ΔAICc>2). Detection probability was highest in autumn at 0.49 (SE 0.05) per visit and lowest in summer at 0.27 (SE 0.06) (Figure 2). The autumn season was fitted as the default detection probability covariates for all subsequent occupancy models investigated.

Given detection probabilities for eastern pygmy possum in the study area were influenced by season, based on the model outputs four nest box checks in the autumn season would be required to be >90 % confident of detecting an EPP at an occupied site.

Table 3: Model selection table for detection probability of eastern pygmy possum (*Cercartetus nanus*) in peri-urban northern Sydney.

Model	ΔAIC	w	npar	neg2ll
psi(.),p(Autumn)	0	0.4269	3	559.7
psi(.),p(Season)	0.97	0.2633	5	556.67
psi(.),p(Month)	1.85	0.1692	14	539.55
psi(.),p(Summer)	4.59	0.043	3	564.29
psi(.),p(Spring)	5.05	0.0341	3	564.76
psi(.),p(.)	6.08	0.0204	2	567.78
psi(.),p(Year)	6.25	0.0187	9	553.95
psi(.),p(Rain)	6.48	0.0168	3	566.18
psi(.),p(Winter)	8.08	0.0075	3	567.78

*P= probability of detection; ΔAIC = delta AIC score; w=model weight; npar=number of parameters; neg2ll= -2*log-likelihood. Supported models shaded in grey.*

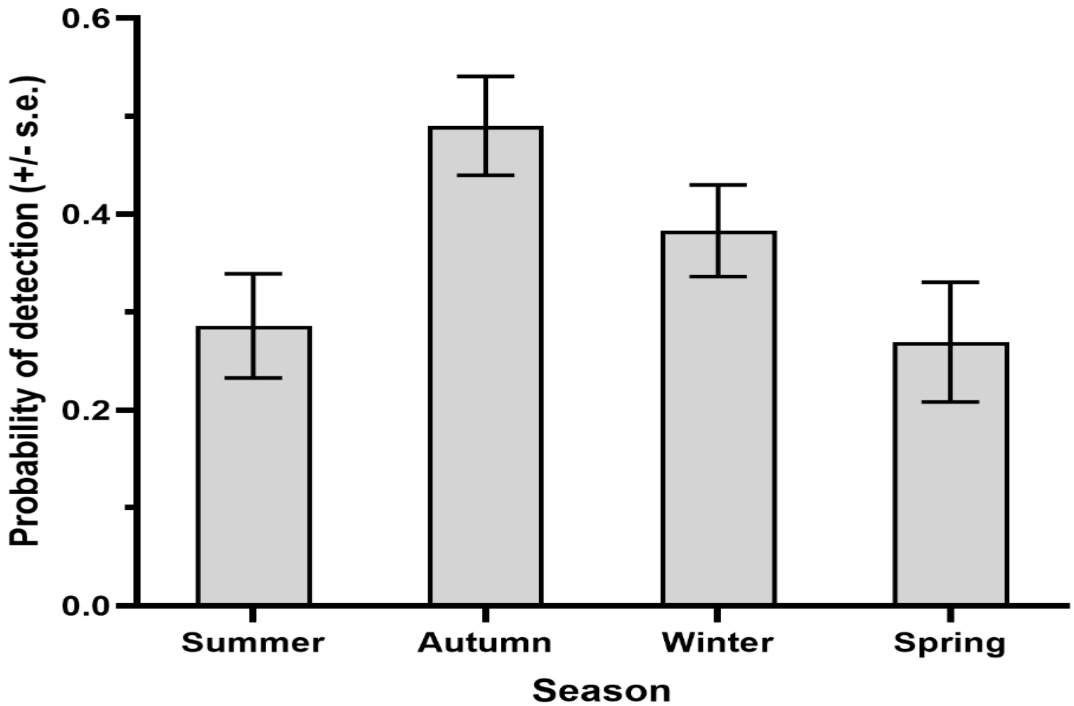

Figure 2: Probability of detection per monthly nest box survey of eastern pygmy possums (*Cercartetus nanus*) for each season.

Occupancy probability

Thirty occupancy models were fitted within the model set, with up to two-covariates in each model, as introducing a third covariate did not improve model performance (ΔAICc>2). The AICc difference between the top model and others was >2, indicating it was the most informative model and provided the best explanatory power without overfitting. The occupancy estimate under median conditions was 49% (SE 0.18), indicating widespread occupancy by this threatened species in the peri-urban study area.

Unburnt vegetation in the landscape (500 m buffer) was negatively associated with occupancy and had the highest individual covariate AIC weight (Figure 3). The extent of native vegetation in the landscape (500 m buffer) was positively associated with occupancy (Figure 4). The top performing model incorporates both these covariates as additive elements (Table 3).

For ≥70 % probability of a site being occupied, at least 50 % of the native vegetation (500 m buffer) had been burnt in the 30 years prior to survey. This increases to 100 % of the native vegetation being subjected to fire to achieve a 90 % probability of occupancy. We also investigated several fire interval covariates (extent of vegetation burnt 0-5, 5-15 and 15-30 years prior to survey) and pyrodiversity indices, but these did not improve model fit, as was the case for local (home range) variables. Each site had on average, been burnt four times by fire in the 30 years prior to survey (1980-2017), with some sites subjected to up to 12 fires (Figure 5). Two wildfires occurred during this time (1994 and 2002), however most fires in the study sites were prescribed burns of varying severity. Fire severity data were not available to be modelled. Of the sites burnt in the 1994 and 2002 wildfires, 34 % of sites (n=41) had over 70 % of native vegetation burnt in the 500 m buffer. In comparison, only one of the sites (2 %) surveyed had over 70 % of the 500 m buffer burnt during prescribed burns, with on average 96 % of sites experiencing less than 10% burnt when subjected to a prescribed burn in any year.

Table 4: Model selection table for occupancy probability of eastern pygmy possum (*Cercartetus nanus*) in peri-urban northern Sydney.

Model	ΔAIC	w	npar	neg2ll
psi(NotBurnt500+Native500),p(Autumn)	0	0.4794	5	539.07
psi(NotBurnt500+LongUnburnt500),p(Autumn)	3.42	0.0867	5	542.49
psi(NotBurnt500+No.Fires),p(Autumn)	3.48	0.084	5	542.55
psi(NotBurnt500),p(Autumn)	3.66	0.0767	4	544.73
psi(NotBurnt500+PYRO200),p(Autumn)	3.74	0.0739	5	542.8
psi(NotBurnt500+IntermediateFire200),p(Autumn)	5.25	0.0348	5	544.31
psi(NotBurnt500+Patch),p(Autumn)	5.4	0.0323	5	544.46
psi(NotBurn500+YSLF),p(Autumn)	5.56	0.0297	5	544.63
psi(NotBurnt500+PMI500),p(Autumn)	5.6	0.0291	5	544.67
psi(NotBurnt+RecentFire200),p(Autumn)	5.62	0.0288	5	544.69
psi(NotBurnt200),p(Autumn)	5.72	0.0274	4	546.79
psi(Native500),p(Autumn)	8.25	0.0078	4	549.31
psi(LongUnburnt500),p(Autumn)	9.19	0.0048	4	550.26
psi(LongUnburnt200),p(Autumn)	11.3	0.0017	4	552.36
psi(IntermediateFire200),p(Autumn)	13.85	5e-04	4	554.92
psi(No.Fires),p(Autumn)	13.98	4e-04	4	555.04
psi(PYRO200),p(Autumn)	14.12	4e-04	4	555.19
psi(IntermediateFire500),p(Autumn)	14.52	3e-04	4	555.58

Model	ΔAIC	w	npar	neg2ll
psi(PYRO500),p(Autumn)	14.64	3e-04	4	555.7
psi(Native200),p(Autumn)	15.4	2e-04	4	556.47
psi(Heath200),p(Autumn)	15.94	2e-04	4	557
psi(PMI500),p(Autumn)	16.26	1e-04	4	557.33
psi(YSLF),p(Autumn)	17.59	1e-04	4	558.66
psi(Patch),p(Autumn)	17.97	1e-04	4	559.04
psi(Heath500),p(Autumn)	18	1e-04	4	559.07
psi(PMI200),p(Autumn)	18.07	1e-04	4	559.14
psi(Roads500),p(Autumn)	18.15	1e-04	4	559.21
psi(RecentFire200),p(Autumn)	18.57	0	4	559.63
psi(Roads200),p(Autumn)	18.6	0	4	559.67
psi(RecentFire500),p(Autumn)	18.63	0	4	559.7

*P= probability of detection; ΔAIC = delta AIC score; w=model weight; npar=number of parameters; neg2ll= -2*log-likelihood. Supported models shaded in grey.*

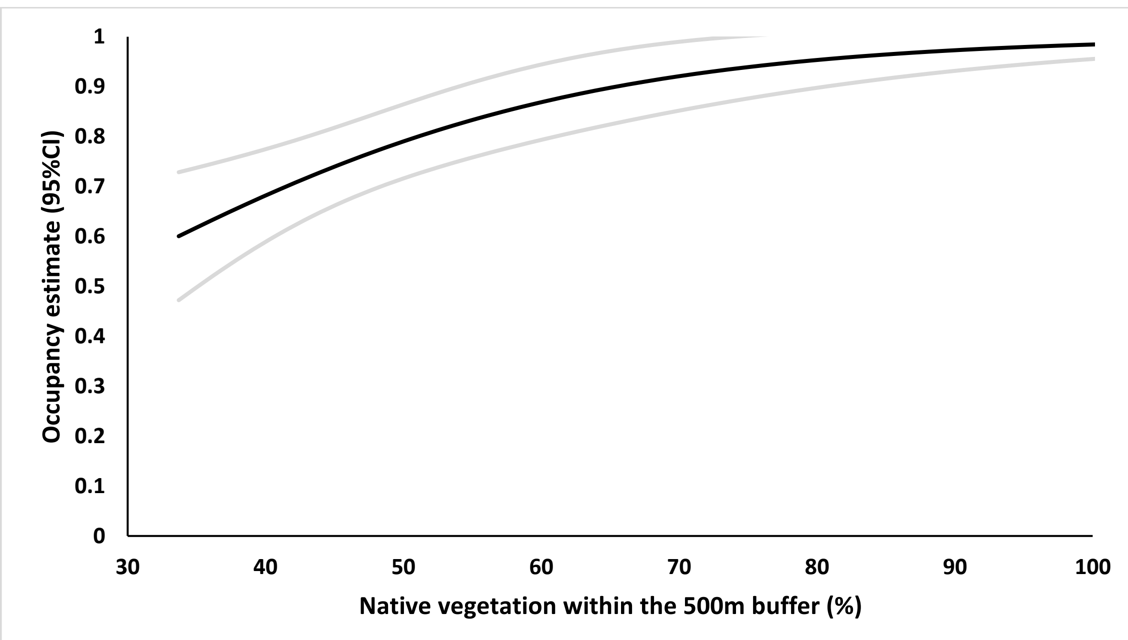

Figure 3: Probability of occupancy for eastern pygmy possums (*Cercartetus nanus*) at a site within the study area based on amount of native vegetation within the 500 m buffer surrounding nest boxes.

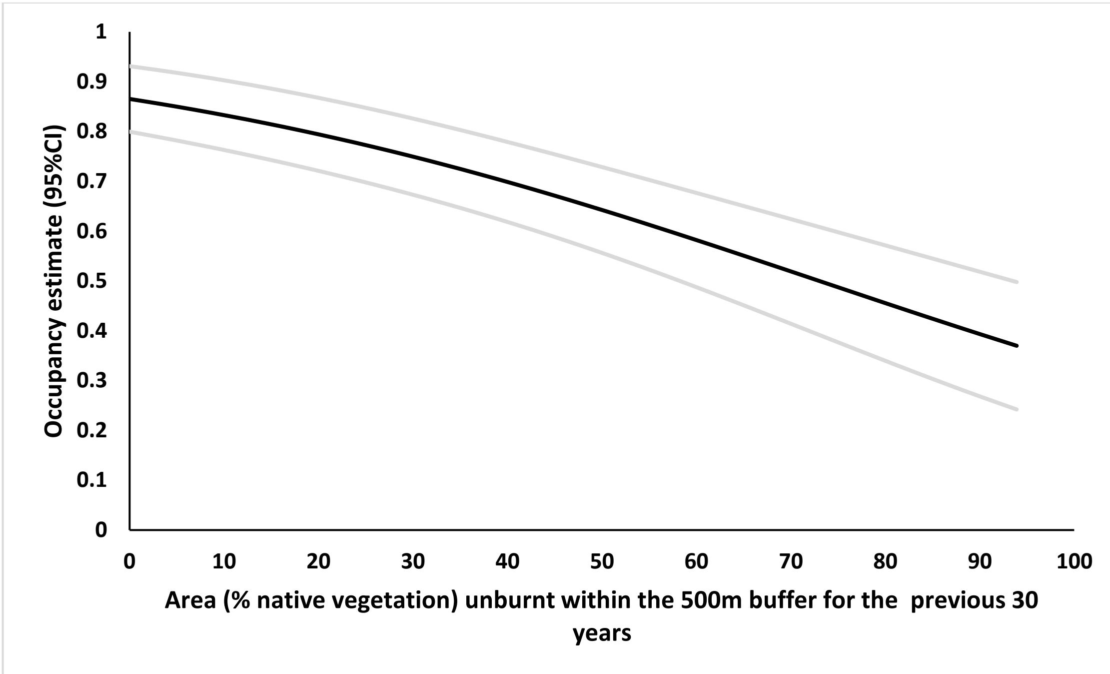

Figure 4: Probability of occupancy at a site within the study area of eastern pygmy possums (*Cercartetus nanus*) based on the area of native vegetation (% of 500m buffer) unburnt in the 30 years prior to survey (1980-2017).

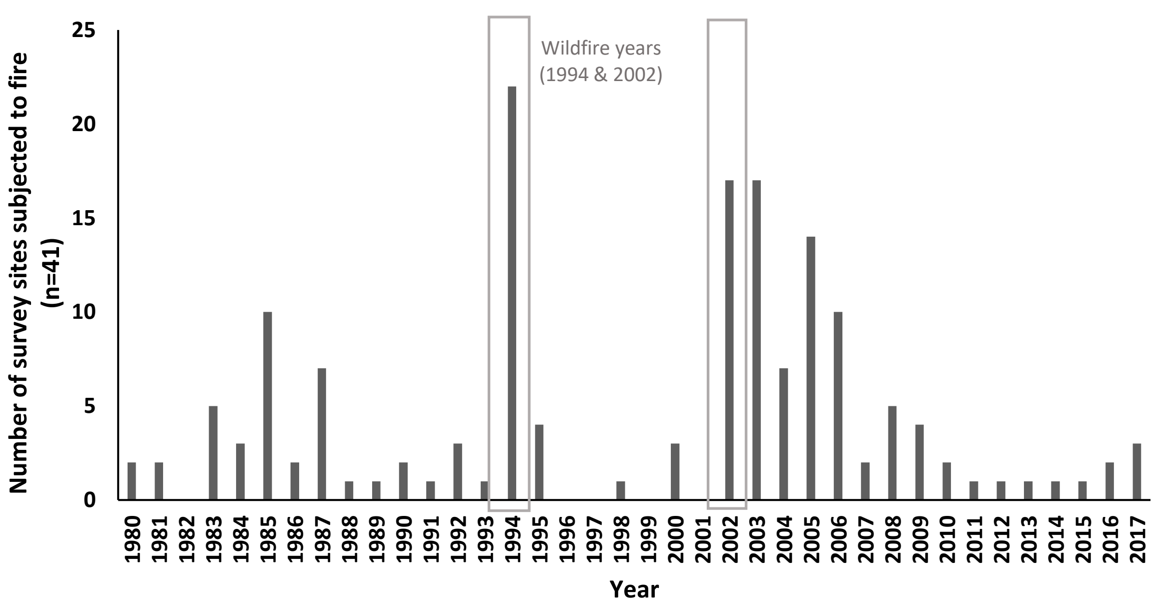

Figure 5: Number of eastern pygmy possum (*Cercartetus nanus*) study sites subjected to fire within the 500m buffer per year (1980-2017) in peri-urban Sydney.

Discussion

This study provides the first investigation of eastern pygmy possum occupancy within bushland on the peri-urban edge, offering insights into factors influencing this urban-sensitive species. At a landscape level, fire history and the extent of native vegetation were key drivers of possum occupancy. Occupancy probability increased with greater areas of historically burnt vegetation and larger extents of native vegetation. In an urban context, fire plays a critical role in managing fuel loads to protect property from wildfires but may also indirectly influence wildlife occupancy and habitat features. The results underscore the importance of remnant vegetation within the peri-urban matrix for sustaining urban-sensitive species.

Detection of Eastern Pygmy Possum

Detection probability was most influenced by survey season, with autumn being optimal for surveying eastern pygmy possums using nest boxes. During this season, a minimum of four surveys per site (with five nest boxes) is required to achieve a 90% detection probability when the species is present. Survey effort must nearly double in summer to achieve comparable detection rates. Interestingly, the use of cameras trained on banksia inflorescence within the study area had the highest probability of detection in winter (Thompson *et al.* 2025), as did a nearby study in similar habitat using thermal cameras and spotlighting (Madani and Gonsalves 2025).

The high detection probability in autumn coincides with the peak flowering of *Banksia ericifolia* (March–July) (McFarland 1985; Goldingay 2023), a key food resource for eastern pygmy possums in nearby populations (Tulloch and Dickman 2006; Harris *et al.* 2007; Goldingay and Keohan 2017). Flowering intensity of *Banksia ericifolia* correlates with breeding activity in female possums (Goldingay and Rueegger 2018). Other key banksia species in the study area, though less abundant, include *Banksia spinulosa* (flowering autumn–winter), *Banksia integrifolia* (autumn–spring), *Banksia paludosa* (autumn–winter), and *Banksia serrata* (summer–autumn). Across its range, eastern pygmy possums are strongly associated with banksia species, often being considered nectar/pollen specialists

(Turner 1984; Tulloch and Dickman 2006, 2007; Goldingay and Rueegger 2018; Law *et al.* 2018). However, they also consume nectar and pollen from other sources, including eucalypts, *Lambertia* spp., and sap (van Tets 1998; Hockey *et al.* 2019), with increased invertebrate consumption during spring and summer when key nectar sources decline (van Tets and Hulbert 1999). These seasonal dietary shifts have been found to coincide with movement away from banksia-dominated heath, leading to lower detection probabilities outside the main flowering period and potential population troughs (Ward 1990; Bladon *et al.* 2002; Tulloch and Dickman 2006; Rueegger *et al.* 2012), which may have occurred in our study area.

Long-term radio-tracking has shown that while home ranges increase in spring, especially for males, there is no evidence of extensive movement within heathy forests (Law *et al.* 2013). However, increased mobility in spring and summer may expose dispersing individuals to anthropogenic barriers, predation by exotic species, and vehicle collisions, as observed in other urban-edge mammals (Forman 2000; Banks 2004; Burgin and Lunney 2004; Garden *et al.* 2006). Roadkill records from the study area (Government 2025) and other regions (Harris *et al.* 2007) suggest these threats are significant for the species. Further research is needed to understand movement patterns on the peri-urban edge and inform management strategies to mitigate risks. Until more data are available, land managers should assume individuals move between habitat patches and implement appropriate mitigation measures.

Suitable Habitat and Connectivity

In peri-urban environments, habitat patch size and connectivity influence small mammal abundance and diversity (Garden *et al.* 2006). The persistence of urban-sensitive species depends not only on patch size and isolation, but also on the availability of high-quality habitat within the landscape matrix (Brady *et al.* 2011). Previous studies reported significant eastern pygmy possum population declines following habitat clearance that reduced native vegetation patches from 4 ha to 2.6 ha (Bladon *et al.* 2002). In our study, the smallest occupied patch was 2.5 ha, but it was within 50 m of a larger remnant. Rather than patch size alone, occupancy was positively related to the extent of native vegetation within a 500 m buffer, suggesting larger vegetation extents facilitate movement and recruitment. Sites with higher native vegetation cover were typically closer to larger areas of high-quality habitat, whereas the most isolated sites, such as coastal headlands, had the lowest occupancy probabilities.

To enhance habitat quality and availability for eastern pygmy possums in peri-urban areas, management should focus on increasing native vegetation cover. When native vegetation comprises at least 50 % of a 500 m buffer (~40 ha), occupancy probability reaches 70 %. While increasing vegetation extent may not always be feasible, connecting patches via habitat corridors and enhancing floristic diversity, particularly nectar-rich Proteaceae and Myrtaceae species, and providing den habitat by installing nest boxes, could improve habitat suitability.

Influence of Fire on Occupancy

Fire history influenced eastern pygmy possum occupancy in our study area. Low-intensity prescribed burns are commonly used in the area to manage fuel loads and mitigate wildfire risks to property. The recommended fire interval to minimise impacts on eastern pygmy possums is 15 years (NSW Rural Fire Service 2021). Most fires in the study area were low-intensity prescribed fire, although higher-intensity wildfires did affect around 20 % of the sites in 1994 and 2002 (Figure 5), with burning intervals generally much less than recommended. Notably, in nearby *Banksia serrata* and *Banksia spinulosa* forests, possum occupancy was higher at sites affected by moderate wildfire (Chew *et al.* 2024). While fire can stimulate important foraging resources (e.g., *Lambertia formosa*; (Pyke 1983)), frequent low-intensity fires (<7–8 years) reduce long-lived woody shrubs, altering habitat composition and decreasing arboreal habitat quality (Morrison *et al.* 1996). This is concerning given projections of increasing wildfire frequency under climate change (Pendall *et al.* 2022), and likely responses of prescribed fire regimes by land managers in the peri-urban environment.

The dominant banksia species in our study, *Banksia ericifolia*, is an obligate seeder with a canopy-stored seed bank, with high-intensity fire killing plants, and new plants requiring 6 years post-fire to reach flowering maturity (Bradstock and O'Connell 1988). Conversely, *Banksia serrata* and *Banksia spinulosa*, are fire tolerant and resprout after fire, but decline when low-intensity fire intervals are less than 7 years (Bradstock 1990). Although time since last fire was not supported in our models, observed increases in occupancy in burnt areas must be considered in the context of appropriate fire intervals to sustain habitat resources.

Eastern pygmy possums can recolonise burnt habitats rapidly (Chew *et al.* 2024). Following prescribed burns, individuals were detected in nest boxes and on infrared cameras within three weeks (Thompson *pers obs*). Similarly, radio-tracking confirmed denning and foraging in burnt habitat within six months (Law *et al.* 2013), and other studies report increased detectability post-wildfire (Dickman and Happold 1988; Wilson *et al.* 1990). However, responses vary, with some studies finding similar abundances in burnt and unburnt areas 7–10 years post-fire (Tulloch and Dickman 2006, 2007) or a preference for unburnt habitat (Thompson *et al.* 1989; Whelan *et al.* 1996). These differences suggest resilience to single fire events, but fire severity, patchiness, and frequency remain critical factors, particularly in peri-urban areas with frequent prescribed burns (Burns and Phillips 2019).

After the 2019–2020 Australian megafires, there have been calls to increase prescribed burning to mitigate future wildfire severity (NSW Government 2020). Ensuring appropriate fire intervals to sustain foraging resources will be crucial for eastern pygmy possum conservation in urban-edge habitats. The availability of unburnt refugia may also influence post-fire population dynamics and recolonisation (Lindenmayer *et al.* 2016). Further research is needed to determine optimal fire intervals and intensities. Incorporating Aboriginal cultural burning practices could enhance habitat diversity and species conservation while reducing wildfire risk (Bliege Bird *et al.* 2018; Mariani *et al.* 2022).

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Chapter 4: Summary

In this chapter, I use dynamic occupancy modelling to investigate how local and landscape-scale habitat configuration, vegetation extent, and fire history influence the distribution and persistence of the eastern pygmy possum in peri-urban northern Sydney. The results provide insight into the species' habitat requirements and inform targeted management actions for remnant bushland patches. Occupancy modelling revealed that eastern pygmy possums are more likely to occur in larger patches with higher surrounding native vegetation cover, and areas with longer intervals since fire. Seasonal variation in detection highlighted the need for targeted survey timing, while the influence of landscape-scale factors emphasised the importance of functional connectivity.

Building on these results, the next chapter (Chapter 5) investigates how camera trapping can enhance detection of cryptic species, like the eastern pygmy possum, and address identified gaps in monitoring, particularly in the context of fauna crossing structures.

Chapter 5: Assessing the detectability of a cryptic arboreal marsupial using a novel survey approach

Assessing the detectability of a cryptic arboreal marsupial using a novel survey approach

Cassie THOMPSON [a*], Leroy GONSALVES [b], Brad LAW [b] and Peter B. BANKS [a]

[a] School of Life and Environmental Sciences, University of Sydney, Sydney, 2006, Australia. [b] NSW Department of Primary Industries (Forest Science), Sydney, 2150, Australia

Summary text: Cryptic and declining species can be difficult to detect and may require considerable survey effort using standard techniques such as trapping and spotlighting. We investigated detectability for the cryptic eastern pygmy possum (*Cercartetus nanus*) using wildlife cameras in novel ways. Focusing cameras on flowering banksia effectively detected the species and improved capture rates when compared with typical survey methods or focusing cameras on nest boxes. Cameras may offer a low-cost alternative to standard techniques for cryptic and hard to detect species and improve detection probability by supplementing existing survey approaches.

Abstract

Non-detection of a species arising from inadequate sampling effort or ineffective techniques may have serious consequences for its conservation, particularly those that are declining. The threatened and cryptic eastern pygmy possum (*Cercartetus nanus*), despite its widespread distribution, is infrequently detected using standard trapping techniques (e.g. Elliott traps and spotlighting). There are no survey guidelines for the species, and published literature suggest detection often requires significant survey effort and therefore cost. In this study, we investigated the detectability of the eastern pygmy possum using wildlife cameras focussed on nest boxes and nectar food resources. We collected detection data in bushland remnants in northern Sydney over five years using these methods and modelled detection probability. Detection probability was highest during winter in each year, which coincided with banksia flowering and breeding events, but detectability varied across survey years. We found that cameras targeting flowering banksia achieved a 95% detection probability from an average trapping effort of 117 camera nights, compared to 237 camera nights at nest boxes. We conclude that targeted use of wildlife cameras may be a cost-effective alternative to labour-intensive standard survey methods or to supplement existing survey approaches (e.g. nest box checks) and improve detection probability.

Introduction

Accurate detection of presence or absence is important for the conservation and management of fauna species (Burns *et al.* 2019). Environmental impact assessments rely on accurate species detection to ensure that species are properly assessed, appropriate mitigation measures are applied, and any biodiversity losses resulting from development are effectively offset (Brownlie *et al.* 2013). In such instances methods need to be species-specific, with a high-level of accuracy, and appropriate to employ in a cost-efficient manner. The level of survey effort required to determine presence or true absences at a location can be significant for some species, particularly for those that are considered cryptic (Burns *et al.* 2019; Harley and Eyre 2024). Small arboreal mammals are often difficult to survey using conventional techniques like trapping and spotlighting (Moore *et al.* 2021). These methods have known sampling biases (Tasker and Dickman 2001), even for terrestrial fauna (Johnstone *et al.* 2021), and high rates of false absences for arboreal mammals (Wintle *et al.* 2005).

The arboreal eastern pygmy possum (*Cercartetus nanus*) is a threatened species native to eastern Australia and is commonly considered cryptic and hard to survey (Bladon *et al.* 2002; Harris and Goldingay 2005b; Harris *et al.* 2007b). It generally occurs in low density populations (Bowen and Goldingay 2000), which may be missed and assumed absent due to inappropriate survey techniques or survey effort. Despite its seemingly widespread occurrence, the eastern pygmy possum is rarely recorded during fauna surveys, particularly using standard mammal survey techniques (e.g. Elliott trapping and spotlighting) (Bowen and Goldingay 2000). For example, Suckling (1978) used spotlighting, ground-based Elliott trapping and trialled the use of baited hair tubes (30mm wide PVC pipe open at both ends) where the species was known to occur at the survey site, yet no individuals were recorded. Bowen and Goldingay (2000) reviewed previous surveys in New South Wales (NSW) and found at that time, that only five extensive surveys recorded more than 10 individuals. Similarly, other Burramyids like the little pygmy possum (*Cercartetus lepidus*) and long-tailed pygmy possum (*Cercartetus caudatus*) appear difficult to detect, due to the lack of studies recording them, however pitfall traps have shown success for surveying the western pygmy possum (*Cercartetus concinnus*) (Pestell and Petit 2007) and the little pygmy possum (Ward 1992; Duncan and and Taylor 2001).

Traditional methods for detecting eastern pygmy possums, such as Elliott and pitfall trapping, have shown limited and inconsistent success. Elliott trapping appears more effective when used repeatedly in the same location (Ward 1990; Laidlaw 1996), particularly near flowering banksias (Bowen 2000; Harris 2005), though this increases effort and the standard bait mix may be inadequate as it competes with natural foods. Pitfall traps have reported higher capture rates than spotlighting or Elliott trapping in some studies (Bennett et al. 1989; Bowen and Goldingay 2000; Tasker and Dickman 2001; Tulloch and Dickman 2006), yet large-scale surveys in NSW State Forests recorded only one capture from over 10,000 pitfall trap nights (Bowen and Goldingay 2000). Spotlighting, while common for arboreal mammals, is considered less reliable for this species (Kavanagh and Webb 1998; Davey 1990). The literature offers little clear guidance on the most effective survey methods, and the species is regarded as "very difficult to detect, especially via spotlighting" (eastern pygmy possum – Ecological Data, BioNet Atlas, NSW). Traditional trapping techniques are also labour-intensive and potentially harmful to animal welfare (Garden et al. 2007). Given this and the low detectability of the eastern pygmy possum with these methods, alternative reliable approaches are needed.

There has been an increase in the use, and it has been suggested that the species is most reliably detected in nest boxes (Bowen and Goldingay 2000; Goldingay 2023). Nest boxes have been successfully applied to study the effects of fragmentation on demography of the eastern pygmy possum (Bladon *et al.* 2002), life history (Ward 1990) and the effects of habitat, movements and social organisation (Harris and Goldingay 2005b; Law *et al.* 2013; Harris *et al.* 2014; Goldingay and Keohan 2017; Goldingay and Rueegger 2018; Law *et al.* 2018; Goldingay 2019, 2023). Previous studies have also investigated optimal box design to record eastern pygmy possums (Beyer and Goldingay 2006; Rueegger *et al.* 2012). A recent study found a detection probability of 0.35 per survey visit for a cluster of five nest boxes (Chew *et al.* 2024). However, like conventional techniques, nest boxes can represent a labour-intensive survey method due to the ongoing need for physical checks at a site, ongoing maintenance and these have not always proven effective for detection, particularly in areas where hollows are abundant (Harris and Goldingay 2005a).

Camera traps are rapidly becoming a preferred tool for recording mammal species (Bowler *et al.* 2017). Camera traps have been found to be more effective than live trapping (Bondi *et al.* 2010) and hair tunnels (Paull *et al.* 2011) for detecting small mammals, and despite high initial costs, camera traps have been found to be less costly than labour-intensive conventional methods in the long term (Welbourne *et al.* 2015). While camera trapping can be as effective and efficient in determining occupancy for some arboreal mammals as it is for terrestrial species (Harley and Eyre 2024), few studies have specifically used camera traps to detect the eastern pygmy possum. Cameras have been used to identify behaviour and assess nest box preferences of eastern pygmy possums (Rueegger *et al.* 2012). Another study in Jervis Bay (NSW) used a range of standard methods (Elliott and cage traps) but only detected the eastern pygmy possum on camera traps on the ground using drift netting (Welbourne *et al.* 2015). Cameras with infrared triggers have also been used to record pollinators on banksia inflorescence and successfully recorded eastern pygmy possums (Carthew 1993). Infrared cameras also found eastern pygmy possums were the most frequent small mammal visitor spending significant periods at inflorescence of heath-leaved banksia (*Banksia ericifolia)* in bushland reserves in northern Sydney (Saul 2013; O'Rourke *et al.* 2020), suggesting cameras focussed at resources may optimise detections.

In this study we assess the efficacy of camera traps for detecting the eastern pygmy possum at nest boxes and flowering food resources, in all seasons and over a 5-year period that spanned a range of climatic conditions, including a significant drought that resulted in the 2019/2020 megafires across eastern Australia, though the study area was not burnt. The use of cameras has the potential to reduce field effort to detect cryptic species, given these can be set up to collect several months of data from one installation. We modelled detection probability using camera traps focused on banksia inflorescences and nest boxes (artificial hollows), to assess which method is best to maximise likelihood of detection for eastern pygmy possums when using cameras. We also assessed how detection probability varied among seasons and years. Cumulative detection probability curves were generated to guide survey effort required to achieve a high degree of confidence in absences for the species at surveyed sites.

Materials and methods

Our study area was comprised of seven bushland remnant localities on the urban edge in northern Sydney, Australia, located within the Northern Beaches local government area on the Ingleside escarpment (Fig. 1). This area is dominated by a mixture of urban and semi-rural land uses interspersed with remnant vegetation. Vegetation at the seven localities comprised heathland or heathy woodland on sandstone dominated by flowering Myrtaceae and Proteaceae species, with an abundance of banksia spp. Cameras were installed at or near nest boxes which had successfully captured eastern pygmy possums as part of an ongoing population study (C. Thompson et. al, unpublished) (Table 1). Nest boxes were made from salvaged hollows of varying sizes (but with a minimum internal diameter of 10 cm), end caps made of steel, and a drilled opening of 3 cm near the top of the box. These were installed between 0.5 m and 1.8 m from the ground on banksia or eucalypt trees, to allow for checking without a ladder. Groups of 5 or 10 nest boxes were installed at each bushland remnant, along a transect at a spacing of 50-100 m apart. Given a typical home range for an eastern pygmy possum is approximately 3-4 ha, (Harris *et al.* 2007a; Law *et al.* 2013) each transect was separated by at least 500 m, where there was no impermeable barrier (e.g. large roads, development and fences). Not all nest boxes were used for this study, as these were installed and are being monitored for an ongoing population study (C. Thompson et. al, unpublished) associated with road crossing structures that were being built as part of the road upgrade (‘underpass’ and ‘overpass’). The crossing structure study had not recorded any tagged eastern pygmy possums crossing the road over the eight years of survey, thus the ‘north’ and ‘south’ localities associated with the crossing structure locations were considered independent for this study. Similarly, Goldingay (2023) recorded only two road crossings (road width of approximately 10 m) between nest boxes from >100 tagged individuals.

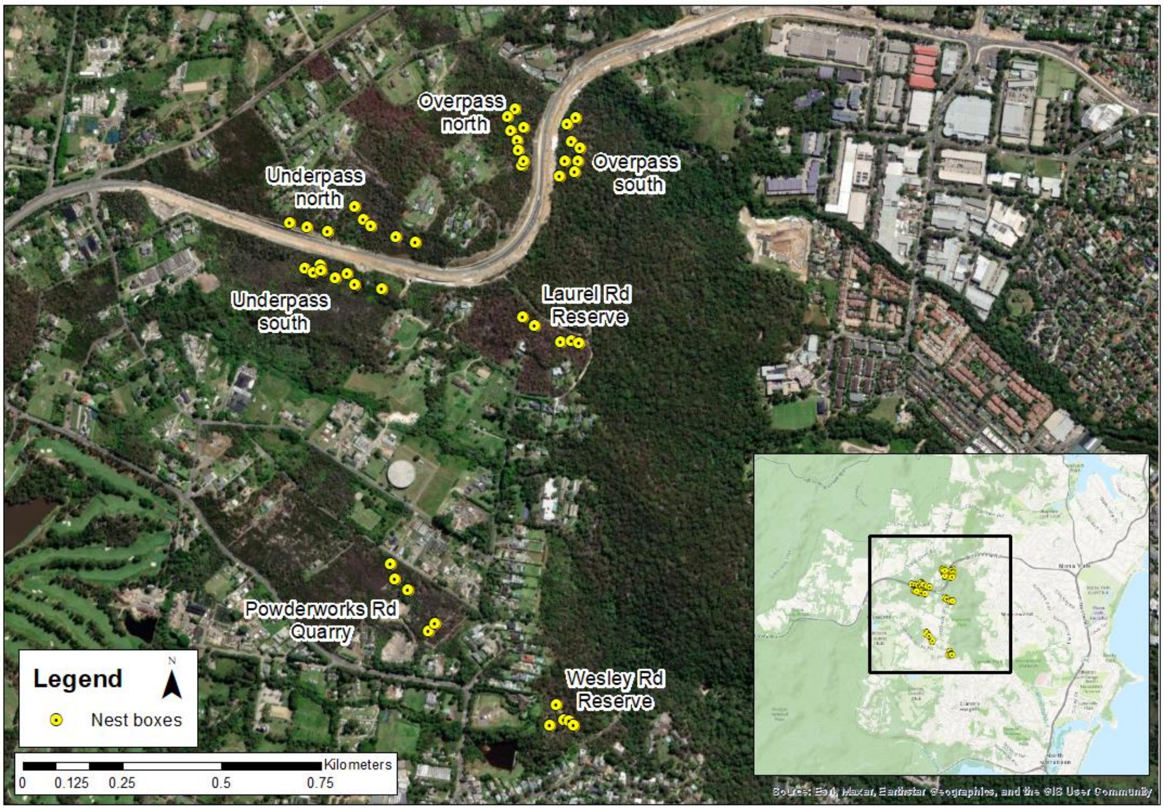

Fig. 1. The study area and the location of nest boxes in northern Sydney.

Reconyx hyperfire (HC600 and PC800) covert infrared cameras were set to detect eastern pygmy possums by directing them at either: flowering banksias or nest boxes. A total of 16 camera locations at nest boxes, and 12 camera locations at banksia inflorescence in proximity to nest box locations were established. Cameras sampling flowering banksia were installed in proximity to installed nest boxes (within 20 m), however both camera types were not used concurrently. The cameras have inbuilt passive infrared LED motion sensors and were installed ~0.5 m in front of the boxes or banksia inflorescence on trees and secured with straps, with a high PIR sensitivity. Cameras were not changed for close proximity photos. Based on pilot testing of the methods, this appeared to be a sufficient distance to avoid overexposed images and to allow for identification of all mammals to species level. Cameras were set to record a sequence of 10 photos, but this was reduced to 5 photos after the first year of survey given the number of false triggers and number of images to process. A quiet period of 1 min was set before they could be triggered again. For each site (nest box/banksia inflorescence), a detection of at least one eastern pygmy possum per night was scored as ‘presence’. This was done to reduce counting the same individual multiple times and to provide a 'detection' or 'non-detection' score per survey night per camera for detection modelling. Capture rates at each camera location were calculated based on the number of detections (i.e. one image of an eastern pygmy possum per camera per night) divided by the total camera nights and expressed as a percentage.

Heath-leaved banksia was the dominant banksia species in the study area. This species is a known important local food resource for eastern pygmy possums (Tulloch and Dickman 2007; Goldingay and Keohan 2017; Goldingay 2023) and its inflorescences produce copious amounts of sugary nectar at night (Carpenter 1978). Heath-leaved banksia flowers in autumn through winter and sometimes into spring (Copland and Whelan 1989; Goldingay 2023), thus cameras were only installed within these seasons for the banksia cameras. Flowering duration of individual inflorescences vary considerably, but may be less than four weeks (Copland and Whelan 1989). Banksia inflorescences were targeted for survey based on flower maturity and nectar secretion, and to allow a minimum recording period of 30 days to allow for the highest nectar availability to be sampled over the camera’s deployment across autumn, winter and spring (Fig. 2). Nest box cameras were not limited to the flowering season of heath-leaved banksia and recorded across several seasons. Images were individually processed using an image viewer and tagged. Detected fauna were identified to species level where possible, using reference images and texts (Van Dyck and Strahan 2008). Data were then collated as a detection history (presence/absence) for input into the modelling.

Fig. 2. (a) Covert infrared camera trained on a flowering Heath-leaved Banksia (*Banksia ericifolia)* inflorescence in northern Sydney (b) Covert infrared camera trained on a salvaged log nest box in northern Sydney.

Fig. 3. Example images of the eastern pygmy possum (*Cercartetus nanus*) using Covert infrared cameras (a) trained on nest boxes and (b) flowering banksia inflorescence.

An occupancy model was used to assess detection probabilities using data generated by cameras at the seven bushland localities, with data collected over 5 years. As camera numbers for the study were limited, cameras were not installed consistently within localities over the survey period but shifted among nest boxes and banksia flowers across the survey period, resulting in detection/non-detection data and periods of no survey for each camera location (Table 2). To ensure independence, detection histories were prepared for each camera location for input into the model. A 'site' was included for each camera type (banksia or nest box) within a locality (eg Laurel Rd Reserve), resulting in one record (detection/non-detection) or NA if the camera was moved to another site, per night. A total of 32 sites were sampled. Data analyses were run using the RPresence package (MacKenzie and Hines 2023) using a single season occupancy modelling framework, as we were only modelling detection probabilities. Site occupancy modelling accounts for the potential for imperfect detectability (i.e., false absences that result when a species is present but not detected), by using information from repeated observations at sample sites occupied to estimate detection probabilities (Mackenzie 2005).

Table 1. Study sites and camera number and types in each site in northern Sydney

Site name	**Approximate patch size (ha)**	**Number of nest boxes installed at site**	**Number of camera locations included in analysis per method (across all years)**	
			Nestbox	**Banksia**
Laurel Rd Reserve	98	5	3	0
Overpass north	25	10	4	2
Overpass south	98	10	4	3
Powderworks Rd Quarry	9	5	0	1
Underpass north	25	10	4	3
Underpass south	12	10	2	4
Wesley Rd Reserve	98	5	2	0

Table 2: Survey year and number of detection nights (where at least one image of an eastern pygmy possum was captured in a night) and total camera nights by camera trapping method for the eastern pygmy possum in Northern Sydney

Year	**Number of eastern pygmy possum detection nights (total camera nights) by camera survey method**	
	Nestbox	**Banksia**
2018	12(98)	0(0)
2019	55(590)	0(0)
2020	61(1239)	12(60)
2021	5(1130)	9(113)
2022	0(107)	9(396)

Detection probability (p) was modelled with occupancy (psi) held constant. Detection was modelled to compare covariate models with a null model. We assessed single covariates and built upon the most supported single covariate model by adding an additional covariate until there was no improvement (i.e., adding an additional variable did not improve on the top model by >2 AIC points). We included year as a covariate in all detection models to account for any changes in detection probability associated with year of sampling. Variables assessed were season (austral spring, summer, autumn and winter) and survey method (camera on nest box or camera on banksia inflorescence). Banksia inflorescence could only be sampled during particular seasons, ie when flowering, however nest boxes were sampled across a range of seasons. Detection models were ranked from the lowest to highest based on Akaike's information criterion (AIC) (Burnham and Anderson 2004), with the difference in AIC (ΔAIC) calculated between each model and the top-ranked model. Models with ΔAIC <2 were considered equally plausible to explain the data (Burnham and Anderson 2004).

To evaluate the minimum survey effort required to confidently detect the eastern pygmy possum at a site when present, we calculated the cumulative detection probability (P) using the standard formula from MacKenzie and Royle (2005):

$P = 1 - (1-p)^k$

Where:

P is the cumulative probability of detecting the species at least once over k independent surveys,

p is the estimated single-survey detection probability, and

k is the number of surveys (or camera-nights in this context).

This approach allowed us to estimate the number of repeated surveys required to achieve a desired cumulative detection probability (typically ≥ 0.95) under different conditions and detection methods, in this case for different detection methods (e.g., cameras trained on banksia inflorescences vs. nest boxes), stratified by season and year to reflect observed variability. Given the strong effect of year of survey on detection probability, estimates from 2020 were chosen to guide minimum survey duration to achieve high detection probability (95%) for eastern pygmy possum. This year was chosen because it provided an intermediate detection probability value (i.e., not the extreme ends) and represented a year where both banksia and nest box cameras were used.

Results

During 2018-2022, 133 eastern pygmy possum detection nights were recorded using cameras at nest boxes (during 2,147 camera nights), while cameras at banksia inflorescences recorded 30 eastern pygmy possum detection nights (703 camera nights) (Fig. 3). Detection nights decreased across the survey period for nest box cameras and banksia cameras (Fig. 4).

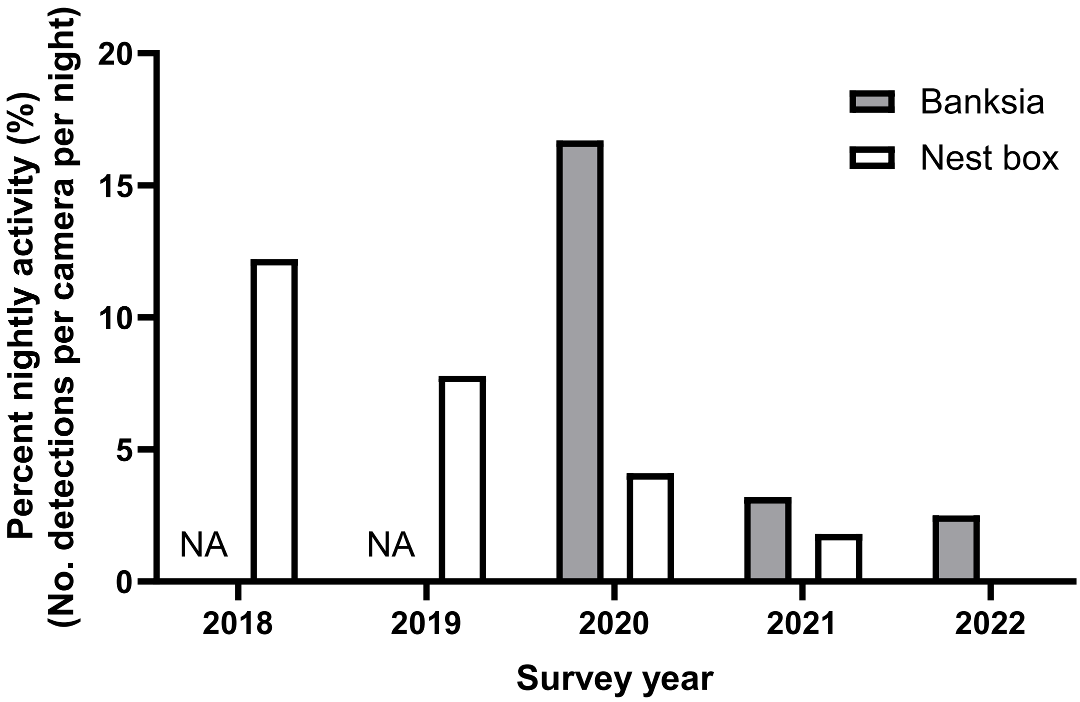

Fig. 4. Nightly eastern pygmy possum (*Cercartetus nanus*) activity (total detections/total camera type x100 per year) across years for both banksia and nest box cameras in northern Sydney. NA - No banksia cameras were used in 2018 and 2019 and only one nest box camera was used in 2022 but did not get any detections in the period deployed.

Modelling revealed a single model explained the data better than any other (Table 4). This additive model allowed detection probability to vary among years of survey, between seasons and between surveys using cameras trained on banksia inflorescences or nest boxes. The detection probability was highest in winter and lowest in spring (Fig. 5a).

Modelled detection probability was more than 3-times (3.33) higher for cameras on banksia inflorescences compared to cameras on nest boxes (Fig. 5b). This trend was found for all years in which both surveys methods were employed (Fig. 6). Detection probability for banksia cameras in winter ranged from 0.10 in 2000, to 0.01 in 2022, and for nest box cameras in winter from 0.14 in 2018 to 0.01 in 2022 (Fig. 6).

Table 3: Published capture rates for eastern pygmy possum (*Cercartetus nanus*) by survey methods

Method	**Average capture rate for method (%)**	**Average capture rate (detections per method night) (%) when detected, study and location**
Nestboxes	9.13%	33.5% (Bladon et al 2002 pre-clearing) Dorrigo NSW 7.8% (Bladon et al 2002 post-clearing) Dorrigo NSW 4.46% (Chew *et al.* 2024) Central Coast, NSW 3.82% (Rueegger *et al.* 2012) Royal NP, NSW 3% (Harris and Goldingay 2005b) Barren Grounds NR, NSW 2.2% (Harris et al 2014) Barren Grounds NR, NSW
Pitfall traps	0.93%	2.22% (Shelly 1998) Central-west, NSW 0.33% (Goldingay and Daly 1998) Queenbeyan, NSW 0.5% (Braithwaite 1983) Eden, NSW 0.65% (Rueegger *et al.* 2012) Royal NP
Elliott traps	1.7%	0.07% (Whelan *et al.* 1996) Royal NP, NSW ground Elliots 2.24% (Goldingay *et al.* 1987) Barren Grounds NR, NSW 1.94% (Goldingay *et al.* 1991) Budderoo NP, NSW 0.44%.(Rueegger *et al.* 2012) Royal NP, NSW 3.8% (Harris et al 2014) Barren Grounds NR, NSW adjacent to flowering banksia and with honey-water mixture near trap (none in ground traps) 4.03% (Harris and Goldingay 2005b) Barren Grounds NR, NSW 0.3% (Laidlaw 1996) Otways, VIC with ground Elliots 2.5% (Evans and Bunce 2000) Wilsons Promontory NP, VIC in banksia trees 0.02% (Tasker and Dickman 2001) North-eastern NSW
Pitfalls and Elliots	0.84%	0.68% (Tulloch and Dickman 2006) Royal and Heathcote NP, NSW 0.14% (Harris *et al.* 2007a) Ground and tree Elliots and pitfalls in Jervis Bay, NSW

Method	Average capture rate for method (%)	Average capture rate (detections per method night) (%) when detected, study and location
		1.7% (Tulloch and Dickman 2007) Royal and Heathcote NP, NSW
Spotlighting	1.29%	1.29% (Davey 1990) South coast, NSW
Baited hairtubes	0.17%	0.04% (Dickman and Happold 1987) ACT 0.3% (Tulloch & Dickman 2006) Royal NP, NSW

Table 4: Final detection models for eastern pygmy possum using banksia and nest box cameras for detection.

Model	DAIC	AIC	wgt	npar	neg2ll
psi(.),p(Year+Season+Banksia)	0.00	1183.1	0.99	10	1163.10
psi(.),p(Year+Season)	10.76	1193.86	0.01	9	1175.86
psi(.),p(Year+Spring)	13.91	1197.01	0.00	7	1183.01
psi(.),p(Year+Winter)	17.44	1200.54	0.00	7	1186.54
psi(.),p(Year+Banksia)	28.93	1212.03	0	7	1198.03
psi(.),p(Year)	48.97	1232.07	0	6	1220.07
psi(.),p(Year+Autumn)	50.94	1234.04	0	7	1220.04

Note: DAIC: The difference in Akaike Information Criterion (AIC) values between a given model and the model with the lowest AIC. Season = Autumn+Winter+Spring+Summer, where only one season (eg Winter) is included in a model, that season is being contrasted to all others which are equal. Abbreviations - wgt: Model Weight; npar: Number of Parameters; neg2ll: Negative Log-Likelihood; psi: Occupancy; p: Detection Probability.

Minimum camera night requirements at an occupied site to be 95% confident of detecting eastern pygmy possum with a camera trained on a banksia inflorescence in winter was 28 nights (assuming detection probabilities recorded in 2020) or on average 117 nights (when detection probability was averaged across the 3 years of surveys) for one camera. The minimum level of sampling needed to achieve a similar detection probability for a camera on a nest box was 85 nights in winter (assuming detection probabilities recorded in 2020) or 237 nights, when averaging across the 5 years of survey for one camera (Fig. 7).

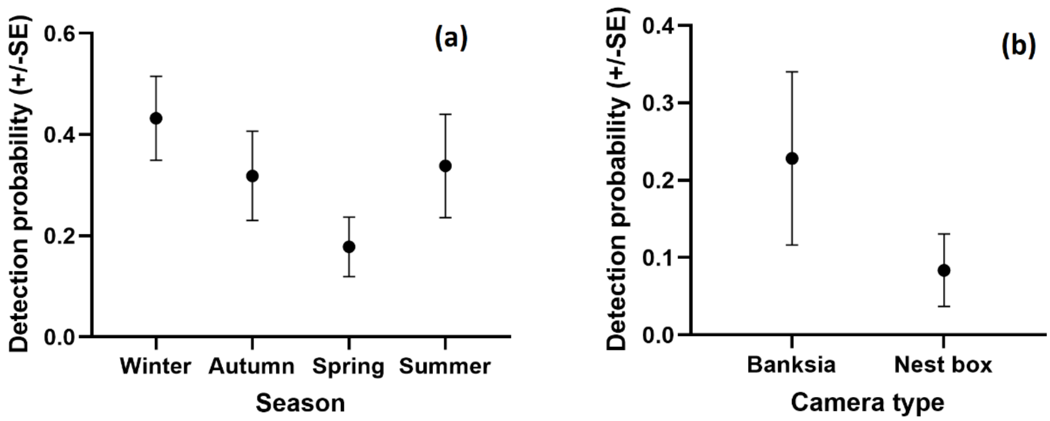

Fig. 5. (a) Detection probability versus season for eastern pygmy possum (*Cercartetus nanus*) in northern Sydney using cameras (based on data from 2020 + banksia, with occupancy held constant) (b) Detection probability versus camera type for eastern pygmy possum in northern Sydney (based on 2020 + season data, with occupancy held constant).

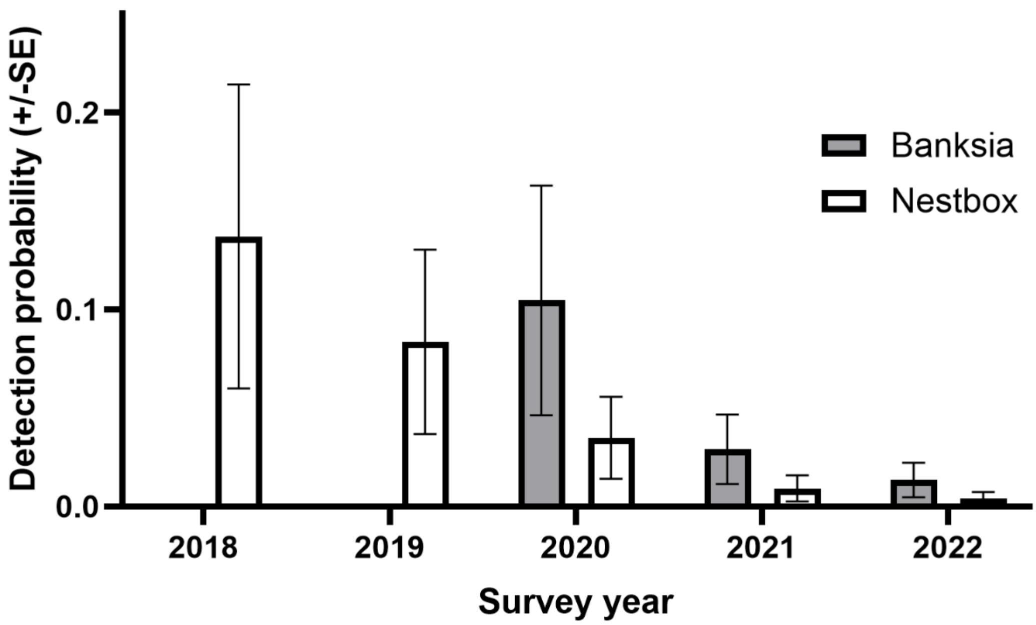

Fig. 6. Influence of survey year on detection probabilities for eastern pygmy possum (*Cercartetus nanus*) in northern Sydney using banksia and nest box cameras.

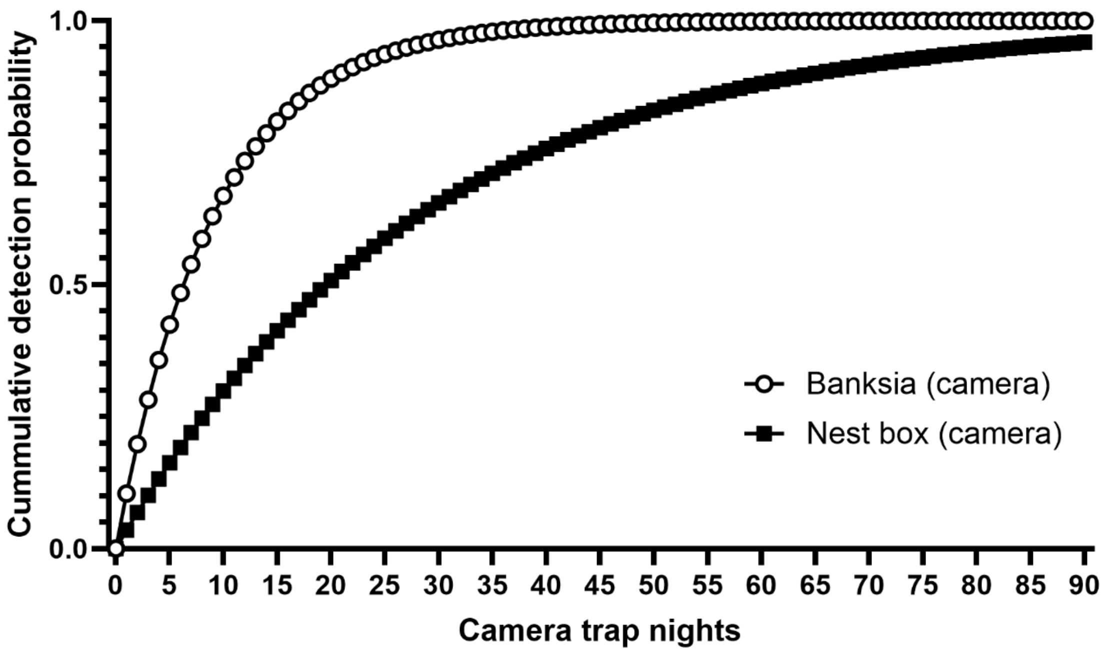

Fig. 7. Survey duration vs cumulative detection probability (2020) for the eastern pygmy possum (*Cercartetus nanus*) in northern Sydney bushland remnants based on single-visit detection using nest box cameras or banksia inflorescence cameras in winter.

Discussion

Influence of survey method on detection probability

Wildlife cameras are an invaluable tool for the detection of rare and elusive arboreal species if used correctly, particularly for species that are small and nocturnal, making observations from the ground at night extremely difficult (Bowler *et al.* 2017). We found that camera traps trained on banksia inflorescence yielded a 95% detection probability on average from 117 camera nights (with a range of 28 to 222 camera nights across the three survey years) and outperformed cameras trained on nest boxes. Compared with traditional survey methods that require daily trap checks, camera traps may provide a more cost-effective alternative, particularly for cryptic species with reported low capture rates (Bowen and Goldingay 2000). While image processing adds to effort, advances in artificial intelligence (AI) may reduce this burden (Vélez *et al.* 2023).

Few studies have estimated detection probabilities for eastern pygmy possums while accounting for imperfect detection. However, studies on similar cryptic marsupials have demonstrated the value of modelling detectability, including sugar gliders (*Petaurus* *notatus*) where bait type significantly influenced detection rates (Owens *et al.* 2024), and broader vertebrate communities in northern Australia where occupancy models accounted for low detection probabilities in small mammals (Einoder *et al.* 2018). For the eastern pygmy possum, a recent study found a detection probability of 0.35 per visit for clusters of nest boxes when spread across all seasons on the Central Coast, NSW (Chew *et al.* 2024). This translates to an estimated survey effort of seven nest box checks to a cluster of five nest boxes at a site to be 95% confidence of detecting the species. Another study reported detection probability of 0.21 per visit in autumn/winter (translating to 13 nest box checks) and 0.48 or five nest box checks for any age-sex-class males (Goldingay 2023). Both studies were completed in similar habitat to the study area within myrtaceous-rich woodland with a dominance of banksia. We found comparable detection probabilities using single cameras trained on banksia flowers, though this was year-dependent (0.01-0.10 per night per camera) and for cameras trained on nest box cameras (0.01-0.14 per night per camera).

A potential limitation of remote methods is the species' use of torpor (Nowack *et al.* 2016; Geiser *et al.* 2018), which may lead to non-detection on camera but not during physical checks. However, repeat sampling using cameras may offset this limitation. Eastern pygmy possums can enter deep, multi-day torpor throughout the year (Geiser 1993; Turner *et al.* 2012), yet in some areas torpor was rare (e.g. one in 499 observations near Sydney; Goldingay and Rueegger 2018). Increased survey duration can help mitigate false absences due to torpor. Additionally, cameras may be less intrusive for torpid individuals, avoiding energetic costs associated with disturbance (Sørås *et al.* 2022).

Capture rates vary widely across survey methods: 0.93% (pitfall traps), 1.7% (Elliot traps), and 9.1% (nest boxes). The nest box capture rate drops to 4.3% by excluding an outlier Bladon et al. (2002), which had pre-clearing trapping rates of 33.5% that have not been replicated in any other study (Bowen and Goldingay 2000) (Table 3). In our study, single cameras on nest boxes had a 5.2% capture rate, while banksia cameras reached 7.5%. Rueegger et al. (2012) found positive detections at 72.5% of nest boxes using cameras, including 10 with no records from manual checks. This supports the value of cameras in improving detection and cost-effectiveness.

Influence of season on detection probability

Detection probability peaked in winter and was lowest in spring for nest box cameras. While Chew et al. (2024) found no seasonal effect in nest box detectability, in this study sample imbalances may have masked trends. Goldingay (2023) reported a fourfold increase in detection of breeding females in nest boxes in autumn/winter compared to spring/summer. This trend likely aligns with flowering of key banksia and breeding periods, as has been observed elsewhere (Ward 1990; Bladon *et al.* 2002; Tulloch and Dickman 2006; Law *et al.* 2013; Goldingay 2023). We recommend camera surveys coincide with peak flowering of dominant local nectar resources.

Influence of year on detection probability

Detectability can reflect population abundance (Kéry and Schmidt 2008; McCarthy et al. 2012). As populations decline, previously adequate survey effort may no longer suffice (Burns et al. 2019), highlighting the need to account for detection probability when monitoring trends (Gonsalves et al. 2024). We observed declining detectability over time, which resulted in increased survey effort required for 95% detection, from 21 to 715 camera nights (nest box cameras) from 2018 to 2022, and 28 to 222 camera nights (banksia cameras) from 2020 to 2022. This may be reflecting possum abundance over the survey period. The study period coincided with a prolonged drought and the 2019–2020 bushfires. Though the study area was unburnt, drought likely affected local possum populations. Heathlands dominated by banksia are vulnerable to declining precipitation and climate change (Fitzpatrick et al. 2008; Yates et al. 2010), and banksias show reduced reproduction in drought (Poot et al. 2012). Thus, declines in detectability may reflect drought-driven reductions in plant productivity and subsequently possum abundance. Eastern pygmy possums may have also shifted to alternative habitats outside camera detection zones, which is known to occur in western pygmy possums, which can travel long distances in times of drought to forage (Morrant and Petit 2012). It is also possible that individuals may move out of the typical banksia-rich microhabitat areas where cameras were located on both nest boxes and on banksias, in search of other food resources.

Implications for survey effort

Targeting nest boxes or flowering banksias with cameras offers a low-cost alternative to live trapping, especially with advances in AI and cheaper equipment. Welbourne et al. (2015) found camera trapping used ~62% fewer consumables and only ~27% of the labour compared to live trapping. Achieving 95% detection using nest box checks of clusters of five boxes requires seven monthly visits (Chew et al. 2024), while our study indicates similar confidence with three months of banksia camera data or eight months from nest box cameras, requiring only two site visits if using suitable cameras with sufficient storage. Use of more than one camera per site would reduce this sampling effort.

Traditional methods for sampling small arboreal mammals often require canopy access (Moore et al. 2021). Our cameras were deployed at 0.5–1.8 m and were easily accessible. This aligns with known height preferences of eastern pygmy possums (Evans and Bunce 2000; Law et al. 2018). Remote cameras are increasingly used to detect arboreal mammals and can be more cost-effective than traditional approaches (Gonsalves et al. 2024). Accurate placement within the camera's PIR detection band is essential, as most are designed for large terrestrial species (Debruille et al. 2020). While baits and lures may improve detection, their success varies as they compete with background natural foods (Garvey *et al.* 2020; Johnstone *et al.* 2021). Banksia flowers, being both abundant and a known food resource, acted as an effective natural lure. Unlike standard baits (e.g., peanut butter/oats or sugar spray), native floral resources may be more attractive to nectarivores like the eastern pygmy possum, but this remains untested. This method could benefit other hard-to-detect nectarivores including other Burramyids and cryptic small mammals such as feathertail gliders, though none were detected in our study.

The efficacy of cameras in habitats lacking heath-leaved banksias (e.g., inland regions or other banksia species like *B. serrata* or *B. integrifolia)* is not clear. The species also uses other food plants (e.g., *Xylomelum pyriforme*, *Doryanthes excelsa*, *Corymbia gummifera*, *Lambertia formosa*) (Carthew 1993; Tulloch and Dickman 2007; Law et al. 2018). Future studies could test floral resources with seasonal blooms, though camera placement height may pose challenges. Outside banksia-dominated habitats, alternative natural food sources and bait strategies should be investigated. We recommend pilot studies in different regions and habitats to determine appropriate survey effort and reduce false absences. Long-term monitoring should account for changes in detectability related to survey method, camera technology, and population changes (Gonsalves et al. 2024).

In conclusion, wildlife cameras offer a non-invasive, cost-effective method to detect cryptic arboreal species when used to tap into natural behaviours. We tested a novel camera-based approach for eastern pygmy possums targeting natural food sources and found comparable or higher detection probabilities and capture rates than those reported using standard methods, particularly in banksia-rich areas. However, cameras provide limited data on demography or population dynamics essential for management. Modelling techniques may compensate for this to some extent (Gracanin and Mikac 2022). Nonetheless, we recommend continued use of nest box checks to complement cameras, especially outside banksia-dominated habitats.

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Chapter 5: Summary

In Chapter 5, I evaluate the effectiveness of camera trapping as a non-invasive tool for monitoring the eastern pygmy possum, explore seasonal influences on detection probability, and identify key methodological considerations for improving survey reliability in fragmented urban bushland. Camera trap surveys revealed that eastern pygmy possum detection varied seasonally and was highest when flowering resources were available, with detectability higher when training cameras on flora resources as a lure, when compared with cameras trained on nest boxes.

While survey methods can provide valuable insight into current distribution and habitat use, understanding the longer-term impacts of fragmentation requires genetic evidence. The final data chapter (Chapter 6) applies landscape genetic analysis to assess how fragmentation and roads influence gene flow and population structure in eastern pygmy possums across the study landscape.

Chapter 6: Assessing genetic connectivity of eastern pygmy possums across a peri-urban landscape: Insights for conservation planning

Assessing genetic connectivity of eastern pygmy possums across a peri-urban landscape: Insights for conservation planning

Cassie THOMPSON[a]*, Matthew Lott[b], Catriona D. Campbell[c], Peter B. Banks[a] and Greta Frankham[b]

a School of Life and Environmental Sciences, University of Sydney, Sydney, 2006, Australia.

b Australian Centre for Wildlife Genomics, Australian Museum Research Institute, Sydney, 210, Australia.

c Southern Ranges Branch, NSW National Parks & Wildlife Service, Jindabyne, 2228, Australia.

Summary text: Urbanisation is fragmenting native habitats and placing increased pressure on vulnerable species. Using DNA analysis, we showed that eastern pygmy possums maintain moderate genetic diversity and connectivity across fragmented urban bushland in northern Sydney, with some differentiation between habitat patches but only limited impacts from a major road. By identifying key habitat corridors, our research shows how genetic data can guide the placement of wildlife crossings and help planners design cities that better support species at threat.

Abstract

Urban fragmentation threatens biodiversity, particularly for species dependent on continuous habitat corridors. In peri-urban environments, fragmentation can disrupt gene flow and isolate wildlife populations, especially for arboreal mammals, but these effects are not well known for many species. We investigated the impacts of fragmentation on the eastern pygmy possum (*Cercartetus nanus*) a threatened small arboreal marsupial, using SNP-based genotyping of 119 individuals across eight habitat patches including across a major arterial road, in northern Sydney. Genetic diversity remained across the population, with low inbreeding and subtle population structure, suggesting ongoing gene flow. While some habitat patches showed minor genetic differentiation, the major arterial road was not a complete barrier to movement. Principal Coordinates Analysis, F_{ST} values, and Wilcoxon tests supported patch-level structuring shaped by landscape features rather than distance alone. Least-cost path modelling identified key corridors that will continue to support

connectivity and inform the placement of wildlife crossing structures. Genetic methods can guide conservation actions in fragmented urban environments. Our findings suggest that with strategic planning, including the preservation of corridors and the implementation of targeted mitigation such as wildlife crossings, functional connectivity for sensitive species can be maintained, even in heavily modified landscapes.

Introduction

In peri-urban areas, urban development and the associated fragmentation of habitat presents a significant challenge for wildlife, particularly for species that rely on continuous habitat corridors for movement and genetic exchange (Garden *et al.* 2006). For example, 20% of Australian mammals of conservation concern have distributions that overlap with cities (Ives *et al.* 2016). Many species that once occurred in urban areas now only persist in fragmented bushland patches (Fitzgibbon *et al.* 2011; Nelson *et al.* 2021). It has been estimated that between 2000 and 2017, over 20,000 hectares (ha) of forested habitat for nationally listed threatened species were destroyed within Australia's cities and towns (Foundation 2020), with many small mammal species now lost from urban areas (How 2000; Garden *et al.* 2010).

Within and on the urban edge, the effects of fragmentation may be compounded by urban barriers, which can act as both physical and behavioural obstacles to wildlife movement (Fahrig 2009). Roads, particularly major arterial roads, have been recognised as barriers to wildlife movement (Goosem 2001; Taylor and Goldingay 2012; Stephens *et al.* 2013). This is particularly the case for small mammals, which often avoid road surfaces and gaps in vegetation (McGregor *et al.* 2008; Peter 2013) and arboreal species that rely on canopy connectivity for movement (Soanes *et al.* 2013). This disruption of movement can lead to reduced gene flow, increased genetic differentiation, and ultimately population decline (McKinney 2006; Goldingay *et al.* 2013; Knipler *et al.* 2022b). However, these urban processes are not well understood for small arboreal mammals.

The emergence of cost effective, high-throughput next-generation sequencing platforms now allows researchers to identify thousands or even millions of hypervariable genetic markers, such as single nucleotide polymorphisms (SNPs), that can be used to investigate fine-scale population structure (Allendorf *et al.* 2010). Increasingly, SNP genotyping approaches are being used in landscape genetics, and have a range of different applications for assessing population level impacts from roads (Balkenhol and Waits 2009). Such methods provide a powerful tool in identifying how linear infrastructure and other effects of fragmentation and population connectivity impact genomic diversity over time in fragmented populations (Soanes *et al.* 2018; Gracanin *et al.* 2023). Studying associations between habitat fragmentation and genetic connectivity, especially for small arboreal mammals for which few studies exist in urbanised landscapes, is crucial for developing effective conservation strategies to help mitigate the negative impacts of urbanisation (Proft *et al.* 2018; Lee *et al.* 2023).

The eastern pygmy possum (*Cercartetus nanus*), a cryptic, arboreal marsupial, is an ideal species for studying the impacts of fragmentation and roads on wildlife in peri-urban landscapes. Found in south-eastern Australia, the factors threatening the survival of the eastern pygmy possum include isolated sub-populations with little opportunity for dispersal which increases the risk of local extinction, clearing that results in habitat loss and fragmentation, inappropriate fire regimes that remove nectar-producing understorey plants, the loss of nest sites due to past intensive forestry and firewood collection, and predation by foxes and cats (NSW Scientific Committee 2001). While this species is capable of dispersing across relatively large distances (up to 500m per night) despite its size (15-40g) (Bladon *et al.* 2002; Law *et al.* 2013), its reliance on specific habitat features, such as the shrub-layer and mid-storey vegetation for foraging and nesting, makes it particularly vulnerable to habitat fragmentation (Tulloch and Dickman 2006; Law *et al.* 2018). Given its mainly arboreal nature, and requirement for connected vegetation for movement, it may be especially sensitive to the impacts of fragmentation caused by urban infrastructure (Bladon *et al.* 2002). Thus, urban fragmentation and particularly roads, have the potential to pose a significant and real threat to the genetic health of the local population of this vulnerable species in peri-urban Sydney.

This study aims to assess the impact of habitat fragmentation on the genetic connectivity of eastern pygmy possums in a peri-urban landscape. Using molecular genetic tools (SNP-based analysis) we investigated: population structure and the extent of genetic differentiation of the eastern pygmy possum across habitat patches in the peri-urban landscape; assessed overall genetic diversity within specific patches that may indicate emerging genetic isolation; whether a large two lane heavy-use arterial road acts as a barrier to gene flow in eastern pygmy possums; and if there are areas that management should focus on to maintain genetic connectivity. By answering these, we aim to provide important insights into the impacts of landscape fragmentation and road barriers on the genetic connectivity of this urban-sensitive species. The results of this study will help inform conservation management strategies, including the placement of wildlife crossing structures and the identification of critical corridors for the long-term viability of the eastern pygmy possum population.

Materials and methods

Study area and sample collection

This study was conducted in fragmented bushland remnants on the urban edge of the northern Sydney region, which is characterised by a mix of semi-rural, industrial, and residential land uses. The study area includes several arterial roads, including Mona Vale Road, that bisects the landscape and several large habitat areas including contiguous vegetation within Garigal and Ku-ring-gai Chase National Parks (Fig. 1). Mona Vale Road is a two-lane road approximately 10-25 m wide and subjected to heavy traffic loads (36,000 vehicles per day/night in 2013), particularly at night by trucks (NSWGovernment 2016). Mona Vale Road is being upgraded in sections to a dual carriageway (four lanes – doubling the pavement width) with an impermeable median barrier, which has the potential to exacerbate barrier effects on wildlife movement. Genetic data were collected in the study area based on monitoring locations for a before-after-control-impact study being completed to investigate the efficacy of the wildlife crossing structures. Thus, habitat patches to investigate the genetic structure of the eastern pygmy possum population, aimed to test whether Mona Vale Road may currently be a potential barrier to movement as a ‘before’ sample, and chose sites on both the north and south of the road where native vegetation provided potential movement corridors (72% of samples), as well as nearby control sites.

Groupings of genetic samples into habitat patches were chosen based on the eastern pygmy possum’s known demographic and movement characteristics, and a long-term study monitoring individuals and movement of the local population by the author (Thompson *unpublished data*). The eastern pygmy possum generally has a small short-term home-range of <0.5ha (Ward 1990; Laidlaw 1996; Bladon *et al.* 2002; Tulloch and Dickman 2006; Law *et al.* 2013), and has typically been recorded moving small distances overnight on the urban edge (up to 80 m (Harris *et al.* 2007)), however some longer-term movements, particularly between seasons have been recorded in forested areas (up to 5 km (Harris 2010)). For this study on the urban edge, habitat patches comprised sampling locations in contiguous vegetation, only separated by gaps in native vegetative cover greater than 100 m, or where there was more than 500 m between sites in semi-connected vegetation. Data from an ongoing mark-recapture study shows that no individual eastern pygmy possums have been recorded as having moved between the identified habitat patches in the study area in the last 8 years (Thompson *unpublished data*). Samples collected in larger conservation areas of interconnected native vegetation such as Garigal South and Ku-ring-gai Chase North were considered to be part of the same patch (Fig. 1). Sampled patches in the study area ranged from 4.8 ha (Minkara) to >15,000 ha (Ku-ring-gai Chase) in patch size.

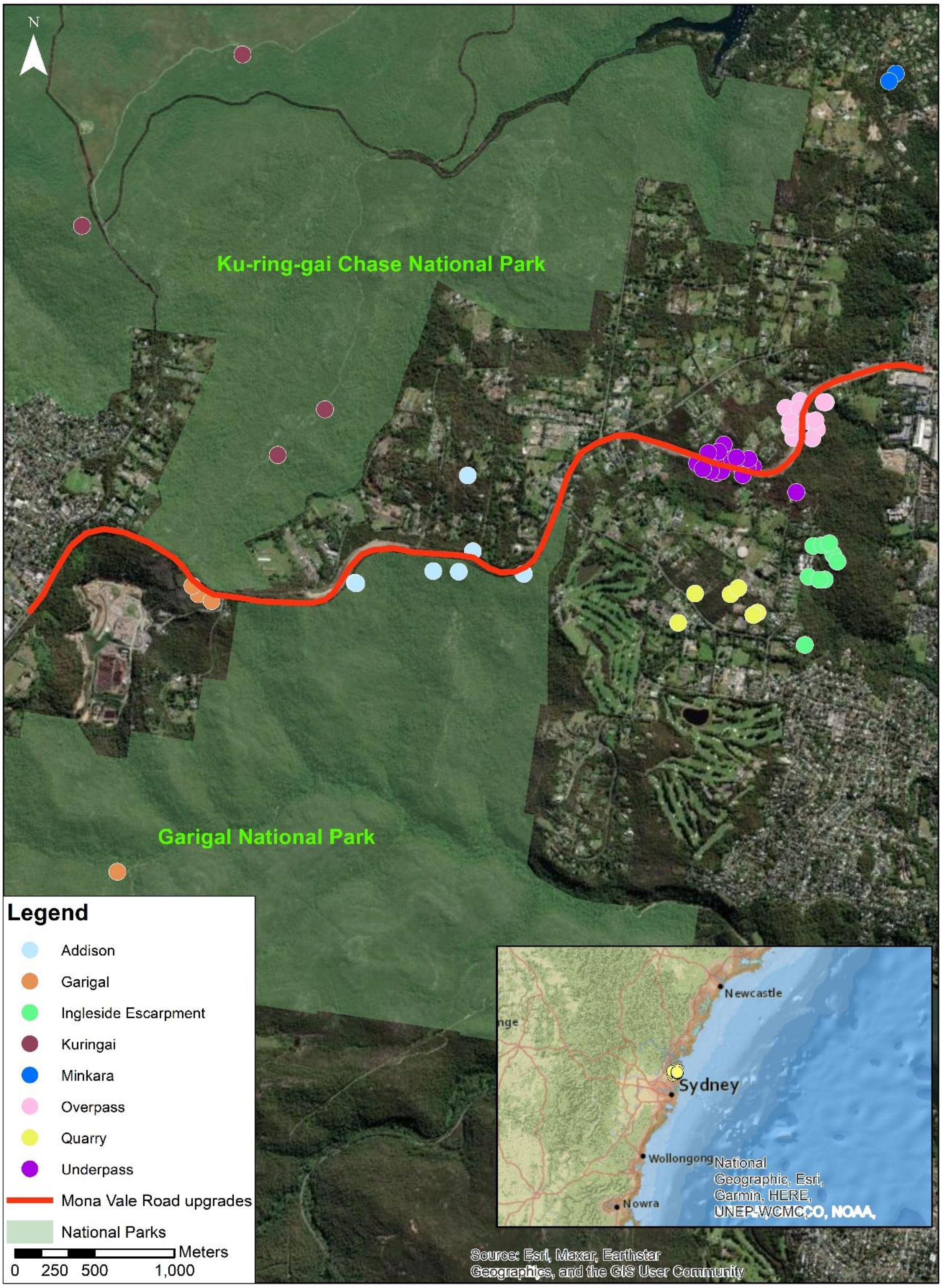

Figure 1: Study area in peri-urban northern Sydney showing the genetic sampling locations for the eastern pygmy possum (*Cercartetus nanus*), habitat patches and location of Mona Vale Road.

Eastern pygmy possums were sampled from nestboxes installed on both sides of Mona Vale Road and in nearby habitat patches (up to 4 km from the road), representing a gradient of habitat connectivity (Figure 1). Nest boxes were made from hollow branches with 3 cm openings specifically for eastern pygmy possum. They were checked monthly for 60 months, with genetic samples collected between 2015 and 2020. Animals were sexed and weighed, with condition reported including breeding status, and a unique sub-cutaneous microchip was inserted into each individual for identification. Only sub-adult and adult individuals (weighing >10g) were sampled for genetics. Tissue samples were collected using sterilised metal ear punches (ethanol and flaming), stored in 90% ethanol and kept at -20°C until DNA extraction.

DNA Extraction and SNP Genotyping

DNA extraction was completed by the Australian Centre for Wildlife Genomics at the Australian Museum (Sydney, Australia). Genomic DNA was extracted from 119 tissue samples using the Isolate II Genomic DNA Kit (Meridian Biosciences) or a high-salt precipitation method (Sunnucks and Hales 1996). DNA concentrations were quantified using a Qubit fluorometer using the Qubit dsDNA HS Assay Kit (ThermoFisher Scientific Waltham). Genotyping for SNP discovery was performed at Diversity Arrays Technology (Canberra, Australia) using the DArTseq™ platform, a restriction enzyme-based genome complexity reduction method that has been used to successfully identify SNPs in a wide range of vertebrate species (Kilian *et al.* 2012). Samples were processed according to standard DArTSeq protocols, optimized for the eastern pygmy possum. After demultiplexing and adapter trimming, the resultant short-read sequence data were processed using Stacks v2.64 (Catchen *et al.* 2013). Briefly, sequencing reads were standardised by truncating them to 68bp in length, and low-quality data (based on a PHRED score of <30) were discarded using the process_radtags function (Catchen *et al.* 2013). The denovo_map.pl function was then implemented, with the maximum number of mismatches allowed between stacks within and between individuals when building the catalogue set to 2 (-M) and 4 (-n), respectively. All other parameters were set to the default values, with one exception: the alpha threshold required to call a SNP was reduced to 0.01 (i.e., a greater number of sequence reads were required to make a SNP call statistically significant at each locus). The

SNPs were then extracted from the catalogue using the populations program in Stacks. To reduce the probability of linkage between markers, a single random SNP was extracted from each locus. Prior to downstream analyses, the genotypes were further filtered in PLINK v 1.9. Variant sites with call rates of <90% and minor allele frequencies (MAF) of <0.01 were removed. This MAF threshold was selected to minimise the risk of including false alleles that originate from sequencing errors by ensuring that each allele was sampled in ≥2 individuals independently (as shown by the formula 3/2 N: 3/(2 × 119) = 0.01) (Wright *et al.* 2019; Lott *et al.* 2020). Finally, to accommodate downstream genetic analyses requiring a neutral set of markers, loci that deviated from Hardy-Weinberg equilibrium were identified based on 10,000 Monte Carlo permutations using the package pegas v0.12. for the R software (Paradis 2010) and removed from the data set. The protocol described above resulted in a panel of 6,885 high quality, biallelic SNPs.

Genetic analysis

To assess the potential barrier effect of Mona Vale Road, individuals were grouped based on their location relative to the road (north vs. south). A separate analysis was conducted to examine the genetic differences across the eight habitat patches within the study area.

Genetic diversity metrics (observed heterozygosity, Ho; expected heterozygosity, He; and inbreeding coefficient, FIS), were calculated for each patch and across the north-south groups (ie north and south of Mona Vale Road) using the dartR package (Gruber *et al.* 2018) in R (version 4.3.0) (RCoreTeam 2024). To visualise genetic similarities and differences among individuals and patches, we performed a principal coordinates analysis (PCoA) using the adegenet (Jombart 2008) R package. Discriminant Analysis of Principal Components (DAPC) was also completed to explore population structure and highlight differentiation between groups. Pairwise F_{ST} values were calculated to quantify genetic differentiation among patches and between the north and south groups relative to Mona Vale Road. Confidence intervals (95%) for F_{ST} were calculated with 1000 bootstraps in the R package dartR (Gruber *et al.* 2018). Additionally, hierarchical partitioning of genetic variation was assessed using an Analysis of Molecular Variance (AMOVA) implemented in the Poppr package version 2.7. (Kamvar 2014). SNP genotype data were converted from a genlight object to a genind object and pairwise genetic distances were calculated using the bitwise.dist function, and AMOVA was run with populations defined by habitat patches. Statistical significance of variance components was tested using 999 permutations.

To further explore patterns of genetic structure, we calculated pairwise Euclidean genetic distances using the gl.dist() function in dartR (Gruber *et al.* 2018), based on SNP genotype data. Individuals were then grouped by their patch assignment or by their location relative to Mona Vale Rd. We extracted subsets of pairwise distances representing within-group comparisons (e.g. within the same patch or road side) and between-group comparisons (e.g. across patches or road sides). To statistically evaluate differences in genetic distances between groups, we used Wilcoxon rank-sum tests (Wilcoxon 1992), a non-parametric method suitable for or skewed or non-normally distributed data. The tests assessed whether between-group distances were significantly greater than within-group distances, indicating elevated genetic differentiation across the road or between isolated patches. P-values were used to assess statistical significance, and results were visualised using boxplots of genetic distance distributions. skewed or non-normally distributed data. To examine the influence of spatial distance and habitat type on genetic structure, Mantel tests were conducted using 10,000 permutations to assess correlations between genetic and geographic distances. These tests were performed separately for comparisons between habitat patches and across Mona Vale Road.

To assess potential functional connectivity between genetically differentiated habitat patches, we constructed a landscape resistance model using a binary raster derived from the Native Vegetation of the Sydney Metropolitan Area (Version 3.1) dataset. This raster reflected habitat permeability based on native vegetation presence. Native vegetation (classified using the Plant Community Type attribute) was assigned a low resistance value (1), representing areas likely to facilitate movement. Non-native and anthropogenic areas including roads, backyards, and urban development, were assigned high resistance values (100), representing barriers to movement and gene flow. Using the gdistance package in R (van Etten & Hijmans 2010), we modelled least-cost paths (LCPs) between the identified habitat patches. This allowed us to identify potential movement corridors by calculating cost-distances that incorporate landscape resistance and spatial configuration of habitat. Corridor classification was based on quantitative least-cost path outputs and pairwise genetic differentiation between habitat patches. Paths with low cumulative resistance and low pairwise FST values were classified as 'strong connections,' those with intermediate resistance or moderate differentiation as 'moderate connections,' and paths with high resistance or high genetic differentiation as 'substantial barriers.' These thresholds were defined relative to the range of values observed across all patch pairs

Sampling and modelling limitations

Sample sizes varied among habitat patches (e.g., Minkara n = 2; Ku-ring-gai Chase n = 7; Quarry n = 23). While the SNP dataset was large (6,885 loci), low sample sizes in some patches may reduce the precision of genetic diversity estimates and may obscure fine-scale structure or rare dispersal events.

While the resistance surface used for least-cost path (LCP) modelling captured broad patterns of habitat permeability, it relied on a simplified binary classification of the landscape in which all native vegetation was assigned low resistance (1) and all non-native, cleared, or urban land was assigned high resistance (100). This approach follows common practice in landscape genetics but does not account for finer-scale variation in vegetation structure, canopy connectivity, or sub-canopy resources that may influence eastern pygmy possum movement behaviour. Importantly, non-native vegetation, private gardens, and semi-modified peri-urban habitats although assigned high resistance, may still offer some degree of permeability for the species. The exclusion of these more nuanced habitat states may therefore underestimate potential movement pathways.

Results

Genetic diversity among habitat patches

Genetic diversity across the eight habitat patches varied with observed heterozygosity (Ho) ranging from 0.198 to 0.279, while the expected heterozygosity (He) ranged from 0.130 to 0.231 (Table 1). Across all patches, Ho exceeded He, resulting in consistently negative inbreeding coefficients (FIS) under the Hardy-Weinberg equilibrium. This excess of heterozygotes suggests the absence of inbreeding and may reflect ongoing gene flow, recent admixture, or outcrossing among patches. The highest genetic diversity was found in Ku-ring-gai Chase (Ho = 0.279, He = 0.231), while Minkara exhibited the lowest diversity (Ho = 0.198, He = 0.130).

Genetic differentiation among habitat patches

Pairwise genetic differentiation (F_{ST}) values among the eight habitat patches ranged from 0.000 to 0.030, with the highest values observed between more distant patches, such as the Quarry and Minkara patches (F_{ST} = 0.03) (Fig. 2). The lowest F_{ST} values were observed between Overpass and Underpass patches (F_{ST} = 0.000), suggesting that despite more than 500 m distance, these were not distinct 'patches', and indicating that geographic proximity likely plays a role in reducing genetic differentiation (Fig. 2). F_{ST} values less than 0.05 indicate little genetic variation, with greater than 0.15 indicative of significant differentiation and genetic structure (Frankham et al. 2002). The mean F_{ST} across the patches was 0.014, with a standard deviation of 0.008, suggesting little genetic variation and that overall, gene flow is occurring between the patches, but with some degree of differentiation. An Analysis of Molecular Variance (AMOVA) indicated that the majority of genetic variation in eastern pygmy possums was found within habitat patches (96.7%), whereas variation among patches accounted for only 3.3% of the total genetic variation (Φ_ST = 0.033, p = 0.001). This low but statistically significant differentiation suggests that while patches are largely connected, subtle structure exists across the landscape.

Population structure and clustering across habitat patches

Principal Coordinates Analysis (PCoA) and Discriminant Analysis of Principal Components (DAPC) revealed weak but measurable population structure across habitat patches, however there was no clear partitioning of samples into distinct clusters that corresponded to the collection localities (Fig. 3). The first two axes of the PCoA explained a modest proportion of the genetic variation, with Axis 1 explaining 2.61% and Axis 2 explaining 2.01% of the total variance. Although the first two PCoA axes explained relatively small proportions of the genetic variance (<3% each), this is typical for large SNP datasets where variance is distributed across many loci. Despite the low percentages, the ordination clearly identified subtle clustering consistent with geographic distance and patch isolation. A slight clustering was observed, particularly for patches such as Quarry and Garigal, where more distinct clustering was detected (Fig. 3).

Table 1: Genetic diversity metrics for the eastern pygmy possum (*Cercartetus nanus*) across habitat patches in peri-urban northern Sydney. Observed heterozygosity (Ho), expected heterozygosity (He), and inbreeding coefficients (FIS).

	Ho		**He**		**FIS**	
Patch	Ho	SD	He	SD	FIS	SD
Addison (n=9)	0.268	0.202	0.211	0.140	-0.141	0.131
Garigal (n=15)	0.261	0.190	0.209	0.136	-0.150	0.123
Ingleside Escarpment (n=12)	0.261	0.199	0.207	0.140	-0.149	0.129
Ku-ring-gai Chase (n=7)	0.280	0.227	0.215	0.152	-0.150	0.153
Minkara (n=2)	0.198	0.313	0.130	0.191	-0.119	0.213
Overpass (n=19)	0.267	0.188	0.214	0.133	-0.156	0.125
Quarry (n=23)	0.245	0.195	0.196	0.142	-0.163	0.124
Underpass (n=32)	0.255	0.176	0.207	0.128	-0.153	0.115

Table 2: Pairwise FST values among habitat patches for the eastern pygmy possum (*Cercartetus nanus*) in per-urban northern Sydney.

Patch	**Overpass**	**Underpass**	**Minkara**	**Ingleside Escarpment**	**Quarry**	**Addison**	**Garigal**	**Ku-ring-gai Chase**
Overpass	-	-	-	-	-	-	-	-
Underpass	0	-	-	-	-	-	-	-
Minkara	0.02	0.02	-	-	-	-	-	-
Ingleside Escarpment	0	0.01	0.02	-	-	-	-	-
Quarry	0.02	0.02	0.03	0.02	-	-	-	-
Addison	0.01	0.01	0.01	0.01	0.02	-	-	-
Garigal	0.01	0.01	0.02	0.01	0.02	0.01	-	-
Ku-ring-gai Chase	0.01	0.02	0.02	0.01	0.03	0	0.01	-

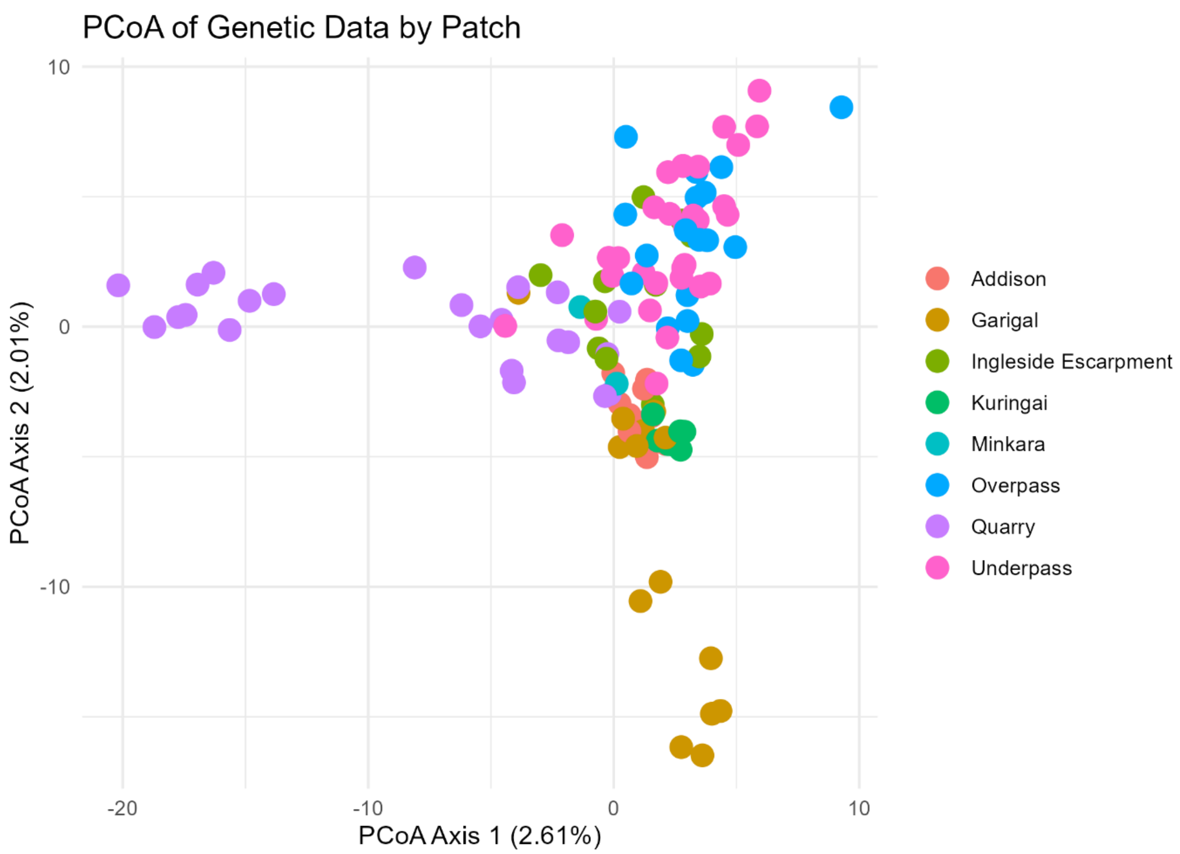

Figure 3: PCoA plot showing patch-level genetic structure in the eastern pygmy possum (*Cercartetus nanus*) in peri-urban northern Sydney.

Genetic differentiation across Mona Vale Road

Genetic diversity was similar between the two road groups: observed heterozygosity (Ho) was 0.259 for the northern group (n=42) and 0.257 for the southern group (N=77), while expected heterozygosity (He) was 0.212 in the north and 0.211 in the south. Inbreeding coefficients (FIS) were low and negative for both groups (North: FIS = -0.1505, South: FIS = -0.1508), suggesting an excess of heterozygotes and possibly indicating gene flow or outcrossing between the populations.

The pairwise F_{ST} between the north and south populations was low but statistically significant (F_{ST} = 0.004, p = 0.001), indicating only a subtle genetic differentiation across Mona Vale Road. Overlapping genetic clusters between individuals on either side of the road are shown on the PCoA, with a slight divergence along the first principal component axis (PC1: North = 0.018, South = –0.021) (Fig. 4). A Wilcoxon test comparing within- vs.

between-group genetic distances was significant (p < 2.2e-16), indicating that individuals within the same side of the road were more genetically similar than those separated by the road.

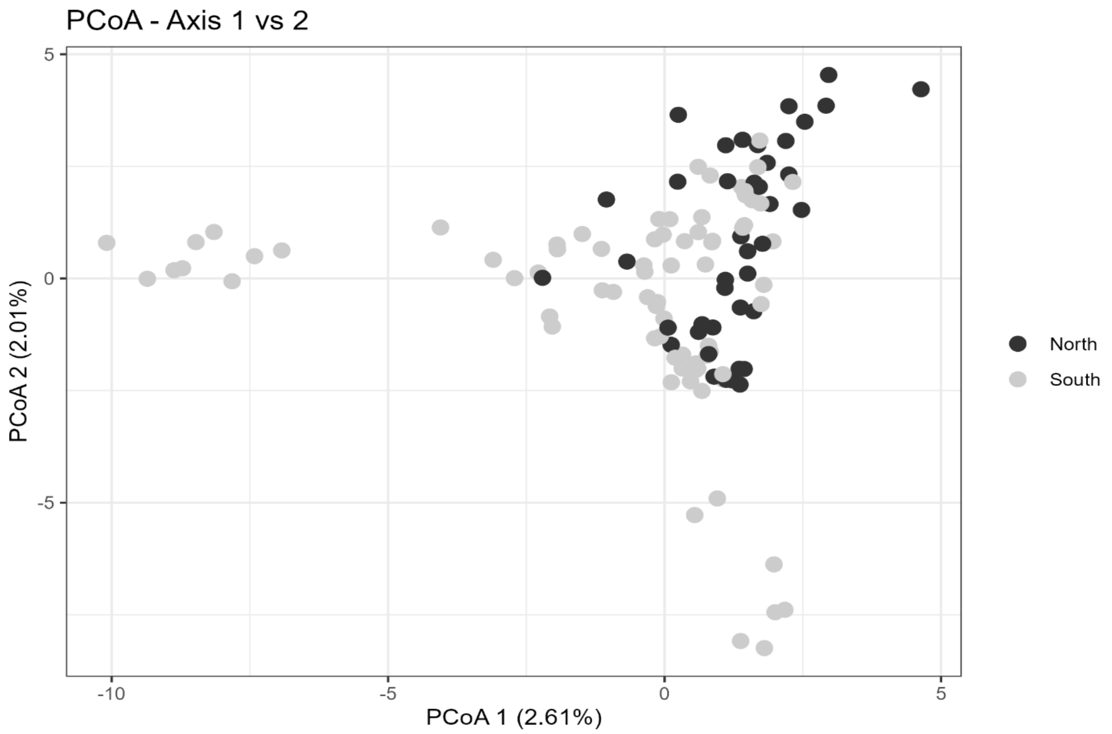

Figure 4. PCoA between north and south of Mona Vale Road for the eastern pygmy possum (*Cercartetus nanus*) in peri-urban northern Sydney.

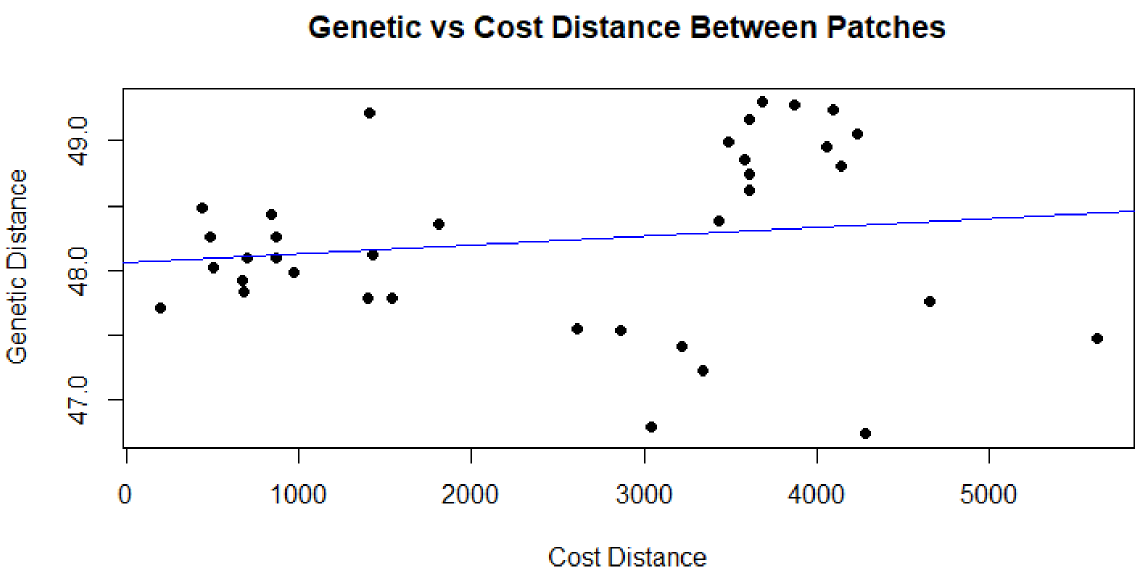

Figure 5. Scatterplot of genetic vs. Euclidean cost distance for the eastern pygmy possum (*Cercartetus nanus*) in habitat patches in peri-urban northern Sydney.

Isolation by distance and landscape resistance

The Mantel test for isolation by distance (IBD) showed a weak correlation between genetic distance and Euclidean distance among habitat patches ($r = 0.2618$, $p = 0.001$) (Fig. 5). This result suggests that genetic differentiation increases with geographic distance, with more isolated patches showing greater differentiation. However, when landscape resistance was incorporated into the analysis, the correlation with genetic distance became stronger ($r = 0.31$, $p = 0.005$). This indicates that landscape features, such as the presence of roads, urban areas, and non-native vegetation, have a more pronounced effect on gene flow than simple geographic distance alone.

Least-Cost Path analysis and connectivity

Least-cost path (LCP) analysis identified several important corridors of connectivity among habitat patches, with two individual-level paths crossing Mona Vale Road, suggesting occasional dispersal events despite the road's potential as a barrier. Notably, the Overpass patch, which extends both north and south of Mona Vale Road, serves as a potential corridor that facilitates gene flow across the road. At the patch level, key corridors for regional gene flow were identified between Ku-ring-gai Chase and Addison patches on the north of Mona Vale Rd, connecting to the Ingleside Escarpment patch on the south of Mona Vale Rd (Fig. 6). The apparent absence of predicted LCPs between Ku-ring-gai Chase and Garigal, despite their geographic proximity and low FST differentiation (0.01), may partially reflect uneven sampling intensity across the study area or the lower statistical weight of sparsely sampled patches.

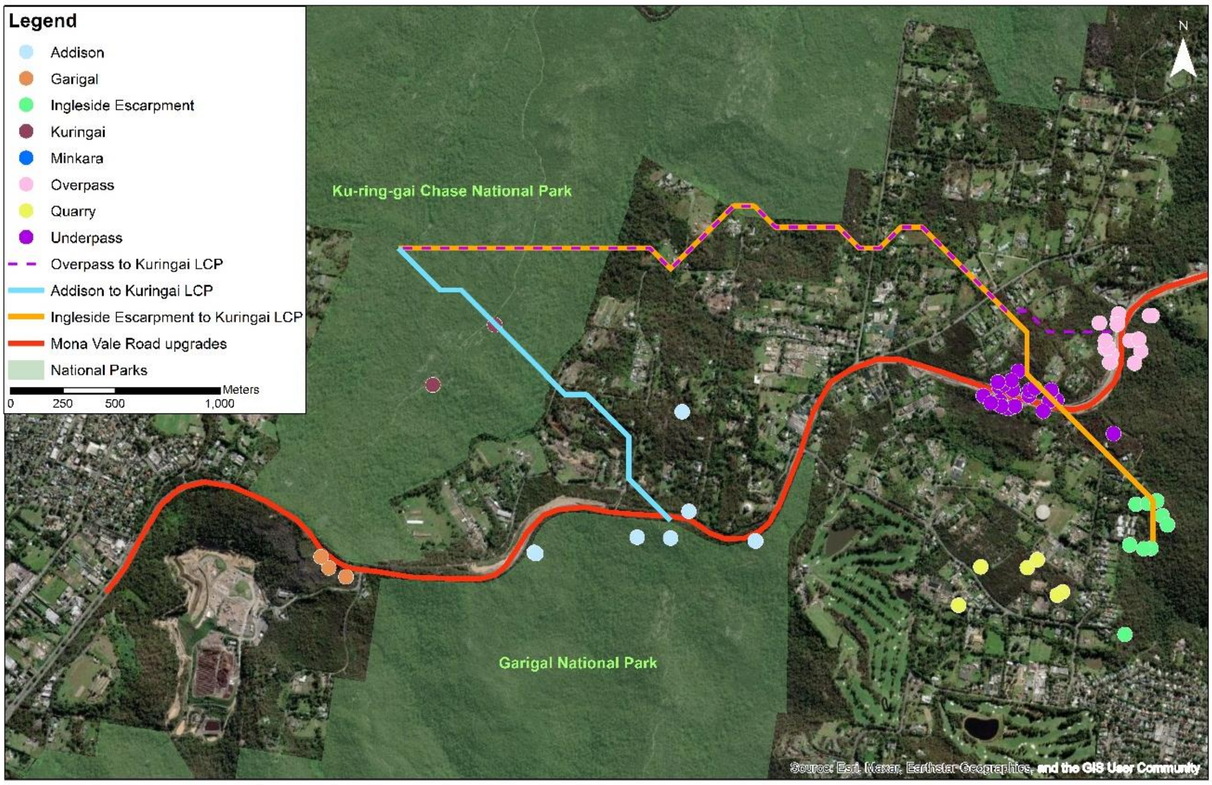

Figure 6: Least-Cost Paths between sampled individuals and patches for the eastern pygmy possum (*Cercartetus nanus*) in peri-urban northern Sydney.

Discussion

Genetic diversity and population structure in fragmented habitats

An understanding of genetic diversity and population structure in fragmented habitats is important to direct conservation and management actions for wildlife populations and their habitats (Hohenlohe *et al.* 2021). When linked with landscape analysis, genetic methods become a powerful tool to guide conservation management actions (Hohenlohe *et al.* 2021). The results from genetic and landscape analyses of the eastern pygmy possum population in peri-urban northern Sydney show a single eastern pygmy possum population in the landscape (approximately an area of 12km^2), with low levels of inbreeding and high genetic diversity across the population, despite the challenges posed by fragmentation and barriers to movement. Our results suggest that Mona Vale Road may be contributing to some level of population structure, but it is not a complete barrier to gene flow. This suggests ongoing gene flow facilitated by remnant vegetation corridors or occasional successful road crossings (Soanes *et al.* 2013; Yokochi *et al.* 2016). With the upgrade of

Mona Vale Road and associated traffic volumes and thus risk of road strike (Balkenhol and Waits 2009), the potential for increased isolation exists for the local population. These results provide an important baseline genetic diversity metric prior to the fragmentation impacts of the road upgrades, so that such impacts can be accurately assessed (Hedrick 2005; Soanes *et al.* 2018).

Behavioural considerations and road impacts

Little is known about the behaviour of the eastern pygmy possum associated with roads (Harris *et al.* 2007), as most research has been completed in large forested areas (Tulloch and Dickman 2006; Harris *et al.* 2014; Law *et al.* 2018; Goldingay 2023; Chew *et al.* 2024). The eastern pygmy possum's reliance on tree hollows for shelter and its arboreal nature make the species particularly vulnerable to disruptions in both canopy and understorey connectivity (Law et al., 2018). However, the behaviour and movement of the species through cleared areas and open space on the urban edge also remain largely unknown. Previous studies have shown that males tend to travel greater distances in forests areas and that some levels of habitat disturbance are tolerated (Law *et al.* 2013).The results of our study suggest that some eastern pygmy possum individuals move across the roads in the study area, however it is not clear how often this occurs and why. Perhaps this may be linked to dispersal of young, resource availability, or the search for mates or ephemeral food sources (Chapter 4 (Thompson *et al.* 2025a)), however further study is needed to clarify this.

Patch-level genetic structure and AMOVA

Genetic structuring was more evident at the patch scale, particularly among those separated by urbanised, cleared areas, indicating that habitat fragmentation is still influencing gene flow within the landscape. As expected, genetic diversity was highest in the largest remnant patches associated with conservation reserves (e.g., Ku-ring-gai Chase) and lowest in smaller, more isolated patches such as Minkara. This is to be expected as large habitat areas enable larger populations sizes and reduce the threat of genetic drift, by providing dispersing individuals. While no discrete genetic clusters were detected, the observed gradient in genetic differentiation highlights the influence of landscape configuration which includes patch size, connectivity, and matrix permeability, on genetic diversity in eastern pygmy possums.

In our study, genetic differentiation among eastern pygmy possum habitat patches was generally low (mean F_{ST} of 0.014 (SD = 0.008)), suggesting moderate gene flow across the fragmented peri-urban landscape. While some isolated patches showed higher differentiation, most were weakly structured, indicating that residual habitat connectivity is still supporting genetic exchange. The Analysis of Molecular Variance (AMOVA) supported this pattern, showing that 92.4% of genetic variation occurred within patches, while only 7.6% was attributable to differences among patches ($p < 0.001$), reinforcing the conclusion that most variation is maintained within local populations rather than being partitioned between habitat patches.

The genetic differentiation values place the eastern pygmy possum population at the lower end of the spectrum of population differentiation observed in similarly fragmented landscapes, particularly when compared to both ground-dwelling and other arboreal marsupials. For instance, ground-dwelling species with limited mobility such as the long-nosed potoroo (*Potorous tridactylus*) and southern brown bandicoot (*Isoodon obesulus*) have shown much higher F_{ST} values across fragmented landscapes (e.g., 0.03–0.08 between nearby populations, and up to 0.361 between distant patches) (Frankham *et al.* 2014; Li *et al.* 2016). While these are not directly comparable with our results due to differences in markers, they illustrate strong genetic structuring due to poor dispersal across cleared or urbanised areas.

Arboreal marsupials also exhibit a wide range of FST values depending on fragmentation and landscape context. For example, pairwise FST values in greater gliders (*Petauroides volans*) ranged from 0.041 to 0.666 (Knipler *et al.* 2023); in sugar gliders (*Petaurus breviceps*) from 0.011 to 0.131 (Knipler *et al.* 2022a) and from 0.011 to 0.090 (Gracanin *et al.* 2023); and in squirrel gliders (*Petaurus norfolcensis*) from 0.014 to 0.335 (Knipler *et al.* 2022b), with additional values observed around Mackay (0.019–0.227) and Brisbane (0.003–0.180) (Goldingay *et al.* 2013). These studies highlight how urbanisation and loss of continuous canopy cover are associated with higher genetic differentiation.

Our findings suggest that while eastern pygmy possums share many of the movement constraints seen in other arboreal marsupials, their relatively low genetic differentiation indicates that functional connectivity persists in this landscape, likely supported by remnant vegetation corridors in the area, midstorey structure, and occasional successful dispersal across gaps and roads. By comparison, the higher FST values observed in greater gliders, sugar gliders, and squirrel gliders in highly fragmented or urbanised areas illustrate how canopy disruption and habitat isolation can strongly limit gene flow.

Patches exhibiting low differentiation (e.g., Ku-ring-gai Chase and nearby Garigal/Addison patches) show minimal genetic separation, likely due to contiguous vegetation and occasional dispersal, and therefore were not highlighted as critical least cost pathways in the modelling. Conversely, patches with slightly higher differentiation, typically in the eastern fragmented areas, generated pathways identified as “strong connection” or “moderate barrier” because genetic differences among them provided signal for the model to detect potential corridors. This indicates that the least cost pathway analysis is influenced not only by landscape configuration but also by the sampling distribution and the degree of observed genetic differentiation.

Overall, the low FST of eastern pygmy possums relative to other arboreal marsupials supports the idea that functional connectivity exists, but the least cost pathways should be interpreted with caution. They identify where gene flow is measurable rather than necessarily representing the shortest or most optimal physical pathways across the landscape. This interpretation is particularly important when designing road-crossing structures, as corridors with minimal genetic differentiation may still warrant conservation attention due to their potential role in facilitating movement. The wider locality retains close to 40% native vegetation, with large areas within conservation reserves with vegetated corridors throughout (Bangalay and Eastcoast Flora 2012). Thus, the eastern pygmy possum’s resilience may reflect a combination of behavioural flexibility and landscape features that still facilitate movement, but it also highlights the species’ potential vulnerability to further fragmentation.

Isolation by distance and landscape resistance

The isolation by distance analysis identified genetic differentiation increasing with geographic distance between patches, across a mosaic of bushland and urban areas. While gene flow may still occur across shorter distances, sub-populations in more isolated patches such as between the Quarry and Minkara, or Quarry and Ku-ring-gai Chase patches, appear to show reduced connectivity. Not unsurprisingly, habitat is highly fragmented with areas of urbanisation, Mona Vale Rd and cleared paddocks between these patches (Figure 1). The Mantel test results confirmed that landscape features, such as the presence or absence of connected native vegetation, are more strongly correlated with genetic distance than Euclidean distance alone, showing that landscape resistance plays a significant role in shaping gene flow for the eastern pygmy possum.

Uneven sample sizes across patches may influence estimates of genetic diversity and differentiation. While population-level patterns were generally robust, the smallest patches (e.g., Minkara, n = 2) produced less reliable diversity estimates and may have reduced power to detect subtle structure. However, the overall low FST values (mean 0.014) and strong within-patch variation (92.4% of variance) suggest that general conclusions about moderate connectivity remain valid. Despite these limitations, the integration of SNP-based genetic structure, IBD patterns, and resistance-based modelling provides a conservative and ecologically meaningful indication of where functional connectivity currently exists and where it may be most vulnerable to future fragmentation. Continued monitoring—genetic and movement-based—will be essential to validate corridor function, particularly following completion of the Mona Vale Road upgrade and installation of crossing structures.

Identifying key corridors

These results reinforce the idea that landscape permeability, particularly the availability of connected vegetation, can mitigate genetic isolation, even in urbanised settings. Although many other small mammal species have been lost from urban areas in Australia (How and Dell 2000; Garden *et al.* 2010; Foundation 2020), our study reveals eastern pygmy possum population are persisting on the urban edge as genetically connected and diverse. The key to this population's long-term survival will be the ability to preserve and enhance connectivity between habitat patches to maintain genetic diversity and prevent the loss of genetic resilience (Christie and Knowles 2015).

Least-cost path (LCP) analysis identified key corridors for connectivity between the Overpass and Ku-ring-gai Chase patches (strong connection with moderate resistance), Ingleside Escarpment to Ku-ring-gai Chase patches (substantial genetic barrier identified but potential for gene flow), and Addison to Ku-ring-gai Chase patches (strong connection with lower landscape resistance) (Figure 5). The identified links align with the planned wildlife crossing structures, located at the Overpass and Underpass patch locations (Figure 5).

However, there are limitations to this analysis that must be considered. The identified pathways extend through highly fragmented habitat, rather than along the seemingly more intuitive route between the two National Parks where they closely abut Mona Vale Road to the west. This may reflect the influence of the higher number of samples collected in the eastern, more fragmented habitat, compared to the western, more continuous areas. Similarly, the spacing and distribution of samples can bias LCP results, particularly if genetically differentiated patches are modelled preferentially, while patches with low differentiation—such as Ku-ring-gai Chase relative to Garigal and Addison—carry little weight in the model (Table 2). Consequently, pathways through these less differentiated, but potentially important, habitat areas may not appear in the LCP results. This limitation highlights that LCP results represent the combination of observed genetic differentiation and landscape resistance, and do not necessarily indicate the only or shortest functional pathways for movement. Indeed, if road crossing becomes more perilous after the Mona Vale Road upgrade, the shortest distance between contiguous high-quality patches may represent a valuable crossing opportunity, even if it is not strongly supported in the current genetic dataset. Future monitoring should therefore integrate movement data and sample more evenly across both continuous and fragmented habitat to validate and refine the identification of key corridors for conservation and mitigation measures. Despite this, the identified least-cost pathway corridors are critical for maintaining gene flow and should be prioritised for conservation and enhancement, as they represent the best opportunities for facilitating genetic movement across the fragmented landscape.

Wildlife crossing structures and management implications

Planned wildlife crossing structures within the Underpass and Overpass patches will support and facilitate the movement of possums between identified key patches. These have been designed based on knowledge of small, arboreal mammals, as the use of wildlife crossing structures by the species is largely unknown (Thompson *et al.* 2025b). This includes vegetated approaches, canopy connectivity features (e.g., timber rails and/or vegetated crossing structures), and exclusion fencing to enhance their effectiveness (van der Ree *et al.*

2015; Rytwinski *et al.* 2016; Soanes *et al.* 2024). In particular, the fauna overpass (located at the Overpass patch) will allow for continuity of habitat, which appears to be an important component for genetic flow for the species, as it will be planted with eastern pygmy possum preferred foraging plants to mimic surrounding habitat and maintain connectivity. Continued genetic monitoring at suitable intervals will be required into the future to measure how effective these measures are at mitigating the barrier effects of the road upgrade. Where possible, public and private (backyards and rural properties) lands within the identified least-cost paths should be improved with planting food resources to provide pollen and nectar for the species to assist in the functionality of these corridors into the future.

Genetic methods are increasingly playing a vital role in understanding wildlife populations (Hohenlohe *et al.* 2021) and restoration ecology (Mijangos *et al.* 2015). Despite the potential for use in assessing effectiveness for connectivity of populations, genetic methods have rarely been applied in the context of road upgrade projects in Australia (Soanes *et al.* 2024). Our study provides a method to identify key locations for installing wildlife crossing structures and enhancing wildlife corridors as a practical management action to maintain the genetic diversity of species. This method can be applied in any area where development pressure may fragment or create barriers for movement. While currently the fragmented landscape has not yet caused genetic isolation for the eastern pygmy possum in peri-urban northern Sydney, it will be important to continue genetic sampling after the Mona Vale Road upgrade, along with spatial monitoring of movement and crossing structure usage. Using this study as a baseline, the efficacy of mitigation can be monitored against this through time to ensure the long-term viability of the eastern pygmy possum population on Sydney's urban edge.

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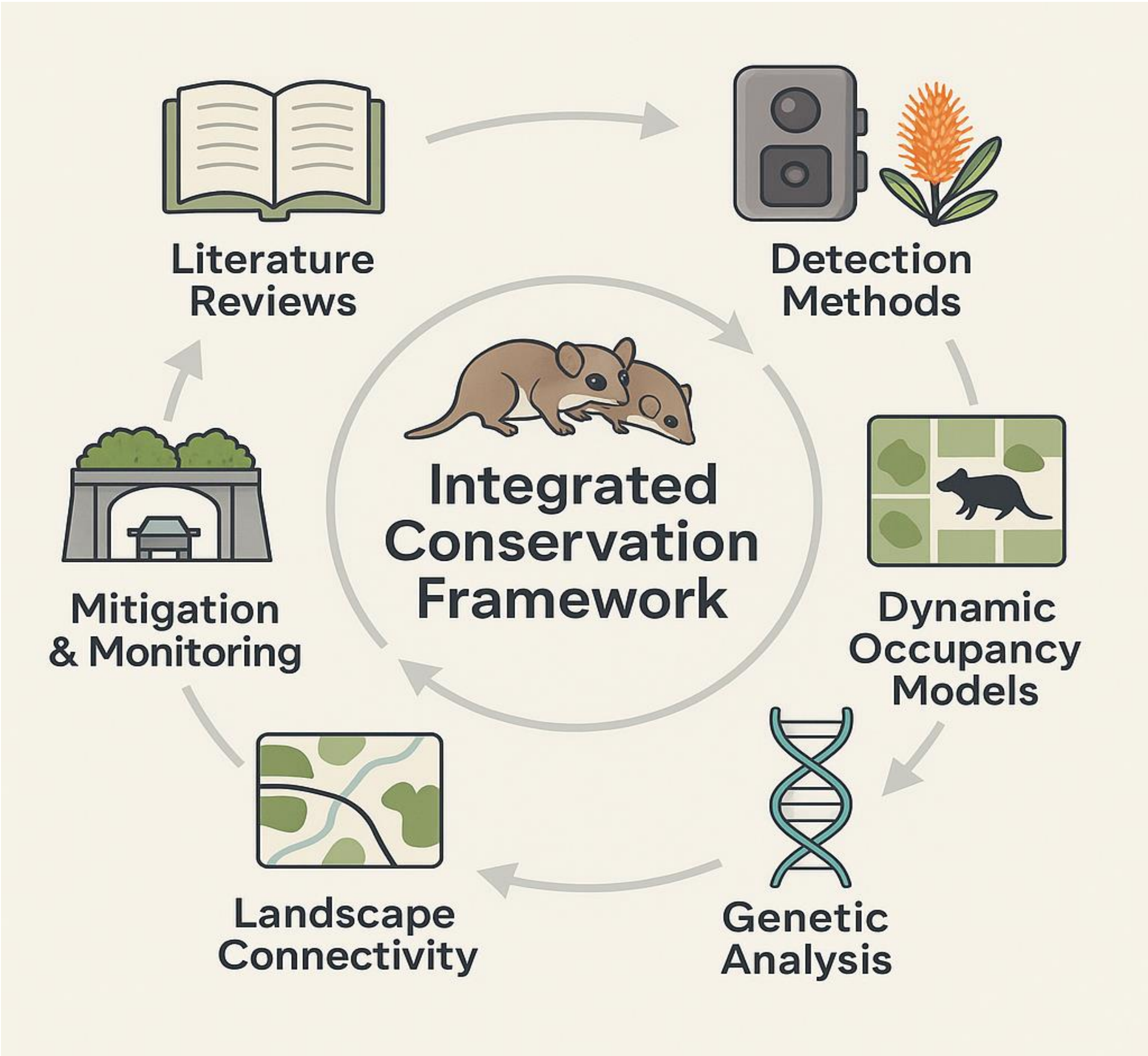
Chapter 6: Summary

In this chapter, I use SNP-based landscape genetics to assess the extent of gene flow among eastern pygmy possum populations in a fragmented urban matrix. It also evaluates the influence of roads and habitat connectivity on genetic differentiation and identifies least-cost pathways that could inform the placement of wildlife crossings and restoration corridors. Genetic analyses identified moderate diversity between habitat patches but limited genetic separation across Mona Vale Road, suggesting the road has not yet become a complete barrier to gene flow. Least-cost path modelling highlighted key corridors for maintaining connectivity, and patch-level variation in heterozygosity pointed to emerging isolation in some areas.

In the final chapter (General discussion), I synthesise the findings from all chapters, spanning ecological, genetic, and applied management dimensions, to identify key themes and discuss their implications for conserving urban-sensitive species in fragmented landscapes.


Literature Reviews
Detection Methods
Integrated Conservation Framework
Dynamic Occupancy Models
Genetic Analysis
Landscape Connectivity
Mitigation & Monitoring

Summary

My thesis examined how habitat fragmentation and road infrastructure affect the persistence, movement, and detectability of the eastern pygmy possum (*Cercartetus nanus*), a small, arboreal, and urban-sensitive marsupial. Despite extensive fragmentation and the high-risk urban edge environment, the eastern pygmy possum persists in the peri-urban bushland of northern Sydney. Understanding the ecological processes enabling persistence in the face of these threats is central to conservation goals for urban wildlife in general. By integrating high-resolution genetic data and landscape connectivity modelling (Chapter 6), dynamic occupancy models (Chapter 4), detection method evaluation (Chapter 5), , alongside two comprehensive literature reviews (Chapters 2 and 3), this study adopted an applied, multidisciplinary approach to help understand the conservation needs of cryptic species in urbanised environments.

Collectively, my two review chapters and three data chapters reveal numerous key insights. A review of road ecology in Oceania (Chapter 2) contextualised the thesis within regional trends, identifying knowledge gaps in multi-species and ecosystem-scale road mitigation. Then camera-based detection methods (Chapter 5) improved detectability of the eastern pygmy possum when targeted at flowering banksia, offering a more cost-effective and efficient survey tool than traditional methods. Second, results from occupancy modelling (Chapter 4) demonstrate that habitat availability, particularly the extent of native vegetation (within a 500m radius), significantly influences possum occupancy, while extensive historical fire reduces occurrence. Third, genetic analyses (Chapter 6) showed moderate genetic diversity within the local population and subtle differentiation among different habitat patches, with some patch-level structuring driven by vegetation connectivity. Importantly, the main arterial road Mona Vale Road which dissected eastern pygmy possum habitat, did not act as a complete genetic barrier to the species, suggesting permeability under certain conditions. Based on the genetic sampling, least-cost path analysis identified habitat corridors critical for maintaining gene flow. Fourth, a review of wildlife crossing structure use (Chapter 3) by arboreal mammals highlighted the lack of structures targeted for eastern pygmy possum and identified the need for more strategic monitoring methods to be employed to quantify effectiveness, rather than simply use. From these investigations,

distinct thematic insights emerge. The remainder of this discussion is organised around these emerging themes.

Bridging the gaps in road ecology

Globally, studies in road ecology and mitigation efforts have primarily focussed on large taxa such as ungulates (e.g., deer) and carnivores (e.g., bears, wolves) (van der Ree *et al.* 2015; Soanes *et al.* 2024). In Australia, emphasis has been placed high-profile species that are of conservation concern like the koala (*Phascolarctos cinereus*) and various glider species in both published research and road mitigation design (Taylor and Goldingay 2012; Soanes and van der Ree 2015; Goldingay and Taylor 2016; Soanes *et al.* 2024). While these species face legitimate threats from roads, smaller, more cryptic fauna such as rodents, small dasyurids, and smaller arboreal marsupials have been overlooked, creating a critical knowledge gap in understanding the broader ecological impacts of linear infrastructure. As noted in Chapter 6, small mammals and cryptic arboreal species are especially vulnerable to the effects of roads. Many have short-range movements, high site fidelity, and a strong aversion to open or disturbed areas (Goosem 2001; McGregor *et al.* 2007). Yet despite these traits, these species are rarely the target of road impact assessments or mitigation design. Surveys of species such as bush rats (*Rattus fuscipes*), brown antechinus (*Antechinus stuartii*), or small gliders like the feathertail glider (*Acrobates pygmaeus*), are seldom targeted in either monitoring programs or crossing structure evaluations. Despite this, they may provide evidence of the most beneficial outcomes from wildlife crossing structures, due to some of the highest reported rates of crossing structure use (Chapter 3). Further, the data are often buried in grey literature or not reported at a species level (often due to inability to distinguish species with monitoring methods used), and are of a lesser conservation concern making synthesis and meta-analysis difficult.

This gap was particularly evident in the case of the eastern pygmy possum, despite being listed as a vulnerable species, it was not a target species for any of the reviewed wildlife crossing structures in monitoring reports from the last 25 years (Chapter 3). Thus, conservation status may not always be a determining factor for mitigation. As demonstrated in Chapters 2 and 3, small-bodied and cryptic species are virtually absent from published road ecology research in Oceania. The peer-reviewed literature (Chapter 2) showed a dominant focus on koalas, wallabies, and gliders, with minimal inclusion of non-listed or less detectable species. This trend continued in the grey literature (held by the NSW transport department) reviewed in Chapter 3, where over 150 monitored wildlife crossing structures were assessed, yet only one confirmed the detection of the eastern pygmy possum, despite many of the wildlife connectivity structures being in suitable habitat and even recording the species in surrounding habitat (eg Woolgoolga to Ballina Pacific Highway Upgrade). This underrepresentation may be partly due to low detection probability using traditional survey and monitoring methods. Many of the grey literature reports lacked systematic survey protocols suitable for small nocturnal species, such as targeted camera traps set up to be able to distinguish small mammal species or tagging individuals (eg microchipping). However, design features of the crossing structures also appear to play a role. Structures were rarely tailored to the ecology of smaller arboreal mammals, often lacking dense vegetative cover, ground cover and woody debris, or suitable refuge spaces on approaches, although this was also underreported. Such shortcomings likely hindered use for understorey-dwelling species like the eastern pygmy possum, even if structures were otherwise physically accessible. Interestingly, the one structure the eastern pygmy possum was recorded in, a bebo arch with a natural substrate that has retained vegetation up to the opening, is likely to have regenerated into dense shrubs at the entrances over time, encouraging use (Figure 1).

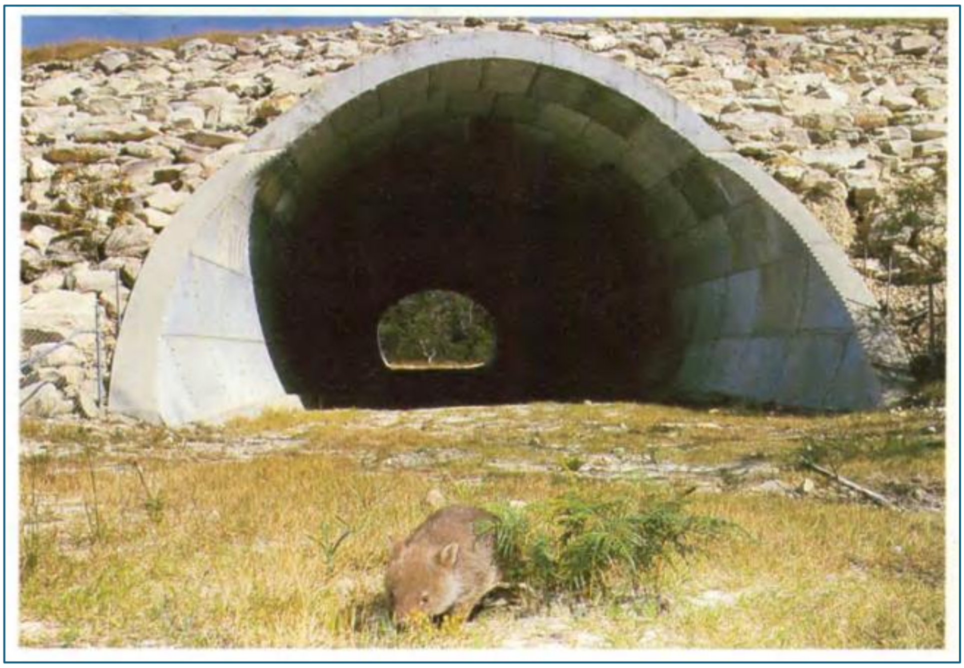


Figure 1: *The bebo arch constructed under the F3 freeway (Pacific Highway) north of Sydney which recorded the* eastern pygmy possum *on the concrete ledge. This is 10m wide and 7m high and connects two areas of national park under the highway, providing habitat for a range of species such as the bare-nosed wombat (Vombatus ursinus) pictured. Source:* https://www.ozroads.com.au/NSW/Freeways/F3/F3_c2s_brochure.pdf

In chapter 3 I synthesised over two decades of unpublished monitoring reports for NSW, identifying trends in structure use, underrepresented species, and gaps in reporting consistency. This dataset is a largely untapped resource offering the potential for large-scale meta-analysis and evidence-based adaptive management. However, my review highlights that road ecology studies are often limited in scope and duration, despite the need for larger temporal and spatial scales. Further, the data reported often lacks important information to allow for quantitative analysis. Compounding the issue is underrepresentation by certain species, because of the survey methods used, and the common assumption that all arboreal mammals will use the same types of mitigation structures that only targeted certain species. Rope bridges and glider poles were used extensively by species such as squirrel gliders (*Petaurus norfolcensis*) but were not used by more sedentary species like greater gliders (*Petauroides volans*) or cryptic species like the eastern pygmy possum. Vegetated overpasses, while theoretically useful for understorey-foraging species, were also rarely built, underreported and under evaluated. These findings highlight the limitations of generic structure design based on use by target species. Species-wide integration of ecological requirements, for all potential species potentially using a structure based on local species assemblages and not just species of conservation concern, is needed as is ensuring monitoring methods capture the full suite of species using structures. Because even though some species are thought to be ‘common’ and of lesser conservation concern and with the increasing threats of urbanisation and climate change, they still have an important role to play in ecosystem processes.

The analysis of wildlife crossing structures use was often hindered by gaps in crossing structure design details, descriptions of covariates associated with the structure locations, or inappropriate monitoring methods, especially for small cryptic fauna. My review also highlighted that structure presence alone does not equate to connectivity unless it translates into successful use, movement, and gene flow. Without adequate methods and goals to evaluate efficacy, mitigation efforts risk being ineffective or, worse, misleading. For example, it is possible that vegetated overpasses may provide one of the most suitable mitigation options for a wide range of different species, particularly if additional measures such as glide poles or specific plantings are incorporated in their design. However, these are rarely installed due to high costs, and where these have been installed in NSW, monitoring has been inadequate to determine efficacy by the Transport department. It is clear, dedicated monitoring methods need to be developed to take a whole of ecosystem approach and move away from targeting single-species to ensure that the full effects of wildlife crossing structures can be measured. Further, control sites and an understanding of wildlife populations surrounding wildlife crossing structures is needed to understand the role that structures are having on minimising the barrier effect of the road, preventing road strike and improving wildlife connectivity and gene flow.

To improve conservation outcomes for small and cryptic species, a shift in road ecology practice is needed. Monitoring protocols must be standardised where possible, and expanded to include taxa that have been historically neglected to allow for structured meta-analysis using effect sizes. Emerging technologies such as eDNA sampling, automated imaging, and acoustic sensors, offer scalable solutions for detecting elusive fauna

(Gonsalves *et al.* 2024). These tools should be embedded into infrastructure projects from the outset, rather than treated as optional add-ons. Furthermore, the design of mitigation structures must move toward adaptive, evidence-informed models going beyond the demonstration of structure use toward structure efficacy. This includes trials of different structure types based on known movement behaviour and habitat preferences not just where native vegetation will be bisected, with follow-up studies that link structure use to demographic or genetic outcomes, not just use data. Reconnecting landscapes, not only mitigating connectivity impacts, should also be a goal particularly in urban areas. Long-term genetic monitoring, as planned for the Mona Vale Road upgrade (Chapter 6), provides a powerful means of assessing whether connectivity has been functionally restored or the road barrier minimised and thus proving the efficacy of the wildlife crossing structure.

My reviews (Chapter 2 & 3) highlight the need for a national open-access repository for studies on the use of wildlife crossing structures by fauna. If reporting was standardised, this would enable monitoring metrics and reporting formats to be standardised across jurisdictions, species and structure types, allowing for robust statistical comparison and meta-analysis. A consistent repository would also help avoid duplication of effort, improve transparency, and enable adaptive management by facilitating data sharing between agencies, researchers, and practitioners (Lesbarrères and Fahrig 2012). It could also be used by research groups to identify knowledge gaps and focus future studies. Another key insight is the importance of fostering stronger collaboration between transport agencies and academic and industry researchers. Such partnerships can improve the scientific rigour of monitoring and evaluation programs, ensure that appropriate detection methods are used to capture a full suite of species, and expand evaluations beyond simple usage to include demographic and genetic outcomes (Clevenger and Sawaya 2010; Soanes *et al.* 2013). This type of co-produced, outcome-focused science is essential for evaluating whether wildlife crossing structures are delivering meaningful conservation benefits, not just use records. While the call for integrating road ecology with applied planning is not new (van der Ree *et al.* 2007), implementation has lagged behind, particularly for small or cryptic species. Embedding research partnerships in infrastructure projects from the outset would help ensure monitoring is fit-for-purpose, adaptive, and aligned with ecological and conservation goals.

My review also highlights the need to promote synthesis work that incorporates both published and unpublished datasets to inform national guidelines for connectivity measures and their monitoring. Importantly, this should include adaptive management practices when these aren't effective, as this is often overlooked, a problem that is not unique to Australia (Soanes *et al.* 2024). Failures need to be reported so that others can learn (Meek *et al.* 2015). Finally, cross-disciplinary collaboration is essential. Planners, engineers, ecologists, and local communities must work together to ensure that mitigation reflects both ecological and practical realities. By designing wildlife crossing structures and monitoring programs that account for detection bias, meet a range of species-specific needs, and target long-term outcomes, road mitigation can move from symbolic intervention to effective conservation.

Persistence under pressure — but it's a fragile balance

Urbanisation is widely recognised as a major driver of habitat degradation, with profound consequences for biodiversity (Bianchini *et al.* 2021; Seifollahi-Aghmiuni *et al.* 2022). In peri-urban environments, land conversion and infrastructure expansion lead to the direct loss of native vegetation, increased edge effects, and the disruption of ecological processes such as dispersal, gene flow, and population recovery (Garden *et al.* 2010). These effects are particularly pronounced for species that depend on connected habitat networks, including my focal species the eastern pygmy possum. Evidence from across Australia indicates ongoing localised declines for other urban sensitive taxa such as the koala (*Phascolarctos cinereus*), southern brown bandicoot (*Isoodon obesulus*), and squirrel glider (*Petaurus norfolcensis*) in peri-urban areas (How and Dell 2000; Maclagan *et al.* 2018).

A key takeaway from Chapter 4 is that the ability of native species, like eastern pygmy possums, to persist in fragmented landscapes is shaped not only by habitat amount but by its configuration and quality (Fahrig 2003). For some species, the extent of contiguous vegetation is critical (Astrom and Bengtsson 2011), while others, particularly ground-dwelling or small mammals, may be more sensitive to the connectivity of habitat patches (Bennett 2003; LaPoint *et al.* 2015). Functional connectivity, or the degree to which a landscape facilitates movement, often plays a more important role than mere proximity or patch size, especially in heavily urbanised environments where dispersal barriers are pervasive (FitzGibbon *et al.* 2007). Despite the ongoing pressure from urbanisation, the eastern pygmy possum exemplifies how small, urban-sensitive mammals can persist in fragmented peri-urban systems. In chapter 4 I show that occupancy was strongly associated with the extent of native vegetation within a 500 m radius, particularly in areas retaining unburnt refugia (Chapter 4, Fig. 3). Occupancy was also significantly higher in patches that had experienced frequent fire in recent decades, indicating that fire history may affect habitat quantity and recolonisation potential, and thus shape site-level persistence.

However, in Chapter 6 I found that habitat extent alone was insufficient to maintain long-term genetic viability, with connectivity playing an important role between habitat patches and increasing genetic diversity within and between patches. Single nucleotide polymorphism (SNP)-based genetic analysis detected subtle but consistent differentiation between habitat patches (mean F_{ST} = 0.014), indicating slightly reduced gene flow. While this value reflects moderate levels of connectivity, it also highlights that functional isolation is emerging, which is a warning consistent with the concept of extinction debt, where genetic erosion precedes observable population decline (Tilman et al., 1994; Spear et al., 2010). Least-cost path analysis further identified key corridors that maintain permeability across the landscape, many of which follow vegetated riparian strips or remnant corridors of native vegetation between larger reserves (Chapter 6, Fig. 5). Such pathways are likely to support the conservation of other small arboreal mammals, such as sugar gliders and common ringtail possums (*Pseudocheirus peregrinus*), that also rely on canopy or understorey connectivity to traverse fragmented landscapes (Malekian *et al.* 2015; Soanes and van der Ree 2015).

However urban environments are not static and other forms of disturbance can exacerbate the impacts of fragmentation. Eastern pygmy possum occupancy was higher in areas with a high proportion of burnt habitat within the past 30 years (Chapter 4, Fig. 4), suggesting that fire may influence habitat suitability for eastern pygmy possums on the urban-edge. This likely reflects changes in floral resources, and the creation of habitat features such as tree hollows (Tulloch and Dickman 2006; Law *et al.* 2018). While some fire may promote habitat complexity and nectar production, the results here point to a complex relationship. Chew *et* *al.* (2024) found positive associations between occupancy and recent fire in forested areas for the eastern pygmy possum, but the frequency and spatial extent of burns in urban bushland may reduce the potential for recolonisation or limit food plant recovery in a fragmented landscape. A mosaic fire regime, interspersing long-unburnt refuges with carefully managed burn patches, may better support small mammal persistence in fragmented peri-urban landscapes (Lindenmayer *et al.* 2016). But it will be important to manage fire intervals in patches to ensure that the potential for recolonisation and habitat restoration is maximised. More research is needed in this area to guide the planning, intensity and scale of prescribed fires in eastern pygmy possum habitat on the urban edge.

Taken together, the findings from my thesis suggest that the eastern pygmy possum population retains functional connectivity across this fragmented urban landscape, yet this connectivity is fragile and at risk of future collapse if additional pressure from urbanisation is applied. Unlike many other small arboreal mammals, the eastern pygmy possum has persisted on the peri-urban edges despite extensive vegetation loss and road development in the Sydney Basin. Dynamic occupancy models and SNP-based genetic data indicate moderate gene flow between patches, but with clear signs of emerging isolation. This contrasts with findings for squirrel gliders and bandicoots (*Isoodon spp.*), which often exhibit sharp declines in occupancy and stronger genetic structuring in urban environments (FitzGibbon *et al.* 2007; Goldingay *et al.* 2013).

The ability of eastern pygmy possums to persist may be due to their nectarivorous-based but flexible diet (van Tets 1998; Hockey *et al.* 2019), and flexibility in den selection (Law *et al.* 2013; Goldingay 2019). It may also relate to the unique study area in northern Sydney, which still contains over 30% of pre-European settlement native vegetation within the landscape (Bangalay and Eastcoast Flora 2012), which is high in comparison to other urban edges (Ives *et al.* 2016). However, this apparent resilience must not be mistaken for long-term security. As with other species subject to extinction debt, populations may appear stable until thresholds are crossed, after which recovery becomes more difficult or impossible. This has shown to be the case at North-Head in Sydney, where the eastern pygmy possum has been re-introduced (O'Rourke *et al.* 2020) , due to the lack of connectivity to allow for natural recolonisation from nearby known populations (eg Manly Dam).

To protect functionally-connected populations before they fragment further, a proactive, multi-scale approach is needed. Future research should investigate the traits that enable persistence in urban remnants. This includes examining traits such as home range size, dispersal ability, diet breadth, and nesting plasticity in peri-urban areas (van der Ree and McCarthy 2005; Garden *et al.* 2006). Detectability is a critical yet often overlooked factor to inform such occupancy studies, particularly in urban landscapes where many fauna species persist at low densities (Garden *et al.* 2007). In Chapter 5, I demonstrate that camera traps effectively detect eastern pygmy possums. While detection probability remained low and varied seasonally and annually, highlighting the challenge of monitoring cryptic, nocturnal species in fragmented urban bushland, these findings raise broader concerns for other small mammals that may still persist undetected in peri-urban areas. Without reliable detection, these species risk being excluded from conservation planning and mitigation strategies, potentially leading to unnoticed declines in biodiversity on the urban fringe (McKinney 2009; Soanes and Lentini 2019). Using remote and cost-effective methods, comparative studies of co-occurring small mammals could identify why species like the eastern pygmy possum continue to persist where others have disappeared. Finally, urban planning must treat the urban edge as an active conservation frontier. Protecting vegetated corridors, managing fire regimes strategically, and restoring functional connectivity is essential if cities are to support viable wildlife populations. Peri-urban remnants may be the last refuge for many species but only if they retain the ecological processes that support persistence (Bennett 2003; Fischer and Lindenmayer 2007; Wintle *et al.* 2019).

Integrating genetics, occupancy, and connectivity for informed conservation

Urban conservation planning faces the complex challenge of identifying areas where intervention will yield the greatest biodiversity benefit, especially in landscapes altered by development and fragmentation (Soanes and Lentini 2019). This thesis presents a novel, integrative method that combines genetic analysis, occupancy modelling, and least-cost path connectivity assessment that can inform conservation planning. The Mona Vale Road upgrade, situated within the core of the study area, offers a real-world case through which this integrative method can be applied to guide more effective and evidence-based conservation planning for a small, urban-sensitive species: the eastern pygmy possum.

Mona Vale Road case study

I have argued that in order to understand and mitigate the impacts of habitat fragmentation caused by urban roads, multiple lenses to quantify fragmentation impacts are needed: genetic analysis reveals long-term population structure and gene flow; occupancy models provide present-day insight into habitat use and extinction risk; and landscape connectivity analysis identifies spatial priorities for intervention. The upgrade of Mona Vale Road in northern Sydney, Australia, represents an opportunity for a real-world case study to apply and test the integrated method developed throughout this thesis. Specifically, the testing of structure designs tailored to small, cryptic, and arboreal mammals on the urban edge, an area in which little empirical data currently exists (Chapter 3), such as those completed for gliding mammals and koalas (Goldingay and Taylor 2016, 2017). This was in fact an original aim of my PhD research. Mona Vale Road bisects numerous bushland reserves and conservation areas which provide habitat for the eastern pygmy possum and other small mammals, within a mosaic of rural and residential areas on the peri-urban edge. This arterial road is a two-lane road approximately 10-25 m wide and subjected to heavy traffic loads (36,000 vehicles per day/night in 2013), particularly at night by trucks (NSWGovernment 2016). As part of a major infrastructure upgrade, Mona Vale Road is being widened from two to four lanes, with a central median barrier which is expected to act as a complete barrier to small mammal movement. However, results from this thesis revealed that Mona Vale Road, while not yet a complete genetic barrier, may already be reducing gene flow between populations north and south of the alignment (Chapter 6, FST = 0.008).

Based on the potential increased barrier effect from the upgraded road on the eastern pygmy possum, wildlife crossing structures were incorporated into the final design of the road upgrade. Since the eastern pygmy possum moves through the understorey, often coming to the ground when moving between vegetation (Law *et al.* 2018), a vegetated overpass was considered for the upgrade which would potentially provide movement for this as well as a range of other more common species in the area. Overpasses have proven effective for the movement of a range of species globally, particularly in Europe for large ungulates and ground-based mammals (Corlatti *et al.* 2009). Though vegetated fauna overpasses have not been widely implemented or studied for use by semi-arboreal and arboreal fauna in the international literature, with more of a focus on canopy/rope bridges for such species(Gregory *et al.* 2022). Further, the only known structure to ever record use by the eastern pygmy possum was a bebo underpass with a concrete ledge. As such, the two wildlife connectivity structures installed comprised:

- A 5 m-wide vegetated overpass designed as a pedestrian bridge planted with *Banksia ericifolia* and other nectar-rich shrubs essential for pygmy possum ecology, costing over $5 million (see Figure 2, 3 and 4).
- A dedicated fauna underpass (1.5m high x 2.1m wide with a concrete ledge along one side 30x30cm) with timber rails designed to facilitate movement of small, non-gliding arboreal mammals, costing over $250k.

These structures have been placed at locations where vegetation corridors link the north and southern sides of Mona Vale Road, which also were identified in the least-cost path modelling (Chapter 6, Fig. 5). This represents an alignment between functional connectivity and infrastructure design which is something rarely achieved in peri-urban road upgrades. Importantly, through implementing these connectivity mitigation measures, the assessment concluded that the project would not significantly impact on the eastern pygmy possum under state legislation, though no monitoring program was required by the project approval process to determine their efficacy (NSWGovernment 2016).

Figure 2: *The Mona Vale Road pedestrian-style vegetated overpass being installed. Credit Jacqui Marlow.*

Figure 3: *The Mona Vale Road pedestrian- style vegetated overpass following installation and planting and showing the duplicated Mona Vale Rd beneath.*

Figure 4: *Mona Vale Road overpass, showing planted Banksia ericifolia and other shrubs to facilitate the movement of the eastern pygmy possum*

Although my project intended to use a before–after control–impact (BACI) monitoring design to evaluate the efficacy of the crossing structures in mitigating the barrier effect of the Mona Vale Road upgrade, delays due to COVID-19 and weather-related disruptions meant post-construction data could not be collected within the thesis timeframe (the operational project phase only commenced in early 2025). The aim was to use a multi-method approach to determine efficacy; aiming to combine population-level, individual-level and genetic monitoring to identify use, crossing events and quantify the impacts of the road upgrade on the local eastern pygmy possum population. Nest boxes were installed on both sides of the road at crossing locations and in matched control sites to support long-term demographic population analysis and have been inspected annually since 2017. The extensive baseline and during construction monitoring data collected will support long-term monitoring and demographic tracking, and lays the groundwork for studies post-construction (planned through 2025–2030 and potentially beyond).

This case study highlights a key limitation in road ecology: monitoring timeframes rarely match ecological timeframes. As shown in Chapter 3, few transport agency monitoring reports in NSW extend beyond five years, and most are less than two because of funding and approval condition requirements. The Mona Vale Road project offers a valuable precedent for long-term, integrative monitoring, combining camera trapping, genetics, and demographic data to assess mitigation effectiveness over multiple generations.

Towards broader application and standardisation

While the eastern pygmy possum served as a model species, the approach I took here is applicable across a range of taxa in fragmented urban systems. Its applicability for other urban-sensitive mammals, such as antechinus, bandicoots, or small gliders, offers a valuable tool where dispersal is constrained by infrastructure, vegetation gaps, or fire history. Further, it has potential for integration into environmental impact assessments (EIAs) and monitoring, biodiversity offset frameworks, and road planning guidelines.

To facilitate broader application, future work should:

- Apply this methodology across multiple species and landscapes, testing its generality and cost-effectiveness in different ecological contexts.
- Incorporate emerging detection tools and survey methods such as eDNA, passive acoustic monitoring, and AI-powered image recognition, to improve detection of cryptic or nocturnal fauna in urban areas and using crossing structures.
- Embed species-specific ecological data from genetic and occupancy monitoring into infrastructure projects from inception, ensuring that mitigation strategies are focused and targeted.
- Encourage collaboration among ecologists, planners, and engineers to design wildlife crossings, corridors, and mitigation strategies informed by robust, species-specific ecological evidence.

The methodology presented in this thesis offers a scientifically rigorous approach for assessing and mitigating the impacts of fragmentation, particularly those caused by infrastructure projects, on biodiversity in increasingly urbanised regions. Using these collaborative methods, my study has informed the installation of two wildlife crossing structures along Mona Vale Road. The translation of fine-scale genetic and spatial data into infrastructure design represents one of the few published examples where urban ecology research directly shaped on-ground mitigation for an urban-sensitive species. It also aligns with international best practice for conservation planning for road ecology (Soanes et al., 2018).

The value of genetic monitoring in this context is particularly noteworthy. While occupancy models capture current habitat use, they may mask underlying declines in functional connectivity. The integration of genetic data allowed early detection of potential future isolation, offering a proactive conservation strategy before demographic collapse occurs. This approach is especially relevant for cryptic or low-density species where traditional monitoring may fail to detect early warning signs of fragmentation (Tilman *et al.* 2017).

Conclusion

This thesis set out to investigate the impacts of habitat fragmentation and road infrastructure on the eastern pygmy possum, an urban-sensitive species persisting in Sydney's northern fringe. Through a combination of field surveys, genetic analysis, occupancy modelling, and literature review, this research provides a comprehensive assessment of how landscape change affects small mammal populations and how these impacts can be mitigated through evidence-based planning.

The effects of fragmentation on species persistence were explored through both occupancy modelling and genetic analysis, revealing that vegetation extent, fire history, and landscape connectivity strongly influence eastern pygmy possum distribution and gene flow. The role of roads as movement barriers was assessed using spatial genetics and least-cost path analysis, demonstrating that while arterial roads like Mona Vale Road are not yet complete barriers, they are contributing to emerging genetic structure, highlighting the need for mitigation for future upgrades. Detection challenges for small, cryptic species were addressed firstly for wildlife crossing structure use through a critical review of grey literature revealing significant gaps and the need for more targeted survey methods and secondly, identifying novel cost-effective survey methods that improve detection. My thesis provides practical recommendations for future conservation strategies, including the integration of species-wide wildlife crossing designs, long-term monitoring, and the embedding of genetic tools into infrastructure planning to reduce the impacts of fragmentation on small urban-sensitive mammals.

By developing and applying a replicable, integrated methodology, this research offers not only insights into the persistence of one vulnerable species, but a transferable model for managing biodiversity in fragmented, urban landscapes. It shows that while species like the eastern pygmy possum can persist under pressure, their future will depend on proactive, science-led conservation that addresses both structural and functional connectivity across urban systems.

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