

THE ECOLOGY AND CONSERVATION OF THE LARGE-FOOTED MYOTIS (*MYOTIS MACROPUS*) IN AN URBAN ENVIRONMENT

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**Bachelor of Applied Science
Master of Science (Honours)**

Submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

School of Biology and Environmental Science

Science and Engineering Faculty

Queensland University of Technology

2020

Keywords

Australia, artificial roost, bat, breeding site, bridge, Brisbane, Chiroptera, colony, conservation, culvert, ecology, genetics, green space, habitat, harem, mammal, movement, *Myotis*, *Myotis macropus*, philopatry, polyoestrous, polygynous, promiscuous, relatedness, riparian, roost, roosting ecology, roost selection, selection, urban, urban design, urban planning.

Abstract

Urbanisation creates unique landscapes for fauna, and species that cannot adapt will be lost. Understanding the mechanisms that enable species to persist in urban areas is limited and our understanding of them is essential for effective conservation and management of urban wildlife populations. The large-footed myotis (*Myotis macropus*) is a specialist trawling bat that is found throughout urban environments along the east coast of Australia and can be found roosting in concrete culverts under roads. However, little is known about the selection and use of these roost sites. I examined roost selection at two spatial scales, identified habitat selection preferences and home range size, and used population genetics to investigate gene flow and genetic diversity in an urban population of *M. macropus* in Brisbane, Australia.

I examined roost selection at the landscape scale by using a stratified sampling design, generalized additive models and a presence-absence distribution model. Modelling identified that roost presence in a concrete culvert can be predicted by the dimensions of the culvert and its position in the landscape. Bats preferred culverts >1.2 m in height, and while a preference for box culverts was detected, both design types (box and pipe) were occupied. Culvert variables are intrinsically linked to landscape variables, which is determined by landscape context. Stream orders determine the distribution of landscape variables such as channel dimensions (width and depth) and waterway density, which in turn determine the distribution of culverts required across an urban road network. Culverts meeting the probability of occurrence threshold were a limited resource in this urban landscape with only 5.5% of culverts identified as potential roosts.

I examined roost selection at the roost scale by comparing roost culverts to available culverts. Roost culverts differed significantly from available culverts and the primary difference was the availability of microhabitat (lift holes and crevices). Roost culverts had lift holes that had greater cavity dimensions than available culverts and crevices were only found at roost culverts. Culverts containing microhabitat were a limited resource in this urban landscape.

Through the use of radiotelemetry, I examined roost use, home range and patterns of land use selection. I captured bats roosting in road culverts and radio-tracked 13 non-reproductive females over two seasons. I tracked bats to a total of three day-roost sites: two culverts and a bridge. Bats switched roosts every 4.8 ± 3.7 (1-11) days. Home ranges (95% Kernel Density Estimate) did not differ between seasons and ranged from 5-277 ha. Core use areas (50% Kernel Density Estimate) differed between seasons with median winter areas (22 ha) significantly larger than median summer

areas (1 ha), although activity for both seasons concentrated on five elongate pools. This finding suggests that *M. macropus* uses foraging behavioural plasticity to exploit food resources available in urban environments and that elongate pools are vital core use areas for urban *M. macropus*. Land use selection was investigated using compositional analysis and *M. macropus* showed a significant preference for the recreation land use type at the landscape and home range scale. Transport, industrial, railways and public services land use types were used in proportion to their availability at the landscape scale. Maintaining open green space and spatial heterogeneity in urban planning and design will provide a landscape mosaic for urban *M. macropus* to persist.

I used population genetics to investigate gene flow in the Brisbane culvert roosting population of *M. macropus*. I found genetic differentiation between all roosts sampled. Gene flow was moderate among peri-urban populations and restricted between urban populations. I found evidence of female philopatry and pairs of related females within roosts indicating philopatry to natal colonies. Urban roosts had more related pairs than peri-urban roosts. The limited shared paternal ancestry found within roosts suggests *M. macropus* has a harem social structure and a promiscuous mating system. My results suggest that female dispersal distances are less than 30 km and female-biased gene flow is locally restricted while gene flow between populations is dependent on males dispersing along riparian corridors.

My results demonstrate that roost selection by *M. macropus* in concrete culverts is limited at two spatial scales by the availability of suitable culverts and not all culverts provide equal roosting habitat in an urban landscape. Disturbance to culvert roosts, especially during the breeding season, may cause a significant disturbance to an urban roost and culvert roosts should be considered sites of high conservation value to urban *M. macropus*. Roosting opportunities for *M. macropus* can be increased by leaving lift holes unsealed in existing culverts and installing culverts >1.2 m in height on waterways on all new road projects.

Myotis macropus displayed a preference for particular urban habitat types and avoidance of others, had small home ranges and geographically isolated populations were also genetically isolated. Despite these limitations, my findings suggest that this specialist species is able to persist in urban landscapes, provided roosts are available and elongate pools, riparian corridors and open green space are maintained within urban planning and design.

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Statement of Original Authorship

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Signature: [QUT Verified Signature](#)

Date: 8/12/20

Acknowledgments

The research undertaken for this PhD would not have been possible without the support and encouragement from so many people. I would like to take this opportunity to thank the people who have helped me be able to finish this PhD in 3.5 years. Firstly, I want to thank my primary supervisor Stuart Parsons for giving me the space to manage my project, my way. I cannot thank you enough for giving me the opportunity to do this PhD in the first place and for your constant support and encouragement. You have been an amazing supervisor for an independent student who knew what they wanted to do from the beginning. When my plan wasn't going to plan and panic set in, thank you for making yourself available for a phone call to talk things through to put my mind at ease. Thanks for coming to the field and showing me how to take wing biopsies and then keeping my kids occupied so I could process bats. A huge thank you for also teaching me about how not to get stuck to a bat when attaching a radio transmitter. Thanks for listening to me rant about bats and roads over these last few years. I always enjoy an animated bat chat with you and I will really miss these!

Thanks to each of my co-supervisors who have also provided support, encouragement and advice when I needed it- Ramona Maggini, Moni Rhodes and Carrie Hauxwell. To Ramona Maggini, thank you for teaching me how to use R and ArcGIS. You taught me so much by showing me how to use these programs and you have really helped develop my skills as a researcher. I absolutely could not have finished this PhD without your mentoring and advice. Thank you for painfully going through all the early R scripts until I worked out what this whole coding thing was about and for providing such thorough reviews on chapter drafts. To Moni Rhodes, thank you for being a world of support and encouragement across all aspects of my life over the many years we have known each other. Thank you for teaching me all about tracking bats in a city and for spending cold winter nights with me tracking trawling bats flying over houses, shopping centres and everywhere else a trawling bat wasn't supposed to fly over! I am indebted to you for standing in for me when my family had a medical emergency. To Carrie Hauxwell, thank you for stepping into a supervisory role so close to the end of a PhD. Your energy and enthusiasm for all things science is infectious and I feel blessed to have had your involvement during the final stages of a PhD when my energy was wanning. Thank you for always being available to talk bats and genetics, for including me in your molecular lab and for always having a cup of tea on offer!

I will be forever grateful to the friends who kept cheering me on throughout this PhD. I would like to thank a few people in particular. Thanks to Luke Hogan for always being willing to join me in the field, dealing

with activating tiny transmitters and spending countless nights tracking bats with me. Thanks to Leroy Gonsalves for his enthusiasm and willingness to listen to each of my findings about this fascinating bat. Thank you to Lisa Cawthen for always hearing me out, for your constant support and positivity and making me laugh many times during the final write-up. My mental health thanks you. A huge thank you to Leroy, Lisa, Liz Williams and Kerry Borkin for reading over chapters for me and providing such great feedback. A big thanks go to four special non batty friends, Stan Bellgard, Virginia Dale, Libby Matapo and Claire Chapman, who have provided continual support despite the challenges they themselves have faced. Thank you for always believing in me!

I would like to thank my family because without their unconditional support I could not have completed this PhD. To my sister Steph, I thank you for your constant words of encouragement and your absolute certainty that I would get this PhD finished helped keep me focussed on completing. To my parents, Paul and Vanda, thank you for everything. You have supported me emotionally, academically and financially and I am in awe that you managed to complete your PhDs with a young family with a fraction of the support you have given me. Thank you for reviewing everything I have written and for always thinking it was amazing even when it wasn't, and for the constant cups of tea that kept me caffeinated each weekend during the final write up. To my husband Micah, thank you for supporting me to undertake a PhD and for always believing that pursuing something that makes me happy is worth more than an actual income, even when I doubted it myself. Thank you for running the house and taking care of the kids so I could disappear and get this thesis finished, and for still loving me at the end of all this! To the other love of my life, my horse Cedar Ridge Leeroy Brown, thank you for teaching me the meaning of endurance. With you I have learned the value in persisting and persevering. Remembering the long hours spent preparing for, and competing in, 160 km endurance rides helped keep me going with this thesis. One step at a time.

Lastly, to my precious children, Noah, Lola and Anya. Thank you for coming on this PhD journey with me. Thank you for spending your school holidays searching for bats in culverts in the middle of a Brisbane heatwave, for coming radiotracking in the middle of winter, for taking notes, for jumping in to hold equipment whenever I needed help and being just excited as I was every time we found a bat. Thank you for understanding that finishing this thesis was really important to me even though it meant I have been away writing each night and on weekends over the last couple of months. It is done now. Now it's time to have some fun again. Let the adventures begin.

This research was supported by an Australian Government Research Training Program Scholarship.

Chapter 1: Introduction



Large-footed myotis (*Myotis macropus*) with a just furred pup roosting in a lift hole in the ceiling of a culvert under a road. Photo by V. Gorecki.

1.1 THE URBAN ENVIRONMENT AND BIODIVERSITY

Contemporary urbanisation is rapidly transforming the global environment at such a rate that the current period in Earth's history is defined as the era of the Anthropocene (Seto *et al.* 2010). The human population living in urban areas has increased in the last fifty years from 1 to 4 billion (Santini *et al.* 2019) and current trajectories anticipate that by 2025, there will be more than 600 cities of one million or more people worldwide (Seto *et al.* 2010). Urbanisation involves the transformation of natural environments to anthropogenic landscapes. It is one of the most ecologically damaging change to land use globally and as a result, is a significant threat to biodiversity (Jung and Threlfall 2015).

Urban environments have traditionally been viewed as homogenous because of an increase in impervious surfaces, loss of vegetation and construction of artificial features (McKinney 2006, Cadenasso *et al.* 2007). Compared to natural areas, urban environments typically have increased temperatures, noise and light pollution, habitat fragmentation, road mortality and barriers to connectivity (Russo and Ancillotto 2015, Moretto and Francis 2017, Gili *et al.* 2020). However, urban environments are unique in that they retain components of the natural environment, such as topography, soil and waterways, and include anthropogenic features which vary in intensity from high density residential housing to open green space (Li and Wilkins 2014). This combination of artefacts from the natural environment and human design features result in spatially heterogeneous environments containing patches of natural and artificial features (McKinney 2002, Threlfall *et al.* 2013b, Li and Wilkins 2014). Within these urban environments are complex biotic and abiotic processes operating at a variety of scales, resulting in ecological patterns and processes unique to urban environments (Shochat *et al.* 2006, Cadenasso *et al.* 2007). Understanding these unique spatial and temporal ecological patterns is vital to the management and conservation of urban wildlife populations.

To date, research guiding the management and conservation of urban wildlife populations has largely focussed on examining how different taxa use remnants of the natural environment. Understandably, research priorities have focussed on ways to make cities more sustainable for wildlife by quantifying the significance of natural landscape features to be incorporated into urban planning and design (Adams *et al.* 2005, Pataki 2015). The priority for biodiversity conservation within urban environments has been to maintain functional landscape connectivity through the retention or restoration of vegetation corridors and functional habitat patches (Threlfall *et al.* 2016), and the retention of mature trees (Rhodes and Wardell-Johnson 2006, Le Roux *et al.* 2014). Mature trees provide breeding sites for fauna such as insectivorous bats and birds, and are a limited

resource in most urban environments (Davis *et al.* 2013b, Le Roux *et al.* 2014). However, many taxa have been able to adapt to, or exploit, unique roosting and nesting opportunities that exist in urban environments (McKinney 2002).

Some species of insectivorous bats roost in bridges and culverts. Undoubtedly, the most famous urban bat roost is the Ann W. Richards Congress Avenue Bridge in Austin, Texas. Approximately 1.5 million Mexican free-tailed bats (*Tadarida brasiliensis*) emerge every night between March and October (Keeley and Tuttle 1999). The most comprehensive study on bats roosting in transport infrastructure is by Keeley and Tuttle (1999), who surveyed 2421 highway structures across 25 states in America to document 24 species of bats roosting in 211 structures. Monadjem *et al.* (2015) have been able to conduct a 10-year study on population dynamics of Egyptian slit-faced bats (*Nycteris thebaica*) because of the availability of a permanent roost site located in culverts. Several species of swallows have adapted to the breeding and dispersal opportunities provided by vast transport infrastructure networks available in urban environments (Adams 2016). Cliff swallows (*Hirundo pyrrhonota*) have expanded their range across America over the last 100-150 years (Brown and Brown 1995) and the cave swallow (*Hirundo fulva*) has experienced a range expansion in Texas and colonised southern Florida (West 1995). These range expansions have been attributed to the widespread construction of highway culverts, bridges, tunnels and buildings that provide alternative, cliff- and cave-like nesting sites (Brown and Brown 1995). Movement into new territory has been enabled by the exploitation of bridges and culverts for nesting sites, evidenced by new colonies occurring along highways (West 1995). Our understanding of how to manage and conserve species that may not be dependent on natural breeding sites to persist within highly modified, urban environments, is limited. Urban environments may provide unique roosting and nesting sites but connectivity between these sites is vital for gene flow to maintain viability of urban wildlife populations.

Urban environments can restrict gene flow between populations due to habitat fragmentation and barriers to movement, resulting in genetic differentiation between isolated populations (Miles *et al.* 2018). Knowledge on how genetic variation is distributed across a species' range is vital to understanding how contemporary population dynamics are shaped by spatial and temporal environmental variation (Botero-Delgadillo *et al.* 2020). High genetic differentiation was identified between populations of white-footed mice (*Peromyscus leucopus*) in urban parks due to the amount of impervious surface between populations (Munshi-South 2012). Major roads create such significant barriers to badger (*Meles meles*) movement that they can act as territorial boundaries (van Tuijl *et al.* 2019). However, even highly mobile species are affected by the heterogeneity within

urban environments. Bats are considered a highly mobile species and therefore less likely to be affected by landscape barriers and habitat fragmentation, but a 30-fold reduction in effective population size and corresponding levels of decline in heterozygosity estimates were found for Sunda fruit bats (*Cynopterus brachyotis*) in Singapore and were attributed to due to habitat loss and fragmentation (Chattopadhyay *et al.* 2019). Biological factors can also contribute to genetic differentiation due to behaviours such as philopatry (Miles *et al.* 2019). Population differentiation and genetic structure is often found in avian species due to natal philopatry which reduces gene flow, and promotes genetic differentiation (Hill *et al.* 2012). The impact of low genetic diversity is a contributing factor to the extinction debt, in which extinction occurs long after unsustainable population thresholds have been passed (Kuussaari *et al.* 2009). An understanding of how anthropogenic and biological factors contribute to the genetic structure of urban wildlife populations is an essential part of effective population management.

1.2 URBAN ECOLOGY OF INSECTIVOROUS BATS

Bats are a diverse group of mammals found throughout urban environments globally (Jung and Threlfall 2018). There are over 1400 species described to date, comprising 20% of all mammals in the world. Insectivorous bats (hereafter referred to as bats) are small, placental mammals that form colonial groups during the breeding season (Kunz *et al.* 2011), living in groups that range over several orders of magnitude from a few individuals to several million (Kerth 2008). In many urban environments, bats represent the highest diversity in mammal fauna (van der Ree and McCarthy 2005, Santini *et al.* 2019). Bats also have the potential to colonize urban environments (Moretto and Francis 2017), yet the impact of urbanisation on bats worldwide is poorly understood. Gehrt and Chelsvig (2003) suggest the relationship between urbanisation and bats is context specific and that some heterogeneous urban environments may act as islands of habitat for some species. Urban tolerant species typically have high mobility such as open and edge aerial foragers and trawling bats (Jung and Threlfall 2015). Additionally, species with flexible roosting strategies able to utilise roosting opportunities provided within urban environments are more able to persist in modified environments (Jung and Threlfall 2015).

Bats are highly mobile due to their ability to sustain flight and this ability enables them to move great distances (Moussy *et al.* 2013). Despite this ability to disperse, many species show strong site fidelity and philopatry to their natal colony (Kerth 2008, Moussy *et al.* 2013). Roost permanency has been suggested to affect roost fidelity (Kunz 1982, Brigham 1991). Evidence suggests bats have higher fidelity to permanent roost sites such as buildings, bridges and culverts, than to ephemeral

roost sites like trees (Brigham 1991, Evelyn *et al.* 2004). These permanent, anthropogenic roost sites are valuable to bats because they provide thermally stable roosts and have reduced interspecies competition compared to natural roosts like tree hollows. Thermally stable roosts such as buildings, bridges and culverts, minimise energetic costs associated with thermoregulation, gestation, embryonic development and lactation (Bennett *et al.* 2008, Smith and Stevenson 2013, Lutsch 2019). Anthropogenic roost sites have reduced interspecies competition when compared to natural roost sites like tree hollows, which are limited resources in urban environments (Rhodes and Wardell-Johnson 2006, Davis *et al.* 2013, Moretto and Francis 2017). However, the use of anthropogenic roost sites such as buildings, bridges and culverts increases the likelihood of bat-human conflict (Soulsbury and White 2016). This increases the likelihood of displacement and disruption to social groups as colonies are excluded from artificial roost sites (Russo and Ancillotto 2015).

Roost exclusion could be a significant conservation issue to urban bat populations. O'Shea *et al.* (2011) documented the loss of 35% of big brown bat (*Eptesicus fuscus*) colonies roosting in buildings over a four-year period. Tracking of *E. fuscus* after exclusion from a building revealed high site fidelity with individuals returning to the roost using previously unused access points (Brigham and Fenton 1986). After complete eviction, individuals moved to new roosts up to 100 m away, where they produced fewer offspring, demonstrating that roost exclusion has the potential to lower reproductive success and this impact is magnified if an exclusion occurs in the breeding season (Brigham and Fenton 1986). If roost availability limits a bat population, providing suitable alternative roosting opportunities should be a conservation priority.

Few studies have examined bat roost selection and availability in artificial roosts in urban environments to quantify the potential impact of the loss of these roosts. The few studies which have investigated building roosts have found bats have a preference for those with specific attributes, and not all buildings should be considered suitable (Brigham and Fenton 1986, Brigham 1991, Neilson and Fenton 1994, Evelyn *et al.* 2004, Ellison *et al.* 2007, Neubaum *et al.* 2007, Berková *et al.* 2014). Similarly, some species of bats have a preference for concrete arch and girder bridges although little is known about the availability of these designs in urban environments (Keeley and Tuttle 1999, Adam and Hayes 2000, Feldhamer *et al.* 2003, Ferrara and Leberg 2005, Bennett *et al.* 2008, Bektas *et al.* 2018). Research on culvert roosts has also indicated bats have a preference for box culverts taller than 1.5 m (Walker *et al.* 1996, Schulz 1998, Keeley and Tuttle 1999, Bender *et al.* 2010, Wojtaszyn *et al.* 2013, Meierhofer *et al.* 2019), although like bridges, there is a paucity of information on the availability of culverts preferred by bats in urban environments.

Culverts assist with drainage by enabling water to flow under roads (Meierhofer *et al.* 2019). Culverts vary in shape, design and construction material although they typically have reduced light, stable microclimates, and water in or around the culvert (Humphrey and Gore 1992, Katzenmeyer 2016, Meierhofer *et al.* 2019). All of these factors contribute to creating suitable roosting environments for many species of bats (Walker *et al.* 1996, Trousdale *et al.* 2008, Bender *et al.* 2010, Katzenmeyer 2016, Meierhofer *et al.* 2019). Some studies have demonstrated permanent roost occupancy in culverts (Monadjem *et al.* 2015) and others have recorded seasonal variation in occupancy (Gonsalves and Law 2018), suggesting that culvert roosts differ in their conservation significance. Consistent findings of roosts located within box culverts over 1.5 m in height suggests culvert roosting bats may be actively selecting particular culvert attributes (Walker *et al.* 1996, Schulz 1998, Keeley and Tuttle 1999, Bender *et al.* 2010, Wojtaszyn *et al.* 2013, Meierhofer *et al.* 2019). Whilst evidence exists for roost selection preferences, little is known about the availability of potential roost culverts in urban environments. If culvert roosting bats are selecting particular culverts, research is needed to identify drivers of culvert roost selection and availability within urban environments for effective management and conservation of urban bat roosts. This research is vital to guide management of bat roosts within transport infrastructure networks when culverts require upgrading, repair or maintenance.

1.3 AUSTRALIA'S FISHING BAT: THE LARGE-FOOTED MYOTIS (*MYOTIS MACROPUS*)

Myotis macropus, the only species from this genus found in Australia, is a small bat weighing on average 8 g (Churchill 2008). This species has a patchy distribution across northern and south-eastern Australia, and is listed on state legislation as threatened across much of the southern part of its range (Campbell 2009). *Myotis macropus* maintain relatively stable, resident populations and high roost fidelity has been documented (Campbell 2009, Gonsalves and Law 2017, Law *et al.* 2020a). Harem roosting behaviour has been recorded in *M. macropus* (Dwyer 1970 b, c, Lloyd *et al.* 1999, Barclay *et al.* 2000, Law *et al.* 2001) and the species is seasonally polyoestrous, having two to three litters of single pups per breeding season (Dwyer 1970b, Lloyd *et al.* 1999).

Myotis macropus is a trawling bat displaying morphological adaptation for foraging over water by having long, pointed wings with slightly high aspect ratio and low wing loading (Norberg and Rayner 1987, Jones and Rayner 1991, Campbell 2011), as well as large feet to rake the surface of the water for aquatic prey (Campbell 2011, Aizpurua and Alberdi 2018, Law *et al.* 2020). Studies have revealed a diet dominated by aquatic insects and to some extent, fish (Dwyer 1970a, Thompson and Fenton

1982, Robson 1984, Jansen 1987, Jones and Rayner 1991, Law and Urquhart 2000, Burns 2005). Fish scales and shrimp legs have been identified in *M. macropus* faecal pellets (Robson 1984, Jansen 1987, Law and Urquhart 2000, Burns 2005). The dietary dominance of surface-dwelling aquatic insects which are pollution tolerant (Law and Urquhart 2000), suggests *M. macropus* may be tolerant to low water quality although the distribution of the species has been affected by heavy metal contamination and sediment pollution in some studies (Clarke-Wood *et al.* 2016, Straka *et al.* 2016, Gonsalves and Law 2017).

While the morphology of *M. macropus* demonstrates a specialist adaptation to trawling behaviour, evidence of foraging behavioural plasticity has also been documented. Moth scales have been found in faecal pellets (Jansen 1987) and aerial hawking has been observed by several authors (Dwyer 1970a, Jones and Rayner 1991, Burns 2005). Faecal pellets analysed in northern Australia revealed a broad diet of termites (Isoptera), spiders (Araneae), ants (Formicidae) and plant hoppers (Fulgoroidea) (Milne *et al.* 2016), indicating this species has flexible foraging behaviour and is able to exploit a variety of seasonal food sources.

In addition to flexible foraging behaviour, flexibility in roost types used has also been documented. In 1961, *M. macropus* were recorded roosting in a limestone cave (Seebeck and Hamilton-Smith 1967). Colonies have since been recorded roosting in palm fronds (Dwyer 1970a), rocky overhangs (Kirkely 1996, Campbell 2009), disused railway tunnels (Dwyer 1970b), aqueduct tunnels (Seebeck and Hamilton-Smith 1967, Campbell 2009), trees (hollows and stags) (Caddle 1998b, Campbell 2009, Law *et al.* 2020a), a jetty (Gonsalves and Law 2017), bridges (Law and Urquhart 2000, Anderson *et al.* 2005, Law *et al.* 2020), road culverts and stormwater drains (Schulz 1998, Milne and Pavey 2011, Clarke-Wood *et al.* 2016), and caves (McKean and Price 1967, Jansen 1987). Despite the documented flexibility in roost use, the Action Plan for Australian Bats identifies the loss of roost sites as a threatening process to the survival of *M. macropus* populations (Duncan *et al.* 1999).

Mobility in foraging and flexibility in roosting strategies are traits that enable *M. macropus* to persist in modified environments (Jung and Threlfall 2015). Despite this flexibility, *M. macropus* are only recorded along waterways and species occupying specialist niches are considered more susceptible to extinction (Safi and Kerth 2004a). This dependence on waterways places *M. macropus* at higher risk of extinction. *Myotis macropus* have been recorded foraging over fresh water (Anderson *et al.* 2005, Campbell 2009), brackish water (Campbell 2011, Clarke-Wood *et al.* 2016) and saline water (Gonsalves and Law 2017). Roosts have only been located within 100 m of water (Campbell 2009) and genetic differentiation has been found between roosts on a connecting waterway only 15 km apart (Campbell *et al.* 2009). Banded *M. macropus* were not trapped beyond 6 km upstream or 6 km

downstream of a roost, nor on adjacent waterways, suggesting that movement across landscapes is restricted to riparian corridors (Campbell *et al.* 2009, Law *et al.* 2020a). Although *M. macropus* displays flexible roosting strategies using a range of roost types, the species is restricted by the location of suitable roosts. Roost specialisation is recognised as an extinction risk in bats because species with specialised requirements may decline as they are unable to switch to other available resources (Sagot and Chaverri 2015). *Myotis macropus* is restricted to roosts within riparian corridors, despite apparent flexibility regarding the type of roost used.

1.4 THESIS AIMS AND STRUCTURE

Myotis macropus is a trawling bat with specialised foraging and roosting requirements and this seemingly contradicts the documented flexibility in foraging and roosting strategies used by this species. Flexibility in roost types used by *M. macropus* has led to an assumption that the availability of roosts is not a limiting factor for the distribution of this species (Campbell 2009, Clarke-Wood *et al.* 2016). However, the Action Plan for Australian Bats (Duncan *et al.* 1999) identifies the loss of roost sites as a threatening process to the survival of *M. macropus* populations. Additionally, despite the occurrence of *M. macropus* throughout urban areas, no studies have investigated the roosting ecology of *M. macropus* in this landscape and our knowledge on how to manage this species in modified urban landscapes is limited. This paucity of information results in a limited ability to make evidence-based decisions on how to manage *M. macropus* roosts in artificial structures such as road culverts. The aim of this thesis was to examine culvert roost selection and urban landscape use by *M. macropus* to contribute towards the ecology and conservation of this unique species, with the intention of improving conservation outcomes for colonies roosting in road culverts.

Due to the diverse subject matter of this PhD, each chapter of this thesis has been written as a standalone research journal article, so there is some repetition between each chapter to establish context. Although diverse in scope, each chapter contributes towards a better understanding of the ecology and conservation of urban *M. macropus*. One chapter has been accepted for publication and three are in preparation for submission to different journals:

Chapter 2: A multiscale analysis of roost selection in concrete culverts by the large-footed myotis (*Myotis macropus*): implications for roost management of a specialist trawling bat in an urban environment.

The aim of this chapter was to investigate culvert roost selection at multiple scales by identifying which landscape and culvert variables could predict the presence of a *M. macropus* roost in a concrete culvert at a landscape scale. This approach was used to identify the distribution of culvert roosts within the study area and to provide guidance to road managers on culverts most likely to contain a roost to prevent unplanned disturbance to culvert roosts. This chapter used a stratified sampling design using landscape and culvert variables that most likely influence culvert suitability. The stratifying predictors used were land use, waterway permanency, culvert design and culvert size. I completed seasonal field surveys of 308 concrete culverts over one summer and one winter, and generalised additive modelling was used to model the distribution of *M. macropus* roosts in culverts and predict the potential roosting habitat available. This chapter identifies landscape and culvert variables associated with culvert roosts of *M. macropus* and I discuss these findings regarding urban culvert roost management. This chapter is in preparation to be submitted to Ecology and Evolution.

Chapter 3: Roost selection in concrete culverts by the large-footed myotis (*Myotis macropus*) is limited by the availability of microhabitat.

This chapter extends the findings of Chapter 2 by investigating culvert roost selection at two spatial scales. In this chapter, I investigate roost selection at a roost scale by comparing roost culverts to locally available culverts. Roosts identified during field work for Chapter 2 were revisited once again in summer to target the distribution and selection preferences for maternity culvert roosts. A total of 57 culverts were inspected and multivariate statistics were used to identify the similarity and dissimilarity between roost types and occupied and unoccupied culverts. I discuss the availability of suitable culvert roosts and identify an approach to increasing permanent roosting opportunities within concrete culverts in an urban environment. This chapter is published in *The Australian Journal of Zoology* for a special edition on Australasian Bat Research. Received 5 May 2020, accepted 10 August 2020, published online 25 August 2020.

Chapter 4: Landscape use in an urban environment by a specialist trawling bat, the large-footed myotis (*Myotis macropus*).

This chapter builds upon the findings of Chapters 2 and 3 by examining roosting ecology of culvert roosting *M. macropus* and investigating aspects of the urban ecology of this species. The aim of this chapter was to determine roost use, home range size and how urban land uses are used by a specialist trawling bat. This chapter uses radiotelemetry to study fine-scale spatial movement of thirteen adult

female *M. macropus* over one summer and two winters. I captured bats roosting in culverts and tracked them to explore movements between day roosts to examine aspects of roost switching and roost fidelity. I provide the first estimation of home range size for this species. I explore patterns of land use selection at two spatial scales to provide guidance to urban biodiversity planning. This chapter is in preparation to be submitted to Biological Conservation.

Chapter 5: Population structure and gene flow between culvert roosts of a specialist trawling bat in a subtropical city.

This chapter moves away from ecological methods and uses molecular genetic methods to examine patterns of gene flow in a culvert roosting population of *M. macropus*. The aim of this chapter was to provide insight into movement in an urban environment at a molecular level. This chapter uses nuclear and mitochondrial DNA to study gene flow between culvert roosts. DNA was sequenced for 72 *M. macropus* and used to identify genetic differentiation between roosts and patterns of genetic structuring. I discuss aspects of population genetics and discuss the implications of these findings for the conservation of an urban *M. macropus* population. This chapter is in preparation to be submitted to Molecular Ecology.

Chapter 7: General discussion

This chapter summarises the preceding research chapters and provides a general discussion of the urban ecology of *M. macropus*. The thesis concludes by identifying areas for future research and recommendations for the management and conservation of urban *M. macropus* populations. Due to the format of chapters being standalone research journal articles, I use ‘we’ throughout the experimental chapters in this thesis. I also refer to each chapter by its chapter number, instead of by manuscript details. During this PhD, I designed and completed all the work, performed all the analyses and writing of all the chapters. My supervisors have provided input to study design and contributed towards manuscripts. Details of co-authors are given at the start of each chapter.

1.5 OTHER PUBLICATIONS AND CONFERENCE PRESENTATIONS

Other manuscripts have been prepared that include data collected as part of this thesis but are not included in this thesis. Research conducted during this PhD has been presented at domestic and international conferences. These publications and conference presentations are listed below.

Publications

Kwak, M., Markowsky, G. and **Gorecki, V.** Impacts of urbanisation on the ectoparasites of the large-footed myotis *Myotis macropus* (Gould, 1854) (Vespertilionidae). Submitted to Parasitology Research on 15 May 2020.

Gorecki, V., Parsons, S. All roads lead to home: a review on the use of transport infrastructure as roost sites by bats. In preparation to be submitted to Sustainability.

Conference presentations

Gorecki, V., Rhodes, M and Parsons, S. (2020) Landscape use in an urban environment by a trawling bat, the large-footed Myotis (*Myotis macropus*). Spoken presentation: *Virtual Ecological Society of Australia Conference*, 30 November- 3 December, 2020.

Gorecki, V., Tarlinton, B., Parsons, S. and Hauxwell, C. (2020) Population structure and gene flow between culvert roosts of a specialist trawling bat in a subtropical city. Spoken presentation: *Virtual Australasian Mammal Society Conference*, 17- 18 November, 2020.

Gorecki, V., Parsons, S., Maggini, R., Rhodes, M., Tarlinton, B. and Hauxwell, C. (2020) From caves to culverts: Roost selection and roosting ecology of the large-footed myotis (*Myotis macropus*). Spoken presentation: *19th Australasian Bat Society Conference*, Te Anau, New Zealand, 8-10 March, 2020.

Gorecki, V., Parsons, S., Maggini, R. and Rhodes, M. (2019). Roost selection in concrete culverts by the large-footed Myotis, *Myotis macropus* in Brisbane, Australia. Spoken presentation: *18th International Bat Research Conference*, Phuket, Thailand, 28 July- 1 August, 2019.

Gorecki, V., Parsons, S., Maggini, R. and Rhodes, M. (2019). Roosting ecology of bats in road structures in Brisbane. Spoken presentation: *Ecological Consultants Association of NSW*, Quorrobolong, Australia, 25-26 July, 2019.

Gorecki, V., Parsons, S., Maggini, R. and Rhodes, M. (2018) Large-footed Myotis *Myotis macropus* roost selection in concrete culverts in Brisbane. Spoken presentation: *Ecological Society of Australia*, Brisbane, Australia, 25-29 November 2018.

Gorecki, V., Parsons, S., Maggini, R. and Rhodes, M. (2018) Large-footed Myotis (*Myotis macropus*) roost selection in concrete culverts in Brisbane. Spoken presentation: *Australian Mammal Society*, Brisbane, Australia, 1-5 July, 2018.

Gorecki, V., Parsons, S., Maggini, R. and Rhodes, M. (2018) Insectivorous bat roosts in road structures in Brisbane. Spoken presentation: *Australasian Network for Ecology and Transportation*, Creswick, Australia, 29 April- 2 May, 2018.

Gorecki, V., Parsons, S., Maggini, R. and Rhodes, M. (2018) Insectivorous bat roosts in road structures in Brisbane. Spoken presentation: *18th Australasian Bat Society Conference*, Sydney, Australia, 3-6 April, 2018.

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Chapter 2: A multiscale analysis of roost selection in concrete culverts by the large-footed myotis (*Myotis macropus*): implications for roost management of a specialist trawling bat in an urban environment



The distribution of large-footed myotis (*Myotis macropus*) roosts in concrete culverts was modelled in this chapter. Photo by V. Gorecki.

This chapter is in preparation for submission to the journal *Ecology and Evolution*.

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Abstract

Urbanisation affects the distribution and abundance of biodiversity as different species occupy environments that are unique to towns and cities. Many species of insectivorous bats have been able to adapt to novel environments provided in urban landscapes and are found in artificial roost sites such as buildings, bridges and culverts. The large-footed myotis (*Myotis macropus*) is a specialist trawling bat found roosting in culverts under roads in urban environments. Little is known about how this species selects culvert roosts or how widely these unique roosts are distributed across a city. We used an experimental design to stratify landscape and culvert variables to complete seasonal surveys of 308 culverts across Brisbane City Council (BCC). We modelled the distribution of *M. macropus* and modelled the predicted available culvert habitat across a large subtropical city. The distribution of *M. macropus* roosts in concrete culverts can be predicted at a landscape level using the landscape variables stream order, channel width and waterway density, and the culvert height. Stream orders determine the distribution of landscape variables such as channel dimensions (width and depth) and waterway density, which in turn determine the distribution of culverts required across an urban road network. *Myotis macropus* preferred culverts taller than 1.2 m in height, and while a preference for box culverts was detected, both design types (box and pipe) were occupied. Culverts potentially suitable for roosting by *M. macropus* are limited in this urban landscape. We discuss the implications of these findings in the context of managing *M. macropus* roosts in urban environments.

Keywords

Subtropical urban environment, culvert, *Myotis macropus*, roosting habitat.

2.1 INTRODUCTION

Urban environments are spatially heterogeneous landscapes that uniquely combine natural landscape features such as topography, vegetation and waterways with anthropogenic features like road networks and housing at different densities (Cadenasso *et al.* 2007). As a consequence, urbanisation affects the distribution, composition and abundance of biodiversity differently, yet our understanding of the processes structuring urban populations is limited (Evelyn *et al.* 2004).

Insectivorous bats occur in urban environments (Jung and Threlfall 2015). Day roosts are essential to the survival of urban bat populations as they provide protection from predators, have stable

microclimates and are locations of social interactions (Kunz 1982, Lewis 1995). However, urban environments have reduced availability of natural roost sites and this represents a limited resource to urban bat populations (Moretto and Francis 2017). Many species of bats have been able to adapt to the novel environments provided in urban setting, seeking out artificial roosts in buildings (Fagan *et al.* 2018), bridges (Keeley and Tuttle 1999) and culverts (Meierhofer *et al.* 2019). The Congress Avenue Bridge in Austin, Texas, houses 1.5 million Mexican free-tailed bats (*Tadarida brasiliensis*) (Keeley and Tuttle 1999). A targeted search of culverts in Poland identified 7,293 bats representing four species of *Myotis* (Wojtaszyn *et al.* 2013), suggesting that culvert roosts may provide important roosting opportunities to urban bats.

Recent research has suggested culverts are suitable roost sites for some species because they have reduced light, stable microclimates and are associated with surface water (Trousdale *et al.* 2008, Katzenmeyer 2016, Meierhofer *et al.* 2019). While much effort has been placed on understanding bat roost selection in bridges (Smiddy 1991, Perlmetter 1996, Shiel 1999), selection of culverts by bats has received limited attention. A preference for box culverts higher than 1.5m has been recorded in various studies reporting on bat roosts in culverts (Walker *et al.* 1996, Schulz 1998, Keeley and Tuttle 1999, Bender *et al.* 2010, Wojtaszyn *et al.* 2013, Meierhofer *et al.* 2019). Consistent findings in the descriptions of culvert roosts suggests culvert roosting bats may be actively selecting particular culvert attributes, and this has implications for urban bat conservation. However, little is known about how urban bats select artificial roost sites such as culverts, how widely culvert roosts are distributed across a city, or if it is possible to predict the distribution of culvert roost habitat to prevent disturbance.

Roosts are essential to the survival of bats and roost preferences can influence a species' distribution and abundance (Kunz and Lumsden 2003). The most urban tolerant bat species are characterised by greater flexibility in roosting strategies (Jung and Threlfall 2018). Urban tolerant bats also display high mobility such as open and edge aerial foragers, and trawling bats (Jung and Threlfall 2018). Trawling bats have manoeuvrable flight, developed for foraging over water, and this has lead to morphological adaptations that reflect their specialised foraging behaviour (Campbell 2011). Specialist species with specialist adaptations have a narrow ecological niche, and are therefore considered more prone to extinction (Safi and Kerth 2004b). The large-footed myotis (*Myotis macropus*) is an example of a specialist bat as it is a trawling species that selects roost sites in the vicinity (generally within 100 m) of waterways (Campbell 2011, Churchill 2008) but also displays flexible roosting behaviour. *Myotis macropus* has been recorded roosting in bridges, tunnels, jetties, road culverts and stormwater drains (Barclay *et al.* 2000, Anderson *et al.* 2005, Churchill 2008,

Campbell 2009, Gonsalves and Law 2017, Law *et al.* 2020) and provides a unique opportunity to study culvert roost selection by a specialist bat in an urban environment.

The aim of our study was to investigate culvert selection for roosting by *Myotis macropus* in an urban environment. We used a stratified sampling to identify the landscape variables and culvert attributes that make a culvert suitable for roosting. We then used the model to predict the distribution of culverts potentially suitable for roosting across BCC. This information will guide management decisions by road management authorities with *M. macropus* roosts in transport infrastructure networks.

2.2 METHODS

Study area

The study was conducted in the Local Government Area (LGA) of Brisbane City in south-east Queensland, Australia (Figure 2-1). The Brisbane City Council (BCC) area covers 117,000 hectares and is characterised by a subtropical climate (Brisbane City Council 2012). The urban landscape of BCC is dominated by residential housing, industrial and commercial areas covering 62% of the LGA (ABARES 2016). Conservation and natural areas are located on the western fringe of the city and cover 27% of the LGA, while production landscapes, including dryland agriculture, irrigated agriculture and plantations, are disseminated and cover 5% (ABARES 2016). Water covers 6% of the LGA due to the meandering Brisbane River and its tributaries, as well as several large waterbodies that include water supply reservoirs and dams. The distribution of population densities and degrees of urbanisation reflect the spatial arrangement of natural features within urban environments such as rivers and harbours, as well as historical development patterns and transport infrastructure (McKinney 2002, Li and Wilkins 2014). Brisbane has an average population density of 18 people per hectare (ppl/ha), which is lower than the population densities in the cities of Sydney (29 ppl/ha), Vancouver (31 ppl/ha) and London (64 ppl/ha) (Spencer *et al.* 2015). Approximately 75% of the Brisbane population lives in areas defined as low-medium population density, with densities between 4 and 30 people per hectare, compared to 38% in Sydney, 29% in Vancouver and 9% in London (Spencer *et al.* 2015).

Stratified sampling

A database of culverts present in the study area was obtained from BCC and included a total of 2666 concrete culverts located on council owned roads (which excludes highways due to safety restrictions). This study focuses on concrete culverts only as insufficient replicates of culverts

constructed in different materials (steel, brick, cast iron and timber) were available. The database provided unique asset identification codes for each individual culvert and information about culvert height, width, length, number of barrels, construction age, material, design (box or pipe) and location. This database was used to select sites (culverts) for the survey.

Following the approach of Maggini et al. (2002), sampling was stratified according to landscape and culvert variables that most likely influence culvert suitability. The stratifying factors were land use, waterway permanency, culvert design and culvert size. Bat species assemblage and distribution is strongly influenced by landscape structure (Norberg and Rayner 1987) so land use was used as a proxy for landscape structure in this study. *Myotis macropus* is a specialist trawling bat highly associated with waterways and large water bodies (Barclay et al. 2000, Anderson et al. 2005, Campbell 2009), waterway permanency was therefore used as an indicator of water availability and permanency through time at the site. To our knowledge, the influence of design and size on culvert suitability as a roost site has not been assessed in previous studies.

Land use was derived from the corresponding ALUM GIS file (ABARES 2016). A total of five broad categories were considered, comprising the following ALUM tertiary level classes: (i) natural environments, (ii) managed environment, (iii) agricultural environment, (iv) suburban environment, and (v) urban environment (see descriptions in Table 2-2). Waterway permanency was derived from stream order classifications provided in the GIS file ‘Queensland Drainage 25k’ (Management 2010). Three waterway permanency categories were defined: (i) ephemeral, (ii) semi continuous and (iii) continuous (see descriptions in Table 2-2). Estuarine waterways were not included in this study. Culvert design was based on the shape of the culvert and the two categories considered were (i) box (square or rectangular), and (ii) pipe (circular). Culvert size categories were derived from the diameter of culverts and were classified into four categories: (i) small, (ii) medium, (iii) large and (iv) extra-large (see descriptions in Table 2-2).

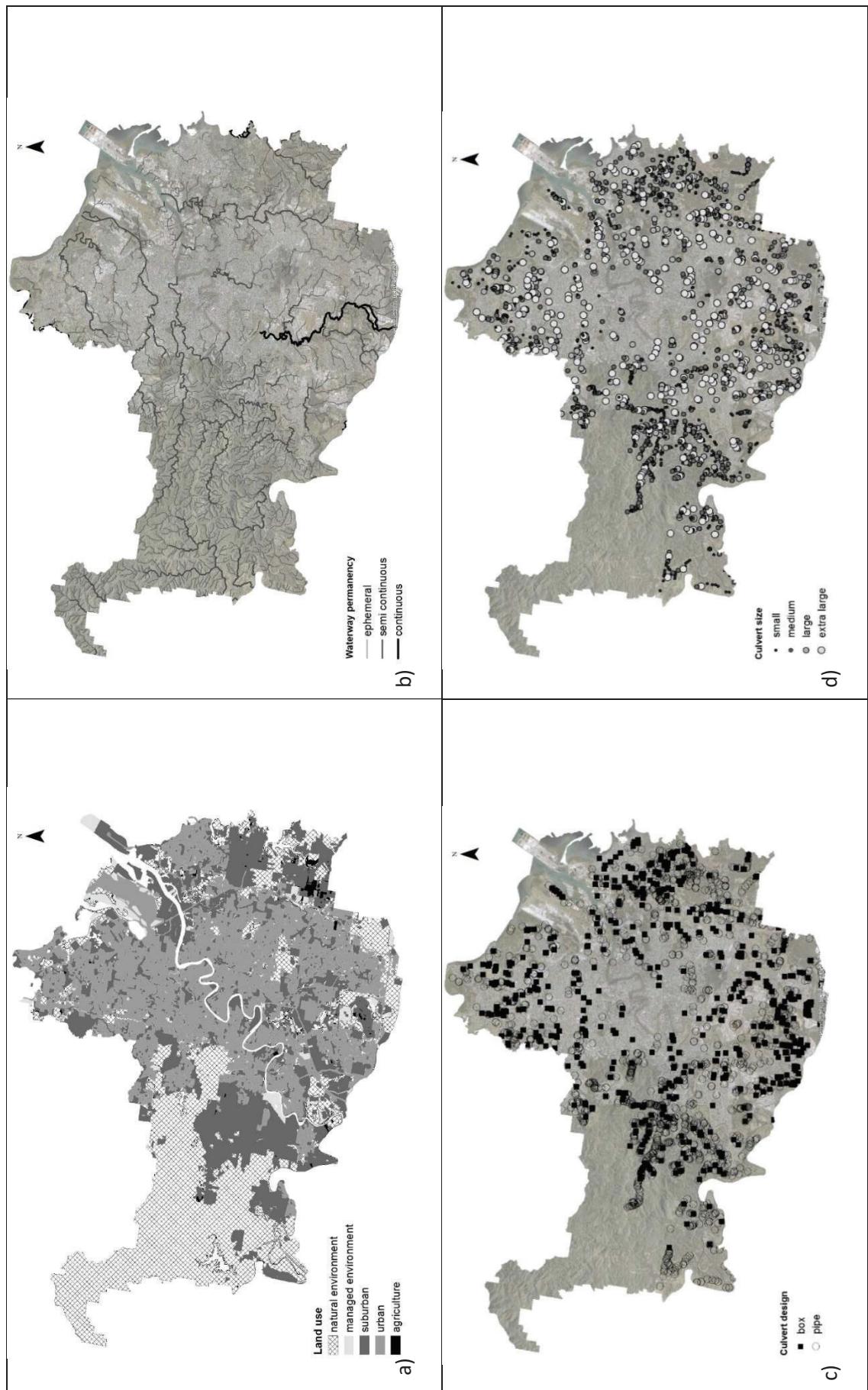


Figure 2-1 Variables used to stratify the sampling and relative distribution across BCC: (a) land use; (b) waterway permanency; (c) culvert design; (d) culvert size.

Site (culvert) selection

Stratification of predictor categories resulted in a total of 120 potential combinations (strata) to be sampled (5 land use x 3 waterway permanency x 2 culvert designs x 4 culvert size classes). A number of combinations did not occur in reality resulting in a total of 83 unique combinations to survey. Each predictor category was given a code so that each combination (stratum) had a corresponding unique ID number (Table 2-1). For example, a 1.5 m high box culvert on an ephemeral waterway in a national park is designated by code 1114. Sampling sites (concrete culverts) were selected within ArcGIS version 10.5 (ESRI 2019) by considering each stratum separately so as to inspect the spatial spread of the sites.

Table 2-1 Predictor category codes used to define combinations of landscape variables and culvert attributes to sample

Predictor	Category	Code
Land use	Natural environment	1000
	Managed environment	2000
	Agricultural environment	3000
	Suburban environment	4000
	Urban environment	5000
Waterway permanency	Ephemeral	100
	Semi continuous	200
	Continuous	300
Culvert design	Box	10
	Pipe	20
Culvert	Small	1
	Medium	2
	Large	3
	Extra large	4

For strata with fewer than five sites, all available sites were selected for field sampling. For strata with more than five sites available, a pseudo-random choice of five sites was made by randomly selecting an ID and by ensuring that a minimum distance of 1 km was maintained between sites finally selected. Some strata contained sites that were naturally clustered (e.g. the agricultural land use type is geographically restricted in Brisbane) and so a minimum spatial separation could not always be guaranteed.

Field sampling

Field sampling was conducted during the austral summer of 2017 and 2018 (December to April, October to November), and winter of 2018 (May to September). Each culvert was surveyed for the

presence of *M. macropus* once in summer and once in winter. A headlamp was used to light the space within the culvert and inspect structural features such as lift holes, structural deformities such as cracks and crevices, and other potential roosting habitat such as mud nests built by fairy martins (*Hirundo ariel*) and welcome swallows (*Hirundo neoxena*). An endoscopic camera was used to inspect deep or partially blocked structural deformities. A maximum of 20 minutes was spent searching each structure to standardise search effort. If *M. macropus* was present, the number of bats was recorded, and the culvert was defined as occupied. If evidence of bat occupancy such as staining, guano and bat fly pupae was present, the culvert was defined as unoccupied due to the uncertainty of which species may have occupied the culvert.

Additional site-specific characteristics were recorded at each culvert and investigated to determine if site-specific features would contribute to roost selection preferences. Site-specific characteristics included: distance to nearest streetlight, channel width, channel depth, vegetation height, and projected foliage cover (%) over a 25 m transect centred on the culvert. Culvert orientation was recorded by taking a compass bearing at the upstream end of the culvert and by converting it into west-east (eastness) and south-north (northness) gradients. Distance to nearest streetlight was included due to evidence that artificial lighting at night (ALAN) has significant, negative impacts on the movement of bats in urban environments (Moretto and Francis 2017, Haddock *et al.* 2019, Laforge *et al.* 2019, Russo *et al.* 2019). Channel width and depth was included to test for preferences in waterway size (Anderson *et al.* 2005) and vegetation height and cover was included to test for preferences in vegetation structure. Culvert orientation was recorded to test for a preference in orientation as bat roosts are often orientated towards the west to maximise solar exposure to provide suitable microclimates for unfurred pups due to the warming effect of solar radiation (Mering and Chambers 2014).

Surveys were carried out under permits issued by Queensland Department of Environment and Science (Scientific Purposes Permit WA0001898) and Queensland University of Technology Animal Ethics (AEC1700000540) and Biosafety Committees (1700000368).

GIS variables

We derived additional GIS-based landscape variables to be used for modelling. The density of potential available roosting habitat in the form of road structures such as culvert and bridges within 1 km, 5 km, 10 km and 15 km (search radius from each culvert) was calculated in ArcGIS v10.5 using the BCC asset database (Table 2-2: RSdensity). The density of potential foraging resources in the form of waterways (wwaydensity) at 1 km, 5 km, 10 km and 15 km (search radius from each culvert) was calculated using the GIS file Queensland Drainage 25k (Management 2010). Distance from each

culvert to the nearest small waterbody (size <625 m²; NearestSmallWater) and large waterbody (=> 625 m²; NearestLargeWater) were calculated in ArcGIS v10.5 using the spatial layers Small Water Bodies and Large Water Bodies, accessed through the Queensland Government QSpatial portal (QSpatial 2017).

A layer of percent vegetation cover was created by merging the Queensland Regional Ecosystem spatial layer, which identifies polygons of remnant vegetation and high-value regrowth; BCC's significant landscape tree layer, which identifies mature landscape trees; and BCC's parklands layer, which identifies council owned parks and green spaces. This resulted in a single spatial layer of polygons identifying patches of all vegetation within the study area.

Table 2-2 Descriptions of culvert attributes and landscape variables used for the stratification of the sampling and the modelling of culvert suitability for roosting by *Myotis macropus* for two models ((1) ecology and (2) culvert).

Abbreviation	Description	Model
Land Use	Land use category- (i) natural : conservation areas and native vegetation, (ii) managed : production landscapes with structured vegetation and low intensity uses, (iii) agricultural : production landscapes with limited structural vegetation and high intensity uses, (iv) suburban : low-medium density urban areas and industrial areas with modified natural areas and (v) urban environment : high density urban areas and commercial areas with limited natural areas.	Ecology, culvert
Waterway permanency	(i) ephemeral : stream orders 1-2, streams that may have either a continuous or a discontinuous channel whereby intermittent base flows occur episodically after rain but generally there is no permanent water source, (ii) semi continuous : stream orders 3-4 with continuous base flows or semi-permanent pools that are not dependent on episodic rainfall and (iii) continuous : stream orders 5-6 with a continuous channel with well-defined banks and floodplain with permanent, or semi-permanent, base flows	Ecology
Design	Shape of the culvert- (i) box culverts: square or rectangular tunnels and (ii) pipe culverts: circular pipes.	Ecology, culvert
Size	Culvert size- (i) small : <500 mm, (ii) medium : 500 <=x< 1000 mm, (iii) large : 1000 <=x<1500 mm and (iv) extra-large : =>1500 mm.	Ecology, culvert
Height	Height of culvert (mm)	Ecology, culvert
Width	Width of culvert (mm)	Ecology, culvert
Length	Length of culvert (m)	Ecology, culvert
Barrels	Number of barrels (sections) at a culvert	Ecology, culvert
Channel Width	Bankfull width of waterway (m)	Ecology

Abbreviation	Description	Model
Channel Depth	Bankfull depth of waterway (m)	Ecology
Stream order	Stream order culvert is located on, derived from stream order classifications (1-5)	Ecology, culvert
VegHeight	Height of ecologically dominant layer (EDL) over 25 m transect centred on culvert	Ecology
VegCover	Projected foliage cover over a 25 m transect centred on culvert	Ecology
NearestLight	Distance from centre of culvert to nearest streetlight (m)	Ecology
RSdensity	Density of road structures (culverts and bridges) within 1, 5, 10, 15 km (number of structures per square kilometre)	Ecology
wwaydensity	Density of waterways within 1, 5, 10, 15 km (length of waterways (km) per square kilometres)	Ecology
NearestSmallWater	Distance to small waterbody (< 625 sq m in size)	Ecology
NearestLargeWater	Distance to large waterbody (=> 625 sq m in size)	Ecology
Northness	Orientation of culvert along a south-north gradient (-1= south, 1= north)	Ecology
Eastness	Orientation of culvert along a west-east gradient (-1= west, 1= east)	Ecology
PercentVeg	Percent cover of vegetation in each 25m pixel	Ecology

Statistical modelling

Collinearity between predictor variables was investigated prior to modelling. When a correlation above 0.7 was detected, univariate models were fitted for each of the correlated variables and the variable that had the lowest contribution was removed. Response to variables calculated at different scales (road structure density, waterway density) was also modelled separately to identify the relevant scale for each predictor. Road structure density at 10 km and waterway density at 5 km had the strongest relationships with the presence of a roost.

We used Generalised Additive Models (GAM) (Hastie and Tibshirani 1990) to investigate relationships between the presence of a roost in a concrete culvert and landscape variables and culvert attributes. Models were built in R version v.3.4.2 (R Development Core Team, 2011) using the multiple generalised cross-validation ‘mgcv’ package (Wood 2007). The presence/absence (binomial distribution) of a roost in a culvert was modelled using penalised regression splines with smoothing parameters selected by UBRE for each variable.

Two different models were defined. The first model is an ‘ecological model’ describing *M. macropus* roost selection in an urban environment and fitted using landscape variables and culvert attributes, that were either used to stratify the sampling, GIS derived or collected in the field. The second model is a ‘culvert structural model’ calibrated using only culvert attributes available from the asset

database (height, width, length, number of barrels, design and stream order ; Table 2-2) and aimed at informing road managers about characteristics that would make a culvert suitable for roosting.

The models were selected using the backward selection strategy as described by (Wood 2007). The backward selection strategy involves removing each non-significant predictor variable (highest *p*-value) in sequential order until obtaining a final model with significant (*p*<=0.05) variables only, and the lowest Akaike's Information Criterion (AIC) (Akaike 1973). The `gam.check` function in the 'mgcv' package was used to inspect the residual plot vs. fitted values, the Quantile-Quantile plot (QQ-plot), the residual plot against the original explanatory variables, and the histogram of residuals. A three-dimensional perspective plot was generated to assess interactions between final variables using the `vis.gam` function.

Models were evaluated using the area under the curve (AUC) of a Receiver Operating Characteristic (ROC) plot (Fielding and Bell 1997). AUC value provides a measure of model performance whereby a value of 0.5 represents a random model and a value of 1 indicates a perfectly discriminating model (Fielding and Bell 1997). Model performance was further evaluated using the correlation between model predictions and presence/absence of *M. macropus* roosts (observed data).

Culverts suitable for roosting across BCC

We used the final 'culvert structural model' and the full BCC asset database to identify the spatial distribution of potentially suitable culverts for roosting across Brisbane. To do so, we first used the `predict.gam` function in the 'mgcv' package (Wood 2007), the culvert model and the full BCC database to generate the probability of occurrence of *M. macropus* for each culvert. Using the 'PresenceAbsence' package (Freeman and Moisen 2008), we then compared the predicted probability of occurrence to the observed presence/absence to determine the optimal threshold to convert all probabilities. We tested different methods (MaxKappa, PredPrev=Obs, MaxSensSpec, ReqSens (Freeman and Moisen 2008) to determine the optimal threshold of occurrence for the conversion, and compared them based on, confusion matrices, Kappa values and predicted prevalence. Predicted probabilities equal to or above the threshold were classified as presences, while values below the threshold were classified as absences.

Among the different methodsthat were tested for determining the optimal threshold for converting the predicted probabilities of occurrence from the 'culvert model' into presences/absences, MaxKappa was the method that gave the best results: highest Kappa value (0.59) and a predicted prevalence (0.072) that was closest to the observed prevalence (0.065).

2.3 RESULTS

Survey effort

Of the 338 sites initially planned for field sampling, only 308 were actually inspected. Reduced sampling was primarily due to (i) safety issues such as deep water in a culvert or busy roads with narrow road verges, and (ii) errors in the information provided in the asset database and no alternative sites available for a given stratum.

Myotis macropus roost distribution

Of the 308 concrete culverts inspected, a total of 20 *M. macropus* culvert roosts were identified over the two seasonal visits (Figure 2-2). Photographs of culverts used by *M. macropus* as roost sites are shown in Appendix 1. Colony sizes displayed seasonal variation with larger colonies identified in summer. A total of 14 *M. macropus* roost sites were located in summer with a mean colony size of 15 individuals (range 1-65), and 14 roosts with a mean colony size of 8.3 (range 1-34) were identified in winter. Roosts were located across the entire study region although not in a uniform pattern. Due to the low number of roosts detected, data for both seasons was pooled to proceed with presence-absence modelling.

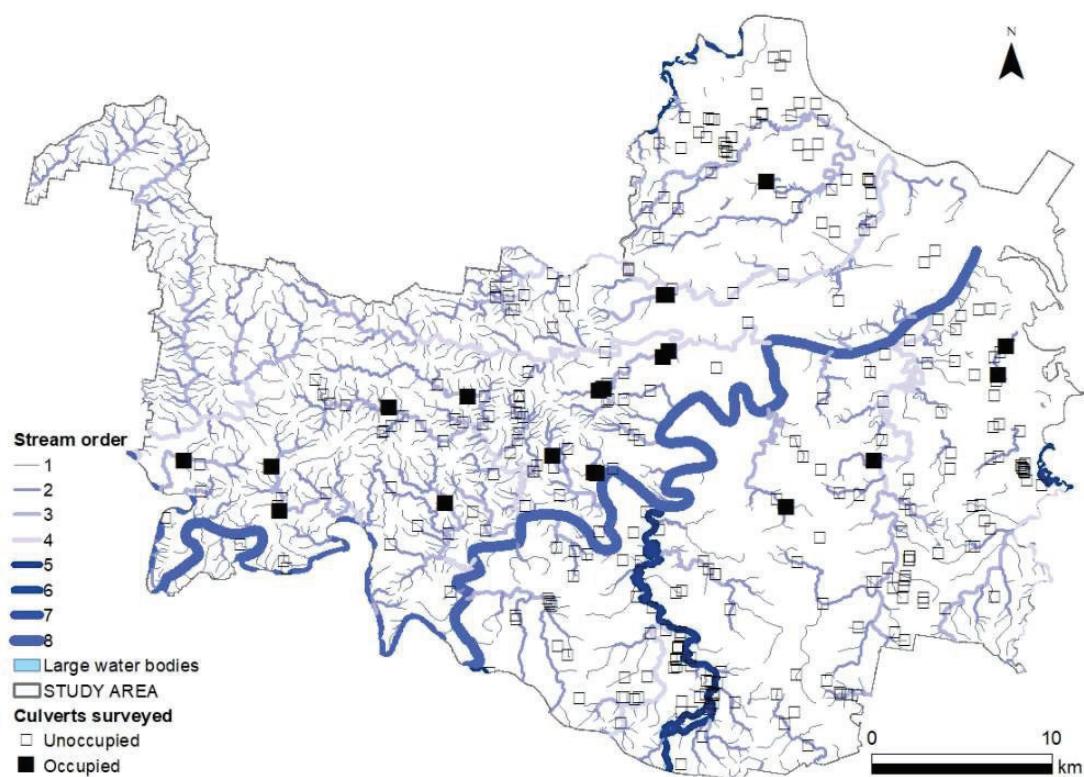


Figure 2-2 Distribution of culverts surveyed for the presence of *Myotis macropus* roosts

Ecological model

Three models showed high explanatory power (high percentages of explained variance) and high discrimination power (high AUC value and high correlation value) (Table 2-3). The best fitting (model 3) was finally selected for its lowest AIC, highest percentage of explained variance (77.60%), highest AUC value (0.99) and highest correlation between model predictions and observed data (0.85). Across all models, the variables culvert height, waterway density at 5 km and distance to nearest large waterbody were consistently retained. A preference was displayed for the box design. The plot in Figure 3 shows the smooth functions (on the scale of the linear predictor) describing the relationship between the probability of a *M. macropus* roost and each variable retained in the best GAM model (Model3).

Culvert height proved to be the most significant predictor ($p= 0.0001$, $edf= 2.4$) of a roost (Table 2-3; Figure 2-3). *Myotis macropus* displayed a strong preference for taller culverts with the spline plot showing an increase in the predicted presence of a roost as culvert height increases. Roosts were indeed only found in sampled culverts of 1.2 - 3.0 m in height. During the sampling, roosts were found in 6 pipe culverts and 17 box culverts, and the final model identified a negative relationship with pipe culverts ($p= 0.02$, estimate= -2.98) suggesting *M. macropus* have a preference for box culverts. However, culverts over 1.2 m in height were typically box culverts located on higher order streams (Figure 2-4).

Channel width was a significant predictor of a roost ($p= 0.04$, $edf= 2.36$) with an increasing probability of the presence of a roost associated with channels up to 10 m wide. After this value, the relationship seems to become negative, although there is a large error associated with the estimate in this part of the curve (Figure 2-3). The probability of a roost occurring in a culvert increases with increasing waterway density ($p= 0.003$, $edf= 2.14$) and with increasing distance from the nearest large waterbody ($p= 0.008$, $edf= 2.97$) (Figure 2-3). Note however, that large waterbodies (reservoirs, dams) are located high in the catchment (Figure 2-2) and culverts closest to large waterbodies are smaller than culverts that are located further away from large waterbodies (Figure 2-5).

Roosts are associated with a higher degree of vegetation cover at the culvert scale but not at the landscape scale, since the final model retains the predictor related to the amount of vegetation available immediately surrounding a culvert as significant (projected foliage cover, $p= 0.01$, $edf= 3.00$).

Table 2-3 GAM models describing the relationship between the presence of a *Myotis macropus* roost in a concrete culvert and predictive landscape variables and culvert attributes. Model 3 shows the best fit.

Model and variables	Estimate/edf	p-value	Sig.	Dev. explained (%)	AIC	AUC	Cor
Model 1				65.00	75.13	0.98	0.76
Intercept	-9.33	0.00018	***				
Box	2.05	0.02061	*				
Height	2.22	0.00814	**				
ChannelWidth	2.85	0.03634	*				
Waterway5km	1.94	0.00540	**				
NearestLWater	2.74	0.01929	*				
Model 2				66.30	72.17	0.98	0.76
Intercept	-7.98	0.000406	***				
StreamOrder5	2.16	0.000264	***				
Height	2.12	0.015984	*				
VegetationCover	3.00	0.001151	**				
NearestLWater	1.00	0.049621	*				
RoadStructure10km	1.00	0.007464	**				
Waterway5km	2.06	0.000264	***				
Model 3 - best				77.60	62.85	0.99	0.85
Intercept	-11.026	0.00840	**				
Design (pipe)	-2.977	0.02710	*				
Height	2.390	0.00145	**				
ChannelWidth	2.362	0.04550	*				
VegetationCover	3.000	0.01135	*				
NearestLWater	2.975	0.00854	**				
Waterway5km	2.141	0.00387	**				

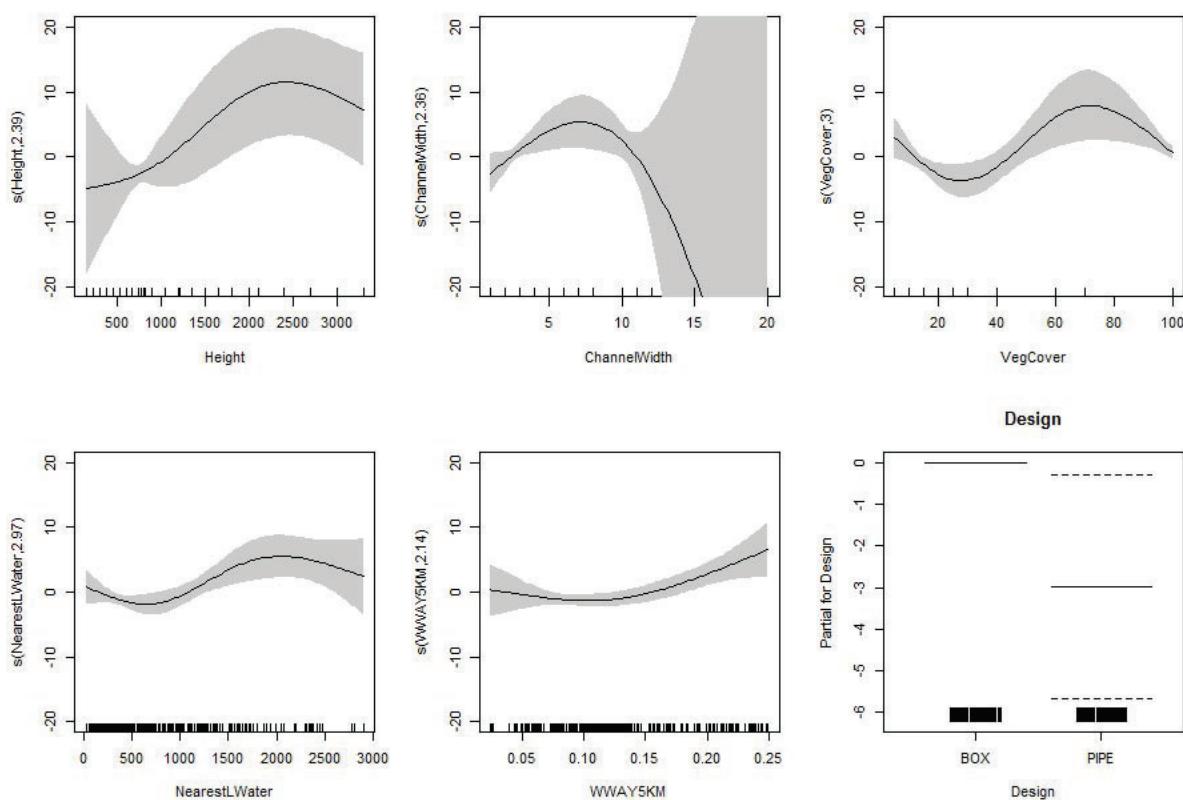


Figure 2-3 Estimates (solid lines) and 95% confidence intervals (shading) of a binomial logit link Generalised Additive Model for presence/absence of *Myotis macropus* roosts in concrete culverts as a function of continuous predictor variables: height (edf= 2.39), channel width (edf= 2.36), vegetation cover (VegCover) (edf= 3.00), distance to nearest large waterbody (NearestLWater) (edf= 2.97), waterway density at 5 km (WWAY5KM) (edf= 2.14,) and categorical predictor variable design.

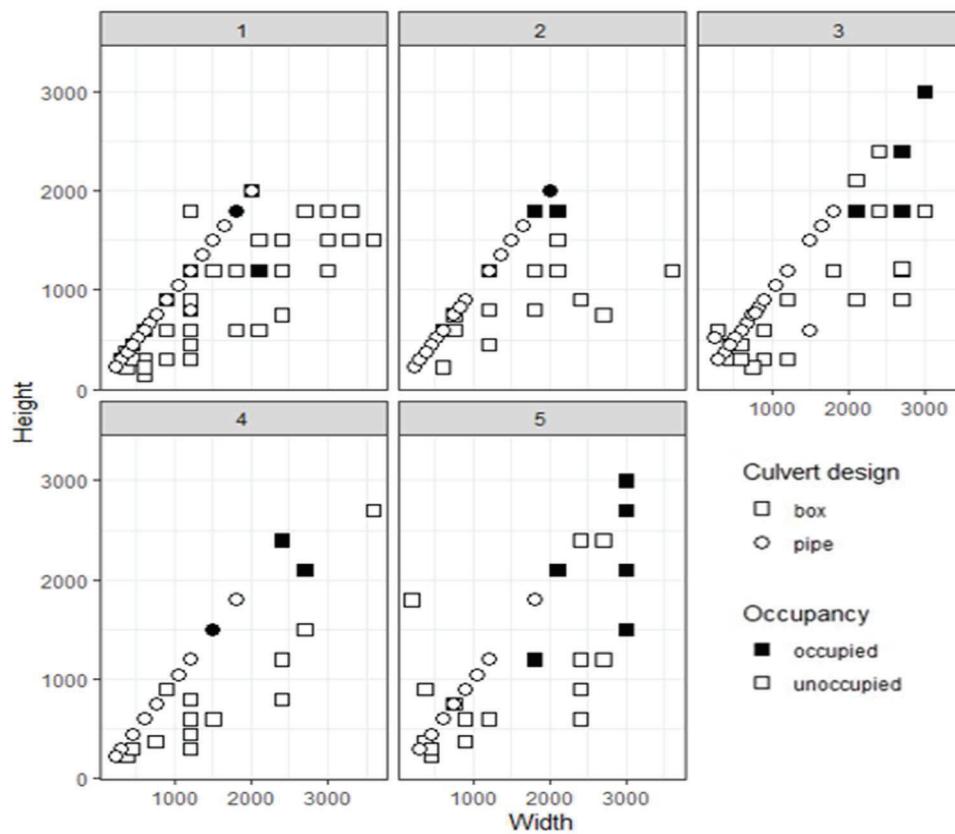


Figure 2-4 Culvert height (mm) and culvert width (m) increases with increasing stream order (1-5), and culverts occupied by *Myotis macropus* >1.2 m in height and >1.5 m in width are more available on stream orders 3-5.

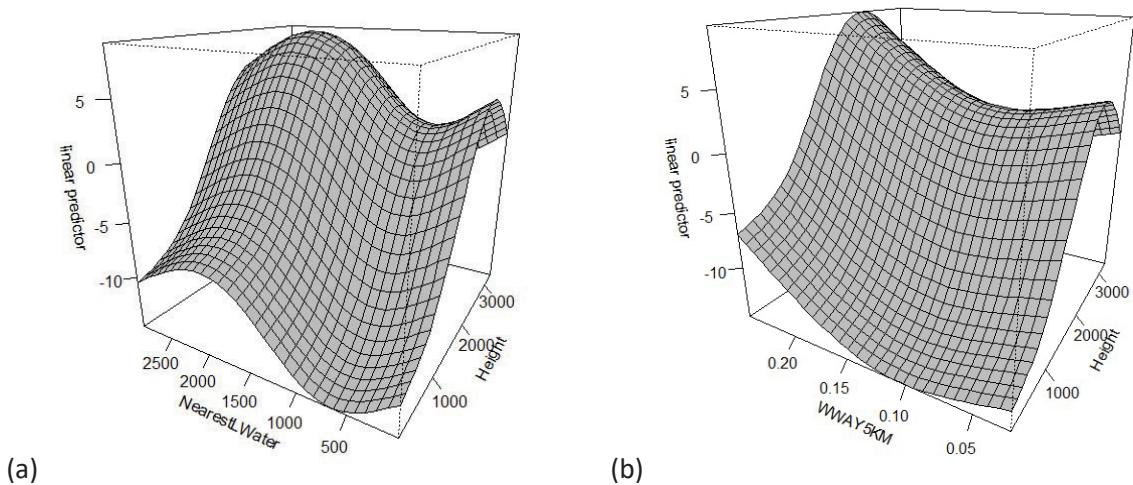


Figure 2-5 Three-dimensional interaction plots of (a) culvert height and distance to nearest large waterbody, showing that culverts closest to large waterbodies are smaller than culverts that are further away , (b) culvert height and waterway density at 5 km, showing that landscapes with higher densities of waterways have taller culverts.

Culvert structural model

Three models showed evidence of reasonable explanatory power and good discrimination power with high AUC values and correlation between model predictions and observed data (Table 2-4). The best model (model 3; Table 2-4, Figure 2-6) was selected for its lowest AIC (95.84), highest amount of variation explained (46.10%), highest AUC value (0.944) and highest correlation (0.6).

Culvert height proved once again to be the most significant predictor of a roost ($p<0.0001$, edf= 2.102). The model retained pipe culverts in a negative fashion although not significantly (Design (pipe), $p=0.158$, estimate= -0.946) suggesting *M. macropus* avoid pipe culverts or have a preference for box culverts. Stream order is the second variable retained significantly in the final model with *M. macropus* showing a preference for stream order two ($p=0.03$, estimate=2.01) and indication of a preference for stream order five although this variable was not significant ($p=0.07$, estimate= 1.77).

Table 2-4 GAM models describing the relationship between the presence of a *Myotis macropus* roost in a concrete culvert and culvert attributes.

Model and variables	Estimate/edf	p-value	Sig.	Dev. explained (%)	AIC	AUC	Cor
Model 1				38.90	96.17	0.92	0.51
Intercept	-4.79	0.000000136	***				
Height	1.97	0.0000588	***				
Model 2				44.40	96.14	0.94	0.59
Intercept	-6.01	0.000000535	***				
Height	2.00	0.000214	***				
StreamOrder2	1.84	0.0439	*				
StreamOrder3	0.50	0.6112					
StreamOrder4	1.69	0.1261					
StreamOrder5	1.86	0.0574					
Model 3 - best				46.10	95.84	0.94	0.60
Intercept	-5.89	0.0000132	***				
Height	2.102	0.00102	**				
Design (pipe)	-0.946	0.1588					
StreamOrder2	2.0199	0.0302	*				
StreamOrder3	0.3948	0.6932					
StreamOrder4	1.7098	0.1327					
StreamOrder5	1.7744	0.0750	.				

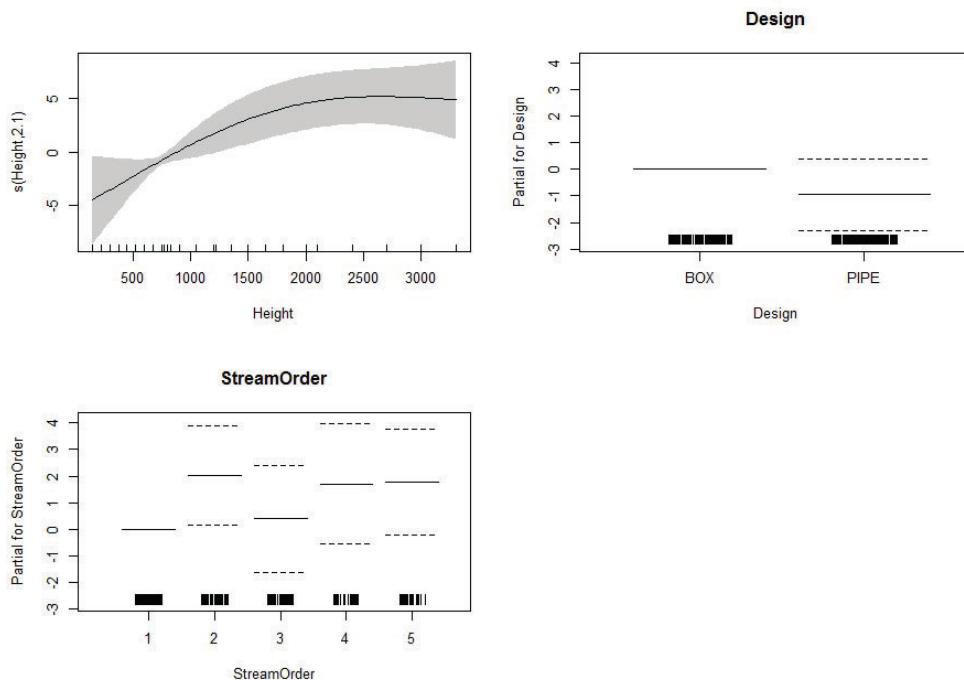


Figure 2-6 Estimates (solid lines) and 95% confidence intervals (shading) of a binomial logit link Generalised Additive Model for presence/absence of *Myotis macropus* roosts in concrete culverts as a function of continuous predictor variable height (edf=2.10) and categorical predictor variables design and stream order.

Culverts suitable for roosting

An assessment of the predicted probabilities from the ‘culvert structural model’ indicates strong alignment to the observed data since the bins are positioned close to the diagonal on the plot (Figure 2-7).

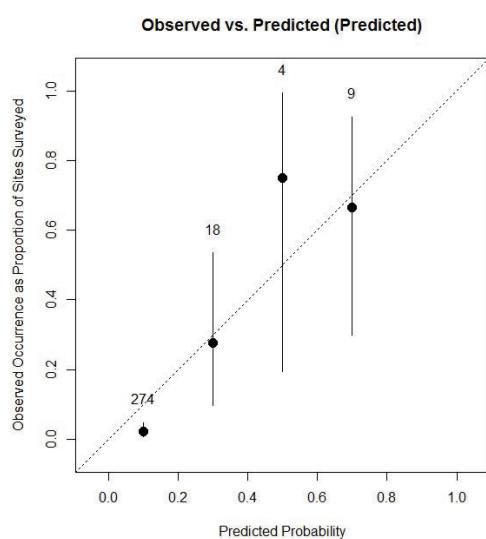


Figure 2-7 Plot showing the proportion of observed occurrences against the predicted values by the culvert structural model with 95% confidence intervals.

Using the MaxKappa method, 0.32 was identified as the optimal threshold for converting the predicted probabilities of occurrence from the ‘culvert model’. The map in Figure 2-8 shows that although a multitude of culverts is available across the study area, culverts that are potentially suitable for roosting by *M. macropus* (i.e. predicted presence) are limited (146 out of 2666 concrete culverts, i.e. 5.5%). The limited availability of suitable culverts is driven by a reduced number of tall and box culverts. Within BCC only 754 culverts (28.0%) are higher than 1.2 m and only 882 (33.1%) are box culverts. These structural requirements combined with water requirements (stream order) determine a very restricted number of suitable culverts.

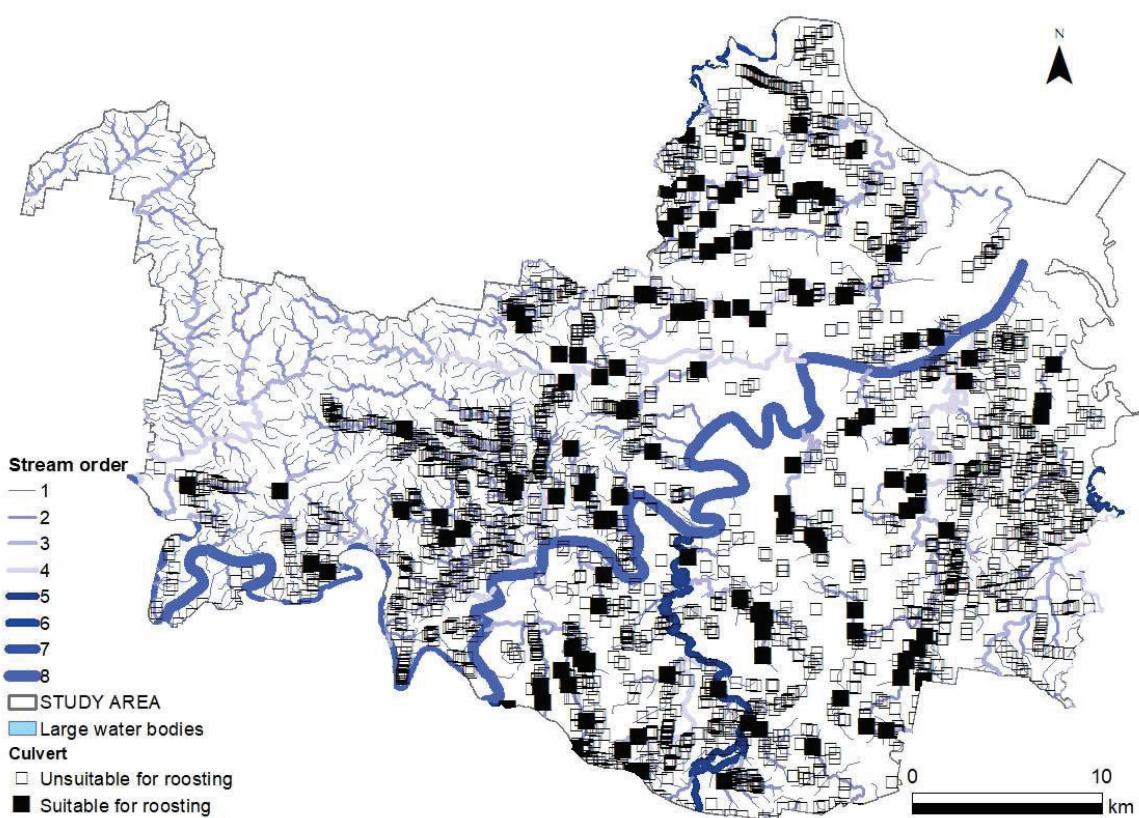


Figure 2-8 Map of potentially suitable culverts for roosting by *Myotis macropus* across Brisbane.

2.4 DISCUSSION

This multiscale study investigated roost selection by *Myotis macropus* in an urban environment using a stratified sampling design to identify landscape variables and culvert attributes that are most suitable. The model was then used to predict the distribution of culverts potentially suitable for roosting across BCC. *Myotis macropus* preferred culverts >1.2 m in height, and while a preference for box culverts was detected, both design types (box and pipe) were occupied. Culvert roosts were located in landscapes with a high density of waterways and on higher order streams. The occurrence

of *M. macropus* roosts in concrete culverts in our study was low, with 20 roosts identified from 305 surveyed culverts (6.56%) over two seasons. This finding can be explained by our sampling design and culvert roost preferences displayed by *M. macropus*. The low occupancy rate recorded in our study is likely due to the inclusion of all culvert size classes in the survey to determine which size class was preferred. *Myotis macropus* also displayed a preference for tall culverts which were limited in this study. Other studies on bat roosts in culverts have had occupancy records of 20% (Meierhofer *et al.* 2019) to 53% (Walker *et al.* 1996), however these studies concentrated on highways which generally have larger culverts than suburban roads like those in our study. It is possible that occupancy rates in our study might have been higher if highways were able to be included in our study design. The colony sizes for *M. macropus* found in concrete culverts in our study is similar to those recorded both in natural roosts and in other artificial roosts. We found colony sizes ranging from 1-65 (av= 15) in summer and 1-34 (av= 8) in winter. As a comparison, 40-60 *M. macropus* were recorded in a tree roost (Dwyer 1970a), fewer than 30 individuals in a cave roost on a river (Campbell 2009), 50 roosting in a jetty, (Gonsalves and Law 2017) and 21 individuals in a roost in an abandoned rail tunnel (Barclay *et al.* 2000).

Culvert attributes that determine culvert suitability for roosting

Culvert height was the most significant predictor of the presence of a roost. In our study, roosts were only located in culverts 1.2 - 3 m in height, which is similar to the size of culvert roosts reported by other researchers (Walker *et al.* 1996, Keeley and Tuttle 1999, Meierhofer *et al.* 2019). Meierhofer *et al.* (2019) postulate this is due to taller culverts being easier for a bat to find, enter and navigate. Tall culverts may also provide roost sites with increased protection from predators. *Myotis macropus* roosting in a tunnel emerged from crevices twenty minutes earlier than conspecifics in nearby tree cavities yet did not exit the tunnel until a similar time after sunset (Campbell 2009). This twenty minutes of safe foraging time within the tunnel provided tunnel roosting *M. macropus* with an opportunity to exploit the peak in insect activity around sunset (Fukui *et al.* 2006, Campbell 2009). The tunnel also provided protection from aerial predators and a safe environment for young bats learning to fly (Campbell 2009). The tall culvert roosts found in our study would provide similar predator free protection to urban *M. macropus* and may be a contributing factor to the selection of tall culverts.

Our results indicate that culvert design was also a significant predictor of the presence of a roost, although this result could be explained by the height of a culvert. Culverts over 1.2 m in height were box culverts located on higher order streams. Box culverts were taller than pipe culverts so the preference for a culvert design is likely driven by the height of the culvert, rather than the shape.

Similarly, Schulz (1998) did not find a relationship between the presence of bats and ceiling roundness, but did find a significant positive relationship with culvert height. Our study did not find that the length of the culvert was a significant predictor of a *M. macropus* roost. Several studies have however identified culvert length as a significant driver of roost selection in culverts, suggesting that longer culverts increase roosting potential by increasing the surface area available to the bats (Keeley and Tuttle 1999, Katzenmeyer 2016, Meierhofer *et al.* 2019). Katzenmeyer (2016) found the number of bats roosting in a culvert increased with increasing culvert length and concluded similarly to Keeley and Tuttle (1999), that culverts over 100 m in length are preferred by bats. That said, our finding is supported by other studies that have reported roosts in culverts < 100 m. A total of 182 individuals comprising 17 species from four subfamilies of Phyllostomidae in Peru were captured in one culvert which was only 15 m long and 2 m height (Hice *et al.* 2004). Similarly, Bender *et al.* (2010) found occupied culverts had lengths of 13-19 m. We did not find that *M. macropus* preferred longer culverts nor avoided short culverts, therefore we do not advise the use of culvert length as a predictor of the presence of a bat roost in targeted culvert surveys.

Landscape variables surrounding culvert roosts

Roosts in culverts were located at lower positions in the landscape, on larger waterways. Channel width, distance to nearest large waterbody and waterway density are all linked to stream orders which are located in specific positions in the landscape. The stream order classification system provides a hierarchical approach to classifying the physical template (hydrology and geomorphology) of streams (Strahler 1952) and can explain ecological patterns. Generally, as stream orders increase, stream dimensions such as cross-sectional area (channel width and depth), flow volume, flow velocity and substrate roughness also increase and flora and fauna communities diversify (Vannote *et al.* 1980). In our study, culvert roosts were located at lower positions in the landscape, on wider waterways which require larger culverts. We found an increasing probability of the presence of a roost associated with channels up to 10 m wide. This is consistent with the findings of waterway preferences of the trawling Daubenton's bat (*Myotis daubentonii*), which has displayed a preference for waterways 5-10 m wide (Warren *et al.* 2000, López-Baucells *et al.* 2017).

We found a negative relationship between culvert roosts and proximity to large waterbodies and this can be explained by the location of taller culverts in the landscape, relative to large waterbodies. Large waterbodies have been recognised as important foraging sites for *M. macropus* by numerous studies (Dwyer 1970a, Thompson and Fenton 1982, Barclay *et al.* 2000, Campbell 2009). *Myotis macropus* have been tracked foraging at 4.9 km (Gonsalves and Law 2017), 10 km (Barclay *et al.* 2000) and 22 km (Caddle 1998) from roost sites so our finding is unexpected. Our finding can be

explained by the location of tall culverts in the landscape. Tall culverts are inherently located at increasing distances from water supply sources. However, this result could also indicate that in our study, urban *M. macropus* populations are not reliant on large waterbodies as foraging sites.

Tall culverts are located further down the catchment in landscapes with a higher density of waterways which could provide *M. macropus* with sufficient foraging resources, so they are not required to commute to large water bodies. Anderson *et al.* (2005) found that *M. macropus* were significantly more likely to be recorded foraging on stream orders 4-6 which occur at lower elevation and contain large, smooth pools with limited riffle zones. This habitat preference is consistent with habitat use by other trawling bats Daubenton's bat (*Myotis daubentonii*), the pond bat (*Myotis dasycneme*) and long-fingered bat (*Myotis capaccinii*). Large, elongate pools with smooth surfaces are preferred foraging grounds for trawling bats due to high insect activity and reduced clutter associated with rough water surfaces that interfere with echolocation (Warren *et al.* 2000, Lintott *et al.* 2015, Todd and Williamson 2019). The distribution of *M. daubentonii* was associated with a preference for smooth water sections of the river, located at on wide rivers at lower altitudes, with well-structured riparian forests (López-Baucells *et al.*, 2017, Todd and Williamson 2019). Similarly, *M. dasycneme* displayed an affinity for medium to large waterways and large lowland ponds (Van De Sijpe *et al.* 2004) and *M. capaccinii* selects large rivers with smooth surfaces (Almenar *et al.* 2006). Our finding of a preference for roost sites in similar landscape positions also indicates that *M. macropus* could be roosting in close proximity to sufficient foraging resources in this urban environment.

Our results suggest the degree of vegetation cover at the culvert scale, not percent vegetation cover at the landscape scale, is an important variable in roost selection. However, this result can be explained by the clustering of culvert roosts in the western fringe of Brisbane, close to national parks where tree cover is higher compared to the highly urbanised areas of the city centre and suburban areas (Hourigan *et al.* 2010)

Our finding that percent vegetation cover at a landscape scale is not a significant predictor of a roost is consistent with other studies that found *M. macropus* may not be as dependent on patches of vegetation as significantly as riparian corridors. A fourteen-year study on population dynamics of *M. macropus* in a plantation forest found that riparian vegetation buffered *M. macropus* from the impacts of timber harvesting (Law *et al.* 2020). The importance of riparian corridors to trawling bats in urban environments has been well documented with studies demonstrating both higher bat activity within riparian habitats (Dixon 2012, Lintott *et al.* 2015) compared to adjacent residential areas and the value of these linear landscape features in providing functional landscape connectivity

(Hale *et al.* 2012, Russo and Ancillotto 2015). The absence of a relationship between culvert roosts and percent vegetation cover at the landscape scale could also be an artefact of the GIS data layers we used which may not specifically, nor accurately reflect riparian vegetation cover.

We measured distance between each culvert and the nearest streetlight expecting to find a negative relationship between roost selection and artificial light, however, we found no significant effect of distance to nearest streetlight on roost selection within culverts. Streetlights were located at the entrance of several culvert roosts. This finding is in contrast to the growing evidence that artificial lighting at night (ALAN) has significant, negative impacts on some species of bats through modifying landscape connectivity in urban environments (Moretto and Francis 2017, Haddock *et al.* 2019, Laforge *et al.* 2019, Russo *et al.* 2019). Several studies have demonstrated ALAN sensitivity of bats in the genus *Myotis* along riparian corridors (Haddock *et al.* 2019, Russo *et al.* 2019) and in regards to activity around culverts and bridges (Barré *et al.* 2020, Bhardwaj *et al.* 2020). We postulate our finding reflects the limited availability of roosts in this urban environment and urban *M. macropus* may be accepting unfavourable conditions to be able to utilise an available resource. Our modelling identified potential culvert roosting habitat was limited across Brisbane. Natural roosts like cavities, fissures and hollows associated with mature trees are also a limited resource in urban environments where mature trees are sparse (Le Roux *et al.* 2014).

The availability and distribution of roosts can influence the abundance and distribution of urban bat populations (Moretto and Francis 2017). Additionally, the most urban tolerant bats have been identified as those with flexible roosting strategies able to take advantage of roosting opportunities in urban environments (Jung and Threlfall 2018). The limited availability of both natural and artificial roosts may result in some highly flexible urban bats persisting in roosts despite being artificially lit (Russo and Ancillotto 2015). Light was not found to affect the number of *M. daubentonii* emerging from a priory roost in England, however the emergence time was delayed compared to emergence times recorded when artificial light was not on (Shirley *et al.* 2001). Delayed emergence time reduces foraging duration, and this can have implications for health and overall fitness (Boldogh *et al.* 2007). Our result could also reflect differences between ALAN impacts on roosting and foraging behaviour, similarly to Russo *et al.* (2017) who suggest ALAN affects drinking behaviour more significantly than foraging behaviour. Likewise, Russo *et al.* (2019) found ALAN had an effect on the foraging behaviour of *M. daubentonii* yet Spoelstra *et al.* (2018) found no effect of ALAN on commuting behaviour of *M. daubentonii*. We recommend further research to quantify the effect of ALAN on roost selection, emergence behaviour and health of urban bat populations.

Management implications

We expected roosts to be negatively correlated with increasing urbanisation, but no significant association between culvert occupancy and land use type was identified in our study. Despite the availability of 2666 concrete culverts across our study area, only 146 (5.48 %) met the preferred culvert dimensions within the optimal landscape position to provide potential roosting habitat. Our finding highlights how limited these roost sites are in an urban environment and suggests that culverts containing large colonies may be crucial sites for urban biodiversity conservation.

Tall culverts provide suitable roost sites for urban *M. macropus* populations, enabling this species to persist in a highly modified landscape. The limited availability of tall culverts can be linked to the scarcity of hollow bearing trees for both hollow roosting bats (Rhodes and Wardell-Johnson 2006) and birds (Davis *et al.* 2013a) in urban environments. Culverts >1.2 m in height, on stream orders 2-5 and located in landscapes with medium to high densities of waterways are critical urban habitat for *M. macropus* and sites of high conservation value for urban trawling bats. Disturbance to a culvert that results in roost exclusion or abandonment, has the potential to displace an urban bat population since culverts suitable for roosting are not highly available in urban environments and natural roost sites are scarce. Disturbance to this type of culvert, especially during the breeding season, may cause a significant disturbance to a roost. Roosting opportunities for *M. macropus* can be increased by installing culverts >1.2 m in height on waterways on all new road projects. Trawling bats are able to use flexible roosting strategies to persist in urban environments, but their existence depends on the identification, preservation and creation of urban day roosts.

Acknowledgments

We would like to thank Brisbane City Council for provision of the asset database and permission to access all culverts.

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Chapter 3: Roost selection in concrete culverts by the large-footed myotis (*Myotis macropus*) is limited by the availability of microhabitat



An empty lift hole in a culvert. Culverts containing microhabitat were identified as a limited resource in this chapter. Photo by V. Gorecki.

This chapter is published as Gorecki, V., Rhodes, M., & Parsons, S. (2020). Roost selection in concrete culverts by the large-footed myotis (*Myotis macropus*) is limited by the availability of microhabitat. *Australian Journal of Zoology*. Received 5 May 2020, accepted 10 August 2020, published online 25 August 2020.

Abstract

The large-footed myotis (*Myotis macropus*) is a specialist trawling bat with flexible roosting behaviour, being able to switch between caves, tree hollows and artificial roosts such as bridges, tunnels and culverts. However, little is known about how this species selects culvert roost sites in urban landscapes where hollows may be limited or absent. We surveyed 57 concrete culverts and found 21 *M. macropus* roosts comprising day and maternity roost sites; 305 bats were captured. Colony sizes averaged 20.6 ± 17.7 (range 4-49) for maternity roosts and 2.0 ± 0.8 (range 1-3) for day roosts. Roost culverts differed significantly from available culverts predominantly in terms of availability of microhabitat (lift holes and crevices). Roost culverts had lift holes that had greater cavity dimensions than available culverts and crevices were only found at roost culverts. Culverts containing microhabitat were a limited resource in this urban landscape and so increasing their availability may provide more urban roost sites for this specialist species.

Keywords

Myotis macropus, culvert, roost selection, microhabitat, urban, artificial roosts

3.1 INTRODUCTION

Urbanisation causes biodiversity decline on a global scale. Multiple studies have found negative impacts of urbanisation on biodiversity through habitat loss and fragmentation, road mortality, barrier effects, introduction of predators, chemical pollution and artificial lighting (van der Ree *et al.* 2015; Kingston and Voigt 2016). Despite these significant impacts, some species have adapted to human modified landscapes, and some even benefit from the increased temperatures, unique sheltering sites and predator avoidance opportunities provided in urban areas (Russo and Ancillotto 2015).

Bats are a globally diverse group of mammals with over 1400 species currently recognised (Simmons and Cirranello 2020). They are important components of the ecosystems they inhabit due to their

roles as pollinators, seed dispersers and control agents for pest insect species. The importance of these roles are magnified as not all faunal groups that fulfil similar roles have been able to adapt to urban landscapes (Kasso and Balakrishnan 2013; Kolkert *et al.* 2020). Bats are also threatened through loss of habitat and disturbance to roosts and are slow to recover from population declines (Evelyn *et al.* 2004). Bats commonly occur in urban areas (Russo and Ancillotto 2015; Jung and Threlfall 2018) and studies of roosting ecology have focused on selection of roost trees (Evelyn *et al.* 2004; Rhodes and Wardell-Johnson 2006; Threlfall *et al.* 2013), bat boxes (Rhodes and Jones 2011; Griffiths *et al.* 2017), bridges (Allen *et al.* 2011) and buildings (Evelyn *et al.* 2004; Ellison *et al.* 2007; Neubaum *et al.* 2007). These studies have found that urban landscapes have different patterns of roost availability, abundance and selection (Threlfall *et al.* 2013). Urban areas have reduced availability of roost sites and the availability and diversity of roosts directly influences the abundance and diversity of bats (Moretto and Francis 2017).

Protection of roost sites is dependent on knowledge of roost selection criteria. Roost site selection has consequences for the survival of bat populations and is influenced by a range of factors including thermoregulation, parasite avoidance, proximity to foraging resources, roost availability and abundance (Kunz *et al.* 2003). Bats may also use different roost sites throughout the year, depending on season and reproductive condition. Maternity roosts are considered the sites of greatest importance for the conservation of urban bat populations (Threlfall *et al.* 2013), as they are sites where females congregate to raise young (Neubaum *et al.* 2017).

The large-footed myotis (*Myotis macropus*) is a trawling bat that has a patchy distribution across northern and south-eastern Australia and is threatened across much of the southern part of its range (Churchill 2008; Campbell 2011). One of the recognised threats to the species is disturbance to roosts located in roads (Duncan *et al.* 1999). Due to its specialised foraging behaviour, the species is closely associated with waterways (Campbell 2009). In forested landscapes, it roosts in tree hollows on average 6.5 m above the ground with average cavity dimensions of 1.5-30 cm wide and 9-93 cm

long (Campbell 2009), usually within 100 m of a waterway. In landscapes where tree hollows and artificial roosts are available, *M. macropus* can switch between trees, bridges and tunnels, interchangeably (Campbell 2009; Law *et al.* 2020). However, little is known about how this species selects artificial roost sites in urban landscapes, especially where hollows may be limited or absent. Such information is important for understanding factors that may limit the success of this species in urban habitats and how conservation managers and those associated with the design and installation of culverts can aid in their conservation.

We investigated selection of concrete culverts for roosting by the large-footed myotis (*Myotis macropus*) in an urban landscape in Brisbane, Australia. The aim of our study was to characterise culverts used for roosting and identify how these differed to locally available culverts. We predicted that culverts selected as roost sites would be taller than available culverts and be surrounded by high densities of hollow-bearing trees to facilitate roost switching. A preference for culverts >1.5 m in height have been recorded in various studies investigating bat roosts in culverts (Keeley and Tuttle 1999; Meierhofer *et al.* 2019; Schulz 1998; Bender *et al.* 2010). We predicted *M. macropus* to select culvert roosts surrounded by high tree hollow densities due to the species' documented ability to switch between natural and artificial roost sites (Campbell 2009; Law *et al.* 2020) and we postulate that this roost switching behaviour is driven by predator avoidance. Additionally, we predicted that maternity roost selection would be preferential towards culverts with a western orientation to maximise solar exposure to provide suitable microclimates for unfurred pups due to the warming effect of solar radiation (Mering and Chambers 2014). We provide morphometric data for culvert roosting *M. macropus* as the first study to capture and record weights and measurements for this species.

3.2 METHODS

Study area

The study was conducted in the Local Government Area (LGA) of Brisbane City Council (BCC) in south-east Queensland, Australia (Figure 3-1). Brisbane has a predominantly urban environment with intensive land uses such as residential housing, industrial and commercial areas covering 67% of the LGA (ABARES 2016). Natural environments are located on the western fringe of the city and comprise 27% of the LGA. Water covers 6% of the LGA due to the meandering Brisbane River and its tributaries, as well as several large water supply reservoirs and dams (ABARES 2016). Brisbane is classified as having a humid subtropical climate in the Köppen climate classification system, which is characterised by hot and humid summers and mild winters (Peel *et al.* 2007). During the time of this study, the minimum daily temperature was 21.5°C, the maximum daily temperature 31.2°C, and average daily relative humidity was 58.6% (Australian Bureau of Meteorology 2020).

Culvert surveys

Field sampling was conducted during early Austral autumn in March 2019. The timing of the survey was intended to locate maternity roosts without disturbing non-volant young. Known *M. macropus* roost sites located in concrete culverts (V.Gorecki, unpublished data) were inspected during the day. If a culvert was occupied, it and the four nearest concrete culverts >1 m in height were also surveyed. The nearest culverts surveyed were restricted to concrete culverts >1 m in height to standardise the construction material and size class of culverts inspected and so reduce the variation between culvert variables.

Each culvert was assessed for the presence of bats. Culverts were categorised as roost or available culverts. Roost culverts included day roosts and maternity roosts and available culverts were culverts that were not occupied by *M. macropus*. Day roosts were defined as roosts without reproductive females or juveniles and maternity roosts were defined as roosts with pregnant and/or lactating females and juveniles.

Culvert height, width and length were measured, the number of barrels counted and the culvert design (box or pipe) were recorded. The presence of lift holes and crevices (i.e. microhabitat) were recorded and dimensions (radius and depth) measured using a measuring tape. Lift holes are circular holes on the inside of the ceiling of a culvert and are manufacturing features used to lift the culvert into location during road construction. Crevices are structural deformities within a culvert such as cracks or seals that have deteriorated.

Orientation of the culvert was recorded by taking a compass bearing along the log axis of the culvert, at the upstream end. Paired ambient and internal relative humidity and temperature were recorded by placing one HOBO Pro v2 U23-001 data logger (One Temp Pty Ltd, Australia) 10 m from the entrance to a culvert and one at the centre point within the culvert. Each HOBO was programmed to take simultaneous readings at one-minute intervals. Internal temperature and relative humidity were highly correlated ($r=0.73$, $p<0.0001$) and as relative humidity is of greater significant to bats (Mering and Chambers 2014), we removed temperature from further analysis. As humidity data was collected from different culverts at different times of the day, we used the difference between internal and ambient measurements ($\text{RH}_{\text{ambient}} - \text{RH}_{\text{culvert}}$) to standardise the microclimate data (Rhodes and Jones 2011).

Channel width at the downstream end of a culvert was recorded due to greater variation in channel width located at the downstream end of culverts (Liriano *et. al.* 2002). Vegetation height, vegetation cover and the number of stems per m^2 were measured using the point quadrant method with the point centred on the culvert on a 50 m sampling transect (Krebs 1989). The proportion of trees within each quadrant that were hollow bearing was also recorded to determine hollow bearing tree density (Threlfall *et al.* 2012). Diameter at breast height (DBH) for each tree in each quadrant was measured using a DBH tape.

Bat survey

Bats found in each culvert were captured by removing them from the roost site by hand or using a hand-net. Bats were placed into a cloth holding bag and weighed (with bag weight subtracted) using a 20 g Pesola spring scale (Pesola®, Switzerland). The right forearm length was measured to the closest 0.5 mm using Vernier calipers. Bats were aged as juvenile or adult based on ossification of the metacarpal-phalangeal joint as defined by Brunet-Rossini and Wilkinson (2009) and reproductive status was recorded in accordance with classes identified by Racey (2009). Bats were released at the location of capture on the same night. Bat capture and manipulation was carried out under permits from the Queensland Department of Environment and Science (Scientific Purposes Permit WA0001898), the Queensland University of Technology Animal Ethics Committee (AEC1700000540) and Biosafety Ethics Approval (1700000368).

Data analysis

Normality of morphometric data were tested using the Shapiro Wilk's test. Data for forearm measurements satisfied the assumptions of normality so parametric tests were used for this response variable. Raw and log transformed Log(X+1) data for weight did not satisfy the assumptions of normality. Thus, non-parametric tests were used for this variable. A two-way ANOVA with Bonferroni adjustment for multiple comparisons was used to test for differences in forearm measurements between sex, reproductive status and the interaction of these variables. We compared weights between male and female bats in reproductive and non-reproductive status using a one-way PERMANOVA. Each PERMANOVA was based on 9999 permutations under a reduced model.

A Mann-Whitney U test was used to compare between roost (day and maternity) and available culverts for all continuous variables, whereas a Fisher's exact test was used to assess the difference for categorical variables (culvert design (box or pipe) and stream order). A Rayleigh's test of

uniformity was conducted to determine if the orientation of each culvert was significantly different from random.

A Canonical Analysis of Principal Coordinates (CAP) was conducted to explore culvert roost variables (height, width, length, number of barrels, orientation, presence/absence of microhabitat (lift holes, crevices), difference in humidity) and landscape variables (channel width, vegetation cover, tree density, hollow density, tree diameter at breast height) in relation to culvert types (roost and locally available culverts). Normality of data were tested using the Shapiro Wilk's test. The data were log transformed ($\text{Log}(X+1)$) and normalised for the analysis and a Euclidean distance dissimilarity matrix was used.

To determine if there was a difference between roost types we used a one-way PERMANOVA based on 9999 permutations under a reduced model. The Similarity Percentages Procedure (SIMPER) with a Euclidean dissimilarity measure was used to identify variables contributing the most to the dissimilarity between roost and available culverts. A one-way analysis of similarities (ANOSIM) with a Euclidean similarity index was used to test the significance of the pairwise dissimilarities.

Differences were considered significant at the threshold of $p=0.05$. Normal data are presented as mean \pm one standard deviation (SD), minimum (min.) and maximum (max.) values and skewed data are presented as median \pm interquartile range (IQR). Statistical analyses were conducted in SPSS, PAST (Hammer et al. 2001) and R version v.3.4.2 (R Core Team 2013) using the 'vegan' (Oksanen et al. 2010) and 'circular' packages (Agostinelli and Lund 2013).

3.3 RESULTS

A total of 57 culverts were surveyed and 21 *M. macropus* roost sites were identified (Figure 3-1), comprising seven day and 14 maternity roosts. Colony sizes averaged 20.6 ± 17.7 (range 4-49) for maternity roosts and 2.0 ± 0.8 (range 1-3) for day roosts. *Myotis macropus* were absent from 36 culverts.

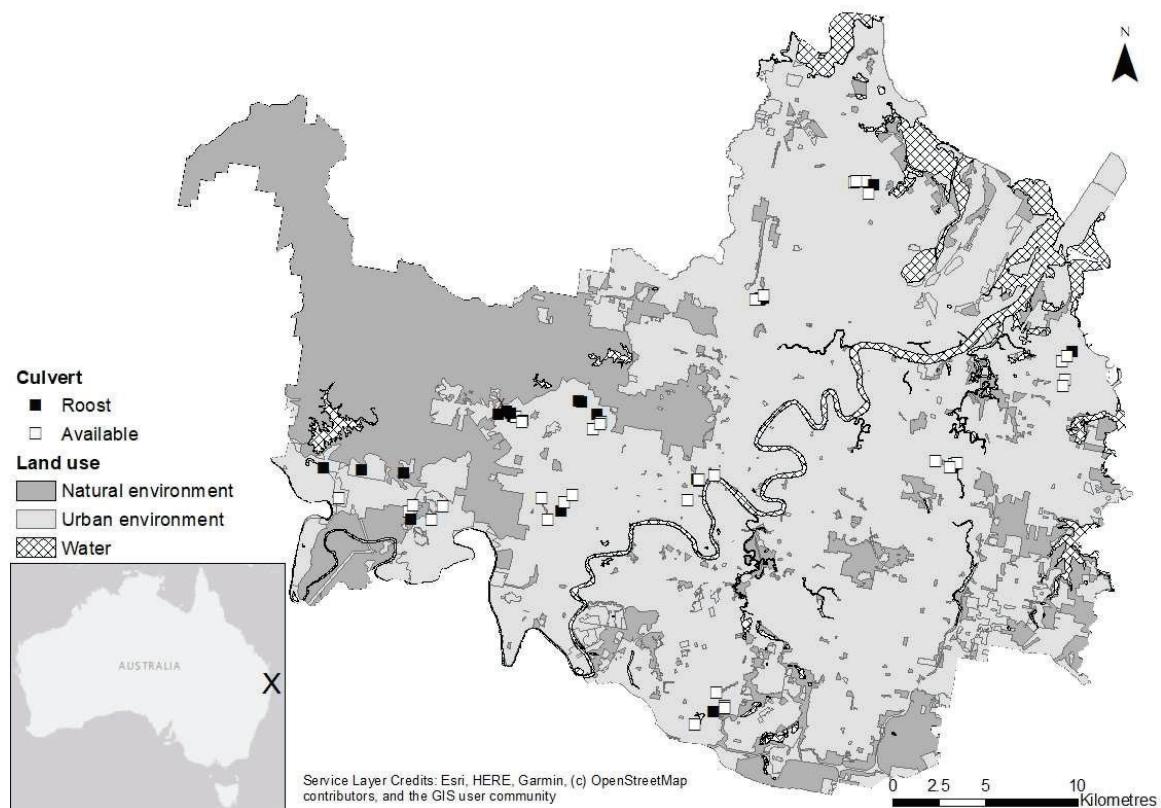


Figure 3-1 Location of *Myotis macropus* culvert roost sites showing a higher density of roost sites located along the western urban fringe of Brisbane in close proximity to natural environments comprising national parks and reserves.

Myotis macropus morphometrics

A total of 305 *M. macropus* individuals were captured from concrete culverts across Brisbane (Table 3-1). The sex ratio was skewed with females comprising 65.3% (females n=199, males n = 106) of captures. Pregnant females were the largest and heaviest bats captured while juvenile males were the smallest and lightest. Non-reproductive females were larger and heavier than males. Statistical analysis did not identify a significant interaction effect of sex by reproductive status on forearm length ($F_{(1,1)}=2$, $p=0.34$). However sex ($F_{(10,4)}=1$, $p=0.001$) and reproductive status ($F_{(26,9)}=2$, $p<0.0001$) significantly affected forearm length. Adult females had larger forearms than adult males and females of all reproductive conditions had larger forearms than males (Table 1). The PERMANOVA identified a significant interaction between weight and sex (total sum of squares = 23.76,

pseudoF_(19,15)=1; p=0.0001) and reproductive status (total sum of squares = 65.65, pseudoF_(52,9)=2; p=0.0001) on weight. No other comparisons detected statistically significant differences. We identified four non-volant pups during our survey.

Table 3-1 Forearm and weight measurements from *Myotis macropus* captured in concrete culverts in Brisbane.

Sex/age/rep status	n	Forearm (mm)			Weight (g)		
		Min	Max	Mean	Min	Max	Median±IQR
Adult females	184	37.1	43.0	40.0±0.9	7.6	15.0	9.9±1.1
lactating	2	39.5	40.6	40.0±0.7	10.1	11.0	10.5±0.5
post lactating	38	37.1	43.0	39.9±1.1	9.0	12.1	10.0±1.2
pregnant	11	39.0	42.0	40.3±0.9	10.1	15.0	12.2±1.7
non reproductive	133	37.8	42.6	40.0±0.9	7.6	11.7	9.9±0.6
Juvenile females	15	36.0	40.0	38.7±1.2	5.8	9.5	7.4±1.7
Adult males	88	36.0	42.0	39.7±1.0	6.4	10.9	9.5±0.9
reproductive	61	36.9	42.0	39.8±1.0	8.4	10.9	9.6±0.9
non reproductive	27	36.0	40.9	39.4±1.1	6.4	10.9	9.6±0.9
Juvenile males	18	32.8	39.8	38.1±1.6	5.74	9.3	7.9±1.9

Characteristics of culvert roosts

Bat roosts were located across the study region, however the distribution of roost sites in culverts was not uniform (Figure 3-1). The density of culvert roosts was higher along the western fringe of the city with few roosts located in the centre and to the east. Roost and available culverts differed significantly in height, number of lift holes and their dimensions, the presence of crevices and tree diameter at breast height (Table 3-2). Roost culverts were significantly taller (height) than available culverts ($U= 279$, $p=0.05$) (Table 3-2). One of the most obvious differences between roost and available culverts was the presence of lift holes ($U= 26$, $p<0.0001$). The number of lift holes at roosts culvert ranged from 6-260 (mean 54±66) while the number of lift holes at available culverts ranged from 0-136 (mean 5±24). Lift holes were both significantly deeper ($U= 46$, $p<0.0001$) and wider ($U= 36$, $p<0.0001$) at roost culverts. Lift holes at roost culverts averaged 14.5±3.3 cm (range 3.8-21.5 cm) in depth and 5.6±0.5 cm (range 4.5-6.3 cm) in width. The number of crevices at roost culverts averaged 1±3 (range 1-15) and no crevices were recorded at available culverts ($U= 297$, $p=0.003$).

Crevices averaged 48.6 ± 224.2 cm (range 0-1100 cm) in depth, 0.8 ± 1.5 cm (range 0-4 cm) in width and 81.8 ± 285.2 cm (range 0-1400 cm) in length.

Tree DBH was significantly larger at roost culverts compared to available culverts ($U= 249$, $p=0.01$) averaging 1.5 ± 2.0 m compared to 0.9 ± 0.5 m, respectively. Roost and available culverts did not differ in culvert design (box or pipe) ($p=0.08$), or stream order ($p=0.19$). The orientation of culverts did not differ significantly from a uniform distribution, meaning there was no relationship between a bat roost and the orientation of a culvert ($Z = 0.13$, $p=0.66$).

Table 3-2 Comparison of culvert and landscape variables between roost culverts and available culverts.

Variables	Roost culvert		Available culvert		Significance
	Range	Mean \pm SD	Range	Mean \pm SD	
Height (cm)	1.5-3.0	$1.9.0 \pm 0.4$	1.5-3.0	1.7 ± 0.5	0.05
Width (cm)	1.5- 3.6	$2.4.0 \pm 0.5$	0.3-3.6	2.1 ± 0.7	0.07
Length (cm)	0.7- 7.3	0.2 ± 0.2	0.1-0.1	0.2 ± 0.2	0.95
Barrels (n)	1-8	3 ± 2	1-7	3 ± 1	0.89
Humidity difference	2.0-25.3	13.0 ± 7.4	0.9-25.3	12.2 ± 7.2	0.61
Number lift holes (LH)	6-260	54 ± 66	0-136	5 ± 24	$p < 0.0001$
LH average depth (cm)	3.721.5	14.4 ± 3.2	0-20.0	1.4 ± 4.6	$p < 0.0001$
LH average width (cm)	4.5-6.2	5.5 ± 0.5	060.0	2.0 ± 10.5	$p < 0.0001$
Number of crevices	0-15	1 ± 3	0	0	0.003
Crevice average depth (cm)	0-1100.0	48.5 ± 224.2	0	0	0.003
Crevice average width (cm)	0-4.0	0.8 ± 1.5	0	0	0.003
Crevice average length (cm)	0-1400.0	81.8 ± 285.2	0	0	0.003
Channel width (m)	2.0-30.0	10.0 ± 8.0	2.0-20.0	8.0 ± 6.0	0.4
Diameter at breast height (m)	0.3-10.7	1.4 ± 2.01	0.2-2.9	0.9 ± 0.5	0.02
Vegetation cover (%)	23.0-95.0	58.0 ± 18.0	14.0-96.0	57.0 ± 20.0	0.92
Vegetation height (m)	4.6-17.2	9.8 ± 3.6	3.6-18.2	8.6 ± 3.7	0.21
Tree density (trees/km ²)	0-0.2	0.04 ± 0.08	0-0.6	0.08 ± 0.1	0.08
Hollow density (trees/km ²)	0-4.0	0.4 ± 0.9	0-4.0	0.4 ± 1.1	0.25

Statistical significance of Mann-Whitney U test

Roost culverts compared to available culverts

CAP analysis identified differences between roost (day, maternity and vacant) and available culverts (Figure 3-2). Ordination showed that available culverts were clearly separated from roost culverts and this was associated with the presence or absence of microhabitat. There was no discrimination between roost types based on the variables measured, and roost culverts were positively associated

with the presence of microhabitat, culvert height, culvert width and DBH. The greatest difference between roost and available culverts was the presence of microhabitat which contributed 13.6% to the dissimilarity between the two groups. Examples of microhabitat features are shown in Appendix 2. When combined, five variables contributed 50% of the differences between roost and available culverts: microhabitat (13.6%), DBH (10.3%), height (8.7%), channel width (8.7%) and number of barrels (8.5%). The dissimilarity between roost and available culverts was significant ($R=0.08$, $p=0.04$) although the dissimilarity was low. A difference between roost types (i.e. day and maternity) was not detected (total sum of squares 180.6, pseudoF=0.6; $p=0.7$).

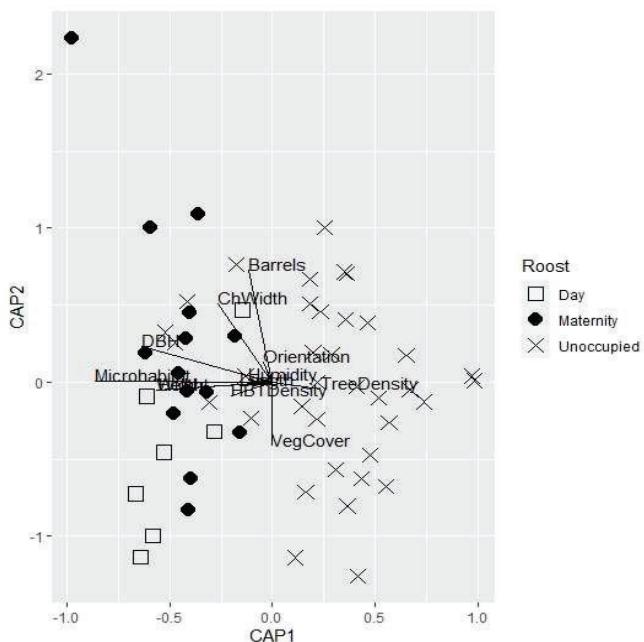


Figure 3-2 Canonical analysis of principal coordinates (CAP) showing associations with culvert and landscape variables for roost and available culverts. Roost and available culverts are separated by the presence or absence of microhabitat (lift holes, crevices). Roost culverts are positively associated with the presence of microhabitat, culvert height, culvert width and DBH.

3.4 DISCUSSION

This study demonstrated that *M. macropus* use concrete culverts as day and maternity roosts in an urban environment and that roost selection is explained by the availability of lift holes and crevices within culverts. We predicted that culverts selected as roost sites would be taller than available culverts and be surrounded by high tree hollow densities to facilitate roost switching. We also

predicted that maternity roost selection would be preferential towards taller culverts with a western orientation to retain afternoon heat. Our results revealed that roost culverts were taller than available culverts, but we found no evidence of a relationship with the availability of hollows or culvert orientation. We also found no evidence of differentiation in selection criteria between roost types.

Culvert roosting *Myotis macropus*

The population of *M. macropus* captured in our study shared morphometric similarities to populations measured in other studies. The weights and forearm measurements of adult bats were within the range recorded in southern Australia (Churchill 2008). During our survey we discovered non-volant young in late March which suggests that *M. macropus* in Brisbane may have a third breeding event. Reproduction in *M. macropus* varies with latitude with the species having one litter in Victoria (November-December, Churchill 2008), two litters in northern New South Wales/southern Queensland (October and January) (Dwyer 1970; Law *et al.* 2020) and three litters in central Queensland (September, December and March) (Dwyer 1970).

Our discovery of non-volant young in culvert roosts in Brisbane in March is a novel finding. Dwyer (1970) studied the reproductive cycles of *M. macropus* roosting in a disused rail tunnel near Brisbane and a storm water tunnel in Rockhampton, central Queensland. Two breeding events were recorded in Brisbane (October and January) and three breeding events were recorded in Rockhampton (September, December and March). Lloyd *et al.* (1999) studied reproductive strategies of *M. macropus* (= *Myotis moluccarum*) at the same tunnel roost as Dwyer (1970) and identified two breeding events of similar dates to Dwyer (1970). Polyestry is reported in subtropical Vespertilionid bats (Wilson and Findley 1970; Myers 1977) but does not appear to be related to latitude for all polyestrous vespertilionids. Indeed, latitude would not explain our result as our study location is at a higher latitude than that described by Dwyer. The breeding event we detected may be due to the fact that culverts, by design, are located on waterways and may provide a specialist trawling bat a

roost location in close proximity to foraging resources. Allen et al. (2010) compared populations of Brazilian free-tailed bats (*Tadarida brasiliensis*) roosting in bridges and caves and found that pups born at bridge roosts were heavier and larger at birth and grew faster than those born at cave roosts. This difference was attributed to the bridge roost having higher roost temperatures, being closer to foraging areas and larger mothers at the bridge roost having larger pups at birth. Similarly, Blanco et al. (2015) did not find that polyoestry in mouse lemurs (genus *Microcebus*) was associated with seasonal conditions, rather the ability of females to access high quality food or food throughout the year. It is clear that this pattern of reproduction in *M. macropus* is worthy of further study.

***Myotis macropus* roost selection in concrete culverts**

We observed differences between roost culverts and available culverts. Roost culverts were taller, had more available microhabitat and were surrounded by trees with greater DBH. Roosts were located in culverts >1.5 m in height, and this is consistent with findings in other studies (Schulz 1998; Keeley and Tuttle 1999; Bender et al. 2010; Meierhofer et al. 2019;). A comprehensive survey across North America reported that bats had a preference for concrete culverts 1.5-3 m in height (Keeley and Tuttle 1999). Similarly, Schulz (1998) found a significant positive relationship with bat roosts and culvert height. Our study found bats select similar culvert dimensions, which could be due to several factors. Meierhofer et al. (2019) suggest taller culverts are easier for bats to find, enter and navigate, especially in a landscape where other roost sites are limited. Culverts may also provide roost sites with increased protection from predators. Concrete surfaces constructed to facilitate the flow of water under a road are generally smooth, and taller culverts may provide more protection from predators unable to scale smooth surfaces. Finally, lift holes are manufacturing elements associated with lifting and moving culverts into location during road construction. Lift holes are generally only manufactured for culverts 1.2-3.6 m in height (Department of Transport and Main Roads, pers. com). As such, lift holes are only present in taller culverts although specifications require lift holes to be sealed over on completion of construction (Department of Transport and Main Roads 2017).

The presence of lift holes and crevices (microhabitat) was a clear difference between roost and available culverts. Roost culverts had lift holes that were either not sealed or had seals which had deteriorated or fallen out, providing gaps for bats to roost within. Roost culverts also had a greater mean number of accessible lift holes, and lift holes at roost culverts were both deeper and wider, providing more cavity space for bats to roost in. Available culverts had lift holes which were sealed over or only partially deteriorated. Crevices were only located at roost culverts near deteriorated seals at the end of joins within a culvert. Our findings are consistent with several studies that have found that bats, especially of the genus *Myotis*, have adapted to roosting within culvert manufacturing features like lift and weep holes, and deterioration features like crevices (Pottie *et al.* 2005; Bender *et al.* 2010; Gonsalves and Law 2017; Lutsch 2019).

Gonsalves and Law (2017) studied foraging habitat of a colony of *M. macropus* roosting in lift holes in a jetty in Sydney, Pottie *et al.* (2005) note the most frequent roost sites for *M. aduersus* in Singapore were the weep holes of stormwater drains, and Lutsch (2019) consistently found *Perimyotis subflavus* and *M. austroriparius* roosting in weep holes in culverts on roads in Georgia. Similar to our study, Bender *et al.* (2010) found three bat species, including *M. austroriparius*, roosting in crevices and weep holes in culverts on roads around Alabama and found that unoccupied culverts did not contain any weep holes or crevices. Lutsch (2019) studied microclimates in culverts and found that weep holes have higher average daily temperatures than the rest of a culvert and they are able to provide thermal stability. Thermally stable roosts can minimise energetic costs associated with thermoregulation, gestation, embryonic development and lactation (Smith and Stevenson 2013).

In addition to microhabitat variables, the immediate landscape surrounding a culvert appeared to have an influence on roost selection. Roost culverts had significantly larger trees (DBH) surrounding the culvert than available culverts. There was no difference between roost and available culverts for all other vegetation associated variables. This result could indicate vegetation variables may not be

important factors in roost selection in highly modified urban landscapes where roost sites are limited. Meierhofer *et al.* (2019) found bats were more likely to be present in culverts with limited surrounding vegetation and explain their finding as an artefact of the highly urbanized nature of their study area. Roost culverts in our study were clustered along the western fringe of Brisbane in close proximity to natural environments such as national parks and conservation reserves. Our findings could reflect the clustering of sites on the urban fringe where tree cover is higher than densely built up areas of the city centre and the east coast (Hourigan *et al.* 2010).

Larger trees at roost entrances have the potential to assist with predator avoidance on emergence. Fenton *et al.* (1994) observed raptors hunting bats emerging from bridge and building roosts into open air space in South Africa. Bats would emerge from their roost into open air space and were subjected to aerial hunting by a range of raptor species. Keeley and Tuttle (1999) found *Corynorhinus rafinesquii* roosted in low bridges surrounded by thick vegetation and one colony abandoned their roost immediately after vegetation was removed. Culverts with large trees in the immediate landscape could provide more protection for predators to bats emerging from roost sites lower in the landscape into clutter space than roost sites which exit into high, open air space such as roosts inside bridges and buildings. Roost culverts with larger trees present in the immediate landscape could provide additional protection from predators.

We found no difference between roost types. We targeted maternity season to capture the selection criteria for this critical time period, and it is likely that the absence of differentiation between roost types reflects the limited availability of microhabitat (lift holes and crevices) within culverts in our study. This finding could also reflect our small sample size and a higher number of day roosts sampled may have been able to detect a difference between roost types. However, our finding is consistent with the results of Caddle (1998) and Campbell (2009) who found no preference in roost selection by *M. macropus*. These authors did not find differences between *M. macropus* roost trees and available trees, only a preference for roost sites located in close proximity to water.

The absence of clear roost selection behaviour by *M. macropus* in Campbell (2009) was attributed to the uniform age of vegetation surrounding the reservoir and this is similar to our finding that microhabitat features such as lift holes and crevices are limited within culverts in our study. Although this specialist species may demonstrate flexible roosting behaviour by roosting in both natural and artificial roosts, roost sites being a limited resource in both natural and urban landscapes will contribute to their vulnerability.

Management and conservation implications

Myotis macropus is a specialist trawling bat that has managed to persist in highly modified, urban landscapes. Their specialised foraging behaviour increases their risk of extinction as they occupy a specific ecological niche and are susceptible to disturbance to waterways (Safi and Kerth 2004). This specialisation may also benefit the species as riparian corridors buffer waterways from surrounding landscape disturbance (Law *et. al.* 2020). While the species has been able to persist in anthropogenic landscapes, this study has shown that roost selection is limited by the availability of microhabitat (lift holes and crevices) within culverts. Lift holes are restricted to culverts 1.2-3.6 m in height and lift holes are intentionally sealed post construction. As a result, not all culverts >1.2 m provide an opportunity for roost selection.

Disturbance to culvert roosts should be avoided. Any disturbance to a roost site within a culvert has the potential to have a significant impact on an urban population of *M. macropus* due to the limited availability of microhabitat within other locally present culverts, further contributing to the risk of extinction of a specialist species. If a roost is abandoned due to disturbance, there are limited culvert roost alternatives available in the local landscape. Disturbance to roosts in culverts should be avoided because of their significance to urban *M. macropus* populations due to the limited availability of microhabitat within culverts. Roost culverts should be prioritised for retention in urban environments and appropriate management plans developed where culvert repair, maintenance and replacement are required for culverts >1.2 m in height. Management plans should document

procedures to ensure disturbance does not occur at critical time periods such as when non-volant young are present. Where disturbance to roost culverts cannot be avoided, targeted surveys of culverts >1.2 m in height should be undertaken to identify the presence and distribution of roosts and confirm the availability of alternative roost sites.

Additional culvert roost sites in the urban environment may be created by providing microhabitat in existing culverts. For example, lift holes may be unsealed. Incorporating lift holes into new culverts or during repairs to existing culverts, would increase the availability of urban roosting habitat for this specialist species. Successful colony relocations have occurred for *M. macropus* roosts in bridges through the provision of timber bat boxes prior to demolition of a bridge or portions of the original roost site being secured to new structures (Williams 2006; Hoye and Hoye 1999; Marshall 2011; Thomson 2014). However, the provision of bat boxes only has temporary conservation applications (Rhodes and Jones 2011). Bat boxes require regular maintenance to continue to provide habitat (Rueegger 2016; Griffiths *et al.* 2017) and flooding events are likely to dislodge and remove boxes installed in waterways. A permanent roost alternative would be to leave lift holes unsealed to increase the amount of microhabitat available to *M. macropus* in urban areas.

Increasing the amount of potential roost habitat available to this specialist species will contribute to their ability to persist even in highly modified landscapes. Managing artificial *M. macropus* roost habitat is complex and this study has shown that not all culverts provide equal roosting habitat in an urban landscape.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgements

The authors thank Brisbane City Council for the provision of the asset database and for permission to access council owned culverts. Thank you to Leroy Gonsalves for assistance with the CAP and to Lisa Cawthen for providing feedback on an early draft of this manuscript. VG was supported by a Research Training Program Stipend Scholarship. This research did not receive any specific funding.

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Chapter 4: Landscape use in an urban environment by a specialist trawling bat, the large-footed myotis (*Myotis macropus*)



The large-footed myotis (*Myotis macropus*) has enlarged hind feet which is a morphological adaptation for capturing prey directly from, or beneath, water surfaces. Photo by V. Gorecki.

This chapter is in preparation for submission to the journal *Biological Conservation*.

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Abstract

Urban environments are spatially heterogeneous landscapes comprising remnants of the natural environment and features unique to anthropogenic environments. Some species are able to persist and even thrive in urban environments while others are not, and detailed investigations of behaviour and ecology are required to distinguish life history and other traits that differentiate them. The most urban tolerant insectivorous bat species are those with greater mobility like open and edge aerial foragers and trawling bats, and species with flexible roosting strategies. However, despite greater mobility, trawling bats are also specialist species due to the unique ecological niche they occupy. Little is known about how trawling bats use urban environments. We studied roost use, home range and patterns of land use selection in an urban population of a specialist trawling bat with flexible roosting strategies, the large footed myotis (*Myotis macropus*). We captured bats roosting in road culverts and radio-tracked 13 non-reproductive females over two seasons. We radio-tracked bats to a total of three day-roost sites: two culverts and a bridge. Bats switched roosts every 4.8 ± 3.7 (1-11) days. Home ranges (95% Kernel Density Estimate) did not differ between seasons and ranged from 5-277 ha. Core use areas (50% Kernel Density Estimate) differed between seasons with median winter areas (22 ha) significantly larger than median summer areas (1 ha). Land use selection was investigated using compositional analysis and *M. macropus* showed a significant preference for the recreation land use type at the landscape and home range scale. Transport, industrial, railways and public services land use types were used in proportion to their availability at the landscape scale. Our result suggests that *M. macropus* uses foraging behavioural plasticity to exploit food resources available in urban environments. Maintaining spatial heterogeneity in urban planning and design will provide a landscape mosaic for urban adapted *M. macropus* to persist.

Keywords

Myotis macropus, urban environment, home range, trawling, artificial roost, behavioural plasticity

4.1 INTRODUCTION

Urban environments have traditionally been viewed as homogenous patches that differ from natural environments due to anthropogenic modification (Cadenasso *et al.* 2007, Li and Wilkins 2014). Urban landscapes are unique in that they retain components of the natural environment, such as topography, vegetation and waterways, and include anthropogenic features which vary in intensity from high density residential housing to open green space. This combination of artefacts from the natural environment and human design features result in spatially heterogeneous environments

containing patches of natural and artificial features (McKinney 2002, Threlfall *et al.* 2013b, Li and Wilkins 2014). Urban species able to utilise the heterogeneity that is unique to urban environments may persist (McKinney 2002, Threlfall *et al.* 2013b, Lintott *et al.* 2015), while species unable to utilise urban space will be lost from urban environments.

There is growing evidence that some species are not just persisting in urban environments but are thriving. A wide range of bird and mammal species, such as northern goshawks (*Accipiter gentilis*) (Rutz 2006), red squirrel (*Sciurus vulgaris*) (Jokimäki *et al.* 2017), and red fox (*Vulpes vulpes*) (Kauhala *et al.* 2016) are well documented in their ability to exploit urban environments. Many species of insectivorous bats (referred to as bats from here on) have also persisted in urban environments due to the provision of permanent or seasonal roost sites in the form of buildings (Brigham 1991, Evelyn *et al.* 2004), bridges (Keeley and Tuttle 1999) and culverts (Wojtaszyn *et al.* 2013). Day roosts are essential to the survival of bats as they provide protection from predators, have stable microclimates and are locations of social interactions (Kunz 1982, Lewis 1995). Urban environments may also provide bats with higher densities of food resources such as insects attracted to lights (Rydell 2006). These resources can even enable some species to increase the number of offspring produced each year (Ancillotto *et al.* 2015).

The capacity of bat species to persist in urban environments is trait dependent. The most urban tolerant bat species are correlated with greater flexibility in resource requirements (e.g. roosting strategies) and mobility (e.g. open and edge aerial foragers and trawling bats) (Jung and Threlfall 2018). However, trawling bats display morphological, echolocation and behavioural adaptations due to their specialisation of foraging over water (Campbell 2011). All trawling bats display enlarged hind feet, hairy legs, strengthened calcars and relatively short tails which are morphological adaptations for capturing prey directly from, or beneath, water surfaces (Campbell 2011, Aizpurua and Alberdi 2018). Species with specialist adaptations occupy a narrow ecological niche, and are not considered flexible in their resource requirements, therefore making them more prone to extinction (Safi and Kerth 2004). Habitat specialisation places bats at higher risk of extinction than dietary specialisation, however, some trawling species are commonly found in urban environments (Safi and Kerth 2004). How trawling bats use the spatial heterogeneity found in urban environments and are able to persist is unknown.

Myotis macropus, the only species of the genus found in Australia, is a specialist trawling bat often found in urban environments. It has a patchy distribution across northern and south-eastern Australia and is threatened across much of the southern part of its range (Campbell 2009). *Myotis macropus* forages directly over waterways and has a diet of predominantly aquatic insects and fish

(Dwyer 1970a, Thompson and Fenton 1982, Robson 1984, Jansen 1987, Jones and Rayner 1991, Law and Urquhart 2000, Burns 2005). *Myotis macropus* has only been found roosting within 100 m of water (Dwyer 1970, Campbell 2009, Gonsalves and Law 2017, Law *et al.* 2020). However, it roosts in a range of structures and has been recorded roosting in bridges, tunnels, jetties, road culverts and stormwater drains (Barclay *et al.* 2000, Anderson *et al.* 2005, Churchill 2008, Campbell 2009, Gonsalves and Law 2017). *Myotis macropus* also switches interchangeably between roosting in trees and artificial roost sites such as bridges and tunnels (Campbell 2009, Law *et al.* 2020). However, little is known about roost switching and roost fidelity of this species and how this may contribute to its ability to persist in an urban environment. Additionally, home range sizes for this species are unknown and it is unclear how this specialist bat uses landscapes within an urban environment.

We studied fine scale movements of *M. macropus* to examine how a specialist trawling bat uses an urban environment. Specifically, we used radio-tracking to study roost use, home range and patterns of land use selection. We predicted *M. macropus* would switch between using tree roosts and artificial roosts, as reported by other authors (Campbell 2009, Law *et al.* 2020). We also predicted that bats would show a preference for the land use type associated with waterways due to their specialised foraging behaviour and show avoidance of all other land use types. We use our findings to suggest conservation and management strategies for this species in urban environments.

4.2 METHODS

Study area

The study was conducted in the Local Government Area (LGA) of Brisbane City Council (BCC) in south-east Queensland, Australia. Brisbane has a humid subtropical climate (according to the Köppen climate classification system; (Peel *et al.* 2007) . The climate has hot, humid summers (October-April), with mean daily temperatures of 25°C and mild winters (May-September) with mean daily temperatures of 18°C (Australian Bureau of Meteorology 2020). Mean yearly rainfall is approximately 1100 mm. The urban landscape of BCC is dominated by residential housing, industrial and commercial areas covering 62% of the LGA (ABARES 2016). Conservation and natural areas are located on the western fringe of the city and cover 27% of the LGA, while production landscapes, including dryland agriculture, irrigated agriculture and plantations, are disseminated and cover 5% (ABARES 2016). Water covers 6% of the LGA due to the meandering Brisbane River and its tributaries, as well as several large waterbodies that include water supply reservoirs and dams. Brisbane has an average population density of 18 people per hectare (ppl/ha), which is lower than the population densities in the cities of Sydney (29 ppl/ha), Vancouver (31 ppl/ha) and London (64

ppl/ha) (Spencer *et al.* 2015). Approximately 75% of the Brisbane population lives in areas defined as low-medium population density, with densities between 4 and 30 people per hectare, compared to 38% in Sydney, 29% in Vancouver and 9% in London (Spencer *et al.* 2015).

The study site selected for radiotelemetry was located in an urban mosaic landscape in northern Brisbane (-27.418129° 152.999217°) (Figure 4-1). We selected this location because it contained three culverts that were known to be *M. macropus* roost sites over multiple seasons and years (V Gorecki, unpublished data). The study area had permanent water and potential roost sites in the form of hollow bearing trees, bridges and culverts in an urban landscape comprising eight types of land uses (example aerial imagery shown in Appendix 3).

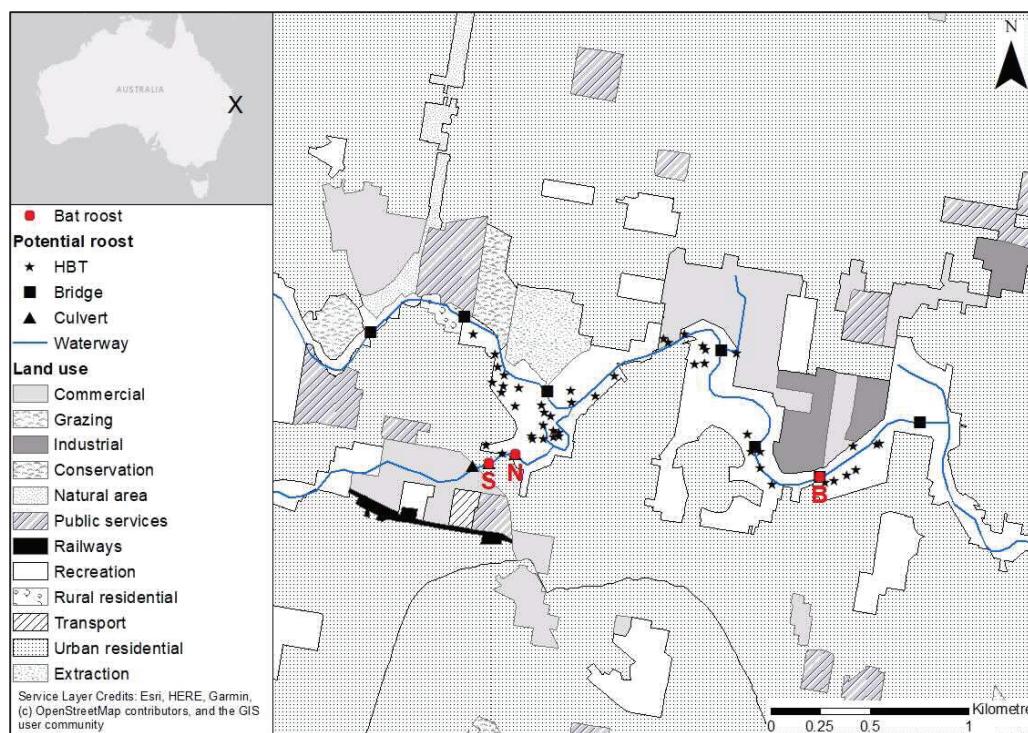


Figure 4-1 Land use types present in the study area. Availability of hollow bearing trees (HBT), bridges and culverts are shown along waterways. Day roost sites are shown in red and B= bridge, N= northern culvert S= southern culvert.

Eight urban land use types were identified using the Australian Land Use and Management Classification (ALUM) ArcGIS file (ABARES 2016):

1. Commercial: land with shops and markets, high density of building and lighting with limited natural areas.
2. Industrial: factories, workshops, industrial uses, moderate density of buildings and lighting with moderate natural areas in the form of landscaped gardens.

3. Public services: schools, community services, cemeteries, low density of buildings with moderate-high natural area cover in the form of playgrounds and gardens.
4. Railways: permanent rail transport tracks and associated infrastructure, low density of buildings, high density of lighting and limited natural areas.
5. Recreation: parklands, sportsgrounds, outdoor greenspace, low density of buildings, low density of lighting and high natural area cover in the form of waterways, riparian zones and green space.
6. Transport: land allocated to infrastructure used for transportation of goods or people, moderate density of buildings and lighting with moderate natural areas in the form of landscaped gardens.
7. Urban residential: land with houses, apartments, roads and streetlights, high density of buildings, high density of lighting and limited natural areas.
8. Extraction: water extraction, purification, treatment or supply of fresh water for public, domestic and commercial use, low density of housing and lighting and high natural area cover.

Bat capture and radiotelemetry

A total of 13 non-reproductive female bats were captured and tagged in August 2018 ($n= 5$), February 2019 ($n= 4$) and August 2019 ($n= 4$). A capture session was planned for Feb 2020 but high rainfall resulting in flooding of the study site and meant this second summer survey was unable to be completed. We defined August as a winter survey and February as a summer survey. Bats were located roosting in lift holes, which are circular holes located on the ceiling of a culvert and are used to lift the culvert into place during construction. For each capture event, a hand net was placed over roost holes and bats were captured as they emerged at dusk. This ensured that we were certain that captured *M. macropus* had selected a culvert roost at least once. We recorded sex, weight, and forearm measurement, and bats were aged as adult or juvenile based on ossification of the metacarpal-phalangeal joint (as defined by Brunet-Rossini and Wilkinson 2009).

We identified the reproductive status of females as pregnant if a foetus was present in the abdomen as determined by gently palpation; lactating if milk was secreted from the nipples or if no hair was present around the nipples; post-lactating if no hair was present around the nipples but no milk was secreted; or non-reproductive if none of the previous criteria applied (Racey 2009). All non-reproductive females present within the roost were tagged for each survey. Sample sizes were limited by the number of non-reproductive females present within the roost at the time of capture.

We trimmed a small patch of hair around the upper scapulae and attached a radio-transmitter (Model LB-2X; Holohil Systems Ltd., Carp, Ontario, Canada) to each individual using VetBond (3M, Animal Care Products, St. Paul, USA). Bats were released at the capture site. Transmitters weighed 0.31 g and were <5% of the weight of the tagged animals (Aldridge and Brigham 1988). Bat capture and manipulation were carried out under permits from the Queensland Department of Environment and Science (Scientific Purposes Permit WA0001898), the Queensland University of Technology Animal Ethics Committee (AEC1700000540) and Biosafety Ethics Approval (1700000368).

We tracked tagged bats using three-element Yagi antennae and Australis 26k scanning receivers (Titley Scientific, Brendale QLD). Bats were located during flight by homing in on the signal or through triangulation from teams located at different locations (White and Garrott 1990). Fixes were recorded by taking a compass bearing of the location of a tagged bat. Fixes were taken every 5 minutes and used to triangulate their locations using Locate III (Nams 2005). Accuracy of home range estimates improve at short time intervals (De Solla *et al.* 1999). Fix locations were considered unreliable if the size of the 95 % error ellipse was > 1 ha (Nams 2005). When a tagged bat was not detected during a simultaneous reading, no data was recorded. Calibration of signal strength and distance to the transmitter were tested on tagged bats in day roosts and were repeated throughout the study to ensure consistency. Day roosts were 3 m tall culverts that were at parkland level (refer Appendix 3 for photographs) and provided the only opportunity to calibrate signal strength between observers. If the signal was at full strength, the transmitter was within 50 m of the receiver while weak strength was approximately 150 m. We tracked individual bats for an average of 8 nights (range 3-12) the day after capture and release and attempted to follow individuals from the time they exited day roosts at dusk until they returned to their roost. We defined a day roost as the location of a tagged bat during the day.

Roost use

We calculated use for each day-roost as the number of days spent at that site by each tagged bat divided by the total number of tracking days, multiplied by 100 (Rhodes 2007). We calculated roost fidelity by dividing the number of days spent by each bat in a particular roost by the total number of tracking days, and then expressed this as a percentage. We calculated roost switching as the number of consecutive days an individual returned to a day roost divided by the number of tracking days. To determine roost switching we only included individuals that had a minimum of three consecutive days of tracking (Russo *et al.* 2005).

Home range and core use area estimation

We used the fixed kernel density estimate (KDE) method to estimate the 95% (home range) and 50% (Core use area) probability contours for each radio-tagged individual. Our data were clustered so we used the *ad hoc* bandwidth (h_{adhoc}) rather than the least-squares cross-validation (h_{lsvc}) or reference bandwidth (h_{ref}) because h_{adhoc} and h_{lsvc} tend to result in over-smoothed densities, inflated home range size estimates and increased Type I errors for clustered data (Seaman *et al.* 1998, Kie 2013, Anthony and Sanchez 2019). The *ad hoc* method is also less sensitive to sample size variation between individuals (Schuler *et al.* 2014), and is not affected by spatially correlated points (Kie 2013). We used OpenJUMP HoRAE to estimate kernel density polygons for each bat (Steiniger and Hunter 2012) and ArcGIS v10.6 to measure the home range span by measuring the distance from one edge of the 95% KDE home range to the other. We calculated the minimum convex polygon (100% MCP) using the minimum bounding geometry tool in ArcGIS to enable comparisons to older studies.

Landscape use

We assessed land use selection by compositional analysis (Aebischer *et al.* 1993). Compositional analysis identifies land use selection at multiple scales and each tagged animal makes a contribution to the analyses, regardless of the number of location fixes obtained. This approach sits at an animal level, taking animals rather than location fixes as the sampling unit and thereby avoiding issues with autocorrelation typically associated with radiotelemetry data (Fattorini *et al.* 2017). We assessed land use selection at two scales; placement of home ranges on the landscape (second-order) and use of habitats within home ranges (third-order) (Johnson 1980).

We defined the second-order study area in ArcGIS v10.6 by dissolving the 95% KDE for all bats into one polygon encompassing the area used by all bats. We calculated the proportion of each land use type within the study area. We then calculated the proportion of each land use type used by each bat and compared this to the proportion of each land use type within the study area (Fattorini *et al.* 2017). We defined the third order study area in ArcGIS v10.6 using 95% KDE for each individual and calculated the proportion of each land use type available to each bat. We then calculated the percentage of location fixes occurring within each land use type, for each bat (Fattorini *et al.* 2017).

Non-parametric tests of land use selection were assessed in the “*phuassess*” package for R 3.6.3 due to lack of normality in the data (Fattorini *et al.* 2017).

Statistical analysis

A one-way permutational multivariate analysis of variance (PERMANOVA) was performed to test for differences between seasons. The PERMANOVA was based on 9999 permutations under a reduced model with a Bonferroni adjustment for multiple comparisons. We used a one-way PERMANOVA to test for the effect of including individuals with fewer than 30 fixes on 95% and 50% KDE's between seasons because a minimum sample size of 30 is recommended for kernel density estimation (Seaman *et al.* 1999). To assess the difference in Core use area size between seasons we used a Mann-Whitney *U* test.

To determine whether variability in the number of fixes between individuals influenced home range size we used Spearman's correlation coefficient to assess the integrity of the data by testing for correlations between the number of tracking nights and 100% MCPs, 95% and 50% KDEs, and between the number of fixes and 100% MCPs, 95% and 50% KDEs. Significance levels were set at the threshold of $p<0.05$. Parametric results are presented as mean \pm standard deviation and non-parametric results are presented as median and interquartile range (IQR). Statistical tests were performed using the software PAST (Hammer *et al.* 2001).

4.3 RESULTS

A total of 13 non-reproductive adult female bats were tracked for 3-12 days (Table 4-1). The average number of fixes per individual was 83.1 ± 41.6 (Table 4-1). No significant difference was found between winter and summer in 95% KDE (home range) (Sum of squares= 1.04E05, Pseudo-*F*= 3.5, $p=0.07$) so data were pooled for home range analysis across seasons. A difference was found between winter and summer in 50% KDE (Core use area) (Sum of squares= 5932, Pseudo-*F*= 4.9, $p=0.04$), however a post-hoc test failed to differentiate between seasons.

Three bats did not meet the recommended sample size of 30 fixes for kernel density estimation (fixes of 11, 18 and 26). We removed these three individuals and tested for a difference between winter and summer and no significant difference was found for home range (Sum of squares= 9.8E04, Pseudo-*F*= 3.24, $p=0.09$) or core use area (Sum of squares= 5249, Pseudo-*F*= 3.62, $p=0.07$). We have included these individuals in our analysis as they provide insight into relative use in a highly urban landscape (Patriquin *et al.* 2019).

Table 4-1 Tracking results for non-reproductive female *Myotis macropus* showing season, number of tracking nights, number of fixes, range spans, home ranges (100% MCP and 95% KDE) and core use areas (50% KDE).

Bat code	Season	Number of tracking nights	Number of fixes	Range span (km)	100% MCP (ha)	95% kde (ha)	50% kde (ha)
1	Winter1	11	111	2.3	77	95	17
2	Winter1	3	18	1.8	30	68	17
3	Winter1	11	70	3.1	79	202	39
4	Winter1	12	104	2.5	62	103	21
5	Winter1	11	89	1.9	64	105	22
6	Summer	7	115	1.7	33	5	0.8
7	Summer	10	110	1.6	29	6	1
8	Summer	7	97	1.2	19	7	1
9	Summer	4	26	1.2	15	106	22
10	Winter2	11	149	1.9	39	9	4
11	Winter2	4	78	3	30	248	60
12	Winter2	11	103	3.1	79	277	65
13	Winter2	4	11	2.6	73	173	49

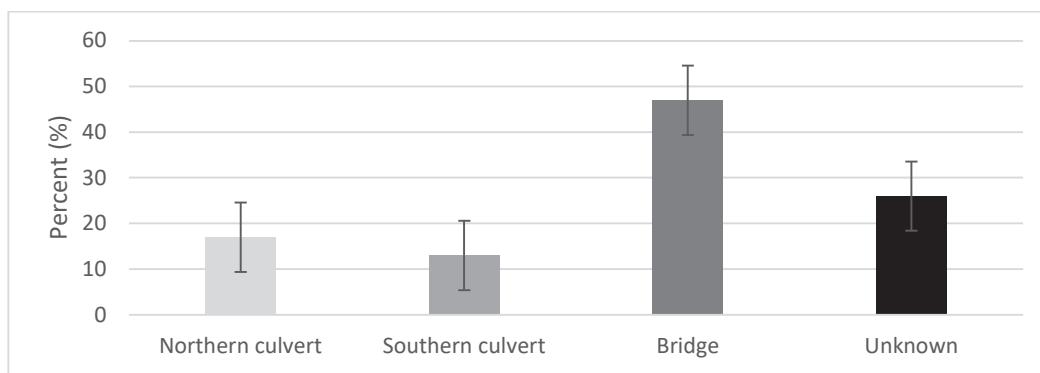
Day-roost use

Bats were captured from two adjacent culverts; northern culvert ($n = 4$) and southern culvert ($n = 9$) (Figure 4-1). Photographs of roost culverts are provided in Appendix 3. Over the three survey periods, we tracked bats to a total of three day-roosts; northern culvert, southern culvert and a bridge. Both culverts were box culverts 2.1 m in height with widths of 2.7 m and 3.0 m, and lengths of 24.0 m and 25.0 m, respectively. Bats were located roosting in lift holes. The multi-span concrete bridge used by bats was a large vehicle bridge on a main road that crossed the creek the bats used for foraging (Figure 4.3, Appendix 3). The bridge was 20.0 m in width and 68.0 m in length. The roost was located in a drainage pipe.

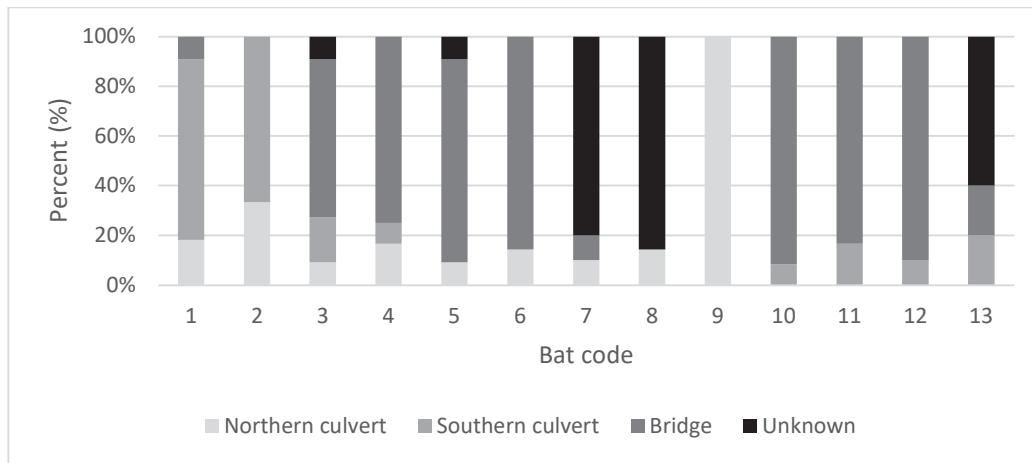
Day-roost locations for four bats could not be determined despite extensive searching of all culverts, bridges, hollow bearing trees and surrounding houses. Tagged bats used 2.5 ± 0.8 (1-3) roosts over a mean tracking period of 8 ± 3.1 (3-12) days. Despite being caught in two different culverts, bats spent

most tracking days in the bridge roost (47%±40%), followed by unknown roosts (19%±33%), northern culvert (17%±27%) and southern culvert (17%±25%) (Figure 4-2a).

Roost fidelity varied between individuals. All captured bats, apart from Bat 9, were radio-tracked to at least one different roost site post capture (Figure 4-2b). Bat 9 continued roosting at the capture culvert and displayed 100% roost fidelity. Bats 6, 10, 11 and 12 moved from the capture culvert to the bridge and displayed 100% roost fidelity to the bridge roost for the duration of the tracking period (Figure 4-2b). Bats 1-5 displayed fidelity ranging from 8-82% (Figure 4-2b). Tagged bats continued sharing roost sites and switched roosts ($n=9$) every 4.8 ± 3.7 (1-11) days.



(a)



(b)

Figure 4-2 Roost use by 13 non-reproductive female *Myotis macropus* in an urban landscape. a) Overall use of different roost sites expressed as a percentage (means ± standard deviation) of total days tracked. **b)** Roost fidelity per individual bat. The shading in b) corresponds to the roosts identified in Figure 4-2a. Locations of roosts are shown in Figure 4.3.

Home range

There was no correlation between the number of tracking nights and 95% KDE home range ($r=-0.04$, $p=0.88$) while a relationship was detected for 100% MCP ($r=-0.4$, $p=0.05$). There was no correlation

between the number of fixes and 95% KDE home range ($r=-0.3$, $p=0.28$) or 100% MCP ($r=-0.06$, $p=0.8$). Home range size varied considerably between individuals with median home ranges of 39 ha for 100% MCP (IQR 29.5-75 ha) and 103 ha for 95% KDE (IDR 8-187.5 ha) (Figure 4.3). The median range span was 1.9 km (IQR 1.6-2.8 km).

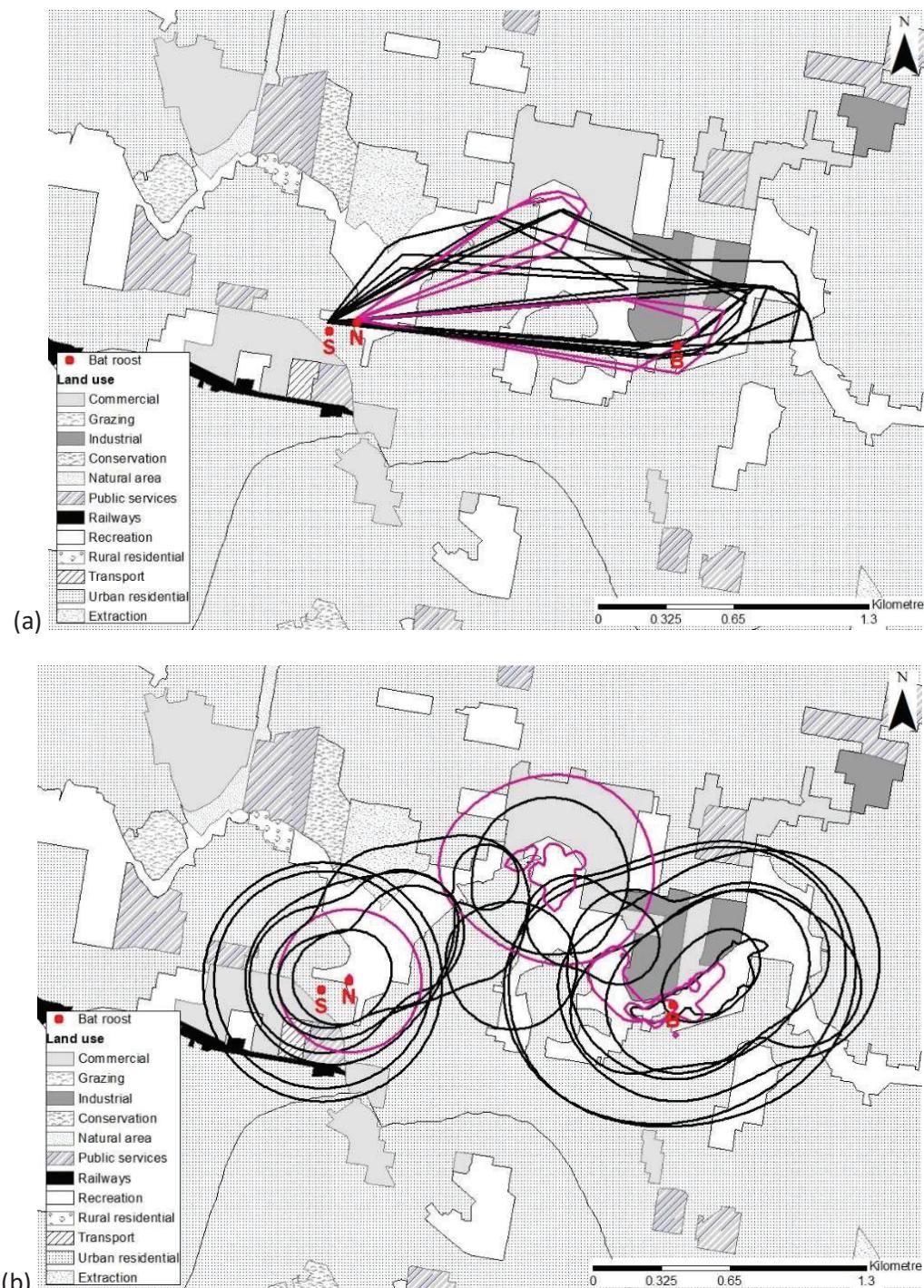


Figure 4-3 Roost sites and home ranges based on (a) 100% MCP (black) and (b) 95% kernel density estimates (pink) of 13 non-reproductive female *Myotis macropus* radio-tracked in Brisbane in 2018-2019. Roost sites are shown in red; B= bridge, N= northern culvert, S= southern culvert.

Core use area

There was no correlation between the number of tracking nights and 50% KDE home range ($r=-0.1$, $p=0.6$), and no correlation between the number of fixes and 50% KDE home range ($r=-0.3$, $p=0.2$).

Winter core use areas were significantly larger than summer core use areas ($U= 4.5$, $p=0.03$). Winter core use areas had a median size of 22.0 ha (50% KDE IQR range 17-54.5 ha) and included waterways, riparian zones, parkland and landscapes immediately adjacent to riparian zones.

Summer core use areas had a median size of 1 ha (50% KDE IQR range 0.9-16.7 ha) and were located on waterways and riparian zones.

Core use areas across both seasons were clustered on five elongate pools (Figure 4-4, Appendix 3). Nine elongate pools were available within the home range of all tagged bats, yet four pools did not comprise core use areas by the tagged *M. macropus* in this study. The five pools had the following approximate measurements; pool A 58.0 m long and 13.0 m wide, pool B 145.0 m long and 6.0 m wide , pool C 210.0 m long and 12.0 m wide, pool D 105.0 m long and 13.0 m wide and Pool E 140.0 m long and 13 m wide. Pool A was only used by Bat 1 during winter 2018, pool B was used by Bat 9 in summer 2019 and Bat 13 in winter 2019 and pools C, D and E were used by all bats during each season.

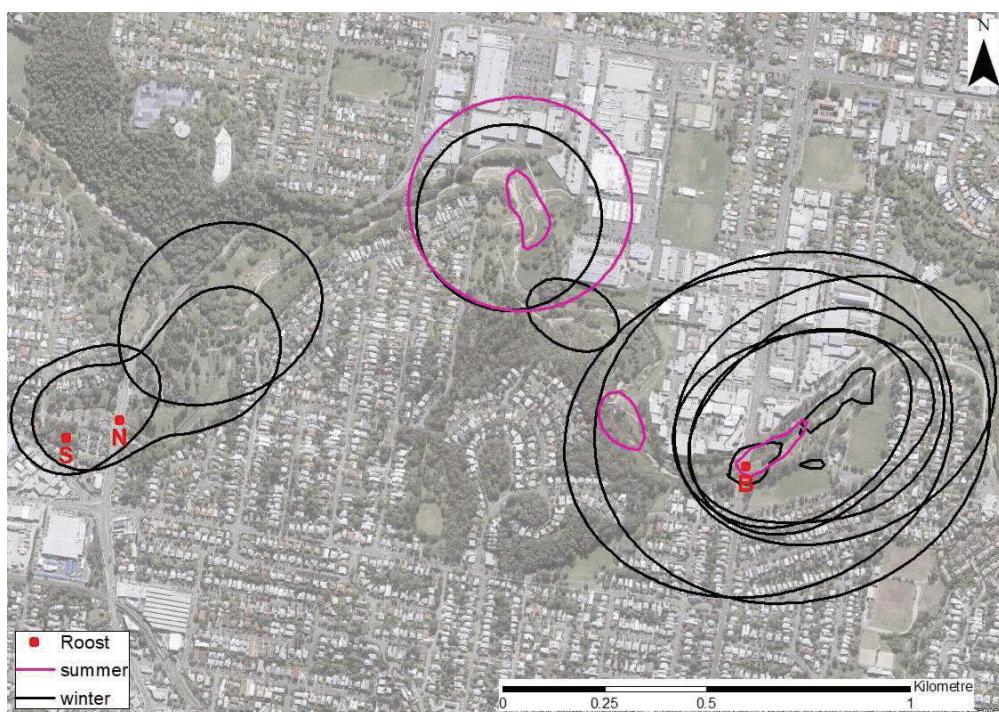


Figure 4-4 Roost sites and core use areas based on 50% kernel density estimates of 13 non-reproductive female *Myotis macropus* radio-tracked in Brisbane in 2018-2019. Colours correspond to different seasons; black= winter ($n= 9$) and pink= summer ($n=4$). Roost sites are shown in red; B= bridge, N= northern culvert, S= southern culvert.

Land use availability and land use selection within the study area

The area of the combined 95% KDE comprising the communal home range for all tagged bats and the second order study area was 361 ha. The second order study area is shown in Figure 4-5.

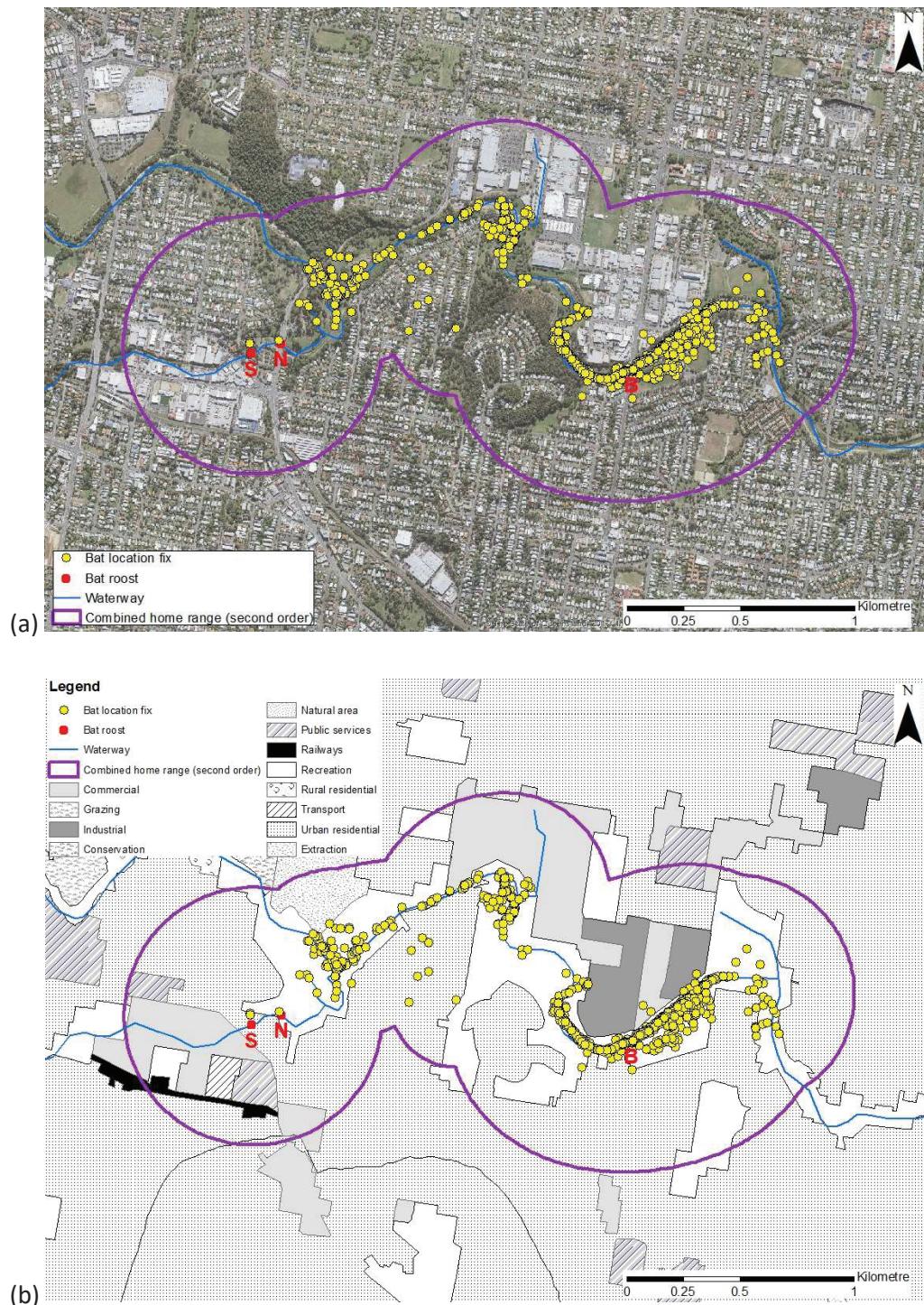


Figure 4-5 Combined home range and second order study area shown with location fixes on (a) aerial imagery and (b) land use types.

The overall percentage composition of land use types available in the study area were 48% urban residential, 29% recreation, 17% commercial, 4% industrial, 2% public services, 2% water storage, 1% railways and 1% transport (Figure 4-6).

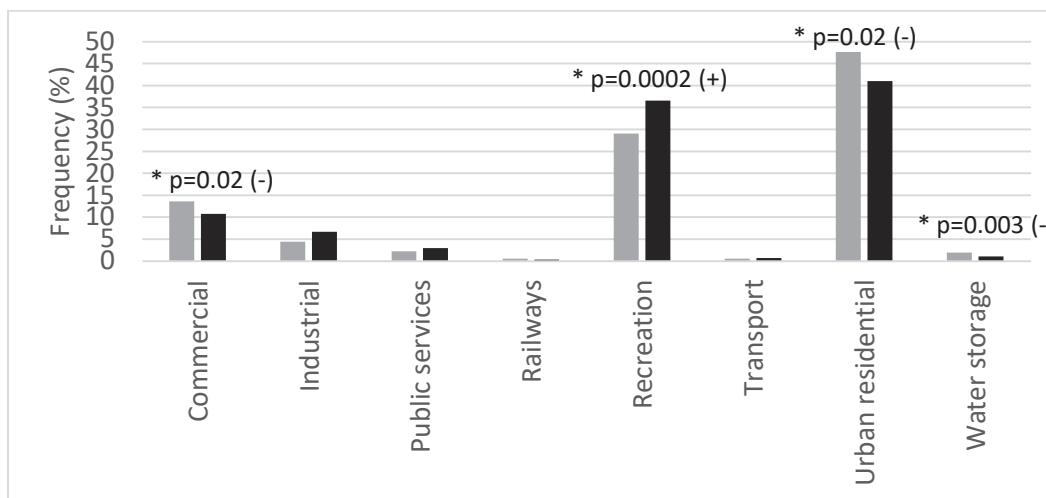


Figure 4-6 Land use within the study area showing land use available (grey) and land use selected by radio-tracking *Myotis macropus* (black). The y-axis shows percent use. Asterisks indicate significant differences with signs in brackets showing a positive or negative preference.

Compositional analysis showed *M. macropus* used land uses in the study area in a non-random manner ($p=0.001$, 8192 permutations). Proportional use of the recreation land was significantly higher than all other available land use types ($p=0.0002$, Table 2). The land use types transport ($p=0.09$), industrial ($p=1$), railways ($p=1$) and public services ($p=0.09$) space were selected at random and used in proportion to their availability. Commercial ($p=0.02$), urban residential ($p=0.02$) and extractive industries ($p=0.003$) land use types were avoided (Table 4-2).

Table 4-2 Second-order land use selection comparing proportion of land use available versus habitat used (overall $p= 0.001$, 8192 permutations). Values in parenthesis represent the proportion of animals which use the land use in greater proportion than its availability, diagonal values represent the p values for each land use type and non-diagonal values represent p values for paired comparisons among land use types; significant results are shown in bold. R= recreation, T= Transport, I= Industrial, R= Railways, PS= Public services, C= Commercial, U= Urban residential, E= Extraction.

Land use type	R	T	I	R	PS	C	U	E	Decision preferred
R (1)	0.0002								
T		0.09							proportionally used
(0.769)			23						
I				1					proportionally used
(0.461)									

Land use type	R	T	I	R	PS	C	U	E	Decision
R (0.461)				1					proportionally used
PS (0.231)					0.0923				proportionally used
C (0.153)						0.0225	1	1	avoided
U (0.153)							0.0225	1	avoided
E (0.077)								0.0034	avoided

Land use selection within home ranges

The mean percentage composition of land use types available within individual bat home ranges were 75% recreation, 8% urban residential, 1% commercial and 1% industrial (Figure 4-7).

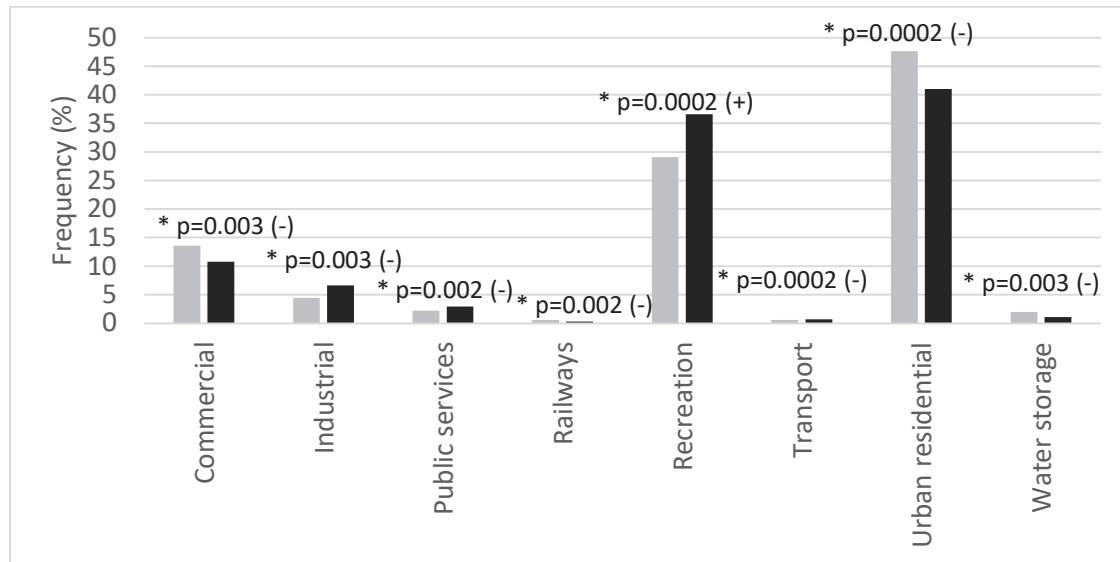


Figure 4-7 Land use within the study area showing land use available (grey) and land use selected by radio-tracking *Myotis macropus* (black). The y-axis shows percentuse. Asterisks indicate significant p-values with signs in brackets showing a positive or negative preference.

Compositional analysis showed *M. macropus* used land uses within individual home ranges in a non-random manner ($p=0.0007$, 8192 permutations) with all bats displaying a significant preference for recreation space ($p=0.0002$). All other land use types were avoided (Table 4-3).

Table 4-3 Third-order land use selection comparing proportion of land use available versus percent of location fixes within land use types (overall $p= 0.0007$, 8192 permutations). Values in parenthesis represent the proportion of animals which use the land use in greater proportion than its availability, diagonal values represent the p values for each land use type and non-diagonal values represent p values for paired comparisons among land use types; significant results are shown in bold. R= recreation, T= Transport, I= Industrial, R= Railways, PS= Public services, C= Commercial, U= Urban residential, E= Extraction.

Land Use Type	R	I	E	C	PS	R	T	U	Decision
R (1)	0.0002								<i>preferred</i>
I (0.076)		0.0034	1	1	1	1	1	1	<i>avoided</i>
E (0.076)			0.0034	1	1	1	1	1	<i>avoided</i>
C (0)				0.0002	1	1	1	1	<i>avoided</i>
PS (0)					0.0002	1	1	1	<i>avoided</i>
R (0)						0.0002	1	1	<i>avoided</i>
T (0)							0.0002	1	<i>avoided</i>
U (0)								0.0002	<i>avoided</i>

4.4 DISCUSSION

The aim of this study was to investigate roost use, quantify home range size and understand land use selection by a specialist bat in an urban environment. This is also the first study to investigate roost use, home range size and land use selection in an urban population of *M. macropus*. The results provide a unique insight into the ecology and behaviour of urban bats.

We predicted that *M. macropus* would switch between natural and artificial roost sites. We only radio-tracked *M. macropus* to artificial roost sites in culverts and bridges. As predicted, all bats showed a preference for the recreation land use type at the two spatial scales we assessed. However, bats also used some urban land use types in proportion to their availability while avoiding others.

Day roost use

The number of roosts used by bats in our study was higher than reported in other studies investigating the roosting behaviour of *M. macropus*. We detected 2.5 roosts used per bat on average while Caddle (1998a) detected an average of 1.9 roosts and Campbell (2009) 1.3. The differences between studies could be explained by the differences in landscape context and reproductive condition of the animals used in different studies. Our study was located in an urban environment and Caddle (1998a) and Campbell (2009) were located in rural environments. Our study only used non-reproductive females and the Caddle (1998a) and Campbell (2009) studies included all reproductive conditions. Different stages of the reproductive cycle require bats to modify their activity according to energetic demands (Speakman *et al.* 2003).

Myotis macropus have been recorded switching between natural and artificial roost sites (Campbell 2009, Law *et al.* 2020a), but we only radio-tracked *M. macropus* to artificial roost sites in culverts and bridges. It is possible that the unknown roosts could be located in trees although our result may reflect the reproductive status of the bats we tagged. Non-reproductive female bats of the ecologically similar Daubenton's bat (*M. daubentonii*) only used tree roosts in small Scottish towns, while reproductive females and males switched between tree and building roosts (Ngamprasertwong *et al.* (2014)). However, Lučan and Radil (2010) reported female *M. daubentonii* roost switched every 2-3 days, and they did not detect an association between roost switching and reproductive status. The bats in our study switched roosts every 4.5 days although we found considerable range in roost fidelity (8-82%). The frequency of roost switching found in our study is much lower than the non-reproductive female bats who switched on average every 1.5 days reported for *M. daubentonii* by Ngamprasertwong *et al.* (2014). Our range of roost fidelity might be lower if we had located all day roosts. Low levels of roost switching and high roost fidelity were found by Gonsalves and Law (2017) who found 100% roost fidelity in a colony of *M. macropus* roosting in a jetty, although the roost in their study contained pregnant and lactating females. Roost permanency affects roost fidelity and switching behaviour (Lewis 1995).

Roost permanency has been suggested to affect roost fidelity (Kunz 1982, Brigham 1991). Evidence suggests bats have higher fidelity to permanent roost sites such as buildings, bridges and culverts, than to ephemeral roost sites like trees (Brigham 1991, Evelyn *et al.* 2004). These permanent, anthropogenic sites are valuable to bats because they provide thermally stable roosts and have reduced interspecies competition (Rhodes and Wardell-Johnson 2006, Davis *et al.* 2013). Thermally stable roosts minimise energetic costs associated with thermoregulation, gestation, embryonic development and lactation (Bennett *et al.* 2008, Smith and Stevenson 2013, Lutsch 2019). Tree hollows in urban environments are characterised by significantly higher usage rates and significantly more aggressive interactions between hollow-dependent fauna (Davis *et al.* 2013).

Globally, bats in the genus *Myotis* have successfully adapted to roosting in artificial structures in urban areas (Brigham 1991, Evelyn *et al.* 2004, Coleman and Barclay 2012, Wojtaszyn *et al.* 2013, Thomas and Jung 2019). Dalquest (1947) noted that Yuma myotis (*M. yumanensis*) had adapted to anthropogenic features such as buildings and bridges so successfully, that no records of use of natural roosts could be found. The endangered little brown bat (*M. lucifugus*) had greater activity in rural villages compared to surrounding boreal forests due to increased roosting opportunities provided in the villages (Thomas and Jung 2019). It is possible that the bridge and culvert roost sites used by bats in our study provide this urban population with sites that meet thermal requirements

and have limited interspecies competition such that *M. macropus* have adapted to roosting only in artificial structures. This finding is unexpected as *M. macropus* roost sites are usually located in trees (Dwyer 1970, Caddle 1998, Campbell 2009).

***Myotis macropus* home range and core use areas**

The size of an animal's home range provides insight into the quality of habitat and resources available (Kniowski and Gehrt 2014). The MCP home range sizes we found were comparable to the sizes reported for other trawling bats in anthropogenic environments that fill a similar ecological niche. The small home ranges found in our study (5-202 ha) suggest that the urban environment in our study area provided sufficient resources for this local population of *M. macropus*. For example, in a mixed agricultural landscape, *M. daubentonii* home ranges varied from 1-163 ha (Ngamprasertwong *et al.* 2014) while the same species in an extensive deciduous forest had much larger home ranges (970-4730 ha; (Encarnaçao 2012). Home ranges for another trawling species, the long-legged bats (*Macrophyllum macrophyllum*), roosting in a half-sunken ship, varied from 7.3-150.7 ha (Meyer *et al.* 2005). Similar patterns have been reported in non-trawling bats. Chaverri *et al.* (2007) found that individuals of tent-making bats (*Artibeus watsoni*) from sites with greater food resources had smaller foraging ranges than at less productive sites, suggesting that resource abundance was the most important determinant of home range size and that animals that find sufficient resources have smaller home ranges.

Within home ranges, individuals concentrate activity in core use areas and these are usually focussed around key foraging grounds or roosts (Kunz *et al.* 2003). Core use areas in our study clustered for all individuals at elongated pools. Elongated pools are known preferred foraging grounds for *M. macropus* and other similar trawling bats due to smooth surfaces and reduced vegetation clutter (Warren *et al.* 2000, Anderson *et al.* 2005, Almenar *et al.* 2009). Vegetation clutter interferes with prey detection by masking echoes, and trawling bats show a preference for pools with smooth surfaces (Boonman *et al.* 1998, Almenar *et al.* 2009, Downs *et al.* 2016). Our results suggest high fidelity to foraging grounds and this behaviour has been observed in other *Myotis* sp. *Myotis daubentonii* showed a preference for particular pools along a river with foraging activity concentrated on four out of eleven pools available with two pools exploited more intensively than the others (Kapfer *et al.* 2008). Elongated pools generally have high insect productivity (Warren *et al.* 2000, Anderson *et al.* 2005). The number of foraging *M. daubentonii* foraging at a pool has been correlated with the area of surface water available and this has been attributed to both the increased amount of food available and the increased size of the foraging space (Moeschler and Ruedi 1995, Dietz *et al.* 2006).

The home range spans in our study (1.2-3.1 km) were comparable to range spans of *M. daubentonii* roosting in agricultural landscapes (where small range spans of 0.68-3.21 km were recorded; (Ngamprasertwong *et al.* 2014). Range spans are generally larger than foraging distance because range spans are defined as the maximum distance across a home range, and include movements such as commuting and foraging (Kenward 2000). However, in our study, range spans were smaller than foraging distances recorded for *M. macropus* in other studies. Bats in our study typically stayed within 1-3 km of their roosts, whereas in other studies they have been tracked foraging at least 4.9 km (Gonsalves and Law 2017), 10 km (Barclay *et al.* 2000) and 22 km (Caddle 1998) from roost sites.

Although the range span in our study was smaller than foraging distance found in other studies on *M. macropus*, they are similar to foraging distances reported for other trawling bats. The long-fingered bat (*M. capaccinii*) was recorded foraging on average 5.6 km from roost sites with 90% of all foraging activity concentrated within 10 km (Almenar *et al.* 2009) and studies on foraging distance in *M. daubentonii* have found distances of 0.6-6.3 km (Dietz *et al.* 2006), 0.5-3.8 (Kapfer *et al.* 2008) and 0.6-7.6 km (Encarnação *et al.* 2005). Variation in foraging and roosting behaviour between two populations of the big brown bat (*Eptesicus fuscus*) was attributed the difference to the distribution of insect prey relative to the location of roost sites (Brigham 1991). Small home ranges and movement restricted to less than 300 m was also found in urban roosting Gould's long-eared bat (*Nyctophilus gouldi*) (Threlfall *et al.* 2013b). The small home ranges and range spans found in our study suggest that foraging resources for non-reproductive female bats can be met in close proximity to roost sites in urban areas. The home range size for males and females in different reproductive stages should be investigated to fully comprehend the use of space by *M. macropus* in an urban environment.

Land use selection

Bats in our study used urban land use types in a non-random manner. At both scales we examined, *M. macropus* showed a significant preference for recreation areas which contain waterways, riparian zones and open green space. This finding is consistent with the ecology of the species and continues to confirm the importance of riparian zones to this specialised trawling bat, potentially even more so in highly modified, urban environments (Anderson *et al.* 2005, Campbell 2009, Campbell *et al.* 2010, Lintott *et al.* 2015, Straka *et al.* 2016). Law *et al.* (2020a) suggest that due to their ecological specialisation, *M. macropus* populations are buffered from landscape impacts occurring outside of the riparian zone. However, this species is vulnerable to landscape changes that affect the riparian corridor. The genetically isolated colonies and population structuring found in *M. macropus* in southern-Australia was attributed to reduced riparian connectivity in rural areas (Campbell *et al.*

2009). Our findings demonstrate urban *M. macropus* have high fidelity to elongate pools and riparian areas and display a preference for urban green space that contains waterways. This highlights the vital importance of riparian health and connectivity to this specialist species.

While the importance of riparian zones to *M. macropus* is evident, our results suggest *M. macropus* also displays behavioural plasticity. Some urban land use types adjacent to riparian zones were used in proportion to their availability, indicating *M. macropus* can utilise highly modified landscapes. This finding is similar to results of Law *et al.* (2001) who observed *M. macropus* foraging on farm dams located 300 m across cleared cattle paddocks with no trees. Our finding that *M. macropus* may forage away from waterways suggests that both riparian zones and adjacent land use types are important for the persistence of urban populations of *M. macropus*. Our results contribute to the growing evidence that some species of insectivorous bats respond to the spatial heterogeneity provided within urban environments (Rhodes and Catterall 2008, Jung and Kalko 2012, Threlfall *et al.* 2013a, Li and Wilkins 2014).

Insights into the diet and foraging behaviour of *M. macropus* suggest further evidence of behavioural plasticity. Dietary analyses and observations of foraging behaviour confirm a diet dominated by aquatic insects and fish (Dwyer 1970a, Thompson and Fenton 1982, Robson 1984, Jansen 1987, Jones and Rayner 1991, Law and Urquhart 2000, Burns 2005). However, aerial insects have also been identified in dietary analysis and aerial hawking has been observed (Jones and Rayner 1991, Burns 2005). A broad diet of termites (Isoptera), spiders (Araneae), ants (Formicidae) and plant hoppers (Fulgoroidea) in faecal pellets was collected during early wet season mass emergence of winged insects, indicating this species has flexible foraging behaviour and is able to exploit a variety of food sources (Milne *et al.* 2016). Aerial hawking has been observed with *M. macropus* successfully capturing prey whilst in flight (Jones and Rayner 1991, Burns 2005). This ability to exploit resources could explain the significant difference between winter and summer core use areas identified in our study. Winter core use areas covered the waterway, riparian zone and adjacent land use types while summer core use areas were largely restricted to elongate pools and the riparian zone. Our results show that in winter, *M. macropus* cover a larger core area and we surmise this is due to the use of flexible foraging behaviour to exploit aerial insect resources because aquatic insect activity is reduced. Season was one of the main factors contributing to the structure of the insect communities in pools in urban green space in Buenos Aires with summer rainfalls distributing taxonomic groups more evenly compared to other seasons (Fontanarrosa *et al.* 2009).

Behavioural plasticity has been recognised in other trawling bats. The pond bat (*M. dasycneme*) (Britton *et al.* 1997) and *M. macrophyllum* (Weinbeer *et al.* 2013) are trawling species able to switch

foraging mode between trawling and aerial hawking. The greater bulldog bat (*Noctilio leporinus*) is a true fishing bat but the species is capable of exploiting insect resources if fish availability is reduced (Schnitzler *et al.* 1994). The behavioural plasticity by *N. leporinus* was documented in the seasonal shift from a diet of insects (55-58% of the dry weight of guano) and fish (12-45% of the dry weight of guano) in the wet season to a fish dominated diet (fish 41-84%, insect 16-59% of the dry weight of guano) in the dry season (Brooke 1994). The ability of trawling species to switch foraging strategy is due to the relative similarity between uncluttered, smooth water and uncluttered, open space (Weinbeer *et al.* 2013) and this flexibility is what enables them to persist in urban environments.

Myotis macropus is a trawling bat that persists in an urban environment by roosting in culverts and bridges and using behavioural plasticity to exploit food resources available in urban environments. Behavioural plasticity is necessary for species to be able to exploit urban environments (Luniak 2004). This trait is an important attribute for a species' ability to persist in anthropogenically modified environments (Jung and Kalko 2012) as it enables individuals to be opportunistic and flexible, maximising on resources available. Equally important is the preservation of heterogeneity in urban environments to enable species with behavioural plasticity to utilise these anthropogenic environments.

Conservation and management implications

Our research suggests *M. macropus* will be able to persist in highly modified environments provided roosts and foraging grounds are present, and riparian connectivity is maintained in urban environments. This study found that culverts and bridges are important roost sites to urban *M. macropus*. Some individuals displayed high roost fidelity which highlights the importance of culvert and bridge roosts to urban bats. Disturbance to, or loss of, a culvert or bridge roost has the potential to be a significant impact to an urban bat population. Road construction projects involving roost sites in urban transport infrastructure should consider that *M. macropus* populations may not utilise tree hollows and that culvert and bridge roost sites are important breeding sites for urban *M. macropus*.

We found that elongate pools were vital core use areas for urban *M. macropus*. Our results suggest that *M. macropus* use predominantly trawling behaviour to forage over elongate pools throughout the year and these pools are critical core use areas in summer. Our results show that core use areas during winter are larger and *M. macropus* likely increases aerial hawking behaviour to forage over the riparian zone and adjacent land use types. Management of urban *M. macropus* populations should consider both riparian zones and adjacent land use types as important foraging grounds for this species.

Myotis macropus has been able to persist in the urban environment of Brisbane largely due to the presence of elongate pools and riparian areas, and the location of suitable roosts within this green space land use type. Retention of green space in urban environments will be crucial to enabling this trawling species to persevere in modified environments. Maintaining spatial heterogeneity in urban planning and design will provide a landscape mosaic for urban adapted *M. macropus* to navigate and persist.

Although *M. macropus* displays behavioural plasticity, this species is still reliant on riparian systems to survive. Riparian systems are particularly vulnerable to the impacts of climate change (Capon *et al.* 2013). Climate change predictions suggest a drier climate through higher annual average temperatures, increased evaporation and reduced rainfall (Hughes 2003). A drier climate will impact on the permanency of waterways and therefore, spatially available food resources for trawling bats.

Acknowledgements

Special thanks go to the many volunteers who spent countless hours radio-tracking *M. macropus*, especially L. Hogan, M. Rhodes and S. Taylor. We thank S. Fuller, J. Dawson and L. Beard for the loan of tracking equipment and L. Gonsalves and K. Borkin for providing critical feedback on this manuscript. This work was supported by the Ecological Consultants Association of New South Wales and the Australasian Bat Society. VG was supported by a Research Training Program Stipend Scholarship.

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Chapter 5: Population structure and gene flow among culvert roosts of a specialist trawling bat in a subtropical city



A female large-footed Myotis (*Myotis macropus*) feeds her pup in the lift hole of a concrete culvert under a road in Brisbane. Photo by V. Gorecki.

This chapter is in preparation for submission to the journal *Molecular Ecology*.

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Abstract

Contemporary population dynamics are linked to spatial and temporal environmental variation and the movement of individuals promotes genetic diversity and gene flow among populations. Urban environments can fragment habitats and introduce barriers to movement between populations, and this can have a profound effect on the population structure and viability of urban wildlife populations. We used single nucleotide polymorphisms and the mitochondrial cytochrome b gene to determine the effects of urbanisation on genetic structure and gene flow in a culvert roosting population of a specialist trawling bat. The large-footed myotis (*Myotis macropus*) is a trawling bat with a specialisation for foraging directly over water surfaces, and with movement predominantly located within riparian corridors. We found genetic differentiation between all roosts. Gene flow was moderate between peri-urban populations and restricted between urban populations. We found evidence of female philopatry and pairs of related females within roosts. The urban roosts in our study had more related pairs than the peri-urban roosts. Our results suggest *M. macropus* has a harem social structure and a promiscuous breeding system due to limited shared paternal ancestry. Our results suggest that female dispersal distances are less than 30 km and female biased gene flow is locally restricted.

Keywords

Philopatry, dispersal, harem, promiscuous, single nucleotide polymorphisms, peri-urban

4.6 INTRODUCTION

Knowledge on how genetic variation is distributed across a species' range is vital to understanding how contemporary population dynamics are linked to spatial and temporal environmental variation (Botero-Delgadillo *et al.* 2020). The spatial distribution of genetic variation differs between species. Genetic variation can be influenced by biological factors such as philopatry, that is fidelity to a natal and breeding site or group (Greenwood 1980). Philopatry can minimise gene flow among populations and lead to increased genetic differentiation. Species such as birds generally have high gene flow among populations as they are highly mobile and able to avoid the effects of geographical distance and barriers. However, avian population differentiation occurs in various species ranging from the highly mobile seabird the Galapagos Nazca boobies (*Sula granti*) (Botero-Delgadillo *et al.* 2020), to the small saltmarsh sparrow (*Ammodramus caudacutus*) (Walsh *et al.* 2012). When population differentiation and genetic structure is found in avian species it is often due to natal philopatry and high site fidelity which reduces gene flow, and promotes genetic differentiation (Hill *et al.* 2012). Similarly, many bat species display a generalised pattern of female philopatry and male-

biased gene dispersal (Kerth *et al.* 2002a, Dixon 2011, Laine *et al.* 2013, Park *et al.* 2019). Genetic differentiation is also promoted by anthropogenic factors such as habitat fragmentation and artificial barriers like roads, which can reduce connectivity among populations and restrict gene flow. Habitat fragmentation can reduce population size and increase isolation among populations (Schlaepfer *et al.* 2018). Major roads create such significant barriers to badger (*Meles meles*) movement that they act as territorial boundaries in some urban environments (van Tuijl *et al.* 2019). Maintaining genetic diversity within urban populations is vital for the viability of these populations and reduces genetic differentiation among populations.

Urbanisation impacts on the patterns of genetic diversity and gene flow in different taxa in different ways and this has implications for the viability of urban wildlife populations. Urban environments are one of the greatest drivers of ecological change (Seto *et al.* 2010). Within some cities, there can be patches of remnant native vegetation and human-engineered novel niches (Littleford-Colquhoun *et al.* 2017). Some taxa are able to successfully navigate these patches and dispersal pathways have increased, with these species displaying high genetic diversity and low genetic differentiation within urban populations (Miles *et al.* 2019). Conversely, other species experience a decrease in genetic diversity within populations, and an increase in genetic differentiation due to reduced movement among populations (Hurtado and Mabry 2019).

Insectivorous bats (referred to as bats from here-on) provide a model to study genetic variation in urban environments. Bats are social animals, living in groups that range over several orders of magnitude from a few individuals to several million (Kerth 2008), and are highly mobile due to their ability to sustain flight (Moussy *et al.* 2013). Despite this ability to move and potentially disperse, many bats show strong philopatry to their natal colony and high fidelity to roosts (Kerth 2008, Laine *et al.* 2013, Moussy *et al.* 2013, O' Donnell *et al.* 2016). Day roosts are essential to the survival of bats as they are locations of social interactions and provide protection from predators (Kunz 1982, Lewis 1995). Many species of bats have adjusted to artificial roosts provided in urban environments such as buildings (Brigham 1991, Evelyn *et al.* 2004), bridges (Keeley and Tuttle 1999), culverts (Wojtaszyn *et al.* 2013) and jetties (Gonsalves and Law 2017). In a global meta-analysis on trait-dependent tolerance of bats to urbanisation, the most urban tolerant bat species were identified as primarily open and edge aerial foragers and trawling bats with flexible roosting strategies (Jung and Threlfall (2018).

The large-footed myotis (*Myotis macropus*) is a trawling bat with flexible roosting strategies and is found roosting in urban environments in concrete culverts under roads. It has specialised foraging

behaviour and only roosts within 100 m of waterways, and movement across landscapes is restricted to riparian corridors (Campbell *et al.* 2009). Harem roosting behaviour has been deduced from observations of one male roosting with a group of females by several authors (Dwyer 1970a, b, Lloyd *et al.* 1999, Barclay *et al.* 2000, Law *et al.* 2001). The species is seasonally polyoestrous, giving birth to a single pup two to three times per breeding season (Dwyer 1970b, Lloyd *et al.* 1999). High roost fidelity has been documented in *M. macropus* (Campbell 2009, Gonsalves and Law 2017) and roosts just 15 km apart were found to be genetically different, suggesting limited movement of individuals among roosts (Campbell *et al.* 2009). No studies have assessed patterns of genetic variation and gene flow in urban environments and it is unknown how urban *M. macropus* populations are structured.

Knowledge on how urban wildlife populations are structured is vital for the development of appropriate management measures to support genetic viability. The aim of our study was to examine genetic variation, gene flow and population structure in a bat species occupying culvert roosts, as a model for the study of genetic variation in urban environments. Contrasting patterns in gene flow have been found between nuclear and mitochondrial markers (mtDNA) (Castella *et al.* 2001, Ibáñez *et al.* 2006). Therefore, we used nuclear (single nucleotide polymorphisms (SNPs)) and mitochondrial (mtDNA) markers to examine genetic variation, gene flow and population structure in a culvert roosting population of a specialist trawling bat in a subtropical city. We predicted gene flow among roosts would be restricted to riparian corridors and, because of this specialised behaviour, we expected genetic differentiation to occur among roosts. We predicted *M. macropus* would display the generalized pattern of female philopatry and male biased gene dispersal found in other *Myotis* sp. (Kerth *et al.* 2002a, Dixon 2011, Laine *et al.* 2013, Park *et al.* 2019).

4.7 METHODS

Study area

The study was conducted in the Local Government Area (LGA) of Brisbane City Council (BCC) in south-east Queensland, Australia (Figure 5-1). Brisbane has a subtropical climate by the Köppen climate classification system, characterised by hot and humid summers and mild winters (Peel *et al.* 2007). During the time of this study (2017 – 2019), the minimum daily temperature was 21.5°C, the maximum daily temperature 31.2°C, and average daily relative humidity was 58.6% (Australian Bureau of Meteorology 2020).

Brisbane is a city with variable land uses such as residential housing, industrial and commercial areas which cover 67% of the LGA (ABARES 2016). Natural environments are primarily located on the

western fringe of the city and comprise 27% of the LGA. Water covers 6% of the LGA due to the meandering Brisbane River and its tributaries, as well as several large water supply reservoirs and dams (ABARES 2016).

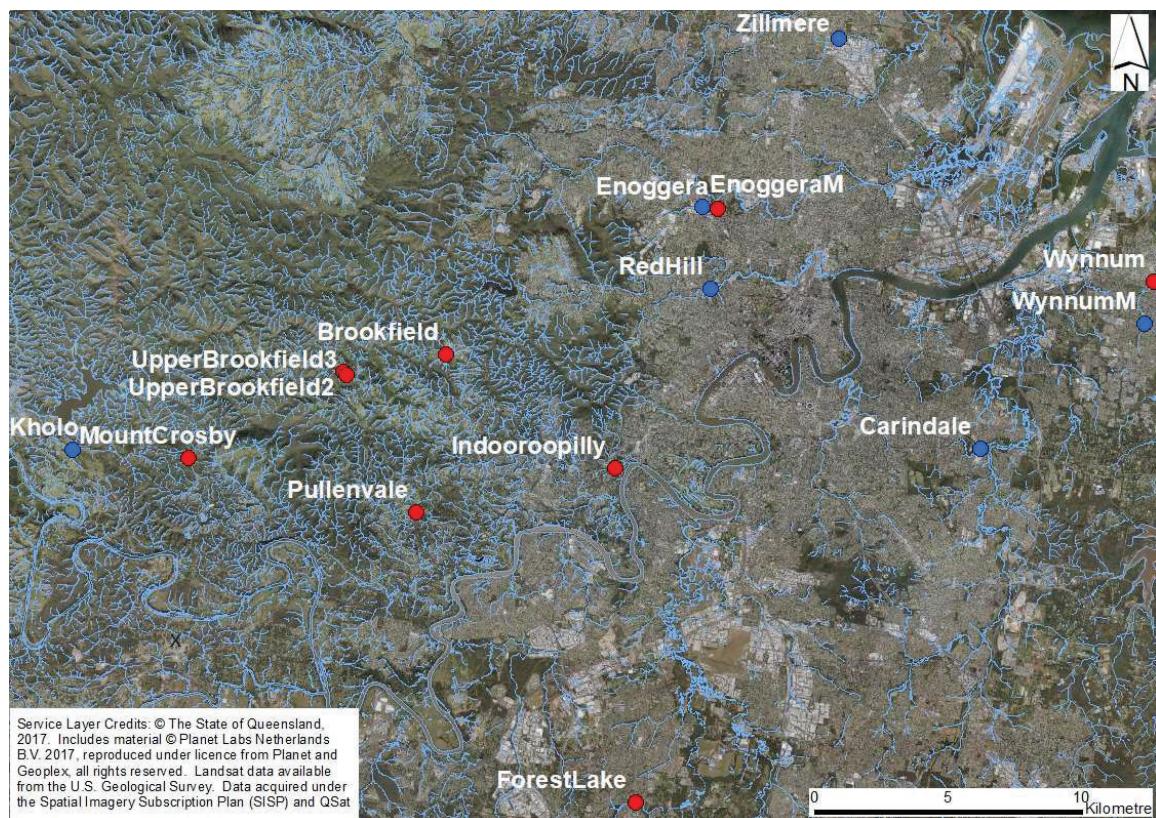


Figure 5-1 Sample locations of *Myotis macropus* from 15 roosts across Brisbane. Red circles show colonial roosts and blue circles show solitary roosts. Waterways are shown in light blue.

Bat sampling

Bat capture and manipulation were carried out under permits from the Queensland Department of Environment and Science (Scientific Purposes Permit WA0001898), the Queensland University of Technology Animal Ethics Committee (AEC1700000540) and Biosafety Ethics Approval (1700000368). Bats roosting in culverts were captured in August 2018 and August 2019 by removing them from the roost site with a hand-net. Tissue samples were taken from the plagiopatagium using a 4 mm punch-biopsy kit (Campbell *et al.* 2009). Biopsy equipment was sterilised by flaming the biopsy punch between individuals and each bat wing was sterilised with a clean alcohol wipe to prevent cross contamination. Bat morphometrics were recorded (sex and weight) and bat were released on the night of capture at the location of capture. Bats that had been previously sampled were identifiable due to healed circular marks at the base of the wing, near the ankle. Biopsy

samples were placed into an Eppendorf tube containing 100% ethanol for transportation back to the laboratory.

A total of 198 bats were captured from 15 roosts comprising colonial roosting groups of males and females and solitary roosting males. From this total, 72 bats were subjected to genetic analyses. Roosts with more than 10 individuals were randomly subsampled for genetic analysis and all bats from roosts with fewer than 10 individuals were analysed (Table 5-1). Five bat datasets were used in our analyses:

- (1) All bats from which genetic information was collected including colonial roosting males and females and solitary roosting males (n=72; male = 24, female= 48),
- (2) colonial roosting males and females only (no solitary roosting males) (n= 63; male= 15, female= 47),
- (3) females only (n=48),
- (4) colonial roosting males only (n= 15) and
- (5) solitary roosting males (n=9).

Table 5-1 *Myotis macropus* roosts sampled and number per roost analysed for genetic characteristics.

Roost	Total collected per roost	Colony or Solitary	Sub-sampled		Total bats subsampled for genetic analysis
			M	F	
Brookfield	67	Colony	2	5	7
Carindale	1	Solitary	1	0	1
Enoggera	7	Colony	1	7	8
EnoggeraM	2	Solitary	2	0	2
Forest Lake	7	Colony	1	6	7
Indooroopilly	7	Colony	1	6	7
Kholo	2	Solitary	2	0	2
Mount Crosby	23	Colony	3	4	7
Pullenvale	39	Colony	2	6	8
Red Hill	1	Solitary	1	0	1
Upper Brookfield2	27	Colony	2	6	8
Upper Brookfield3	9	Colony	1	7	8
Wynnum	3	Colony	2	1	3
WynnumM	1	Solitary	1	0	1
Zillmere	2	Solitary	2	0	2
Total	198		24	48	72

DNA extraction and sequencing

DNA extraction and SNP discovery and genotyping were carried out by Diversity Arrays Technologies, Canberra. DNA was extracted using a NucleoMag 96 Tissue Kit (Macherey Nagel, Düren, Germany). Sequencing for SNP genotyping was done using DArTseq™ technology described in Kilian *et al.* (2012). DArTseq™ technology is optimized for the particular study species and minimizes the percent of repetitive elements, skewed size ranges, or nonideal numbers of fragments (Piza-Roca *et al.* 2019). PCR products were standardized in concentration and pooled for sequencing on a single HiSeq 2500 (Illumina) lane at a 2.5 million read depth.

Single nucleotide polymorphisms and data filtering

SNPs are sequences of DNA where by two alleles of a particular locus differ by one base pair (Weinman *et al.* 2015). SNPs are powerful markers because they allow genome-wide comparisons which can identify small genetic differences between individuals that are otherwise quite similar (Larsen *et al.* 2018). As SNPs are biallelic, heterozygosity cannot exceed 0.5 (Tokarska *et al.* 2009). Kaiser *et al.* (2017) demonstrated that for fine scale genetic studies of relatedness and paternity, 40 SNPs with a mean heterozygosity of 0.4 was adequate for assigning paternity in a bird population. In a simulation study on population genetics, Willing *et al.* (2012) were able to demonstrate that population differentiation using F_{ST} (minimum 0.0102) can be detected using a sample size of 4-6 individuals per population provided more than 1000 SNPs are used. The advantage of using a large number of biallelic markers is identifying a greater representative sample of the entire genome and reduced interlocus sampling variance (Morin *et al.* 2004).

DArTseq™ sequenced a total of 12,992 polymorphic SNP loci with 3.53% missing data for the 72 individual *M. macropus*. We used R version 4.0.0 (R Core Team 2013) and the DARTR package (Gruber *et al.* 2018) to filter and the outcomes of each filtering step are show in Table 5-2. The post-filtering dataset used for SNP analysis consisted of 7,091 polymorphic SNP loci with 0.33% missing data from 15 populations (n= 72).

Table 5-2 Filtering outcomes for nuclear-SNP loci from the DART P/L pipeline

Filtering step	Number of SNP loci (before filtering)	Number of SNP loci (after filtering)
1. Repeatability 100%	12992	9324
2. Call rate/SNP locus of >95%	9324	8079
3. Call rate/individual of >90%	8079	8079
4. Filter for secondary loci (when sequence-tags contained more than one SNP locus, one was retained at random)	8079	7873
5. Removed loci with minor allele frequencies < 2%	7873	7091

Filtering step	Number of SNP loci (before filtering)	Number of SNP loci (after filtering)
6. Filters loci that show significant departure from Hardy-Weinberg Equilibrium using a p-value of 0.05	7091	7091

Mitochondrial sequencing and data handling

Mitochondrial DNA was used to examine patterns of differentiation in female-inherited DNA among roosts. A 761 base-pair (bp) fragment of the mitochondrial cytochrome b gene (*cyt b*) was chosen for comparison to other *Myotis* studies (Ibáñez *et al.* 2006, Wright *et al.* 2018, Park *et al.* 2019).

Mitochondrial *cyt b* was amplified from the extracted DNA using the primers identified by Ibáñez *et al.* (2006), Molcit-F (5'-AATGACATGAAAAATCACCGTTGT-3') and MVZ-16 (5'-AAATAGGAARTATCAYTCTGGTTTRAT-3'). The amplification procedure follows the method outlined by Ibáñez *et al.* (2006). Sanger sequencing was performed by Macrogen INC. (Korea Sequencing Service, Seoul).

Forward and reverse sequences per individual were assembled and manually checked for quality and errors in SANGERSEQR (Hill *et al.* 2014). Consensus sequences per individual were then aligned by ClustalW and trimmed to 571bp using MEGA X (Kumar *et al.* 2018).

Genetic diversity

Genetic diversity was assessed by calculating number of loci (L) per roost using DARTR (Gruber *et al.* 2018), number of alleles per roost (A) using ADEGENET (Jombart 2008) and number of private alleles per roost (P) using GenAIEx (Peakall and Smouse 2006). We calculated observed heterozygosity (H_o) for each roost obtained from allele frequencies in DARTR (Gruber *et al.* 2018) and expected heterozygosity (H_E) was calculated using ADEGENET (Jombart 2008). The mitochondrial dataset was tested for neutrality using Tajima's D tests (Tajima 1989) using POPART (Leigh and Bryant 2015). Mitochondrial diversity within the population was estimated by computing haplotype diversity (H) and nucleotide diversity (π) using HAPLOTYPES (Aktas and Aktas 2015). Haplotype diversity represents the probability that two randomly sampled alleles are different, while nucleotide diversity is defined as the average number of nucleotide differences (Nei 1987).

Population structure and gene flow

Population structure among roosts was examined using principal coordinates analysis (PCoA) calculated in dartR (Gruber *et al.* 2018). We used an analysis of molecular variance (AMOVA) to calculate the level of genetic differentiation within roosts among different populations. The SNP AMOVA was calculated in POPPR (Kamvar *et al.* 2014) and the mtDNA AMOVA was calculated using

GenAIEX (Peakall and Smouse 2006). Both AMOVAs were calculated using the dataset with colonial roosts; AMOVA results are affected by small sample sizes so we removed solitary roosting males from this analysis. We used F-statistics to identify how genetic variation was partitioned across the hierarchical levels of individuals and populations. F-statistics consist of the inbreeding co-efficient (F_{IS}) and fixation index (F_{ST}) (Hartl and Clark 1997). Global F-statistics using SNPs were calculated using GenAIEX (Peakall and Smouse 2006).

We calculated pair-wise Weir-Cockham F_{ST} among colonies using 999 permutations in DARTR (Gruber *et al.* 2018). Pairwise phiPT (an analogue of F_{ST}) were calculated based on 999 permutations in GenAIEX (Peakall and Smouse 2006), using the dataset of colonial roosting bats only, for comparison with the F_{ST} tests completed with SNPs. F_{ST} is a statistic expressing the proportion of the total genetic variance within populations (Hartl and Clark 1997) and is affected by small sample sizes. Thus, we removed solitary roosting males from this analysis to examine differentiation between colonies only.

To further explore the pattern of genetic differentiation among roosts (i.e. population structure analysis), roosts were classified into peri-urban (Brookfield, Mount Crosby, Pullenvale, Upper Brookfield2, Upper Brookfield3, Kholo, n = 40) and urban (Enoggera, Forest Lake, Indooroopilly, Wynnum, Red Hill, Carindale, Zillmere, n = 32). Population structure among peri-urban and urban colonies was examined using sparse non-negative matrix factorization (sNMF) (Frichot *et al.* 2014) to compute least-squares estimates of ancestry coefficients in LEA (Frichot and François 2015). This method follows the Bayesian method described by François (2016), is robust to many demographic situations, and does not make equilibrium population genetic assumptions required for likelihood models like STRUCTURE (Pritchard *et al.* 2003). The number of ancestral populations tested ranged from K = 2 to K = 8, with 10 replicate runs for each value of K. A cross-entropy calculation generated masked genotypes to predict ancestry assignment error with lower values indicating a better fit of the number of clusters to the data (François 2016). Cross-entropy values were plotted and K was selected based on the point of inflection (François 2016).

Isolation by distance

Mantel tests (999 permutations) were used to explore the pattern of genetic isolation by distance (IBD) using the dataset with all bats sampled and only colonial roosting bats. Evidence of sex-biased dispersal was tested using Mantel tests (999 permutations) using the dataset of only females and

then only males. We did not test for IBD using mtDNA because using a single-marker locus is known to be unreliable in detecting IBD (Teske *et al.* 2018).

IBD and sex-biased dispersal was examined using two different distance matrices: (1) Euclidean geographic distance as a measure of direct flight pathways, and (2) distance along connecting waterways because *M. macrourus* is a specialist trawling bat and is highly associated with waterways. Firstly, Euclidean geographic distances between each roost site were calculated using longitude and latitude co-ordinates. The ‘shortest path’ (point to point) tool in the QGIS Network Analysis Toolbox 3 (QNEAT3 plug-in) (QGIS Development Team 2016) was used to calculate pairwise distance matrices of distance between roosts along connecting waterways.

Spatial autocorrelation

The relationship between genetic structure and distance along connecting waterways between colonial roosting males and females was explored by testing for spatial autocorrelation using correlograms (Legendre and Legendre 1998). Correlograms measure genetic similarity between pairs of individuals within distance classes and calculate an autocorrelation coefficient bounded by -1 to 1 (Legendre and Legendre 1998).

Distance classes displaying positive r values indicate that pairs of individuals within that class are more genetically similar than would be expected for random and is a test for fine-scale genetic structure created by philopatry (Banks and Peakall 2012). Mantel tests were conducted in VEGAN (Oksanen *et al.* 2007) and correlograms in MPMCORRELOGRAM (de la Cruz *et al.* 2017).

Relatedness among individual bats

We used the GROUPREL function in RELATED (Pew *et al.* 2015) to assess the relatedness coefficient within groups and the COANCESTRY function to determine the relatedness coefficients between individuals within and among roosts. For both relatedness measures, we used the Wang likelihood estimator (Wang 2002). The Wang likelihood estimator is suitable for small datasets that consist of polymorphic loci and unknown relatives (Wang 2002). In an outbreeding population, coefficients of relatedness for parents and offspring are 0.5, 0.5-0.25 for full siblings and 0.25-0.125 for half-siblings or first-cousins (Wang 2002, Ackiss *et al.* 2019). The RELATED package compares molecular markers of those found in individuals against the markers found within the specified dataset to enable within population relatedness comparisons (Pew *et al.* 2015). Individuals are then assigned to groups. We examined relatedness within all roosts and by sex by testing for relatedness within females and

males separately. We compared adult male-female relatedness in this harem roosting species by removing all single-sex pairwise comparisons from the dataset.

To further explore relatedness among colonial roosts, genetic similarity was assessed by calculating a genomic relatedness matrix using the gl.grm function in DARTR (Gruber *et al.* 2018) and then visualised using the gl.grm.network function in DARTR (Gruber *et al.* 2018). The matrix is calculated by centring the allele frequency matrix of the second allele by subtracting 2 times the allele frequency as described by Yang *et al.* (2010). It is a relative measure of the covariance among individuals averaged over all loci. The relatedness network is a qualitative visualisation dependent on a sensitivity threshold. The network threshold was identified by adjusting the threshold for relationships to be displayed until links appear while the bulk of the population remains as sparse connections (Gruber *et al.* 2018). Using this method, stronger than average relationships are visualised (Gruber *et al.* 2018).

4.8 RESULTS

To provide context for the distance among each colonial roost, distances using Euclidean distance and distance along connecting waterways are shown in Table 5-3. The Wynnum roost is the most geographically distant ranging from 44 km to 118 km along connecting waterways from other colonial roosts. The peri-urban roosts (Enoggera, Pullenvale, Brookfield, Upper Brookfield2, Upper Brookfield3, Mount Crosby) cluster within a 25 km radius. Upper Brookfield2 and Upper Brookfield3 are located just 100 m apart on the same waterway (Table 5-3).

Table 5-3 Distance matrices showing Euclidean distance among colonial roosts above the diagonal and distance along connecting waterway (km) below the diagonal. B= Brookfield, E= Enoggera, FL= Forest Lake, I= Indooroopilly, MC= Mount Crosby, P= Pullenvale, UB2= Upper Brookfield 2, UB3= Upper Brookfield 3, W= Wynnum.

	B	E	FL	I	MC	P	UB2	UB3	W
B		9.44	9.93	9.06	9.37	8.82	8.39	8.36	10.31
E	15.10		10.14	9.37	10.09	9.78	9.73	9.72	9.86
FL	35.68	40.74		9.56	10.08	9.64	10.00	9.99	10.34
I	26.52	41.23	28.95		9.80	9.06	9.41	9.39	10.09
MC	37.84	95.68	50.87	72.14		9.20	8.92	8.92	10.63
P	18.52	38.23	26.74	23.98	25.67		8.81	8.78	10.39
UB2	20.40	26.60	39.20	29.30	26.20	20.50		5.38	10.44
UB3	20.30	26.50	39.10	29.20	26.10	20.40	0.10		10.44
W	78.90	44.21	72.36	45.80	118.60	57.70	52.65	52.98	

Genetic diversity

Population median observed heterozygosity was H_o 0.28 (min 0.23- max 0.30) and median expected heterozygosity was H_E 0.25 (min 0.12- max 0.28). The Enoggera roost had the highest number of private alleles (9) while Upper Brookfield3 had the highest number of alleles per population (13, 110) (Table 5-4).

Table 5-4 Population statistics for *Myotis macropus* roosting in culverts in Brisbane. The number of individuals sampled at each roost is indicated (n). Mean values are indicated for the number of loci per population (L), number of alleles per population (A), number of private alleles per population (P), observed heterozygosity (H_o) and expected heterozygosity (H_E).

Roost	n	L	A	P	H_o	H_E
Brookfield	7	7092	12927	2	0.28	0.27
Carindale	1	7073	8853	0	0.25	NA
Enoggera	8	7092	12499	9	0.30	0.26
Enoggera M	2	7092	10722	1	0.28	0.21
Forest Lake	7	7092	12666	3	0.29	0.26
Indooroopilly	7	7092	12258	4	0.29	0.25
Kholo	2	7091	10758	1	0.27	0.21
Mount Crosby	7	7092	12852	3	0.29	0.27
Pullenvale	8	7092	12857	1	0.28	0.27
Red Hill	1	7087	8989	0	0.27	NA
Upper Brookfield2	8	7092	13110	4	0.28	0.28
Upper Brookfield3	8	7092	12818	5	0.30	0.27
Wynnum	3	7092	10294	5	0.24	0.18
Wynnum M	1	7081	8831	0	0.25	NA
Zillmere	2	7091	10526	0	0.23	0.20

Mitochondrial genetic differentiation

Mitochondrial diversity was low with only four segregating sites and four haplotypes identified, so statistical power is limited. Nucleotide diversity was low ($\pi = 0.001$) and haplotype diversity was high ($h = 0.83$), and although statistical power is low a negative Tajima's D result of -0.15 indicates recent population expansion. The mtDNA AMOVA identified higher variation among populations with 29.52 % of variation among roosts and 70.48 % of variation within roosts (Table 5-5).

Table 5-5 Results from AMOVA based on mtDNA (*cyt b*) for 72 *Myotis macropus*.

Source	df	SS	MS	Est. Var.	%
Among Pops	8.00	8.51	1.06	0.11	29.52
Within Pops	54.00	14.67	0.27	0.27	70.48
Total	62.00	23.17		0.39	100.00

Population structure and genetic differentiation

The PCoA on 72 individuals from 15 roosts revealed structuring by sampling location suggesting genetic differentiation among roosts. The eastern-most roost (Wynnum), the northern roosts (Enoggera and Enoggera M), the centrally located roost (Indooroopilly), and Upper Brookfield3 separated between the three ordination axes, while the remaining roosts clustered together (Figure 5-2). The PCoA explained 10.20% of variation among populations on three ordination axes, with axis 1 explaining 3.50 % of the total variance, and axes 1 and 2 explaining 6.90 % of the total variance (Figure 5-2).

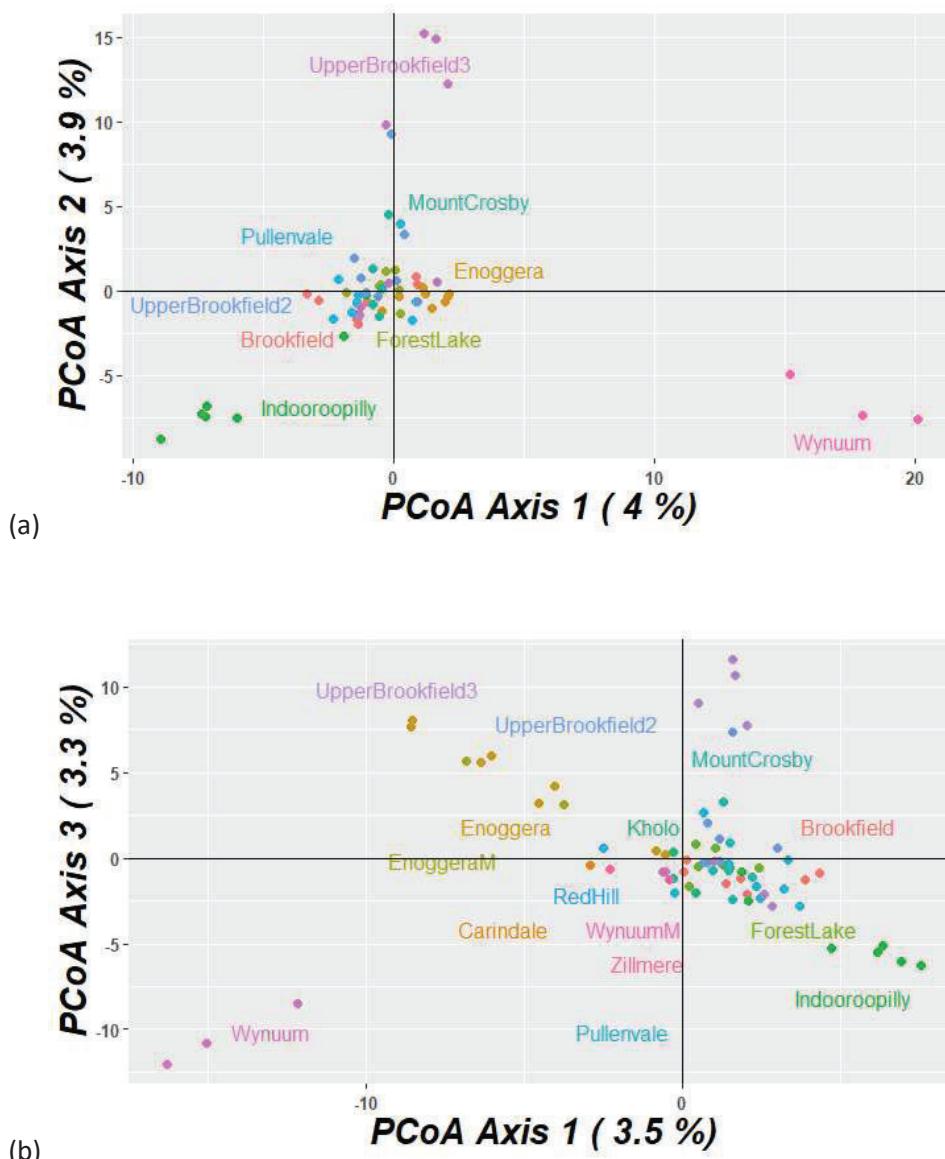


Figure 5-2 Genetic similarity among individuals from each roost shown by principal coordinate analysis. A total of 10.20% variation were explained by the first three ordinated axes (a) axes 1 and 2 explaining 6.90 % of the total variance and (b) axes 1-3 combined explain 10.20 % of the total variance.

The SNP AMOVA showed 5.38% of the total genetic variation was explained by population differences and 93.60% corresponded to differences among individuals (Table 5-6).

Table 5-6 Results from AMOVA based on 7091 SNP loci genotyped for 72 *Myotis macropus*.

Source	df	SS	MS	Est. Var.	%
Among Pops	8.00	14601.71	1825.21	57.59	5.38
Among Indiv	54.00	55272.50	1023.56	10.87	1.02
Within Indiv	63.00	63115.50	1001.83	1001.83	93.60
Total	125.00	132989.71		1070.29	100.00

Global F-statistics revealed no population level inbreeding (F_{IS} 0.01, $p=0.3$) and 5% differentiation across the population (F_{ST} 0.05, $p=0.001$). Global phiPT was high and significant (0.30, $p=0.001$), indicating reduced mitochondrial gene flow.

Genetic differentiation among colonial roosts, measured by pairwise F_{ST} -values, ranged from 0.02-0.19 indicating variable patterns of gene flow (Table 5-7). All of the pairwise comparisons were significantly different ($p<0.0001$), indicating genetically differentiated populations at the time of collection. The peri-urban cluster of roosts (Brookfield, Upper Brookfield, Pullenvale, Mount Crosby) showed little differentiation among one-another ($F_{ST} <0.05$) while the most centrally located roost (Indooroopilly) showed moderate to high differentiation among all other roosts (F_{ST} 0.05-0.19).

The southern-most roost (Forest Lake) showed little differentiation from the western roosts (Upper Brookfield, Pullenvale, Mount Crosby and Brookfield) (F_{ST} 0.03-0.05), but did show: moderate differentiation with the northern-most (Enoggera, F_{ST} 0.07) and central roosts (Indooroopilly F_{ST} 0.07), and high differentiation with the western roost Wynnum (F_{ST} 0.16). The northern roost (Enoggera) showed little differentiation from Upper Brookfield2 (F_{ST} 0.04), and moderate to high differentiation from all other roosts (F_{ST} 0.05-0.16), including Upper Brookfield3 (F_{ST} 0.06) which is located only 100 m downstream (Table 5-7). The eastern-most roost (Wynnum) was the most genetically different roost with all F_{ST} statistics over 0.1 (Table 5-7). Pairwise phiPT values ranged from 0 among several of the western peri-urban roosts, to 0.9 ($p=0.004$) between Indooroopilly and Wynnum (Table 5-7). The eastern-most roost (Wynnum) was the only one to show significant differentiation from other roosts using mtDNA.

Table 5-7 Pairwise comparisons of genetic differentiation among colonial roosts of *Myotis macropus* using nuclear (SNPs) and mitochondrial (cyt b) markers. Pairwise SNP F_{ST} statistics shown below the diagonal and pairwise mtDNA phiPT statistics shown above the diagonal. Significantly different ($p < 0.05$) comparisons are shown with an asterisk indicating the strength of the significance (* $p \leq 0.05$, ** $p \leq 0.01$, * $p \leq 0.001$, **** $p \leq 0.0001$). B= Brookfield, E= Enoggera, FL= Forest Lake, I= Indooroopilly, MC= Mount Crosby, P= Pullenvale, UB2= Upper Brookfield 2, UB3= Upper Brookfield 3, W= Wynnum.**

	B	E	FL	I	MC	P	UB2	UB3	W
B			0.33	0.33					0.73
		0.07	**	**	0.00	0.00	0.00	0.00	**
E	0.05		0.56	0.00					0.76
	****		**	**	0.03	0.02	0.11	0.00	*
FL	0.04	0.07		0.78				0.39	0.69
	****	****		**	0.33	0.19	0.27	**	*
I	0.05	0.08	0.07						1.00
	****	****	****		0.33	0.20	0.40	0.26	***
MC	0.02	0.05	0.04	0.06					0.74
	****	****	****	****		0.00	0.00	0.00	**
P	0.03	0.06	0.05	0.06	0.03				0.58
	****	****	****	****	****		0.00	0.00	*
UB2	0.002	0.04	0.03	0.05	0.01	0.02			0.72
	*	****	****	****	****	****		0.00	**
UB3	0.03	0.06	0.05	0.07	0.03	0.04	0.02		0.76
	****	****	****	****	****	****	****		**
W	0.14	0.17	0.16	0.19	0.14	0.15	0.13	0.15	
	****	****	****	****	****	****	****	****	

To further explore the differentiation among the peri-urban cluster of roosts and the urban roosts, we used a structure analysis to compare peri-urban and urban culvert roosting *M. macropus*. The most probable number of ancestral clusters was $K = 2$ as the largest rate change in entropy occurred from two to three populations (Figure 5-3). Assignment to two ancestral populations was highly supported with a cross entropy value of 0.736. However, $K = 3$ was also supported with a cross entropy value of 0.739, while $K = 4$ had a cross entropy value of 0.742 (Figure 5-3, Appendix 4). Genetic structure among peri-urban and urban roosts showed differentiation in ancestry and admixture among individuals in peri-urban colonies compared to urban colonies (Figure 5-3).

Analysis of peri-urban roosts shows assignment of 37 individuals (96%) to three ancestral clusters with only three individuals (4%) showing limited admixture (Figure 5-3). The assignment of individuals in urban roosts shows assignment of 25 individuals (81%) to three main ancestral clusters with seven individuals (9%) showing limited admixture (Figure 5-3). The ancestral structure in individuals in the urban roosts shows less admixture than in the peri-urban roosts and this reduced diversity could indicate a founder effect. The peri-urban roost providing the greatest genetic

admixture was Upper Brookfield3 and the urban roost providing the greatest genetic admixture was Enoggera (Table 5-3).

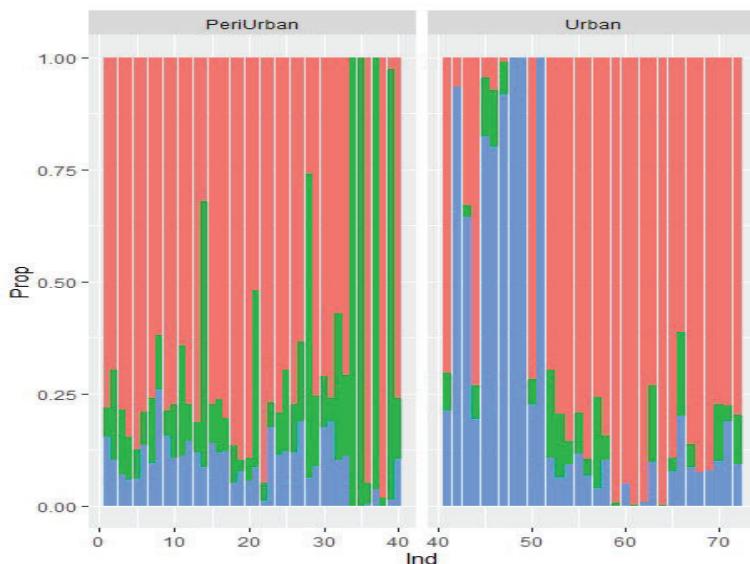


Figure 5-3 Genetic differentiation among peri-urban (Brookfield, Upper Brookfield, Pullenvale, Mount Crosby, Kholo) and urban (Enoggera, Forest Lake, Indooroopilly, Wynnum, Red Hill, Carindale, Zillmere) *Myotis macropus* culvert roosts showing results for sNMF using $K=3$ with each ancestral cluster shown in a different colour. Each vertical bar represents an individual. Peri-urban colonies show higher admixture than urban colonies indicating greater genetic exchange among peri-urban roosts.

Isolation by distance

To test the prediction that bats roosting in geographically close roosts were more genetically similar, and to test for sex-biased movement, we tested for IBD. We detected a weak correlation between genetic distance and waterway distance ($p=0.06$), while no significant correlation for Euclidean distance was found (Table 5-8). When we removed the solitary roosting males from the analysis, we detected a significant correlation for colonial roosts between genotypic and both Euclidean geographic distance ($r = 0.55$, $p=0.01$), and distance along connecting waterways ($r = 0.62$, $p=0.03$) (Table 5-8).

When the two sexes were examined separately, we found a significant correlation between genotypic distance and distance along connecting waterways for female *M. macropus* ($r=0.63$, $p=0.01$), suggesting female-biased movement is geographically restricted along connecting waterways. We did not find a correlation between either colonial roosting males or solitary roosting males and the two distance matrices we used (Table 5-8). For solitary roosting males, the Mantel statistic was negative for both Euclidean distance and waterway distance suggesting solitary males move greater distances than colonial roosting males and females.

Table 5-8 Mantel statistic showing Pearson correlation between genotypic and geographic distance using two distance matrices (1) Euclidean distance and (2) distance along connecting waterways. Results show that female-biased movement is restricted along connecting waterways. Significant ($p \leq 0.05$) values are shown in bold.

Dataset	Euclidean distance		Waterway distance	
	Mantel statistic	p-value	Mantel statistic	p-value
Colonial roosts+solitary roosts	0.07	0.32	0.27	0.06
Colonial roosts	0.55	0.01	0.62	0.03
Females	0.35	0.08	0.63	0.01
Colonial roosting males	0.46	0.06	0.11	0.39
Solitary roosting males	-0.25	0.71	-0.04	0.54

Spatial autocorrelation

To further test the prediction that bats roosting in geographically close roosts were more genetically similar, and to test for sex-biased movement, we tested for spatial autocorrelation. The spatial autocorrelation analysis revealed that genetic relatedness was significantly correlated with geographic distance for females but not males, supporting the findings of the Mantel tests (Table 5-9). Correlograms identified females within 29.72 km were significantly ($r = 0.50$, $p=0.04$) more likely to be genetically similar than expected compared with random, indicating reduced female movement beyond ~30 km and possibly female philopatry to roosts (Table 5-9). Female genetic similarity beyond 44.53 km was significantly negative ($r = -0.56$, $p=0.02$), suggesting that female genetic similarity beyond ~45 km was not different from random. Comparatively, male *M. macropus* did not show significant genetic similarity at any of the distance classes, indicating that dispersal in *M. macropus* is male-biased.

Table 5-9 Spatial genetic autocorrelation (rM) with distance ranges showing female *Myotis macropus* were more genetically similar within 29.72 km, while male *M. macropus* did not show significant genetic similarity at any of the distance classes. Significant ($p \leq 0.05$) values are shown in bold.

Distance range (km)	Female		Male	
	rM	p-value	rM	p-value
0.1 - 14.91	0.17	0.03	-0.02	0.41
14.91 - 29.72	0.50	0.04	0.26	0.15
29.72 - 44.53	0.12	0.38	-0.20	0.23
44.53 - 59.35	-0.56	0.02	-0.15	0.36
59.35 - 74.16	-0.20	0.02	-0.08	0.34
74.16 - 88.97	-0.30	0.16	0.01	0.45
88.97 - 103.78	0.08	0.43	0.07	0.32
103.78 - 119.60	-0.32	0.08	0.07	0.27

Relatedness

To test the prediction that females would display philopatry to a natal roost, we tested for relatedness among individual bats. Mean colony relatedness within groups of two or more bats varied depending on geographical region. The urban roosts had higher overall within-roost relatedness ($r = 0.113$) than the peri-urban roosts ($r = -0.030$). However, urban roosts varied in their degree of relatedness. Wynnum had the highest within roost relatedness overall ($r = 0.394$), followed by Indooroopilly ($r = 0.163$) and Enoggera ($r = 0.151$), where values above 0.125 indicate high levels of related individuals (citation to this factoid needed). The remaining urban roosts had overall relatedness values under 0.125 indicating fewer related individuals within each group (Forest Lake $r = 0.063$, Zillmere $r = -0.208$). All the peri-urban roosts had low relatedness levels (Brookfield $r = -0.023$, Kholo $r = -0.091$, Mount Crosby $r = 0.003$, Pullenvale $r = -0.008$, Upper Brookfield $r = -0.032$).

Pairwise coefficients of relatedness across all individuals, regardless of roost identity, ranged from -0.391 to 0.529. Most pairs of bats were unrelated or distantly related (97.7%) due to non-significant levels of estimated relatedness ($r \leq 0.125$) among individuals. However, 56 related pairs were identified with 34 being female-female pairs. The highest number of within-roost related pairs occurred in Enoggera (18 pairs with $r = 0.137$ -0.488), followed by Indooroopilly (11 pairs with $r = 0.144$ -0.499) and Upper Brookfield (11 pairs with $r = 0.127$ -0.485) (Figure 5-4). The female-male pair at Indooroopilly with an estimated relatedness of $r = 0.499$ were likely mother and son. Additional related pairs were found at Forest Lake (4 pairs, $r = 0.241$ -0.478), Pullenvale (4 pairs, $r = 0.206$ -0.455), Brookfield (1 pair, $r = 0.521$) and Mount Crosby (1 pair, $r = 0.148$) (Figure 5-4). The three bats co-roosting at the one culvert in Wynnum (Figure 5-4) were identified as two-parents (father $r = 0.529$, mother $r = 0.520$) co-roosting with their male offspring. The parents had a pairwise coefficient of $r = 0.135$ indicating they were half siblings or first cousins. Three pairs of related individuals were identified between the roosts of Brookfield-Upper Brookfield ($r = 0.451$), Enoggera-Zillmere ($r = 0.190$) and Mount Crosby-Upper Brookfield ($r = 0.336$) (Figure 5-4).

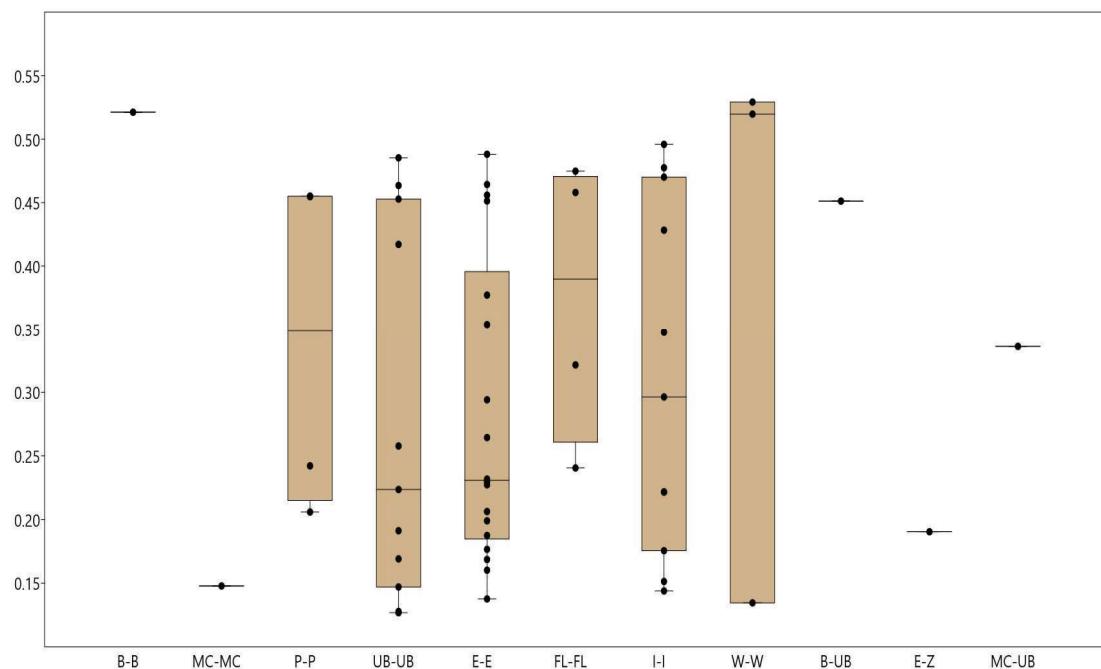


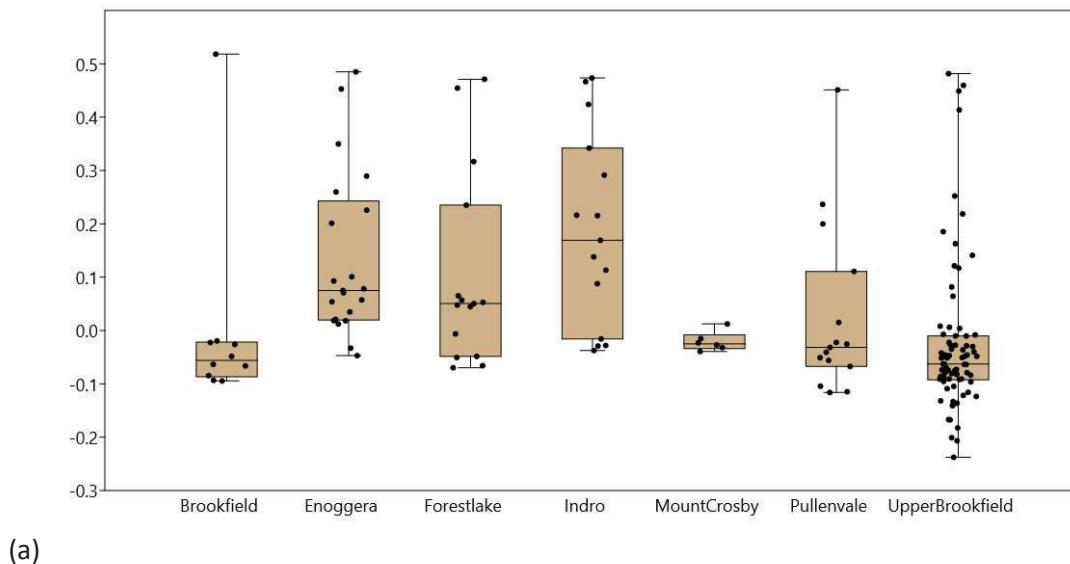
Figure 5-4 Pairwise coefficients of relatedness showing within and between roost relationships for pairs with relatedness coefficient $> r = 0.125$. Each black dot shows a pairwise comparison. Box plots shows the range, median and standard errors. Wang coefficient thresholds are parent-offspring = 0.5, full siblings = 0.5-0.25 and half-siblings/first-cousins = 0.25-0.125. The x-axis shows roosts (B= Brookfield, E = Enoggera, FL= Forest Lake, I= Indooroopilly, MC= Mount Crosby, P= Pullenvale, UB= Upper Brookfield, W= Wynnum) and the y-axis shows the relatedness coefficient.

Most female pairwise comparisons were unrelated or distantly related (97.1%) due to non-significant levels of estimated relatedness ($r \leq 0.125$) between individuals. However, 34 related pairs were identified (Figure 5-5a). Ten related female pairs were found at Upper Brookfield ($r = 0.128\text{-}0.482$) and nine at Indooroopilly ($r = 0.138\text{-}0.473$). Additional pairs of related females were identified at Enoggera (7 pairs, $r = 0.201\text{-}0.485$), Forest Lake (4 pairs, $r = 0.235\text{-}0.471$), Pullenvale (3 pairs, $r = 0.199\text{-}0.451$) and Brookfield (1 pair, $r = 0.518$).

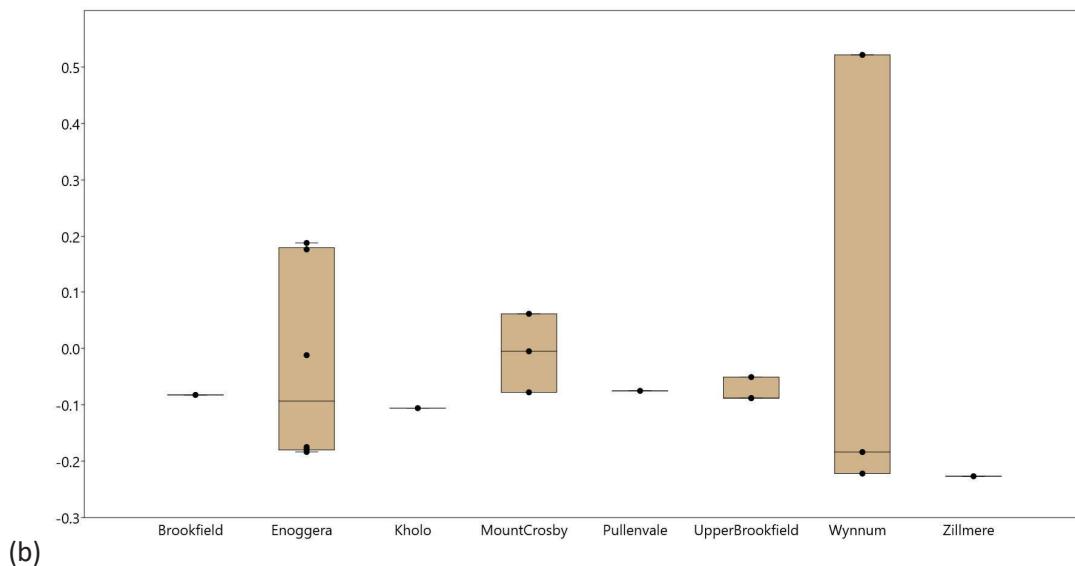
Pairs of males were mostly unrelated (98.9%) with only three related pairs of males identified with two occurring at Enoggera ($r = 0.177\text{-}0.188$) and the previously identified father-son pair at Wynnum ($r = 0.521$) (Figure 5-5b).

Male-female pairwise comparisons identified 87.9% were not related or distantly related. A total of 19 related male-female pairs were identified (Figure 5-5b); two were previously identified mother-son pairs at Indooroopilly and Wynnum. No further parent-offspring pairs were identified. Nine related male-female pairs were identified at Enoggera ($r = 0.138\text{-}0.464$), two at Indooroopilly ($r =$

0.151-0.496), one at Pullenvale ($r = 0.455$), one at Mount Crosby ($r = 0.455$) and the three highly related individuals at Wynnum ($r = 0.135$ -0.529). Seventeen related male-female pairs were identified as half-siblings or full siblings ($r = 0.336$ -0.464).



(a)



(b)

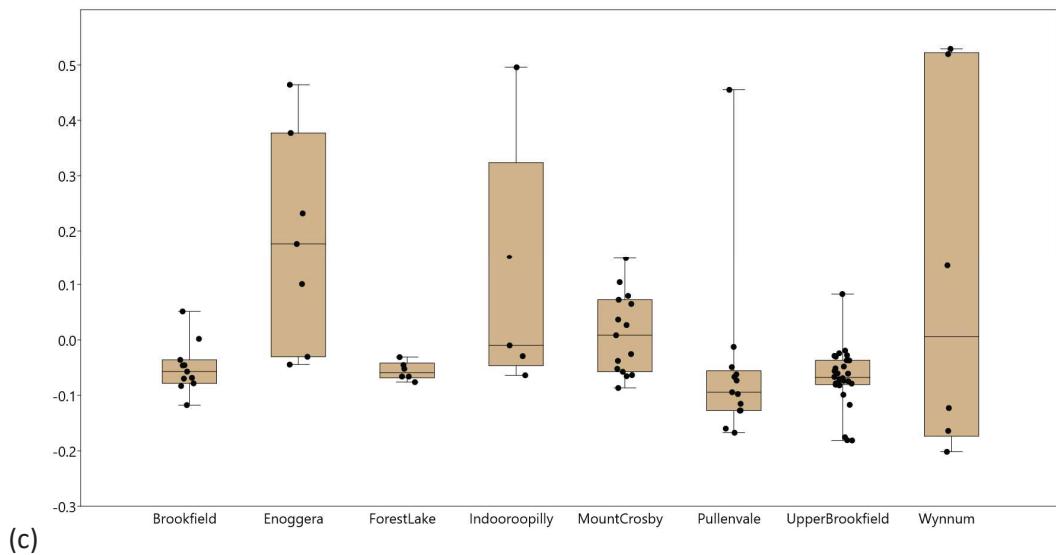


Figure 5-5 Pairwise coefficients of relatedness showing more related females (a) than males (b) within roosts of culvert roosting *Myotis macropus* in Brisbane. Male and female pairwise comparison (c) shows limited relatedness among males and females. Each black circle shows a pairwise comparison, box plot shows the range, the median is indicated by a line within the box plot and standard error bars are shown. Wang coefficient thresholds are parent-offspring = 0.5, full siblings = 0.5-0.25 and half-siblings/first-cousins = 0.25-0.125. The x-axis shows roosts and the y-axis shows the relatedness coefficient.

We built a genomic relatedness network to compare the results to the findings of the pairwise comparisons. The genomic relatedness network identified clusters using a threshold of 0.14, supporting the clusters of related individuals within the same roosts identified using pairwise comparisons of individuals; Brookfield, Enoggera, Forest Lake, Indooroopilly, Pullenvale, Upper Brookfield2, Upper Brookfield3 and Wynnum (Figure 5-6). In contrast to the pairwise comparison, no related individuals were identified at Mount Crosby. Between-roost related individuals were identified in the genomic relatedness network with pairs between Brookfield and Upper Brookfield2 and Upper Brookfield3 and Mount Crosby. The genomic relatedness network did not identify the related pair between Enoggera and Zillmere which were identified in the pairwise comparison.

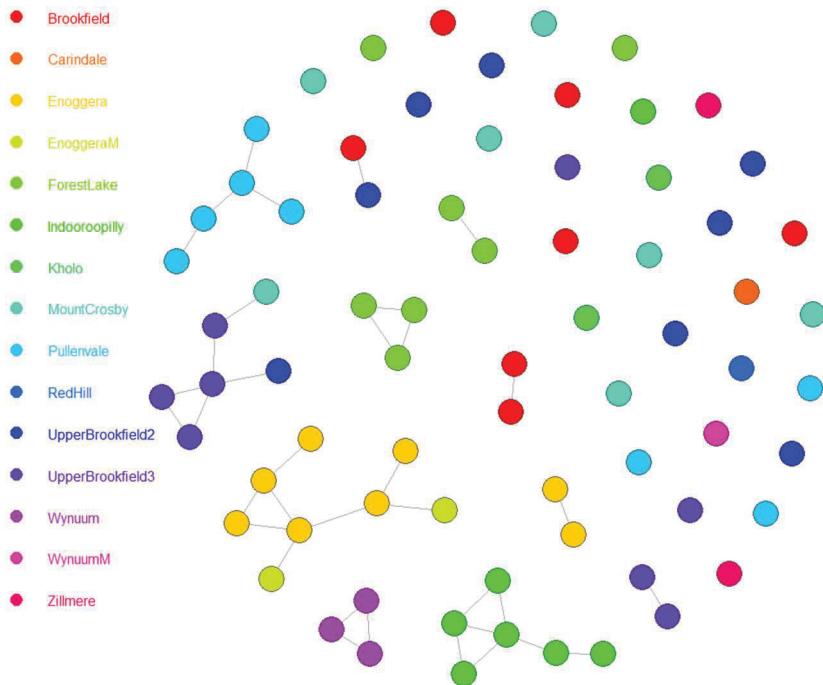


Figure 5-6 The genomic relatedness network shows clusters of related individuals within and between roosts in a culvert roosting population of *Myotis macropus* in Brisbane.

4.9 DISCUSSION

This study is the first study to assess population genetics of a trawling bat in an urban environment. Our findings demonstrate genetic structure in the *M. macropus* population in Brisbane with higher gene flow among local peri-urban populations and reduced gene flow among geographically distant populations. We confirmed *M. macropus* movement across the landscape predominantly occurs along riparian corridors. Our results indicate females show philopatry to roosts and roosts are comprised of related females. Gene dispersal is male-biased, and males show low relatedness to other sampled individuals in the same roost.

Genetic diversity

The levels of genetic diversity found in our study (0.23-0.30) are within the ranges of those recorded for the Coiban mastiff bat (*Molossus coibensis*, 0.11-0.34), velvety free-tailed bat (*M. molossus*, 0.13-0.96) and Miller's mastiff Bat (*M. milleri*, 0.38-0.50) in a study using SNPs on island populations (Loureiro *et al.* 2020). Our values are similar to the those reported for studies on other mammals which range from 0.02 for black bears (*Ursus americanus*) to 0.30- 0.31 for deer (*Odocoileus spp.*), as summarised in Kjeldsen *et al.* (2016). We therefore consider genetic diversity in the culvert roosting population we sampled is high and reflects an outward breeding population. We suggest future research compare genetic diversity among urban and nonurban populations of *M. macropus* to

validate levels of genetic diversity to quantify long-term genetic viability of urban *M. macropus* populations.

Population structure and gene flow

The population structure and genetic differentiation identified in this study demonstrates the *M. macropus* population in Brisbane is not a single, panmictic population. Although we only sampled culvert-roosting individuals, the level of structure and differentiation found over a small geographic distance suggests that gene flow is moderate among peri-urban populations and restricted among urban populations. Structure analysis suggested evidence of urban founder populations with reduced genetic diversity compared to peri-urban populations, suggesting *M. macropus* may be colonising culvert roosts as a mechanism for persisting in urban environments, although our evidence for this is limited. We found high haplotype diversity and low nucleotide diversity across the population, which is indicative of demographic expansion from a small effective population size (de Jong *et al.* 2011). However, the limited statistical power in our mtDNA data is unable to test this. We recommend further research comparing non-urban and urban *M. macropus* population genetics to determine the viability of urban *M. macropus* populations. Additionally, further research on urban *M. macropus* persisting in artificial roosts provides an opportunity to study urban adaptation which will contribute to our understanding on the mechanisms of adaptation and persistence in urban environments by this specialist species.

There are both natural and anthropogenic factors contributing to population structure in urban *M. macropus*. Natural factors shaping the genetic structure in Brisbane are primarily dispersal and philopatry and anthropogenic factors are geographic isolation and between roost connectivity. Our results suggest that dispersal distances are about 30 km and that gene flow is locally restricted. The PCoA clustering of individuals to their roost of capture suggests that gene flow is locally limited. The results of the Mantel tests identified isolation by distance for colonial roosts using both the Euclidean and waterway distance measure. Correlation between genetic difference and different distance measures is expected when individual dispersal distances are smaller than the geographical scale investigated (Slatkin 1993). Although dispersal distances are unknown for *M. macropus*, the species have been tracked foraging 10 km (Barclay *et al.* 2000) and 22 km (Caddle 1998a) from roost sites. Population segregation has been identified among populations 12-15 km apart (Campbell *et al.* 2009, Law *et al.* 2020a) and our spatial autocorrelation results suggest that significant genetic relatedness was not detected beyond 30 km for females.

We found evidence that female *M. macropus* display philopatric behaviour. Evidence for female philopatry was identified in spatial autocorrelation and through pairs of related females within

roosts. We identified several pairs of related females (half siblings or greater) within each colonial roost although mean colony relatedness was low (median $r = -0.011$, range $r = -0.208 – 0.394$). Mean colony relatedness was also low ($r = 0.02$) in closed maternity colonies of *M. bechsteinii* whereby 75% of the colony were close female relatives ($r \geq 0.25$) (Kerth *et al.* 2002b). This result can be explained by a polygynous breeding system with females mating with unrelated males that disperse between colonies (Kerth *et al.* 2002b). *Myotis macropus* are seasonally polyoestrous, having two to three pups per breeding season (Dwyer 1970b, Lloyd *et al.* 1999), display harem roosting behaviour, and are thought to have a polygynous breeding system (Dwyer 1970a, b, Lloyd *et al.* 1999, Barclay *et al.* 2000, Law *et al.* 2001). Increased seasonal reproduction and a polygynous breeding system could explain the low mean colony relatedness and low pairwise relatedness found in our study. Female philopatry has been recorded in several bat species (Petri *et al.* 1997, Kerth *et al.* 2002a, Laine *et al.* 2013, Flanders *et al.* 2016, Monks and O'Donnell 2017, Scott *et al.* 2018) and is a common behaviour for mammals, primarily due to kin selection benefits (Wilkinson *et al.* 2019) or group living benefits (Scott *et al.* 2018). In most polygynous mammals that display philopatric behaviour, roost mates are females which are matrilineal relatives (Lukas and Clutton-Brock 2011, Patriquin *et al.* 2013).

Pairwise coefficients of relatedness were low in our study and displayed spatial variation. Several studies have found low levels of relatedness and high allelic diversity attributing the low genetic structure to high levels of mixing during mating (Kerth *et al.* 2002b, Patriquin *et al.* 2013, Scott *et al.* 2018). The urban roosts in our study had more related pairs than the peri-urban roosts. This finding supports the evidence of a founder effect whereby small founder colonies of related individuals are colonising culvert roosts and the peri-urban roosts are part of a larger outward breeding population. Genetic source-sink dynamics have been documented in several mammal populations (Andreasen *et al.* 2012, Draheim *et al.* 2016, Stillfried *et al.* 2017) and imply that some roosts are more important than others to the viability of the urban *M. macropus* population.

Our results show different patterns in genetic differentiation for nuclear SNP and mitochondrial marker sequence data. Overall F_{ST} statistics for nuclear DNA show significant differentiation among roosts. In contrast, phiPT statistics for mtDNA reveal high gene flow among geographically close roosts, and differentiation among geographically distant roosts. This suggests that to a lesser extent than males, females also contribute to gene flow among roosts potentially through fission-fusion dynamics or extra-colony copulation (Hua *et al.* 2011). The low differentiation in the mtDNA structure found in geographically close roosts in our study could indicate fission-fusion dynamics associated with female movement among roosts. Although little is known about social structure in *M. macropus*, adult females in the polygynous short-nosed fruit bat (*Cynopterus sphinx*) move

among roosts within the same colony and harems experience periodic fission-fusion (Storz *et al.* 2001a). Females may move among a number of roosts in a network, and display philopatry to a network rather than a specific roost (Scott *et al.* 2018). Extra-colony copulation could also explain differentiation among roosts without females dispersing from natal colonies (Burland *et al.* 1999). Advantages of extra-colony copulation include increasing genetic diversity in offspring as well as avoidance of inbreeding (Hua *et al.* 2011).

Although *M. macropus* displays harem roosting behaviour and are considered a polygynous species (Dwyer 1970c, b, Lloyd *et al.* 1999, Barclay *et al.* 2000, Law *et al.* 2001), our findings suggest *M. macropus* may have a promiscuous breeding system. Harem roosting males are thought to monopolize mating opportunities with harem females, and mating opportunities within harems are monopolized by a small number of territorial males (Storz *et al.* 2001b, Wilkinson *et al.* 2019). However, if female *M. macropus* mated exclusively with resident males, a higher level of relatedness would be expected within colonial roosts among male and female offspring, particularly in small urban roosts in areas with few individuals. Our results only revealed the presence of one father-offspring pair in our study and eight male-female sibling pairs that would indicate a common paternal ancestry. The oriental fruit bat (*Cynopterus sphinx*) behaviourally appears to have a harem-based mating system but the species is genetically promiscuous, and the colony, rather than the harem, is the social unit (Garg *et al.* 2012). Kerth *et al.* (2002a) suggest that Bechstein's bat (*Myotis bechsteinii*) is likely to have a promiscuous breeding system despite females displaying high philopatry. We recommend further research into the breeding system of *M. macropus* to provide insight into the significance of colonies within roost networks.

Anthropogenic factors shaping the genetic structure of the culvert roosting *M. macropus* population in Brisbane are geographic isolation and among roost connectivity. The most genetically similar roosts in our study were the peri-urban roosts clustered on the western fringe of Brisbane, which most likely maintain connectivity through a high density of waterways and riparian corridors. The most genetically dissimilar roosts were the most geographically distant, which were also located in landscapes with lower waterway densities and reduced riparian connectivity. Disturbance to geographically isolated roosts that are not part of a geographically close network are likely to have a greater impact on the survival of displaced bats. In the first year following a population crash that forced two long term populations of Bechstein's bat (*Myotis bechsteinii*) to merge, surviving colony mates were still detected roosting together within the new combined colony structure (Baigger *et al.* 2013). Similarly, after a colony disturbance due to predation, eleven surviving long-tailed bats (*Chalinolobus tuberculatus*) integrated into a neighbouring colony but maintained high association

rates with individuals from their original colony (Monks and O'Donnell 2017). Visits among roosts within a network are thought to maintain communal knowledge about neighbouring colonies and there is evidence of acceptance of immigrants and rejection of unknown individuals into colonies (Kerth *et al.* 2002b, Kerth and Van Schaik 2012, Monks and O'Donnell 2017). We found female *M. macropus* in roosts within 30 km of each other are more likely to be more related than expected by random. Roost disturbance within this distance is more likely to result in integration of a disturbed colony into surrounding colonies. However, disturbance to isolated roosts could result in the loss of an already isolated population.

Riparian corridors are vital for connectivity among urban *M. macropus* roosts. We found a correlation with genetic and geographic distance along connecting waterways. Law *et al.* (2020) suggest that due to their ecological specialisation, *M. macropus* populations are buffered from landscape impacts occurring outside of the riparian zone. Similarly, the population structuring found in *M. macropus* populations in southern-Australia was attributed to reduced riparian connectivity in rural areas (Campbell *et al.* 2009). Riparian corridors contained within urban green space, provide dispersal pathways to connect geographically isolated urban *M. macropus* populations. Retaining and maintaining riparian corridors in urban landscapes will enable urban *M. macropus* populations to continue to navigate the spatial heterogeneity provided within urban environments. Given the focus of our study was *M. macropus* roosting in concrete culverts under roads, it is plausible that *M. macropus* may be benefiting from urban-facilitated gene flow (Miles *et al.* 2019). Urban areas can facilitate population establishment and provide corridors for species to spread both within and out of cities (Reed *et al.* 2020). Culvert roosts located under road networks could provide *M. macropus* with roosting opportunities in urban environments where natural roost sites like hollow bearing trees are limited. However, the ability of *M. macropus* to be able to locate and utilise culvert roosts provided in road networks is still dependent on riparian connectivity in urban environments. Further research is needed on population genetics and gene flow in urban populations of *M. macropus* to fully understand how urban populations are structured and how gene flow occurs among natural and artificial roosts.

Conservation implications

This study revealed natural and anthropogenic factors shape the genetic structure of the culvert roosting urban *M. macropus* population in Brisbane. The detection of genetic differentiation and isolation by distance within a single population of *M. macropus* over a relatively small scale has considerable conservation implications for this species. Moderate levels of gene flow are maintained among roosts < 30 km apart and in landscapes with a high density of waterways and riparian

connectivity, highlighting the importance of these landscape features for connecting urban *M. macrourus* populations. The exchange of individuals among roosts across the broader urban population needs to be enhanced to prevent the loss of genetic diversity and this is achieved through the retention and maintenance of riparian corridors.

This study identified that culvert roosts have variable levels of genetic exchange and consequently, have different levels of conservation value to urban *M. macrourus*. Significant research has explored understanding aspects of roost use and roost selection to inform roost protection measures as a means of conserving bat populations, but the impact of roost loss to bats is still poorly understood (Silvis *et al.* 2016). We recommend that disturbance to isolated urban roosts is avoided as these roosts are not supported by a network of neighbouring roosts to which displaced bats could disperse. The loss of roosts that are important for genetic exchange and isolated roosts, could contribute to the decline in genetic diversity of a specialist species currently persisting in an urban environment.

Acknowledgements

Special thanks go to Jenny Ovenden for her guidance, advice and critical feedback on early drafts of this manuscript. This research was supported by a Holsworth Wildlife Research Endowment. VG was supported by a QUT Research Training Program Stipend Scholarship.

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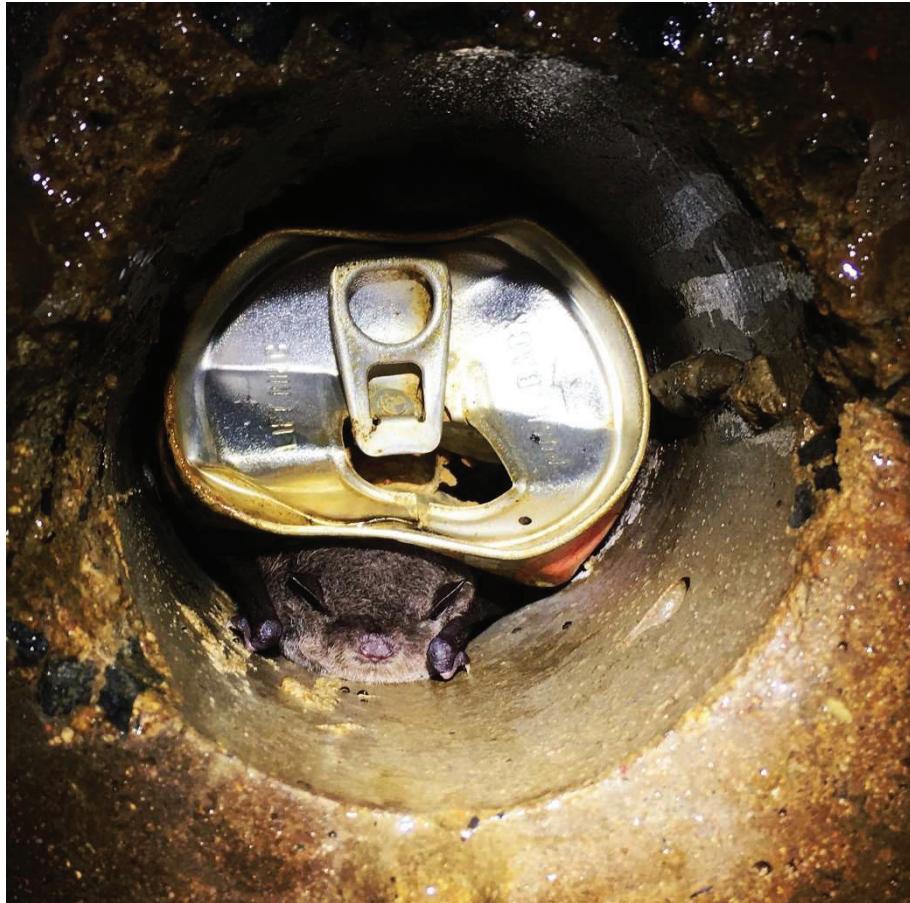
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Chapter 6: Summary and Conclusions



The large-footed myotis (*Myotis macropus*) has flexible roosting strategies but is still dependent on roosts located in riparian areas, such as a lift hole in a concrete culvert. Photo by V. Gorecki.

5.1 SUMMARY OF FINDINGS

Urbanisation is considered to have a negative impact on diversity as the development and expansion of urban environments destroys, degrades and fragments natural habitat. This study used a multidisciplinary approach to investigate roost selection and urban landscape use in a subtropical city by a specialist trawling bat, the large-footed myotis (*Myotis macropus*). Using a range of both ecological and molecular methods, the findings of this study have identified that culvert roosts are limited by design in this urban environment and that selection of a culvert as a roost is limited by the availability of microhabitat within a culvert. This study has also extended the current knowledge on how a specialist-insectivorous bat uses land use types within an urban environment, providing further evidence that *M. macropus* movement predominantly occurs within riparian corridors. Furthermore, the results showcased that in an urban environment, population genetic structuring is due to both natural and anthropogenic factors. It was identified that females are philopatric and individuals within a 30 km radius are more related than random. Gene dispersal across the population occurs through males. In this urban environment (Brisbane, Australia), several genetically distinct populations were identified, suggesting that to manage this species into the future, connectivity among populations needs to be maintained for the viability of the urban *M. macropus* population. The broader management implications are also discussed and provides a summary of the major findings of this thesis and identifies the scope for future research.

5.2 ROOST SELECTION AND MANAGEMENT

Urbanisation is considered to have a negative impact on biodiversity as the development and expansion of urban environments destroys, degrades and fragments natural habitat (McKinney 2002, McKinney 2006). However, urban environments retain artefacts of the natural environment and also create novel niches, enabling some species to persist. One of these novel niches is the distribution of concrete culverts under roads, which are used as roost sites by a specialist trawling bat, the large-footed myotis (*M. macropus*). *Myotis macropus* were first recorded roosting in a limestone cave in 1961 (Seebek and Hamilton-Smith 1967) and colonies have since been recorded roosting in a range of natural and artificial roosts, demonstrating flexibility in roost selection strategies (Schulz 1998, Barclay *et al.* 2000, Campbell 2009, Law *et al.* 2020a). This flexibility in roost use, particularly artificial roosts, has resulted in an assumption that the availability of roosts is not a limiting factor for the distribution of this species (Clarke-Wood *et al.* 2016).

The research in this thesis demonstrates culvert roosts are limited at two spatial scales in this urban environment. At a landscape scale, culvert roosts are limited by engineering design (Chapter 3). Tall culverts are only located on higher order streams, spatially restricting the distribution of available roosts

across an urban landscape (Chapter 3). At a roost scale, culvert roosts are limited by the availability of microhabitat within culverts (Chapter 4). Culverts are manufactured to have smooth surfaces to allow water to drain under roads (Department of Transport and Main Roads 2017). Culverts selected as roost sites had microhabitat in the form of lift holes and crevices, providing a degree of roughness for *M. macropus* to hang from. Lift holes are manufacturing elements associated with lifting and moving culverts into location during road construction and are only manufactured for culverts 1.2-3.6 m in height (Department of Transport and Main Roads, pers. com). As such, lift holes are only present in tall culverts, which are located on higher order streams, so culverts that are available to be selected as a roost by *M. macropus* are limited. Disturbance to, or removal of, a culvert containing a roost has the potential to be a significant impact to an urban population of *M. macropus*.

Recognising that culvert roosts are a limited resource has implications for the management of culverts and suggests that if a roost is disturbed or displaced, an alternative roost may not exist in some areas. Furthermore, disturbance to, or removal of, a culvert containing a roost has the potential to be a significant displacement-stress to an urban population of *M. macropus*. To guide the management of *M. macropus* roosts in culverts, culvert-roosting bats were fitted with radio transmitters to investigate the use of day roosts and rates of roost switching between natural and artificial roosts (Chapter 5). This research revealed a high continued use of culvert and bridge roosts by culvert roosting *M. macropus*, with a low rate of roost switching, indicating a preference for artificial roosts even when natural roosts in the form of mature, hollow bearing trees were available. To further guide management, this study investigated the population structure of culvert roosting *M. macropus* in Brisbane (Chapter 6). This aspect of the research identified that populations within culvert roosts were genetically different over short geographic distances (<30 km); however, urban roosts were genetically distinct from each other and from peri-urban roosts, while peri-urban roosts showed higher gene flow and less differentiation among them. Urban roosts contained higher numbers of related pairs of individuals than peri-urban roosts, with more pairs of related females than related female-male pairs. Culvert roosts may therefore be important breeding sites for urban populations in landscapes where both natural and artificial roosts are limited.

In addition to being important breeding sites, culvert roosts may be the dominant roost type used by *M. myotis* in the study location due to their stability in the urban landscape. Roost permanency has been suggested to affect roost fidelity (Kunz 1982, Brigham 1991). Several studies have documented bats displaying higher fidelity to permanent roost sites such as buildings, bridges and culverts, than to ephemeral roost sites like trees (Brigham 1991, Evelyn *et al.* 2004). These permanent, anthropogenic sites are valuable to bats because they provide thermally stable roosts and have reduced interspecies

competition (Rhodes and Wardell-Johnson 2006, Davis *et al.* 2013, Ancillotto *et al.* 2015). *Yuma myotis* (*M. yumanensis*) is thought to have adapted to anthropogenic features such as buildings and bridges so successfully, that limited natural roosts were found as early as 1947 (Dalquest 1947). It is therefore plausible that urban *M. macropus* populations are roosting in culvert roosts exclusively, due to reduced natural roost availability within urban environments (Le Roux *et al.* 2014), reduced interspecies competition (Rhodes and Wardell-Johnson 2006, Davis *et al.* 2013), stable microclimates (Katzenmeyer 2016) and female philopatry resulting in fidelity to natal roosts (Chapter 6).

Culverts may also provide superior roosts for urban populations of trawling bats worldwide. Culverts are inherently located on waterways, providing roosts close to foraging sites, and this could explain the extra breeding event documented in March (Chapter 4). The spatial distribution of tall culverts containing lift holes being located on higher order streams, results in the positioning of roosts near large pools that have higher insect productivity (Warren *et al.* 2000, Anderson *et al.* 2005, Almenar *et al.* 2009). Ancillotto *et al.* (2015) found populations of Kuhl's pipistrelle (*Pipistrellus kuhlii*) roosting in buildings in Italy had advanced parturition and produced more pups than populations in rural areas, due to the availability of permanent water sources and insect activity around artificial lighting. Similarly, *M. macropus* may benefit from thermally stable roosts close to productive foraging sites and the increased resource availability.

Despite the apparent success and benefit gained by *M. macropus* in colonising culverts, culvert roosts could also become an ecological trap for urban *M. macropus* if not managed correctly. Ecological traps are defined as low quality habitat that animals select over other habitats of higher quality (Schlaepfer *et al.* 2002, Battin 2004, Russo and Ancillotto 2015). Low quality habitat that is used less than it is available does not have the same effect on a population as an ecological trap (Donovan and Thompson III 2001, Battin 2004). Ecological traps result in lower reproduction or survival and ultimately, cannot sustain a population (Donovan and Thompson III 2001). They are generally considered in the context of anthropogenic landscapes in which species are not able to adapt behaviourally and so utilise habitats that may lead to their extirpation (Donovan and Thompson III 2001). In this study, higher reproduction (Chapter 4) and evidence of behavioural plasticity (Chapter 5) implies that culvert roosts are not traps, rather optimal artificial roosts allowing *M. macropus* to persist in a highly modified urban environment. However, these unique roosts could represent an ecological trap due to the increased frequency of displacement and disturbance to bats and the associated impacts upon reproductive success.

Culverts are designed to carry water and are subject to flooding. Flooding associated with increased summer rainfall could potentially decimate a maternity roost during the breeding season, thereby affecting

reproductive success if non-volant young are unable to relocate. Following the collapse of a bridge containing a *M. macropus* roost, Law *et al.* (2020a) documented survival rates three times lower than recorded prior to the bridge collapse. Additionally, road maintenance exposes culvert roosts to a higher risk of disturbance as culverts are managed as transport infrastructure, not sites of conservation value. Findings in this study indicate disturbance impacts are not equal across culvert roosts, and the impact of disturbance can be partially alleviated by increasing the number of culverts available for roosting. In particular, my results on population genetics suggest peri-urban roosts may be more robust to disturbance due to greater gene flow among roosts, greater availability of roosts and higher landscape connectivity, while disturbance to an urban roost may result in extirpation of a local, genetically isolated population.

In summary, there is the potential to increase culvert habitat for this specialist trawling bat in urban areas if road managers and urban planners recognise culvert roosts as sites of conservation value, similar to the value provided by mature, hollow bearing trees. Increasing culvert habitat by unsealing lift holes in existing culverts and constructing culverts >1.2 m in height on new roads could also reduce potential ecological trap effects in urban environments by increasing available roost sites. Furthermore, increasing the number of roosts in an area creates the potential to increase a local population size, and therefore genetic diversity and genetic viability, provided riparian connectivity is maintained. The number of culverts available for selection at a landscape scale can be increased with the construction of new culverts that are >1.2 m in height on urban waterways for all new road projects. Additionally, simple modifications to existing culverts can increase the availability of microhabitats across urban environments. Lift holes are currently plugged on the completion of construction of a culvert and the simple act of removing these plugs would make these microhabitat features instantly available to *M. macropus*. Conservation of urban *M. macropus* populations would benefit from culvert roosts being managed as sites of urban conservation significance.

5.3 URBAN LANDSCAPE USE BY A TRAWLING BAT

Urban landscapes are unique in that they retain components of the natural environment, such as topography, remnant vegetation and waterways, and include anthropogenic design features that vary in intensity from high density residential housing to open green space. The combination of artefacts from the natural environment and anthropogenic features results in spatially heterogeneous urban environments (McKinney 2002, Threlfall *et al.* 2013b, Li and Wilkins 2014). However, our understanding on how urban wildlife populations use the spatial heterogeneity in urban environments is limited.

Trawling bats have been identified as urban tolerant bats due to their increased mobility, developed to enable flexibility and manoeuvrability for foraging over water and edge habitats (Jung and Threlfall 2018). All trawling bats display enlarged hind feet, hairy legs, strengthened calcars and relatively short tails, which are morphological adaptations for capturing prey directly from, or beneath, water surfaces (Campbell 2011, Aizpurua and Alberdi 2018). Species with specialist adaptations occupy a narrow ecological niche, and are not considered flexible in their resource requirements, therefore making them more prone to extinction (Safi and Kerth 2004). How trawling bats like *M. macropus* use the spatial heterogeneity found in urban environments, allowing them to persist, is unknown. Understanding this use of space is vital to protecting resources required for survival.

Myotis macropus used urban land use types in this study in a non-random manner, showing a significant preference for recreation areas that contain waterways, riparian zones, and open green space (Chapter 5). Furthermore, my results relating to population genetics indicated movement across landscapes occurred along connecting waterways (Chapter 6). The most genetically similar roosts in this study were peri-urban, which most likely maintain connectivity through the high density of waterways and riparian corridors found in those areas. The findings of the present study further support the importance of retaining riparian corridors in urban environments to maintain functional landscape connectivity (Hale *et al.* 2012, Lintott *et al.* 2015, Russo and Ancillotto 2015, Straka *et al.* 2020). Additionally, maintaining landscape connectivity is vital to provide pathways for males to disperse and maintain genetic diversity, particularly to isolated urban roosts (Chapter 6).

The findings of this research demonstrate that *M. macropus* has been able to persist in the urban environment of Brisbane largely due to the presence of five semi-permanent, elongate pools, and by using behavioural plasticity to exploit food resources (Chapter 5). Elongate pools were vital core use areas for urban *M. macropus*, indicating that the species use predominantly trawling behaviour throughout the year, and particularly in summer. However, the larger core use areas in winter suggests that *M. macropus* likely increases aerial hawking behaviour to forage over the riparian zone and adjacent land use types when aquatic food resources are low. Urban greenness was identified as the key driver of insect availability at a landscape scale and was found to be a good proxy for productivity and insect activity (Straka *et al.* 2020), indicating the green space adjacent to urban riparian corridors in this study provide suitable foraging resources to enable behavioural plasticity to occur. Behavioural plasticity has been recognised in other trawling bats. The pond bat (*M. dasycneme*) (Britton *et al.* 1997) and long-legged bat (*Macrophyllum macrophyllum*) (Weinbeer *et al.* 2013) are trawling species able to switch foraging mode between trawling and aerial hawking. The greater bulldog bat (*Noctilio leporinus*) is a true fishing bat but the species is

capable of exploiting insect resources if fish availability is reduced (Schnitzler *et al.* 1994). Similar patterns have also been reported in non-trawling bats exploiting available resources in modified landscapes (Chaverri *et al.* 2007, Jung and Kalko 2010, Toth *et al.* 2015).

Whilst the winter core use areas included elongate pools within the riparian corridor and adjacent parklands and residential areas, the summer core use areas were restricted to five elongate pools. This study identified significantly larger core use areas in winter compared to summer, however, the use of elongate pools was consistent between seasons. The finding that elongate pools were vital core use areas through both summer and winter is consistent with the preference for elongate pools as foraging grounds for other trawling bats, due to elongate pools having smooth surfaces, reduced vegetation clutter and high insect productivity (Warren *et al.* 2000, Anderson *et al.* 2005, Almenar *et al.* 2009). This dependence on a unique landscape feature is what places specialist species like trawling bats at greater risk of extinction (Safi and Kerth 2004), and this vulnerability is increased due to the dynamic nature of fluvial systems. Fluvial systems are products of the geomorphic and hydrological conditions within a catchment (Vannote *et al.* 1980), and these systems display spatial and temporal variation. The distribution of fluvial features, like elongate pools, is determined by the geomorphic template (Brierley and Fryirs 2013), and while the physical location of elongate pools may not change, the availability of this aquatic resource varies according to season and climatic conditions (Fontanarrosa *et al.* 2009, Amorim *et al.* 2018). Urban environments introduce additional pressures on these habitat features by modifying runoff through an increase in impervious surfaces, the loss of riparian vegetation, increasing sedimentation and altered water quality (Fontanarrosa *et al.* 2009, Lintott *et al.* 2015), and these factors contribute to structuring the composition and abundance of aquatic macroinvertebrate communities (Fontanarrosa *et al.* 2009). There are short-term and long-term implications to the disturbance to elongate pools in urban environments that are likely to affect the resilience and viability of urban *M. macropus* populations.

Short-term impacts to elongate pools in an urban environment primarily include modification to water quality through stormwater runoff and increased sedimentation which affect insect community composition and availability. Several studies have documented a sensitivity to sediment pollution through the absence of *M. macropus* at waterbodies with sediment pollution or heavy metal contamination (Clarke-Wood *et al.* 2016, Straka *et al.* 2016, Gonsalves and Law 2017). However, the diet of *M. macropus* contains air-breathing invertebrates that are pollution-tolerant and poor indicators of water quality, suggesting the species may be tolerant to low water quality (Law and Urquhart 2000, Law *et al.* 2020). Urban *M. macropus* may be resilient to short-term, temporary impacts of poor water quality in urban waterways through a diet of pollution-tolerant invertebrates and behavioural flexibility (Chapter 5)

enabling the pursuit of alternative insect resources in riparian zones surrounding elongate pools (Straka *et al.* 2020). Increased sedimentation can result in reduced geomorphic variability and reduced biological diversity (Bartley and Rutherford 2005), so sedimentation resulting in permanent modifications to heterogeneity within elongate pools is likely to permanently alter the availability of foraging resources to trawling bats.

A changing climate is likely to result in long-term changes to waterways and *M. macropus* may be more vulnerable to long-term or permanent impacts to core use areas like elongate pools. Climate change predictions suggest a generally drier climate through higher annual average temperatures, increased evaporation and reduced rainfall (Hughes 2003). A drier climate will impact on the permanency of waterways and important habitat features like elongate pools, and therefore, spatially available foraging sites for trawling bats (Fontanarrosa *et al.* 2009). Climate predictions also anticipate a change to rainfall events with the frequency of heavy rainfall as well as the proportion of annual rainfall falling in intense events likely to increase (Capon *et al.* 2013). Law *et al.* (2020) found evidence of a *M. macropus* mortality event following intense rainfall and flooding and suggest that this species is vulnerable to intense rainfall events. An increase in the frequency of intense rain events is likely to affect the long-term resilience of this waterway specialist.

Although elongate pools provide critical core use areas to urban *M. macropus*, tagged bats in this study showed a preference for five pools when nine morphologically similar pools were available, suggesting that pools are not used randomly. Nine elongate pools were available within the home range of all tagged bats, yet four pools did not comprise core use areas by the tagged *M. macropus* in this study. Similarly, Kapfer *et al.* (2008) found female Daubenton's bat (*Myotis daubentonii*) showed a preference for specific pools along a river with foraging activity concentrated on four out of eleven pools available with two pools exploited more intensively than the others. The preference was explained by sexual segregation with females defending high-quality pools so the five pools preferred by tagged bats in this study could reflect foraging site fidelity considering the high relatedness among individuals found at this roost (Chapter 6). This study demonstrates urban *M. macropus* core use areas are dependent on elongate pools and it also identifies that available pools are not used equally. This finding has implications for the management of an urban environment for the conservation of this species. Evidence of foraging site fidelity in genetically isolated urban populations (Chapter 6) highlights the need for the retention of riparian connectivity in urban environments to enable urban *M. macropus* to disperse between both roost and foraging sites.

In summary, the results of this research highlight that in this urban landscape, riparian connectivity is vital to maintain functional landscape connectivity between *M. macropus* populations to enable males to disperse and maintain genetic diversity. Elongate pools are a vital habitat resource for *M. macropus*. The importance of elongate pools to this species varies throughout the year, with the pools a critical resource in summer. In winter, *M. macropus* expands its use of the urban landscape and uses aerial hawking behaviour to forage over the riparian zone and adjacent land use types. Management of urban *M. macropus* populations should consider both riparian zones and adjacent land use types as important habitat for this species. Maintaining spatial heterogeneity in urban planning and design will provide a landscape mosaic for urban *M. macropus* populations to navigate and persist in the urban landscape in the long-term.

5.4 MANAGEMENT IMPLICATIONS

The Action Plan for Australian Bats (Duncan *et al.* 1999) identifies the loss of roost sites as a current threatening processes to the survival of *M. macropus* populations. Recommended actions include carrying out ecological research to determine roost and maternity site selection, habitat requirements and population dynamics. The results of my research provide guidance on roost selection, habitat requirements and population dynamics in an urban environment. The following points discuss the most important conservation themes that emerged from this thesis:

- Not all culverts provide equal roosting opportunities to *M. macropus*. Culvert roosts are limited at two spatial scales in this urban environment. At a landscape scale, culvert roosts are limited by engineering design. At a roost scale, culvert roosts are limited by the availability of microhabitat within culverts. Disturbance to, or removal of, a culvert containing a roost has the potential to be a significant impact to an urban population of *M. macropus* because an alternative roost may not exist in some urban areas. Roost disturbance during the breeding season could affect reproductive success and population dynamics in already genetically isolated urban populations.
- If culvert roost disturbance is genuinely unavoidable, temporary alternative roosts, such as bat boxes, must be provided in the vicinity of the roost being disturbed because it cannot be assumed that other roosts are available. The provision of temporary roosts provides an opportunity for displaced *M. macropus* to move to an alternative roost. Temporary roosts must be provided with enough time for displaced *M. macropus* to be given a chance to relocate. The tagged bats in this study switched roosts ($n=9$) every 4.8 ± 3.7 (1-11) days, so the provision of temporary roosts should be provided a minimum of two weeks prior to disturbance. Maternity colonies are unlikely to

relocate due to the presence of non-volant young and roost fidelity. Culverts containing maternity colonies must not be disturbed until non-volant young are able to disperse.

- The availability of culvert roosts can be readily increased by leaving lift holes within culverts open. Increasing the availability of culvert roosts provides an opportunity to increase the urban population size, which in turn increases genetic diversity and genetic viability of urban *M. macropus* populations. The impact of disturbance to, or removal of, a culvert containing a roost would be reduced if a network of roost sites were available in urban areas. Bat boxes only provide a short-term solution to the loss of a roost and efforts should be made to provide permanent roosting opportunities in urban areas.
- *Myotis macropus* are dependent on spatial heterogeneity within urban environments, especially riparian corridors and open green space, and the retention and maintenance of these landscape features is vital for the persistence and viability of urban *M. macropus* populations. Riparian corridors enable connectivity across urban landscapes, providing pathways for males to disperse between populations and reduce genetic isolation. Riparian corridors contain waterways which are critical habitat for *M. macropus* and they also provide alternative foraging resources to enable behavioural plasticity to occur when aquatic resources may be limited.
- Elongate pools are critical core use areas. The conservation of urban *M. macropus* is dependent on maintaining the integrity of elongate pools. This can be achieved through retaining and maintaining riparian vegetation and preventing sedimentation through the use of sediment fences for any construction and earthworks occurring within 1 km of a waterway.
- Females show philopatry and those with 30 km are more related than expected by chance. Females may be philopatric to a network of roosts within 30 km and geographically isolated roosts are also genetically isolated. Isolated urban populations have higher philopatry as indicated by more related individuals. Gene flow among populations occurs through males and is vital to balance female philopatry and inbreeding within isolated populations. Isolated urban populations are vulnerable to the effects of roost disturbance.

Recognising culvert roosts as sites of urban conservation significance is one step towards improving urban environments for *M. macropus*. Reducing disturbance to culvert roosts and increasing availability of roosting opportunities within culverts provides an opportunity to encourage growth and genetic resilience in urban *M. macropus* populations. Maintaining open green space and spatial heterogeneity in urban planning and design is vital to providing an environment for urban *M. macropus* to navigate and persist in the long-term.

5.5 FUTURE RESEARCH

The research undertaken for this thesis has covered a range of aspects pertaining to the ecology and conservation of *M. macropus* in an urban environment. It has also identified areas that would benefit from future research. These are briefly listed below.

- The use of radiotelemetry provided insight into short-term movement of non-reproductive females at an urban roost. Further work is required to fully comprehend *M. macropus* movement in urban environments. Tracking males, as well as females in all reproductive conditions, would provide greater insight into roost use and landscape use in an urban environment.
- Evidence of behavioural plasticity was identified through home range analysis and this finding could be strengthened by the use of acoustic data, insect trapping and dietary analysis. Combining the use of radiotelemetry, acoustic detection, insect trapping and dietary analysis would provide greater interpretation on the interaction between behaviours observed and movement documented within an urban environment. Dietary analysis in conjunction with insect trapping would provide data on dietary preferences of urban *M. macropus*.
- The population genetics approach used in this research is based on sampling a culvert roosting population. Future research should compare urban and non-urban genetic diversity and population structure to assess the long-term vulnerability of urban populations. Additionally, sampling bats roosting in natural habitat (with tree-roosting individuals) is warranted to test for interaction between natural and artificial roosts within urban environments. If founder populations are roosting in culverts as a mechanism for colonising urban environments, it is plausible that divergence is occurring within urban populations.

5.6 CONCLUSION

This research was undertaken with the aim of understanding roost selection and urban landscape use by *M. macropus* to improve conservation outcomes for colonies roosting in road culverts. As a specialist species, *M. macropus* demonstrates high potential to be able to persist in urban environments, provided roosts are available and not disturbed during the breeding season, and elongate pools, riparian corridors and open green space are maintained. These landscape features and their significance to *M. macropus* need to be considered in urban planning and management, to ensure the species continues to persist in urban environments.

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Appendices



Capturing *Myotis macropus* using a hand net in a concrete culvert. Photo by V. Gorecki.

Appendix 1: Examples of culvert roosts

Culverts containing roosts of *M. macropus* across Brisbane. *Myotis macropus* preferred culverts taller than 1.2 m in height, and while a preference for box culverts was detected, both design types (box and pipe) were occupied.

Appendix 2: Examples of microhabitat

Lift holes containing roosting *M. macropus*.



Crevices containing roosting *M. macropus*.

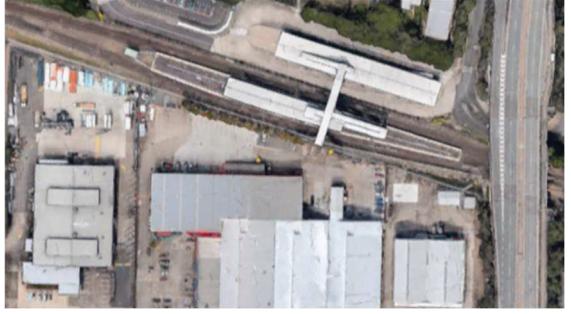


Culverts containing *M. macropus* roosts were only located within culverts that contained microhabitat features such as lift holes and crevices. Lift holes are manufacturing elements associated with lifting and moving culverts into location during road construction. Lift holes are generally only manufactured for culverts 1.2-3.6 m in height (Department of Transport and Main Roads, pers. com). As such, lift holes are only present in taller culverts (Department of Transport and Main Roads 2017). Crevices consisted of deteriorated seals at the end of joins within a culvert. While the species has been able to persist in anthropogenic landscapes, this study has shown that roost selection is limited by the availability of microhabitat (lift holes and crevices) within culverts.

Appendix 3: Radio-telemetry study area land use categories and site photographs

Example aerial imagery of each land use type used in the compositional analysis to study land use selection by *M. macropus* within an urban environment.

Land use category	Description	Example aerial image from Google Earth ©
Commercial	Land with shops and markets, high density of building and lighting with limited natural areas	
Industrial	Factories, workshops, industrial uses, moderate density of buildings and lighting with moderate natural areas in the form of landscaped gardens	
Public services	Schools, community services, cemeteries, low density of buildings with moderate-high natural area cover in the form of playgrounds and gardens	

Land use category	Description	Example aerial image from Google Earth ©
Railways	Permanent rail transport tracks and associated infrastructure, low density of buildings, high density of lighting and limited natural areas	
Recreation	Parklands, sportsgrounds, outdoor greenspace, low density of buildings, low density of lighting and high natural area cover in the form of waterways, riparian zones and green space	
Transport	Land allocated to infrastructure used for transportation of goods or people, moderate density of buildings and lighting with moderate natural areas in the form of landscaped gardens	
Urban residential	Land with houses, apartments, roads and streetlights, high density of buildings, high density of lighting and limited natural areas	

Land use category	Description	Example aerial image from Google Earth ©
Extraction	Water extraction, purification, treatment or supply of fresh water for public, domestic and commercial use, low density of housing and lighting and high natural area cover	

Photographs of the radio-tracking study site.

Northern culvert



Southern culvert



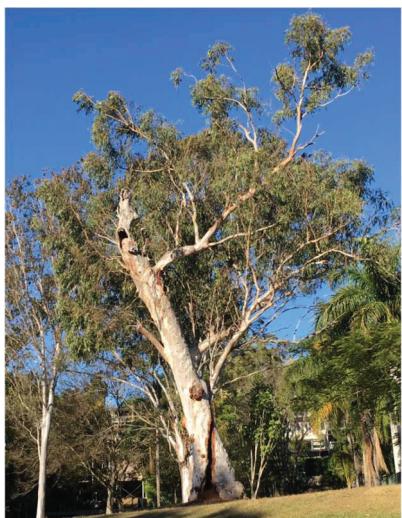
Bridge



Location of roost in pipe in bridge



Hollow bearing tree in parkland



Riparian corridor along creek



Elongate pool A



Elongate pool B



Elongate pool C



Elongate pool D



Elongate pool E



Elongate pool E with bridge upstream



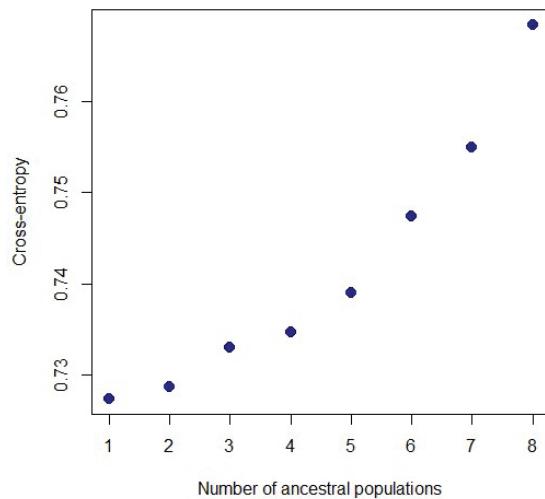
Myotis macropus have been recorded switching between natural and artificial roost sites but in this study, culvert roosting *M. macropus* continued roosting in culverts and bridges so these roost types are important sites for urban *M. macropus*. Core use areas for both summer and winter clustered for all individuals at elongated pools. Elongate pools were identified as vital core use areas for urban *M. macropus*. This study identified that core use areas are larger during winter and include both the riparian zone and adjacent land use types. Management of urban *M. macropus* populations should consider both riparian zones and adjacent land use types as important foraging grounds for this species.

Appendix 4: Population structure cluster selection

Summary of the analysis of sample clustering using the R Bioconductor package LEA with 7,091 high quality SNPs. The sNMF method was used to estimate individual ancestry clusters. Ancestry membership coefficients (K) were estimated to determine subpopulation membership by running 10 replicates of $K = 1-8$ using a cross-entropy criterion (CEC). To select the ancestry coefficient with the highest likelihood, sNMF outputs a cross-entropy score. The most probable number of ancestral clusters was $K = 2$ (Table A3.1). The largest rate change in entropy occurred from two to three populations indicating that $K = 2$ is the optimal number of clusters.

Table A3.1 Cross-entropy criterion scores

$K=$	1	2	3	4	5	6	7	8
min	0.727	0.729	0.733	0.735	0.739	0.747	0.755	0.768
mean	0.733	0.736	0.739	0.742	0.745	0.754	0.764	0.775
max	0.738	0.744	0.745	0.749	0.756	0.763	0.778	0.786

**Figure A3.1 Values of the cross-entropy criterion**

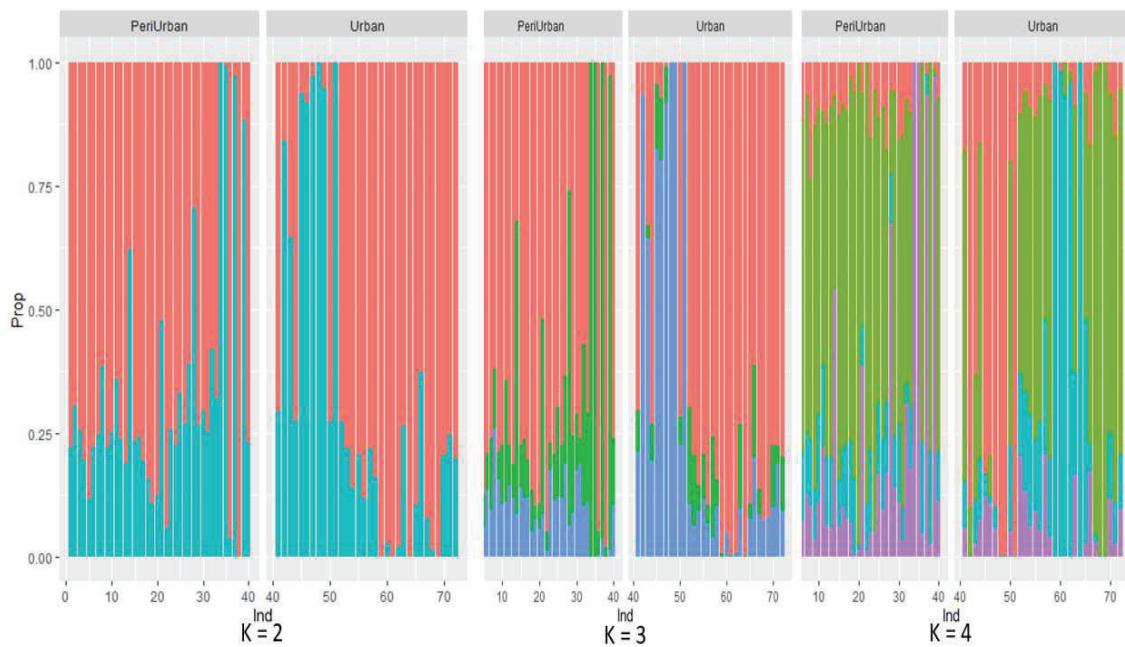


Figure A3.2 Estimated population structure analysis of 7,091 SNPs genotyped for 72 individuals showing genetic differentiation between peri-urban ($n = 40$) and urban ($n = 32$) culvert roosts. Each vertical bar represents one individual which is partitioned into coloured segments that represent the individual's estimated membership fractions in K ancestral clusters.

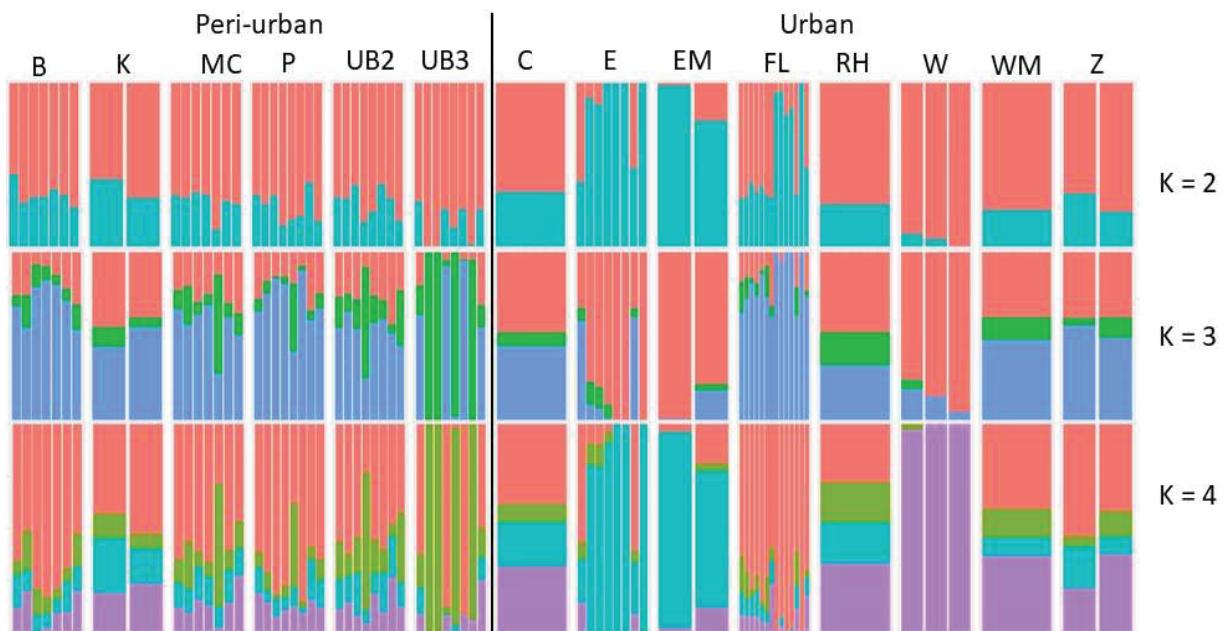


Figure A3.3 Estimated population structure analysis of 7,091 SNPs genotyped for 72 individuals. Each vertical bar represents one individual and samples are sorted according to location of capture. The black line separates peri-urban and urban roosts. Individuals are partitioned into coloured segments that represent the individual's estimated membership fractions in K ancestral clusters. B = Brookfield, K = Kholo, MC = Mount Crosby, P = Pullenvale, UB2 = Upper Brookfield2, UB3 = Upper Brookfield3, C = Carindale, E = Enoggera, EM = EnoggeraM, FL = Forest Lake, RH = Red Hill, W = Wynnum, WM = WynnumM, Z = Zillmere.