

ROOST SELECTION PREFERENCES OF BAT SPECIES PRESENT IN THE YUGAR TUNNEL

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Abstract

The increasing intensity of deforestation and land use change due to urban development and agricultural expansion have significantly impacted on natural habitats. The amount of available natural roosts for bats due to human activities has decreased, increasing the importance of artificial roosts to the survival of many bat species. In South-East Queensland (SEQ), many species of bats have been known to survive within urban and peri-urban settings with increasing use of anthropogenic structures. A better understanding on the roosting ecology of bat species within artificial structures is important for modern bat conservation. Within the peri-urban areas of the Samford region, the Yugar tunnel was known to provide roosting sites for three bat species: *Myotis macropus*, *Miniopterus australis* and *Rhinolophus megaphyllus*. However, little is known about their roosting selection preferences and the utilisation of the surrounding landscape. I examined the bat species and population size, along with the emergence and return patterns, and the roosting selection preferences of each species inhabiting the tunnel.

I examined the bat species and their relative population size by conducting walkthrough surveys in summer and winter. Three bat species including *Myotis macropus*, *Miniopterus australis* and *Rhinolophus megaphyllus* were found occupying the Yugar tunnel in both seasons. There was a minimum of 73 *M. macropus*, 131 *M. australis* and 3 *R. megaphyllus* occupying the tunnel in summer, and 77 *M. macropus*, 15 *M. australis* and 18 *R. megaphyllus* in winter.

I examined the emergence and return patterns of each tunnel bat species by conducting an exit count and acoustic monitoring survey at the tunnel entrance in summer and winter. The pattern of emergence and return were similar across species in summer but varied in winter. Overall, bats emerged earlier in winter than in summer relative to official sunset. In summer, *M. australis* showed the earliest emergence peak, followed by *M. macropus* and then *R. megaphyllus*; in winter, it was *M. australis* first, *R. megaphyllus* second and *M. macropus* last. The final peak return

of all species occurred earlier in summer than in winter relative to sunrise. In summer, all three species' final peak return occurred at similar times between 150 – 160 minutes before sunrise; in winter, the final peak return time varied among species with *M. macropus* first, followed by *R. megaphyllus* and then *M. australis*. The seasonal variation of species' peak emergence and return times was likely driven by multiple physical and biological factors such as predation pressure, reproductive state, foraging needs, resource abundance and weather conditions.

I conducted a microclimate monitoring survey and a walkthrough survey to investigate the roosting selection preference of each species inhabiting the tunnel in summer and winter. My results showed that the three tunnel bat species roosted in different locations and exhibited different roosting strategies in the tunnel. *Myotis macropus* roosts were located inside the drain holes across each zone of the tunnel, *M. australis* roosts were located on the tunnel wall surface in the middle and deep zones of the tunnel, and *R. megaphyllus* roosts were located on the tunnel ceiling in the deep zone of the tunnel in both summer and winter. None of the species shared their roosting locations with other species in either season.

For the roosting microclimate (temperature and humidity), results showed a clear pattern of difference between the tunnel internal ambient microclimate and external ambient (outside the tunnel) conditions; with temperature and relative humidity greatly buffered inside the tunnel, and a lower daily diurnal temperature and a higher daily diurnal relative humidity inside the tunnel compared with external ambient. All three tunnel bat species also showed preferences for roosting locations with higher daily diurnal temperatures and relative humidity and lower daily diurnal temperature and relative humidity fluctuations in the tunnel in both seasons. A variety of physical and environmental factors such as the composition of wall materials, roost location, height, width, length and number of bats within roosts were suggested to have impact on the microclimate within roost.

I conducted an acoustic monitoring survey at a landscape scale to understand the utilisation by bats of the tunnel's surrounding environment. I sampled two habitats within three vegetation types within a 3 km radius from the tunnel in both

seasons. A total of 2100 hours of acoustic recordings were collected and 420 hours were analysed. My results showed that a minimum of 21 bat species were detected in summer and 19 in winter. I investigated the bat species diversity across all sampled sites using a multivariate analysis and showed that there were no significant interactions between bat species diversity across habitats, vegetation types and seasons, and no significant difference within each factor. The overall bat diversity was highest in riparian forest creek in summer, and in open forest edge in winter. My results on the three tunnel bat species in the surrounding landscape showed a significant interaction between search and feeding activity among species, seasons, habitat and vegetation types. Both search and feeding activity of the three tunnel bat species were dominated by *M. macropus*, followed by *M. australis* and then *R. megaphyllus* in both seasons. The searching and feeding activity of the three species were highest in riparian forest in both seasons. It was likely that seasonal effects on water availability (number of watercourses) had shaped the distribution of important resources (i.e. food and water) and consequently foraging bats across the landscape.

The findings of this study suggest that the Yugar tunnel is important to bats in the Samford region, particularly for species occupying the tunnel. Protection of the tunnel and conservation of bats inhabiting it should be considered for the long-term survival of the bats.

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List of Abbreviations

EXA	External ambient
A _z	The ambient reading of the corresponding zone in the tunnel (i.e. Az1 = Tunnel ambient in zone 1)
RH _{avg}	Daily average diurnal relative humidity
RH _{day}	Daily diurnal relative humidity
RH _{fluc}	Daily diurnal relative humidity fluctuation
RH _{max}	Daily maximum diurnal relative humidity
RH _{min}	Daily minimum diurnal relative humidity
TA _{local}	Tunnel local ambient
TA _{non-local}	Tunnel non-local ambient
T _{avg}	Daily average diurnal temperature
T _{day}	Daily diurnal temperature
T _{fluc}	Daily diurnal temperature fluctuation
T _{max}	Daily maximum diurnal temperature
T _{min}	Daily minimum diurnal temperature
Z	The zone inside the tunnel (i.e. Z1 = zone 1)

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: _____

Date: _____

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Chapter 1: General Introduction

This chapter provides a brief overview of the project and a general background of relevant literature to provide the rationale, content and significance of this study. This section also provides an overview and justification for each chapter of the thesis.

1.1 Introductory Statement

An increasing amount of research has been done recently to develop a better understanding of the biology, ecology, and behavior of bats and how this relates to their functions within ecological systems. Studies have shown that bats play an important role in sustaining a healthy ecosystem through pollination, seed dispersal and balancing insect diversity and abundance. Many species of bats roost in anthropogenic structures including houses, tunnels, and mines including several Australian species such as *Vespadelus troughtoni*, *Vespadelus finlaysoni*, *Austronomus australis*, and *Chalinolobus gouldii*. This project studied the bat species present in the Yugar tunnel, near Samford close to Brisbane in Australia. Past studies of bats using this tunnel have reported that *Rhinolophus megaphyllus*, *Myotis macropus*, and *Miniopterus australis* are present. These studies focused on species composition and richness, meaning little is known about their roosting behavior, ecology, and the utilisation of surrounding landscape for foraging. My research will investigate the present bat species in the Yugar tunnel in terms of microhabitat roosting selection, species richness, population size, emergence and return pattern, and relative activity patterns in the surrounding landscape.

1.2 Background

Bats belong to the Order Chiroptera, and with over 1400 species described to date this order has the second highest diversity of all mammals (Welch & Leppanen 2017). Bats represent more than 20% of living mammal species and are the only

mammal capable of true flight (Wang et al. 2014). The common ancestor of bats can be traced back approximately 60 to 70 million years before present (Fenton & Ratcliffe 2010). Recent molecular and phylogenetic studies show that bats (Chiroptera) can be divided into two suborders: Yinpterochiroptera and Yangochiroptera (Agnarsson et al. 2011). Yangochiroptera includes families such as Miniopteridae, Myzopodidae and Vespertilionidae, while Yinpterochiroptera includes Rhinolophidae, Hipposideridae and Craseonycteridae, and all flying-fox families such as Pteropodinae, Nyctimeninae and Epomophorinae.

Bats are distributed in every continent except Antarctica, with higher diversity in tropical and subtropical regions, which decreases towards the poles (Peixoto et al. 2018). Bats live in a variety of habitats including rainforests, deserts, grasslands, woodlands, and wetlands (Fenton & Ratcliffe 2010). They are almost exclusively nocturnal, although diurnal activity has been reported in several species such as the Azores noctule (*Nyctalus azoreum*) and Noack's roundleaf bat (*Hipposideros ruber*) (Russo et al. 2011).

More than 1000 species (~70%) of bats rely on echolocation while the others (~30%) utilise their senses of smell and vision for navigation and foraging (Boonman et al., 2013; Corlett 2011). Echolocating bats use auditory analysis of echoes from their own emitted sounds to identify different surrounding objects and prey (Griffin & Grinnell 1958). For example, bats analyse the echo delay of their calls to calculate distance information to nearby objects in their environment (Simmons 1973). They are also able to determine information on objects in their path such target classification, vertical location, and localisation for single or multiple reflecting targets (Moss & Surlykke 2001; Surlykke et al. 2009).

Bats occupy many sensory-ecological niches, which are reflected in morphological adaptations such as wing shape, body size, and pinna shape (Dong et al. 2013; Stoffberg et al. 2011). For example, bats that forage within forests (also referred to as cluttered habitats) have different wing morphologies when compared with open space foraging species (Marinello & Bernard 2014). Bats that forage in cluttered habitats require good maneuverability to avoid collisions while hunting

prey, and thus generally have lower wing loading and aspect ratios (Maine et al. 2014). Bats that fly in more open spaces generally have higher wing loadings and aspect ratios which allow fast and more energetically economic flight over longer distances (Maine et al. 2014; Ober et al. 2017). Differences in flight morphology among bat species helps to facilitate diverse foraging niches such as gleaning and aerial hawking and allowing for resources partitioning (Denzinger & Schnitzler 2013).

Bats prey on a diversity of food items including insects, fruit, pollen, nectar, flesh, and blood. However, each species tends to express a particular dietary preference with approximately 70% of bats being solely insectivorous; majority of the remainder are frugivorous with less than 1% carnivorous and only three species sanguivores (Fenton & Ratcliffe 2010; Fenton et al. 2012). Bats' broad ecological and dietary niches provide significant ecosystem services such as arthropod suppression, pollination, and seed dispersal. Insectivorous bats largely consume arthropods and airborne insects, many of which are significant agricultural pests or harmful to humans (Kunz et al. 2011). Insectivorous bats play an important role in insect suppression. For example, the Brazilian free-tailed bat (*Tadarida brasiliensis*) can consume up to 70% of their body mass in insects each night and it is estimated that a large colony of over one million individuals could consume up to 8.4 metric tons of insects in one night (Kunz et al. 1995). Frugivorous bats disperse seeds within and across different ecosystems and introduce foundation plants into disturbed landscapes (Corlett 2017). Fifty to ninety percent of tropical plants grow fleshy fruits adapted to be consumed by vertebrates such as bats, indicating the significance of frugivorous species for dispersing the seeds (Galetti et al. 2011). Furthermore, through flight bats can travel a longer distance over shorter time than other mammals thus increasing their effectiveness as seed dispersers.

Over the last decade, many scientific studies have shown that anthropogenic activities such as deforestation and urbanisation have contributed to fragmentation and deterioration of many ecosystems. Such changes have seriously impacted bat species richness and abundance, especially in tropical regions (Bader et al. 2015; Barlow et al. 2007). However, not all bat species are vulnerable to anthropogenic change. For example, some large frugivorous species such as the Jamaican fruit bat

(*Artibeus jamaicensis*), Seba's short-tailed bat (*Carollia perspicillata*) and Ryukyu flying fox (*Pteropus dasymallus inopinatus*) appear to benefit from an increase in exotic vegetation, and show an increase in abundance in areas with human impacts (Williams-Guillen & Perfecto 2010; Jung & Threlfall 2016; Nakamoto et al. 2007). Conversely, insectivorous bats could be more threatened by anthropogenic impacts. Multiple surveys on insectivorous bat species richness and population in Singapore have found that human population increases, and land-use changes have increased the local extinction rate of bats by up to 69-75% (Brook et al. 2003); Rhinolophidae and Hipposideridae represent over half (62%) of the bats documented as locally extinct in Singapore (Pottie et al. 2005). This pattern of decline is claimed to be symptomatic of urbanisation (Russo & Ancillotto 2015). However, some species that are adapted to forage in open space with lower frequency echolocation are more likely to benefit from urban settings (Threlfall et al. 2012). For instance, the Mexican free-tailed bat (*Tardariada brasiliensis*) (Krauel & LeBuhn 2016), Little brown bat (*Myotis lucifugus*) (Coleman & Barclay 2012), and Ride's free-tailed Bat (*Ozimops ridei*) (Threlfall et al. 2012) have shown a higher foraging activity and abundance in urban environments. Urban environments where canopy cover is reduced has increased the prey availability for these species especially in parklands and backyards (Threlfall et al. 2012).

Among the many contemporary anthropogenic changes, habitat fragmentation is likely to be one of major factors impacting biodiversity, including bat communities (Meyer et al. 2015). Habitat fragmentation is a form of landscape disturbance caused by expanding human activities, such as the increasing intensity of agriculture practices, that gradually breaks up large areas of native vegetation into smaller, more isolated and discontinuous patches (Wilson et al. 2016). The decline in habitat patch size is thought to decrease overall species diversity and population size, and disproportionately impact rare species (Cagnolo et al. 2009). Bat species that have low tolerances for edge effects such as Gould's long-eared bat (*Nyctophilus gouldii*) and Little forest bat (*Vespadelus vulturnus*) are likely to experience a population decline in disturbed habitats (Threlfall et al. 2013; Meyer et al. 2008; Haddock et al. 2019). Insectivorous bats have diverse roosting habit (Edirisinghe et al. 2018), but the

removal of hollow-bearing trees due to land-use change and habitat fragmentation decreases roost availability which reduces bat abundance (Seltmann et al. 2017). Therefore, it is important to understand bats' roosting habits and how they interact with the surrounding environment to better inform conservation management (Fabianek et al. 2015b; Morris et al. 2010).

Bats roost in a variety of naturally occurring and anthropogenically created structures including caves, mines, house attics, rock crevices, tunnels, tree hollows, and foliage (Santana et al. 2011). Roosting sites are often found within 10 km of riparian habitats and have good foraging conditions in the surrounding environment; it has been suggested that distance to resources is critical for bats' roost site selection (Fabianek et al. 2015a). Roost selection by bats depends on multiple ecological factors such as microhabitat, temperature, and protection from weather and predators (Moretto & Francis 2017). For example, natural cave roosts are relatively cool and provide a stable thermal environment during the day, while some anthropogenic structures such as house attics and barns provide more variable environments where temperature fluctuations occur daily and seasonally (Marquard & Choate 2009). Furthermore, bats are thought to have multiple roosting sites with a range of microclimates that suit their seasonal needs throughout a year. For instance, within many bat species females and young prefer a warm and humid microclimate that allows them to maintain temperature and reduce water and energy loss in summer (Snoyman & Brown 2011). During winter, when resource and insect abundance are limited, species such as *Myotis lucifugus* (Jonasson & Willis 2012) and New Zealand lesser short-tailed bat (*Mystacina tuberculata*) (Czenze et al. 2017) will lower their resting metabolic rates and enter torpor (Liu & Karasov 2012). As such, many bats prefer roosting in a cool and stable microclimate condition. Species with poor hibernation abilities such as *Tadarida brasiliensis* (Bayless 2019) migrate to other roosts that provide suitable microclimates within their thermoneutral zone (Churchill 1991). The microclimate of cave and cave-like artificial structures such as tunnels and mines vary with height and depth from the surface, distance to water table, air flow within the structure, distance to the entrance, shape and size of the chambers, and the presence of other bat species (Churchill 1991; Perry

2013). The microclimates provided by these structures are a key consideration when assessing the roosting habits of bats.

Bats constitute a large part of Australia's mammal fauna. There are approximately 90 species throughout the continent with the majority belong to the Yangochiroptera and several to the Yinpterochiroptera. For species within the Yinpterochiroptera, one species from the family of Megadermatidae, two species from another bats' family of Rhinolophidae, six species from the family of Hipposideridae and 11 species from family of Pteropodidae are found in Australia (Australian Bat Society 2021). Within the Pteropodidae family seven flying-fox species are found in Australia including the little Red flying fox (*Pteropus scapulatus*), Black flying fox (*Pteropus alecto subsp. gouldii*), Spectacled flying fox (*Pteropus conspicillatus subsp. conspicillatus*), and Grey-headed flying fox (*Pteropus poliocephalus*) (Hall et al. 2000). For species within the Yangochiroptera, there are eight species from Emballonuridae, 11 species from Molossidae, two species from Miniopteridae, and 36 species from Vespertilionidae in Australia (Australian Bat Society 2021). Bats can be found throughout Australia where they inhabit woodlands, rainforests, urban areas, coastal regions, deserts, and riparian zones. The richness of insectivorous bat species varies geographically with New South Wales having 34 species, Western Australia 35 species, and the Northern Territory and Queensland having more than 31 species (Pennay et al. 2011; Armstrong 2011; Reardon et al. 2015). Common bat species in Australia such as the Lesser long-eared bat (*Nyctophilus geoffroyi*), Gould's wattled bat (*Chalinolobus gouldii*), and Inland broad-nosed bat (*Scotorepens balstoni*) are distributed throughout Australia. Other species live in highly restricted ranges in Australia such as the Semon's leaf-nosed bat (*Hipposideros semoni*) and Greater Large-eared horseshoe bat (*Rhinolophus robertsi*) only found in northern Queensland, and Troughton's sheath-tail bat (*Taphozous troughtoni*) only in the Mt Isa region (Queensland Government 2019).

Bats in Australia not only provide ecosystem services, but also make significant contributions to economic growth through insect pest control in the agricultural sector. Because insectivorous bats consume large and diverse diets that include Lepidoptera, Hemiptera, Diptera, Orthoptera, Coleoptera, and Homoptera (Foo et al.

2017; Burles et al. 2008), the top-down control of herbivorous insect populations has a direct positive impact on the crop production as well as saving farmers money through the reduced use of insecticides (Newsome & Sheridan 2018; Williams-Guillen et al. 2008; Kolkert et al. 2021). Furthermore, the positive impact of bats extends beyond the local farm scale and across many ecosystems. Species such as the Gould's wattled bat (*Chalinolobus gouldii*) and the white-striped free-tailed bat (*Austronomus australis*) can fly up to several hundred meters above sea level and cover more than 20 km distance each night (Hull & Cawthen 2013; Rhodes & Catterall 2008).

Agricultural expansion has also directly threatened the habitats of many bat species (Farrow & Broders 2011). Within the agricultural landscapes of Australia, trees are often cleared to make way for farming machinery and increased production which can have both direct and indirect effects on bat populations (Lentini et al. 2012; Maron & Fitzsimons 2007; Mogoutnov & Venning 2014). Intensification of agricultural practices and land-use changes have a significant impact on local bats through a reduction in prey availability, increased exposure to toxic compounds, degradation of foraging habitats, and loss of roosting sites (Park 2015). These problems have caused a decline in some bat species; the Large forest bat (*Vespadelus darlingtonia*) and *Scotorepens balstoni* are known to forage in habitats with higher tree density and show a lower occurrence in an agricultural landscape with low tree abundance (Hanspach et al. 2012).

Trees are vitally important for most bats not only for roosting and because they attract a variety of insect prey, but also because they aid in navigation across the landscape (Lacoeuilhe et al. 2018). Declining connectivity between agricultural landscapes and natural habitats may lead to fragmentation and increased isolation making bat populations more vulnerable to local extinction (Park 2015). If conservation efforts do not concentrate on bats as well as natural habitats, it is estimated that 50% of the bat fauna in Australia may disappear due to natural and anthropogenic processes (Fischer et al. 2010).

The roosting habits of bats have a direct influence on their social interactions, energetics, and breeding success (Campbell 2009). Bats spend more than 50% of their

life roosting. Thus, understanding this behavior is significant for ecological conservation of this group (Campbell 2009). Caves, mines, tunnels, and other anthropogenic structures such as house attics, culverts and bridges provide alternative roosting habitats for more than 20 species of bat in Australia (Armstrong 2010). Species such as the Eastern bent-winged bat (*Miniopterus orianae oceanensis*), (*Austronomus australis*), Orange leaf-nosed bat (*Rhinonictis aurantia*), and Ghost bat (*Macroderma gigas*) are examples of cave-dwelling species in Australia, with *Macroderma gigas* and *Rhinonictis aurantia* widespread throughout the Pilbara region in WA, and often found roosting in disused mines (Cramer et al. 2016; Arteaga Claramunt et al. 2018). These latter two species rely on the humid and warm microclimate provided by the subterranean environment within mines to reduce water and energy loss (Cramer et al. 2016; Armstrong 2010). Specific roosting microclimates are critical to the survival the cave-dwelling species *M. gigas*, which requires temperatures above 23°C and high humidity in summer to conserve energy (Augusteyn et al. 2017).

The roosting microclimate preferences of many insectivorous bats vary depending on the season, sex, and reproductive stage (Johnson & Lacki 2014). Small bats are highly sensitive to microclimate fluctuations in a roost site due to their small body size and high surface area to volume ratio (Campbell 2009). In winter, *Miniopterus orianae oceanensis* prefer a microclimate (tunnels & mines) with a steady daily temperature fluctuation and will roost in the coolest part of the structure. This helps them to maintain a low body temperature to minimise body fat loss throughout the winter (White 2011). Interaction between the structural properties of the roosting sites and the external climate determine the microclimate of bats' roosts (Lundberg et al. 2016). For example, the aspect of the roost entrance determines exposure to sunlight which in turn affects thermoregulation (Russo et al. 2017). Other studies have revealed that an increase in roost exposure to solar radiation is one of the reasons many small bats roost in cavities at higher altitudes (Hoeh et al. 2018). Roost entrance dimensions, internal volume and type of roost (e.g. caves, tunnels, roof spaces, tree hollows) may also influence the roost microclimate (Campbell 2009).

Approximately 34 species of bat are known to live in South-East Queensland (SEQ), and many are cave or hollow-roosting species (Rhodes & Rhodes 2015). Bats in SEQ are most often found in local forests, woodlands, riparian habitats (Richards et al. 2012) and urban areas (Hourigan et al. 2010). Many of them are known to occupy the urban and peri-urban environments and increasing use of caves and human-made structures such as tunnels and houses as roosting sites have been recorded (e.g. The little bent-winged bat (*Miniopterus australis*) and *Chalinolobus gouldii*; Richards et al. 2012). Little is known about how insectivorous bats are affected by land-use change. Within the woodland and forest ecosystems in Queensland, hollow-bearing trees are an essential component that shapes the geographical distribution of bat species throughout the region (Richards et al. 2012). However, the increasing intensity of deforestation has depleted natural tree cavity availability which has resulted in many cavity roosting bat species relying on artificial elements including tunnels, bridges, and houses (Meierhofer et al. 2018). Foraging opportunities and roosting habitats are two significant factors that directly affect the persistence of insectivorous bat species in urban and peri-urban areas (Rhodes & Catterall 2008). A study on bat communities on the Gold Coast (QLD) has found that the complex interaction among the available habitats, land-use practices, tree abundance and diversity, and the degree of urban development have structured bat assemblages in fragmented landscapes (Treby & Castley 2016). A better understanding of insectivorous bat species' dependence on artificial structures and habitats along urban and natural gradients is needed to develop management strategies for preserving bats and their habitats.

The Yugar tunnel is located within the Samford Valley, approximately 30 km west of Brisbane's CBD. The Samford Valley is surrounded by mountains to its west, north and south, and covers approximately 150 km² of land area (Moreton Bay regional Council 2021). The Samford valley was developed into agricultural landscape for farming, dairy and beef cattle, during the 1900s. The land use change from agricultural to urban development began in the 1960s with increasing number of farmlands sold for housing development, and today there are no dairy farms in the Samford district. The population has greatly increased since the early 1990s and now

supports a population of approximately 12000 in the Samford region with land use change from predominately rural to residential properties (Moreton Bay regional Council 2021). The Yugar tunnel was the first concrete railway tunnel on the Samford to Dayboro railway line which began operation in 1919, but was decommissioned in 1955. The tunnel is 82 m in length and passes under Mount Sampson and McDowells Roads. Following the line's closure, the northern entrance to the tunnel was used for garbage disposal and has now been completely blocked by garbage. The tunnel is suffering a moderate level of degradation and is frequently visited by graffiti artists, bushwalkers and cyclists (Hall 2015). Previous studies have detected the Eastern horseshoe bat (*Rhinolophus megaphyllus*), the Southern myotis (*Myotis macropus*), and Little bent-winged bat (*Miniopterus australis*) roosting in the tunnel (Hall 2015).

Myotis macropus is distributed along the coastal band in eastern and northern Australia and has a close association with waterways (Churchill 2008). The high intensity utilisation of waterways by *Myotis macropus* reflects their specialised foraging habit (Campbell 2009). This species predominantly preys on lepidoptera and diptera, and sometimes captures small vertebrates (i.e. fish) using their large feet (Churchill 2008; Law & Urquhart 2000). Several studies of *Myotis macropus* across eastern and southern Australia have recorded this species inhabiting caves (Law et al. 1999) and other artificial structures such as disused railway tunnels (Dwyer 1970), aqueduct tunnels (Seebeck & Hamilton-Smith 1968), and bridges (Law & Urquhart 2000). Although the roosting preference of this species in SEQ is not well documented, in New South Wales the microclimate of a roosting site varied during the summer and winter between 36°C and 1°C, respectively (Campbell 2009). This same study also suggested that riparian vegetation, waterways, as well as roost sites are significant in influencing the population size of *Myotis macropus* (Campbell 2009).

Miniopterus australis has the smallest body size in the *Miniopterus* genus and is distributed along the east coast of Australia from Cape York to Wollongong (NSW Government 2019). *Miniopterus australis* typically forages in densely vegetated areas moving between shrub and canopy layers (Dwyer 1966). Studies of their roosting behavior are relatively rare, with more focusing on *Miniopterus orianae*. Although there are biological and geographical distribution differences between these two

species, the thermoregulatory and reproductive characteristics of *M. orianae* and *M. schreibersii* may help to inform studies of the *Miniopterus* genus, hence to *M. australis* (Dwyer 1968; Hall 1982). A study on the thermal preferences of *M. schreibersii* in South Africa found that temperature was a major determinant of roost selection (Brown & Bernard 1994). This species tends to avoid high temperatures, staying below 30°C throughout the year, and can roost in areas with temperatures as low as 1°C during winter (Brown & Bernard 1994). *Miniopterus schreibersii* has been recorded undertaking internal migrations from warm to cooler areas, with caves with high temperature variability (Kuipers & Dean 1970). The species may migrate over relatively long distance or in a local scale (Brown & Bernard 1994). *Miniopterus orianae oceanensis* has been recorded undertaking long distance migrations between overwintering roost and maternity sites in NSW (Dwyer & Hamilton-Smith 1965; Mills 2021).

Rhinolophus megaphyllus is distributed throughout eastern Australia, from Cape York Peninsula to central Victoria (Murphy 2014). The species is commonly found foraging in a large variety of forested and riparian habitats but not in open environments (Murphy 2014). Foraging activities are concentrated between the forest floor up to the canopy level (Pavey & Burwell 2004), and the species hunts fluttering prey in dense vegetation with slow, highly maneuverable flight (Treby & Castley 2016). *Rhinolophus megaphyllus* prefers roosting in warm, humid environments usually in caves and abandoned tunnels and mines (Murphy 2014). *Rhinolophus megaphyllus* in south-east Queensland may enter torpor when exposed to low temperatures (below 16°C), otherwise it is active for most of the year (Stawski et al. 2014; Geiser & Stawski 2011). The roosting microclimate of maternity sites has humidity over 90% as well as a constant temperature of around 20 -22°C within cave-like structures (Young 2001).

1.3 Context

Tunnels are important roosting habitat for bats world-wide. However, there is a limited amount of research that focuses on bats roosting in tunnels in Southeast Queensland. Therefore, the focus this study is to understand the roosting habits of

the insect-eating bat species inhabiting in the Yugar tunnel. While the number and diversity of bats roosting in the tunnel has been described previously (Hall 2015), this was some time ago and numbers may have changed due to anthropogenic disturbance or changes within the surrounding environment. An accurate assessment of bats living in the tunnel will also assist conservation managers to manage the tunnel and its resident bats. While previous studies have surveyed the bats in the tunnel, nothing is known about their roosting preferences or how they use the surrounding habitat for foraging.

1.4 Purposes

The purpose of this study is to understand the roosting ecology and the utilisation of the surrounding landscapes of insect-eating bats in the Yugar tunnel. This study addresses three questions to understand the bats inhabiting the Yugar tunnel

- 1) What species of bats are using this tunnel?
- 2) What factors might influence how bats select their roost locations in the tunnel?
- 3) How do bats use the area surrounding the tunnel for movement and foraging?

Based on these three questions, this project has established three objectives:

- 1) To determine the species inhabiting the tunnel and their relative population sizes
- 2) To determine how temperature and relative humidity might affect the roosting locations of each species within the tunnel, and
- 3) To determine how the species inhabiting the tunnel use the surrounding environment.

1.5 Significance and scope

Anthropogenic change is intensifying in Queensland recently; expansion of deforestation and urbanization are resulting environmental effects such as land-use change, altering biodiversity structure, and habitat fragmentation has caused significant impacts on many bat species (Simmons et al. 2018; Bader et al. 2015). This research is important for bat conservation as it focuses on roosting habitats and foraging opportunities, the two critical factors that directly affect the persistence of insectivorous bat species in a peri-urban setting. Understanding bat species presence, population size, microhabitat preferences and habitat use are significant for the conservation planning of bats in the local area. The collected information will contribute to the Moreton Bay Regional Council's planning for future conservation of the tunnel.

This study investigates the roosting and foraging habits of each insectivorous bat species inhabiting in the Yugar tunnel in terms of microhabitat roosting selection, species richness, population size, and relative activity patterns in the surrounding landscape. This information will help us to better understand the biology and ecology of several important insectivorous bat species and the significance of this tunnel to them.

1.6 Thesis outline:

This thesis has three main objectives, each of which is addressed in the three experimental chapters (2-4). Each chapter contains an introduction, method, results, analysis, and discussion for each objective. An overall discussion and conclusion will then combine each objective to summarize the findings of the study.

Chapter 2: Understanding the bat species, population, and their emergence and return pattern in the Yugar tunnel.

To achieve this objective, three techniques including exit counts, acoustic monitoring and walk-through surveys were conducted over one summer and one

winter. A total of 5 days of exit counts and acoustic monitoring surveys, and 1 day of walk-through survey were conducted in each season. This chapter compares the findings from the three sampling techniques, then integrates the information to reach conclusions on bat species present, their relative population size in the tunnel during summer and winter, and the emergence and return pattern of each species within and between summer and winter.

Chapter 3: Understanding the microhabitat of the tunnel and determine how this influences the roosting locations of each species.

The aims of this chapter were to determine the location and size of each roost in the tunnel and determine the roosting microclimate (temperature and relative humidity) of each roost / species inhabiting the tunnel. This chapter extends the findings of the walk-through surveys of Chapter 2 by investigating the roosting locations of each species and number of bats within each roost. Furthermore, a total of 10 days of microclimate monitoring was conducted to investigate the microclimate within the tunnel (internal ambient), near the bats' roosts, and outside the tunnel (external ambient) in both seasons. This chapter highlights the roosting selection preferences of each species in terms of two important microclimate variables (temperature and relative humidity).

Chapter 4: Understanding the utilisation of the surrounding environment by bats in the tunnel.

The aims of this chapter were to determine bat species composition and relative activity in the landscapes surrounding the tunnel, with a specific focus on the species inhabiting the Yugar tunnel. Using acoustic recorders, a total of 30 field sites were sampled within a 3 km radius of the tunnel over 10 days in summer and winter, with 2100 hours of recordings collected and 420 hours of bat acoustic activity analysed. This chapter highlights the bat species composition and richness between two habitats (edge and forest) within three vegetation types across summer and winter, and the relative activities of the three tunnel bat species within this setting.

Chapter 5: General discussion

This chapter summarises the findings of previous chapters in relation to the three objectives of the thesis. It also provides a general discussion on the significance of the thesis' results for those charged with maintaining the Yugar Tunnel and conserving the bat species it contains. The thesis concludes by offering several insights for future research regarding the bats inhabiting the Yugar tunnel.

1.7 Reference list

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Chapter 2: Understanding the bat species, population and their emergence and return pattern in the Yugar tunnel

2.1 Introduction

Tunnels play an important role in the life history of bats by providing landscape connectivity across significant barriers, connecting populations that might otherwise be isolated or reducing movement costs. Tunnels also provide significant stable protected roosting habitat for a number of species (Bach et al. 2004). Studies on insectivorous bats around the world have recorded many bats species including *Myotis lucifugus* (Keen & Hitchcock 1980), *Myotis leibii* (Johnson & Gates 2008), *Rhinolophus euryale*, *R. ferrumequinum*, *Barbastella barbastellus* (Uhrin et al. 2010), and *Pipistrellus pipistrellus* (Kerbiriou et al. 2015) roosting in artificial tunnels. Meanwhile, insectivorous bat species such as *Myotis macropus* (Campbell 2009), *Chalinolobus dwyeri* (Dwyer 1966), *Miniopterus orianae oceanensis* (White 2011) have also been found occupying abandoned tunnels in Australia.

Many studies on bat roosting sites have documented physical and environmental factors that affect the timing and behaviour of bats as they emerge or return to tunnel or cave roosts (Campbell 2009; García Ruiz et al. 2017; Pretorius et al. 2020; Gonsalves & Law 2017; Maltagliati et al. 2013; Thies et al. 2006; Funakoshi & Maeda 2003). Emergence and return behaviour are strongly correlated with sunrise and sunset, and influenced by day – night cycle which is synchronised with the endogenous rhythm of bats (Russo et al 2007; Erkert 1978). Bats typically emerge from roosts after sunset and return before sunrise when light levels are low (Rydell et al. 1996; Arndt et al. 2018; Zhang et al. 2015). However, this is not always the case with species such as *Tadarida*

brasiliensis (Reichard et al. 2009; Frick et al. 2012), *Pipistrellus pygmaeus* (Frafjord 2021), and *Chalinolobus tuberculatus* (Griffiths 2007) emerging before or soon after sunset. Factors such as light intensity, resource availability, climate conditions, foraging strategies, reproductive stages and predation risk are suggested to influence bat emergence and return patterns and foraging behaviours (Frick et al. 2012; Russo et al. 2007; Thomas & Jacobs 2013; Arndt et al. 2018; Kunz 1974). Resource availability and predation risk can reflect the cost-benefit trade-off faced by foraging bats (Thies et al. 2006). Bats may emerge earlier, risking predation by diurnal predators, for better foraging opportunities during low resource availability or times of high energetic demand such as during reproductive periods (Frick et al. 2012; Reichard et al. 2009). In contrast, some bats may emerge later after sunset under low light to avoid predators when resources are abundant (Frick et al. 2012; Jones & Rydell 1994; Russo et al. 2007).

As most bat species do not build their own roost and so are heavily dependent on pre-existing structures, roost sites are a crucial but limited resource (Kunz and Lumsden 2003; Kerth 2008). Therefore, artificial structures may play an important role as substitutes for natural roosts (Lisón et al. 2013). Many cave-dwelling bat species in Australia including *Hipposideros semoni*, *Taphozous troughtoni*, *Miniopterus orianae*, *Rhinolophus capensis* and *Rhinolophus philippinensis* are found roosting in tunnels (Brown & Bernard 1994; Thomson et al. 2005). Artificial structures likely reduce exposure to predators and offer protection for bats under severe weather conditions such as heavy rain or during low ambient temperatures (Lausen & Barclay 2006; O'Malley et al. 2020). Artificial structures may also provide stable roosting microclimate which enhance the energetic benefits for roosting bats, particularly during reproductive stages and hibernation (Vivier and van der Merwe 2007; Lausen & Barclay 2006; Johnson et al. 2019). However, some cave-dwelling species such as *Myotis macropus* (Campbell 2009) and *Chalinolobus morio* (Turbill 2006) do not only roost in cave-like structures, but also roost in tree hollows unlike other species such as *Rhinolophus megaphyllus* (Murphy 2014; Pavey 1998) and *Miniopterus schreibersii* (Ramos Pereira et al. 2009; Mispagel et al 2004) which primarily roost in caves or cave-like structures.

The value of the Yugar tunnel for local bats is more likely to be as a roosting site in both summer and winter, as it can no longer act as a movement corridor - the northern entrance to the tunnel is blocked and there are no other known entrances apart from the southern portal. A past study of bats in the tunnel has documented that *Miniopterus australis* was usually found roosting during February to April, whereas *Myotis macropus* and *Rhinolophus megaphyllus* were found throughout the year, but numbers were lower when *M. australis* was present (Hall 2015). However, it is uncertain whether other insectivorous bat species are also using the tunnel; other species may be present but have not been recorded due to being missed during earlier surveys or due to taxonomic redescriptions (e.g. *Miniopterus orianae oceanensis*). In addition, little is known about why or how bats are using the tunnel. For example, the emergence and return behaviour of these three species remain relatively undocumented. Understanding the timing of emergence and return of these bats maybe useful for providing information on factors influencing which species occupy the tunnel and provide a baseline for monitoring the health (size) of the populations.

The aim of this study was to determine the number and species of bats using the tunnel through the application of three techniques: exit counts, acoustic monitoring and walk-through surveys. Using each of these techniques we sought to fulfil the following objectives: 1) to determine the species inhabiting the tunnel and their relative population sizes, 2) to determine the emergence and return pattern of each species in the tunnel.

2.2 Methods and analysis

Acoustic monitoring surveys, exit counts, and walk-through surveys were conducted to determine the composition, emergence / return timing and size of the bat population occupying the Yugar tunnel. Under the Köppen climate classification system, Brisbane is classified as a subtropical region characterised by humid and hot summers and mild winters (Peel et al. 2007). To align the findings from a study by Hall (2015), two

seasons (summer and winter) were used as the sampling periods throughout the study. The exit counts and acoustic surveys were conducted simultaneously in summer (15th – 19th January 2020) and winter (17th – 21st July 2020). Walk-through surveys were conducted two days later to minimize disturbance could potentially affect the emergence pattern of the bats.

To determine the species and temporal pattern of activity of bats emerging and returning through the southern portal of the tunnel, an SM3 acoustic monitoring unit (Wildlife Acoustics, Maynard Massachusetts) was used. The SM3 was set up approximately 5 m from the entrance to the tunnel and configured to make full spectrum triggered recordings at a sampling rate of 384 kHz with 16-bit precision. The SM3 recorded from 30 minutes prior to official sunset until 30 minutes after official dawn, for 5 consecutive nights. After each 5 days deployment, the SM3 was retrieved from the site and all recording data transferred from the SD cards to a computer for back up and analysis. In summer the recorder was in the field for 5 days. However, it experienced a fault on the 4th day of the recording meaning only three nights of data were available for analysis. In addition, there was heavy rain over the 3 sampling nights in summer, and no pattern of bat emergence under normal weather condition was recorded. Recordings were visualised and echolocation calls parameterised using Raven v1.5 software for Windows (Cornell Laboratory of Ornithology, Ithaca, New York). Reinhold et al. (2001) was used to key out species based on their calls, with some call identifications reviewed by a bat expert (Dr. Roger Coles) to improve accuracy. Any calls whose identify remained uncertain were not used in further analyses. Acoustic data was analysed in terms of species present, types of echolocation calls (search phase or feeding buzz), and number of passes per minute. We defined a pass as a series of calls separated from another series of calls by a period of silence lasting at least 1.5 s. If many bats exited at once, the echolocation calls recorded during that period of time were examined at high (millisecond) temporal resolution. It is difficult to distinguish whether recorded echolocation calls from the same species belong to the same individuals or other conspecifics (Hogue & McGowan 2018). Thus, this analysis aimed to quantify the relative

activity of bats leaving the tunnel. However, I acknowledge that due to the placement of the SM3 we cannot rule out that some echolocation calls came from bats flying in the vicinity of the cave (i.e. bats recorded were not roosting in the tunnel).

To determine the timing of bat emergence from the southern portal of the tunnel, exit count surveys were conducted simultaneously with the above acoustic surveys in both summer and winter. Two observers undertook simultaneous counts outside the tunnel entrance. Each observer used two hand-held counters to determine the number of bats emerging from the southern portal. The first counter was used to record the number of bats exiting the tunnel while the second was used to record the number of bats returning into the tunnel. For each observer, the number of bats leaving the tunnel was determined by subtracting the number of the exit counter by number of return counter. The counting session began 30 minutes before official sunset. Each observer was situated near the tunnel entrance such that emerging bats were backlit against the sky. Continuous counts were made and subsequently tallied into 15-minute time intervals. Counting stopped when the observers could no longer reliably see the bats (after approximately 1 hour of observation). The final exit count for each night was calculated by averaging the count of bats leaving the tunnel, as determined by each observer. This result was averaged over 5 nights and compared between summer and winter. Analysis of emergence count data was used to determine the time of first bat emergence, and the time of peak emergence (the highest number of bats recorded in a 15-minute interval). Data on emergence time are presented relative to the time of official dusk, thus allowing meaningful comparisons across nights and seasons.

Walk-through surveys were conducted a day after the exit count and acoustic sampling survey to determine the species, location, and number of bats roosting within the tunnel. A 100 m measuring tape was placed along the centreline of the tunnel from the entrance to the back (82 m). The transect line was followed at a slow walk and the species were observed, identified and counted with the assist of prepared photos of cave roosting bat species in Queensland; distance from the entrance and height on the tunnel

wall was recorded. Direct counting was used to determine the number of bats in small clusters. For larger clusters, the number of bats in a sub-area was counted, and then extrapolated to represent the number of bats in the entire cluster. Any specific roosting structures (e.g. holes in the tunnel wall) used by the bats was also recorded. A red light was used during the surveys to minimise disturbance to the bats and to maximise the accuracy of the count. Photos of bat clusters were taken (under red light) for a supplementary count for checking against visual counts.

Walkthrough surveys were approved by the QUT Animal Care and Biosafety committees (approval number: 1900000687), and conducted with permission of Moreton Bay Regional Council.

A statistical analysis was completed using Rstudio Desktop version 1.2.5042. A two-way non-parametric ANOVA using an *a priori* significance level of 5% was conducted to examine seasonal effects of the acoustic data on first emergence and peak emergence time (relative to sunset; leave) and the peak return time (relative to sunrise; return) for each species in the tunnel across summer and winter. Multiple plots were created to examine the nightly activity pattern of bats, and a peak in activity was identified by observing these plots. Results from exit counts and walk-through surveys did not undergo statistical tests but were used to compare the estimated bat population in the tunnel. The exit count results also assisted in determining the first emergence and were used to compare the time of first emergence peak with the acoustic data.

2.3 Results

Acoustic Surveys

Acoustic surveys at the tunnel entrance were conducted for a total of 57.5 hours in summer and winter. This resulted in the detection of 12 species; all 12 were recorded in summer but only 3 in winter (Table 2-1). Among the 12 species detected in summer, three cave-roosting species, *M. australis*, *M. macropus* and *R. megaphyllus*, were the major contributors to total bat activity. Other species showed relatively low levels of activity. *Miniopterus orianae oceanesis*, another cave-roosting species, had between 9 and 19 passes recorded each night in summer with activity usually occurring within the first 3 hours after sunset.

Table 2-1 Total number of species identified from the SM3 near the tunnel entrance in summer and winter.

Number	Species	Summer	Winter	Cave roosting species
1	<i>Austronomus australis</i>			
2	<i>Chalinolobus gouldii</i>			
3	<i>Miniopterus australis</i>			
4	<i>Miniopterus orianae oceanesis</i>			
5	<i>Myotis macropus</i>			
6	<i>Nyctophilus gouldi/ Nyctophilus bifax</i>			
7	<i>Ozimops lumsdenae</i>			
8	<i>Ozimops ridei/ Ozimops petersi</i>			
9	<i>Rhinolophus megaphyllus</i>			
10	<i>Saccolaimus flaviventris</i>			
11	<i>Scotorepens greyii</i>			
12	<i>Vespadelus darlingtoni</i>			

Relative levels of bat activity throughout the night, as determined by acoustic surveys at the tunnel entrance, showed contrasting patterns in summer and winter, although the level of bat activity recorded across the two seasons was similar with peaks between 200 - 300 passes per hour. In summer, the first peak in activity occurred within the first hour

after sunset, then a second larger peak occurred 2 hours before sunrise; activity level between these two peaks was variable across nights (Figure 2-1). In winter, the first peak also occurred within the first hour after sunset, but the second peak was smaller and occurred 6 hours before sunrise; the level of activity between these two peaks also varied across nights (Figure 2-2). The largest activity peak occurred earlier in winter (shortly after sunset) than in summer (several hours before sunrise). Bats stayed active longer in winter than in summer relative to official sunset; the last nightly activity in summer occurred 2 hours before sunrise, while in winter it occurred 30 mins after sunrise.

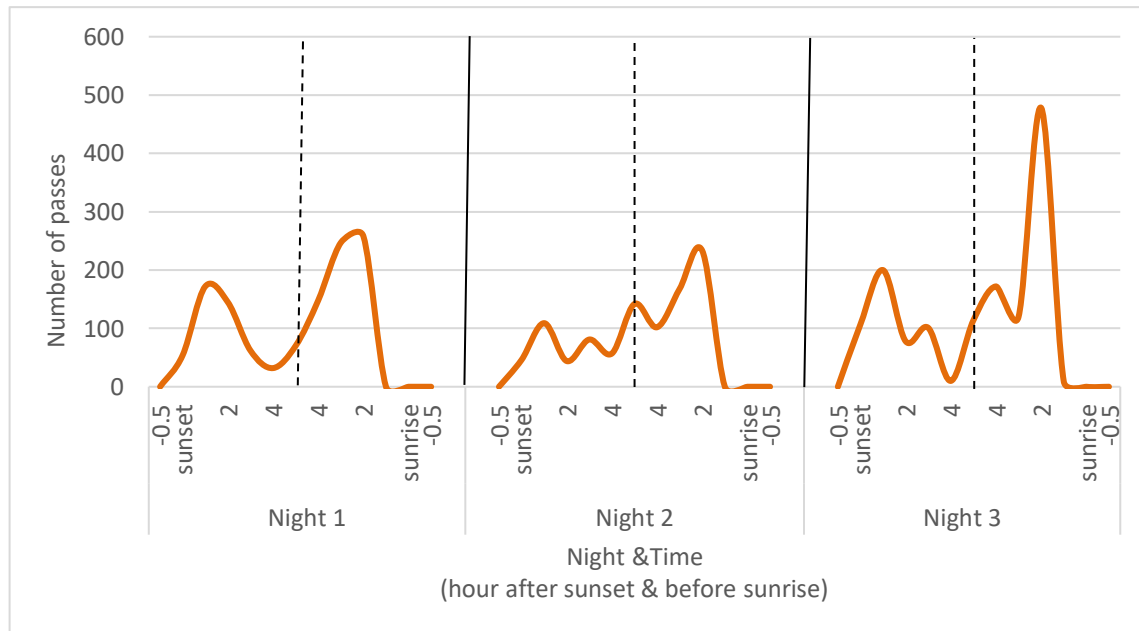


Figure 2-1 Results from 5 nights of acoustic recording of bats' activity approximately 5 m away from the tunnel entrance from 30 minutes before official sunset to 30 minutes after official sunrise, over 3 nights in summer. (Nightly time was divided into 2 perspectives: 1. hours after official sunset and 2. hours before official sunrise, where the dotted line marks the transition between two perspectives) (solid line separate each night and -dotted line indicates the 5th hour after official sunset and before official sunrise) (Note: The recorder was in the field for 5 days. However, it experienced a fault on the 4th day of the recording meaning the final 2 days of recordings were lost). Bats' activity over 3 nights present similar patterns; the first peak in activity occurred within the first hour after official sunset, and the last peak (highest activities of each nights occurred between 2 to 3 hours before official sunrise. On 2 out of 3 days minimum activity occurred between 3 – 4 hours after sunset. Activity never reached 0 between the beginning of first peak in activity and the end of last peak activity.

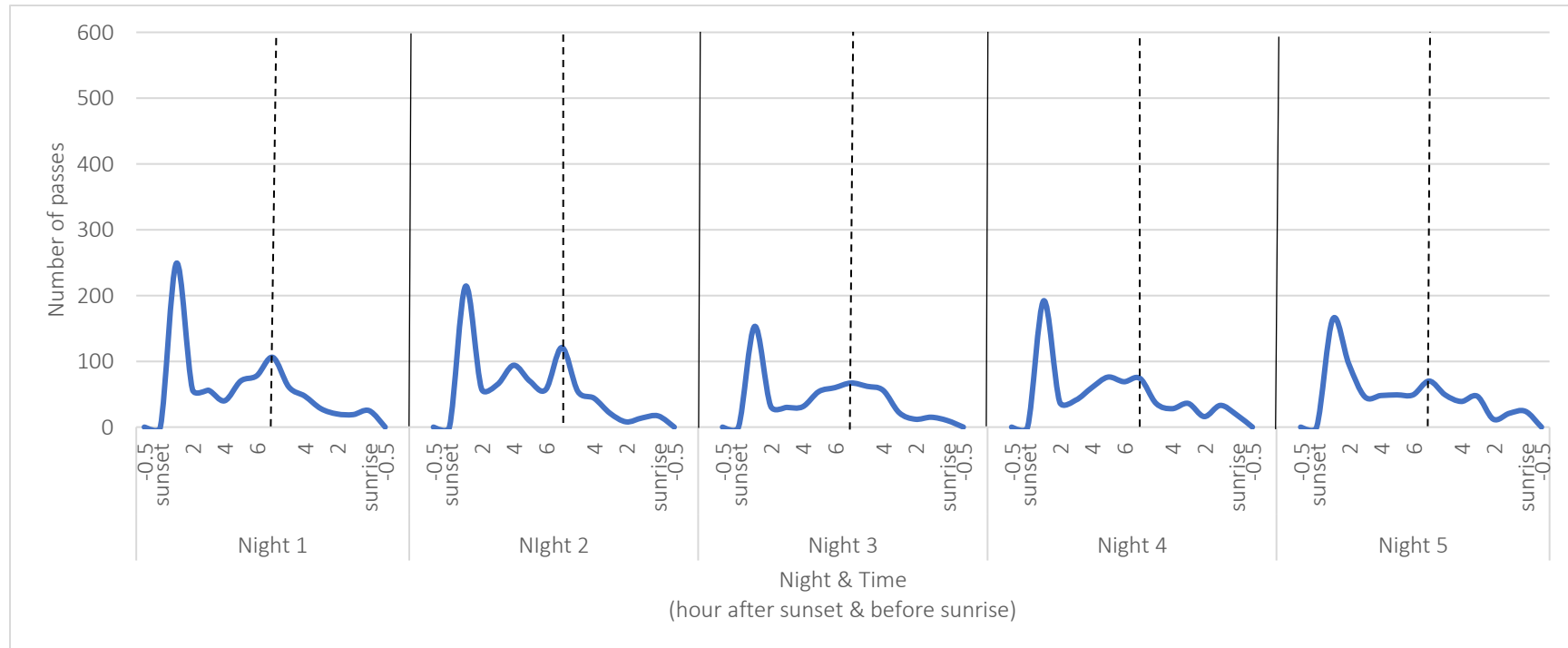


Figure 2-2 Results from 5 nights of acoustic recording of bats' activity approximately 5 m away from the tunnel entrance from 30 minutes before official sunset to 30 minutes after official sunrise, over 5 nights in winter. (Nightly time was divided into 2 perspectives: 1. hours after official sunset and 2. hours before official sunrise, where - - - dotted line marked the transition between two perspectives) (--- solid line separate each night and - - - dotted line indicates 7.3 hours after official sunset or 6 hours before official sunrise). First peak of bats activity occurred within the first hour and the second peak occurred at 6 hours before official sunrise, these patterns remained the same throughout the 5 nights in winter. Last activity was decided between sunrise and 30 minutes after sunrise each day.

The pattern of acoustic activity at the tunnel entrance in the first 4.5 hours after sunset varied between species, both daily and seasonally (Figures 2-3 and 2-4). In summer, all four species (*M. macropus*, *M. australis*, *M. oriana oceanesis* and *R. megaphyllus*) were first detected within 0.5 hours before sunset, but each species exhibited peak activity at different times and at different levels (Figure 2-3). Both *M. oriana oceanesis* and *M. macropus* peaked first but only at very low levels of activity, followed by *R. megaphyllus* and *M. australis*. Both *M. macropus* and *R. megaphyllus* had a second larger peak between 2 and 3 hours after sunset, excepting day 1 when the second peak was smaller for *R. megaphyllus*. In terms of activity levels, recordings were dominated by *M. australis* passes, followed by *M. macropus* and *R. megaphyllus*; activity from *M. oriana oceanesis* was relatively low. In winter, bat activity began later when both the first and peak activity of three species (*M. oriana oceanesis* was not detected in winter) was detected within the first hour after sunset (Figure 2-4). All three species presented a similar activity pattern with the major peak 1 hour after sunset and secondary peaks 3 to 4 hours after sunset. The relative activity levels differed among species with recordings dominated by *M. macropus* followed by *R. megaphyllus* and then *M. australis*. Overall levels of activity were similar between seasons although as described above, the relative contribution of each species differed. Activity of *M. australis* was first detected in summer (within 30 minutes before sunset) earlier than in winter (within 1 hour after sunset), and both *M. macropus* and *R. megaphyllus* emerged later in summer than winter.

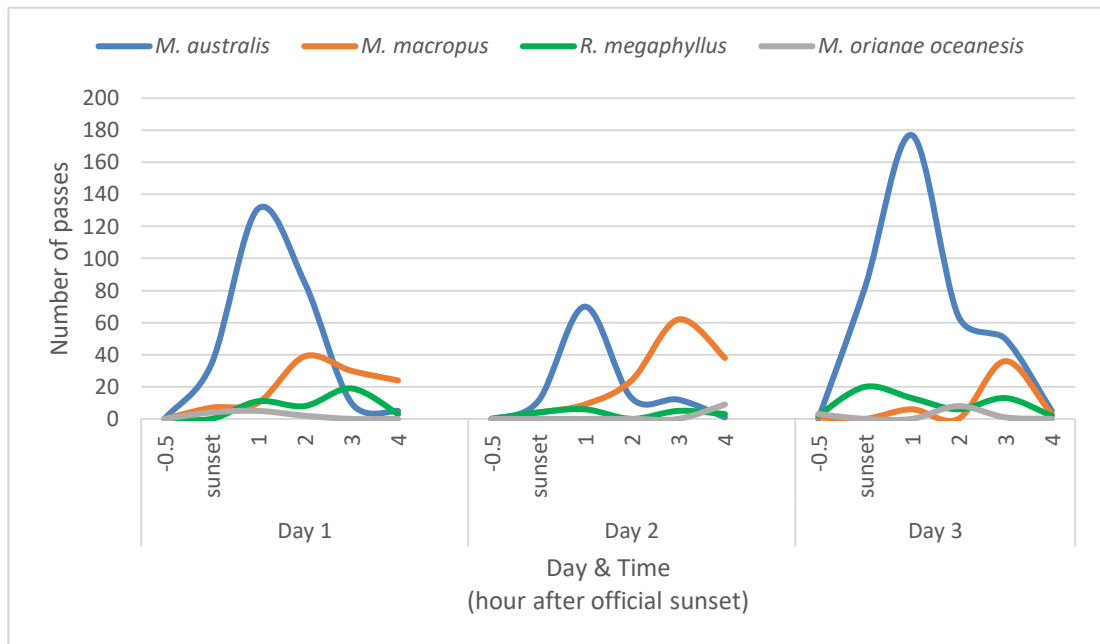


Figure 2-3 Acoustics recording of each tunnel roosting bat species at the tunnel entrance from 30 minutes before official sunset to 4 hours after official sunset over 3 consecutive days in summer. *Miniopterus australis* has the earliest first peak activity (within the first hour after sunset). On 2 out of 3 days *M. macropus* reached the first in peak activity between 2-3 hours after sunset. *Rhinolophus megaphyllus* does not have a regular pattern of first peak activity. *Miniopterus orianae oceanesis* activity remained very low throughout each sampling night compared to the other three species. The time and level of activity of the three species differ among one another. Activity detected was dominated by *M. australis*.

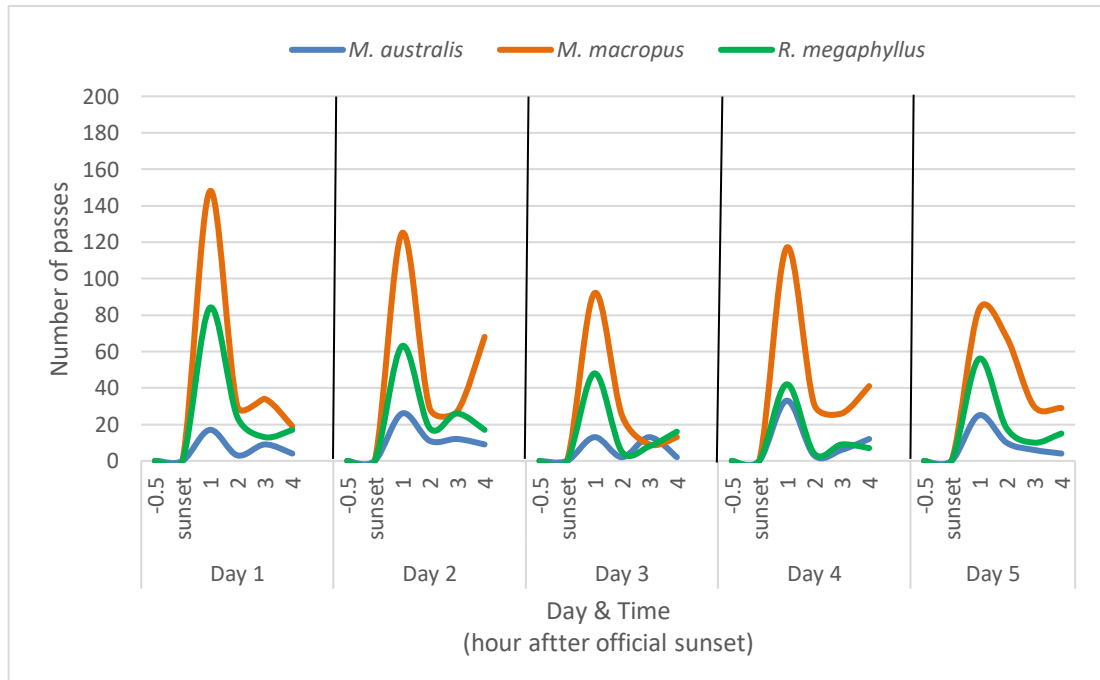


Figure 2-4 Acoustics recording of each tunnel roosting bat species at the tunnel entrance from 30 minutes before official sunset to 4 hours after official sunset over 5 consecutive days in winter. The first activity of each species was detected within the first hour after official sunset, when all three species also reached the first peak in activity which then greatly reduced in the following hour. The three species had similar times of peak activity, but the level of activity differed from one another. Majority of the detected activity was from *M. macropus*. No activity was detected from *M. orianae oceanesis* in winter.

The time of first emergence peak (highest level of bat activity detected at sunset) was significantly different among the three species, and the differences were dependent on season (Aligned Rank Transformation ANOVA, $F = 86.710$, $df = 2$, $p = 5.7487e-10$) with a large effect size ($\eta^2 = 0.90597$; Figure 2-5, Table 2-2). Overall, summer bat emergence reached its peak 106.9 ± 63.5 minutes after official sunset ($n = 3$). *Miniopterus australis* had the earliest peak emergence time (25.7 ± 4.7 mins after sunset) when compared with *M. macropus* (133.0 ± 19.3 mins after sunset) and *R. megaphyllus* (162.0 ± 16.5 mins after sunrise). In winter, the time of peak emergence was 25.1 ± 5.6 minutes after official sunset ($n = 5$). *Miniopterus australis* had a peak emergence time (18.7 ± 0.4 mins after sunset) earlier than *R. megaphyllus* (25.0 ± 1.8 mins after sunset) and *M. macropus* (31.2 ± 3.7 mins after sunset) (Figure 2-5).

Post-hoc comparisons between summer and winter for the time of first activity peak (emergence) of the same species indicated that there were significant differences. Within each species, *M. macropus* and *R. megaphyllus* emerged significantly earlier in winter than summer ($p < 0.0001$ — Table 2-2). While *M. australis* showed no significant difference between two seasons ($p = 0.3079$ — Table 2-2).

Comparing seasonal difference across species, it was found that seasonal interactions on the time of first activity peak (emergence) across different species were significant ($p < 0.05$ — Table 2-2), except for the interactions between *M. australis* in summer and *M. macropus* in winter ($p = 0.3906$) and between *M. australis* in summer and *R. megaphyllus* in winter ($p = 0.9359$, Table 2-2).

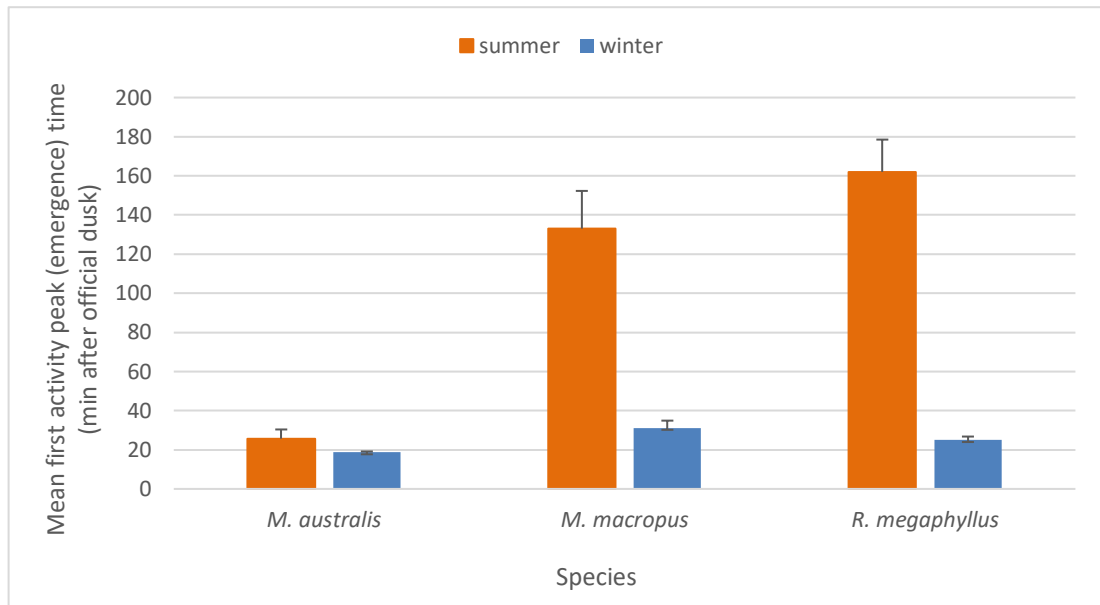


Figure 2-5 The mean of first activity peak (emergence) time relative to official sunset (mean and SD) of each species in the Yugar tunnel. The analyses were on means for each species over 3 days in summer and 5 days in winter. It showed that all three species emerged earlier, and the emergence pattern was less varied, in winter than summer, especially for *M. macropus* and *R. megaphyllus*. Mean peak emergence of all species occurred within 40 minutes after official sunset in winter. For summer, the pattern varied across species, with *M. australis* emerged closest to sunset, *M. australis* and *R. megaphyllus* emerged much later during the night.

Table 2-2 Pos-hoc comparisons for interactions in a two-way model for the Aligned Rank Transformation ANOVA for the first activity peak (emergence) times relative to official sunset of each species between summer and winter. (Results were analysed from the average of first peak emergence times relative to official sunset of each species over 3 days in summer and 5 days in winter).

	Contrast	estimate	SE	df	p. value
same species across two seasons	<i>Miniopterus</i> (Summer) – <i>Miniopterus</i> (Winter)	6.80	6.48	18	0.3079
	<i>Myotis</i> (Summer) – <i>Myotis</i> (Winter)	101.60	6.48	18	< .0001*
	<i>Rhinolophus</i> (Summer) – <i>Rhinolophus</i> (Winter)	136.53	6.48	18	< .0001*
Different species across two seasons	<i>Miniopterus</i> (Summer) – <i>Myotis</i> (Winter)	-5.70	6.48	18	0.3906
	<i>Miniopterus</i> (Summer) – <i>Rhinolophus</i> (Winter)	0.53	6.48	18	0.9359
	<i>Myotis</i> (Summer) – <i>Miniopterus</i> (Winter)	101.60	6.48	18	< .0001*
	<i>Myotis</i> (Summer) – <i>Rhinolophus</i> (Winter)	107.83	6.48	18	< .0001*

<i>Rhinolophus</i> (Summer) – <i>Miniopterus</i> (Winter)	142.80	6.48	18	< .0001*
<i>Rhinolophus</i> (Summer) – <i>Myotis</i> (Winter)	130.30	6.48	18	< .0001*

The time of final activity peak (return) (the time with the highest level of bat activity at sunrise) was significantly different among the three species, and the differences were dependent on season (Aligned Rank Transformation ANOVA, $F = 10.708$, $df = 2$, $p < 0.001$) with a large effect size ($\eta^2 = 0.54333$; Figure 2-6, Table 2-3). In summer, overall bat peak return time was 155.3 ± 12.1 minutes before official sunrise ($n = 3$). *Miniopterus australis* had the earliest final peak return time (158.3 ± 21.5 mins before sunrise) when compared with *R. megaphyllus* (157.7 ± 7.6 mins before sunrise) and *M. macropus* (150 ± 2.6 mins before sunrise) (Figure 6). In winter, overall bat final peak return time was 78.4 ± 49.3 minutes before official sunrise ($n = 5$). The final peak return time indicated that *M. macropus* was the earliest top return (113.5 ± 34.8 mins before sunrise), followed by *R. megaphyllus* (87.7 ± 60.1 mins before sunrise) and *M. australis* (34.2 ± 1.4 mins before sunrise) (Figure 2-6).

Post-hoc comparison of the same species between the two seasons for time of the final activity peak (return) indicated that there was a significant difference for *M. australis* ($p < 0.0001$) *R. megaphyllus* ($p < 0.0001$) but not *M. macropus* ($p = 0.0595$). However, it was found that all three species returned earlier before sunrise in summer than winter (Table 2-3 & Figure 2-6). Comparing different species between the two seasons, the seasonal interactions on the time of first activity peak (emergence) of among all species were significant ($p < 0.05$, Table 2-3).

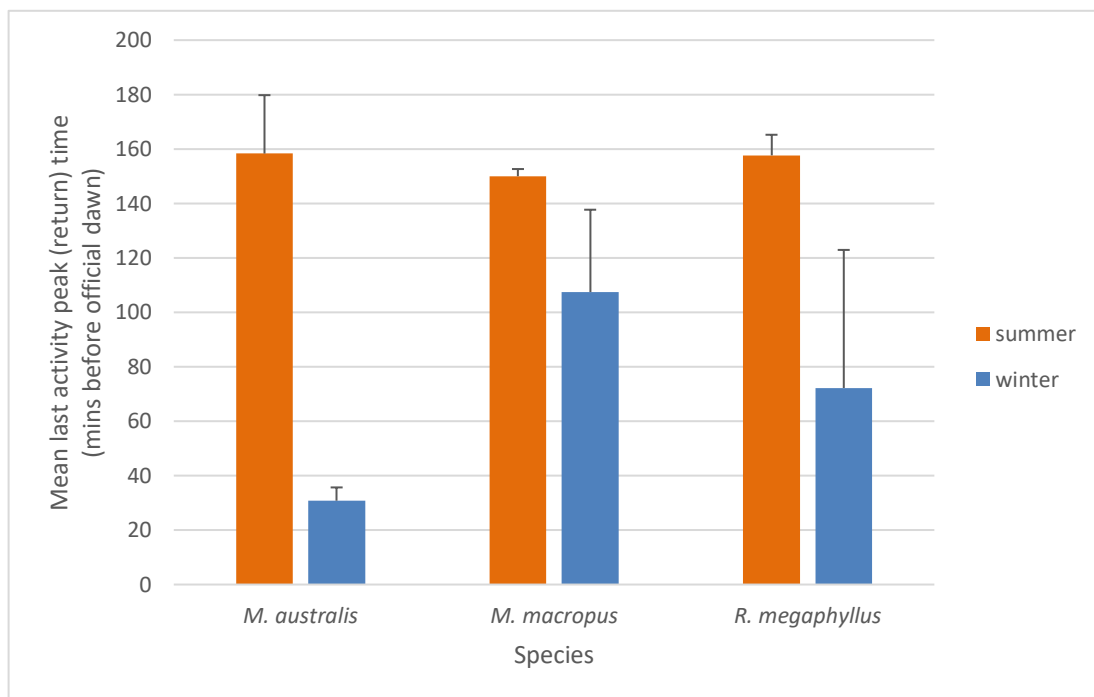


Figure 2-6 The mean of final activity peak (return) time relative to official sunrise (\pm SD) of each species in the Yugar tunnel. The results were calculated by taking the mean for each species over 3 days in summer and 5 days in winter. It showed that all three species returned earlier in summer than winter. The time of mean peak return of all species occurred between 150 – 160 minutes before official sunrise in summer. For winter, the returning pattern varied across species with *M. australis* returned closest to sunrise, *M. australis* and *R. megaphyllus* returned earlier during the night.

Table 2-3 Pos-hoc comparisons for interactions in a two-way model for the Aligned Rank Transformation ANOVA results on the final activity peak (return) times relative to official sunrise of each species between summer and winter. (Results were analysed from the average peak return time relative to official sunrise of each species over 3 days in summer and 5 days in winter).

Contrast		estimate	SE	df	p. value
same species across two seasons	<i>Miniopterus</i> (Summer) – <i>Miniopterus</i> (Winter)	127.53	21.1	18	< .0001*
	<i>Myotis</i> (Summer) – <i>Myotis</i> (Winter)	42.55	21.1	18	0.0595
	<i>Rhinolophus</i> (Summer) – <i>Rhinolophus</i> (Winter)	85.45	21.1	18	< .0001*
<i>Miniopterus</i> (Summer) – <i>Myotis</i> (Winter)		50.88	21.1	18	0.0271*

Different species across two seasons	<i>Miniopterus</i> (Summer) – <i>Rhinolophus</i> (Winter)	86.12	21.1	18	0.0007*
	<i>Myotis</i> (Summer) – <i>Miniopterus</i> (Winter)	119.20	21.1	18	< .0001*
	<i>Myotis</i> (Summer) – <i>Rhinolophus</i> (Winter)	77.79	21.1	18	0.0017*
	<i>Rhinolophus</i> (Summer) – <i>Miniopterus</i> (Winter)	126.87	21.1	18	< .0001*
	<i>Rhinolophus</i> (Summer) – <i>Myotis</i> (Winter)	50.22	21.1	18	0.0289*

Emergence Counts

Emergence counts were conducted over a total of 7.5 hours in both summer and winter. Bats first emerged from the tunnel 15 minutes after official sunset in both summer and winter and continued to emerge until 60 minutes after sunset when low light prevented further counts (Figure 2-7). In summer, daily peak emergence occurred 30 minutes after sunset with 133 ± 20 bats leaving; numbers greatly reduced to 21 ± 23 45 minutes after sunset. In winter, bats emerged at a more regular pace giving a smaller peak in numbers, but over a longer duration; 37 ± 7 bats departed 30 minutes after sunset and 31 ± 20 departed 45 minutes after sunset. Cumulative counts show that in summer majority of bats appear to have emerged from the tunnel 60 minutes after sunset, while in winter it is likely that bats were still leaving the tunnel (Figure 2-8) when counting was halted. The number of exit counts was significantly different between summer and winter (Independents sample t-test, $t = 5.4385$, $df = 7.26$, $p < 0.001$). At least 161 bats were present in the tunnel in summer and 81 in winter (Figure 2-8).

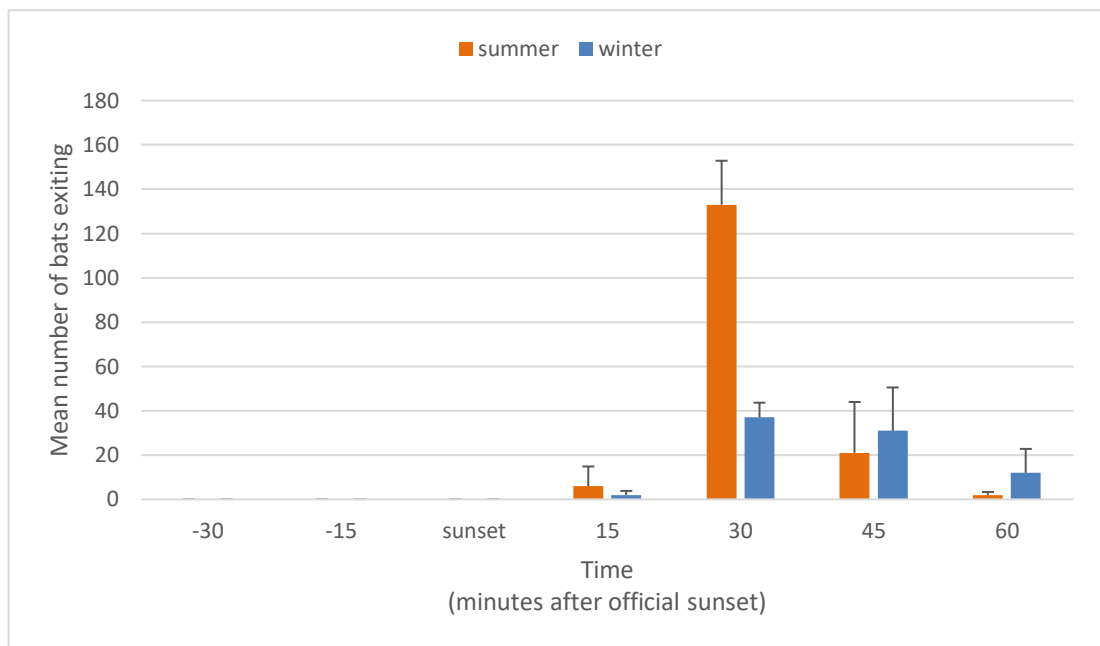


Figure 2-7 Mean exit count (\pm SD) over 5 days in both summer and winter in 15 minutes interval from 30 minutes before sunset to 1 hour after sunset. An hour after official dusk low light levels prevented accurate counting of the bats. First emergence was observed within 15 minutes after official sunset in both summer and winter. In summer, peak emergence occurred between 15 to 30 minutes after sunset. In winter, peak emergence occurred between 15 to 45 minutes after sunset. Number of exit counts during the peak emergence was higher in summer than winter.

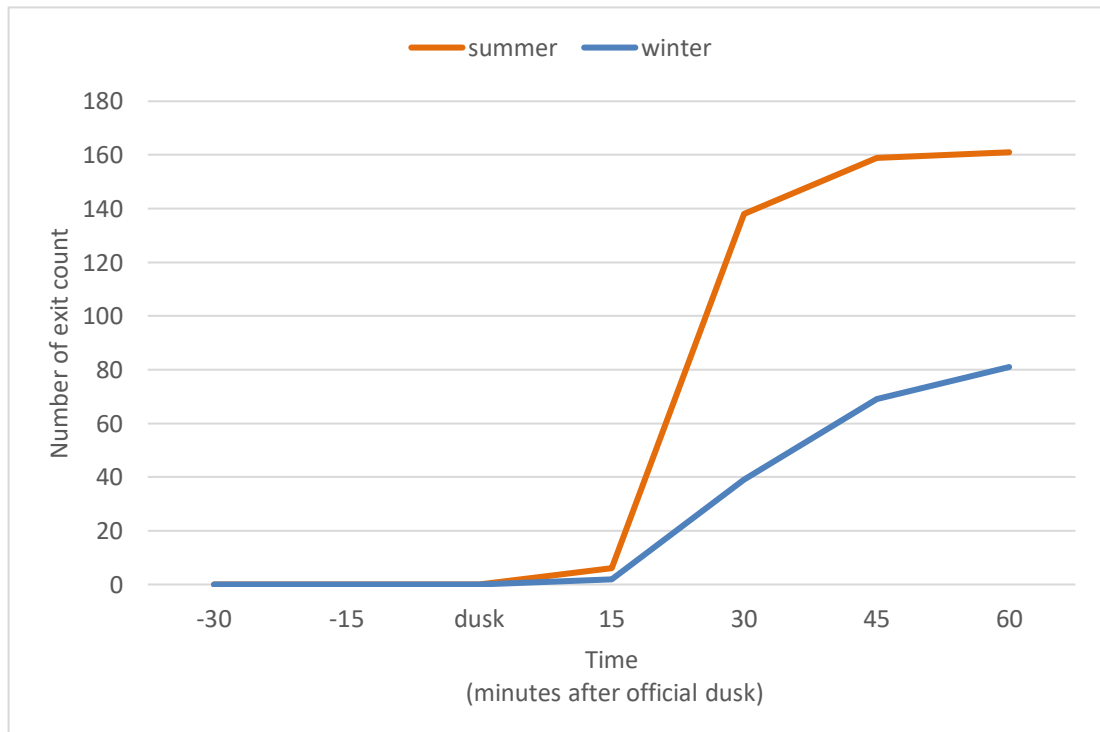


Figure 2-8 Cumulative mean exit counts over 5 days in both summer and winter in 15 minutes intervals from 30 minutes before sunset to 1 hour after sunset. Peak emergence started from 15 minutes after sunset in summer and winter. The emergence rate was higher, but the duration of peak emergence was lower, in summer than winter.

Comparing exit and acoustic counts

The pattern of bat emergence from exit counts and acoustic survey in the first 1.5 hours after sunset was similar within summer and winter (Figures 2-9 and 2-10). In summer, bat activity was first detected from the acoustic survey (15 mins before sunset) before the emergence of first bat was observed from the exit counts. Acoustic recordings showed that there were two activity peaks, the first peak occurred during sunset and the second larger peak occurred 30 minutes after sunset (Figure 2-9). However, exit counts only presented one peak event which occurred at the same time as the second larger peak in activity from the acoustic survey at 30 minutes after sunset (except day 3). The number of bat passes recorded during peak activity level (between 80 to 90 passes per day) was lower than the peak emergence counts (between 100 – 155 counts per day).

In winter, both acoustics and exit counts data showed similar patterns of bat activity and number of emergence (Figure 2-10). Both bat initial activity and emergence, began 15 minutes after official sunset. The level of activity and number of emergences showed one major peak within the first hour after sunset. Bat acoustic activity showed a consistent daily peak pattern occurring 30 minutes after sunset, whereas the peak emergences differed between days, occurring between 30 or 45 minutes after sunset. Both acoustic activity and exit counts greatly reduced shortly after the peak. During the time of peak events from both surveys, the number of bat passes recorded (between 100 to 150 passes per day) was greater than the number of bat emergences observed (between 30 – 60 counts per day) (Figure 2-10).

Comparing the results between summer and winter, the number of exit counts was greater than bat passes detected in summer, and winter was the opposite. However, both surveys indicated similar pattern when peak emergence and activity generally occurred at 30 minutes after sunset in both seasons (Figures 2-9 & 2-10).

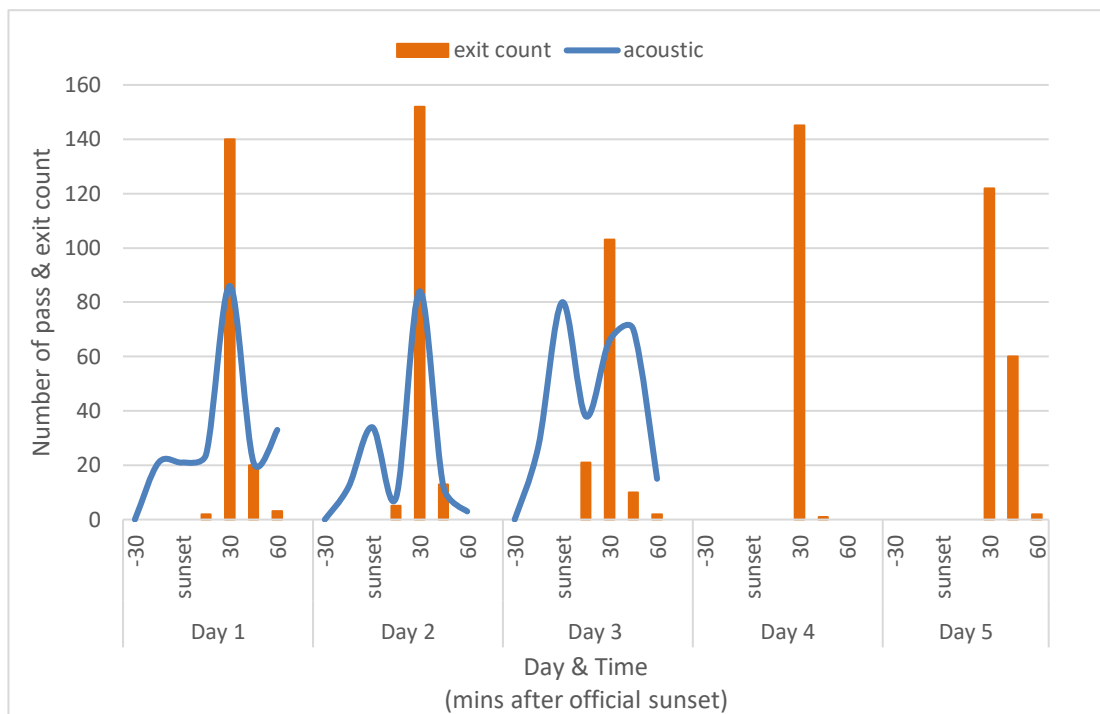


Figure 2-9 Number of exit counts and acoustic passes from the tunnel entrance acoustic survey in 15 minutes interval from 30 minutes before official sunset to 60 minutes after official sunrise over 5 days in summer. Acoustic activity was detected before the observation of the emergence of first bat each day. Peak activity and exit count happened within 15 to 30 minutes after official sunset, except day 3.

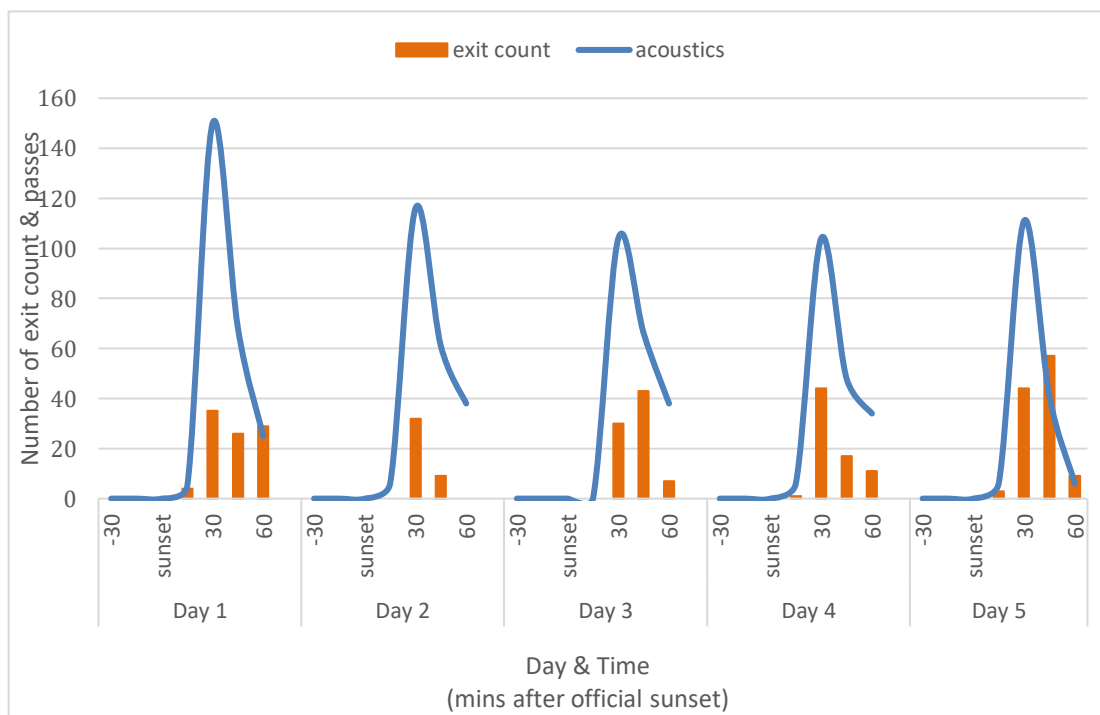


Figure 2-10 Number of exit counts and acoustic passes from the tunnel entrance acoustic survey in 15 minutes interval from 30 minutes before official sunset to 60 minutes after official sunrise over 5 days in winter. First acoustic activity and exit count were generally occurred within 15 minutes after official sunset. Both surveys showed bats' activity greatly increased between 15 to 30 minutes after sunset, when peak number of passes and emergence were generally detected.

Walkthrough Surveys

During the summer walkthrough surveys, it was estimated that there were at least 208 bats roosting in the Yugar tunnel, comprised of individuals from three different species: *Myotis macropus*, *Miniopterus australis*, and *Rhinolophus megaphyllus*. *Myotis macropus* were present within 10 drain holes located approximately half-way (2.5 m) up the tunnel walls and along its entire length. The number of individuals within each hole ranged from 3 to at least 14 individuals (Figure A 1 & A 2). It was estimated that there was a minimum of 73 *M. macropus* occupying the tunnel. There was no obvious difference in the number of *M. macropus* over summer. It was estimated that there were 131 *M. australis* roosting in the tunnel. A total of three clusters of *M. australis* were found on both sides of the tunnel wall. The number of bats in each cluster was 15, 30 and 86, respectively. There was a small decrease in the population of *M. australis* throughout the summer sampling period (January to March); the size of the biggest cluster of *M. australis* dropped to 56 individuals during the last visit in summer (around late February) (Figure A 3 & A 4). For *R. megaphyllus*, only 3 individuals were found hanging on the top of the tunnel ceiling at the back end of the tunnel (Figure A 5). The population of *R. megaphyllus* remained unchanged throughout the summer sampling period.

Up to 110 bats were found during the winter walkthrough surveys, belonging to the same three species found in summer. However, during the winter walkthrough survey (31st July, 2020) only two species were found: *M. macropus* and *R. megaphyllus*. Although *M. australis* were not present during the walkthrough survey, 15 individuals were seen roosting on the tunnel wall 2 days later (Figure A 6). It is estimated that there was a minimum of 77 *M. macropus* roosting in winter. Twenty-two drain holes were found to be occupied throughout the tunnel; 16 holes had only 1 to 5 individuals and 6 holes hosted more than 6 individuals. For *R. megaphyllus*, there were 18 individuals roosting at the same location as summer (Figure A 7). The population of *M. macropus* and *R. megaphyllus* remained similar between the period of walkthrough survey until the end of the roosting microclimate survey (mid-July – late August). Comparing the population between two seasons, the population of *M.*

macropus and *R. megaphyllus* were slightly increased, and *M. australis* was greatly reduced from summer to winter.

2.4 Discussion

Species Inhabiting the tunnel

In this study, acoustic surveys detected a total of 12 species near the Yugar tunnel entrance over three sampling days in summer and five in winter. Of these, only 4 species are known to roost in caves and tunnels: *Miniopterus australis*, *Myotis macropus*, *Rhinolophus megaphyllus* and *Miniopterus orianae oceanesis* (Campbell 2009; Dwyer 1968; Young 2001). All four cave roosting species were detected in summer, but *Miniopterus orianae oceanesis* was absent in winter. Bat activity was dominated by three cave roosting species: *Miniopterus australis*, *Myotis macropus*, *Rhinolophus megaphyllus* in both seasons. For *M. orianae oceanesis* activity in summer, there was only between 9 – 19 passes detected each night. Walk through surveys found that *M. orianae oceanesis* did not use the tunnel as a diurnal roost site, and so their presence in acoustic recordings likely represents activity near the tunnel entrance. However, studies report that *M. orianae oceanesis* will cluster together with *M. australis* (Dwyer 1968). However, differentiating between the species would require handling, which was not part of this study. The results of exit counts and walkthrough surveys generally agreed, with estimates of minimum population sizes for the *M. australia*, *M. macropus* and *R. megaphyllus* being 73, 131 and 6 in summer and 15, 77, 18 in winter, respectively.

Exit counts versus acoustic monitoring

Exit counts and acoustic monitoring were conducted to understand bat emergence patterns and to estimate size of the bat population occupying the Yugar tunnel in summer and winter. Exit count results indicated that bats first emerged from the tunnel 15 minutes after sunset and continued until at least 60 minutes after sunset when low light prevented further counts in both seasons. In summer, daily

peak emergence happened 30 minutes after sunset, after which the number of bats emerging reduced greatly. In winter, bats emerged over a longer period of time but in smaller numbers, with peak emergence occurring from 30 minutes after sunset until 45 minutes after sunset, after which numbers reduced greatly. For acoustic surveys, the sampling began at 30 minutes prior to official sunset until 30 minutes after official sunrise in both seasons. Acoustic results indicated similar emergence patterns for tunnel roosting bats during the first 90 minutes in winter, but not in summer. In summer, initial bat activity occurred 15 minutes before sunset and first reached a small activity peak during sunset, followed by a second larger activity peak 30 minutes after sunset. Bat activity greatly reduced from 45 minutes after sunset. It is important to understand the benefits and limitations of each of these methods for estimating emergence patterns and bat population estimates.

Exit count surveys have been used widely to monitor bat population sizes and emergence times at roosting sites. Exit counts also allow for emergence behaviours of bats, such as light sampling or looping prior to actual emergence, to be described (Berkova & Zukal 2009; Fure 2006; O'Donnell 2002b). By directly counting the number of bats emerging from the tunnel, it is also possible to estimate the minimum number of bats occupying the roost, the time of first bat departure, and the time of peak emergence (Sedgeley & O'Donnell 1999; Pettit & O'Keefe 2017; Rydell et al. 1996; Geipel et al. 2019). However, exit counts cannot accurately determine the species present (for mixed-species roosts) and the total population of bats occupying the roost site. Available light levels have a strong influence on the accuracy of exit counts as bats may continue to emerge after observers are no longer able to see them without the aid of additional technology such as thermal or infra-red detectors (Downs et al. 2003; Elliott et al. 2005). Furthermore, observers cannot necessarily identify bat species during flight (Betke et al. 2008; Agosta et al. 2005; Thomas & Jacobs 2013; Goodenough et al. 2015) without the aid of other equipment such as bat detectors.

Acoustic sampling can determine bat species presence and relative level of activity throughout the night (Skalak et al. 2012; Law et al. 2015). However, acoustic sampling cannot determine the absolute size of the roosting population and number

of bats emerging over time. Acoustic recorders cannot determine flight direction of bats, and it can be very difficult to differentiate calls between individual bats or species (Obrist 2020; Law et al. 2015). Because the direction of flight of bats cannot be determined, those recorded may be leaving, entering, or simply flying near the tunnel. Bats flying within the tunnel (but not yet departing) may also be detected and counted. The high intensity of bat calls detected when multiple bats flew near the tunnel entrance simultaneously may also affect the accuracy of the acoustic analysis.

Despite the shortcomings of each technique, when combined they provide a useful insight into bat activity around the Yugar tunnel. The exit counts and level of acoustic activity showed similar patterns within the first hour after official sunset. This indicated that acoustic activity peaks were likely caused by the emergence and return of bats. With the acoustic result, the emergence and return peaks of each tunnel-roosting species could also be estimated.

Emergence time and sunset

The results of this study have shown that there were significant differences in peak emergence time among the three species, and the differences were dependent on season. Bats overall emerged earlier in winter than in summer relative to official sunset. In summer, *M. australis* had the first peak in emergence, followed by *M. macropus* and then *R. megaphyllus*. This pattern changed in winter, with *M. australis* first, *R. megaphyllus* second and *M. macropus* last.

Bat emergence can be affected by numerous biological and physical environmental factors. This includes factors such as predation risk, light intensity, resource competition, weather conditions, and species foraging niche (Dwyer 1964; Russo et al. 2007; Thomas & Jacobs 2013; Jones & Rydell 1994). The seasonal change in the environmental factors such as ambient temperature, wind, precipitation, ambient light levels and insect abundance may also influence emergence behaviour (Milne et al. 2005; Kalcounis & Brigham 1994). The emergence and return of the three tunnel roosting species in this study are not well documented, necessitating the use

of comparison of results from studies on species in the same family or genus which are considered ecologically similar.

Emergence time of *M. australis* is likely to be driven by foraging needs and predation risk. In this study, *M. australis* reached peak emergence earlier in winter than summer relative to official sunset, and pre-emergence behaviour was detected in summer when the bats became active within the tunnel before sunset but only emerged after sunset. Dwyer (1964) studied the behaviour of *Miniopterus orianae blepotis* and reported that bats became active 30 minutes before emergence and never emerged earlier than 19 minutes before sunset, nor later than 22.5 minutes after sunset throughout the year. Studies on other *Miniopterus* species such as *M. schreibersii* (Alves et al. 2017; Vincent et al. 2011; Garcia-Ruiz et al. 2017) and *M. natalensis* (Pretorius et al. 2020) reported similar emergence time between 20 – 50 minutes after sunset in summer and winter. Thus, the peak emergence time of *M. australis* generally agree with majority of studies on similar *Miniopterus* species.

Little is known about the difference in emergence time of *M. australis* and *M. orianae* between summer and winter. Keith's (2020) study on *M. natalensis* in South Africa reported evening emergence occurred earlier in summer than in other seasons. By emerging earlier in summer, *M. natalensis* optimised foraging time and so maximized food intake when resources were most abundant (Keith 2020; Pretorius et al. 2019). Emergence time is a trade-off between foraging needs and predation pressure. Intensity of sunset illumination is closely associated with predation risk which influences bat emergence behaviour (Russo et al. 2007; Rowse et al. 2016). A delay in emergence time to lower predation risk is widely observed for many bat species (Reichard et al 2009; Jones & Rydell 1994; Thomas & Jacobs 2013). Bats' flight inside the roost close to the entrance allows them to evaluate the light levels outside during the pre-emergence phase (Twente 1955) to ensure predators are no longer a threat (Erkert 1982). This behaviour also appeared in *M. australis* during summer, when acoustic activity was detected before emergences were observed. However, *M. australis* in this study emerged significantly earlier in winter than in summer relative to official sunset. This suggests that resource availability was higher in summer than in winter, meaning bats might not require early emergence to fulfill

daily foraging needs (Stawski & Geiser 2010; Frick et al. 2012; Reichard et al. 2009). However, in winter, cold temperatures likely reduce insect abundance and activity, thus limiting bats' foraging success (Barros et al. 2014). With potential of increased competition due to limited resource availability, *M. australis* may emerge earlier to better access foraging opportunity.

The mating of *M. australis* occurs during winter from June to July, and females give birth and start lactating during summer in December (Dwyer 1968). This study might have coincided with the mating period in winter but probably not with the time of *M. australis* giving birth in summer. Although the reproductive status, sex and age of the bats in the Yugar tunnel is not known, the emergence of *M. australis* was likely not influenced by pregnancy over the sampling period. However, some activity could be from juvenile bats born in summer.

Emergence of *M. macropus* is likely driven by predation risk, reproductive status and weather conditions. *Myotis macropus* peak emergence occurred significantly earlier in winter than in summer relative to official sunset. Studies on *M. macropus* report that emergence occurred between 25 to 45 minutes after official sunset (Campbell 2009; Gonsalves & Law 2017); tunnel roosting species usually emerged earlier than tree roosting conspecifics (Campbell 2009). When compared with published studies, emergence of *M. macropus* in this study occurred at a similar time in winter but not in summer. Arndt et al. (2018) suggested that bats are sensitive to the risk of predation when emerging from a day roost. Bats foraging in open and riparian habitats with exposed feeding conditions might face higher predation risk from aerial predators (Jones & Rydell 1994). Previous studies have agreed that emergence time of *M. macropus* is possibly influenced by the risk of predation (Haddock 2018; Arndt et al. 2018), with Campbell (2009) stating that emergence delay was strongly influenced by the increased aerial predation risk near exposed waterways. Although the effect of sunset illumination on *M. macropus* emergence is not well documented, light intensity is important for triggering onset of activity and emergence of many bat species (Griffiths 2007; Petrzalkova et al. 2009; Erkert 1978). Bat species such as *Myotis dasycneme*, *Myotis myotis*, *Myotis nattereri*, *M.*

daubentonii (Jones & Rydell 1994), *Chalinolobus tuberculatus* (Griffiths 2007), and *Barbastella barbastellus* (Russo et al. 2007) were found to emerge at lower ambient light levels when predation risk was higher. Bats have been shown to emerge later on hotter days in dry and moist conditions (Frick et al. 2012). As foraging success should be higher with higher ambient temperatures due to increased insect activity and abundance (Frick et al. 2012), reducing the risk of predation may be of higher priority for *M. macropus*. This might explain the later emergence of *M. macropus* in summer than in winter.

In northern New South Wales, *M. macropus* may give birth to two young per year, one in October and one in January (Hall 2009). This study may have coincided with pregnancy and lactation of this species in summer. Little is documented on the emergence time of other *Myotis* species. Dietz & Kalko's (2007) study of *M. daubentoni* found that lactating females emerged later than pregnant females, but the difference was not significant (between 46 – 58 minutes after sunset). However, Reichard et al. (2009) indicated that lactating females were likely to emerge earlier than pregnant females due to higher energy demands. Contrastingly, pregnant and juvenile bats have higher energetic costs of flight due to greater wing loading or inexperience, and so they may emerge later to reduce predation risk (Kunz & Anthony 1996; Jones & Rydell 1994). Although it is not known if the Yugar tunnel is a maternity roost for *M. macropus*, the reproductive stages of bats may potentially influence their emergence behaviour.

In this study, *M. macropus* was recorded emerging 133 minutes after official sunset in summer, possibly the latest ever documented for this species. Some studies suggest that delayed emergence may be related to unfavourable weather conditions such as strong winds, rainfall and storms which negatively affect food availability and energetic cost of flight (Voigt et al, 2011; Erickson & West 2002; Thomas & Jacobs 2013; Clark et al. 2002; Geipel et al, 2019). Other bat species, including *Chalinolobus tuberculatus* (Griffiths 2007), *Micronycteris microtis* and *Molossus molossus* (Geipel et al. 2019) also delay emergence under poor weather conditions; the duration of the delay varies among species. Although little is documented on how *M. macropus* responds to severe weather conditions, this might have influenced their emergence

time in summer when there was precipitation over the sampling nights. In this study, there were heavy rains over the 3 sampling nights and no pattern of bats emergence under normal weather condition was recorded in summer. Therefore, there was no comparison for *M. macropus* emergence time between days with rain and no rain in summer. Although little is documented on how *M. macropus* responds to severe weather conditions, the heavy rains might have influenced their emergence time in summer.

Emergence of *R. megaphyllus* is likely driven by foraging needs and predation risk. *Rhinolophus megaphyllus* peak emergence occurred significantly earlier in winter than summer. Studies on other *Rhinolophus* species such as *R. ferrumequinum* (Duvergé et al. 2000), *R. ferrumequinum nippon* (Funakoshi & Maeda 2003), and *R. hipposideros* (Reiter et al. 2008; Mcaney & Fairley 1988) showed that emergence usually occurred between 15 – 40 minutes after sunset. As mentioned above, bats may emerge earlier to maximise energetic intake which is traded off against increased risk of predation. Lactating females of other *Rhinolophus* species, including *R. ferrumequinum* (Maltagliati et al. 2013) and *R. hipposideros* (Reiter et al. 2008), also showed early emergence. In contrast to these studies, the time of peak emergence of *R. megaphyllus* in this study occurred much later in summer than winter. The breeding season of *Rhinolophus megaphyllus* commences around mid-November through to until December, and lactation lasts for 5 weeks ending around late January (Hall 1989); this coincides with this study in summer. However, it is not known if the Yugar tunnel is a maternity roost for *R. megaphyllus*, or even the sex of the bats in the tunnel. Since male bats generally do not require the same high energetic intake as reproductive females (Grinevitch et al. 2009; O'Donnell 2002a), delays in emergence of *R. megaphyllus* in the Yugar tunnel may indicate that this is not a maternity roost and that the bats were emerging later to minimise predation risk. Other cave-roosting species such as *R. ferrumequinum* arouse periodically and forage in winter when temperatures are above 10°C (Park et al, 2000; Ransome 2009). When active, bats require higher energy for thermoregulation under relatively low ambient temperature in winter (Turbill & Fritz 2008; Johnson et al. 2012). It is

possible that *R. megaphyllus* emerged soon after official sunset in winter to better compete for food resources when the temperature was warmer near sunset, and compromising on higher predation risk.

The activity of *R. megaphyllus* was relatively low compared with *M. australis* and *M. macropus*. It was difficult to determine the emergence pattern of *R. megaphyllus* in summer, as the pattern of activity differed between each night. It was possible that *R. megaphyllus* had two emergence phases, beginning with a smaller emergence activity during the first hour, and a second emergence at the third hour; or it could be a major emergence in the first hour and then a return in the third hour. Lactating females of other *Rhinolophus* species such as *R. ferrumequinum nippon* (Funakoshi & Maeda 2003) and *R. hipposideros* (Knight & Jones 2009) were recorded returning to roost sites between foraging bouts around 2 - 5 hours after sunset. Knight & Jones (2009) also found *R. hipposideros* had used up to 5 different night roosts near their foraging areas. Murphy (2014) also found that *R. megaphyllus* has multiple roosting sites near each other in the Pilliga forest. Thus, it is also possible that the activity peaks of *R. megaphyllus* in this study were caused by visits by individuals from other nearby roost in the Samford region. Since it remains unclear about the activity of *R. megaphyllus* at the tunnel entrance, factors driving the emergence pattern and time of this species cannot be determined.

The emergence patterns of the three tunnel roosting species have been suggested to be influenced by predation risk. Avian predation of bats is mostly opportunistic, as bats departing from roost sites can become target for predatory birds (Brighton et al. 2020). The time of emergence peak by bats relative to sunset has a strong influence on the risk of predation by diurnal avian predators (Reichard et al. 2009; Thomas & Jacobs 2013). In fact, there are more than 107 species of Accipitriformes (eagles, kites, and hawks), 36 species of Falconidae (falcons), and 94 non-raptorial diurnal bird species from 28 other families known to prey on bats around the world (Mikula et al. 2016). According to Queensland Government (2020), more than 20 bird species including aerial predators such as owls, ospreys, falcons and kites exist in the Samford area, and they could be potential predators of bats.

Therefore, bats emerging early during sunset will likely increase the risk of predation by diurnal aerial predators.

Many studies agree that there is a trade-off between predation risk and energetic demands which can influence bats' emergence behaviour, and this generally supports the species emergence pattern in winter but not in summer as *M. macropus* and *R. megaphyllus* emerged much later at night. Jones & Rydell (1994) suggest that emergence time varies depending on foraging niche and flight speed. *Myotis macropus* is known to be an open forager characterised by the use of waterways, with high wing loading and aspect ratio allowing them to fly fast above open water while maintaining good manoeuvrability within clutter (Campbell 2009; Norberg & Rayner 1987). *Miniopterus australis* is an 'edge' specialist characterised by its high wing loading and low flight speed (Hourigan 2011; Rhodes 2002; Norberg & Rayner 1987). Similarly, *R. megaphyllus* is a clutter-tolerant species with slow flight speed and often forages in forest interior with dense vegetation structures (Pavey 1998; Norberg & Rayner 1987). Trawling bats such as *M. dasycneme* was found to emerge later than gleaning bats such as *M. myotis*, *M. nattereri* and *M. orianae* (Jones & Rydell 1994). Russo et al. (2007) indicated that bat species characterised by slow flight often emerge later to compensate for higher predation risk. Conversely, clutter bat species usually emerge earlier than open space and clutter-edge species, because the foraging environment offers better protection to aerial predators (Thomas & Jacobs 2013).

Bat activity between the first and last activity peak

Bat activity at roosts throughout the night may be influenced by the nightly foraging patterns and behaviors of bats (Petrzelkova et al. 2009; Agosta et al. 2005; Zhang et al. 2015). Multiple factors including reproductive status, predator avoidance, weather condition, energy conservation, social interactions and communication can influence patterns of bat activity through the night (Knight & Jones 2009; Perks & Goodenough 2020; Egert-Berg et al. 2018; Voigt et al. 2017). Although bats are known to often emerge near sunset and return near sunrise, many

species return earlier to roost between foraging bouts and sometimes remain within roosts despite it being early in the night (Mickael et al. 2002; Murray & Kurta 2004; Dwyer 1964).

In this study, two smaller activity peaks occurred during the night between the initial peak in emergence and the bats' last return to the roost near sunrise in summer. These peaks were likely indicative of the emergence of *M. macropus* and *R. megaphyllus* and the early return of *M. australis*. Based on the emergence time of each species mentioned above, the first peak in emergence was dominated by *M. australis*, while *M. macropus* and *R. megaphyllus* emerged much later - three hours after sunset. The small peak in activity three hours after sunset likely marked the emergence of *M. macropus* and *R. megaphyllus*. For the second small activity peak before the final peak return, acoustic recordings were dominated by *M. australis* calls. As the activity of *M. australis* remained relatively low for 4 hours through the night, the small activity peak before final peak return was likely caused by the early return of some *M. australis*. The early return of some *M. australis* is likely to be driven by satiation or poor weather. Dwyer (1964) suggested that timing of bat satiation would influence the timing of return, with early satiation of *M. orianae blepotis* resulting in early return. Studies also recorded bats returning to roost earlier under heavy rain (Weinbeer et al. 2006; Frafjord 2013).

Bat activity between first emergence and the last return in winter varied between nights with a smaller peak occurring before and after the second largest peak. It is not known whether bats were emerging or returning to the tunnel during this period. However, these two activity peaks were possibly driven by weather conditions, predator avoidance, energy conservation and social interactions. The smaller activity peak before the main return at midnight was possibly a second emergence, the return of some individuals from the first emergence, or even visits by bats from other roost sites. These activities might be due to weather condition, social communication, and energy conservation. As mentioned above, poor weather conditions may result in delayed emergence and earlier return of bats. Bats sometimes return to roost early or visit other roosting sites to care for young bats, or for social interaction and communication among conspecifics (Chaverri et al. 2018;

Thomson 2020; Swift 1997). Some bat species such as *R. hipposideros*, *R. megaphyllus*, *M. lucifugus* and *M. orianae blepotis* may utilize multiple night roosting sites close to foraging areas for purposes such as digestion, assimilation and predator avoidance (Knight & Jones 2009; Dwyer 1964; Anthony et al. 1981; Murphy 2014). For energy conservation, the high costs of flight or poor foraging success under severe environmental conditions may also result in early return for energy conservation (Anthony et al. 1981).

Species final return peak and sunrise

There were significant differences in the final peak return time among the three species of bats in the Yugar tunnel, and the differences were dependent on season. The final peak return of all species occurred earlier in summer than in winter relative to sunrise. In summer, all three species' final return peaked at similar times between 150 – 160 minutes before sunrise; in winter, the final peak return time varied among species with *M. macropus* first, followed by *R. megaphyllus* and then *M. australis*.

The timing of bats returning to roosting sites has been related to predation risk (Speakman et al. 1995), weather conditions (Griffiths 2007; Pretourius et al. 2020), reproductive needs (Pretourius et al. 2020) and energetic demands (Muthersbaugh et al. 2019). In this study, the time of final peak return of *M. australis* was significantly different between two seasons, when return time occurred much closer to official sunrise in winter than in summer. This was likely driven by variation in food availability between summer and winter. Two studies on *M. schreibersii* reported similar findings for return time in summer, when bats returned between two to three hours before sunrise. Russo et al. (2007) and Salvarina et al. (2018) suggested that the time of bat emergence and return could be influenced by insect abundance. Satiation could trigger bats' earlier return. However, when bats do not obtain sufficient food, this could result in their later return (Dwyer 1964). Bats' requirements to reach satiation could be influenced by insect abundance, distance to foraging grounds, and energy demands (Shen & Lee 2000). Studies indicated that insect abundance was positively correlated with warmer ambient temperature and

dependent on immediate condition of weather (Meyer et al. 2016; Turbill 2008; Sherwin et al. 2013). Thus, insect abundance is likely higher in summer and lower in winter; the better foraging opportunity in summer may trigger bats' earlier return when compared with winter (Fenton & Morris 2011; Heim et al. 2017).

Myotis macropus final peak return time was significantly earlier in summer than in winter relative to official sunrise. Similar to *M. australis*, it was likely driven by the variation of food availability between summer and winter. Studies on other *Myotis* species such as *M. myotis* (Zahn & Dippel 1997), *M. chliolabrum* (Rodhouse & Hyde 2014) and *M. formosus* (Shen & Lee 2000) in summer found that these bats returned to their roost between 2- 6.5 hours after sunset. Shen & Lee (2000) indicated that male bats generally return earlier than female and juvenile bats, and females after parturition would return to the roost earlier than before parturition. Some *M. macropus* individuals regularly return to their roosts during nightly foraging periods (Campbell 2009); others spend significant time away from roosts while lactating (Gonsalves & Law 2017). However, little is documented on seasonal differences in the return time of *M. macropus*. As mentioned above, satiation may motivate bats to return to the roost (Anthony et al. 1981; Dwyer 1964). It has been suggested that foraging activity of bats near waterways or streams is influenced less by changes in ambient temperatures than that in open landscapes and cluttered habitats (Dietz, 2006); ambient temperature of about 5°C did not affect the foraging activity of *M. daubentonii* (Dietz & Kalko 2007). Although it is not known how low temperature affects *M. macropus* foraging activity, the later final return peak in winter might reflect prolonged foraging activity of some individuals. Contrastingly, the earlier final return peak of *M. macropus* in summer might be caused by early satiation due to higher food abundance, or possibly breeding condition.

Rhinolophus megaphyllus final peak time of final return occurred earlier in summer than winter relative to official sunrise, however the difference was not significant. The timing of *R. megaphyllus* return was possibly driven by predation risk associated with light illumination at sunrise. A study on *R. hipposideros* reported similar results in that the bats returned earlier in summer than winter (Mcaney & Failey 1988). Other *Rhinolophus* species such as *R. ferrumequinum* (Maltagliati et al.

2013), *R. mehelyi* and *R. euryale* (Straka et al. 2020) are also influenced by light levels. They exhibit a light sampling behavior undertaking flights within roost before emergence and return associated with predator avoidance (Fure 2006). Macney & Failey (1988) indicated that the return of *R. hipposideros* was correlated with sunrise, as it was for *Pipistrellus pipistrellus* (Petrzelkova et al. 2009), *Tadarida brasiliensis mexicana* (Lee & McCracken 2001) and *Carollia castanea* (Thies et al. 2006). Twilight generally appears earlier and lasts longer at a lower light intensity during summer, thus resulting in earlier return of bats (Erkert 1978; Macney & Failey 1988). It is possible that the later return of *R. megaphyllus* in winter was influenced by the late illumination of sunrise.

It is not clear why the final peak return time of the three bat species occurred at similar time in summer but not in winter. Studies have found that bats delayed emergence and returned earlier to roosting site under poor weather conditions such as heavy rain and storms (Thies et al. 2006; Frick et al. 2012; Reichard et al. 2009; Griffiths 2007). Bats' foraging behavior at night time was expected to be relatively uniform, however, variation across nights due to different weather conditions is possible (Dwyer 1964). Voigt et al. (2011) suggested that rain has negative impacts on ambient temperature and bat thermoregulation. Rain also interferes with bats' echolocation thus increasing the energy required for flight. It is possible that bats would have less foraging activity and exhibit earlier return in summer when there was rain over the sampling nights.

Species found in the tunnel and their population

A variety of techniques can be used to study the use of tunnels by bats. Each technique has advantages and disadvantages and so often the use of a combination yields the most accurate results. In terms of determining species diversity, population size and emergence / return behaviour, the use of acoustic recorders, exit counts and walk throughs are favoured (Azmy et al. 2012; Flaquer et al. 2007; Froidevaux et al. 2020). For bat exit counts, this method can provide relatively accurate number and

timing of bats' emergence during sunset when light is sufficient to see them. However, exit count cannot determine the bat species and likely underestimate the total population of a roost (Oyler-McCance et al. 2018; Elliott et al. 2005). It is because bats' emergence time varies among species and individuals within roost, some bats may emerge late under low light condition when observers are no longer able to see them (U.S. Fish and Wildlife Service 1997). For acoustic monitoring, this method can indicate bats relative activity among species and sample continuously providing quantitative data to understand bats' activity pattern through the night (Skalak et al. 2012; Froidevaux et al. 2014). However, the use of acoustic recorder cannot differentiate the direction of bats echolocation calls which makes the determination on bats' behaviours (i.e. emerging or returning to the tunnel or flying in surrounding area) to be difficult. Furthermore, this method is likely to overestimate the total population of a roosting site as it is difficult to differentiate calls among individuals, especially during activity peaks when many echolocation calls overlap (Law et al. 2015). For walk-through survey, this method provides more accurate estimate on the number of bat colonies and populations within roost. By walking-through the roost site, observers can directly count the number of individuals within each cluster. Although some bats may be hidden from sight, this method provides a more accurate estimate of the total bat population in the tunnel when compared with the other two survey methods. However, this method can only provide estimate on population but not the emergence pattern of bats.

The three bat species found roosting in the Yugar tunnel in summer and winter correspond with the findings of Hall (2015). His study found *M. macropus* forming small clusters inside the drain holes and estimated the number of bats in each cluster could be between 5 to 13 individuals. *Miniopterus australis* was found in larger clusters (86 individuals), while 12 *R. megaphyllus* were found roosting individually. These descriptions of clusters and population sizes are very similar to those observed in this study during summer. However, species composition and population sizes in winter were not reported by Hall (2015). This present study found that the population size of *M. macropus* in the Yugar tunnel was similar between summer and winter. Since *M. macropus* breeding seasons started from October to late January and a

lactation period lasts for 8 weeks completed in mid-March (Dwyer 1970), it was expected to have higher population in summer than winter. The reason of having similar population between two seasons might be due to the timing of the study. As summer survey has coincided with the end of the breeding season in late January when most juveniles were likely born, population estimated in summer may represent the whole colony. If all *M. macropus* were using this tunnel throughout the year, the winter population would likely to be similar to that in summer because it hadn't reached the beginning of another breeding cycle. However, juvenile bats were not observed during the walkthrough in both seasons. Published studies reported that some *Myotis* species such as *M. volans* (Baker & Lacki 2006), *M. sodalist* (Silvis et al. 2014) and *M. lucifugus* (Slough & Jung 2020) could have multiple roosting sites. However, Law et al. (2020) found that *M. macropus* occupied the same roosting site throughout the year with maximum recapture time of 9 years. It is likely to believe that *M. macropus* were occupying this tunnel throughout the year within this study, but it may not be a maternity site for this species since no juvenile bats were found in both seasons.

For *R. megaphyllus*, there were only six individuals observed during the daytime in summer and 18 in winter. The result agrees with previous studies that *R. megaphyllus* non-maternity colonies are often small in size, with usually less than 10 and rarely more than 50 individuals (Dwyer 1966; Young 2001). The males of this species were usually found in small colonies and living between a few nearby roost sites within localised regions, and females often appeared as maternity colonies with population ranging from 15 to more than 1500 individuals (Dwyer 1966; Young 2001; Murphy 2014). Dwyer (1996) reported that maternity colonies of *R. megaphyllus* in northern New South Wales were smallest during March and April, then increase throughout winter. The largest colonies were formed around October to November prior to breeding period. The population of *R. megaphyllus* in the Yugar tunnel throughout summer and winter was very small compared to numbers reported for other maternity colonies. It is likely to believe that the Yugar tunnel is not a maternity

site for *R. megaphyllus*, and they were occupying this tunnel throughout the year within this study.

Due to a lack of evidence, it cannot be determined whether the Yugar tunnel is a maternity site for *M. australis*. *Miniopterus australis* were present in the tunnel in large numbers; 131 bats in summer but only 15 individuals in winter. Hall (2015) reported that *M. australis* were perhaps dispersing from breeding sites near the coastal areas during February to April, and they were not always roosting in this tunnel throughout the year, instead preferring nearby caves and old mines. A study that focused on the roosting behaviour on the Eastern Bent-wing bats (*Miniopterus orianae oceanensis*) indicated that during autumn, winter and spring, majority of bats continuously moved between several nearby roosting sites due to external disturbance and bad weather conditions (White 2011). Their flights during these seasons were either searching for new roosting site or looking for social interactions (White 2011). *Miniopterus australis* migrate to their nursery site in early spring from August to September (Dwyer 1968). The nursery colonies of *M. australis* usually consist of between a few hundreds to several thousand individuals (Hall 2015; Dwyer 1968). The population of *M. australis* in the Yugar tunnel decreased slightly over summer (January - April) followed by a small increase during winter (July – September); numbers were still low compared with summer. Dwyer (1968) found that the majority of males disperse away from the maternity colony to other roosting locations during October and November, the females leave their young around February and March, and then the young leave in April. Thus, maternity roosts are abandoned from March to August (Dwyer 1968). Another study of *M. orianae blepotis* indicated that these bats in north-eastern New South Wales do not return to the over winter roosts until late February or early March (White 2011). Hall (1982) reported that in New South Wales females leave the overwinter roosts and return to maternity sites around August to September. The pattern seen at the Yugar tunnel was therefore similar to that seen at other *Miniopterus* maternity site. However, the time when majority of *M. australis* abandoned and returned to the tunnel is not known.

Summary

The aim of this study was to determine the number and species of bats using the Yugar tunnel. Specifically, it was to: 1) determine the species inhabiting the tunnel and their relative population sizes, and 2) determine the emergence and return pattern of each species in the tunnel. Three species were found to be roosting in the tunnel all at relatively low population sizes, and with seasonal (summer versus winter) differences in numbers. Patterns of activity, including emergence and return times, were similar across species in summer but varied in winter. Species and seasonal variation was likely due to a variety of physical and biological factors such as weather conditions, predation pressure, reproductive state, foraging needs and resource abundance.

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Chapter 3: Understanding the microhabitat of the tunnel and assessing how this influences the roosting locations of each species

3.1 Introduction

Roosts are a critically important resource for bats as they spend most time in their lives roosting (Kunz 1982; Kunz & Lumsden 2003; Kubista & Bruckner 2015). Roosts can benefit bats as sites for social interactions (Chaverri et al. 2018; Furmankiewicz et al. 2011), resting between foraging bouts (Winter et al. 2020), avoiding predators, mating, hibernation, and raising young (Lima & O’Keefe 2013; Ruczynski 2006; Zahn & Dippel 1997; Martínková et al. 2020). Roosts are also important for providing protection against severe weather (Lima & O’Keefe 2013; Breviglieri & Uieda 2014; Paksuz 2017).

Degradation of natural habitats due to human activities such as deforestation, urban development and agricultural expansion have decreased the availability of natural roosts for many bat species consequently impacting on populations and causing an increase of roosting in artificial structures (O’Malley et al. 2020; Sparks et al. 2004; Gehrt & Chelsvig 2003; Park 2015; Russo & Ancillotto 2015). The selection between natural and artificial roosts by bats range from obligatory to opportunistic and likely dependent on the availability of the roosts (Thomson 2013). Although little is documented on the selection between natural and artificial roosts by bats around the world, artificial structures have provided roosting opportunities to compensate for the loss of natural roosts for some species (Mering & Chambers 2014; Fontaine et al. 2021; Hoeh et al. 2018; Griffiths et al. 2020). Studies on bats around the world have documented that many bat species such as *Trachops cirrhosus*, *Phyllostomus hastatus* (Lima et al. 2017), *Myotis goudoti*, *Mormopterus jugularis* (López-Baucells et al. 2017), *Rhinolophus hipposideros* and *Rhinolophus ferrumequinum* (Voigt et al. 2016) have also been found roosting in anthropogenic structures such as tunnels,

mines, buildings, bridges and culverts. These structures provide alternative roosting habitats for more than 20 species of bat in Australia (Armstrong 2010). Species such as *Miniopterus orianae oceanensis*, *Austronomus australis*, *Rhinonictes aurantia*, and *Macroderma gigas* are examples of cave-dwelling species in Australia, with *Macroderma gigas* and *Rhinonictes aurantia* widespread throughout the Pilbara region in WA, and often found roosting in disused mines (Cramer et al., 2016; Arteaga Claramunt et al. 2018). Destruction and disturbance of roost sites is a major factor impacting on bat populations, therefore the protection of bat roosting sites is a significant focus for modern bat conservation efforts (Knight & Jones 2009). It is equally important to understand how bats species select roosting sites so that they can be better protected.

Roosting microclimate is essential for the survival of bats and directly influences their roost site selection (Boyles 2007; Sedgeley 2001; Winter et al. 2020). The roosting microclimate preferences of insectivorous bats vary depending on the season, sex, and reproductive stage (Johnson & Lacki 2014; Ruczyński & Bartoń 2020; Sedgeley 2001). Insectivorous bats are highly sensitive to microclimate fluctuations within roost sites due to their small body size and high surface area to volume ratio (Campbell 2009). For example, *Miniopterus orianae oceanensis* prefer a microclimate (tunnels & mines) with a steady daily temperature fluctuation and will roost in the coolest part of the structure in winter. This helps them to maintain a low body temperature and so minimise body fat loss throughout the winter (White 2011). Roosting microclimate selected by bats also depends on the purpose of the roost (Winter et al. 2020; Leivers et al. 2019; Threlfall et al. 2013). For example, bats maternity roosts often maintain higher and relatively more stable temperatures near their thermoneutral zone to minimise energy loss and promote development of young bats (Lausen & Barclay 2006; Sedgeley & O'Donnell 2004; Chruszcz & Barclay 2002; Zahn 1999).

Interaction between the structural properties of the roosts and the external climate determines the microclimate of bat roosts (Boyles 2007). For example, the aspect of the roost entrance determines exposure to sunlight which in turn affects thermoregulation (Russo et al. 2017). Other studies have revealed that an increase in

roost exposure to solar radiation is one of the reasons many small bats roost in cavities at higher altitudes (Hoeh et al. 2018). The structural properties of bat roosts such as roost entrance dimensions, internal height and width, and type of roost (e.g. caves, tunnels, roof spaces, tree hollows) also influence on roost microclimate (Campbell 2009; Leivers et al. 2019; Torquetti et al. 2017; Otto et al. 2016).

Tunnels are significant roosting sites for many bats in Queensland. For example, the Muntapa (Toowoomba Regional Council 2019), Boolboonda (Van de Wetering 2011) and Dularcha Tunnels (Queensland Government 2019) are known to be the roosting sites for many insectivorous species. However, limited research on bats roosting in tunnel in Queensland has been documented. A study of bats using tunnels in Victoria found they provide a stable thermal microclimate with lower daily temperature fluctuation for roosting (Campbell et al. 2010); bat roosts in tunnel were warmer at night and less variable throughout a day comparing to ambient temperature (Campbell et al. 2010). Tunnel roosts were typically cooler than tree roosts during the day, but warmer during the night (Campbell et al. 2010). Thermally stable in microclimate is thought to be suitable for maternity roosting as pregnant females generally preferred limiting excessive heat loss to promote growth of the young (Neubaum et al. 2017).

Many studies about bat roosting have documented how they occupy different structures such as trees, bridges, mines and natural caves in Australia (Threlfall et al. 2013; Gorecki et al. 2020; Pavey 1998; Armstrong 2000; Campbell 2009; Young 2001; White 2011). However, much less emphasis has been put on bats roosting in tunnels, including in south-east Queensland. A past study of bats in the Yugar tunnel documented that *Myotis macropus* were found roosting inside drain holes on the wall and forming small clusters in small crevices on the ceiling near the tunnel entrance. *Miniopterus australis* were also found in holes and crevices on the wall near the entrance while *Rhinolophus megaphyllus* were found roosting on the ceiling near the rubbish infill area at the back side of the tunnel (Hall 2015). However, the roosting microhabitats of bat roosts in the Yugar tunnel are not known. Therefore, this study aimed to determine the roosting selection preferences of bats inhabiting the Yugar tunnel with a focus on microclimate. By conducting a microclimate monitoring survey

and walk-through surveys aimed to achieve the following objectives: 1) to determine the location and size of each roost in the tunnel, 2) to determine the roosting microclimate of each species inhabiting the tunnel.

3.2 Method and Analysis

To determine the positions of all bat roosts in the tunnel a walk-through survey was conducted. During the walk-through, the location of each bat roost in the tunnel was recorded as described in Chapter 2. Following the three surveys conducted in Chapter 2, a final walk-through survey was conducted on the day of deployment (before bats emerged) to finalize the locations of each roost.

As bat roosting sites were not evenly distributed in the tunnel, replicate roosts were not always available, and the number of HOBO loggers (Onset Computer Corporation; Model: HOBO U23 Pro v2 Temperature/Relative Humidity Data Logger - U23-001) was limited, we adopted an opportunistic sampling approach based on the bat locations from the pre-deployment walk-through survey. To determine the roosting microclimate of each species inhabiting in the tunnel, a total of 13 HOBO temperature and humidity monitoring units were deployed. Three units were fitted with an external sensor at the end of a 5m cable allowing it to be placed close to roosting bats inside the drain holes with minimal disturbance. To minimise disturbance the installations of the HOBO units began approximately 2 hours after official sunset when most bats had departed the tunnel. For the installations of the HOBO units, the tunnel was divided into three zones (entrance, middle and deep), with each zone being 27 m in length.

For *Myotis macropus*, one roost was sampled in each zone of the tunnel with the external sensor from the HOBO placed into the drain holes where the bats were roosting. For *Miniopterus australis*, two HOBO units were placed next to roosting clusters on the wall of the tunnel in the deep zone and 1 in the middle zone. For *Rhinolophus megaphyllus*, two HOBO units were placed next to bats roosting on the tunnel ceiling in the deep zone. Four HOBO units were used to determine tunnel ambient temperature and humidity. One sensor was placed in each of the zones

(entrance, middle and deep) and a unit was placed outside the tunnel. The same sampling regime was used in both summer and winter, except in winter only one *Miniopterus australis* cluster was present and monitored in the middle zone and only one *Rhinolophus megaphyllus* site monitored in the deep zone.

The HOBO units for monitoring the bat roosts were set up approximately 5-10cm away from the bats, while the ambient units were placed on the right side of the tunnel wall at the midpoint of each zone. The HOBO units were set to record the temperature and humidity continuously in 1-minute intervals (starting on the deployment day) until the memory was full or the batteries ran flat. The HOBO units were retrieved after 10 days and the microclimate data transferred to a computer. Unfortunately, the HOBO monitoring unit located outside the tunnel entrance (external ambient) went missing in summer. To conduct analysis on the seasonal results, the tunnel entrance (external ambient) data was replaced by ambient climate data from the Samford Ecological Research Station (SERF) 6 km for both summer and winter. However, the tunnel entrance (external ambient) data in winter was still presented as a reference showing the difference in climate between the tunnel and SERF.

We followed the methods of Sedgeley (2001) to describe changes in roost and ambient temperature and humidity. Microclimate data were categorized into 30 minutes intervals over 7 days in both summer and winter; each day was divided into day and night-time blocks corresponding to official sunset and sunrise. In summer, the time block for each day were 05:00 – 19:00 (day) and 19:00 – 05:00 (night). In winter the blocks were 06:30 – 17:00 (day) and 17:00 – 06:30 (night). Diurnal microclimate data were extracted and used to calculate average, maximum, minimum and fluctuation (maximum minus minimum) for each day in terms of temperature and relative humidity. These data were then averaged over the sampling period (7 days) for both seasons to calculate the mean of each variable for a diurnal period.

Sharpiro-Wilks tests found that temperature data were normally distributed, but relative humidity data were not. Two statistical tests were conducted to compare the microclimate differences between bats' roosts (regardless of species) and tunnel

external and internal ambients; independent two-samples t-test for temperature and Mann-Whitney U for relative humidity. Tests were used to compare roosts with tunnel ambient in each zone and with external ambient, and between seasons. Ambient measurements were classified into two groups: 1) local ambient refers to the ambient readings taken from the same tunnel zone as the roost being analysed; 2) non-local ambient refers to the ambient readings taken from different tunnel zone as the roost being analysed; and 3) external refers to the data from SERF.

3.3 Results

Roosting locations

A total of 15 bat roosts were found in summer and 24 in winter (Figures 3-1 & 3-2). In both seasons, *Myotis macropus* roosts were located inside drain holes, *M. australis* roosts were located on tunnel wall surface, and *R. megaphyllus* roosts were located on the tunnel ceiling. The number of *M. macropus* roosts increased from summer to winter, but *M. australis* and *R. megaphyllus* roosts decreased (Figure 3-1). In summer, 10 *M. macropus* roosts, 3 *M. australis* roosts and 2 *R. megaphyllus* roosts were found in the tunnel; in winter, 22 *M. macropus* roosts, a *M. australis* roost, and a *R. megaphyllus* roost were located.

In both summer and winter, *M. macropus* roosted in all three zones of the tunnel but the majority of roosts and bats were concentrated in zone 2 (Z2) (Figure 3-2). The population of *M. macropus* remained relatively stable from summer to winter (the estimated total population increased by 4 from summer to winter – Table 3-1). The number of roosts and number of bats per roost was higher on the right-hand side (east side) of the tunnel in both seasons (Table 3-2). Roosts used by *M. macropus* in summer were concentrated in a smaller area than in winter (Figure 3-2).

Miniopterus australis roosted in Z2 and zone 3 (Z3) during summer, with the biggest roost containing 86 individuals in Z2 (39.6 m into the tunnel). Two smaller roosts with 15 and 30 bats were located in Z3, 61 m and 63.5 m into the tunnel,

respectively (Figure 3-1). In winter, there was a single roost of 15 bats located in Z2, 44 m into the tunnel.

In summer, two *R. megaphyllus* roosts were located in Z3 (60 m – roost Z3.0 and 80 m – roost Z3.1) in the tunnel. The roost at 60 m was a single bat on the ceiling of a doorway (Figure A 8), while the 80 m roost contained 3 individuals located on the ceiling above the rock pile. In winter, only one roost was present with 18 individuals located at the exact same position at 80 m.

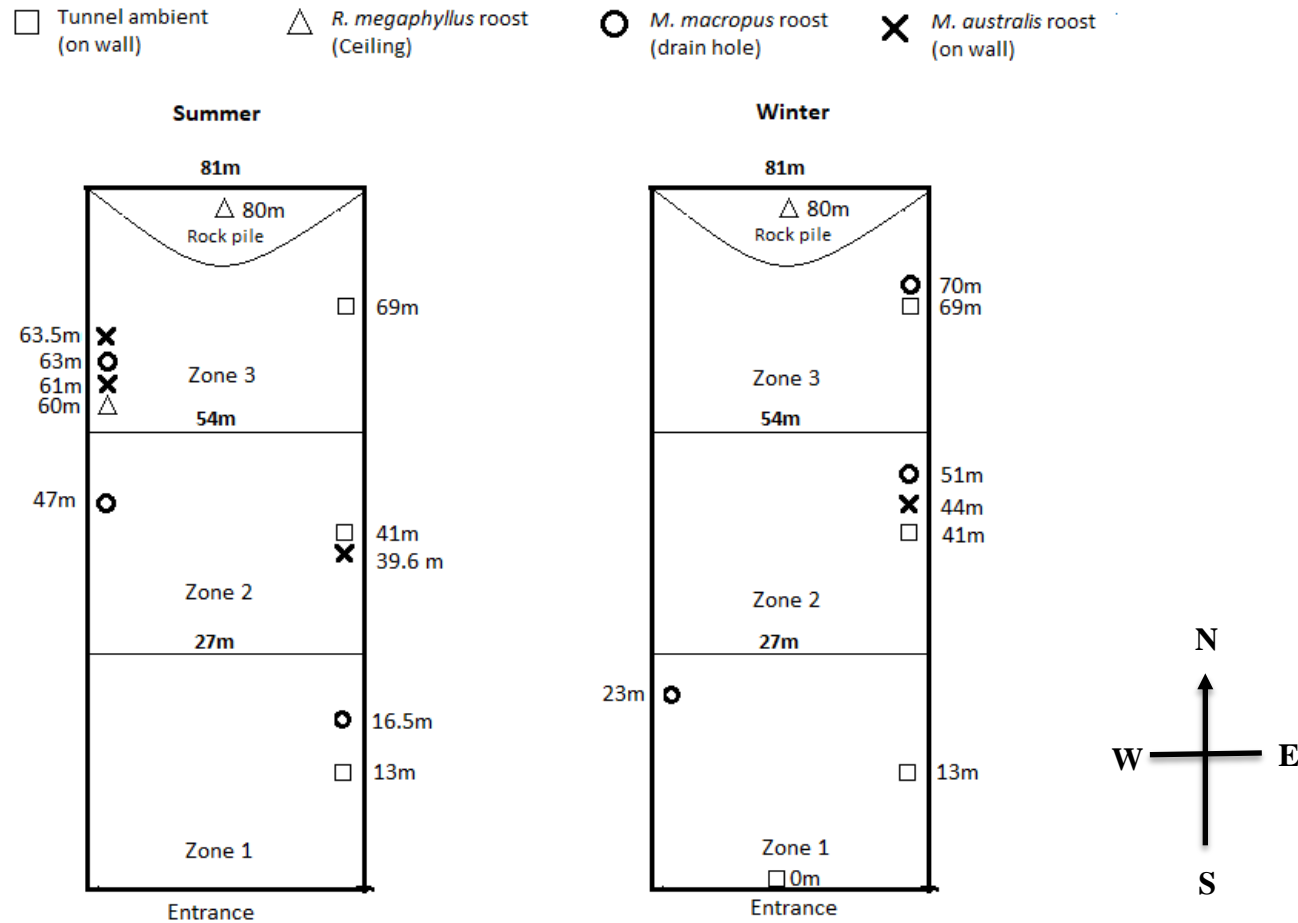


Figure 3-1 Location of each HOBO temperature and humidity logger during summer and winter. A total of 11 HOBO loggers were deployed in summer, and 9 in winter. Three ambient loggers were set at the exact same spot inside the tunnel during both summer and winter. Loggers near bat roosts were located at different positions in the tunnel between summer and winter depending on roost locations, except the *R. megaphyllus* roost at 80 m that remained at the same location.

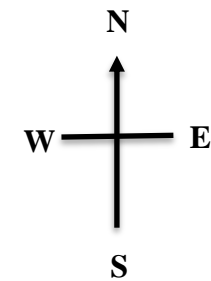
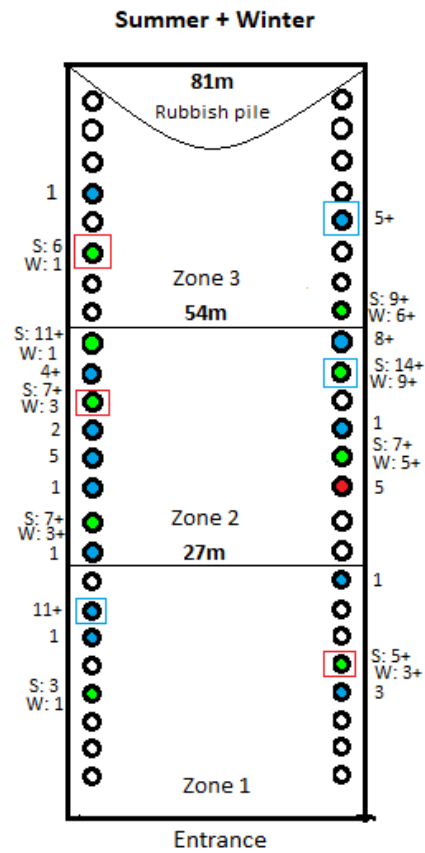
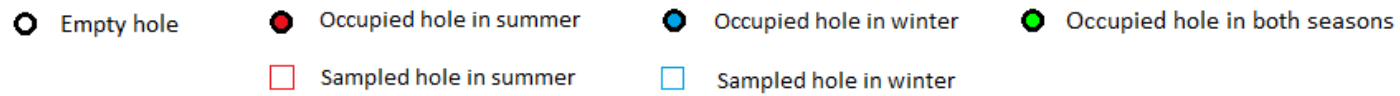


Figure 3-2 Drain hole roost locations of *M. macropus* in summer and winter inside the Yugar tunnel. Drain holes in each zone were occupied, and the majority of bats concentrated in Zone 2 in both seasons. A total of 8 holes were occupied in both summer and winter throughout the tunnel.

Table 3-1 Number of drain holes occupied by *M. macropus* in the Yugar tunnel in summer and winter.

Number of holes in the tunnel occupied by bats in summer and winter		
Variables	Summer	Winter
Holes occupied	10	22
% occupied	21%	46%

Table 3-2 The total population of *M. macropus* inside drain holes and their mean population per hole (\pm SD) along both the left and ride side of the tunnel in summer and winter.

Season	Left		Right		Total
	Total	Bats/ hole (\pm SD)	Total	Bats/ hole (\pm SD)	
Summer	33	6.6 \pm 2.9	40	8.0 \pm 3.5	73
Winter	36	2.8 \pm 3.1	41	4.6 \pm 2.8	77

Roosting microclimate – Temperature

External temperatures and those recorded in the tunnel followed a similar daily pattern, with temperature increasing after sunrise and decreasing in the evening (Figures 3-3 & 3-4). In summer, the daily maximums occurred between 12:00 and 15:00 and minimums occurred between 06:00 and 07:00; within the first hour after official sunrise. In winter, daily maximums occurred between 13:00 and 15:00. However, daily minimums varied more between days with 4 days of minimum temperature occurring between 06:00 – 08:00 and 3 days of minimum temperature occurring between 00:00 – 06:00; within 6 hours before official sunrise.

Tunnel temperatures were more stable than those recorded outside the tunnel, in both summer and winter. In summer, all three tunnel ambients showed daily temperature fluctuations ranging between 21.1°C – 22.5°C (1.4°C), less than that of external ambient (EXA) which ranged between 16.7°C – 29.1°C (12.4°C) (Figure 3-3). In winter, tunnel ambient 2 (A_z2) and ambient 3 (A_z3) showed relatively stable temperature fluctuations ranging between 16.1°C – 18.8°C (2.7°C), smaller than ambient 1 (A_z1) which ranged between 14.6°C – 18.6°C (4.0°C). EXA recorded at tunnel entrance ranged between 12.1°C – 23.5°C (11.1°C) and EXA recorded at SERF with the highest daily temperature fluctuation approximately between 8.7°C – 27.3°C (18.6°C) (Figure 3-4). Each tunnel internal ambient logger showed higher temperatures in summer than winter. A_z2 showed the highest daily temperature followed by A_z3 then A_z1 in both seasons.

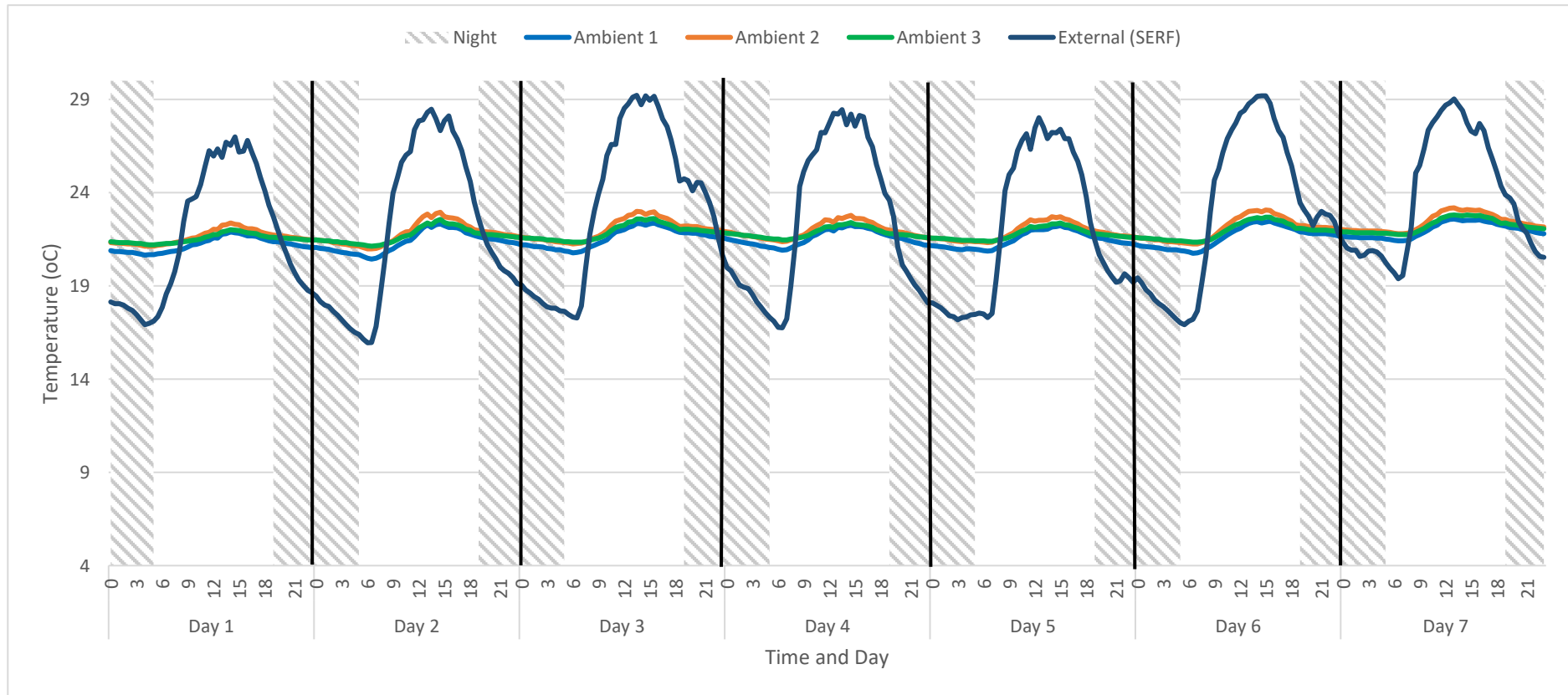


Figure 3-3 Daily temperature recorded by the SERF weather station and tunnel ambient loggers in each tunnel zone over 7 days in summer. The number following the “Ambient” refers to the zone where the logger was located, i.e. Ambient 1 (A_z1) represent the ambient logger in zone 1. Shaded regions represent night time (19:00 – 05:00) and non-shaded regions represent daytime (05:00 – 19:00). Solid vertical lines separate each day (midnight) Note: The tunnel entrance logger was stolen and so temperature at the tunnel entrance was not available for summer. Instead, the SERF weather station values are presented here as “External”. There was a large difference in temperature and temperature fluctuation between the SERF weather station and tunnel ambient in each zone. Temperature at the SERF weather station reached much higher maximums and lower minimum than the tunnel ambients each day. Meanwhile, the SERF weather station was more sensitive towards subtle temperature movement than that of the tunnel ambients. The tunnel ambient in each zone was generally very similar to one another in terms of daily average, maximum and minimum temperatures, and presented more stable day-to-day temperatures than the SERF weather station.

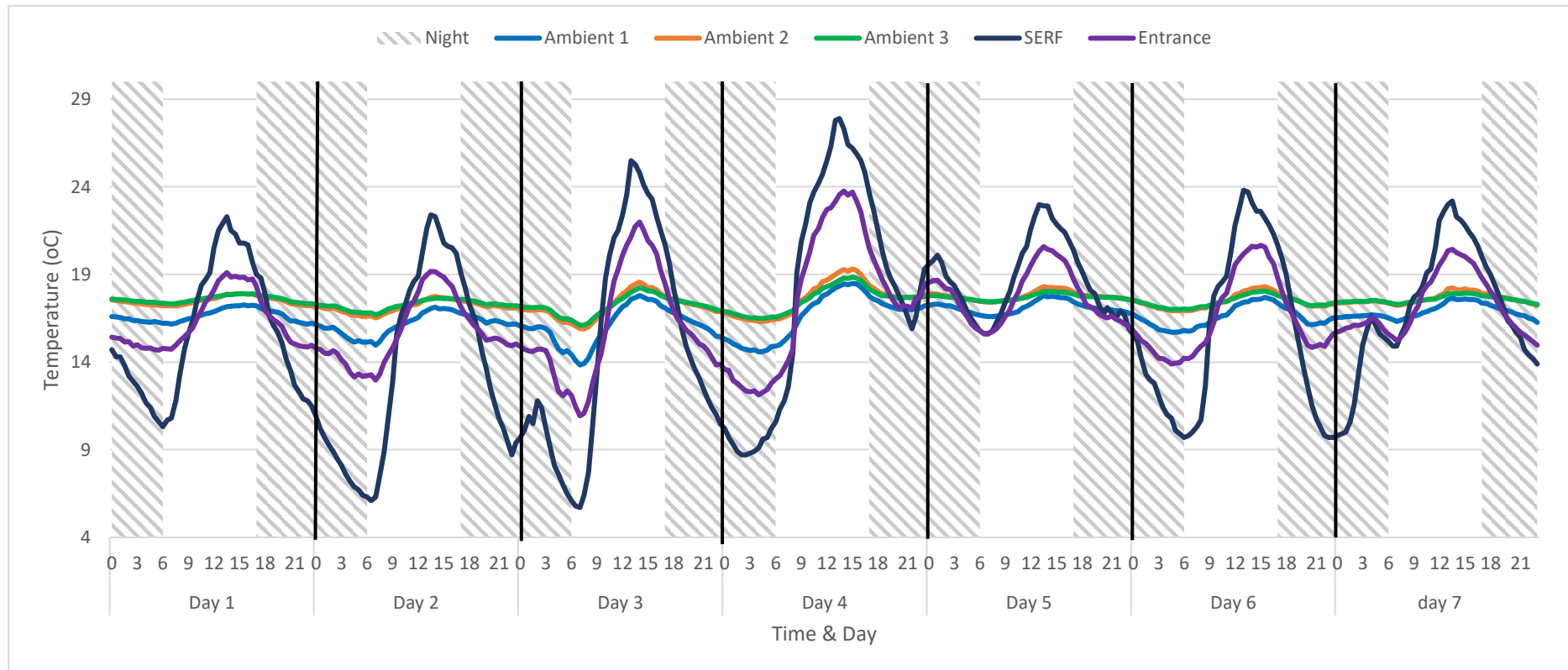


Figure 3-4 Daily temperature recorded by the SERF weather station and tunnel entrance and ambient loggers in each zone over 7 days in winter. The number following the “Ambient” refers to the zone where the logger was located, i.e. Ambient 1 (A₁) represent the ambient logger in zone 1. Shaded regions represent night time (17:00 - 06:30) and non-shaded regions represent daytime (06:30 – 17:00). Solid vertical lines separate each day (midnight). There was a large difference in temperature between the SERF weather station and tunnel ambient in each zone and tunnel external ambient. Temperature at the SERF weather station reached much higher maximums and lower minimum than the tunnel ambients each day. Similar to summer, the SERF weather station was more sensitive towards subtle temperature movement than that of each tunnel ambient. A₁ has the lowest average, maximum and minimum temperature compared with A₂ and A₃. Meanwhile, A₁ has the highest daily temperature fluctuation comparing with A₂ and A₃. The temperature is very similar between A₂ and A₃ in terms of daily average, maximum and minimum temperatures. The tunnel ambient in each zone was more stable than the SERF weather station and the tunnel external ambient.

The mean daily diurnal temperature (T_{day}) differences between roost locations (all species combined) and external ambient readings were compared between summer and winter. Average, minimum, and fluctuation showed significant difference between summer and winter (Independent T-test, $p < 0.05$ – Table 3-3). Meanwhile, there was no significant difference for maximum between summer and winter. Bat roosts showed a lower average (T_{avg}), maximum (T_{max}) and fluctuation (T_{fluc}) of mean daily diurnal temperature than the external ambient reading in both seasons; only minimum diurnal temperature (T_{min}) was higher in bat roosts compared with external ambient (Table 3-3).

The mean T_{day} differences between roost locations (all species combined) and their local ambient reading (TA_{local}) (e.g. roost in Z2 is compared with A_z2) were compared between summer and winter. T_{max} and T_{fluc} showed no significant difference between summer and winter (Independent T-test, $p > 0.05$ – Table 3-4). However, the mean T_{avg} and T_{min} were significantly different with summer showing lower mean temperature differences than winter ($Df = 5.18$, $t = -3.4257$, $p < 0.05$). In summer, bat roosts showed a higher mean T_{avg} and T_{min} than their TA_{local} ; while T_{max} and T_{fluc} were lower at bat roosts than their TA_{local} (Table 3-4). In winter, the mean T_{avg} , T_{max} and T_{min} were higher at bats' roosts than their TA_{local} ; while T_{fluc} was lower at bats' roosts than their TA_{local} (Table 3-4).

The mean daily diurnal temperature differences between roost locations (all species combined) and their non-local ambient reading ($TA_{\text{non-local}}$) were compared (e.g. roost in Z2 is compared with A_z1 and A_z3) between summer and winter. T_{min} and T_{fluc} showed winter had significantly greater differences than summer (Independent T-test, $p < 0.05$ – Table 3-5). Meanwhile, there were no significant differences for T_{avg} and T_{max} ($p < 0.05$). Bat roosts showed a higher mean T_{avg} , T_{max} and T_{min} than their $TA_{\text{non-local}}$ in both seasons; while T_{fluc} was higher in bats' roosts than $TA_{\text{non-local}}$ (Table 3-5).

Table 3-3 Independent T-test results for the difference in mean daily diurnal temperature differences (\pm SD) of each variable between all sampled bat roosts and external ambient between summer and winter. For this analysis all species were combined. Note: +ve mean value = roosts have higher temperature than external ambient; -ve mean value = roosts have lower temperature than external ambient.

Variables	Mean in summer (\pm SD)	Mean in winter (\pm SD)	Df	t-statistic	P-value
Average	-2.40 \pm 0.40	-1.16 \pm 0.32	10.13	-6.14	0.0001**
Maximum	-5.93 \pm 0.68	-5.8 \pm 0.47	10.75	-0.33	0.76
Minimum	4.40 \pm 0.26	7.10 \pm 0.28	8.21	-17.43	8.93e-08**
Fluctuation	-10.34 \pm 0.65	-12.93 \pm 0.45	10.77	8.48	4.34e-06**

Table 3-4 Independent T-test results for the difference in mean diurnal temperature differences (\pm SD) of each variable between all sampled bat roosts and their local tunnel ambient between summer and winter. For this analysis all species were combined. Note: +ve mean value = roosts have higher temperature than local ambient and -ve mean value = roosts have lower temperature than local ambient.

Variables	Mean in summer (\pm SD)	Mean in winter (\pm SD)	Df	t-statistic	P-value
Average	0.14 \pm 0.30	0.45 \pm 0.16	10.84	-2.45	0.033*
Maximum	-0.01 \pm 0.62	0.08 \pm 0.35	10.97	-0.32	0.75
Minimum	0.36 \pm 0.22	1.09 \pm 0.45	5.19	-3.43	0.018*
Fluctuation	-0.37 \pm 0.71	-1.01 \pm 0.69	8.89	1.62	0.14

Table 3-5 Independent T-test results for the difference in mean diurnal temperature differences (\pm SD) of each variable between all sampled bat roosts and their non-local ambient (ambient in the other two zones). For this analysis all species were combined. Note: +ve mean value = roosts have higher temperature than their non-local ambients and -ve value = roosts have lower temperature than their non-local ambients.

Variables	Mean in summer (\pm SD)	Mean in winter (\pm SD)	Df	t-statistic	P-value
Average	0.20 \pm 0.49	0.61 \pm 0.59	16.62	-1.84	0.083
Maximum	0.00 \pm 0.75	0.18 \pm 0.60	22.32	-0.67	0.51
Minimum	0.53 \pm 0.39	1.36 \pm 0.82	11.64	-2.98	0.012*
Fluctuation	-0.53 \pm 0.64	-1.18 \pm 0.58	20.52	2.64	0.015*

Comparing the mean daily diurnal temperature between each individual roost and each ambient, the mean T_{avg} showed greater differences between roosts and EXA than between roosts and TA_{local} and $TA_{non-local}$ in both summer and winter (Figure 3-5 & 3-6). In summer, the difference between roosts and EXA was greater (between 2°C to 3°C) than that in winter (between 1.0°C to 1.5°C). All roosts presented a lower mean T_{avg} than EXA in both seasons. The mean T_{avg} differences between roosts and all tunnel ambients varied more in winter than in summer.

The T_{avg} showed no significant differences between each roost and its TA_{local} between summer and winter (Independent Sample T-test, $p = 0.7547$ - Table 3-3); the difference in the mean T_{avg} between each roost and its TA_{local} was less than 1°C during both seasons. In summer, *M. macropus* roosts in each zone were cooler than their TA_{local} , with the magnitude of differences approaching 0 as roost were located deeper into the tunnel. In winter, roosts showed higher mean T_{avg} than their TA_{local} . For *M. australis* and *R. megaphyllus*, each roost had a higher mean of T_{avg} than its TA_{local} in Z2 and Z3 during summer and winter.

Comparing the mean T_{avg} differences between roosts and $TA_{non-local}$, it was found that there was no significant difference between summer and winter (Independent sample T-Test, $p = 0.0833$ – Table 3-4). Both roost and ambient in Z1 showed the highest differences among 3 zones in both seasons (Figures 3-5 & 3-6). In terms of species, *M. macropus* roost and ambient in Z1 showed a greater difference than with Z2 & Z3. *Miniopterus australis* roosts showed higher mean T_{avg} than $TA_{non-local}$ in both seasons, except roost Z3.1 which was cooler than A_{z2} in summer. *Rhinolophus megaphyllus* roosts were warmer than $TA_{non-local}$ in both summer and winter, except for roost Z3.0 which was cooler than A_{z2} during summer.

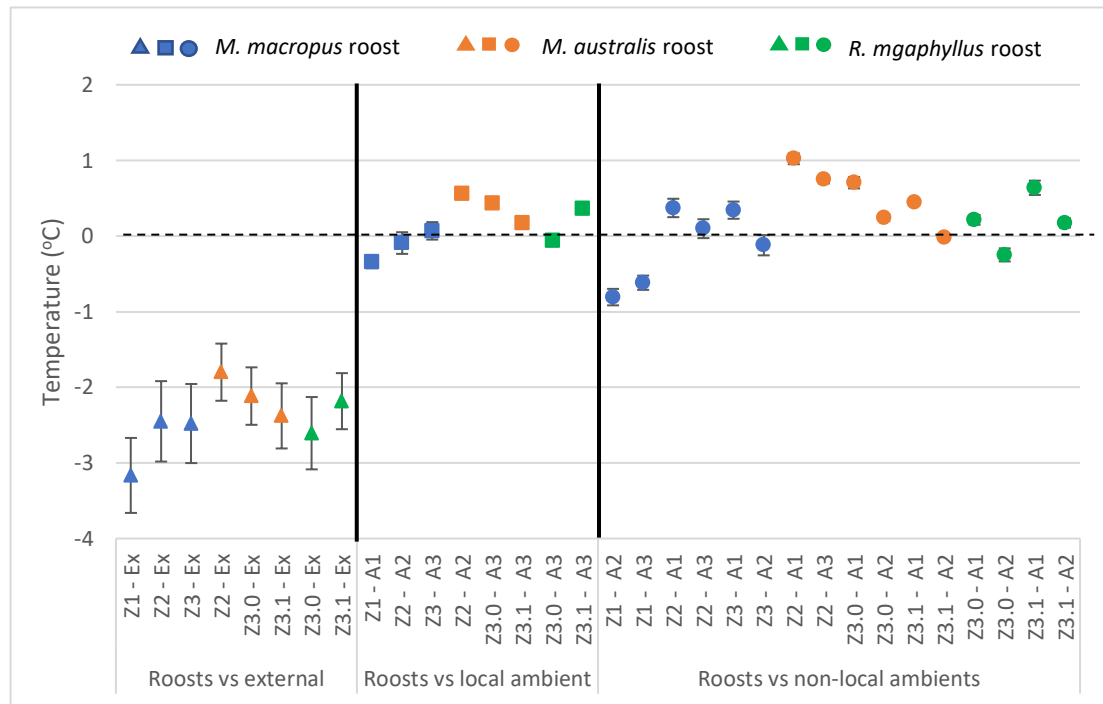


Figure 3-5 The mean daily average diurnal temperature (T_{avg}) difference (\pm SD) of each roost to tunnel external and internal ambients (local and non-local) in summer. Each comparison was named according to the zone where each logger was located, i.e. Ex = External logger, Z1 = roost logger at zone 1, A1 = ambient logger in zone 1, Z3.0 = the closest roost logger to the beginning of zone 3. The triangle icons represent the comparisons between roosts and external ambient, square icons represent the comparisons between roosts and their local tunnel ambient, and circle icons represent the comparisons between roosts and their non-local tunnel ambient. Solid vertical lines separate the roost comparisons between external and tunnel internal ambients. Dotted line highlights the 0°C margin. Error bars showed the standard deviation of the mean difference between each roost and tunnel external and internal ambient. The mean differences were calculated using each roosts minus external or tunnel ambient temperatures. The daily average diurnal temperature between roosts and external showed the biggest difference when comparing with difference to the tunnel ambients. All roosts showed less difference in the daily diurnal average temperature compared with local ambient versus non-local ambients. The difference in the daily diurnal average temperature between roosts and all tunnel ambients was mostly within 1°C.

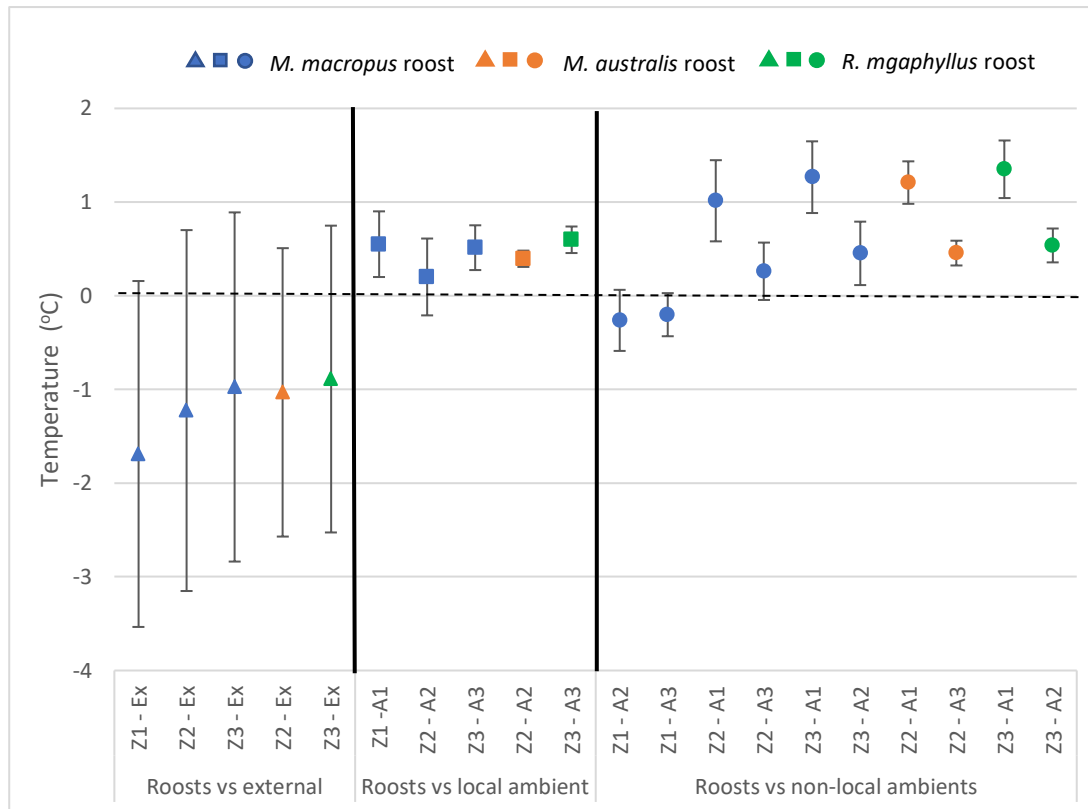


Figure 3-6 The mean daily average diurnal temperature (T_{avg}) difference (\pm SD) of each roost to tunnel external and internal ambients (local and non-local) in winter. Each comparison was named according to the zone where each logger was located, i.e. Ex = External logger, Z1 = roost logger at zone 1, A1 = ambient logger in zone 1. The triangle icons represent the comparisons between roosts and external ambient, square icons represent the comparisons between roosts and their local tunnel ambient, and circle icons represent the comparisons between roosts and their non-local tunnel ambient. Solid vertical lines separate the roost comparisons between external, all tunnel internal ambients. Dotted line highlights the 0°C margin. Error bars showed the standard deviation of the mean difference between each roost and tunnel external and internal ambient temperatures. The mean differences were calculated by roosts minus external or tunnel ambient temperatures. The daily average diurnal temperature between roosts and the external has the biggest difference and varied the most when comparing with difference to all tunnel ambient. The daily average diurnal temperature of roost and A1 presented greater differences when comparing the temperature at A2 and A3.

The mean T_{fluc} showed greater differences between roosts and external ambient (EXA) than that between roosts and tunnel internal ambient in summer and winter (Figures 3-7 & 3-8). In summer, the difference between each roost and EXA was greater (between 9 to 11°C) than in winter (between 12 to 14°C). All roosts showed a lower T_{fluc} than EXA in both seasons.

The mean T_{fluc} showed no significant difference between roosts and their TA_{local} between summer and winter (Independent Sample T-test, $p = 0.1394$ - Table 3-3); differences were within 2°C in both seasons. In terms of species, *M. macropus* roosts showed lower mean T_{fluc} than their TA_{local} in both seasons. *Miniopterus australis* roosts showed higher T_{fluc} compared with their TA_{local} in summer and winter, except roost Z3.1 in summer. *Rhinolophus megaphyllus* roost Z3 fluctuated more than A_z3 in summer but not in winter; both seasons showed less than 0.5°C differences (Figures 3-7 & 3-8).

The mean T_{fluc} differences between roosts and $TA_{non-local}$ showed a significant difference between summer and winter (Independent Sample T-test, $p = 0.0153$ – Table 3-4), with majority of the differences within 2°C. *Myotis macropus* roosts showed lower mean T_{fluc} than $TA_{non-local}$ in both seasons. *Myotis macropus* roost Z2 & Z3 showed bigger differences in winter than summer when comparing with A_z1 (Figures 3-7 & 3-8). For *M. australis*, both roost Z2 and Z3.0 showed higher mean T_{fluc} than $TA_{non-local}$, except roost Z3.1 in summer and roost Z2 in winter (Figures 3-7 & 3-8). *Rhinolophus megaphyllus* roosts showed a lower mean T_{fluc} than $TA_{non-local}$ during both seasons.

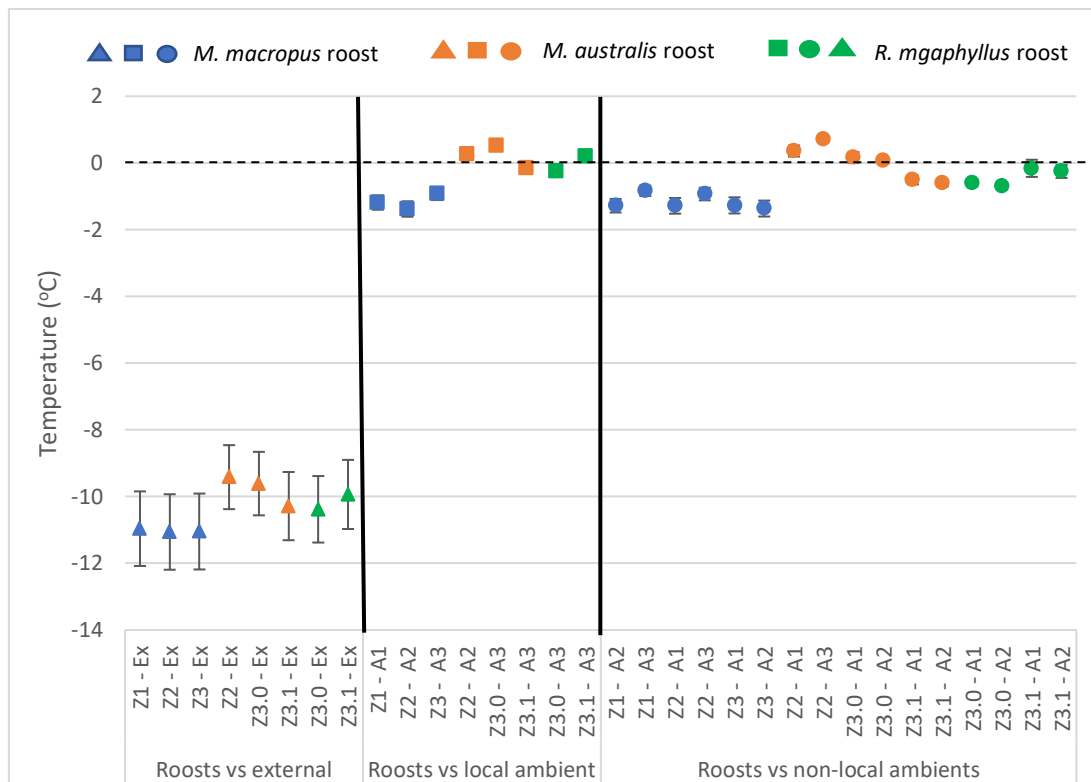


Figure 3-7 Mean daily diurnal temperature fluctuation (T_{fluc}) difference (\pm SD) for each roost compared with tunnel external and internal ambients (local and non-local) in summer. Each comparison was named according to the zone where each logger was located, i.e. Ex = External logger, Z1 = roost logger at zone 1, A1 = ambient logger in zone 1, Z3.0 = the closest roost logger to the beginning of zone 3. The triangle icons represent the comparisons between roosts and external ambient, square icons represent the comparisons between roosts and their local tunnel ambient, and circle icons represent the comparisons between roosts and their non-local tunnel ambient. Solid vertical lines separate the roost comparisons between external and tunnel internal ambients. Dotted line highlights the 0 $^{\circ}$ C margin. Error bars show the standard deviation of the mean difference between each roost and tunnel external and internal ambient temperatures. The mean differences were calculated from roosts minus external or tunnel ambient temperatures. The mean daily diurnal temperature fluctuation difference between each roost and external was largest and varied the most when compared between the tunnel local and non-local ambients in each zone. All three *M. macropus* roosts showed lower diurnal temperature fluctuations than the tunnel ambients in each zone.

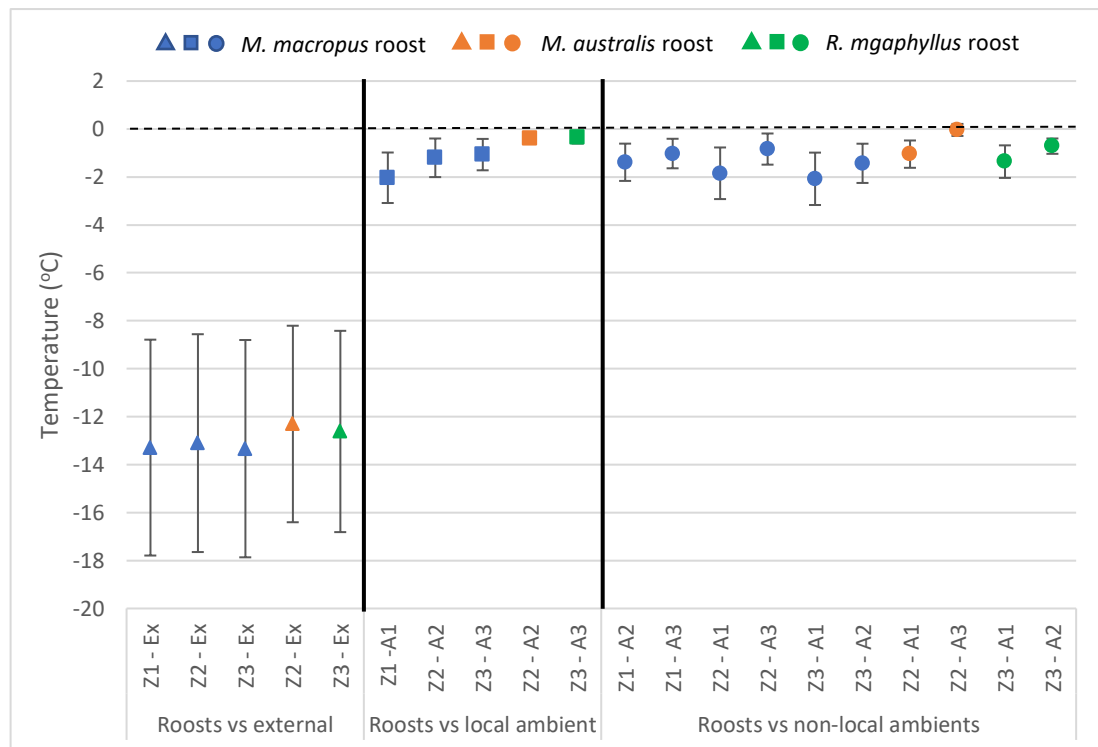


Figure 3-8 Mean daily diurnal temperature fluctuation (T_{fluc}) difference (\pm SD) for each roost compared with tunnel external and internal ambients (local and non-local) in winter. Each comparison was named according to the zone where each logger was located, i.e. Ex = External logger, Z1 = roost logger at zone 1, A1 = ambient logger in zone 1. The triangle icons represent the comparisons between roosts and external ambient, square icons represent the comparisons between roosts and their local tunnel ambient, and circle icons represent the comparisons between roosts and their non-local tunnel ambient. Solid vertical lines separate the roost comparisons between external, local and non-local internal ambients. Dotted line highlights the 0°C margin. Error bars showed the standard deviation of the mean difference between each roost and tunnel external and internal ambient temperatures. The differences were calculated using roosts minus external or tunnel ambient temperatures. The daily diurnal temperature fluctuation between each roost and external showed the largest difference and varied the most when compared with tunnel ambient in each zone. All roosts presented lower mean daily diurnal temperature fluctuation than the tunnel ambient in each zone.

The mean T_{avg} , T_{max} , T_{min} and T_{fluc} in roost temperature for each species was different between summer and winter as shown by winter T_{avg} not being included in the 95% confidence intervals for summer measurements. For almost all comparisons, summer values were higher than those in winter, with the exception of one *M. macropus* roost that showed higher mean of T_{fluc} in winter than in summer (Figures 3-9, 3-10, 3-11). The differences in diurnal roosting temperatures of each species between summer and winter were approximately between 3 – 4°C. The differences in diurnal roosting temperatures among the three species were within 1°C in both seasons.

Comparing the mean daily diurnal roost temperature among three species in summer, *M. australis* showed the highest mean T_{avg} , T_{max} and T_{fluc} of diurnal roosting temperature followed by *R. megaphyllus* and *M. macropus*; the mean T_{min} was highest at *M. macropus* roosts, followed by *M. australis* and *R. megaphyllus* but the differences were small (< 0.3°C) (Figures 3-9, 3-10, 3-11, 3-12). In winter, *M. australis* showed the highest mean T_{max} and T_{fluc} of roosting temperature followed by *R. megaphyllus* and *M. macropus*; T_{avg} was highest in *R. megaphyllus* followed by *M. australis* and *M. macropus*; T_{min} was highest in *R. megaphyllus* followed by *M. macropus* and *M. australis* (Figures 3-9, 3-10, 3-11, 3-12).

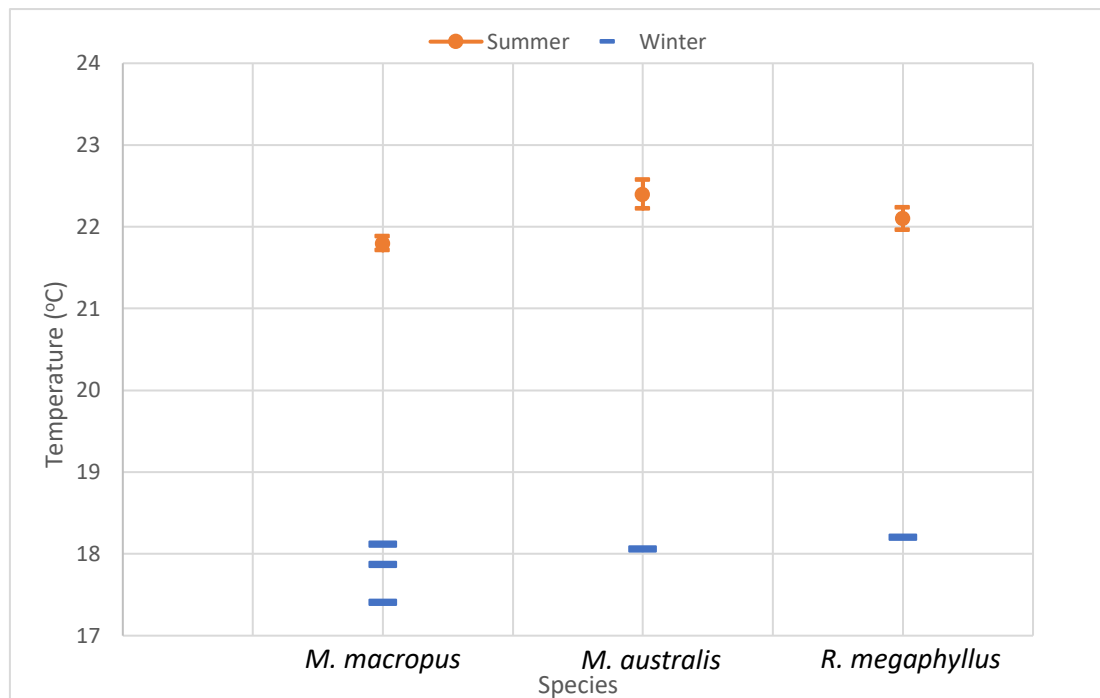


Figure 3-9 Mean and 95% C.I. of the mean for daily average diurnal roost temperature (T_{avg}) for each species over 7 days in summer and winter. The average diurnal roost temperatures in summer ($n = 8$, 3 *M. macropus* roosts, 3 *M. australis* and 2 *R. megaphyllus* roosts) were calculated and presented as 95% C.I.. Winter ($n = 5$, 3 *M. macropus* roosts, 1 *M. australis* and *R. megaphyllus* roosts) were presented using the mean for each roost over 7 days due to low sample sizes for each species. The daily average diurnal roosting temperature of each species was significantly greater in summer than winter i.e. the winter values fell outside of the 95% confidence interval.

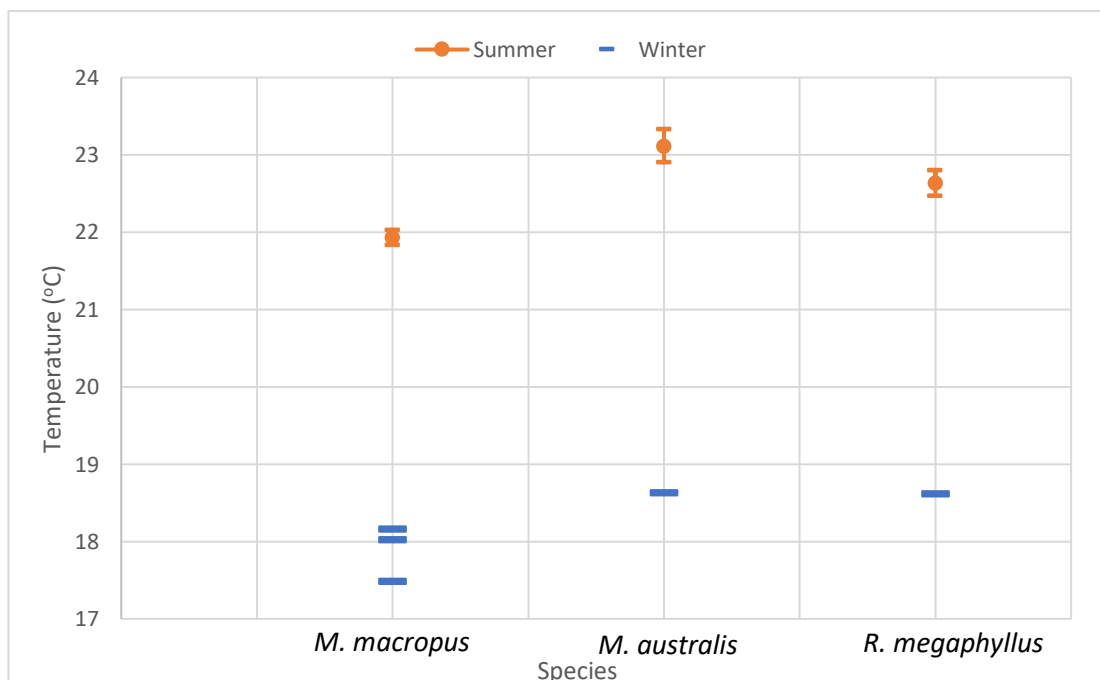


Figure 3-10 Mean and 95% C.I. of the mean for maximum diurnal roost temperature (T_{max}) of each species over 7 days in summer and winter. The maximum diurnal roost temperatures in summer ($n = 8$, 3 *M. macropus* roosts, 3 *M. australis* and 2 *R. megaphyllus* roosts) were calculated and presented as 95% C.I.. Winter ($n = 5$, 3 *M. macropus* roosts, 1 *M. australis* and *R. megaphyllus* roosts) were presented using the mean for each roost over 7 days due to low sample sizes for each species. The daily maximum diurnal roosting temperature of each species was significantly greater in summer than winter i.e. the winter values fell outside of the 95% confidence interval.

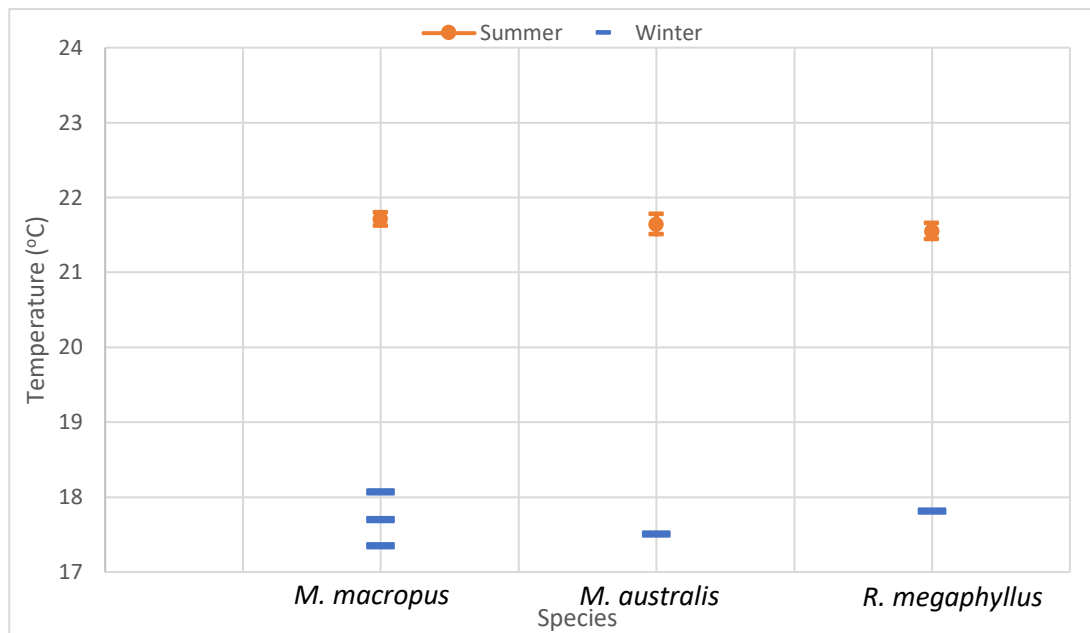


Figure 3-11 Mean and 95% C.I. of the mean for minimum diurnal roost temperature (T_{min}) of each species over 7 days in summer and winter. The minimum diurnal roost temperatures in summer ($n = 8$, 3 *M. macropus* roosts, 3 *M. australis* and 2 *R. megaphyllus* roosts) were calculated and presented as 95% C.I.. Winter ($n = 5$, 3 *M. macropus* roosts, 1 *M. australis* and *R. megaphyllus* roosts) were presented using the mean for each roost over 7 days due to low sample sizes for each species. The daily minimum diurnal roosting temperature of each species was significantly greater in summer than winter.

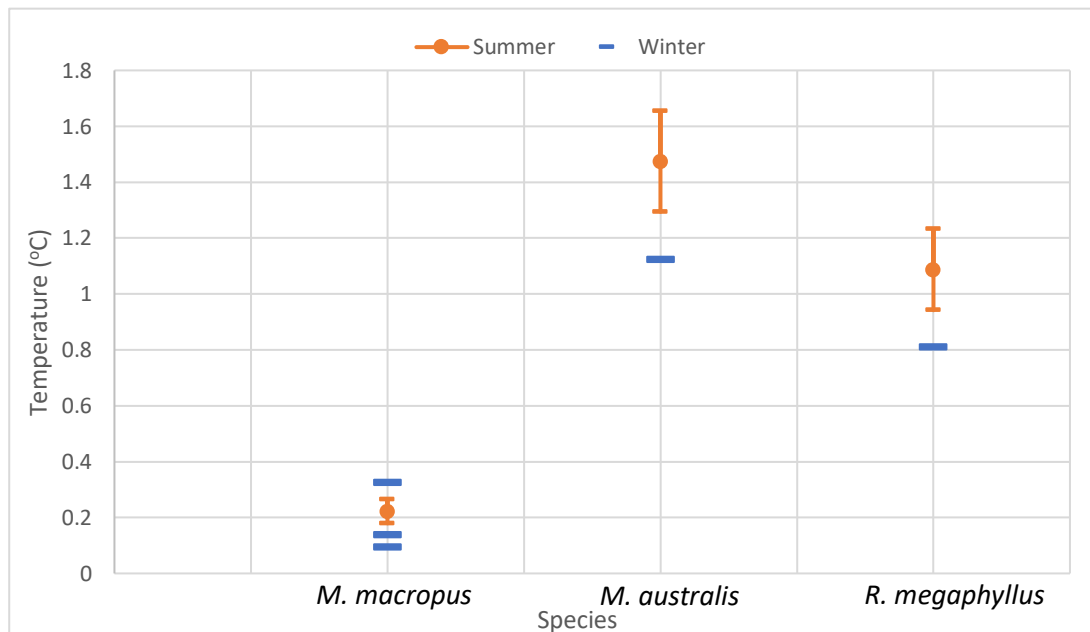


Figure 3-12 Mean and 95% C.I. of the mean for daily diurnal roost temperature fluctuation (T_{fluc}) for each species over 7 days in summer and winter. The diurnal roost temperature fluctuation in summer ($n = 8$, 3 *M. macropus* roosts, 3 *M. australis* and 2 *R. megaphyllus* roosts) were calculated and presented as 95% C.I.. Winter ($n = 5$, 3 *M. macropus* roosts, 1 *M. australis* and *R. megaphyllus* roosts) were presented using the mean for each roost over 7 days due to low sample sizes for each species. Differences for each species between summer and winter were small. *Miniopterus australis* and *R. megaphyllus* have higher daily diurnal roost temperature fluctuation in summer than winter. The mean of daily diurnal roost temperature fluctuation for *M. macropus* is similar between summer and winter. *Myotis macropus* has the lowest daily diurnal roost temperature fluctuation comparing with *R. megaphyllus* and *M. australis* during both seasons.

Roosting microclimate – Humidity

The relative humidity of tunnel internal and EXAs followed a similar daily pattern, with relative humidity decreasing after sunrise and increasing in the evening in both summer and winter (Figures 3-13 & 3-14). Tunnel ambients showed a smaller daily relative humidity fluctuation than EXA in both seasons. The relative humidity of all tunnel ambient loggers and the external logger presented a stable pattern with daily minimums occurring between 12:00 – 15:00 and maximums between 06:00 – 08:00 in both seasons. In summer, the SERF weather station showed a consistent daily relative humidity pattern with maximums of 100% relative humidity and minimums of around 60% relative humidity. In winter, relative humidity from SERF showed maximums of approximately 90% and minimums of approximately 30%. However, these values dropped on day 5 with a lower average and maximum relative humidity until the end of sampling (Figure 3-14). This pattern was also seen in the tunnel ambient readings, but the effect was less than EXA.

The relative humidity of each tunnel ambient was higher in summer than winter (Figure 3-13 & 3-14). The relative humidity of A_z3 was the highest, followed by A_z2 and then A_z1 in both seasons. However, the maximum difference in relative humidity between each ambient was less than 5% in summer and less than 10% in winter. The daily relative humidity fluctuation was higher in winter than that in summer.

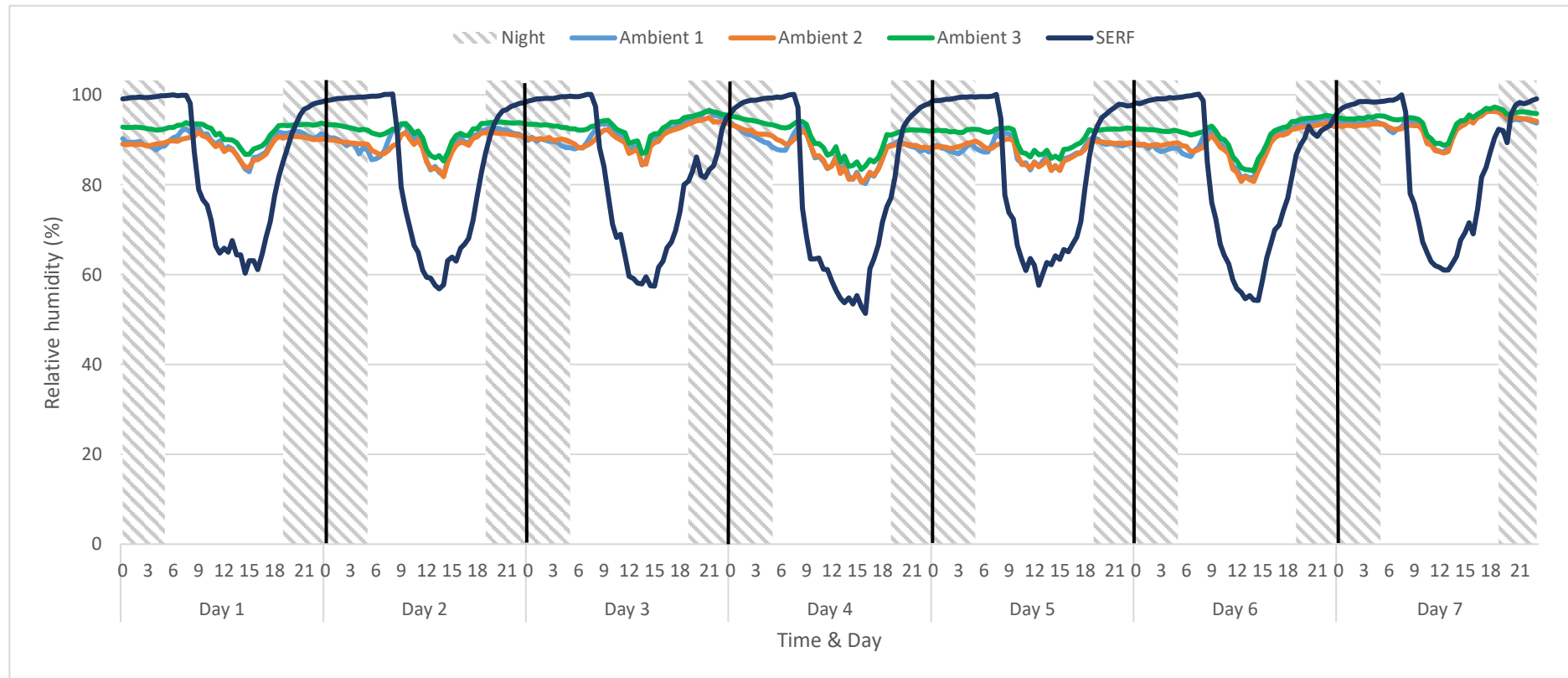


Figure 3-13 Daily relative humidity recorded from the SERF weather station and tunnel ambient loggers in each tunnel zone over 7 days in summer. The number following the “Ambient” refers to the zone where the logger was located, i.e. Ambient 1 represents the ambient logger in zone 1. Shaded regions represent night time (19:00 – 05:00) and non-shaded regions represent daytime (05:00 – 19:00). Solid vertical lines separate each day (at midnight) Note: The tunnel entrance logger was stolen and so relative humidity at the tunnel entrance was not available for summer. Instead, the SERF weather station values are presented here as “External”. The relative humidity of the SERF weather station is more extreme than the tunnel ambients in terms of maximum, minimum and rate of change. The tunnel ambients are very similar to one another in terms of daily average, maximum and minimum relative humidity, and presented more stable day-day relative humidity than the SERF weather station.

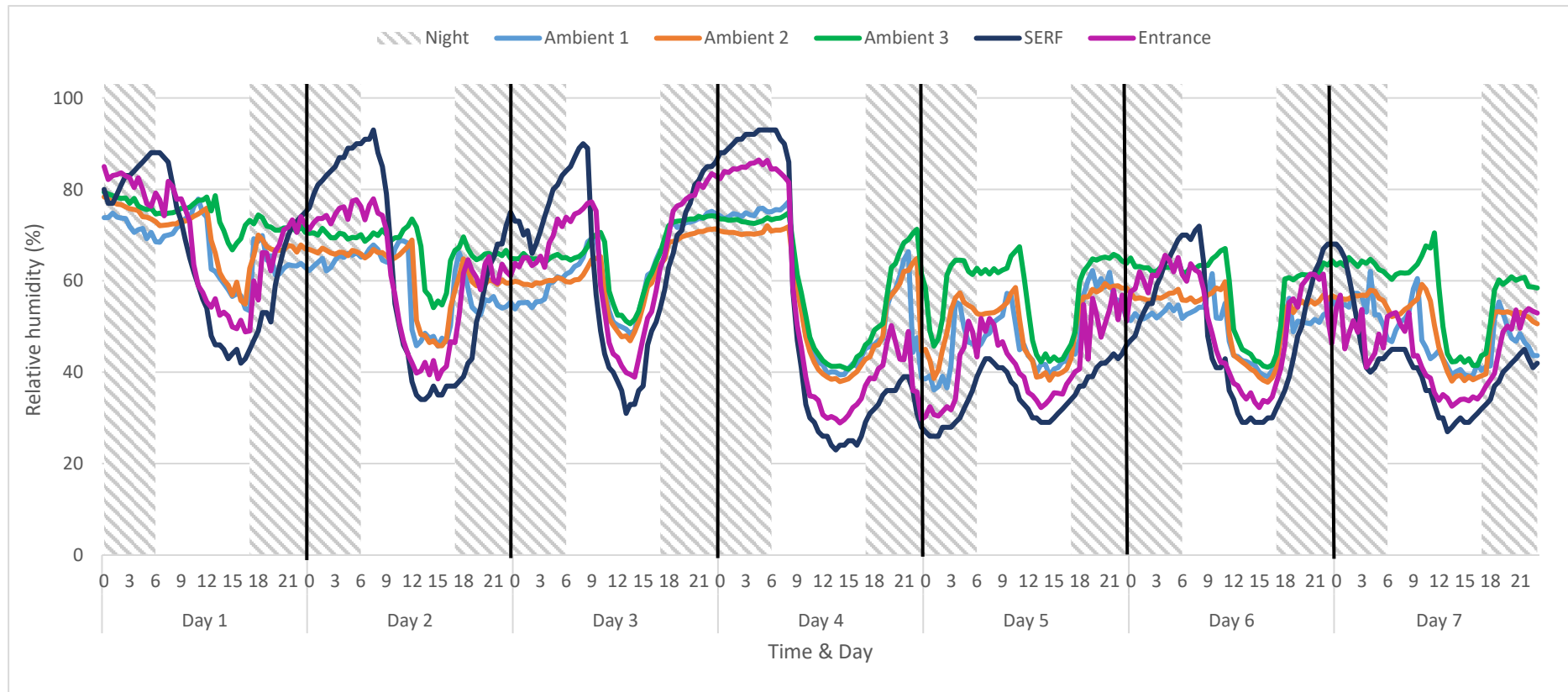


Figure 3-14 Daily relative humidity recorded from the SERF weather station and tunnel ambient loggers in each tunnel zone over 7 days in winter. The number following the “Ambient” refers to the zone where the logger was located, i.e. Ambient 1 represents the ambient logger in zone 1. Shaded regions represent night time (19:00 – 05:00) and non-shaded regions represent daytime (05:00 – 19:00). Solid vertical lines separate each day (at midnight). Note: The tunnel entrance logger was stolen and so relative humidity at the tunnel entrance was not available for summer. Instead, the SERF weather station values are presented here as “External”. There was a big difference on temperature between SERF weather station, and the ambients inside and outside the tunnel. The maximum and minimum relative humidity at the SERF weather station is more extreme than that inside (Ambient 1, 2 & 3) and outside (Entrance) of the tunnel. A3 has the highest average, maximum and minimum relative humidity comparing with A1 and A2. The tunnel ambient in each zone presented more stable day-day relative humidity than the SERF weather station and the ambient outside the tunnel.

The mean daily diurnal relative humidity (RH_{day}) differences between roost locations (all species combined) and external ambient readings were compared between summer and winter. Average, minimum and fluctuation showed no significant differences between summer and winter (Independent T-test, $p > 0.05$ – Table 3-6). However, maximum showed significant difference with winter greater than summer ($p < 0.05$). In summer, bat roosts showed a higher mean of daily average (RH_{avg}) and minimum (RH_{min}) diurnal relative humidity than EXA; the mean daily maximum (RH_{max}), fluctuation (RH_{fluc}) of diurnal relative humidity were lower at bat roosts than EXA. In winter, bat roosts showed a higher mean RH_{avg} , RH_{max} and RH_{min} than EXA, while the mean RH_{fluc} was lower at roosts than EXA (Table 3-6).

Bat roosts showed higher relative humidity than all tunnel ambients in both summer and winter. Differences in RH_{avg} , RH_{max} and RH_{min} between roosts and tunnel ambients were greater in winter than summer. In addition, bat roosts presented much lower mean RH_{fluc} than tunnel ambients in winter than summer (Table 3-7 & 3-8).

The means of RH_{avg} and RH_{max} showed significant differences between roosts (all species combined) and their TA_{local} between summer and winter (Mann-Whitney U test, $p > 0.05$ – Table 3-7). In which, both RH_{avg} and RH_{max} were greater in winter than summer. The means of RH_{min} and RH_{fluc} showed no significant difference between summer and winter ($p > 0.05$).

The means $TA_{non-local}$ of RH_{avg} , RH_{max} , and RH_{min} showed significant differences between roosts and $TA_{non-local}$ between summer and winter (Mann-Whitney U test, $p > 0.05$ – Table 3-8). In which, all three variables (RH_{avg} , RH_{max} , and RH_{min}) showed greater differences in winter than summer. The means of RH_{fluc} showed no significant difference between the two seasons ($p > 0.05$).

Table 3-6 Mann-Whitney U test results comparing the mean diurnal relative humidity differences (\pm SD) of each variable between all sampled bat roosts and external ambient between summer and winter. For this analysis all species were combined. Note: +ve mean value = roosts have higher relative humidity than external ambient; -ve mean value = roosts have lower relative humidity than external ambient.

Variables	n	Mean in summer (\pm SD)	Mean in winter (\pm SD)	W-value	P-value
Average	13	17.1 \pm 6.3	38.4 \pm 21.5	11	0.21
Maximum	13	-4.6 \pm 4.2	15.3 \pm 14.0	5	0.033*
Minimum	13	32.0 \pm 9.5	47.6 \pm 28.8	15	0.51
Fluctuation	13	-36.6 \pm 6.0	-32.3 \pm 15.9	21	0.94

Table 3-7 Mann-Whitney U test results comparing mean diurnal relative humidity differences (\pm SD) between each bat roost and its local tunnel ambient. For this analysis all species were combined. Note: +ve mean = roosts have higher relative humidity than their local ambient and -ve = roosts have lower relative humidity than their local ambient.

Variables	n	Mean in summer (\pm SD)	Mean in winter (\pm SD)	W-value	P-value
Average	13	2.6 \pm 6.7	28.5 \pm 21.7	5	0.030*
Maximum	13	1.7 \pm 4.4	21.7 \pm 13.7	3	0.011*
Minimum	13	4.3 \pm 10.0	33.2 \pm 29.1	7	0.065
Fluctuation	13	-2.57 \pm 6.3	-11.5 \pm 16.0	26	0.43

Table 3-8 Mann-Whitney U test results comparing mean diurnal relative humidity differences (\pm SD) between each bat roost and its non-local tunnel ambient. For this analysis all species were combined. Note: +ve mean value = roosts have higher relative humidity than local ambient and -ve mean value = roosts have lower relative humidity than local ambient.

Variables	n	Mean in summer (\pm SD)	Mean in winter (\pm SD)	W-value	P-value
Average	26	3.6 \pm 6.0	29.1 \pm 20.4	25	0.0040*
Maximum	26	2.2 \pm 4.1	21.9 \pm 13.8	24	0.0034*
Minimum	26	5.4 \pm 9.0	33.6 \pm 27.2	39	0.033*
Fluctuation	26	-3.21 \pm 5.7	-11.7 \pm 15.0	104	0.22

Mean RH_{avg} showed greater differences between roosts and external (EXA) than between roosts and tunnel ambients between summer and winter (Figures 3-15 & 3-16). The difference between roosts and EXA was greater in winter than that in summer, except for *M. australis* roost. All roosts presented a lower mean of RH_{avg} compared with EXA in both seasons.

The RH_{avg} between roosts and TA_{local} was significantly different between summer and winter (Mann-Whitney U test, $W = 5$, $p = 0.02953$ – Table 3-7). The difference in the RH_{avg} between roosts and TA_{local} in summer was generally smaller than that in winter. In summer, *M. macropus* roosts were more humid than their TA_{local} . Meanwhile, *M. australis* and *R. megaphyllus* roosts showed a lower humidity than tunnel local ambient (Figure 3-15). In contrast, all roosts presented a higher mean of RH_{avg} than their TA_{local} in winter (Figure 3-16). In terms of species, *M. macropus* roosts presented higher RH_{avg} differences in winter than summer. *Miniopterus australis* roosts showed little to no difference to their TA_{local} in both seasons. *Rhinolophus megaphyllus* roosts showed higher difference to their TA_{local} in winter than summer. *Myotis macropus* showed the highest mean of RH_{avg} difference between roosts and their TA_{local} when comparing with *R. megaphyllus* and *M. australis* in both seasons.

Comparing the mean RH_{avg} difference between roosts and $TA_{non-local}$, it was found that winter showed a significantly larger difference than summer (Mann-Whitney U test, $W = 25$, $p = 0.004$ – Table 3-8). *Myotis macropus* and *R. megaphyllus* roosts showed higher means of RH_{avg} than their tunnel $TA_{non-local}$ in both seasons (Figures 3-15 & 3-16). Only one *M. australis* roost presented a higher mean RH_{avg} than $TA_{non-local}$, and this was in winter (Figure 3-16). The mean RH_{avg} difference between roosts versus $TA_{non-local}$ was highest for *M. macropus* roosts, followed by *R. megaphyllus*, and then *M. australis* in both summer and winter.

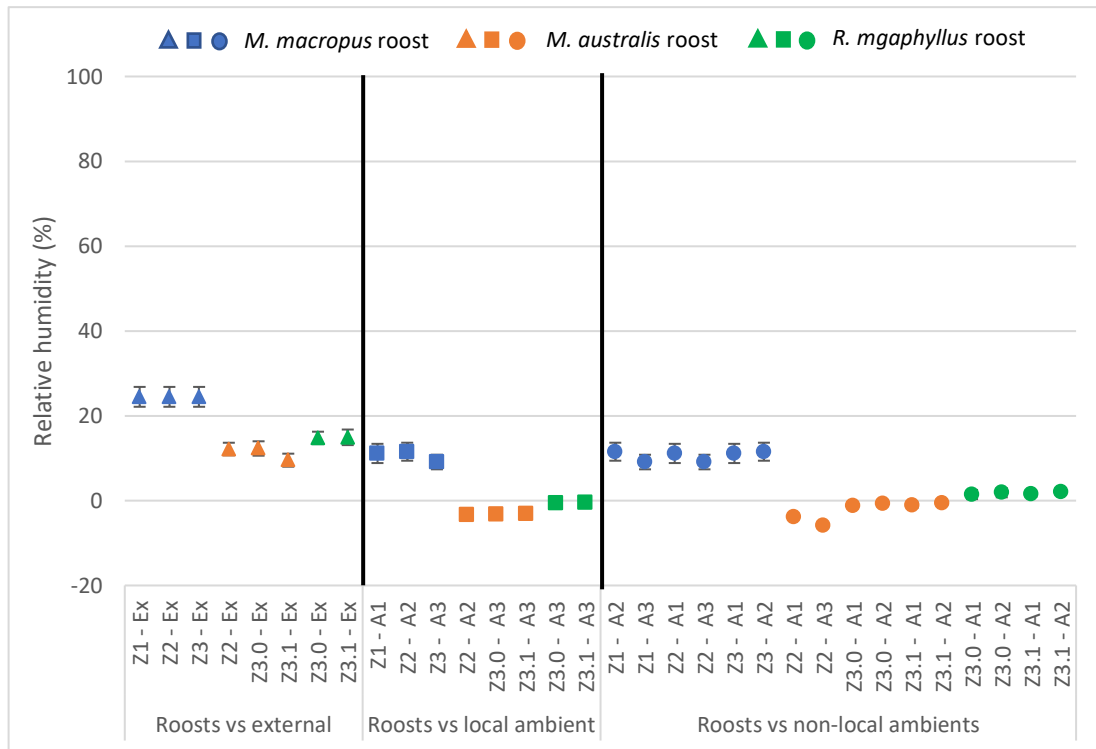


Figure 3-15 Mean daily average diurnal relative humidity (RH_{avg}) difference (\pm SD) of each roost compared with tunnel external and internal ambients (local and non-local) in summer. Each comparison was named according to the zone where each logger was located, i.e. Ex = External logger, Z1 = roost logger at zone 1, A1 = ambient logger in zone 1, Z 3.0 = the closest roost logger to the beginning of zone 3. The triangle icons represent the comparisons between roosts and external ambient, square icons represent the comparisons between roosts and their local tunnel ambient, and circle icons represent the comparisons between roosts and their non-local tunnel ambient. Solid vertical lines separate the roost comparisons with external and tunnel internal ambients. Dotted line highlights the 0% margin. Error bars showed the standard deviation of the mean difference between each roost and tunnel external and internal ambient relative humidity. The differences were calculated using roosts minus external or tunnel ambient temperatures. The mean daily average diurnal relative humidity between all roosts and external showed the biggest differences when compared with tunnel ambients. *Myotis macropus* roosts showed the largest differences in daily average diurnal relative humidity relative to each tunnel ambient when compared with the other two species. Both *M. australis* and *R. megaphyllus* roosts showed little differences compared with the tunnel ambients.

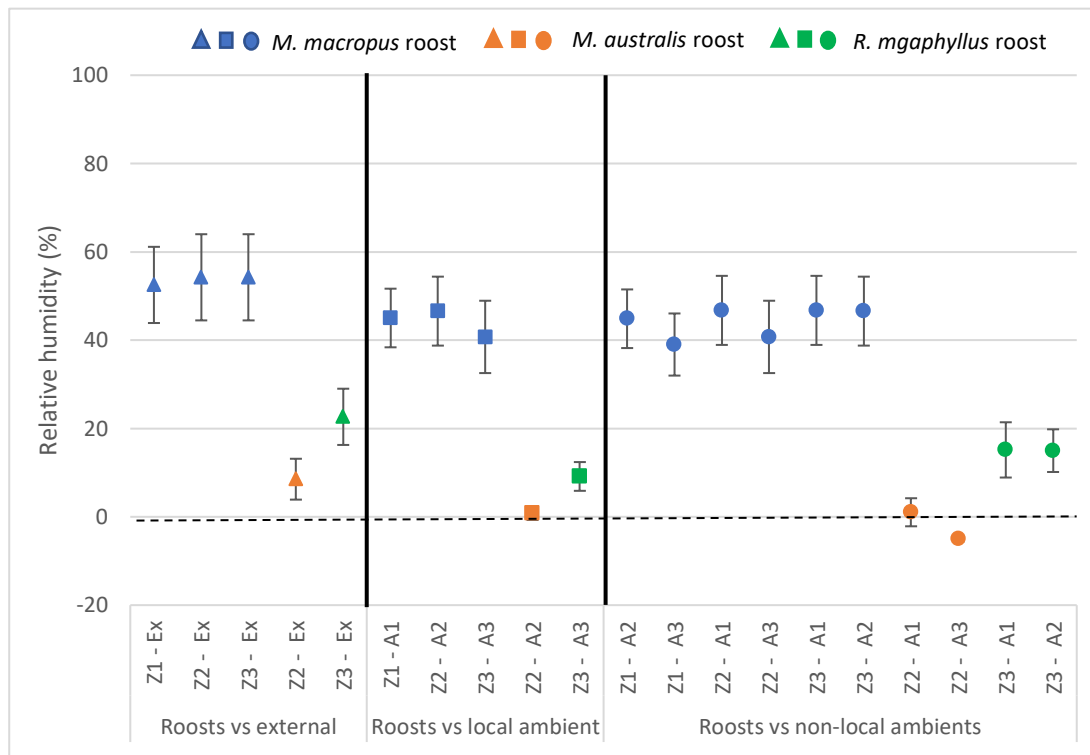


Figure 3-16 Mean daily average diurnal relative humidity (RH_{avg}) difference (\pm SD) of each roost compared with tunnel external and internal ambients (local and non-local) in winter. Each comparison was named according to the zone where each logger was located, i.e. Ex = External logger, Z1 = roost logger at zone 1, A1 = ambient logger in zone 1, Z3.0 = the closest roost logger to the beginning of zone 3. The triangle icons represent the comparisons between roosts and external ambient, square icons represent the comparisons between roosts and their local tunnel ambient, and circle icons represent the comparisons between roosts and their non-local tunnel ambient. Solid vertical lines separate the roost comparisons from external and tunnel internal ambients. Dotted line highlights the 0% margin. Error bars showed the standard deviation of the mean difference between each roost and tunnel external and internal ambient relative humidity. The differences were calculated using roosts minus external or tunnel ambient temperatures. The daily average diurnal relative humidity between roosts and external showed the biggest differences comparing with all tunnel ambients. *Myotis macropus* roosts showed the biggest differences in daily diurnal relative humidity compared with each tunnel ambient among the three species.

Mean RH_{fluc} showed greater differences between roosts and EXA than that between roosts and tunnel internal ambients in summer and winter (Figures 3-17 & 3-18). Both *M. macropus* and *R. megaphyllus* showed greater differences in the mean of RH_{fluc} between roosts and EXA in winter than summer. Only *M. australis* showed similar averages between seasons.

Mean RH_{fluc} showed no significant difference between roosts and all tunnel ambients between summer and winter (Mann-Whitney U test, Roosts vs local ambient: $W = 26$, $p = 0.4351$ – Table 3-7; Roosts vs non-local ambients: $W = 104$, $p = 0.2152$ – Table 3-8). In terms of species, *M. macropus* roosts showed smaller fluctuation than all tunnel ambients in both seasons, but the difference was smaller in summer than winter (Figures 3-17 & 3-18). In summer, both *M. australis* and *R. megaphyllus* roosts showed little to no difference between all tunnel ambients (Figure 3-17). In winter, *M. australis* roosts showed only small differences and *R. megaphyllus* showed slightly higher differences in RH_{fluc} when compared with summer (Figure 3-18).

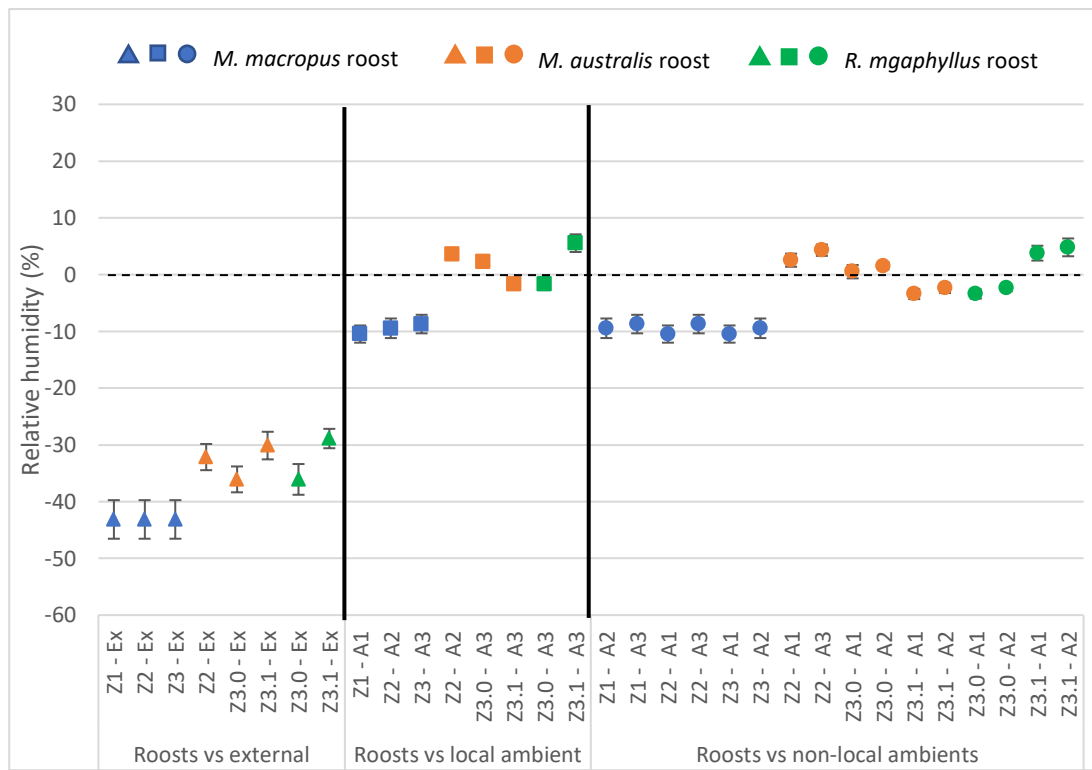


Figure 3-17 Mean daily diurnal relative humidity fluctuation (RH_{fluc}) difference between each roost and tunnel external and internal ambients (local and non-local) in summer. Each comparison was named according to the zone where each logger was located, i.e. Ex = External logger, Z1 = roost logger at zone 1, A1 = ambient logger in zone 1, Z3.0 = the closest roost logger to the beginning of zone 3. The triangle icons represent the comparisons between roosts and external ambient, square icons represent the comparisons between roosts and their local tunnel ambient, and circle icons represent the comparisons between roosts and their non-local tunnel ambient. Solid vertical lines separate the roost comparisons between external and tunnel internal ambients. Dotted line highlights the 0% margin. Error bars showed the standard deviation of the mean difference between each roost and tunnel external and internal ambient relative humidities. The differences were calculated using roosts minus external or tunnel ambient temperatures. The mean daily diurnal relative humidity fluctuation between roosts and external showed the largest differences and varied the most. All three *M. macropus* roosts showed lower diurnal relative humidity fluctuations than tunnel ambients in each zone. Both *M. australis* and *R. megaphyllus* roosts showed little differences to the tunnel ambient in each zone.

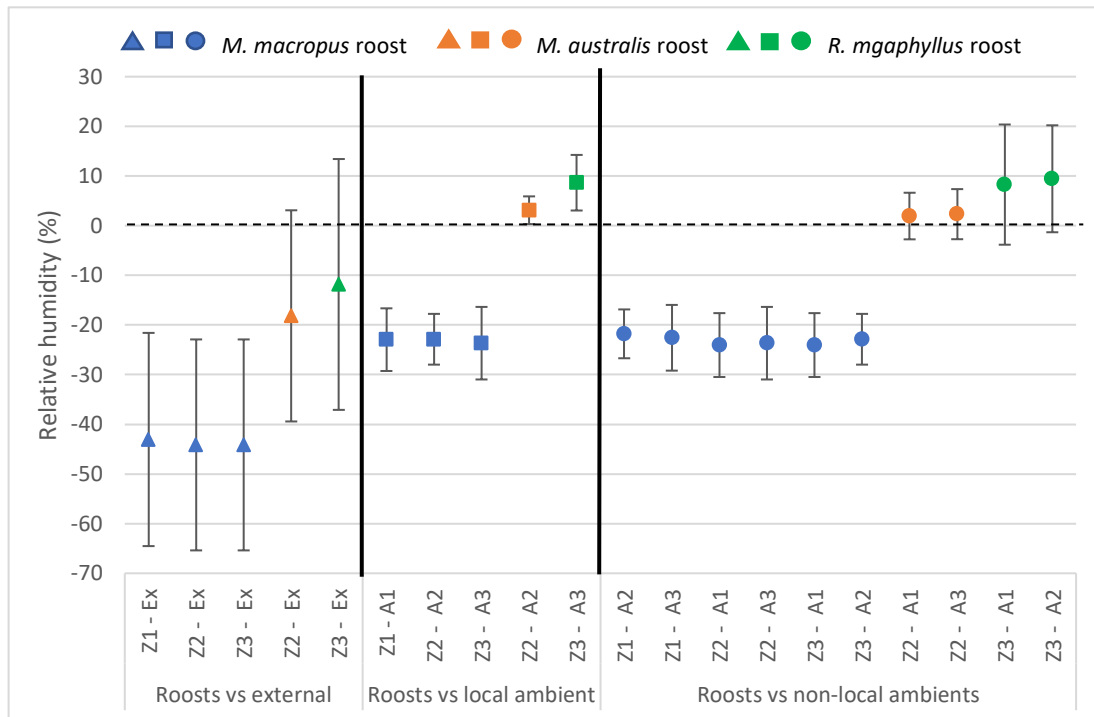


Figure 3-18 Mean daily diurnal relative humidity fluctuation (RH_{fluc}) difference between each roost and tunnel external and internal ambients (local and non-local) in winter. Each comparison was named according to the zone where each logger was located, i.e. Ex = External logger, Z1 = roost logger at zone 1, A1 = ambient logger in zone 1, Z3.0 = the closest roost logger to the beginning of zone 3. The triangle icons represent the comparisons between roosts and external ambient, square icons represent the comparisons between roosts and their local tunnel ambient, and circle icons represent the comparisons between roosts and their non-local tunnel ambient. Solid vertical lines separate the roost comparisons between external and tunnel internal ambients. Dotted line highlights the 0% margin. Error bars showed the standard deviation of the mean difference between each roost and tunnel external and internal ambient relative humidities. The differences were calculated using roosts minus external or tunnel ambient temperatures. The daily diurnal relative humidity fluctuation between each roost to external has the biggest differences and varied the most comparing with difference to the tunnel ambient in each zone. All three *M. macropus* roosts showed lower diurnal relative humidity fluctuations than the tunnel ambient in each zone. Both *M. australis* and *R. mgaphyllus* roosts showed higher relative humidity fluctuations tunnel ambient in each zone.

Among the three species in the tunnel, *M. macropus* roosts showed the highest mean of RH_{avg} , RH_{max} , and RH_{min} followed by *R. megaphyllus* and then *M. australis* in summer and winter. In addition, *M. macropus* roosts showed the lowest mean of RH_{fluc} out of three species in both seasons. In summer, *R. megaphyllus* and *M. australis* presented similar means for RH_{fluc} (10.3% and 10.6%, respectively – Figure 3-22). In winter, the roosting RH_{fluc} was higher in *R. megaphyllus* (32.3%) than *M. australis* (26.0%).

Comparing the mean diurnal roosting relative humidity of each species, *M. macropus* roosts showed almost no differences between summer and winter. It was noted that *M. macropus* roosts remained at 100% humidity throughout the sampling period in both summer and winter, except one roost which had a minimum of 97% relative humidity in winter. There was no relative humidity fluctuation within *M. macropus* roosts. For *M. australis* and *R. megaphyllus* roosts, both showed differences between summer and winter, as the means of RH_{avg} , RH_{max} and RH_{min} between both seasons are not within the 95% C.I. (Figures 3-19, 3-20, 3-21). Both *M. australis* and *R. megaphyllus* roosts showed higher mean roosting RH_{avg} , RH_{max} and RH_{min} , but lower mean RH_{fluc} in summer than winter. The mean of RH_{min} was higher in summer (*M. australis*: 79% and *R. megaphyllus*: 83%) than in winter (*M. australis*: 42% and *R. megaphyllus*: 52%).

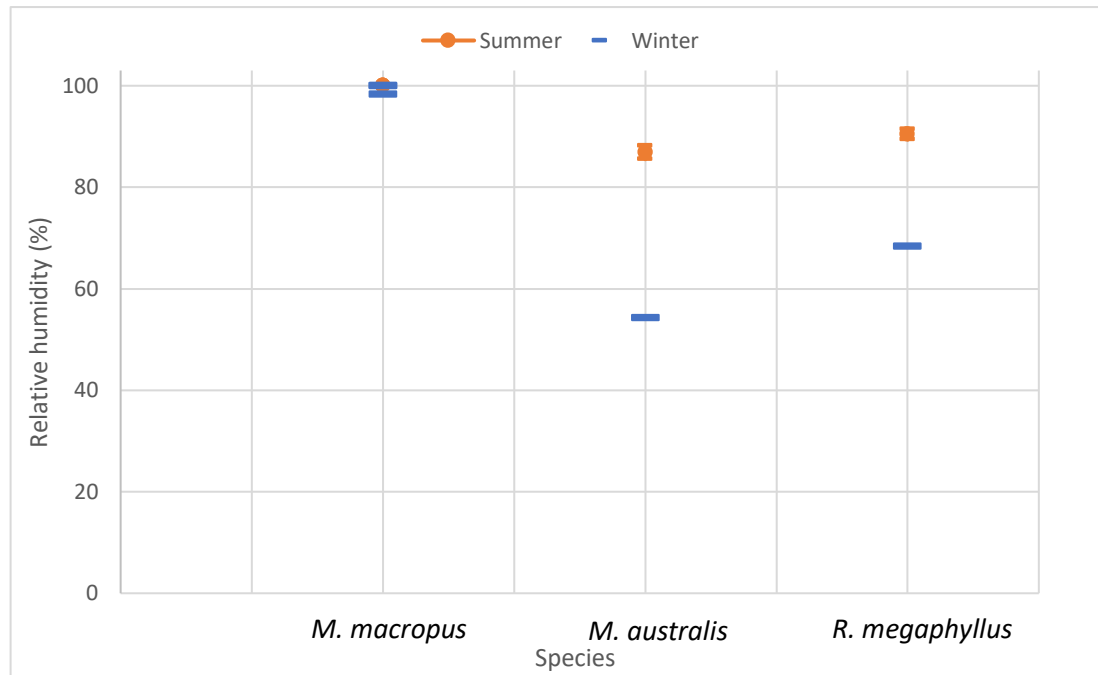


Figure 3-19 Mean and 95% C.I. of the mean for daily average diurnal roosting relative humidity (RH_{avg}) for each species over 7 days in summer and winter. The average diurnal roost relative humidities in summer (n = 8, 3 *M. macropus* roosts, 3 *M. australis* and 2 *R. megaphyllus* roosts) were calculated and presented as 95% C.I.. Winter (n = 5, 3 *M. macropus* roosts, 1 *M. australis* and *R. megaphyllus* roosts) data were presented using the mean for each roost over 7 days due to low sample sizes for each species. The average diurnal roosting relative humidity of *M. macropus* was generally 100% in both seasons. Both *M. australis* and *R. megaphyllus* showed a higher average diurnal roosting relative humidity in summer than winter.

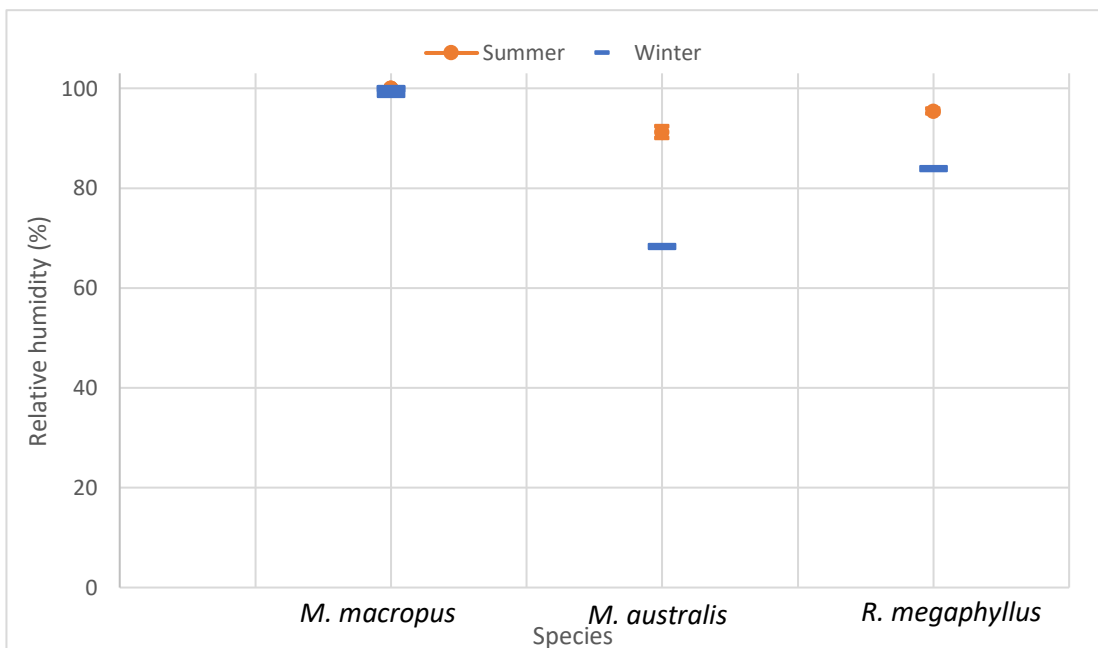


Figure 3-20 Mean and 95% C.I. of the mean for daily maximum diurnal roosting relative humidity (RH_{max}) of each species over 7 days in summer and winter. The mean maximum diurnal roost relative humidities in summer (n = 8, 3 *M. macropus* roosts, 3 *M. australis* and 2 *R. megaphyllus* roosts) was calculated and presented as 95% C.I.. Winter (n = 5, 3 *M. macropus* roosts, 1 *M. australis* and *R. megaphyllus* roosts) data were presented using the mean of each roost over 7 days due to low sample sizes for each species. The maximum diurnal roosting relative humidity of *M. macropus* was generally 100% in both seasons. Both *M. australis* and *R. megaphyllus* showed a higher maximum diurnal roosting relative humidity in summer than winter.

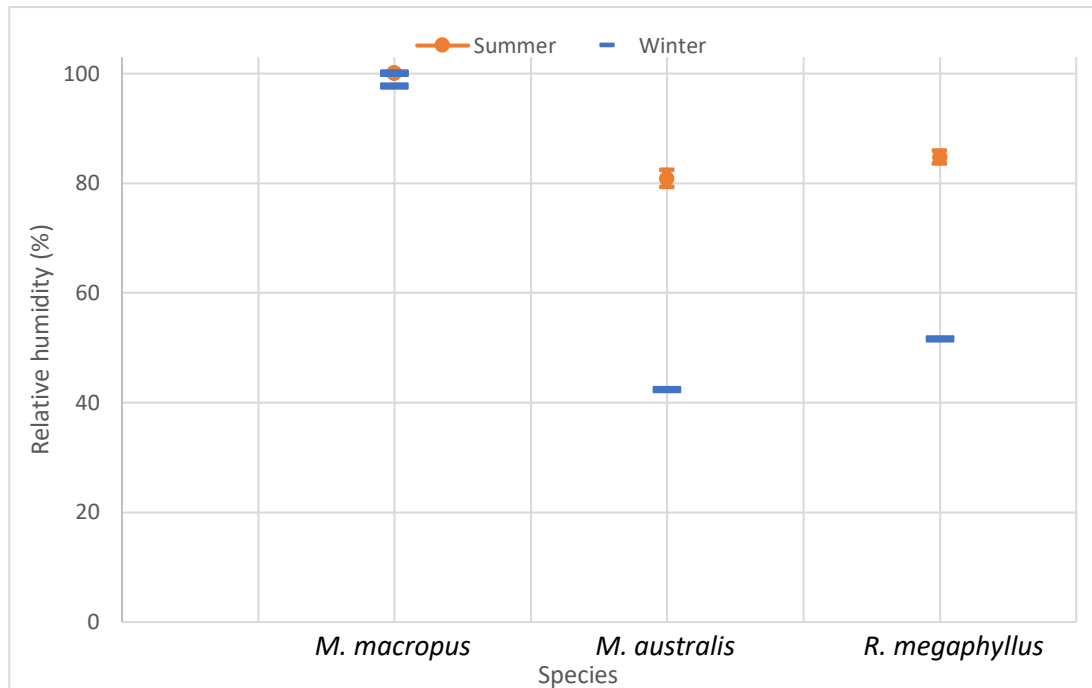


Figure 3-21 Mean and 95% C.I. of the mean for daily minimum diurnal roost relative humidity (RH_{min}) for each species over 7 days in summer and winter. The minimum diurnal roost relative humidities in summer (n = 8, 3 *M. macropus* roosts, 3 *M. australis* and 2 *R. megaphyllus* roosts) were calculated and presented as 95% C.I.. Winter (n = 5, 3 *M. macropus* roosts, 1 *M. australis* and *R. megaphyllus* roosts) were presented using the mean for each roost over 7 days due to low sample sizes for each species. The minimum diurnal roosting relative humidity of *M. macropus* was generally 100% in both seasons. Both *M. australis* and *R. megaphyllus* showed a higher maximum diurnal roosting relative humidity in summer than winter.

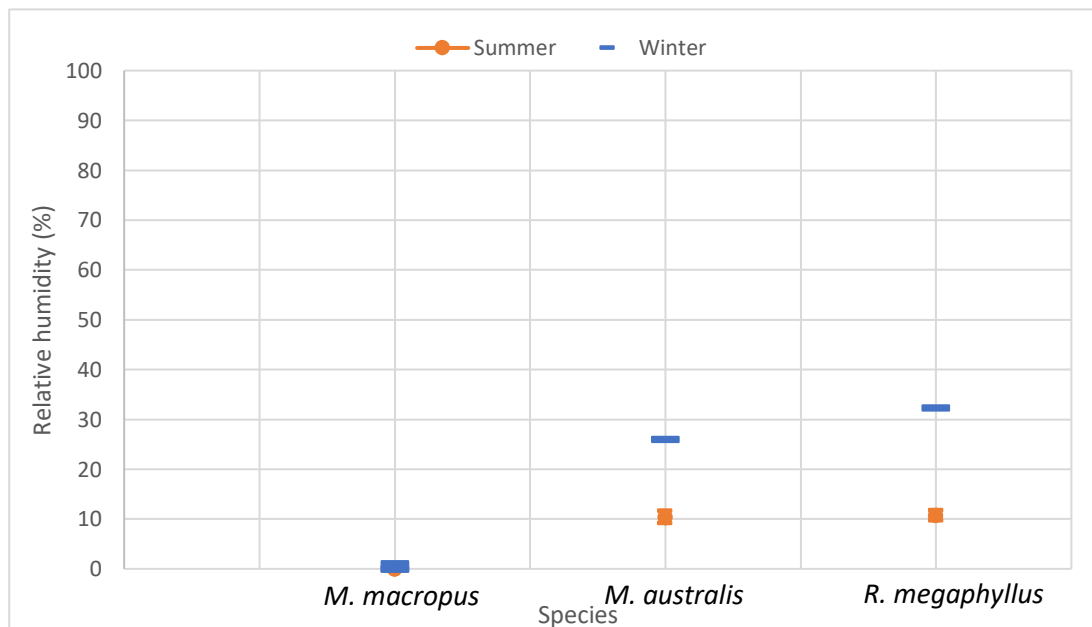


Figure 3-22 Mean and 95% C.I. of the mean daily diurnal roosting relative humidity fluctuation (RH_{fluc}) for each species over 7 days in summer and winter. The fluctuations in diurnal roost relative humidity in summer (n = 8, 3 *M. macropus* roosts, 3 *M. australis* and 2 *R. megaphyllus* roosts) were calculated and presented as 95% C.I.. Winter (n = 5, 3 *M. macropus* roosts, 1 *M. australis* and *R. megaphyllus* roosts) were presented using the mean for each roost over 7 days due to low sample sizes for each species. The relative humidity of *M. macropus* roosts do not fluctuate in either season. Both *M. australis* and *R. megaphyllus* showed a lower daily diurnal roosting relative humidity fluctuation in summer than winter.

3.4 Discussion

Roosting locations

The results of this study show that *M. macropus*, *M. australis* and *R. megaphyllus* roosted in different locations inside the tunnel. *Myotis macropus* roosts were distributed across each zone with majority of roosts and bats concentrated in Z2 in summer and winter. All *M. macropus* roosts were located inside drain holes, 2.8m off the ground. The number of individuals remained stable from summer to winter. However, in winter *M. macropus* were more spread out, occupying more holes but with fewer individuals per hole compared with summer.

Previous studies have found that the *Myotis species* exhibit some degree of plasticity in roost selection; they have been recorded in caves, aqueduct tunnels, abandoned railway tunnels, culverts, bridges and tree hollows (Campbell 2009; Dwyer, 1970; Gorecki et al. 2020, Law et al. 2020). Many *M. macropus* roosting sites were found near waterbodies (Gonsalves & Law 2017; Campbell 2009; Anderson et al. 2006), which highlights their close association with waterways and reflects their specialized foraging strategy. *Myotis macropus* often roost in colonies of 10 – 15 individuals, increasing to around 100 bats in maternity sites (Anderson et al 2006). Campbell (2009) obtained similar results to this study with a colony size in an aqueduct tunnel ranging from 9 – 12; *M. macropus* were roosting in crevices. Meanwhile, Gorecki et al. (2020) reported *M. macropus* colony sizes in culverts around Brisbane ranging between 1 – 3 individuals for day roosts and 4 – 49 individuals for maternity roosts. In terms of total colony size in summer and winter, the Yugar tunnel is of a similar size to previously identified maternity roosts.

Miniopterus australis roosts were found in Z2 and Z3 of the tunnel with the majority of bats clustering in Z2 (55 m from the entrance) during summer. The colony size reduced greatly in winter when only 15 individuals were found roosting in Z2. All *M. australis* roosts were located on the tunnel wall surface, at least 3.3 m above ground in both seasons. Studies on *Miniopterus species* (i.e. *M. schreibersii*, *M. orianae*

blepotis and *M. orianae oceanensis*) reported that roosts were often found in caves, disused tunnels, underground rooms and abandoned mines (Hall 1982; White 2011; Dwyer 1968). These species were also found clustering on flat surfaces, surfaces with shallow crevices, ceilings, and in room corners (Hall 1982; White 2011; Dwyer 1968). *Miniopterus* species in Australia have been recorded roosting in colonies containing from 50 to 60,000 individuals (Dwyer & Hamilton-Smith 1965; Gonsalves & Law 2018). Dwyer (1968) recorded a *M. australis* roost containing about 4000 individuals in a nursery colony in south-eastern Australia. The tropical reproductive pattern of *M. australis* and *M. orianae* are characterized by mating through August with births in December (Dwyer 1968). Hoyer and Spence (2004) reported that *M. orianae* is usually absent from over-winter roosts between September and March each year after they disperse to remote breeding sites. The pattern of population changes seen through this study (presences between January to April – summer & many absence between July to September – winter) suggest that Yugar tunnel could be a potential breeding site for *M. australis*. However, more evidence is required to validate this suggestion.

All *R. megaphyllus* roosts were found in Z3 during summer and winter. The biggest roost was located at the deepest part of the tunnel above the rocks pile and remained at this location across both seasons (roost Z3.1 – summer & roost Z3 – winter). All *R. megaphyllus* roosts were located on the ceiling of the tunnel. Previous studies have reported that *R. megaphyllus* often roosts in caves, tunnels, disused mines, rock piles and culverts (Pavey 1998; Law 2018; Pavey & Burwell 2004; Krutzsch et al. 1992; Murphy 2014). This species is characterized by its roosting position of hanging free from the ceiling (Menkhorst & Knight 2011). *Rhinolophus megaphyllus* usually roost in colonies of less than 10 individuals and rarely more than 50, except nursery roost sites that may have colonies ranging from 15 to over 2000 individuals (Dwyer 1966; Murphy 2014). Law (2018) reported the largest *Rhinolophus* roosts located in Ourimbah State Forest near Sydney contained over 10,000 individuals. As the number of *R. megaphyllus* in the Yugar tunnel was small throughout the year, and no young were seen during walkthrough surveys, it is likely only a day roosting site for this species.

In general, the three bat species in the Yugar tunnel exhibited different roosting strategies and clustered in different locations; they did not share their roosting locations with other species in either season. All roosts were located at least 2.8 m above ground level. It is widely accepted that tunnels and caves benefit bats for mating, hibernation, raising young and offering protection against predators and bad weather (Torquetti et al. 2017; McCracken et al. 2006; Zukal et al. 2017; Lima & O’Keefe 2013; Ruczynski 2006; Zahn & Dippel 1997; Martínková et al. 2020). However, the selection of roosting locations and clustering strategies may be more dependent on microclimate and ambient environment (Sedgeley 2001; Winter et al. 2020; Klys & Woloszyn 2010; Rajasegaran et al. 2018).

Roosting microclimate

Microclimate is an important aspect of roost selection for insectivorous bats. Thermoregulation is critical to the behavior and ecology of bats, and is heavily dependent on roosting microclimate (Campbell 2009; Sedgeley 2006). Insectivorous bats are known to be sensitive to microclimate fluctuation within roosts due to their small size and high surface area to volume ratio (Campbell 2009). Therefore, caves and other human-made structures including tunnels (Campbell 2009), culverts (Gorecki et al. 2020), mines (Brack Jr. 2007) and underground sites (White 2011) can provide thermal stability (Lutsch 2019) by buffering from climatic extremes (Law et al. 2018) and rapid change in temperature and humidity compared with ambient conditions outside the roost (Mitchell-Jones et al. 2007). Variations in roost structures and characteristics are often assumed to have effect on bat roosting microclimate (Boyles 2007). The roosting microclimate of each species in the Yugar tunnel will be discussed in terms of temperature and humidity as below.

Temperature – Tunnel ambient

Based on the results of this study, the mean T_{\max} and T_{\min} of tunnel ambients and external ambient (EXA) were more varied and extreme in winter than summer. Results clearly showed that the temperature inside the tunnel was buffered, with T_{fluc} lower than EXA in both summer and winter. Studies on bats roosting in tunnels and

mines indicated that the internal temperature of these structures could sometimes be warmer or cooler than the ambient environment, but the temperature is often more stable inside the roost sites with less than 1.0°C variation between days (White 2011; Hope & Jones 2013; Meyer et al. 2016). In addition, temperature stability has been shown to increase further away from entrances or exits of tunnels (Hope & Jones 2013). A similar pattern was recorded inside the Yugar tunnel, where temperatures closer to the entrance were more affected by the external ambient temperature. The T_{day} of all tunnel ambients were cooler than EXA temperature in both seasons. The T_{day} of A_{z1} was cooler and fluctuated more than A_{z2} & A_{z3} in both seasons. Interestingly, A_{z2} (mid-way into the tunnel) showed the highest ambient temperature in both seasons. Bats in the tunnel experienced smaller degree of daily temperature fluctuation and a cooler diurnal temperature than EXA.

Temperature - Bats roosts

Bats in this study showed higher T_{avg} and lower T_{fluc} in their roosts (all species combined) than all tunnel ambients during both summer and winter. There was a larger difference in T_{avg} and T_{fluc} between bat roosts and $T_{\text{A}_{\text{non-local}}}$ than between bat roosts and $T_{\text{A}_{\text{local}}}$. Meanwhile, the differences in T_{fluc} between bat roosts and all tunnel ambients were higher in winter than summer. The T_{day} differences between bat roosts and all tunnel ambients were within 1°C in both seasons, excepted when T_{min} occurred in winter.

The T_{avg} and T_{max} showed no significant differences between all sampled roosts (all species combined) and tunnel ambients between summer and winter, only minimum showed significant differences. Meanwhile, minimum temperature increased when roost temperature was less varied between night and day. Willis & Brigham (2007) suggested social organization of bats can affect temperature within roosts. Bats clustering in larger numbers influences energetic requirements which increases roost temperature and reduce the heat loss for both the roosts and resident bats (Otto et al. 2016; Willis & Brigham 2007; Menzies et al. 2016). Roosting bats generating metabolic heat can increase the temperature within occupied roosts by up to 5 – 10°C (Sedgeley 2001). Both temperature and thermal stability are crucial

factors influencing roost selection of bats (Sedgeley 2003; Otto et al. 2016; Klüg-Baerwald et al. 2017). Anthropogenic structures including tunnels, culverts, bridges and mines often offer better thermal stability than external ambient for bats (Menzies et al. 2016; Gorecki et al. 2020; Smith & Stevenson 2013; Meyer et al. 2016; White 2011). Thermal stability enhances bats' ability to minimise energy losses and increase reproductive fitness associated with pregnancy, development and thermoregulation (Sedgeley & O'Donnell 1999; Menzies et al. 2016). Smith & Stevenson (2013) indicated that thermal stability is the major factor influencing bats roost selection rather than high temperatures. It aligns with the results of this study in that majority of the bats were located in Z2 and Z3 where temperatures were relatively more stable than in Z1. In general, bats preferred to select locations with either warmer or more thermally stable (or possibly both) conditions in the Yugar tunnel.

For *M. macropus*, the T_{avg} of each roost was similar to its TA_{local} in summer, and slightly warmer (within 0.5°C) during winter. *Myotis macropus* prefer selecting their roost in thermally stable structures (i.e. drain holes) within roosting sites where temperature is favorable. *Myotis macropus* were recorded roosting where ambient temperature ranging between $12.9 - 6.7^{\circ}\text{C}$ (Campbell 2009), relatively cooler compared with the setting of this study. *Myotis macropus* may tolerate a relatively wide range of temperature. Since the temperature differences between *M. macropus* roosts and all tunnel ambients were small ($< 1.5^{\circ}\text{C}$) in both seasons, roosts selection may be more dependent on thermal stability within the roost. The mean T_{fliuc} of all *M. macropus* roosts were at least 0.8°C less than all tunnel ambients and were lower when compared with *M. australis* and *R. megaphyllus* in both seasons. Gorecki et al. (2020) indicated that *M. macropus* roosting in culverts can maintain thermal stability for breeding and development. This result is similar to other *M. macropus* breeding site such as in a storm water tunnel in Brisbane (Dwyer 1970) and aqueduct tunnels near Melbourne (Campbell 2009), where well-insulated structures offered better thermal stability for bats (Sedgeley 2001; Otto et al. 2016). Meanwhile, other *Myotis* species such as *M. sodalis* (Brack 2007; Boyles et al. 2008), *M. bechsteinii*, *M. nattereri*

(Otto et al. 2016) and *M. formosus* (Kim et al. 2009) have also been reported roosting in more thermally stable roosts.

All *M. australis* roosts were less than 1.2°C warmer than tunnel ambients in both seasons, and the roost in Z2 showed the highest mean T_{avg} . It was likely that *M. australis* prefer to select roosting sites which offer relatively stable diurnal temperature and roost at the warmest location within the tunnel. Bats themselves could be the main contributing factor influencing to the microclimate near their roosts. Codd et al. (2003) indicated that warmer roost temperature indicated higher bat activity, and higher metabolic activity of roosting individuals might contribute to warmer roost temperature. Meanwhile, physical properties of the roost such as the thickness of the wall, composition of wall materials, space and shape of the roost, orientation of entrances, number of entrances, height, width and length, and amount of incident light could all be factors influencing roost temperature and stability (Dwyer 1965; Sedgeley 2001; Ho & Lee 2003; Leivers et al. 2019). The temperature of *M. australis* roosts were likely influenced by tunnel ambients as they showed similar pattern with higher temperature in Z2 than Z1 and Z3. However, the roost temperature could also be influenced by height. The *M. australis* roost in Z2 (4.2 m above ground) is higher than the other two roosts in Z3 (3.5 m and 3.4 m above ground). As heat rises and is trapped at the ceiling of the tunnel, this might cause roost in Z2 to be warmer than the two roosts in Z3. Since the majority of the *M. australis* colony is concentrated at roost in Z2, it is likely that the number of bats and their position high on the tunnel wall contribute to the higher roost temperature.

The mean T_{fluc} differences between *M. australis* roosts and all tunnel ambients were small ($< 0.8^{\circ}\text{C}$) in both seasons. Studies on *M. orianae* recorded similar results for maternity colonies that maintained relatively stable temperatures, with daily variation of around 2 – 4.5°C across seasons (Baudinette et al. 1994; Hall 1982; Dwyer & Harris 1972). Thermal stability of tunnel ambient in Z1 is less than Z2 in winter, likely due to it being closer to the entrance and so more exposed to EXA. *Miniopterus australis* roosting in Z2 and Z3 during summer might be due to better thermal stability. The amount of incident light during the day may also be a contributing factor in *M. australis* not roosting in Z1. Since *M. australis* form clusters on tunnel wall

surfaces, they could be more easily disturbed by solar heat from incident light which likely result in a high daily fluctuation if roosting near the entrance.

For *R. megaphyllus*, the daily diurnal average roost temperatures showed little differences ($< 0.6^{\circ}\text{C}$) to their T_{Alocal} during summer and winter. The biggest roost on the ceiling above the rock pile was warmer than tunnel ambients in both seasons. It was likely that *R. megaphyllus* prefer roosting at relatively high temperature and thermally stable location in the Yugar tunnel. Studies on *R. megaphyllus* have found colonies roosting at temperatures ranging between $12.5 - 35.0^{\circ}\text{C}$ (Dwyer 1963; Upton 2011; Young 2001; Murphy 2014; Slade & Law 2007). *Rhinolophus megaphyllus* colonies often contain relatively high roost temperatures within roosting site (Murphy 2014), and recorded roosting temperature reached $32.0 - 35.0^{\circ}\text{C}$ towards, the upper limit of thermal tolerance of many bat species (Upton 2011). Although the T_{day} difference between *R. megaphyllus* roosts and tunnel ambients may not be significant, it is notable that the largest roost (Z3.1) was warmer than tunnel ambients in both seasons. Since the number of individuals was small during both seasons and they did not huddle together when roosting, it is unlikely that the bats induced a warmer roost temperature. Therefore, the warmer roost temperature was likely influenced by the physical properties of the roost such as height and airflow.

For T_{fluc} , there was less than 1.4°C differences between *R. megaphyllus* roosts and tunnel ambients in both seasons. Roost temperatures generally fluctuated with the tunnel ambient in Z3 but was relatively more stable than Z1 and Z2. Studies on *R. megaphyllus* reported that maternity colonies usually contain warm and stable temperature varying between $2.0 - 3.0^{\circ}\text{C}$ of fluctuation within roosts which aids thermoregulation and development (Young 2001; Dwyer 1963; Slade & Law 2007; Upton 2011). Dwyer (1966) indicated that many roost sites had small and restricted entrances which helped to maintain thermal stability. However, this species was also found roosting in relatively exposed caves as the arrangement of the roost entrance allows air flows into the chamber with greater effect on roost temperature influenced by ambient temperature (Murphy 2014). Park et al. (2000) reported similar results for *R. ferrumequinum*, which selected roosting locations where temperature

fluctuates with EXA, allowing arousal synchronization with sunset to increase foraging opportunities. This study's results on *R. megaphyllus* agree with some studies on *Rhinolophus* species showing they prefer thermally stable roosts.

Relative humidity

External vs tunnel ambient

In general, bats in the Yugar tunnel experienced less RH_{fluc} and a higher RH_{day} than EXA. Relative humidity increased deeper into the tunnel (towards the rock pile) (i.e. $Z3 > Z2 > Z1$) during both seasons. In addition, there was an inverse relationship between diurnal temperature and humidity from external ambient readings, and the relationship became closer when deeper into the tunnel. The RH_{day} of tunnel ambients were higher than EXA in both seasons. The RH_{day} of all tunnel ambients and EXA fluctuated both daily and seasonally. The mean RH_{avg} of each tunnel ambient and EXA in summer was higher and varied less than in winter. Rainy days had caused the relative humidity to remain high inside the tunnel (over 80%) throughout sampling period in summer. Meanwhile, the mean RH_{fluc} of all tunnel ambients were smaller than EXA in both seasons; the differences between tunnel ambients and EXA were greater in summer than winter. Caves and anthropogenic structures including culverts, underground sites and mines occupied by bats often provide high and stable humidity regimes (Leivers et al. 2019; Gorecki et al. 2020; Mitchell-Jones et al. 2008; Hall 1982; Perry 2013).

Bats roosting relative humidity

Bats showed small differences in RH_{avg} , RH_{max} , RH_{min} and RH_{fluc} between roosts and tunnel ambients in summer; much greater differences were found in winter. Roosts were more humid than tunnel ambients in both seasons. The difference of each variable was significant between summer and winter except for RH_{fluc} . The RH_{day} of roosts remained relatively high in both seasons ($> 85\%$ in summer, $> 50\%$ in winter) when compared with tunnel ambients. Bats entering daily torpor often involves water conservation as their small body sizes can easily suffer from rapid evaporative water loss in low humidity and extreme temperature (Solick & Barclay 2007; Perry

2013; Nowack et al. 2017; Marom et al. 2006). Therefore, high humidity is often a contributing factor in roost selection by tropical bats (Perry 2013; de Boer et al. 2013; Usman 1988; Bu et al. 2015). Bat distributions in the tunnel does not reflect such pattern as the majority were located in Z2 rather than Z3, where the highest humidity occurred. In general, bats preferred to select roosting locations with higher and more stable RH_{day} in the Yugar tunnel. However, like roosting temperature, roosting humidity is also species dependent.

Myotis macropus prefer selecting roost locations with high and stable relative humidity in the Yugar tunnel. *Myotis macropus* roosts reached complete saturation with 100% relative humidity throughout the entire sampling period during both seasons. *Myotis macropus* roosted in drain holes and so tunnel ambient conditions will have little effect. Since the size of the drain holes is small, this limited airflow into the roost and provided insulation from the tunnel ambients. Diurnal relative roosting humidity did not fluctuate both daily or seasonally. Many *Myotis* species are sensitive to body dehydration during torpor; evaporative water loss has significant influence on bats physiological condition (Willis et al. 2011; Neubaum 2018). Therefore, high roost humidity is important for bats to reduce energetic cost associated with lowering the risk of dehydration. Studies on other *Myotis* species such as *M. daubentonii*, *M. brandtii* (Siivonen & Wermundsen 2008), *M. sodalis* (Brack 2007) and *M. lucifugus* (Neubaum 2018) reported similar results of having high humidity (> 70%) in roosts.

Miniopterus australis appears to prefer roost locations with high and stable relative humidity in the tunnel. For *M. australis*, RH_{avg} and RH_{fluc} showed small differences (< 6%) between roosts and tunnel ambients in both seasons. *Miniopterus australis* roosts were less buffered in RH_{fluc} from the tunnel ambients compared with *M. macropus*. Several studies on *M. oriana* also highlighted the high humidity in maternity colonies around Australia (Baudinette et al. 1994; Baudinette et al. 2000) with some sites maintained near 100% humidity during summer (Dwyer 1963; Codd et al. 2003; Hall 1982). Baudinette et al. (2000) noted that *M. oriana* is characterized by a high metabolic rate making it prone to evaporative water loss. A high humidity to achieve water balance of roost site is significant for balancing bats' body temperature efficiently (Baudinette et al. 1994; Papadatou et al. 2008). The seasonal

difference in relative humidity may influence the presence of *M. australis* in the tunnel. The lower humidity in winter may have caused them to move to another roosting site to assist with daily torpor.

Rhinolophus megaphyllus appears to select roost locations with higher humidity in the tunnel. The mean RH_{avg} and RH_{fluc} between *R. megaphyllus* roosts and tunnel ambients showed very small differences ($< 5\%$) in summer, while larger differences ($< 20\%$) were found in winter. *Rhinolophus megaphyllus* roosts showed higher daily diurnal relative humidity and humidity fluctuation than tunnel ambients in both seasons (stronger effect in winter). Hall et al. (1975) noted *R. megaphyllus* preferred roosting in warm and humid mines and caves. Roosting sites of *R. megaphyllus* often exhibit high humidity (Slade & Law 2007; Murphy 2014) with some sites exceeding 90% (Hall et al. 1975; n.d.), Upton (2011) similar to other *Rhinolophus* species (Winter & Marntilla 2020; Pandurska & Beshkov 1998; Churchill 1991; Morni et al. 2018). The distribution of *R. megaphyllus* in the tunnel during both seasons agrees with the strong preference for humid roost; they roosted only in Z3 which had the highest humidity in the tunnel. However, roost humidity in winter (ranging between 62% - 84%) is noticeably lower than past studies. The increased number of individuals roosting in the tunnel from summer to winter indicated that *R. megaphyllus* can tolerate a lower diurnal humidity and a greater humidity fluctuation within a roost. High humidity under cold condition increases bats' body heat loss. Since bats in the tunnel do not undergo hibernation and are active throughout a year, maintaining warm temperature may be of greater importance than high humidity within roost during winter.

Summary

The aim of this study was to determine how the microhabitat of the tunnel influence the roosting preference of each bat species inhabiting there. Specifically, it was to: 1) determine the location and size of each roost in the tunnel, and 2) determine the roosting microclimate of each species inhabiting the tunnel. It was found that *M. macropus*, *M. australis* and *R. megaphyllus* roosted in different locations inside the tunnel, and their roosts' population size differed between locations in the tunnel (Z1 vs Z2 vs Z3) and between two seasons (summer vs winter). The roosting microclimate preferences vary across three species in both seasons, but they all exhibit similar preference of roosting in locations with better thermal stability. The differences in roosting microclimate across three species was likely due to a variety of physical and environmental factors such as the composition of wall materials, roost location, height, width, length and number of bats within roosts.

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Chapter 4: Understanding the utilisation of the surrounding environment by bats in the tunnel

4.1 Introduction

The foraging habitats and use of landscape elements by bats varies among species, and also strongly influences their choice of roosting location (Webala et al. 2010; Ciechanowski 2015). Insectivorous bats are highly mobile opportunistic feeders, and their ability to fly enables them to access a variety of resources at a landscape scale (Rainho et al. 2011). For example, *Rhinonictoris aurantia* (Cramer et al. 2016), *Tadarida teniotis* and *Rhinolophus mehelyi* (Rainho et al. 2011) can travel more than 20 km away from their roosts for foraging, while *Austronomus australis* (Rhodes & Catterall 2008) and *Rhinolophus megaphyllus* (Pavey 1998) are often found foraging only a few kilometers away from their roost.

Environmental factors such as insect diversity, vegetation type and structure, water availability, along with morphological features such as wing morphology and echolocation call characteristics also affect the how insectivorous bats exploit their environment. Vegetation type and structure (e.g. open, edge, clutter) have been suggested to influence bat species diversity and abundance (Medellin et al. 2000; Heer et al. 2015; Ciechanowski 2015); environmental heterogeneity impacts the diversity and abundance of feeding resource within habitats and consequently bat diversity (Fahr & Kalko 2011; Heer et al. 2015; dos Santos et al. 2016). Among three habitat types, edge; open and cluttered, edge habitats are expected to have a higher bat diversity. Edge habitats are transitional zones which connect adjacent habitats and offer a higher diversity of food resources and aid bats' navigation across landscapes through the presence of linear elements (i.e. waterways, vegetation edges) (Jantzen & Fenton 2013; Carlier et al. 2019; Kalda et al. 2015; Jensen et al. 2005; Gruebler et al. 2008). Cluttered habitats contain vegetations with a relatively more closed canopy which provide potential roosting sites (Ciechanowski 2015;

Callahan et al. 1997; Sedgely 2003) and favorable foraging condition for clutter adapted species (Wegiel et al. 2019; Patriquin & Barclay 2003). Open habitats such as arable lands, grasslands and above forest canopies are the least attractive to many bat species due to the lack of protection from predators and wind (Ciechanowski 2015; Heim et al. 2015); open-space adapted species are capable of fast and efficient flight across long distances (Peixoto et al. 2018; Heim et al. 2015). Menzel et al. (2005) suggested that insect densities should be higher in cluttered versus open habitats and consequently have higher bat activity. Seasonal variation in climate conditions may also impact resource availability and bat abundance and diversity, and this effect may be habitat specific (Klingbeil & Willig 2010; Martinez-Ferreira et al. 2020; Peixoto et al. 2018; Stevens 2013). For example, in winter bats may hibernate or migrate to overcome a shortage of available resources, and some bats may use prolonged torpor or return earlier from foraging trips to minimise energy loss when food availability is limited, thus reduce overall bat activity (Stawski et al. 2009; Klug-Baerwald et al. 2016; Griffiths 2007; Meyer et al. 2016).

It is important to understand the interaction between bats and habitats within fragmented landscape, and the use of the surrounding environment near their roost. Within the peri-urban landscapes of the Samford region to the west of Brisbane, unmodified environments have been gradually replaced by suburban and managed agricultural areas (Moreton Bay Regional Council 2021). The gradual increase in agricultural expansion, urbanization and land use change may have negative impacts on habitat heterogeneity and biodiversity in the Samford region (Nolte 2011). Bats play an important role in ecosystem services by contributing to ecological services such as seed dispersal, arthropod suppression and pollination (Kunz et al. 2011; Margareta et al. 2008; Kasso & Balakrishnan 2013). Bats exhibit high species diversity in different foraging assemblages associated with their functional guilds and show diverse mechanism for resource partitioning (Kasso & Balakrishnan 2013; Emrich et al. 2014). The space, environment and the response to vegetation structures play important roles in shaping bat communities within habitats (dos Santos et al. 2016). The ability of different species to use habitats surrounding their roosts either for

foraging or as a movement corridor is reflected in their persistence across fragmented landscapes (Heer et al. 2015).

This aim of this chapter was to determine bat diversity and how each of the species inhabiting the tunnel utilize the surrounding landscapes of the Yugar tunnel in summer and winter using acoustic recorders. By conducting acoustic monitoring surveys we will achieve the following objectives: 1) to determine bat species composition in the surrounding landscapes, 2) to determine bats diversity among habitat and vegetation types, 3) to determine the use of vegetation and habitat types in the surrounding landscapes by the bat species inhabiting in the tunnel.

4.2 Method and analysis

Acoustic surveys were conducted in three vegetation types within a 3 km radius of the tunnel (Figure 4-1). This distance was selected as it covers the foraging range of the three species found in the tunnel (Brown & Bernard, 1994; Gonsalves & Law 2017; Pavey & Burwell 2004). The three vegetation types used were remnant open forest of mixed eucalypts (open forest), regrowth open forest of mixed eucalypts (regrowth open forest), and endangered notophyll/ microphyll vine forest (riparian forest) (Queensland Government 2019). Each vegetation type was replicated 5 times approximately 1.5 km apart. Within vegetation types, two vegetation structures were sampled: forest edge and forest interior. Edge was on the outside edge of the vegetation, on the boundary of a large open area while interior was within the vegetation and at least 100 m from the edge. Within the riparian habitat, the edges used were along creeks which may or may not have contained water. We distinguish these from other edges by referring to them as “Creek”.

One SM3 acoustic recorder was deployed in each vegetation structure, within each vegetation type, giving a total of 30 SM3 sampling sites (5 replicates of 3 vegetation types, with 2 vegetation structures in each). Because, there was insufficient SM3 recorders available to sample all sites simultaneously, three

deployments were used during summer and five during winter (due to fewer SM3s being available in winter). In summer, two replicates of each vegetation type was sampled in the first two deployment sessions, and one of each vegetation type was sampled in the last deployment session. In winter, one of each vegetation type was sampled within each deployment session. The SM3 units used automated trigger sampling with a sampling rate of 384 kHz with 16-bit precision. Recordings were made from 30 minutes before dusk until 3 hours after dusk for 10 consecutive days. A total of 30 days was sampled in summer (between 12 January – 22 February 2020) and 50 days in winter (between 13 July – 13 September 2020). Recordings were collected from the field and transferred to computer where they were analysed using Raven Pro (Cornell Laboratory of Ornithology, Ithaca NY). While reviewing recordings, calls from bats were classified to species based on Reinhold et al. (2001) key. Calls recorded were further classified as being either search phase or feeding. As with Chapter 2 recordings, some call identifications were reviewed and assessed by bat expert Dr. Roger Coles to improve accuracy. Calls whose identify remained ambiguous were removed from further analyses. Activity was quantified as the number of passes per minute, with a pass defined as a series of calls, separated from another series of calls by at least 1.5 seconds of silence.

A total of 2100 hours of acoustic recordings were collected from the summer and winter surveys. To facilitate analysis of such a large amount of data, we subsampled at a 1:5 ratio by analysing the first 12 minutes of every complete hour, and the first 6 minutes of every incomplete hour, of each sampling day (e.g. for SM3s recording between 18:18 – 21:48 in summer, recordings made between 18:18 – 18:24 and 21:00 – 21:06 were analysed). The number of searching and feeding passes of each bat species from each acoustic recording file was summed within the same minute for analysis. A species accumulation graph was generated to indicate if the amount of sampling effort was sufficient to detect all the species present at all sampled locations. All reached an asymptote.

Species diversity across each habitat type, within each vegetation structure, was calculated using the Shannon-Weaver diversity index. Evenness was calculated using the two formulas:

$$H = \sum[(p_i \times \ln(p_i))]$$

where p_i = proportion of total searching passes of each species, and H = Shannon-Weaver diversity index (E):

$$E = \frac{H}{\ln(S)}$$

where E = evenness and S = species richness. To determine activity of the three tunnel bat species, the number of search phase and feeding passes of all three species were combined separately for each sampling location to conduct statistical analysis.

Shapiro-Wilk tests and scatterplots were used to test the assumptions of linearity of the original data and the log transformed data; both indicated original and transformed data had non-normal distributions. Therefore, two non-parametric approaches were used to carry out the statistical analyses. A three-ways non-parametric ANOVA using a 0.05 rejection level was conducted on the Shannon-Weaver indices and evenness among seasons, habitat and vegetation types. A four-ways non-parametric ANOVA using a 0.05 rejection level was conducted across the 3 tunnel species (*Myotis macropus*, *Miniopterus australis*, and *Rhinolophus megaphyllus*) on their number of search and feeding passes among seasons, habitat and vegetation types to examine their foraging location in the surrounding landscapes. It should be noted that it is not known if the bats recorded actually came from the Yugar tunnel and so the results of this study should be interpreted accordingly.

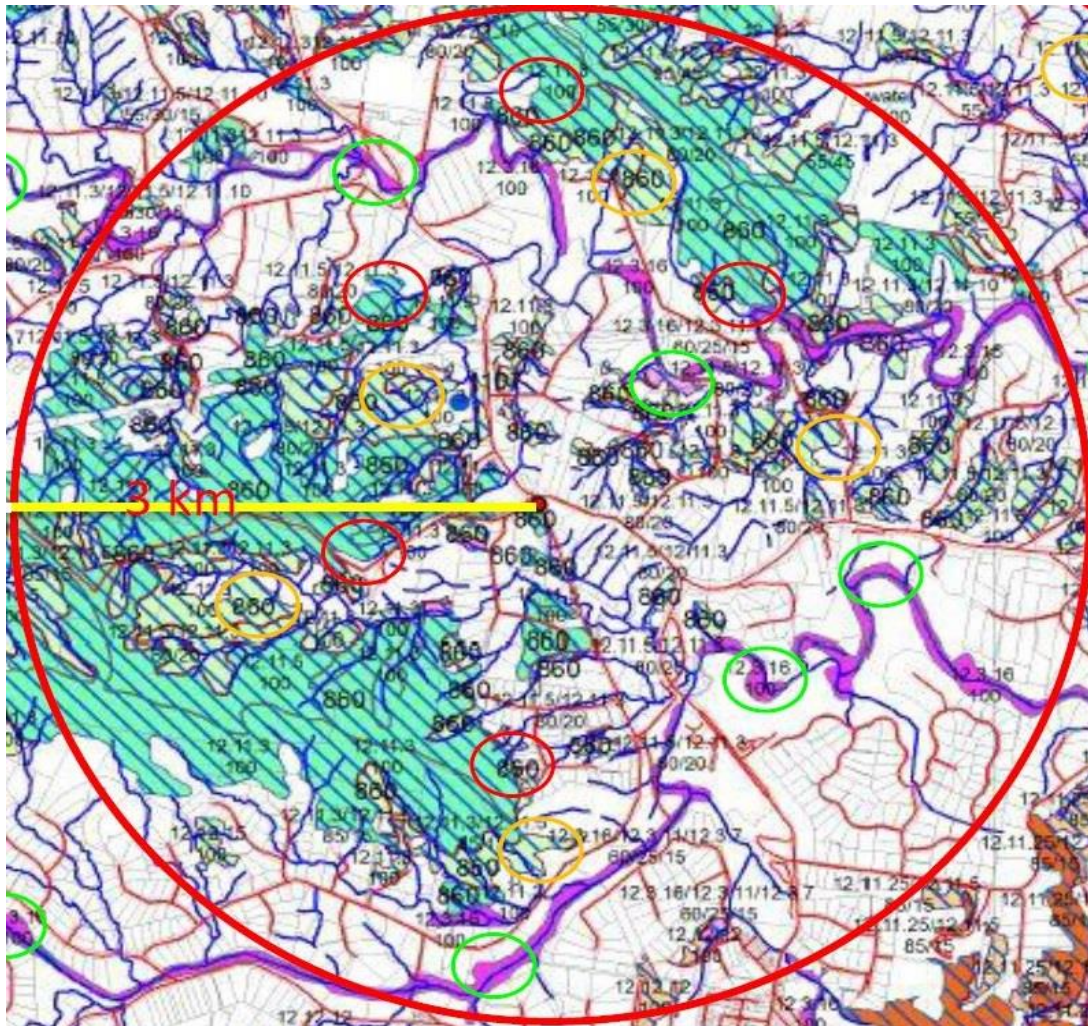


Figure 4-1 SM3 units deployment locations overlaid on the Samford vegetation map provided by Moreton Bay Regional Council. Note: The centre of the red circle represents the location of the Yugar tunnel. (Red circles = Open forest sites, Yellow circles = Regrowth open forest sites, and Green circles = Riparian forest sites). Survey areas were all located within a 3 km of the Yugar tunnel (represented by the yellow line). A total of 5 replicates of each vegetation type were sampled in summer and winter. An SM3 unit was deployed at the edge and interior within each vegetation types. Therefore, a total of 30 sites were sampled within each season.

4.3 Results

Following subsampling, a total of 420 hours of bat acoustic activity were analysed; 140 hours each from riparian, open and regrowth open forest. In terms of habitat type within each vegetation type, 70 hours were analysed in both interior and edge / creek habitats within each forest type.

A total of 22 bat species were detected and identified from acoustic surveys in summer and winter; 21 species were found in summer and 18 in winter (Table 4-1). Total activity in summer (4168 passes) was greater than in winter (1805 passes) (Figures 4-2 & 4-3). Of all species found in summer, the majority of activity was from 4 species: *Ozimops ridei*/*Ozimops petersi* had the highest proportion of activity (37%) followed by *Myotis macropus* (18%), *Scotorepens greyii* (13%), and then *Chalinolobus gouldii* (10%). In winter, the majority of activity was due to *Myotis macropus* (42%), *Vespadelus pumilus* (18%), *Miniopterus australis* (12%), and *Rhinolophus megaphyllus* (8%) (Figure 4-2 & 4-3).

Among the three tunnel bat species in summer, *M. macropus* was the dominant species which consisted approximately 90% of the activity, while *M. australis* (9%) and *R. megaphyllus* (< 1%) activities were much lower. In winter, all three tunnel bat species made major contributions to the bat activity detected (Figures 4-2 & 4-3). The activity of the three tunnel species differed between summer and winter. Activity recorded from *M. macropus* was similar between summer (746 passes) and winter (759 passes); *M. australis* was higher in summer (356 passes) than winter (217 passes); and *R. megaphyllus* was higher in winter (143 passes) than summer (21 passes) (Figures 4-2 & 4-3).

Table 4-1 Total number of species identified across all sites in the surrounding area of the tunnel during summer and winter.

Number	Species	Summer	Winter
1	<i>Austronomus australis</i>		
2	<i>Chalinolobus gouldii</i>		
3	<i>Chalinolobus morio</i>		
4	<i>Chalinolobus nigrogriseus</i>		
5	<i>Falsistrellus tasmaniensis</i>		
6	<i>Micronomus norfolkensis</i>		
7	<i>Miniopterus australis</i>		
8	<i>Miniopterus orianae oceanensis</i>		
9	<i>Myotis macropus</i>		
10	<i>Nyctophilus gouldi/ Nyctophilus bifax</i>		
11	<i>Ozimops lumsdenae</i>		
12	<i>Ozimops ridei/ Ozimops petersi</i>		
13	<i>Rhinolophus megaphyllus</i>		
14	<i>Saccolaimus flaviventris</i>		
15	<i>Scoteanax rueppellii</i>		
16	<i>Scotorepens balstoni</i>		
17	<i>Scotorepens greyii</i>		
18	<i>Vespadelus darlingtoni</i>		
19	<i>Vespadelus pumilus</i>		
20	<i>Vespadelus regulus</i>		
21	<i>Vespadelus troughtoni</i>		

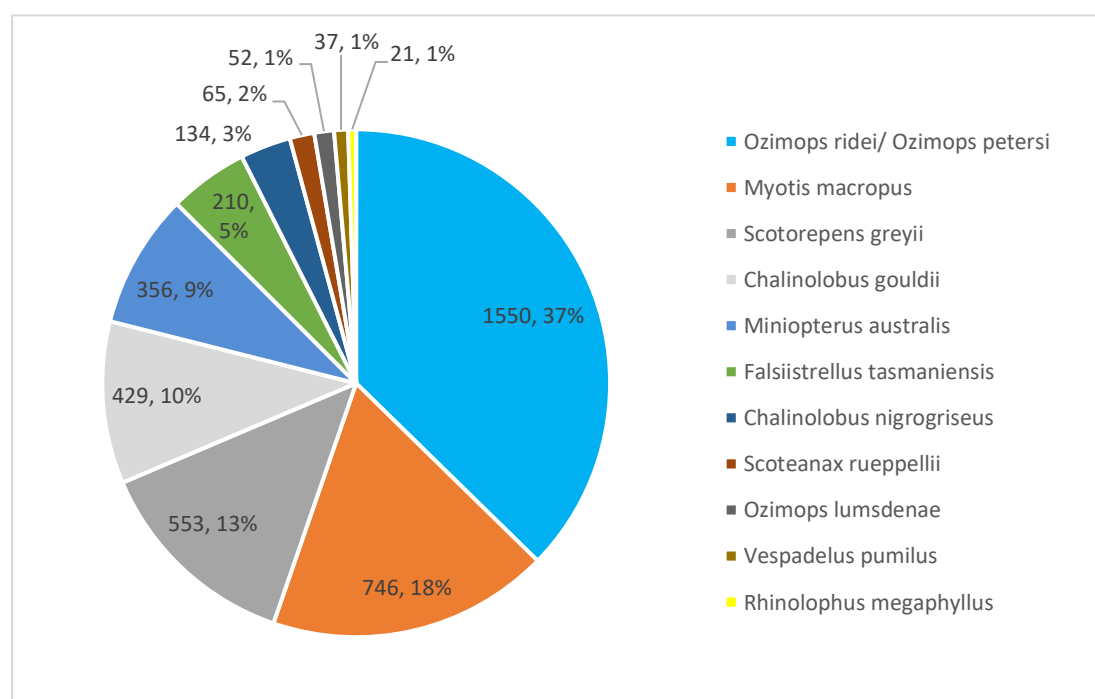


Figure 4-2 Proportion of search-phase echolocation call passes recorded from the 10 most active bat species from all sites in summer. (Numbers within the pie chart indicates the number of search-phase passes and the proportion that each species contributed). Note: the species list legend is sorted in descending order based on the proportion of search-phase echolocation call passes. A total of 4168 bat passes were analysed.

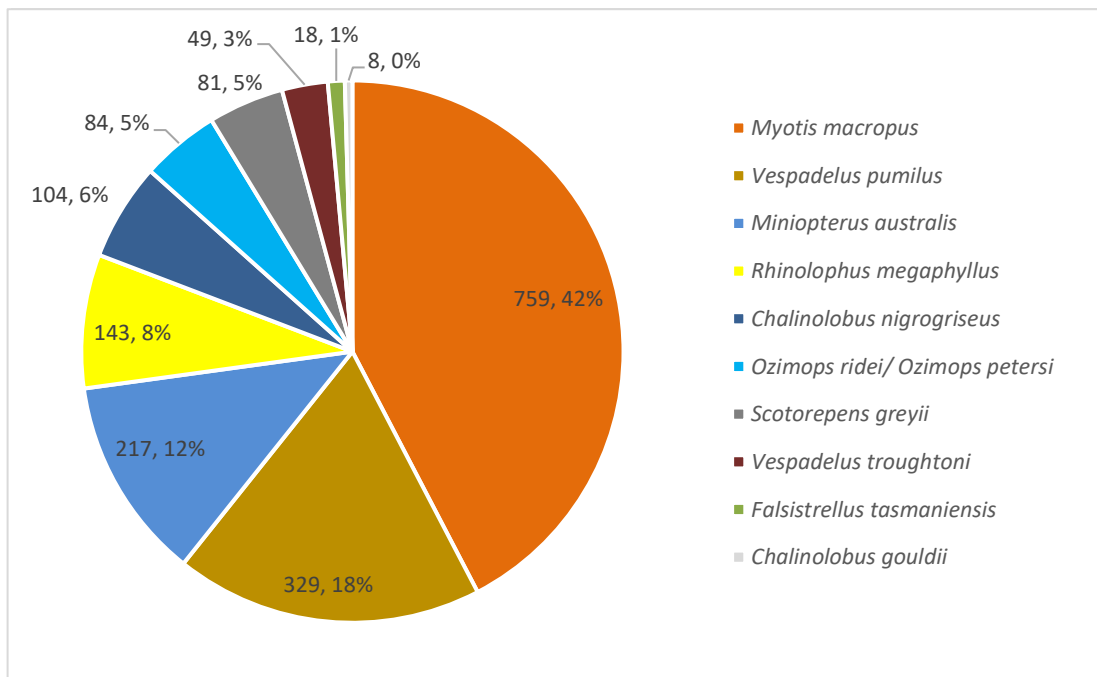


Figure 4-3 Proportion of search-phase echolocation call passes recorded from the 10 most active bat species from all sites in winter. (Numbers within the pie chart indicates the number of search-phase passes and the proportion that each species contributed). Note: the species list legend is sorted in descending order based on the proportion of search-phase echolocation call passes. A total of 1805 bat passes were analysed.

Overall bat species diversity and evenness

Bat diversity and evenness in the environment surrounding the tunnel showed different patterns across vegetation types and structures between summer and winter. There was high variation in diversity within some habitats such as the open forest edge in summer and riparian forest creek in winter; both contained relatively low diversity in the majority of sites but also had the highest species diversity in one site (Figure 4-4). The edge and creek habitat presented higher species diversity than interior in each vegetation types during both seasons, except for open forest in summer (Figure 4-4). In summer, bat species diversity was highest in riparian forest creek; in winter, it was in open forest edge (Figure 4-4). For bat species diversity, there was no significant interaction across vegetation types, habitat, and seasons (3 ways interaction) (Aligned Rank ANOVA, $F = 2.536$, $df = 2$, $p = 0.091$ — Table 4-2). Two-ways interactions were not significant across each factor, and there was no significant difference within each factor (Aligned Rank ANOVA, $p > 0.05$ – Table 4-2). For bat species evenness, there was no significant interaction across habitat types, vegetation types and seasons (3-way interaction) (Aligned Rank ANOVA, $F = 1.624$, df

= 2, $p = 0.209$ — Table 4-2). The two ways interactions were also not significant, and only seasons showed significant difference in evenness with winter showed greater evenness than summer (Table 4-2). Both edge and interior of three vegetation types showed higher species evenness score in winter than summer, except for riparian forest creek (Figure 4-5). In summer, bat species was more even in regrowth open forest edge; in winter, it was open forest edge (Figure 4-5).

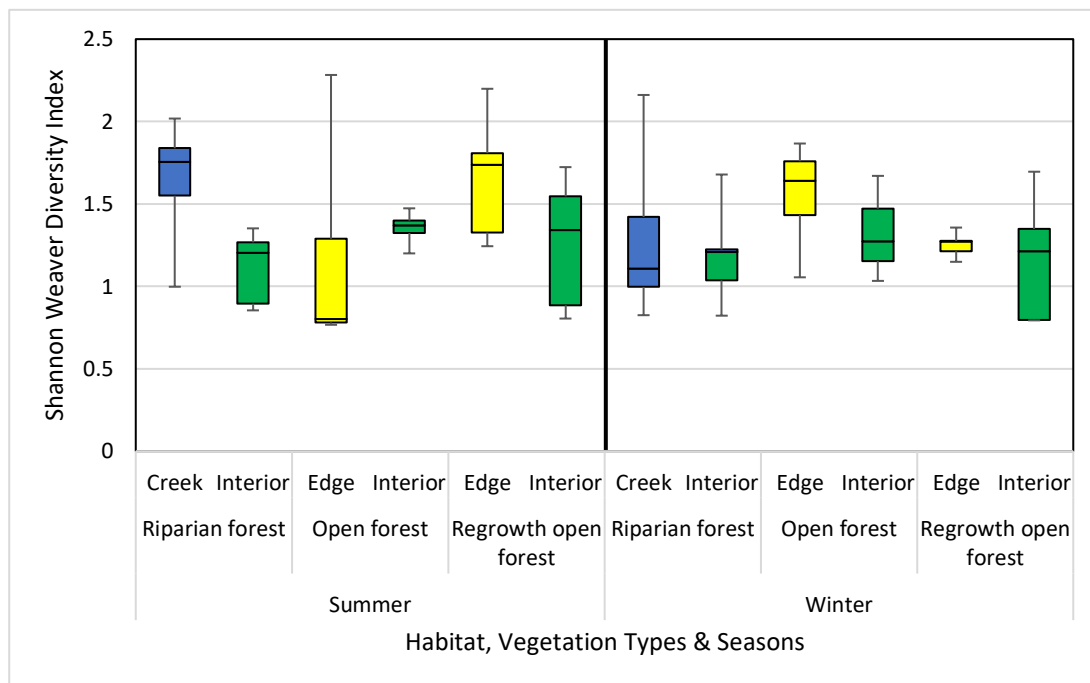


Figure 4-4 Bat species diversity in interior and edge / creek across three vegetation types (Riparian, Open, Regrowth) in summer and winter. (The solid vertical line separates summer and winter). Note: Creek in the Riparian forest was counted as edge. In summer, regrowth open forest edge has the highest species diversity across all habitats within each vegetation; in winter, species diversity was highest in open forest edge.

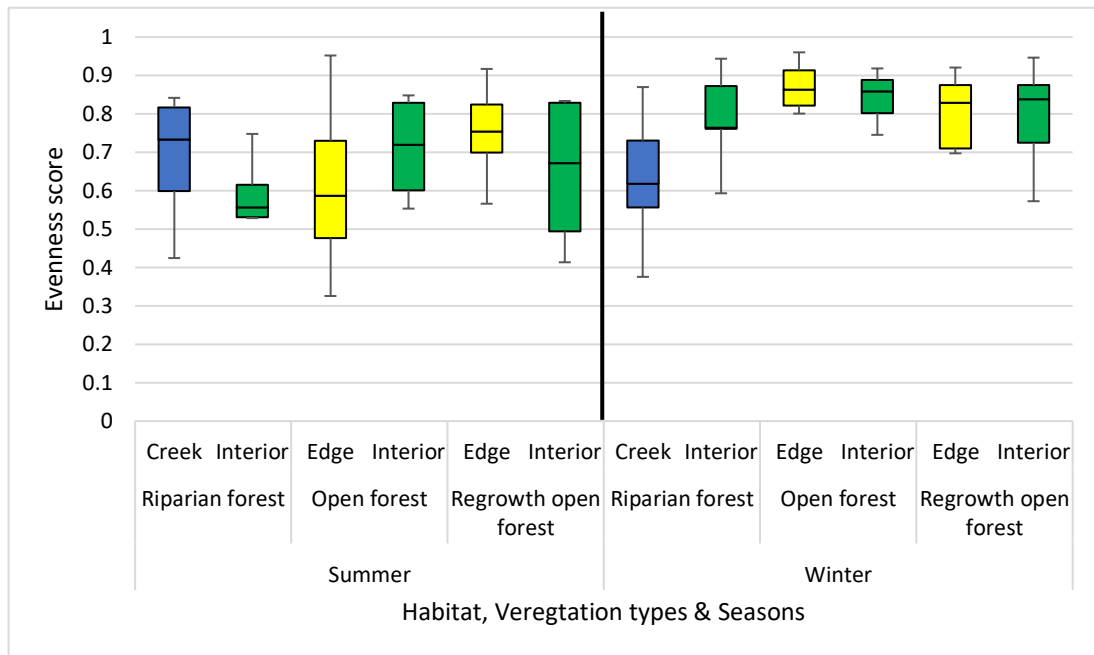


Figure 4-5 Bat species evenness in interior and edge / creek across three vegetation types (Riparian, Open, Regrowth) in summer and winter. (The solid vertical line separates summer and winter). Noted: Creek in the Riparian forest was counted as edge. Species evenness across all habitats within each vegetation is higher in winter than in summer. In summer, regrowth open forest edge had the highest species diversity across all habitats within each vegetation. In winter, species evenness was highest in open forest edge.

Table 4-2 Aligned Rank ANOVA results comparing bat species diversity and evenness scores across two vegetation structures within three vegetation types during summer and winter. This statistical test analysed interactions across 3 factors including season, vegetation and habitat types.

Contrast	Df	Df.res	Diversity		Evenness	
			F value	P-value	F value	P-value
Season	1	43	0.82	0.36	7.78	0.0078*
Vegetation	2	43	0.063	0.94	1.31	0.28
Habitat	1	43	3.25	0.079	0.00011	0.99
Season: vegetation	2	43	2.28	0.11	0.81	0.45
Season: habitat	1	43	0.23	0.64	0.94	0.34
Vegetation: habitat	2	43	1.07	0.35	0.43	0.65
Season: vegetation: habitat	2	43	2.54	0.091	1.62	0.21

Relative activity of the three tunnel species in the surrounding environment

All three bat species showed contrasting patterns in search-phase calling activity between habitat and vegetation types. In both summer and winter, all three bat species showed highest activity in riparian forest and edge habitat (Figures 4-6 & 4-8). *Myotis macropus* searching activity was significantly greater than *M. australis* and *R. megaphyllus* in riparian forest edge in both seasons (Figures 4-6 & 4-8). The searching activity of all three species were relatively low within open forest and regrowth open forest in both seasons, with fewer than 50 passes detected. Activity recorded from *M. australis* and *R. megaphyllus* were similarly low within each vegetation type. Contrastingly, *M. macropus* activity was significantly different between vegetation and habitat types, with nearly all searching activity concentrated in riparian forest and only a few passes detected in the other two vegetation types. Finally, the majority of activity was concentrated in edge habitat in both seasons with the exception of riparian forest interior by *M. macropus*, which showed greater activity in summer (Figures 4-6 & 4-8). Searching and feeding calls from the three tunnel bat species showed significant interactions across species, seasons, habitat and vegetation types (4 ways interaction) (Aligned Rank ANOVA, $F = 18.05$, $df = 4$, $p = 4.89e-12$ – searching activity; $F = 58.47$, $df = 4$, $p = 2.22e-16$ – feeding activity) (Table 4-3).

Myotis macropus had the highest number of feeding buzzes recorded, followed by *M. australis*. No feeding buzzes were detected from *R. megaphyllus* in any site in both seasons (Figure 4-7 & 4-9). In summer, *M. macropus* feeding activity occurred in both edge and interior but only within riparian forest; edge / creek showed a significantly higher number of passes than interior. In winter, feeding activity only occurred in riparian forest edge / creek. Meanwhile, *M. macropus* feeding activity in riparian forest edge / creek was higher in winter than summer. For *M. australis*, feeding activity was detected in each vegetation type and structure during both seasons, except in open forest during summer (Figure 4-7). The amount of *M. australis* feeding activity detected was relatively small, with fewer than 10 feeding buzzes detected in each habitat and vegetation type during both seasons (Figures 4-7 & 4-9)

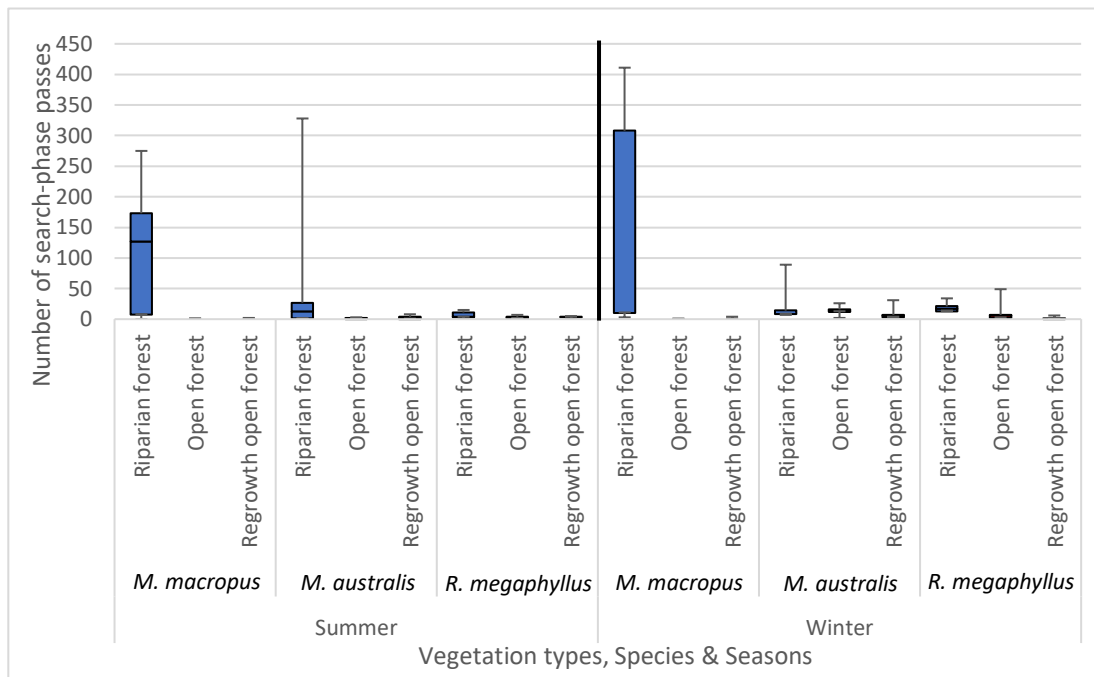


Figure 4-7 Search-phase echolocation call activity (passes) of the three tunnel bat species across each vegetation types in summer and winter (the solid vertical line separates summer and winter). All three bat species showed the highest relative search-phase activity in riparian forest in summer and winter. The amount of search-phase activity from each species in open and regrowth open forest was low in both seasons; it was slightly higher in winter than summer. *Myotis macropus* had the highest number of searching passes in both seasons.

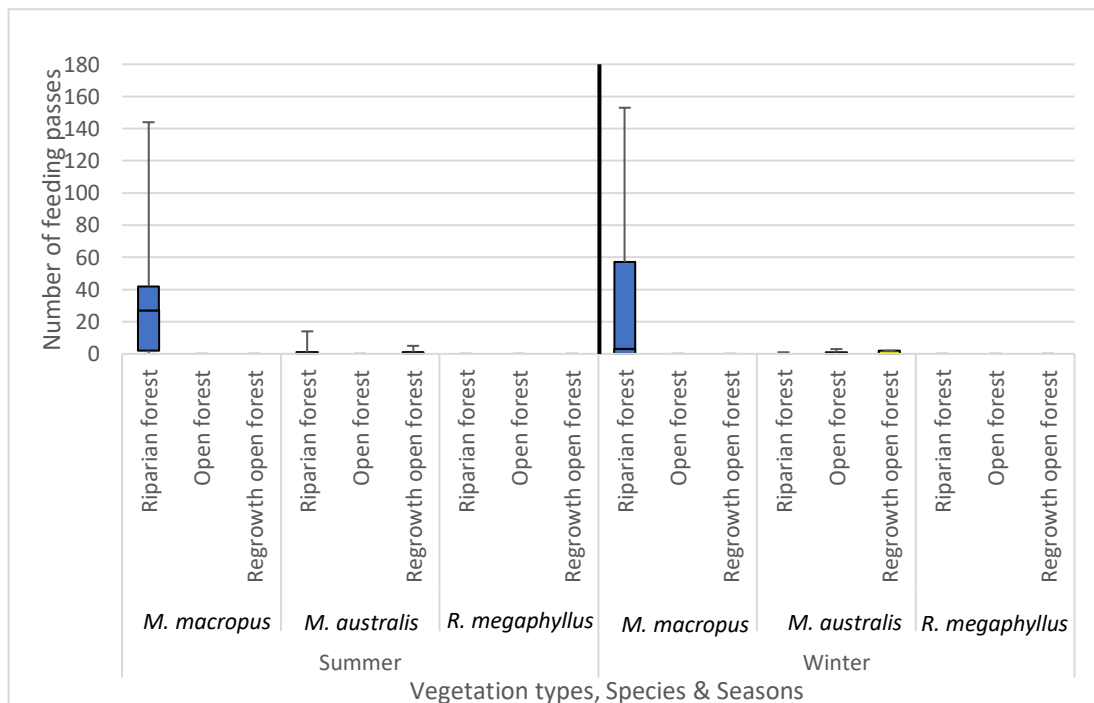


Figure 4-6 Feeding-phase echolocation call activity (passes) of the three tunnel bat species across each vegetation types in summer and winter (the solid vertical line separates between summer and winter). Feed-phase activity was highest in *M. macropus*, followed by *M. australis* and then *R. megaphyllus* in both seasons. *Myotis macropus* had the highest feeding-phase activity in riparian forest in summer and winter; *M. australis* had the highest feed-phase activity in riparian forest in summer, and regrowth open forest in winter; *R. megaphyllus* had no feeding-phase activity in both seasons.

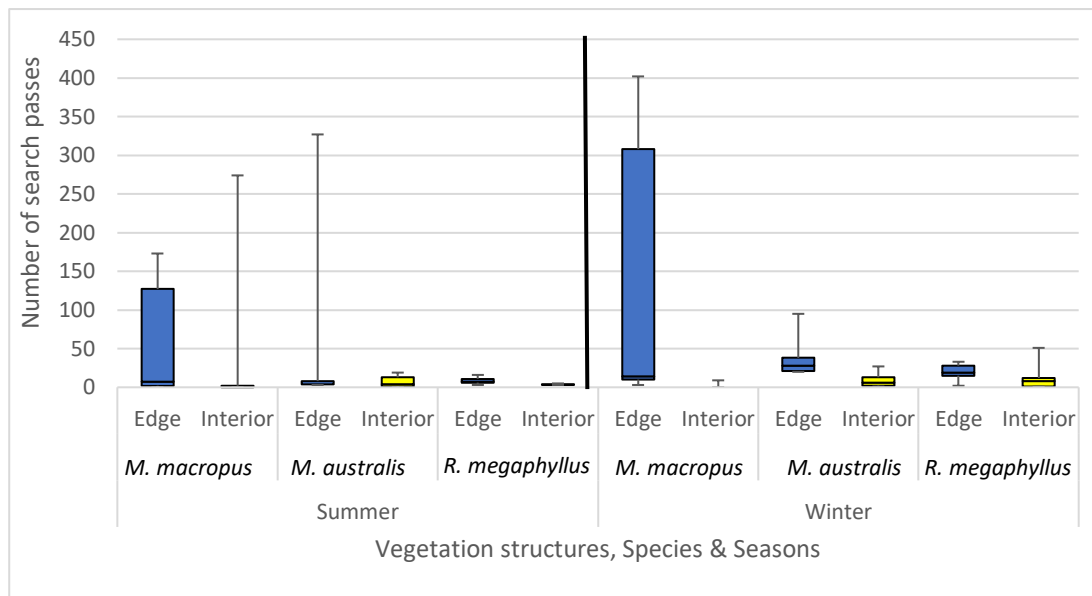


Figure 4-8 Search-phase echolocation call activity (passes) of the three tunnel bat species in two vegetation structures in summer and winter (the solid vertical line separates summer and winter). All three species showed higher search-phase activity in edge / creek versus interior in both seasons, except for *M. australis* in summer. *M. macropus* has the largest difference between edge and interior compared with the other two species. Relatively few passes were detected from *M. australis* and *R. megaphyllus* in both seasons.

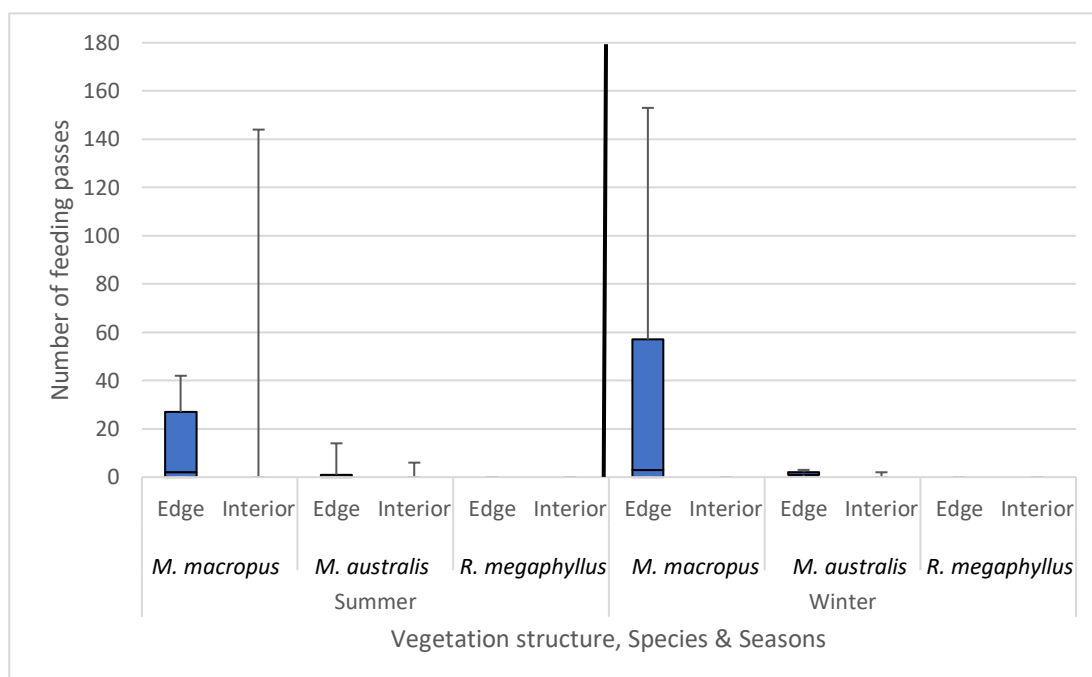


Figure 4-9 Feeding-phase echolocation call activity (passes) of the three tunnel bat species within each vegetation structure in summer and winter (the solid vertical line separates between summer and winter). *Myotis macropus* and *M. australis* have higher feeding-phase activity in edge than interior in both seasons. There was no *R. megaphyllus* feeding-phase activity detected in either structure in summer or winter.

Table 4-3 Aligned Rank ANOVA results comparing searching and feeding activity (number of passes) of the three tunnel bat species across each vegetation type and structure during summer and winter. This statistical test analysed interactions across 4 factors including species, season, vegetation and habitat types.

Contrast	Df	Df.res	Searching		Feeding	
			F value	P-value	F value	P-value
Species	2	144	48.03	< 2.22e-16**	91.50	< 2.22e-16**
Season	1	144	8.02	0.005*	39.95	3.07e-09**
Vegetation	2	144	32.57	2.13e-12**	87.49	< 2.22e-16**
Habitat	1	144	103.94	< 2.22e-16**	176.90	< 2.22e-16**
Species: season	2	144	23.27	1.75e-09**	37.12	9.99e-14**
Species: vegetation	4	144	28.33	< 2.22e-16**	50.19	< 2.22e-16**
Season: vegetation	2	144	3.50	0.032*	24.28	8.19e-10**
Species: habitat	2	144	50.48	< 2.22e-16**	124.94	< 2.22e-16**
Season: habitat	1	144	50.42	5.24e-11**	221.33	< 2.22e-16**
Vegetation: habitat	2	144	75.47	< 2.22e-16**	120.94	< 2.22e-16**
Species: season: vegetation	4	144	17.91	5.89e-12**	22.35	2.26e-14**
Species: season: habitat	2	144	48.20	< 2.22e-16**	137.71	< 2.22e-16**
Species: vegetation: habitat	4	144	28.44	< 2.22e-16**	52.36	< 2.22e-16**
Season: vegetation: habitat	2	144	31.20	5.55e-12**	120.79	< 2.22e-16**
Species: season: vegetation: habitat	4	144	18.05	4.89e-12**	58.47	< 2.22e-16**

4.4 Discussion

This study sub-sampled the first 3.5 hours after official sunset. Therefore, it is possible it may be possible that all species present were not sampled. The surveys also do not show the location of bats from the Yugar tunnel. However, the vegetation and habitat types where these three tunnel bat species may likely occur in the surrounding landscape can be identified.

Bat species in the surrounding environment

The results of this study have shown that a total of 21 bat species were identified in the environment surrounding the Yugar tunnel in summer and winter; 21 species were identified in summer and 18 in winter. There were no significant interactions between bat species diversity across habitats, vegetation types and seasons (3 way and 2 ways interactions), and no significant difference between groups within each factor. Species evenness showed similar results, with no significant interactions across habitats, vegetation types and seasons (3 ways and 2 ways interactions). The only significant difference occurred within the seasonal factor in which species evenness in winter was higher than in summer. The number of bat echolocation call passes detected in summer was much greater than in winter, and it was dominated by different species each season. In summer, activity was dominated by *Ozimops ridei*/ *Ozimops petersi*, followed by *Myotis macropus* and then *Scotorepens greyii*. In winter, *Myotis macropus* was the most active, followed by *Vespadelus pumilus* and *Miniopterus australis*.

Bat diversity and evenness are influenced by multiple factors including habitat and vegetation structures (Adams et al. 2009; Starik et al. 2018; Hourigan et al. 2010), season (Gonsalves & Law 2018), resource availability (Martínez-Ferreira et al. 2020), eco-morphology and foraging plasticity (Webala et al. 2011; Bullen & McKenzie 2001). For example, bat species with low aspect ratio such as *Nyctophilus gouldi*, *Vespadelus pumilus* and *Vespadelus vulturnus* are more maneuverable than species with high aspect ratio such as *Falsistrellus tasmaniensis*, *Saccolaimus flaviventris* and *Austronomus australis* (Norberg & Rayner 1987; Rhodes 2002; Law & Chidel 2001;

Glass 1993). These latter three species exclusively forage in open habitats due to their flight speed and maneuverability are not suited to cluttered habitats (Jung & Threlfall 2018; Senawi & Kingston 2019; Mayberry et al. 2020; Mendes et al. 2017). These patterns are clearly evident in this study, with bat species with high aspect ratio such as *Saccolaimus flaviventris*, *Austronomus australis* and *Ozimops* species occurring in open or regrowth open forest; bat species with relatively lower aspect ratio than these three species such as *Chalinolobus gouldii* and *Nyctophilus gouldi*/*Nyctophilus bifax* occurred in riparian forest.

The results of this study indicated that bat diversity varied between sites across habitat and vegetation types during summer and winter, with the highest diversity in riparian creek during summer, and open forest edge during winter. Bat diversity of this study was likely driven by the seasonal effect on the vegetation and habitat structures, which changed the species foraging distribution across the landscape. In terms of vegetation, a high density of water resources, maintenance of mature plantation and understorey clutter can provide favorable conditions for some bats (Vasko et al. 2020; Cruz et al. 2016). Some studies highlighted that riparian reserves are of great importance for bats, as they promote better connectivity across larger vegetation patches (de la Peña-Cuellar et al. 2015), greater insect diversity and abundance (Straka et al. 2020), and host higher bat diversity and abundance (Johnson et al. 2010) than other vegetation types across different landscapes (Webala et al. 2010). Meanwhile mature forests offer better quality of habitat with higher bat species diversity and activity than young regrowth forests (Webala et al. 2010). Müller et al. (2012) noted that the high vegetation density in cluttered environment often provide higher insect diversity and abundance. These partly agree with the result of this study in that bat diversity was highest in riparian forest during summer, and in open forest than regrowth open forest during winter.

In terms of habitat type, bat species diversity was higher in edge / creek than interior across the three vegetation types during summer and winter, except in open forest during summer. This was likely due to the higher insect species richness and interactions, and diversity of resources offered by edge / creek habitat and the use of linear landscape element by bats. Multiple studies have found that forest edge

often contains higher bat species diversity than interior and open habitats across different landscapes (Carlier et al. 2019; Holland & Fahrig 2000; Alignier et al. 2014; von Hirschheydt et al. 2020). Morris et al. (2010) suggested that an increase in predator – prey interactions associated with insect abundance might increase species diversity along vegetation edges. Edges benefit bats by providing habitat for insects (Krauel & LeBuhn 2016; von Hirschheydt et al. 2020), linear elements for flight navigation to foraging grounds (Kalcounis-Rueppell et al. 2013), which may support a greater diversity of more diverse of bat species in this habitat (Jantzen & Fenton 2013).

Although overall bat activity and diversity varied between seasons, the difference for bat diversity was not significant. This was likely due to the mild temperatures during most winter nights when some bats and insects remained active. Many subtropical bat species such as *Nyctophilus bifax*, *Scotophilus dinganii* and *Chalinolobus gouldii* do not enter hibernation and remain active for foraging during mild nights in winter when insects remain active (Stawski et al. 2009; Kelly et al. 2007; Levin et al. 2015; Stawski & Currie 2016). Even some bat species from the temperate zone such as *Rhinolophus ferrumequinum*, *Myotis nattereri* and *Myotis lucifugus* are active and may forage during warm winter nights (Park et al. 1999; Hope et al. 2014; Bernard & Mc Cracken 2017). Although bat activity is highly variable, bats can remain active and forage on the majority of winter nights (Turbill 2008; Geiser & Stawski 2011). Since subtropical bats remained mostly active in tropical and subtropical regions during winter, this might explain why species diversity was not significantly different between two seasons.

Bat diversity was highest in riparian forest during summer but not in winter. This is possibly influenced by seasonal climate and weather conditions. In the dry season (winter), insect prey is often highest near water sources (Amorim et al. 2015). In fact, water availability is an important factor influencing insect prey diversity and abundance (Seibold et al. 2013; Blakey et al. 2017). Limited water availability throughout the landscape may constrain bats foraging close to larger watercourses that offer more reliable access to this important resource (Starbuck et al. 2020; Hagen & Sabo 2012; Amorim et al. 2018). As the number of available watercourses in

riparian forest differed between two seasons, this may have a strong influence on the distribution of bat species in the study area. In this study, the river of several riparian sites was completely dry in winter perhaps leading some bat species to shift to other nearby foraging areas.

In terms of evenness, there was no significant interactions among habitat, vegetation types and seasons. Similarly, there was no significant differences within each factor, except for season. It was likely that the significant difference for species evenness within seasonal factor was driven by species activity rather than richness. Studies noted that evenness can be negatively related to species diversity and abundance across various taxonomic groups (Bock et al. 2007; Su 2018; Estrada-Vellegas et al. 2012; MacDonald et al. 2017); an increase in species richness, biomass and abundance often reduce species evenness (Symonds et al. 2008; Estrada-Vellegas et al. 2012). Results of this study also found an interaction between diversity and evenness with higher species evenness in winter than summer, and sites with lower diversity such as regrowth open forest edge / creek and interior in winter showed higher species evenness. However, species diversity may not be the main factor influencing evenness on our data. Although bat activity was dominated by 4 different species in both seasons, the amount of total bat activity was smaller in winter than summer. Therefore, the proportion of other species' activity is relatively larger and increased species evenness.

The three tunnel bat species activity in the surrounding environment

Search and feeding activity were significantly influenced by the interaction of species by seasons, habitat and vegetation types. Both search and feeding activity of the three tunnel bat species were dominated by *M. macropus*, followed by *M. australis* and then *R. megaphyllus* in summer and winter. In addition, *M. macropus* had the largest difference for both searching and feeding activities across seasons, habitat and vegetation types. Both *M. australis* and *R. megaphyllus* showed much smaller differences across the three factors as the number of search-phase and feeding-phase passes were lower than that of *M. macropus*. Searching and feeding

activity of the three species was higher in riparian forest than open forest and regrowth open forest, and higher in edge / creek than interior during both seasons.

Identifying the bats' functional guilds is key to understanding their use of the surrounding environment across various landscape elements, vegetation structures and habitat types (Denzinger & Schnitzler 2013; Mancini et al. 2019). Morphological features and echolocation call characteristics are good proxies for predicting the pattern of habitat use and foraging behaviour of bats (Marinello & Bernard 2014; Nunez et al. 2019; Roemer et al. 2019; Nkrumah et al. 2016). Among three tunnel bat species, *M. macropus* is a trawling species which has long and pointed wings with low wing loading and high aspect ratio allowing slow and maneuverable flight within clutter and rapid flight in open environments (Jones & Rayner 1991; Norberg & Rayner 1987; Campbell 2011). This species has large feet for trawling the aquatic insects and small fish that make up the majority of its diet (Law & Urquhart 2013; Dwyer 1970), and forages almost exclusively near permanent waterways along riparian zones (Campbell 2009; Gonsalves & Law 2017; Campbell et al. 2009). *Miniopterus australis* is an 'edge' specialist with low wing loading and aspect ratio, and using high frequency echolocation calls to hunt for prey and avoid obstacles within clutter (Rhodes 2002; Hourigan 2011). This species mainly resides in forest edges but is also found in other habitats such as open macadamia farms (Crisol-Martinez et al. 2017), cacao farms and garden habitat (Davies et al. 2016). *Rhinolophus megaphyllus* is a clutter-tolerant species with low wing loading and aspect ratio, with echolocation calls dominated by constant frequency component which helps them to detect flying insects within clutter environment (Law & Chidel 2002; Norberg & Rayner 1987; Pavey 1998).

Contrasting results were found on the searching and feeding activities among the three tunnel roosting species across habitat and vegetation types between summer and winter. The overall searching and feeding activity were dominated by *M. macropus*, while *M. australis* and *R. megaphyllus* activities were at relatively low levels in both seasons. For *M. macropus*, the pattern of habitat and vegetation used by this species followed its functional guild, with both searching and feeding activities concentrated in riparian forest edge / creek, and no feeding activity detected in open

forest and regrowth open forest in either season. This result supports previous studies on *M. macropus* that have shown this species has a highly specialized foraging behaviour and diet (Thompson & Fenton 1982; Law & Urquhart 2013; Anderson et al. 2006; Gonsalves & Law 2017; Law et al. 2020; Jones & Rayner 1991; Campbell 2011). Activity in riparian forest in summer was distributed more evenly across sites; in winter, activity was concentrated in 2 out of 5 sites. Again, this could be due to the seasonal climate and weather conditions. As mentioned above, water availability offers higher insect abundance for bats which may concentrate bats near water to access food and roosting resources. This is likely to have a strong influence on the distribution of *M. macropus* in the study area. In winter, the weather was relatively dry and less rain fell, leading to waterways being completely dry in some riparian forest sites. This might explain why both searching and feeding activity of *M. macropus* were more even in summer than winter. Although the distribution of location searching and feeding activities might differ between seasons, the number of searching and feeding calls were not significantly different. This suggests that seasonal effects might influence water availability and insect abundance within riparian sites rather than foraging activities of *M. macropus*; with fewer options of foraging sites available, this species might experience higher competition on aquatic resources in winter.

For *M. australis*, the number of search-phase and feeding-phase passes were low across all habitats within each vegetation type, in both seasons. In general, the results supported the functional guild of *M. australis* as an 'edge' specialist with searching and feeding activity highest in riparian forest edge / creek in summer and winter. Vegetation in riparian forest sites is more cluttered with higher insect diversity which provides favorable conditions for bats (Law & Chidel 2002). Contrastingly, open forest and regrowth open forest have relatively sparse and scattered trees that create more open space within the forests. Crisol-Martínez et al. (2017) found that *M. australis* had its highest activity in less fragmented forest. Other studies on *Miniopterus schreibersii* also indicate a high level of activity in riparian areas (Rainho et al. 2011; Russo & Jones 2003; Serra-Cobo et al. 2000). Species specialized in edge habitat often avoid foraging in more open space (Walsh & Harris

1996; Müller et al. 2012). However, *M. australis* were also recorded foraging in various habitats including secondary forest, agricultural farms (Davies et al. 2016) and open woodland (Crisol-Martínez et al. 2017), and urban bushland (Hourigan 2011) across modified habitats (Turner et al. 2000). This may suggest that water availability maybe of greater importance for *M. australis* using riparian forest edge / creek. Water availability and insect biomass have a positive influence on bat activity (Straka et al. 2020; Threlfall et al. 2012; Rainho et al. 2011). In addition, small water bodies also increased foraging activity of 'edge' specialized bats (Crisol-Martinez et al. 2017).

Seasonal comparisons across all habitats within each vegetation type indicated that both searching and feeding activity of *M. australis* were not significantly different between summer and winter, except for one riparian forest edge / creek site. Although the overall search and feeding activity was low across each vegetation and habitat type in both seasons, the majority of the search activity was concentrated in one riparian forest edge / creek site. Among all sampled sites, that riparian forest edge / creek site was possibly the main foraging site for *M. australis* in both seasons. The lower level of *M. australis* activity in winter could be associated with colder temperatures reducing insect activity (Stawski et al. 2009; Griffiths 2007). Another important factor may be the absence of *M. australis* in the Samford region. Dwyer (1968) noted that many *M. australis* will return to their breeding site during winter (between August and September) and forming large nursery colonies. There is a possible breeding site near to coastal areas at Nanago (Hall 2015). This migration pattern agrees with the great reduction of *M. australis* population in the Yugar tunnel during winter. It is possible that the reduction in *M. australis* activity in winter might be caused by the near-absence of this species in the study area.

For *R. megaphyllus*, the number of search passes was very low across all habitats within each vegetation type, and no feeding buzzes were detected in either season. This was likely due to the low number of *R. megaphyllus* present and their foraging locations. The results of this study generally support *R. megaphyllus*'s functional guild as 'clutter' species with the highest searching activity in riparian forest edge in both seasons. Similar results were also found by Lloyd et al. (2006) and

Law & Chidel (2002) with the highest activity in riparian zones and a regular use of regrowth and unlogged forests. Riparian zones not only provide greater resource availability but also function as an environmental structure to aid in navigation during commuting (Scott et al. 2010; Medinas et al. 2013; Greif & Siemers 2010). Since there was a low amount of searching activity and no feeding activity detected in both seasons, it is possible that *R. megaphyllus* might be using riparian forest sites as a flight path to their forage ground rather than foraging. This species is known to travel up to 2.25 km away from their roost to forage (Pavey 1998), and females were recorded travelling over 20 km between maternity and non-maternity sites (Dwyer 1966). If *R. megaphyllus* forage in habitats beyond the study area, this may explain why their activity was higher in edge / creek than interior, and no feeding buzzes were detected in both seasons. In addition, there was more *R. megaphyllus* activity detected in winter which also mirrors the increased number of individuals found in the Yugar tunnel. However, the number *R. megaphyllus* in the Yugar tunnel was low in both seasons compared with *M. australis* and *M. macropus*. The small number of *R. megaphyllus* present in the Yugar tunnel possibly reflects the low level of activity in the surrounding area.

Summary

The aim of this study was to determine 1) bat species composition in the surrounding landscapes, 2) bat diversity among habitat and vegetation types, and 3) the use of vegetation and habitat types in the surrounding landscapes by the bat species inhabiting in the tunnel. A total of 21 bat species were detected in summer and 18 in winter within a 3 km radius surrounding the tunnel. Bat diversity varied between sites across habitat and vegetation types during summer and winter, with the highest diversity in riparian creek during summer, and open forest edge during winter. However, there were no significant interactions between bat species diversity across habitats, vegetation types and seasons. It was likely due to the seasonal activity pattern of bats which they remained active and forage in both seasons. The searching and feeding activity of the three tunnel bat species was higher in riparian forest than open forest and regrowth open forest, and higher in edge / creek than interior during both seasons. It was likely due to several biological and environmental

factors such as bats' morphological features and echolocation call characteristics, vegetation structures, water availability and insect diversity and abundance.

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Chapter 5: General discussion

This chapter provides a general summary of results for each of the three experimental objectives (Chapter 2, 3 & 4), and highlights the significance of the Yugar tunnel in terms of its conservation value for bats in the Samford region. The chapter also provides advice to Moreton Bay Regional Council as the guardians of the tunnel. Finally, the chapter offers some suggestions for future research on bats inhabiting the Yugar tunnel.

5.1 Summary of findings

In the introductory chapter (Chapter 1) of this thesis, three questions were proposed to gain a deeper understanding of the ecology and biology of bats occupying the Yugar tunnel. The questions were: 1) What species of bats are using this tunnel? 2) How do the bats select their roost locations in the tunnel? 3) How do bats use the area surrounding the tunnel for movement and foraging? To answer these three questions, three main objectives were developed and achieved through multiple surveys including exit counts, acoustic monitoring and walkthrough surveys over one summer and one winter. The summary of results are presented below against each objective / chapter.

Objective 1/ Chapter 2: Understanding the bat species, population, and their emergence and return pattern in the Yugar tunnel

For bat species found in the tunnel, three species including *M. macropus*, *M. australis* and *R. megaphyllus* were detected during summer and winter. *Myotis macropus* showed a similar number of individuals in the tunnel between summer and winter, and they were likely inhabiting the tunnel throughout the year (and may be as a maternity site). The similar number of individuals between the two seasons was likely influenced by the sampling time coinciding with breeding in summer but not in winter. For *R. megaphyllus*, the number of bats increased greatly from summer to winter. Although *R. megaphyllus* were inhabiting the tunnel in both seasons, their

numbers throughout summer and winter were small compared to those reported for maternity colonies. Therefore, the tunnel may function as a diurnal roost rather than a maternity roost. For *M. australis*, the number of bats reduced greatly from summer to winter. This seasonal pattern is likely explained by the patterns of *M. australis*, similar to patterns of other *Miniopterus* that are tied to large-scale movements to maternity sites in summer. However, it cannot be confirmed whether the Yugar tunnel is a maternity site for *M. australis* due to lack of evidence.

The emergence and return pattern of bats showed significant differences among species, and the differences were dependent on season; all three species emerged earlier in winter than summer, and returned earlier in summer than winter. Several factors such as predation risk, light intensity, reproductive period, food availability and foraging strategies were suggested to have influenced both emergence and return behavior of the bats. In addition, it was likely that due to poor weather conditions, the peak emergence of bats occurred much later with return flight activity occurring earlier during summer.

For bat activity between the first and last activity peak, two smaller peaks occurred during the night in summer; a smaller peak occurring before and after the second largest peak in winter. In summer, the two smaller activity peaks were likely driven by the emergence of *M. macropus* and *R. megaphyllus* and the early return of *M. australis* due to poor weather or early satiation. In winter, the smaller activity peaks before and after the main return were possibly driven by a second emergence, the return of some individuals from the first emergence, or even by bats visiting from other roost sites due to multiple factors such as weather conditions, social communication, and energy conservation.

Objective 2/ Chapter 3: Understanding the microhabitat of the tunnel and determine how this influences the roosting locations of each species

Myotis macropus, *M. australis* and *R. megaphyllus* roosted in different locations and exhibited different roosting strategies inside the tunnel. Their selection of roosting locations and clustering strategies likely depended on microclimate and the ambient environment. *Myotis macropus* roosts were located inside the drain holes

(2.8 m above ground) and distributed across each zone of the tunnel, with the majority of roosts and individuals concentrated in the middle zone (Z2) during summer and winter. For *M. australis*, all roosts were located on the tunnel wall surface (at least 3.3 m above ground). A total of 3 *M. australis* roosts were found distributed across Z2 and Z3 (deep) with the majority of individuals clustering in Z2 in summer, and only one roost in Z2 in winter (the number of bats reduced greatly from summer to winter). For *R. megaphyllus*, all roosts were found in Z3 during summer and winter, with the largest roost located at the deepest part of the tunnel (above the rock pile) in both seasons. None of the three bat species shared their roosting locations with other species in either season.

For the roosting microclimate preferences of each species in the tunnel, it was found that the three bat species exhibited different roosting microclimate preferences. A variety of physical and environmental factors such as the composition of wall materials; roost location; height, width, length of the tunnel; and number of bats within roosts were suggested to impact the microclimate within roosts. In general, bats roosting in the tunnel experienced smaller daily diurnal temperature and relative humidity fluctuations when compared with external ambient (outside the tunnel). For the roosting microclimate preference of *M. macropus*, it was found that this species prefers to select their roosts in thermally stable structures (i.e. drain holes) with high and stable relative humidity where temperature is favorable. All sampled *M. macropus* roosts showed a relatively stable microclimate with smaller T_{fluc} and RH_{fluc} than all tunnel ambients. In addition, *M. macropus* roosts reached complete saturation with 100% relative humidity throughout the entire sampling period during both seasons. These conditions reduce energetic cost associated with reducing the risk of dehydration, and highlights their preferences for humid roosting environment. For *M. australis*, this species prefers to select roosting sites which offer relatively stable diurnal temperature and relative humidity, and roost at the warmest location within the tunnel during both seasons. All *M. australis* roosts were less than 1.2°C warmer than tunnel ambients in both seasons. Although the bats themselves could be a contributing factor influencing the microclimates within roost, the height of roost in Z2 possibly indicated that they prefer to roost at the warmest part of the

tunnel. *Rhinolophus megaphyllus* prefers to select roost locations with relatively high temperature and relative humidity, and high thermal stability during both seasons. *Rhinolophus megaphyllus* roosts showed little buffering effect towards temperature and relative humidity fluctuation from their tunnel local ambient, which suggests that *R. megaphyllus* maybe able tolerate a higher microclimate fluctuation than the other two species in the tunnel.

Objective 3/ Chapter 4: Understanding the utilisation of the surrounding environment by bats in the tunnel

In summer, a total of 21 bat species were detected in the landscape within a 3 km radius of the tunnel; 18 were detected in winter.

For bats diversity among habitat and vegetation types, it was found that bat diversity varied between sites across habitat and vegetation types between two seasons, with the highest diversity in riparian creek during summer, and open forest edge during winter. However, there were no significant interactions across habitats, vegetation types and seasons, and bat diversity was not significantly different within each of these factors. This was likely due to the mild temperatures experienced on most winter nights when some bats and insects remained active. Bat diversity was highest in edge/ creek habitats likely due to the higher insect species richness and abundance, and water resources offered by edge / creek habitat and the use of linear landscape elements by bats for movement. Bats diversity in different vegetation types was likely driven by seasonal effects (i.e. climate and weather conditions) which influence water availability (number of available watercourses with available water). The same factors also shape the distribution of insect and consequently foraging bats across the landscape. Since the creeks in several riparian sampling sites were completely dry in winter, this may have driven some bat species to foraging in other vegetation types.

The use of surrounding landscapes by the three tunnel bat species was dominated by *M. macropus*, followed by *M. australis* and then *R. megaphyllus* in both seasons. The use of surrounding landscapes likely depended on their morphological features and echolocation call characteristics. The activities of all three tunnel bat

species generally agrees with their functional guilds with *M. macropus* being “riparian specialist”, *M. australis* being “edge specialist” and *R. megaphyllus* being “clutter-tolerant”. Both searching and feeding activity of the three species was higher in riparian forest than open forest and regrowth open forest, and higher in edge / creek than interior during both seasons. For *M. macropus*, their distribution of activity across riparian forest sites was more even in summer than in winter. This was likely driven by the seasonal differences in water availability across the landscape which led to *M. macropus* activity being more concentrated within 2 riparian forest sites in winter. For *M. australis*, their search and feeding activity was higher in summer than winter but both activities were infrequent across all habitats within each vegetation types in both seasons. Since the habitat and vegetation structures are relatively open in open forest and regrowth open forest when compared with riparian forest, *M. australis*’ highest activity in riparian forest reflects their preference of foraging in cluttered environments. The lower level of *M. australis* activity in winter was possibly due to majority of them absented in the Samford region as they returned to their breeding sites, or associated with cold temperatures reducing insect activity. For *R. megaphyllus*, the number of search passes was very low across all sites, and no feeding buzzes were detected in either season. This was likely due to their low numbers at the foraging locations. The activity pattern of *R. megaphyllus* possibly indicates that they were using riparian forest sites as a flight path to foraging grounds.

5.2 Implication for the tunnel custodians, Moreton Bay Regional Council

The Yugar tunnel is a roosting site for three bat species including *Myotis macropus*, *Miniopterus australis* and *Rhinolophus megaphyllus* in summer and winter. In this study, there was a minimum of 73 *M. macropus*, 131 *M. australis* and 3 *R. megaphyllus* occupying the tunnel in summer, and 77 *M. macropus*, 15 *M. australis* and 18 *R. megaphyllus* in winter. The population sizes of the three species were relatively small compared to maternity colonies found elsewhere, and no juvenile bats were observed during the walkthrough surveys. Therefore, it is unlikely that the Yugar tunnel is a maternity site for bats. However, the Yugar tunnel may serve other aspects of bat biology and behaviour such as social interactions, resting between foraging bouts; as protection against weather and predators; and by providing a stable microclimate for day roosting. The significance of the tunnel likely varies by species. For *M. australis*, their population was greatly reduced from summer to winter as they may have migrated to their breeding sites during winter. Therefore, the tunnel is likely to be more important during the breeding season in summer. For *R. megaphyllus*, their population was very low throughout the study. It is likely that the tunnel may serve as an over-winter roost for this species as their population increased from summer to winter. For *M. macropus*, their population remained stable throughout the study. Although it was not certain whether they are permanent residents, the Yugar tunnel likely to be more important for *M. macropus* than the other two species. In addition, the geographical position of the Yugar tunnel is important for the bats as it is surrounded by multiple habitats which allow bats to easily access to important resources such as water and food. This is especially so for *M. macropus* as they forage almost exclusively near permanent waterways, and the short distance between the tunnel and riparian forests (foraging grounds) showed its strategic importance for foraging by this species. The richness and abundance of resources within the surrounding landscape also enables bats to emerge later during the nights thus reducing the predation pressure from diurnal and crepuscular avian predators.

All three tunnel bat species exhibit roosting preferences for warm and humid locations with buffering from daily ambient temperature and relative humidity fluctuations. Such microclimate conditions provided by the Yugar tunnel could also provide roosting sites for other bat species in the Samford region (i.e. *Vespadelus troungtoni* and *Miniopterus orianae oceanensis*). Although it is not known whether bats' use of the tunnel is obligate or facultative, there is no doubt that the tunnel offers alternative roosting opportunities for bats in the Samford region. The importance of the Yugar tunnel to bats may increase as the intensity of land use increases due to urban development. Although all three tunnel bat species: *M. macropus*, *M. australis* and *R. megaphyllus* are classified as the least concern species in the IUCN Red List (Gorecki & Pennay 2021; Armstrong et al. 2019; Armstrong & Aplin 2017), the loss of roost sites may increase the threatening processes to the survival of these species (*M. macropus* in particular; Gorecki 2020). The Yugar tunnel may be able to partially compensate for the loss of natural roosts for not only these three species but also other bat species in the surrounding area.

This study has highlighted the significance of riparian forest for bats in the Samford region. It was found that bat diversity was highest in riparian forest, and the higher diversity in open forest during winter was likely due to the seasonal climate affecting the number of available watercourses across the landscape. Since the foraging of bats is likely opportunistic, the reduced number of watercourses in the study area during winter is likely to have driven some bat species to forage in other nearby habitats. It is possibly that the water availability (number of rivers) has shaped the distribution of bat community in the Samford region. In this study, a minimum of 19 bat species were detected using the riparian forest during summer and 17 species in winter (not mentioned in the thesis). Therefore, the maintenance of the water quality and riparian vegetation will benefit the bat community in the Samford region in the long-term, as well as the bats inhabiting the Yugar tunnel (particularly for *M. macropus*).

Over the course of this study, the Yugar tunnel has been consistently visited by unauthorised people for unknown purposes. New rubbish such as empty beer bottles, graffiti spray bottles, etc was noted each time bat surveys were conducted in

summer. The tunnel gate locking mechanism has also been tampered with several times over a short period of time and obvious signs of disturbance to bats by unauthorised visitors to the tunnel; bats were taking flight in the tunnel in late morning and the tunnel was covered with the smell of spray paint before a visit. The presence of humans in the tunnel likely produces significant disturbance to bats. There is a potential that bats may abandon the Yugar tunnel if they are consistently disturbed. Therefore, I recommend reducing the chance of unauthorized people entering be of primary importance to the conservation of bats in the Yugar tunnel.

5.3 Future research

This study has focused on several aspects of the roosting and foraging ecology of the bat species inhabiting in the Yugar tunnel. However, the study has also served to highlight other areas where knowledge is lacking and additional research would result in a deeper understanding of the bats' biology and behaviour. Some suggested areas for future research are given below.

- The results of this study do not determine the function of Yugar tunnel for the bats that roost within it. Most importantly, it does not confirm whether the Yugar tunnel is a maternity site for the bats. This study does suggest that the Yugar tunnel is likely not a maternity roost is based on the seasonal population patterns and observation from the walkthrough surveys in a distance. Future research should investigate the sex, age and reproductive condition of bats in the tunnel to confirm this conclusion.
- This study only provides information on bat species diversity and relative acoustic activity across the landscape. It is possible that the three tunnel bat species detected in the surrounding environment were from other nearby roosting sites. The results do not explicitly show where the tunnel bats go for foraging at night. Future research should radio track bats from the tunnel to better understand how they utilise the surrounding environment. Such research may also provide more details on the emergence and return pattern of individual bats in the tunnel.
- Future research should focus on the population genetics of bats in the tunnel to determine whether they are permanent residents and how they are related to other colonies within the area. Such results will give context to the importance of the bats in the tunnel from a landscape-scale conservation perspective.

5.4 Reference list

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Appendices



Figure A 1 *Myotis macropus* roost inside a drain hole in the Yugar tunnel in summer



Figure A 2 Another *Myotis macropus* roost inside a drain hole in the Yugar tunnel in summer



Figure A 3 *Miniiopterus australis* roost in Z2 during the last visit to the tunnel in summer (late February).



Figure A 4 *Miniiopterus australis* roost in Z2 during the last visit to the tunnel in summer (late February).



Figure A 5 *Rhinolophus megaphyllus* roost above the rocks pile in summer. (Bats were highlighted in red circles)

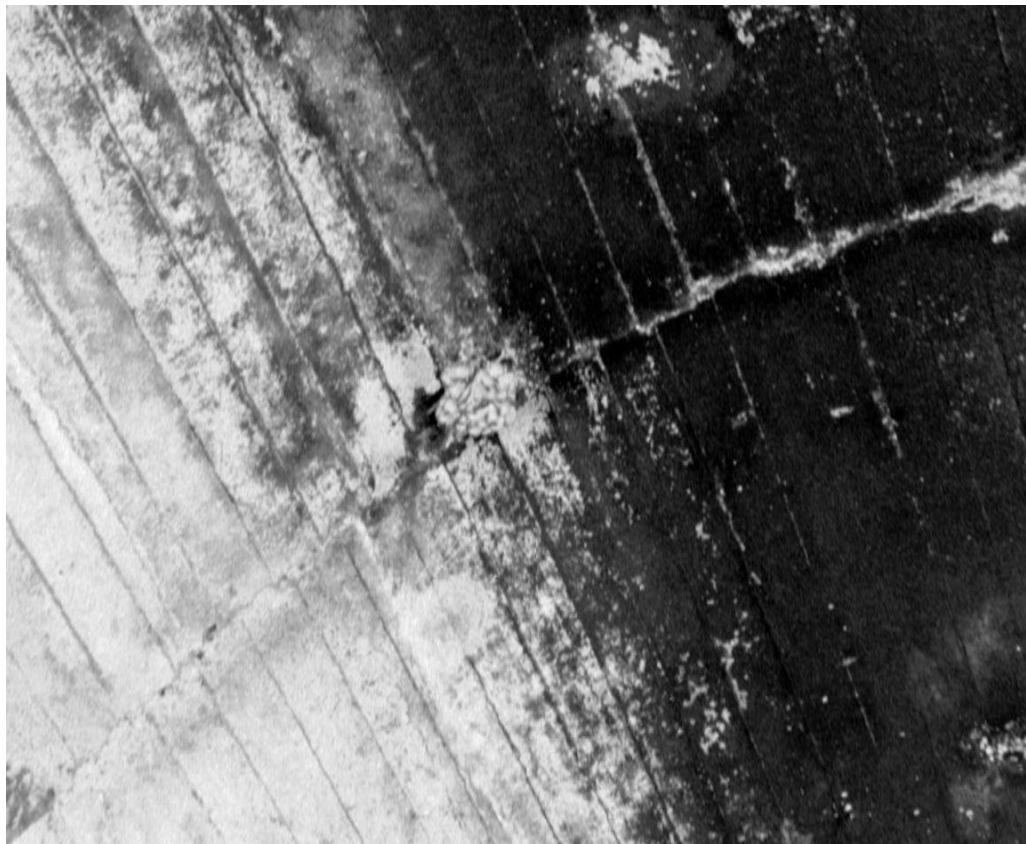


Figure A 6 *Miniopterus australis* roost in Z2 (2 days after walk-through survey) in winter



Figure A 7 *Rhinolophus megaphyllus* roost above the rocks pile in winter.



Figure A 8 A single *Rhinolophus megaphyllus* hanging on the ceiling of a doorway inside the Yugar tunnel in summer.