Bat habitat use of restored jarrah eucalypt forests in south-western Australia.

Joanna M. Burgar

School of Veterinary & Life Sciences

Murdoch University, Perth, Western Australia

Submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

October 2014

Statement of Originality

I declare that this thesis is my own account of my research and contains as its main content work that has not previously been submitted for a degree at any tertiary education institution.

Joanna Burgar

Abstract

Restoration is an important tool in conserving biodiversity, yet passive faunal recolonisation may take decades, or longer, to occur. This is of particular conservation importance in biodiversity hotspots, such as south-western Australia, which are experiencing increasing fragmentation and rapidly drying climates. Within this hotspot, I investigated the response of nine insectivorous tree-dwelling bat species to restored mine-pits in jarrah (*Eucalyptus marginata*) forests. I assessed bat activity in restored, relative to unmined, forests and the suitability of restoration as foraging and roosting habitat. Bat echolocation call surveys measured bat activity in varying ages of restoration and unmined forest across two years (2010-2012) during both maternity and mating seasons. Although all bat species were detected in both forest types, restored mine-pits of all ages had significantly different bat communities and lower overall activity compared to unmined forest. Habitat filters to bat use of restoration were evident for the more manoeuvrable bat species and were predominantly related to midstorey forest structure. Tree density was the most important predictor of bat use of restoration for less manoeuvrable bat species. To determine the suitability of restored forest as foraging habitat I investigated the diet of three species (*Chalinolobus gouldii*, *Nyctophilus gouldi* and *Vespadelus regulus*) over maternity and mating seasons (2010/2011) by examining prey remains in faecal samples. I used high-throughput sequencing and bioinformatics analyses to phylogenetically group prey DNA and found that niche partitioning occurred, with dietary divergence positively related to bat ecomorphological divergence. In addition, I assessed the foraging potential of restored forest and found that prey occurrence did not necessarily equate to prey accessibility for all bat species. There was a synergistic effect of vegetation structure and insect biomass for edge foraging bat species. To determine the suitability of restoration as roosting habitat I used telemetry to radio-track 36 bats from two species (*N. gouldi* and *V. regulus*) to 59 distinct roosts. Not one bat was found roosting in restored forest and individuals preferred roosting in mature, tall trees in intermediate to late stages of decay. My research clearly shows that restored forest does not yet provide suitable foraging or roosting habitat for all jarrah forest bats. Improving habitat suitability through management manipulations, such as thinning and burning, may accelerate bat recolonisation of restored forest. In the interim, retention of mature forest patches is necessary for conserving and maintaining bat populations across restored landscapes.

Table of Contents

Acknowledgements

This thesis arose out of work undertaken since I came to Australia in 2010. This work would not have been possible without the help and support of a great many people along the way. I would never have been inspired to undertake a PhD without the two best role models a daughter could hope for, my parents, who both taught and never asked if, but instead where, I was going to university. To my father, who helped me with my science homework and gave me my aptitude for math, I am so fortunate to have had you for a dad and I only wish you could be here to see me graduate – you are in my heart always. To my mother, who listened to all of my challenges and struggles with fieldwork, analyses and writing, and helped with grammar to the bitter end, you are an inspiration for tackling a PhD while working and yet still managing to ensure a loving home for our family – I aspire to be as selfless and generous as you. To my sister, who gave me unwavering support, always managed to find me work and thankfully even a job that gave me a good start in stats, thank you for being so close even when you were half a world away.

I am forever indebted to my two supervisors, Dr. Michael Craig and Dr. Vicki Stokes, without whose unending assistance this thesis would not have been possible. I am also extremely grateful to Dr. Kyle Armstrong for lending me field equipment, advising me on bat call identification, and providing reference bat calls; to Dr. Allan Wills for the loan of light traps and assistance with invertebrate identification; to Dr. Paul Webala for giving me a quick introduction to jarrah forest bats, answering countless emails thereafter, and providing reference bat calls; and to Sian Contarino for persevering and assisting with sorting thousands of invertebrate samples. I am also grateful to the many Murdoch and University of Western Australia undergraduate students who came out in the field or spent a day in the lab to assist me.

I will also be forever grateful to Richard Hobbs and the entire Ecosystem Restoration & Intervention Ecology Research Group (ERIE) at the University of Western Australia for being such an inclusive lab group, both intellectually and

socially. Having the opportunity to present ideas and rehearse conference papers in a non-threatening and collegial environment certainly improved my public speaking confidence, and perhaps was the reason I was able to take runner-up student presenter at my last conference as a student. Friday afternoon (sometimes progressing into night) beers were also a great way to let off some of the work-week stress, and make weekend working that much more enjoyable.

I would also like to thank all of the people who made Australia feel like home for me and kept me inspired during what, at times, felt like a never-ending journey. I don't know if I would have made it through the day without the morning coffee breaks with the MUCRU group, who made special concessions to include me as another echolocating animal researcher. To the inspiring Krista Nicholson for reminding me of the importance of quality data collection as well as being a wonderful distraction and the perfect person to have sitting just behind me. To Jenny Smith for keeping me sane with yoga, tea study dates, and sustainability, and for reminding me of good ole North America with your unbelievable recollection of pop-culture while making such witty, intelligent remarks. To Emily Fisher for joining me in exploration of south-west surf spots, keeping me company at Murdoch on those weekend work days, and for being such a sweet and understanding friend. To Simon Allen for motivating me to get my PhD submitted on time…come to think of it, am I not owed a dinner, or did we resolve to keep it as a gentleman's bet?

To Maggie Triska for sharing in the fun of dozens of veg surveys, chit-chatting PhD issues during long drives to and from Huntly, being a stats guru and discussing stats even when we were supposed to be out for fun, being my wildlife spotting partner in crime, but most importantly for being that wonderful ray of North Dakota sunshine that I was lucky enough to pick up from the airport on your first day in town and being such a good friend, one that I hope to never lose. To Christine Allen for reminding me of the beauty of the south-west with camping trips and writing retreats, being another odd person in the drive for sustainability and wacky food choices and, of course, for all the fun dancing! To Bridget Johnson for the absolute best Ridgefield experience, sharing in outrageous silliness, making

me laugh, and generously giving me a place to stay and a car to use when I was without. And of course to the (in)famous Dr. Jodi Price for joining me at punk shows (not enough, though!), making me loads of yummy vegan meals and treats, putting together a nearly-there PhD package for the last push, sharing your office with me, and always being up for having a good time.

I acknowledge the support of Murdoch University for funding my scholarship and, through the Murdoch University Animal Ethics Committee, providing the approval to study and handle bats (Approval W234710). I am grateful to the Department of Environment and Conservation (DEC) for providing permits and permission to work in the jarrah forest (CE002999, CE003726, SF007648 and SF008894). This work would not have been possible without the generous financial support from Murdoch University, Alcoa of Australia, Australasian Bat Conservation Fund, Nancy Pallin of the Australian Bat Society, Australian Geographic, Ecological Society of Australia, Holsworth Wildlife Research Endowment, Institute of Foresters of Australia (Maxwell Ralph Jacobs Fund) and the Wildlife Preservation Society of Australia.

Chapter One: General Introduction

Ecological restoration is one tool that can help prevent biodiversity loss, maintain ecosystem functions and services, and mitigate global change, including climate change (Hobbs & Cramer 2008). While restoration may refer to any interventionist activity towards the management or repair of degraded ecosystems, restoration most often comprises the re-establishment of functioning ecosystems with a complement of species similar to those historically present (Hallett et al. 2013; Hobbs & Cramer 2008). As restoration is often economically and physically intensive most projects occur at the local scale (e.g., Matthews et al. 2009), although some extend to the regional scale (e.g., Lengyel et al. 2012). In contrast to restoration, the terms rehabilitation and revegetation most often apply to the active or passive repair of degraded lands to a functioning ecosystem, rather than a historic reference point, and are not specifically focused on conserving biodiversity (Hobbs & Cramer 2008). Restoration ecology has been cited as the future hope for conserving biodiversity, with restoration named as the critical element in managing the world's environment (Dobson et al. 1997). Restoration predominantly focuses on the re-establishment of plants (Brudvig 2011; Ruiz-Jaen & Aide 2005), despite the critical role fauna play in processes such as soil aeration, nutrient cycling, seed dispersal and herbivory (Greenslade & Majer 1993; Lindell 2008; Majer & Nichols 1998). Most restoration projects assume that once the requisite flora species have re-established fauna will passively recolonise (Palmer et al. 1997).

However, there has been a recent move to examine restoration outcomes across a variety of ecosystem attributes and not solely through the vegetative component, focusing on the importance of soil microbial communities (Harris 2009), pollination (Dixon 2009), and fauna (Cristescu et al. 2012) within restored systems.

Where restoration studies have extended to fauna the research has primarily centred on arthropods, and to a lesser extent, birds, fish and other invertebrates (Brudvig 2011). If restoration is aimed at conserving global biodiversity bats are a necessary group to consider. Firstly, bats are highly speciose and found on all continents other than Antarctica (Fenton 2003). Bats also fill a variety of ecological niches and are an integral component of the ecosystem, particularly in terms of insect control and pollination (Kunz et al. 2011; Leelapaibul et al. 2005). Lastly, bats can provide insights into habitat quality at both the site and landscape level and may be useful as bio-indicators (Jones et al. 2009; Stahlschmidt & Bruhl 2012). Bats often require multiple habitats to meet their breeding and foraging needs (Law & Dickman 1998; Lumsden et al. 2002a); their mobility means that bats are able to move between habitats and thus relative levels of occurrence within different habitats provides an indication of habitat use. In addition, the presence or absence of certain prey species in the diet of foraging bats can provide insight into ecosystem health. For example, a bat diet that contained insect species intolerant of pollution indicates a pristine environment where the bats forage (Clare et al. 2011). As our knowledge of bat ecology improves, generalisations may be inferred and lessons learnt in one region may be relevant elsewhere, improving our ability to understand the effects of restoration on bat populations globally.

Study Area & Restoration Process

One particularly relevant area to study the effect of restoration on fauna is across the restored landscape of the northern jarrah (*Eucalyptus marginata*) forest, situated in the biodiversity hotspot of south-western Australia (Myers et al. 2000). South-western Australia is facing rapid environmental change, both in terms of climate and land-use (Batini 2007; Bradshaw 2012). As a result of climate change, within the next century the Mediterranean biomes within Australia are predicted to contract to 77-49% of current size (Klausmeyer & Shaw 2009). Globally, compared to all other biomes, the Mediterranean biome is predicted to experience the greatest proportional loss in biodiversity over the next century, predominantly due to changes in land-use (Sala et al. 2000). The extent of primary vegetation in south-western Australia has been reduced by nearly 90% (Myers et al. 2000), largely due to clearing for agriculture or pasture and grazing (Bradshaw 2012; Yates & Hobbs 1997). The potential direct and indirect threats facing south-western Australia in the upcoming decades makes conservation of this region of the utmost importance for minimising biodiversity loss.

The jarrah forest, a dry sclerophyll eucalypt forest, is managed for multiple-uses, including conservation, timber harvesting, potable water catchment management, recreational activities and mineral extraction (Gardner & Bell 2007). Alcoa of Australia Limited (hereafter Alcoa) currently holds a mining lease comprising ~700 000 ha and nearly all of the northern jarrah forest (Figure 1). Within this lease Alcoa mines bauxite ore, the main source of aluminium, and supplies over 10% of the world market of production alumina (Gardner & Bell 2007). Alcoa has been operating in the jarrah forest for over four decades and has mined >15 000 ha of land (Koch 2007a). Mining has generally occurred in a north-easterly direction, but bauxite ore grade requirements have created a somewhat interspersed mosaic of mine-pits within unmined forest. Alcoa clears, mines and restores between 40-50% of the forest within a mine-site, ~600 ha of land annually (Koch 2007a). The mining process consists of removing a shallow layer of duricrust and bauxite (four to five metres deep) in pits that range in size from ~2-30 ha (Grant 2006) and pits are restored as soon after the ore is removed as feasibly possible. The topsoil that is initially removed is returned directly, either to the same pit or to a nearby pit, to enhance soil seedbank germination (Koch 2007a). In addition to returning topsoil, the restoration process involves returning waste timber and rocks to the mine-pit as habitat for fauna, contour ripping the pit to avoid soil compaction and erosion, seeding with native local vegetation and planting those species unable to return via seed, and vegetation monitoring (Koch 2007a). Over time, adaptions to Alcoa's restoration practices have shifted the focus from land stabilisation to the current objective of restoring a self-sustaining jarrah forest ecosystem (Koch 2007a). Much research has focused on the ability of Alcoa's restoration to meet its objectives in terms of vegetation (e.g., Koch 2007b), which have generally been met (Koch & Hobbs 2007). Recent research has examined the effect of restoration on some faunal groups (e.g., Christie 2011; Craig et al. 2012; Majer et al. 2013), but not yet bats, despite the presence of nine insectivorous, forest-dwelling species (Churchill 2008).

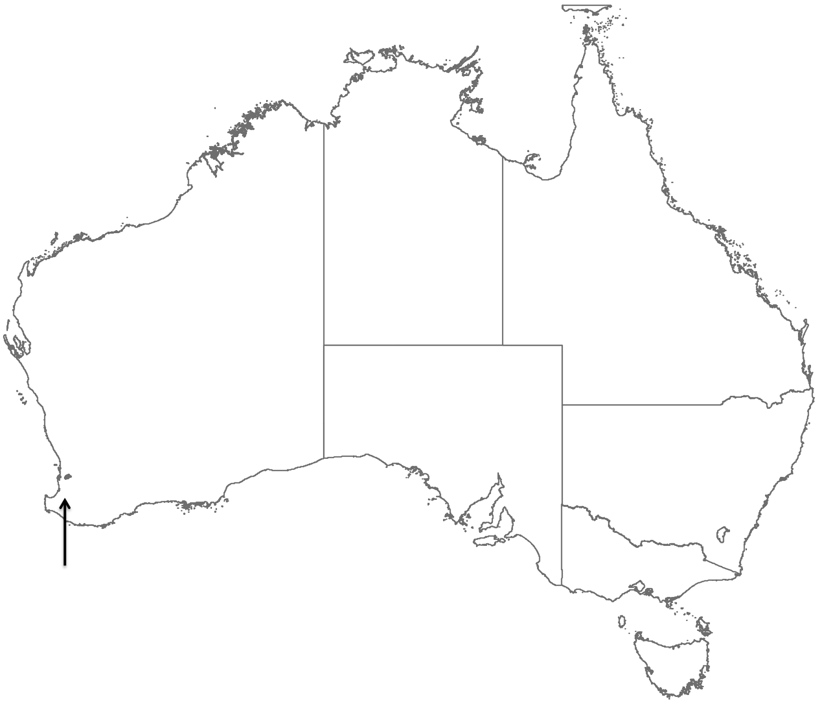


Figure 1: Alcoa's Huntly minesite (~15 000 ha) is situated in the northern jarrah forests of south-western Australia, depicted on the map by the black mark above the arrow.

Bats of the Northern Jarrah Forest

Not only have there been no studies on the effect of restoration on bats in the northern jarrah forest, there is little published literature on the effect of restoration on bats in Australia or elsewhere. The few accounts of revegetated agricultural landscapes in eastern Australia (Law & Chidel 2006; Law et al. 2011) and urban woodland restoration in North America (Smith & Gehrt 2010) suggest bats respond favourably to restoration. An Australian review of the effect of rehabilitation on fauna in mines found that bats were overlooked as a study taxa (Cristescu et al. 2012), with only one study assessing bat responses to forest restored after mining (Knight 1999). Thus, there is an obvious research gap pertaining to the response of bats to restoration in a mining landscape. At the regional scale there are only three published studies on jarrah forest bats: the most recent study examined the impact of silviculture on bats in the southern jarrah forest (Webala et al. 2011; Webala et al. 2010) and the other published work examined jarrah forest bat ecomorphology (Fullard et al. 1991).

Jarrah forest bats belong to the Vespertilionidae and Molossidae families (Table 1). The Vespertilionidae species have either one or two pups, that are born in late spring or early summer, while the Molossidae species have one pup born in mid to late summer (Churchill 2008). All species predominantly roost in trees (Goldingay 2009; Rhodes 2007; Webala et al. 2010) and consume a diversity of insect prey (Fullard et al. 1991). Two of the nine species, *Falsistrellus mackenziei* and *Nyctophilus major*, are restricted to south-western Australia, with the exception of one isolated *N. major* population on the edge of the Nullabor Plain (Churchill 2008). The remaining species are widespread across Australia, typically with disjunct populations in south-western Australia, some of which are taxonomically unresolved (Parnaby 2009) and may be more taxonomically distinct than currently thought. Research suggests that *Tadarida australis*, *Mormopterus kitcheneri*, and *Chalinolobus gouldii* are disturbance tolerant (Law et al., 1999) while the remaining species (*C. morio*, *F. mackenziei*, *N. gouldi*, *N. geoffroyi, N. major,* and *Vespadelus regulus*) are likely disturbance sensitive (Law et al., 1999, Webala et al. 2011).

Table 1. The nine bats of the northern jarrah forest belong to the Vespertilionidae (above line) and Molossidae (below line) families. Common name nomenclature follows Armstrong and Reardon (2006). Wing loading and aspect ratio values were taken from Fullard et al. (1991) with the exception of *F. mackenziei* and *N. major* (N.L. McKenzie and R.D. Bullen, pers. comm) and were measured from live specimens, with the exception of *T. australis* (which were taken from preserved specimens). Mass and forearm length values were taken from live adult specimens measured in the northern jarrah forest throughout the duration of this study (J. Burgar, unpublished data), with the exception of *F. mackenziei, M. kitcheneri,* and *T. australis* (Churchill, 2008). Echolocation call characteristics were taken from a regional (south-western Australia) reference call library of calls recorded primarily during hand release but also through visual identification, particularly for *T. australis*. Habitat type (primary and secondary, where appropriate) is adapted from Fullard et al. (1991). Mean (±SE) are provided for measured values as well as minimum and maximum range values (in brackets) for mass, forearm length and characteristic frequency.

Species names	Common name	Mass (g)	Forearm length (mm)	Aspect Ratio	Wing Loading (m^{-2})	Frequency (kHz)	Duration (ms)	Slope	Habitat (1° / 2°)
Chalinolobus gouldii (Gray, 1841)	Gould's wattled bat	14.9 ± 0.4 (11.2-20.3)	44.4 ± 0.3 (39.6-46.6)	5.5 ± 0.9	9.1 ± 1.9	29.1 ± 0.1 (28-41)	5.5 ± 0.1	52 ± 3	Edge / Open
Chalinolobus morio (Gray, 1841)	Chocolate wattled bat	7.9 ± 0.2 (5.9-10.1)	36.6 ± 0.3 (32.9-38.7)	4.8 ± 0.4	6.7 ± 0.7	49.1 ± 0.1 (48-66)	3.5 ± 0.0	62 ± 2	Edge
Falsistrellus mackenziei Kitchener et al., 1986	Western false pipistrelle	21.0 (17.0-26.0)	50.7 (48.0-53.7)	6.9	8.6	33.6 ± 0.1 (32-49)	5.9 ± 0.1	52 ± 2	Open / Edge
Nyctophilus geoffroyi Leach, 1821	Lesser long-eared bat	7.5 ± 0.5 (5.4-10.9)	37.8 ± 0.6 (34.1-41.6)	5.1 ±0.3	5.7 ± 0.3	41.3 ± 0.4 (40-61)	2.8 ± 0.1	176 ± 5	Closed
Nyctophilus gouldi Tomes, 1858	Gould's long-eared bat	9.7 ± 0.2 (6.9-14.9)	40.9 ± 0.2 (32.3-48)	5.5 ± 0.5	7.0 ± 0.4	48.3 ± 0.5 43-71)	3.0 ± 0.1	223 ± 8	Edge / Closed
Nyctophilus major Gray, 1844	Western greater long-eared bat	14.8 ± 0.7 (12.6-17.8)	45.3 ± 0.3 (44-46.3)	6.0	6.7	40.6 ± 0.7 (37-65)	3.4 ± 0.2	206 ± 10	Edge / Closed
Vespadelus regulus (Thomas, 1906)	Southern forest bat	5.5 ± 0.1 (3.8-7.8)	31.8 ± 0.1 (29.4-35)	5.3 ± 0.4	6.8 ±0.9	42.9 ± 0.1 41-62)	4.0 ± 0.0	59 ± 2	Closed / Edge
Mormopterus kitchenerei Reardon et al., 2014	South-western freetailed bat	10.5 (7.5-10.5)	34.1 (32.6-35.4)	6.1 ± 0.1	12.3 ± 0.2	26.9 ± 0.1 (26-34)	4.2 ± 0.2	96 ± 5	Open / Edge
Tadarida australis (Gray, 1838)	White-striped freetailed bat	37.6 (30.5-47.5)	60.6 (57.2-64.5)	8.3 ± 0.3	16.0 ± 1.3	12.5 ± 0.1 (12-18)	10.6 ± 0.2	34 ± 1	Open

Insectivorous bats possess particular traits, e.g., wing morphology and echolocation call structure, which influence manoeuvrability, foraging and microhabitat use (Norberg & Rayner 1987; Siemers & Schnitzler 2004) and facilitate habitat partitioning (Aldridge & Rautenbach 1987). Trait variability has been assessed for bats from the continental (Stevens et al. 2003) down to the local scale (Kingston et al. 2000) and bat species traits are often used as a way to explain habitat use (e.g., Adams et al. 2009; Buchalski et al. 2013; Hanspach et al. 2012; Jung & Kalko 2011).

Echolocation

All jarrah forest bats utilise echolocation for spatial orientation and foraging, although *Nyctophilus* species also detect prey from prey-generated cues, exhibiting both aerial hawking and gleaning foraging strategies (Grant 1991). Echolocating bats emit high frequency (typically ultrasonic) tonal signals, produced in the larynx (Jones & Teeling 2006), and analyse the returning echoes to perceive their environment (Teeling 2009). The sensitive ears of a bat detect the echoes, which the brain then converts into information about the size, texture and distance of any objects, all the while contending with interfering factors, such as internal or external noise and echolocation calls from other bats (Schnitzler & Kalko 2001). Clutter, a generic term adapted from use with radar and sonar, refers to any object in the background or off to the side of the target of interest that returns additional echoes, which interferes with the processing of echoes from the target itself (Petrites et al. 2009). Thus echolocating bats have adapted to deal with both forward masking, where returning echoes interfere with emitted signals, and backward masking, where background or clutter echoes interfere with target echoes (Schnitzler et al. 2003).

Echolocation calls are generally species-specific with frequency structure, duration, harmonic composition, and sound pressure level differences between species (Jones & Teeling 2006; Schnitzler & Kalko 2001). Consequently, echolocation calls can be used to identify bat species, although echolocation call based species identification is not analogous to identifying bird species by bird songs. The main difference being the selection pressure; bird songs are species-specific and related to the cost associated with conspecifics answering (Barclay 1999; but see O'Farrell et al. 1999b). In contrast, bat echolocation calls are for individual use rather than social communication and have evolved due to habitat selective pressures (Fenton 1994). Indeed, echolocation call diversity and plasticity suggests that habitat has played a more important role than phylogeny in shaping the evolution of echolocation in bats (Jones & Teeling 2006). Typically bat echolocation call evolution follows general patterns of allometric scaling with smaller bats having calls of higher frequencies and shorter duration than larger bats, whose calls are at lower frequencies and of longer duration (Jung et al. 2014). Despite the importance of habitat in shaping echolocation call structure, there are generally enough differences in echolocation calls between species within a region to accurately distinguish between species, or species groups, although accuracy of species groupings may depend on the recording system (Adams et al. 2010; Bullen & McKenzie 2002).

Due to the nature of echolocation calls, they are effective for bats for short and medium distance spatial orientation and foraging (Jung et al. 2014; Petrites et al. 2009; Schnitzler et al. 2003). Bats are able to detect prey at relatively far distances (up to 67 m for Emballonuridae, 30 m for Molossidae, and 28 m for Vespertilionidae) due to the ability of bats to adjust the intensity of their call (Surlykke & Kalko 2008). Recent research examining Molossidae calls indicate that the perceptual range of insects (10-40 mm) changes depending on the peak frequency of the echolocation call, with optimal prey perception ~4-5 m for frequencies between 20-40 kHz (Jung et al. 2014). Consequently, bats rely on other cues, such as sight and memory, for long distance orientation and migration (Schnitzler et al. 2003; Schnitzler & Kalko 2001). Echolocation calls are classified based on their frequency signal components, being either frequency modulated, or constant frequency, or a combination of the two (Schnitzler et al. 2003). In addition, calls can be either narrowband, with limited frequency modulation and long (>5 ms) duration, or broadband, covering a range of frequencies and being of relatively short (<5 ms) duration (Jones & Teeling 2006; Schnitzler et al. 2003).

In the jarrah forest, *T. australis* echolocation calls typify narrowband calls whilst *Nyctophilus* species exemplify broadband calls (Figure 2). Narrowband calls are very capable of detecting echoes but lack the precision to determine exact locations; i.e., these calls are able to detect the presence of prey from further afield but are less well equipped to pinpoint the exact location (Fenton 1990; Schnitzler & Kalko 2001). Conversely, broadband calls excel at determining exact target locations but are less effective at detecting echoes; i.e., these calls require close proximity for prey detection, but when detected are able to pinpoint the location with great accuracy (Fenton 1990; Schnitzler & Kalko 2001). Not surprisingly, bat species that fly in open areas typically emit narrowband calls while those that navigate and forage amongst dense vegetation utilise broadband calls (e.g., Adams et al. 2009). But echolocation is a flexible system, allowing foraging bats to adjust the intensity of their calls to alter prey detection distances (Jung et al. 2014), as well as the frequency of their calls to change directionality and thus improve their sphere of prey detection (Jakobsen & Surlykke 2010). Bats also alter their calls based on the environment, increasing the broadband component and decreasing the narrowband component, or vice versa, depending on the habitat (Broders et al. 2004; Schnitzler et al. 2003). In addition, bats modify the rate of signal emission, also known as the interpulse interval, depending on the clutter density of their surrounding environment to perceive both rapid changes in the immediate vicinity and to probe farther into the surroundings to determine their future flight path (Petrites et al. 2009). The ability to switch between components of calls can complicate bat echolocation call-based species identification when frequency ranges between species overlap (Adams et al. 2010).

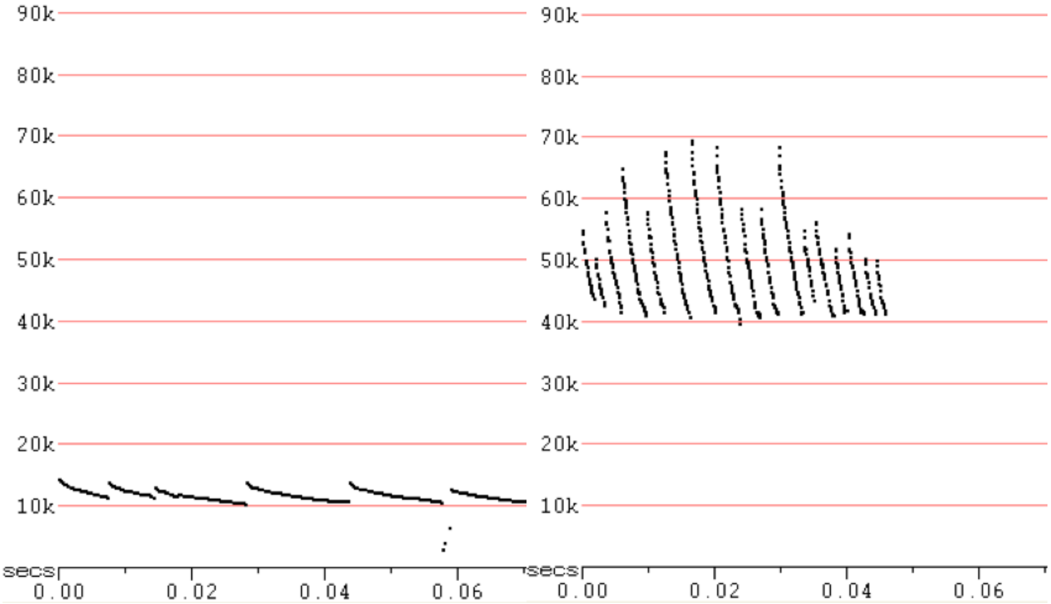


Figure 2: Echolocation calls of two jarrah forest bat species; the left sonogram depicts a typical *Tadarida australis* narrowband call, while the sonogram on the right depicts a typical *Nyctophilus* species broadband call. The x-axis is time (ms) and the y-axis is frequency (kHz).

Aspect Ratio & Wing Loading

Two key characteristics of bat ecomorphology are aspect ratio and wing loading (Fullard et al. 1991). Aspect ratio is calculated by dividing an individual bat's wing area by the square of its wingspan, whereas wing loading is the weight of the bat multiplied by gravitational acceleration, divided by the wing area (Fenton 1990). Higher aspect ratio and wing loading values are indicative of narrow and long wings; these less manoeuvrable bats, such as *T. australis*, are capable of fast flight in open environments (Bullen & McKenzie 2001). Conversely, lower values signify more manoeuvrable bats, such as *N. geoffroyi*, who exhibit slower flight speeds but have the ability to navigate densely vegetated habitats (Brigham et al. 1997; Bullen & McKenzie 2001).

Inferences on bat species habitat use are often based on ecomorphology and echolocation call (Bullen & McKenzie 2001; Fenton 1990). Bats with similar aspect ratio and wing loading can differ markedly in prey capture ability due to differences in echolocation call structure and echo-processing capabilities (Siemers & Schnitzler 2004). Species, such as *N. geoffroyi* and *V. regulus*, with low aspect ratio and wing loading are manoeuvrable and can exploit closed, i.e., densely vegetated, microhabitats (Fullard et al. 1991). However, the difference in their call structure suggests that *V. regulus* would have lower capture success than *N. geoffroyi* when closer to vegetation but as distance to vegetation increases their capture success rates become more similar (Siemers & Schnitzler 2004). On the other end of the spectrum are less manoeuvrable species, such as *T. australis*, with high aspect ratio and wing loading values, adapted for fast flight in open space environments (Fullard et al. 1991). The remaining jarrah forest bat species predominantly exploit the edge environment, i.e., the interface between dense vegetation and open space, although those with higher wing loading and aspect ratio values also utilise the open environment and those species with lower values favour closed environments. Species may be flexible in their use of microhabitat, although this flexibility is unidirectional (Schnitzler et al. 2003). Bats adapted for closed environments may use edge, or even open, environments but not vice versa (Fenton 1990).

Effects of Disturbance on Bats

Published literature on bat use of restoration is often limited to opportunistic observational accounts (e.g., Knight 1999). However, substantial research has investigated the response of bats to other types of human induced disturbances.

These studies typically examine the response of bats to agriculture, urbanisation or timber harvesting, and the majority indicate that disturbance has a negative effect on bat populations (e.g., Berthinussen & Altringham 2012; Farrow & Broders 2011; Fischer et al. 2010; Webala et al. 2011). Findings from these studies enable inferences to be made on the effect of restoration on jarrah forest bat populations.

Restored landscapes comprise areas of restoration embedded within a matrix of vegetation that is, or resembles, the ecosystem to be restored (e.g., Koch 2007a). The northern jarrah forest is a mosaic of restored forest embedded within a matrix of unmined forest. In contrast, timber managed forests are generally on a rotational harvesting system with silvicultural treatments ranging from the retention of some to none of the trees within a site. Consequently, mature tree retention within timber-harvested landscapes may provide suitable habitat for bats (Perry et al. 2007; Webala et al. 2010). In contrast, the extensive clearing of agricultural lands result in little retention of native vegetation; revegetated sites are typically surrounded by an expanse of tree-less paddocks and farmland mosaic landscapes provide few foraging and roosting resources for bats (e.g., Lumsden et al. 2002a). Both the scale and the management intentions differentiate timber-harvesting landscapes and revegetated agricultural lands from restoration projects aimed at restoring fully functioning ecosystems. Restoration differs from timber-harvesting regrowth primarily in that the area to be restored rarely has any remnant vegetation, and differs from revegetated agricultural landscapes in that restoration actively re-establishes a full suite of historical species whereas revegetated lands assume passive repair once overstorey trees are planted (Hobbs & Cramer 2008; Munro et al. 2009).

In the northern jarrah forest, the mining process involves the complete removal of vegetation within a mine-pit, followed by restoration within ~2 years (Koch 2007a). By the time restoration is five years old, the mine-pit contains scrub vegetation dominated by sapling eucalypts and acacias (Grant 2006). In these early years, bat use will likely be significantly lower in restoration than in unmined forest. Bat activity is typically lower where there is no overstorey vegetation, as in the case of treeless paddocks, when compared to remnant forest (Law & Chidel 2006; Law et al. 2011). Species richness is also generally lower in paddocks compared to remnant forest (e.g., Law et al. 2011), although species richness can increase significantly when paddocks contain remnant trees (Fischer et al. 2010). However, similar species richness does not necessarily equate to similar community composition as the activity levels for some species within paddocks suggests infrequent use, representing only a fraction of their total activity across the landscape (Lumsden & Bennett 2005). Large bodied, less manoeuvrable species are often able to exploit structurally simplified environments (Hanspach et al. 2012) and typically have comparable or higher activity levels within open environments, compared to remnant sites (Law & Chidel 2006; Lumsden & Bennett 2005; McConville et al. 2013).

Within ~20 years, restored forest is typified by pole-sized eucalypts with a dense, high fuel-load *Acacia* understorey (Grant 2006; Grigg et al. 2010). A meta-analysis of Australian post-mining restoration faunal succession, albeit not including bats, concluded that faunal species richness and density increased as restoration aged (up to 27 years) but then plateaued without reaching levels found within unmined reference sites (Cristescu et al. 2012). In the agricultural landscape, sites revegetated more than 10 years previously had double the bat activity of treeless paddocks (Law & Chidel 2006), suggesting that bats preferentially choose revegetated sites over treeless paddocks as vegetation ages. Similar results are found with respect to timber harvesting. In the timber-harvesting landscape, young regrowth forest (15-22 years old) had significantly lower levels of bat activity, when compared to unlogged forest (Law & Chidel 2002; Law & Chidel 2001), but older regrowth had significantly higher levels of bat activity, when compared to younger regrowth (Webala et al. 2011). Thus, it may be plausible to assume that bat activity within restoration will increase as restoration matures but may not reach levels comparable to those within unmined forest for many decades.

Vegetation density, or clutter, is typically considered the best predictor of bat activity within a forested environment, both in terms of tree density and vertical stratification (Adams et al. 2009; Armitage & Ober 2012; Lloyd et al. 2006). The more cluttered the vegetation, the lower the species richness and/or overall activity levels. In revegetated agricultural landscapes bat activity has been negatively correlated with understorey clutter (Law & Chidel 2006; Obrist et al. 2011), peaking when tree densities were ~20- 50 stems ha ^{1} (Hanspach et al. 2012; Lumsden & Bennett 2005). Similarly, in timber-harvested landscapes, understorey vegetation clutter was negatively related to bat activity (Webala et al. 2011). When specifically examining the influences of vertically stratified vegetation structure on bat activity levels, research suggests that vegetation complexity dictates species-specific activity patterns for each strata (Adams et al. 2009; Jung et al. 2012; but see Loeb & Waldrop 2008). More manoeuvrable species are more prevalent than less manoeuvrable species in cluttered sub-canopies, but as the sub-canopy opens the species composition changes, emulating the composition above the canopy where less manoeuvrable species are more prevalent (Adams et al. 2009; Armitage & Ober 2012). Adams et al (2009) found that the sub-canopy and canopy were significantly more cluttered in young regrowth than old regrowth, and that both bat activity and insect abundance was lower within these strata in young regrowth. The strong interaction between vegetation openness and insect abundance, combined with the avoidance of cluttered areas by bats capable of traveling through them, suggests that cluttered environments provide poor quality foraging habitat that are not worth the energetic costs of navigating through (Fenton 1990; Sleep & Brigham 2003). Foraging studies often suggest that habitat structure takes primacy over prey occurrence as a driver of bat activity (Armitage & Ober 2012; Dodd et al. 2012b; Morris et al. 2010). As restoration typically has a higher tree density and more cluttered sub-canopy than unmined forest (Grant 2006; Koch 2007b), restoration will likely have lower bat activity, particularly for the less manoeuvrable species, and different bat community composition than the unmined forest.

Bat Foraging Within Restoration

Foraging echolocation calls can be distinguished from search phase navigation calls in that they contain a characteristic rapid succession of pulses at the termination of the call, termed a “feeding buzz” (Jakobsen & Surlykke 2010; Schnitzler & Kalko 2001). Foraging activity may be quantified either as the number of feeding buzzes/foraging calls (e.g., Webala et al. 2011) or bat activity can be used as a proxy (Dodd et al. 2012b). Gleaning bats, such as *Nyctophilus* species, may use prey-generated noise to take their prey directly from a substrate and it has been proposed that they emit feeding buzzes in half, or fewer, of their foraging attempts (Faure & Barclay 1994; Grant 1991). However, more recent research suggests that while all bats likely emit feeding buzzes they may not always be captured by recording equipment as the feeding buzzes of gleaning bats is of very low amplitude (Schnitzler et al. 2003). Thus, there is the potential of under-representing the foraging activity of gleaning bats when using feeding buzzes to quantify foraging activity. Foraging calls typically comprise ~5% of the echolocation calls recorded during a survey (e.g., Law et al. 2011; Webala et al. 2011), although foraging calls can be >20% of all echolocation calls (Adams et al. 2009), depending on the type of habitat surveyed (Law & Chidel 2006). Forest tracks and riparian zones are important foraging habitat for bats, particularly within production landscapes (Hagen & Sabo 2011; Law & Chidel 2002; Lloyd et al. 2006) where foraging activity in recently disturbed and young regrowth sites is lower than the foraging activity in undisturbed sites or those with older regrowth (Law et al. 2011; Webala et al. 2011). The relationship between foraging activity and prey occurrence is not always clear, with some studies finding a positive relationship and others none, and these relationships are often species specific (Adams et al. 2009; Lumsden & Bennett 2005; Ober & Hayes 2008; Webala et al. 2011). The discrepancy between studies may reflect differences in vegetation clutter. In a timber-harvested landscape, nearly three-quarters of bat feeding activity occurred in older, less cluttered regrowth, where insect occurrence was 2.5 times, but not significantly, greater than in younger, more cluttered regrowth. Within the younger regrowth feeding activity was also more prevalent in the less cluttered vegetation strata (Adams et al. 2009). In pine managed forests bat foraging activity patterns were species-specific with bats suited for open space environments foraging along edges (i.e., forest/open clearcut) while manoeuvrable gleaners avoided open areas, preferentially foraging in unmanaged, denser forest (Morris et al. 2010). Consequently, while prey may be present in certain forest types, they may not be accessible to bats due to the clutter from dense vegetation.

Bat Roosting Within Restoration

A meta-analysis of tree-dwelling bats in North America found that cavity roosting bats preferentially roost in larger, taller trees, within areas of relatively open canopy and close proximity to water, compared to non-roost trees (Kalcounis-Ruppell et al. 2005). Conversely, a review of hollow-use by Australian birds and bats found that a general paucity of data limited generalisations on Australian bat roost selection (Goldingay 2009). Australian bats preferentially chose hollows with slit entrances only slightly larger than themselves (Lumsden et al. 2002b; Taylor & Savva 1988), likely to minimise predation risk. Selection may also be based on the energetic benefits of the roost micro-climate, particularly in the maternity season (Boyles 2009). Bats select roosts with stable micro-climates by typically choosing roosts with higher minimum temperatures that reach their maximum later in the day, and stay warmer longer, than available non-roost hollows (Campbell et al. 2010; Sedgeley 2001). However, stable micro-climates do not always translate into energetic benefits; variable temperatures within maternity roosts mean cool night temperatures for the non-volant young but also warmer day temperatures, allowing mothers to provide sustained lactation (Law & Chidel 2007).

Within the jarrah forest, only the roosting preferences of *N. gouldi* and *V. regulus* have been studied (Webala et al. 2010). This study, in addition to studies elsewhere in Australia, suggest that jarrah forest bats prefer large, mature trees for roosting, although most have been found roosting in anthropogenic structures as well (Lumsden et al. 2002b; Lunney et al. 1988; Rhodes 2007; Taylor & Savva 1988; Tidemann & Flavel 1987; Webala et al. 2010). Slit entrance size and proximity to water were important factors in roost site selection for *C. morio*, *N. geoffroyi*, *N. gouldi*, and *M. planiceps* (Tidemann & Flavel 1987). Bats within a disturbed landscape are rarely found roosting outside remnant patches or remnant trees (but see Law & Chidel 2006; Lunney et al. 1988), even though they are capable of travelling great distances from foraging to roosting areas. In Tasmanian forests four bat species travelled between one and five kilometres from trapping to roosting sites, through areas of regrowth, but were not found roosting within the regrowth (Taylor & Savva 1988). Similarly, when eucalypt plantations were established within an agriculture landscape, not one of the four bat species was found roosting anywhere other than remnant forest, even though *N. geoffroyi* was found to roost under decorticating bark, a phenomenon that readily occurred in both the eucalypt plantation and the remnant forest (Law et al. 2011). When mature forest was absent bats roosted in regrowth in eastern Australia, although typically in the few remaining mature trees (Law & Anderson 2000; Lunney et al. 1988). Roost site selection may also depend on bat sex and the season, with males and non-reproductive females showing more flexibility in roosting structures than reproductive females, who are constrained by maternity roosts (Law & Anderson 2000; Lumsden et al. 2002a). Over two-thirds of male *N. geoffroyi* roosted in a range of anthropogenic, albeit mostly wooden, structures across the agricultural landscape, while female *N. geoffroyi*, and all *C. gouldii* individuals, roosted within remnant forest (Lumsden et al. 2002b). Due to the absence of large trees in restoration it is unlikely that restoration will provide roosting habitat for jarrah forest bats, particularly for reproductive females during the maternity season.

Hypotheses for Bat Use of Restoration

The aim of this study was to investigate the effects of restoration on the commuting, foraging and roosting requirements of bats in the northern jarrah forest of south-western Australia. Drawing from research in the southern jarrah forest and elsewhere, I predicted that the nine species of insectivorous, tree-dwelling bats would respond differently to restoration and that individual species responses would be dictated by their ecomorphology. Overall, the study tested two main hypotheses: (1) bat succession would be congruent with vegetation succession within restoration; and (2) current restoration would meet some, but not all, habitat requirements of jarrah forest bats.

Specifically, I tested the following predictions:

- As restoration matures bat activity and community composition becomes more similar to the unmined forest state (Hypothesis 1).
- Bat communities in restoration with high vegetation density will be distinct from unmined forest as the increased vegetation clutter will make the restoration unsuitable for less manoeuvrable bat species (Hypothesis 1).
- Prey accessibility will be greater in older restoration, compared to younger restoration, matching foraging opportunities within unmined forest (Hypothesis 2).

- Restoration does not yet provide roosting habitat for bats and is unlikely to do so for close to a century, i.e., until hollows form (Hypothesis 2).

Thesis Content

During my PhD, I designed the study in collaboration with my supervisors, Michael Craig and Vicki Stokes. With the help of volunteers, I collected and processed all data (with the slight exception of some of the molecular work for Chapter 2), undertook all statistical analyses with the assistance of my supervisors, and drafted and revised all chapters. This thesis has been written as a series of papers (Chapter 3 has been published), written with the support of my supervisors, and as such is written in the plural form.

This thesis consists of six chapters and an appendix. Chapters 2-5 include results of fieldwork undertaken in the northern jarrah forests, south-western Australia. Chapter 6 concludes with an overall discussion of the main findings of the study and their management implications.

Chapter 2 describes jarrah forest bat activity across the landscape in the context of habitat filters, primarily at the species level but also based on bat species traits. I surveyed bat activity (echolocation calls) and vegetation structure at 64 sites within unmined forest and restored forest in multiple age groups (0-4, 5-9, 10-14 and >15 years). Surveys were conducted over two years, during both the maternity and mating seasons, to (i) determine if habitat filters to bat use of restoration were present; (ii) if present, determine if they are unidirectional or dynamic and; (iii) identify the filters.

Chapter 3 examines dietary partitioning in three species of jarrah forest bat (*C. gouldii*, *N. gouldi* and *V. regulus*). I collected faecal samples over the maternity and mating season of one field year and used a combination of molecular (high-throughput sequencing) and bioinformatics analyses to test (i) the occurrence of dietary partitioning between bat species and between sexes and seasons within a species; and (ii) if dietary divergence was related to ecomorphological divergence.

Chapter 4 builds on the knowledge of Chapter 3 and investigates the foraging behaviour of jarrah forest bats across the restored landscape. During the mating season of 2010 I surveyed bat activity (as a proxy for foraging activity), insect biomass (surveyed using light traps) and vegetation structure to examine prey accessibility within three forest types: 10-14 year old restoration, >15 year old restoration and unmined forest. I used this data to determine if there were differences between restored and unmined forest in terms of (i) vegetation structure; (ii) insect prey; and (iii) the accessibility of insect prey to predatory bats.

Chapter 5 examines the roosting preferences of two jarrah forest bat species (*N. gouldi* and *V. regulus*). It uses information from telemetry of foraging bats, tracked to their diurnal roosts, during both the maternity and mating season, in conjunction with vegetation surveys, to determine (i) species specific bat roosting preferences at three spatial scales (i.e., tree, site, and landscape) and two temporal scales (mating and maternity seasons) and (ii) the relative availability of suitable roosting trees and sites in restoration and adjacent intact forest.

Chapter 6 summarises the key results and contextualises them with respect to conserving bats in a restored landscape.

Appendix 1 provides detailed information on the automated echolocation call identification process and compares the automated identification with manual identification.

Appendix 2 provides supplementary figures for Chapter 4, Chapter 5, and Appendix 1.

Chapter Two

Unidirectional and dynamic habitat filters as a predictive framework to explain bat use of restored eucalypt forests in south-western Australia.

Introduction

Restoration is one key tool to conserving global biodiversity, particularly in the face of increasing land-use change (Hobbs & Harris 2001; Suding 2011). To ensure that restoration is effective in conserving biodiversity, restoration must provide suitable habitat for all species, including fauna (Cristescu et al. 2012). This is particularly important as some species may be slow, or fail, to recolonise restoration (Craig et al. 2012; Majer et al. 2013). The concept of habitat filtering has recently been used as a framework for predicting how species will respond to land-use changes, including restoration (Palmer et al. 1997). In essence, filtering identifies habitat features that slow, or limit, recolonisation of restored areas (Poff 1997) and can elucidate why some species move from the regional species pool into restored areas whilst others do not (Palmer et al. 1997). Conceptually a habitat filter is a habitat feature whose presence, or absence, renders the habitat unsuitable for a species. Filters can be unidirectional, decreasing in magnitude over time, or dynamic, fluctuating in magnitude over time (Craig et al., 2012). In newly restored areas, tree hollows may be an example of a unidirectional filter as this habitat feature is initially lacking but as restoration matures, over decades or longer, hollows form (Vesk et al. 2008). The absence of hollows may render the restoration as unsuitable habitat for hollow dependent species, such as bats (Goldingay 2009), but as restoration ages and hollows form, the magnitude of this filter decreases. In contrast, shrub and tree density may be examples of dynamic filters where species are only able to tolerate a certain threshold density. In the northern jarrah forest, abundance of the skink *Morethia obscura* was found to be inversely proportional to overstorey (>3 m) stem density with both species abundance and stem density changing over relatively short time frames (Craig et al. 2012). Newly restored sites have low stem density but density rapidly increases as fast-growing pioneer shrub species dominate restoration and then decreases as these species either die out or are burned (Koch 2007b).

The habitat filter framework has been successfully applied in the field of restoration ecology for flora (e.g., Matthews et al. 2009; Wallem et al. 2010), but only two studies have specifically examined habitat filters in the context of fauna (Craig et al. 2012; Summerville et al. 2006). This is despite mounting evidence that filtering provides a useful framework for understanding how fauna respond to land-use change (e.g., Hanspach et al. 2012; Pereira et al. 2004; Poff et al. 2010) and thus filtering may be a useful model for predicting faunal recolonisation of restoration. The limited use of the habitat filter framework for fauna may be an artefact of the vegetation heavy focus of restoration, which is primarily concerned with re-establishing plant diversity (Brudvig 2011; Wortley et al. 2013). Restoration ecology generally assumes that once vegetation is established fauna will passively recolonise (Palmer et al. 1997). While restored sites may be comparable in species richness to reference sites, community composition rarely, if ever, resembles that of reference sites, particularly for specialist and disturbance sensitive fauna (e.g., Cristescu et al. 2012; Majer et al. 2013; Taillefer & Wheeler 2012). To ensure re-establishment of both flora and fauna, land managers must be able to accurately predict faunal use of restored systems and identify strategies to facilitate and accelerate the return of slowly recolonising species. Habitat filtering is gaining momentum as this predictive framework in restored systems, modelled both on individual species (Craig et al. 2012) and utilising a traits-based approach (Summerville et al. 2006). In restoration, the ability of species to utilise the same site may change over time as vegetation succession progresses, thus filters to recolonisation can be both unidirectional or dynamic (Craig et al. 2012). Land managers, who are tasked with conserving fauna in restored systems, can use the habitat filter framework to identify where active management might be required.

Habitat filtering is a useful framework for studying the response of bats to land-use change (Hanspach et al. 2012), including restoration. Bats are highly mobile, often using multiple habitats to meet their foraging and roosting needs (Borkin & Parsons 2011) and this mobility means bats have the capacity to rapidly respond to filters. In addition, insectivorous bats possess particular traits, e.g., wing morphology and echolocation call structure, which influence manoeuvrability, foraging and microhabitat use (Norberg & Rayner 1987; Siemers & Schnitzler 2004). Variation in the expression of traits facilitates habitat partitioning in bats (Aldridge & Rautenbach 1987; Sleep & Brigham 2003) and consequently makes bats susceptible to a range of habitat filters. Most studies examining bat habitat use of vegetated landscapes conclude that vegetation structure is the predominant predictor of bat use. Large, less manoeuvrable species exploit open space environments and small, more manoeuvrable species are capable of using more densely vegetated environments (e.g., Armitage & Ober 2012; Lloyd et al. 2006; Morris et al. 2010). The habitat within restored forest changes over time as vegetation succession progresses; newly restored sites are open as they contain young plants but as restoration matures, vegetation cover increases, often exceeding the cover in reference sites (e.g., Norman et al. 2006; Vesk et al. 2008). Thus bats may differentially use restoration depending on restoration age and the specific traits possessed by each bat species. Large, less manoeuvrable bat species may favour young restoration and avoid old restoration (e.g., Patriquin & Barclay 2003) while the reverse may apply for those species adapted to closed or edge environments (e.g., Law & Chidel 2006). The use of traits to predict bat habitat use across environmental gradients has been successful in both agricultural (Duchamp & Swihart 2008; Hanspach et al. 2012) and urban (Jung & Kalko 2011; Threlfall et al. 2012) landscapes. Globally, bats are experiencing population declines attributed to the structural simplification of the landscape and habitat loss (e.g., Farrow & Broders 2011; Mickleburgh et al. 2002; Webala et al. 2011). Restoration is one key way to ameliorate land degradation and habitat loss, and the habitat filtering framework may be an effective way to identify conservation strategies for bat populations in restored landscapes.

The purpose of our study was to use the habitat filter framework to identify which habitat features, if any, limit the suitability of restoration as bat habitat. Our study area was the northern jarrah (*Eucalyptus marginata*) forest, restored after bauxite mining within the biodiversity hotspot of south-western Australia (Myers et al. 2000). Alcoa of Australia (hereafter Alcoa) clears, mines and restores ~600 ha of jarrah forest annually (Koch 2007a) with the aim of restoring a fully functioning jarrah forest ecosystem (Grant 2006). Restoration is successful in terms of floristic composition and environmental management (Bell & Hobbs 2007; Koch & Hobbs

2007) but faunal community composition within restored forest does not always converge on unmined forest communities (Craig et al. 2012; Majer et al. 2013). Habitat filtering has been successfully applied as a framework for understanding reptile and small-mammal successional patterns within restored northern jarrah forest (Craig et al. 2012), but not yet for bats. Nine species of insectivorous, forest-dwelling bats inhabit the northern jarrah forest (Table 1), including two endemic, range-restricted species (Churchill 2008). A recent study in the southern jarrah forest investigated the response of bats to logging history and found that jarrah forest bats display differential tolerances to vegetation structure (Webala et al. 2011).

Table 1: Bat species traits. Body mass and forearm length were measured from bats trapped in the study area for all species (J. Burgar, unpublished data) with the exception of *T. australis* (measurements taken from Churchill 2008). Wing loading and aspect ratio values were taken from Fullard et al. (1991) with the exception of *F. mackenziei* (N.L. McKenzie and R.D. Bullen, pers. comm). Echolocation call characteristics were taken from reference library echolocation calls recorded in the study area as well as the southern jarrah forest (J. Burgar, unpublished data).

Species	Body mass (g)	Forearm length (mm)	Aspect Ratio	Wing-loading (N m^{-2})	Call Frequency
T. australis	36.0	60.0	11.1	19.6	Low
F. mackenziei	23.7	50.5	6.9	8.6	Low
C. gouldii	14.9	44.4	5.5	9.1	Low
M. kitcheneri	10.5	34.1	6.1	12.3	Low
V. regulus	5.5	31.6	5.3	6.8	Medium
C. morio	7.9	35.5	4.8	6.7	High
Nyctophilus spp.*	10.7	41.3	5.3	6.6	Linear

* Traits from *N. geoffroyi*, *N. gouldi* and *N. major* were pooled to comprise the *Nyctophilus* spp. group.

Thus, we were interested in determining if ecomorphology would enable predictions of bat habitat use within a restored landscape. Our study objectives were to (i) determine if habitat filters to bat use of restoration were present; (ii) if present determine if they are unidirectional or dynamic and; (iii) identify the filters. For small, manoeuvrable species we predicted the presence of unidirectional filters to habitat use of restoration; i.e., that these bats would have low activity in newly restored forest but activity levels would increase as restoration aged and potentially converge on activity levels in unmined forest. For large, less manoeuvrable species we predicted the presence of dynamic filters; i.e., that these bats would have high activity in young restoration and unmined forest compared to relatively low activity in forest restored ≥5 years previously. We anticipated that bats of the northern jarrah forest would exhibit similar patterns as bats with similar traits elsewhere, and that tree density would be the most influential habitat filter to bat use of restoration (Hanspach et al. 2012).

Methods & Materials

Study area and experimental design

We surveyed bats at Alcoa's Huntly minesite (32°36'S, 116°07'E), located ~80-100 km SSE of Perth, Western Australia (Figure 1). The region is characterised by a Mediterranean climate with cool, wet winters and dry, warm summers. From 1990-2012 Huntly rainfall averaged 1180 mm annually, with >75% falling between May and September. The first year of the study (2010) was exceptionally dry and received barely half the average rainfall (630 mm) while the second year (2011) received average rainfall (1205 mm).

Alcoa mines bauxite in the northern jarrah forest (~700 000 ha), where other land-uses include conservation, timber production, water supply and recreation (Dell et al. 1989). Alcoa has been mining in the forest for >40 years, and due to the spatial distribution of bauxite ore across the landscape, Huntly minesite (~15 000 ha) is a patchy mosaic of unmined and restored forest. Both forest types have an overstorey dominated by two eucalypt species, jarrah and marri (*Corymbia calophylla*). Being within a biodiversity hotspot, the unmined forest is incredibly diverse with 300-400 plant species in areas typically mined, and a recorded maximum of 163 species in 0.1 ha (Koch 2007b). Restoration practices return ~141 plant species to restored sites and the floristic similarity between unmined forest and 5 year old restoration averages 22% (Koch 2007b).

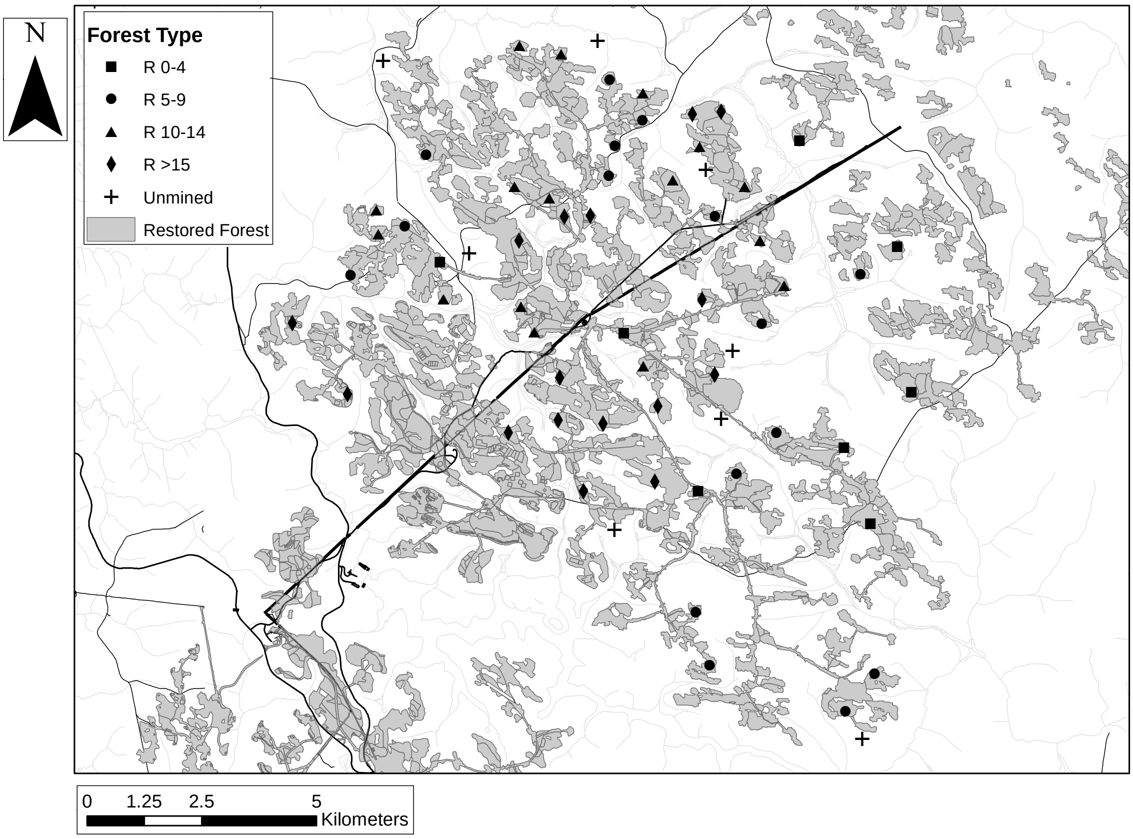


Figure 1: Map showing the spatial arrangement of the 64 sites at Alcoa's Huntly minesite in south-western Australia. Light grey lines denote streams and black lines denote sealed roads.

To assess bat successional patterns, and identify potential filters, we surveyed bats in five forest types: four ages of restoration (0-4, 5-9, 10-14 and >15 years) and unmined forest. These restoration ages represent different stages of vegetation succession within restored mine-pits (Norman et al. 2006). The tree (plant >5 m in height) density of the restored forest is highly variable, particularly in ≥5 year old restoration (range = 44-6787 stems ha^{-1}; mean = 2269 ± 226 SE). Alcoa monitors mine-pits nine-months post-restoration and categorises them as sparse, desirable or dense based on eucalypt density: <500 stems ha^{-1}, 500-2500 stems ha^{-1}, and >2500 stems ha^{-1}, respectively (Grant 2006). To capture tree density variation we selected sites to reflect multiple age classes (above) of various tree densities. Adaptive management of restoration meant that <5 year old restoration had tree densities only within the desirable category while the other restored forest ages had densities in the desirable and dense categories. We selected eight sites each within eight treatments for a total of 64 sites: desirable 0-4, desirable 5-9, dense 5-9, desirable 10-14, dense 10-14, desirable >15, dense >15 and unmined forest. There was no difference in eucalypt stem densities between the desirable and dense treatments for restored forest 5-9 (t_{14}=-1.40, P=0.184), 10-14 (t_{14}=-0.35, P=0.786) and >15 (t_{14}=-0.84, P=0.416) so we used forest type (restored forest 0-4, 5-9, 10-14, >15 and unmined forest) as a variable rather than treatment type. To be included in the study, sites had to meet the following criteria: >4 ha size, >500 m distance between detector locations, at least one edge bordered by unmined forest and detectors >80 m from other ages of restored forest or unmined forest; and <5 stems ha^{-1} of *E. patens* (not included in the seed mix after 1993). Due to the progression of mining in a north-easterly direction, and the paucity of potential sites within some restoration ages, there was limited interspersion between sites of some forest types (Figure 1). This was unlikely to bias the results given the distances that bats travel in one night (Chapter 5).

Bat surveys

Bat surveys were conducted four times at each of 64 sites between October and March in both 2010/2011 and 2011/2012 for a total of 512 survey nights. Each site was surveyed using ultrasonic detectors (Anabat, Titley Electronics, Australia), to record bat echolocation calls, and were set to record from 30 mins before sunset until 30 mins after sunrise. We deployed eight detectors each survey night to simultaneously survey each treatment (forest type / applicable eucalypt density), to evenly distribute any potential effect of night between treatments. All sites were surveyed once before being re-surveyed and we did not survey on nights with rain or high winds. Detectors were placed on PVC poles 1.5 m above the ground and angled at 45°, facing a gap in the vegetation to minimise vegetation attenuation (Law & Chidel 2002), and oriented towards the closest unmined forest edge. Acoustic surveys are limited in that not all species are equally detectable: soft amplitude echolocation calls are less readily detected than loud calls (Jakobsen & Surlykke 2010; Jung et al. 2014; Schnitzler & Kalko 2001). However, as we are not making comparisons between species but rather focusing on patterns of relative bat activity between different forest types within each species, acoustic surveys were the most appropriate method for our study aims.

Vegetation surveys

Vegetation structure was assessed at each site each field year following bat surveys (i.e., between April and July 2011 and 2012). Vegetation surveys consisted of sampling vegetation characteristics in five 5 x 5 m quadrats at each site, one at the detector location and four 30 m from the detector location in each of the cardinal directions. We quantified vegetation structure in each quadrat by visually estimating percent vegetation cover in four strata: overstorey (>15 m), midstorey

(5 to 15 m), shrub (0.75 to 5 m) and ground (<0.75 m). We measured the maximum vegetation height, using a tape measure for the ground and shrub strata and a tree vertex for the overstorey and midstorey strata. Log and litter cover were quantified by visual estimation of percent cover in each quadrat. Canopy cover was measured from canopy photographs taken at the centre of each quadrat (Macfarlane 2011) while canopy height was the average of the five tallest overstorey plants within 10 m of the centre of all quadrats. Tree density was measured differently between years. In the first year we calculated tree density by counting the number of trees in each of the quadrats and extrapolating to stems ha^{-1}. In the second year we measured the distance from the nearest tree to the detector location, and points 10 m, 20 m, and 30 m from the detector location in each of the cardinal directions and used a formula to calculate tree densities: $10\,000 / 2 * x^2$ where x is the average of the nearest tree distances. Tree density measurements were consistent between years despite the different methods used (ANOVA to test the interaction between year and forest type: $F_{4,118} = 1.654$, $P = 0.165$).

Data analysis

Prior to analysis we excluded four bat survey nights when detectors failed, for a total of 508 detector nights from 64 sites. We quantified bat activity as the number of bat call files per night; call files contained a group of echolocation pulses recorded within a 15 second span, which we assumed to belong to one individual bat (Fenton 1999; O'Farrell et al. 1999b). Bat call files were downloaded using CFCRead© software (C. Corben/Titley Electronics) and processed using AnaLook version 3.8 (C. Corben; http://hoarybat.com). All downloaded call files were filtered (see Appendix 2 for filter details) to remove extraneous noise and extract echolocation call parameters for subsequent identification. Three species of *Nyctophilus* occur in the study region (*N. geoffroyi*, *N. gouldi*, *N. major;* J. Burgar, unpublished data) but their calls are indistinguishable when recorded using an Anabat detector and zero-crossing analysis interface module (Adams et al. 2010) and so calls from these three species were pooled as *Nyctophilus* spp. Bat calls were automatically identified to species, or species group, using a random forest model, a type of supervised learning machine, from the caret package (Kuhn 2008) within the statistical program R (R Core Team 2013). We trained the random forest model using a regional call library comprising 142 call files and 7 485 individual pulses, for all species, and a noise class (Appendix 1 for details on the automation process). The model identified individual pulses so we identified a call file as the species / species group with the highest number of pulses in that file. In the case of a tie we identified the call file as unknown. The automation process provided a certainty score, or probability, that each echolocation pulse was assigned to the correct species, enabling us to classify pulses with low certainty scores as unknown. To be identified to a species, or species group, a pulse had to have a certainty score >0.4125 of being correctly assigned to that species or species group. We added a 0.30 buffer to the 1 in 8 random chance (0.125) of a species / species group being assigned to a class. Comparison of automated identification at various certainty scores against manual identification showed that the random forest model identified calls with a certainty score ≥0.4125 as 93% similar to manual identification while retaining a large proportion of identified calls. More conservative certainty scores classified greater proportions of calls as unknown (see Appendix 1 for full details on automation vs. manual identification).

Statistical analysis

We ran Spearman correlations on all explanatory variables and omitted highly correlated (>0.70) variables from the analyses. Final explanatory variables were canopy height, canopy cover, tree density, midstorey cover, shrub height, shrub cover, ground cover, and log cover. We visually checked the final variables to ensure they were normally distributed and transformed those that were not (natural log transformation for log cover; square root transformation for canopy height, tree density, shrub cover, and ground cover).

Vegetation Structure

We took a model-based approach to test the effect of field year and forest type on vegetation structure, using the function manyglm in the R package mvabund (Wang et al. 2012). This approach uses a multivariate generalised linear model (GLM) framework to make community level and vegetation structure / taxon-specific inferences by fitting separate GLMs to each variable, with a common set of explanatory variables, and testing significance through resampling-based hypothesis testing (Wang et al. 2012). We ran negative binomial GLMs with a two-dimensional matrix of the vegetation structure variables as the dependent variable and both field season, forest type, and the interaction between the two as the independent variables. Wald test statistics were constructed assuming correlation (matrix shrunk by parameter 0.84) and P-values were calculated using 999 resampling iterations via PIT trap resampling.

Bat Community Composition

To determine the presence of habitat filters, we again used a model-based approach to test the effect of field year and forest type on bat community composition. We considered a filter to be present if bat species community composition and activity levels differed between forest types and/or field years. We ran the multivariate GLMs with mean annual bat activity (for each species/species grouping at each site) as the dependent variable and field year, forest type, and the interaction between the two as the independent variables. We again used the function manyglm, with a negative binomial distribution, in the R package mvabund (Wang et al. 2012). We assumed that there was no correlation between bat species activity levels so constructed log likelihood ratio test statistics and calculated *P*-values using 999 resampling iterations via PIT trap resampling.

Filters to Bat Habitat Use – Species Level

We identified potential species-specific habitat filters to bat use of restoration by modelling bat activity (for each species / species grouping) against vegetation structure variables for sites in the restored forest. Employing an information-theoretic approach to model selection (Burnham & Anderson 2002) we constructed 18 separate models, a null model, a full model with all of the vegetation structure variables and then two models for each individual vegetation structure variable: the variable in its original form and both the original and quadratic form to test linear and curvilinear relationships, respectively. We ran generalised linear mixed models (GLMMs) for each species / species group with bat activity as the response variable, vegetation structure variables as the explanatory variables and both site and field year as random factors. Models had a negative binomial regression structure and a log-link function using the R package glmmADMB (Fournier et al. 2012; Skaug et al. 2013). Models used the Laplace likelihood approximation (Raudenbush et al. 2000) and type III sum of squares. Models were ranked based on Akaike's information criterion (AIC) score and AIC weights using the R package MuMIn (Barton 2013). From the global set we considered the models with the highest AIC weights as the best model(s) describing habitat filters to bat species use of restoration. We only considered best model(s) that ranked higher than the null model. Where there was only one best model we determined model parameter estimates for that one model but where more than one best model was identified we used model averaging to calculate parameter estimates from these models (Burnham & Anderson 2002). We conducted deviance tests to assess the goodness of fit of each of the best models. We also calculated evidence ratios (ER; Anderson 2008) to weigh support for consideration of each of the vegetation structure variables as habitat filters to bat use of restoration. Evidence ratios are the sum of AIC weights for models that include the vegetation structure variable divided by the sum of AIC weights from models that did not include the vegetation structure variable.

Final models were checked to ensure the overdispersion parameters were less than 1 (Zuur et al. 2009). To assess the potential of spatial auto-correlation between sites we examined correlograms, graphical representations of the spatial correlation between sites at a range of distances, for each species using the R ncf package (Bjornstad 2012). Spline correlograms showed that there was no spatial correlation between sites for any species other than *Vespadelus regulus*, which showed a small amount of positive spatial correlation for sites less than 1 000 m apart. This minimal amount of spatial correlation for *V. regulus* was accounted for by using site as a random factor in the GLMMs.

Results

We recorded 29 027 bat call files over the two field years, with more call files recorded in the second field year than the first (18 781 vs. 10 246) (Table 2). Using the 0.4125 certainty threshold and the criteria for ties, 4668 call files (16%) were classified as unknown while 24 359 calls were identified to species / species group. *V. regulus* was detected most frequently (16 597 call files or 68%) and *Falsistrellus mackenziei* least frequently (237 call files or 1%).

Table 2: Number of calls recorded for each species during each field year. In addition 4668 (16%) calls (550 (5%) and 4118 (22%) for the first and second field year, respectively) were classified as unknown.

	T. australis	*F. mackenziei*	*C. gouldii*	*M. kitcheneri*	*V. regulus*	*C. morio*	*Nyctophilus* spp.
1st Year	1044	56	857	173	6815	391	360
2nd Year	1246	181	1999	303	9782	346	806
Total	2290	237	2856	476	16597	737	1166

Vegetation Structure

Vegetation structure was highly variable across forest types (Figure 2; Table 3). Multivariate analyses indicated that vegetation structure was significantly different between unmined forest and each of the restored forest types (R 0-4 – unmined P = 0.001; R 5-9 – unmined P = 0.001; R 10-14 – unmined P = 0.001; R >15 – unmined P = 0.006) and non-significant between the two field years (P = 0.058). There was a significant interactive effect of forest type and year for restored forest <5 years (P = 0.001) but not for restored forest ≥5 years (R 5-9 –

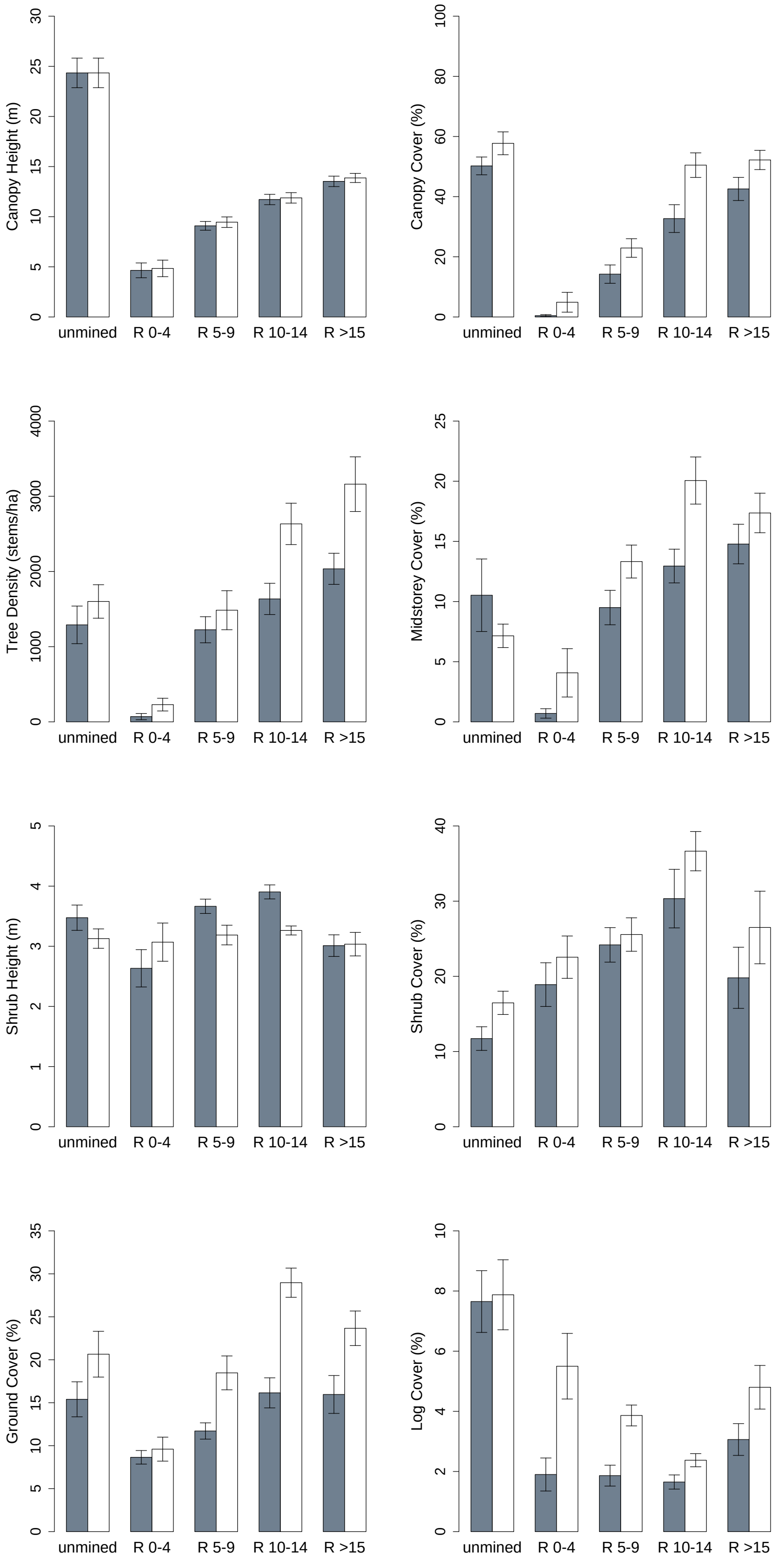


Figure 2. Vegetation structure mean (±SE) for the northern jarrah forests. Dark grey bars indicate the first field year while white bars denote the second field year.

Table 3. Vegetation community structure modelled across a restored landscape in south-western Australia. Response variables were ordered so that tests indicate differences in vegetation community structure between each restored forest type and unmined forest and between the second, compared to the first, field year. Wald scores are provided; P-values were adjusted to account for multiple testing and significance is indicated by MNS ≤0.10, * ≤0.05, ** ≤0.01, and * ≤0.001.**

	Intercept	R 0-4	R 5-9	R 10-14	R >15	Field Yr 2	R 0-4 * Field Yr 2	R 5-9 * Field Yr 2	R 10-14 * Field Yr 2	R >15 * Field Yr 2
Multivariate Statistics	34.62***	10.05***	6.42***	4.85***	4.05**	2.90^{MNS}	4.64***	3.06^{MNS}	2.99^{MNS}	2.18
CanHgt	8.44***	3.10***	2.37*	1.73	1.47	0.00	0.00	0.08	0.00	0.00
CanCov	18.13***	6.89***	4.74***	1.63	0.63	0.48	3.30**	0.90	0.79	0.16
TreDens	29.71***	8.45***	0.35	0.87	1.65	0.74	2.37*	0.11	0.62	0.51
MidCov	12.00***	5.45***	0.43	0.87	1.41	1.46	3.71**	2.20	2.56*	1.75
ShrbHgt	5.07***	0.95	0.41	0.72	0.44	0.30	0.79	0.22	0.24	0.24
ShrbCov	5.07***	1.09	1.68	2.23	1.08	0.83	0.45	0.62	0.42	0.28
GrdCov	6.63***	1.15	0.82	0.16	0.24	0.39	0.13	0.50	0.76	0.56
LogCov	1.75**	2.06***	2.62*	2.45*	1.61	0.20	1.40	1.20	0.62	0.72

unmined *P* = 0.066; R 10-14 – unmined *P* = 0.069; unmined – R >15 years *P* = 0.293). Multivariate, compared to univariate, tests were more powerful in detecting differences in vegetation structure between restored and unmined forest. Vegetation structure specific tests revealed that canopy height, canopy cover, tree density, midstorey cover and log cover were driving these differences as all were significantly lower in <5 year old restoration than unmined forest (all *P* = 0.001; Table 2). However, when examining the interactive effect of field year on vegetation structure we found that while canopy height, canopy cover, and tree density were consistently lower in <5 year old restoration compared to unmined forest during both field years, there was no difference in midstorey or log cover between <5 year old restoration and unmined forest during the second field year (midstorey cover year 1 W = 5.29, *P* = 0.001, year 2 W = 1.88, *P* = 0.073; log cover year 1 W = 2.06, *P* = 0.002; year 2 W = 0.43, *P* = 0.692). As restoration aged, vegetation structure became more similar to that of unmined forest; 5-9 year old restoration was only significantly different from unmined forest with respect to canopy height (*P* = 0.035), canopy cover (*P* = 0.001) and log cover (*P* = 0.030) while only log cover differed significantly between 10-14 year old restoration and unmined forest (*P* = 0.039). There were no significant differences in vegetation structure between unmined forest and >15 year old restoration at the univariate level.

Bat Community Composition

Bat activity was highly variable within and between forest types and years, ranging from a mean of <3 to >80 calls per night depending on the species and forest type (Figure 3; Table 4). Multivariate analyses indicated that, at the community level,

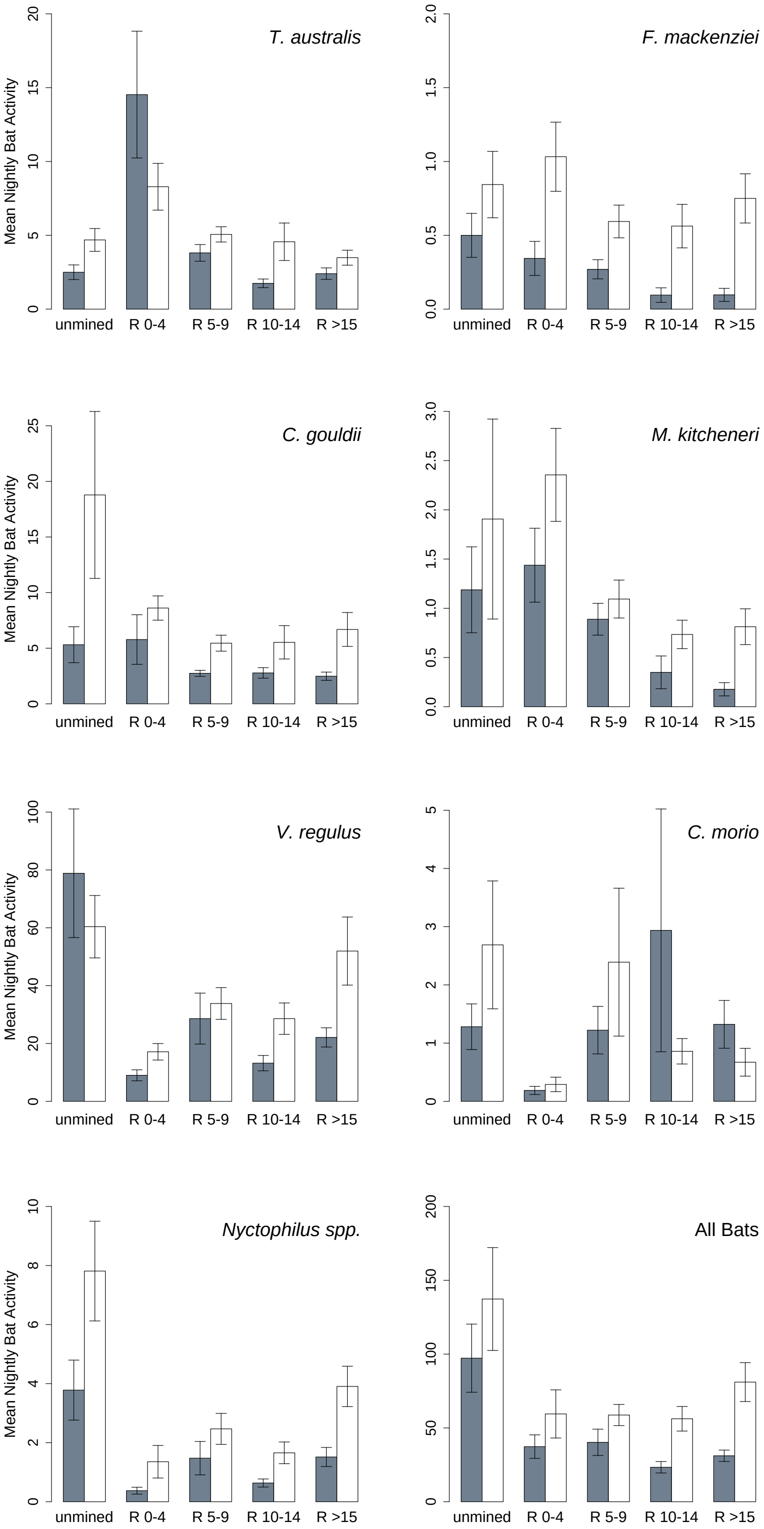


Figure 3. Species specific mean (±SE) nightly bat activity for the northern jarrah forests. Dark grey bars indicate the first field year while white bars denote the second field year. Note the different scales along the y-axis.

Table 4. Bat community activity modelled across a restored landscape in south-western Australia. Response variables were ordered so that tests indicate differences in bat community activity between each restored forest type and unmined forest and between the second, compared to the first, field year. Likelihood ratio scores are provided; P-values were adjusted to account for multiple testing and significance is indicated by MNS ≤0.10, * ≤0.05, ** ≤0.01, and * ≤0.001.**

	Intercept	R 0-4	R 5-9	R 10-14	R >15	Field Yr 2	R 0-4 * Field Yr 2	R 5-9 * Field Yr 2	R 10-14 * Field Yr 2	R >15 * Field Yr 2
Bat Community	464.10	66.15***	20.18 ^{MNS}	57.56***	40.30***	18.23^{MNS}	14.97*	2.83	13.98	14.45 ^{MNS}
T. australis	6.26	20.87***	1.31	1.73	0.09	2.62	5.62	0.48	0.89	0.18
F. mackenziei	3.97	2.62	0.42	4.18	4.18	0.17	2.97	0.05	2.62	3.76
C. gouldii	34.18	0.02	3.96	4.52	5.83	11.16*	2.47	1.39	1.14	0.18
M. kitcheneri	0.07	0.26	0.68	3.81	12.12**	0.60	0.00	0.00	0.09	4.26
V. regulus	407.95***	22.37***	8.78 ^{MNS}	25.3***	13.00**	0.44	2.45	0.79	4.33	4.76
C. morio	0.03	5.48^{MNS}	0.00	1.47	0.05	0.83	0.00	0.00	4.12	2.05
Nyctophilus spp.	11.65	14.54**	5.03	16.54**	5.03	2.42	1.46	0.11	0.79	0.26

bat activity was significantly higher in unmined forest than any of the restored forest types (R 0-4 – unmined *P* = 0.001; R 10-14 – unmined *P* = 0.001; R >15 – unmined *P* = 0.001) with the exception of 5-9 year old restoration where the difference was non-significant (*P* = 0.065). Community level bat activity was not significantly different between the two field years (*P* = 0.074) and there was only a weak interactive effect of field year and forest type on bat activity (interaction between forest type and year: R 0-4 *P* = 0.047, R >15 *P* = 0.074).

For small, manoeuvrable bats we detected the presence of a unidirectional filter for *V. regulus*, potential unidirectional filter for *Chalinolobus morio* and a dynamic filter for *Nyctophilus* spp. *V. regulus* activity levels were consistently lower in restored forest compared to unmined forest (R 0-4 – unmined *P* = 0.001; R 5-9 – unmined *P* = 0.073; R 10-14 – unmined *P* = 0.001; R >15 – unmined *P* = 0.008), supporting the existence of habitat filters to *V. regulus* use of restoration. *C. morio* had marginally non-significant lower activity levels in <5 year old restoration, compared to unmined forest (*P* = 0.087), but then no difference in activity levels for ≥5 year old restoration and unmined forest, hinting at the presence of a unidirectional filter. In contrast, *Nyctophilus* spp. activity patterns support the existence of a dynamic habitat filter to use of restoration as there was significantly lower activity in <5 year old (*P* = 0.003) and 10-14 year old (*P* = 0.002) restoration, compared to unmined forest, but otherwise activity levels were similar between restored and unmined forest.

For large, less manoeuvrable species we did not detect the presence of filters for any species other than *Mormopterus kitcheneri* and this filter was dynamic.

Tadarida australis had significantly higher levels of activity in <5 year old restoration, compared to unmined forest ($P = 0.001$), but then no difference in activity levels for ≥5 year old restoration and unmined forest. Neither *F. mackenziei* nor *C. gouldii* activity levels suggested the presence of habitat filters to their use of restoration. However, *C. gouldii* was the only bat species to have significantly higher activity levels in the second, compared to the first, field year ($P = 0.011$). *M. kitcheneri* had similar activity levels in <15 year old restoration as unmined forest but had significantly lower activity levels in >15 year old restoration as compared to unmined forest ($P = 0.008$).

Filters to Bat Habitat Use – Species Level

We identified one best habitat filter model to describe bat use of restoration for each species / species group, with the exception of *V. regulus* where no models were ranked higher than the null model (Table 5). Models including tree density as the habitat filter best explained bat use of restoration for three of the large bodied, less manoeuvrable species (Table 6). As tree density increased, activity levels for *T. australis*, *F. mackenziei* and *C. gouldii* decreased but while this was a linear relationship for *F. mackenziei* and *C. gouldii, T. australis* activity decreased until tree density reached ~1600 stems ha^{-1} and then activity levels plateaued as tree density increased (Figure 4). Canopy height best explained *M. kitcheneri* activity; as canopy height increased *M. kitcheneri* activity decreased (Figure 4). The quadratic form of midstorey cover was identified as the one best habitat filter model to describe bat use of restoration for two of the smaller and more manoeuvrable species (Table 6). *C. morio* and *Nyctophilus* spp. activity levels increased as midstorey cover increased but only to a point (~15% midstorey cover for *C. morio* and 20% cover for *Nyctophilus* spp.) and then activity levels for both species decreased with increasing cover (Figure 4).

Deviance tests indicated that each of the one best models were better at explaining the data than the full model (drop in deviance (D_{drop}) *P* values ranged from 0.139 to 0.957; Table 6). Evidence ratios (ERs) provided strong evidence to support the vegetation structure variable in the best model as a predictor of bat use of restoration for large bodied, less manoeuvrable species (*T. australis* ER = 5.67, *F. mackenziei* ER = 1.78, *C. gouldii* ER = 4.26, *M. kitcheneri* ER = incalculable or infinity). There was weaker, but still some, evidence to support acceptance of the vegetation structure variable identified by the best model for *C. morio* (ER = 1.17) and *Nyctophilus* spp. (ER = 1.27).

Table 5. Habitat filters to bat use of restoration in the northern jarrah forests. AICc scores for the top models are as follows: *T. australis* 2210.57, *F. mackenziei* 739.23; *C. gouldii* 2276.40; *Mormopterus* sp.4 1052.14; *V. regulus* 3650.40; *C. morio* 1130.11; and *Nyctophilus* spp. 1467.35. The full model included all terms (df=20), the linear models had one variable (df=5), and the quadratic models (indicated by ^2) had each variable and the appropriate squared variable (df=6). Top models (ΔAIC <1.0) are highlighted in bold.

	T. australis		*F. mackenziei*		*C. gouldii*		*M. kitcheneri*		*V. regulus*		*C. morio*		*Nyctophilus* spp.	
	ΔAIC	AIC wgt	ΔAIC	AIC wgt	ΔAIC	AIC wgt	ΔAIC	AIC wgt	ΔAIC	AIC wgt	ΔAIC	AIC wgt	ΔAIC	AIC wgt
Full model	11.38	0.00	16.34	0.00	19.85	0.00	21.52	0.00	19.73	0.00	23.46	0.00	10.07	0.00
CanHgt	5.52	0.05	2.89	0.10	3.46	0.11	**0.00**	**0.73**	0.96	0.07	6.09	0.02	6.06	0.02
CanHgt ^2	7.28	0.02	3.89	0.06	5.46	0.04	2.00	0.27	0.88	0.07	4.86	0.05	5.03	0.03
CanCov	24.3	0.00	7.14	0.01	8.18	0.01	14.68	0.00	0.46	0.09	6.55	0.02	5.38	0.03
CanCov ^2	7.86	0.02	8.45	0.01	9.74	0.00	12.15	0.00	2.48	0.03	5.60	0.03	5.56	0.02
TreDens	6.08	0.04	**0.00**	**0.43**	**0.00**	**0.59**	12.63	0.00	1.00	0.07	5.99	0.03	2.40	0.12
TreDens ^2	**0.00**	**0.81**	1.39	0.21	1.96	0.22	12.60	0.00	2.00	0.04	2.02	0.19	4.22	0.05
MidCov	24.36	0.00	3.92	0.06	10.76	0.00	16.43	0.00	1.02	0.07	6.21	0.02	1.89	0.16
MidCov ^2	5.22	0.06	5.72	0.02	11.36	0.00	15.82	0.00	2.98	0.03	**0.00**	**0.52**	**0.00**	**0.40**
ShrbHgt	32.10	0.00	9.54	0.00	8.70	0.01	25.09	0.00	1.06	0.07	6.64	0.02	7.01	0.01
ShrbHgt ^2	32.22	0.00	10.24	0.00	10.76	0.00	23.66	0.00	2.72	0.03	7.67	0.01	8.73	0.01
ShrbCov	33.06	0.00	8.62	0.01	11.78	0.00	26.37	0.00	0.76	0.08	6.80	0.02	5.11	0.03
ShrbCov ^2	35.04	0.00	9.26	0.00	12.78	0.00	27.49	0.00	0.90	0.07	8.46	0.01	2.96	0.09
GrdCov	33.20	0.00	4.50	0.05	11.88	0.00	20.07	0.00	1.04	0.07	6.89	0.02	7.81	0.01
GrdCov ^2	31.88	0.00	6.09	0.02	13.68	0.00	22.04	0.00	3.10	0.02	6.50	0.02	9.36	0.00
LogCov	33.16	0.00	9.79	0.00	12.36	0.00	27.56	0.00	0.00	0.11	6.80	0.02	7.20	0.01
LogCov ^2	32.4	0.00	11.85	0.00	11.98	0.00	27.98	0.00	0.46	0.09	8.47	0.01	7.77	0.01
Null Model	31.16	0.00	7.89	0.01	10.35	0.00	25.59	0.00	**0.00**	**0.16**	4.92	0.04	5.79	0.00

Table 6. Parameter estimates for top model(s) predicting species specific habitat filters to bat use of the restored landscape. No models ranked better than the null model for *V. regulus*. Model-averaged estimates are provided for *V. regulus* where seven "best" models (habitat filters) were identified. Drop in deviance indicates the drop in deviance from the full model (all parameters) to the model with only the selected parameter(s). Quadratic terms are indicated by ^2.

Species	Parameter	Estimate	SE	z Value	*P*	Drop in Deviance	Drop in Deviance *P*
T. australis	Intercept	2.74	0.30	9.24	<0.001	18.42	0.188
	Tree Density	-0.06	0.01	-4.81	<0.001		
	Tree Density ^2	0.00	0.00	2.80	0.005		
F. mackenziei	Intercept	-0.43	0.56	-0.76	0.444	15.51	0.415
	Tree Density	-0.02	0.01	-3.25	0.001		
C. gouldii	Intercept	1.93	0.33	5.88	<0.001	12.00	0.679
	Tree Density	-0.01	0.00	-3.72	<0.001		
M. kitcheneri	Intercept	2.60	0.58	4.50	<0.001	10.33	0.799
	Canopy Height	-0.96	0.16	-5.86	<0.001		
C. morio	Intercept	-1.25	0.39	-3.24	0.001	6.34	0.957
	Midstorey Cover	0.17	0.06	3.03	0.002		
	Midstorey Cover ^2	-0.01	0.00	-2.87	0.004		
Nyctophilus spp.	Intercept	-0.67	0.40	-1.67	0.094	19.72	0.139
	Midstorey Cover	0.11	0.04	2.80	0.005		
	Midstorey Cover ^2	-0.00	0.00	-2.01	0.045		

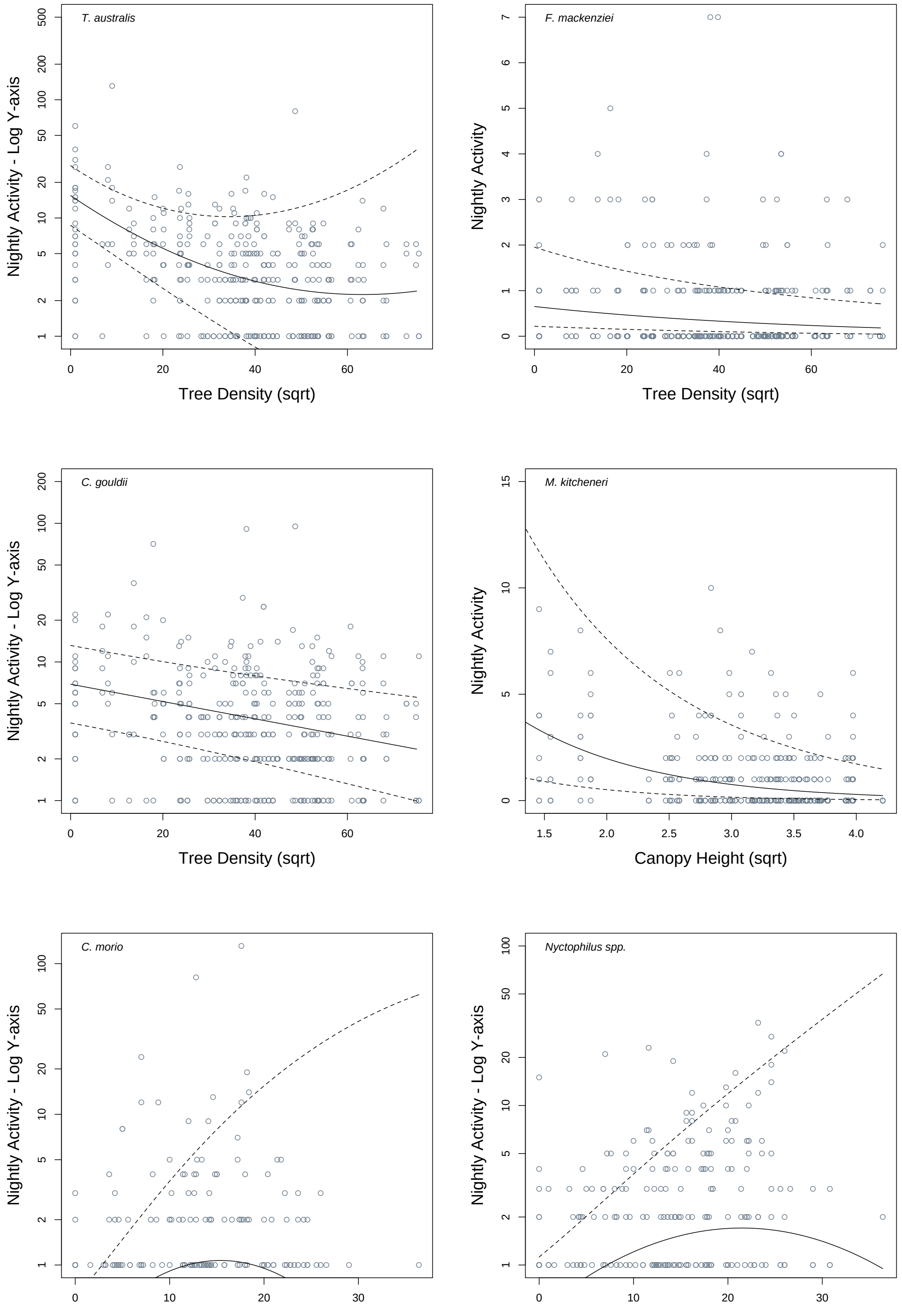


Figure 4. Goodness of fit representation of the observed data and predicted top habitat filter model for each species / species group where the top model ranked higher than the null model.

Discussion

Presence of Filters

Although bat activity was highly variable within and between forest types all bat species / species groups were detected in both restored and unmined forest. Unlike vegetation structure, which became more similar to the vegetation structure of unmined forest state as restoration aged, bat succession towards the unmined forest bat community was not apparent. Overall bat activity was consistently lower in restoration, compared to unmined forest, indicating that restoration limits bat use and that habitat filters are present. Our findings corroborate other bat research, showing different community composition within revegetated compared to remnant sites (Law et al. 2011), and other fauna studies suggesting filters to faunal use of restoration (e.g., Craig et al. 2012; Cristescu et al. 2012; Majer et al. 2013).

We predicted the presence of unidirectional filters to bat use of restoration for small, manoeuvrable species. Instead we detected the presence of both unidirectional and dynamic filters. *V. regulus*, *C. morio* and *Nyctophilus* spp. all avoided <5 year old restoration but while *V. regulus* and *C. morio* activity suggested the presence of a unidirectional filter, *Nyctophilus* spp. activity was indicative of a dynamic habitat filter as *Nyctophilus* spp. activity fluctuated as restoration matured. This group avoided both <5 and 10-14 year old restoration but had comparable activity levels in other years of restoration and unmined forest. These three species share similar ecomorphologies in terms of low wing loading and aspect ratio and are typically considered closed environment species, also capable of using edge environments (Fullard et al. 1991). Our findings are consistent with other studies where *V. regulus* and *Nyctophilus* spp. activity was highest in the least disturbed sites in a system, i.e., remnant or mature forest compared to open paddocks or young regrowth (Law & Chidel 2006; Webala et al. 2011). Somewhat contradictory, *C. morio* is thought to spend less time in the closed environment than *V. regulus* and *Nyctophilus* spp., but the filter to *C. morio* use of restoration was relatively short-lived, dissipating once restoration was ≥5 years old. However, our results are consistent with findings in eastern Australia where *C. morio* was detected in both remnant forest and eucalypt plantings, but not in open paddocks (Law & Chidel 2006).

We predicted the presence of dynamic habitat filters to bat use of restoration for large, less manoeuvrable species and while our results suggested this was true for *M. kitcheneri* we did not detect the presence of a filter for the other three species. Instead we found a preference by *T. australis* for <5 year old restoration compared to unmined forest, but no difference in activity levels between ≥5 year old restoration and unmined forest. This is consistent with findings from the southern jarrah forest where there was no difference in *T. australis* activity between logging histories (Webala et al. 2011). *C. gouldii, F. mackenziei* and *M. kitcheneri* all have relatively low peak echolocation call frequencies (~25-35 kHz; Appendix 1, Fullard et al. 1991) and ecomorphologies suited primarily for edge, but also open, environments (Fullard et al. 1991). Our results are consistent with findings from the southern jarrah forest (Webala et al. 2011) where none of these species showed a difference in activity between logging histories. The presence of a dynamic filter to *M. kitcheneri* use of restoration, i.e, lower activity only in >15 year old restoration compared to unmind forest, is concordant with research elsewhere in Australia finding that bats with low echolocation frequency calls and suited for edge environments had reduced activity in 25-50 year old regrowth, compared to sites >60 years old (Adams et al. 2009).

Multivariate analysis indicted filters might be dynamic over very short time periods, such as between the two years of this study. While we found evidence to suggest a difference in activity levels between unmined forest and both <5 and >15 year old restoration, depending on the field year, examination at the univariate level did not reveal these differences. This is likely due to the increased power of the multivariate analysis, compared to the univariate tests (Wang et al. 2012). The potential for the influence of filters to fluctuate between years underscores the importance of longitudinal, multi-year studies. We suspect that the differences in bat activity between the two years was related to differences in local climate and the subsequent effect this had on vegetation structure. The first field year was the driest on record (12 years of recorded data for Huntly and 80 years for nearby Dwellingup) with 630 mm of rainfall compared to 1180 mm during the second year, the majority of which fell between field years. The first field year also coincided with extreme heat, in the form of continuous warmth and multiple heat waves, culminating in extensive dieback of both jarrah and marri crowns (Brouwers et al. 2013). This is a particular concern as climatic conditions, in conjunction with environmental characteristics, can affect the ability of species possessing particular traits to utilise certain habitats, effectively reducing the suitability of habitat as climate changes (Boucek & Rehage 2014; Poff et al. 2010). Future studies explicitly examining the link between habitat filters and climate will improve the ability of the habitat filter framework to elucidate community responses to global change.

Filters to Bat Habitat Use

Bats with high echolocation frequency calls and ecomorphologies adapted to edge environments are typically the most tolerant of increasing vegetation density (Adams et al. 2009). The increased adaptability of *V. regulus* to closed environments, compared to *C. morio* and *Nyctophilus* spp. (Fullard et al. 1991), may explain why we were unable to identify a habitat filter for *V. regulus* but identified midstorey cover as a filter for both *C. morio* and *Nyctophilus* spp. Our findings are consistent with assumptions based on ecomorphology for *V. regulus* (Fullard et al. 1991). The fact that we were unable to identify any habitat features as filters for *V. regulus*, while detecting their effect on bat use of restoration, may suggest that filtering is complex, related to overall structure rather than specific variables, and/or that we did not measure the habitat filter.

Tall trees, an open canopy structure, and log cover are typical of the unmined forest structure (Bell & Heddle 1989). In Germany, mature forest patches, with multiple vegetation strata, have been positively associated with increased bat activity and occurrence (Jung et al. 2012). Thus, increased *V. regulus* activity in the unmined forest may be reflective of the more heterogeneous structure, in terms of vegetation height, cover, and tree density of the unmined jarrah forest (Bell & Heddle 1989), compared to restoration. In the southern jarrah forest *V. regulus* activity was negatively correlated with understorey cover (Webala et al. 2011) while in eastern Australia *V. regulus* activity was positively correlated with tree density (Hanspach et al. 2012). Neither of these habitat features were related to *V. regulus* activity in this study, despite measuring the same variables. These mixed results, in combination with consistently lower *V. regulus* activity in disturbed systems, compared to remnant forest (Hanspach et al. 2012; Webala et al. 2011), may suggest that filtering is related to the cumulative effect of multiple vegetation variables and that not any one feature is a filter in its own right. Conversely, it may also suggest that some unmeasured component of the forest may be influencing activity. Although we did not measure tree hollow availability in this study we speculate that hollow availability may be a habitat feature influencing *V. regulus* activity. In an agricultural landscape in eastern Australia *V. regulus* activity was highest in an area close to a known roost in remnant forest (Law & Chidel 2006). In the jarrah forest, *V. regulus* preferred roosting in the hollows of large, mature trees (J. Burgar, Chapter 5; Webala et al. 2010). It is equally plausible that we did not measure the habitat feature driving *V. regulus*' activity and/or that multiple filters are at play, including a complex amalgam of features that together comprise the filter. Future studies are necessary to clearly elucidate the mechanism filtering *V. regulus* use of restoration.

The curvilinear relationship between bat activity and midstorey cover for *C. morio* and *Nyctophilus* spp. suggests that while both bat species may be ecomorphologically adapted to navigate dense vegetation, the energetic costs of doing so may well outweigh the benefits (e.g., Sleep & Brigham 2003). This was an example of a unidirectional filter for *C. morio* because when midstorey cover was low, in <5 year old restoration, *C. morio* activity was also low but then activity increased as midstorey cover increased to a threshold of ~15% midstorey cover.

Although we only weakly detected the presence of a habitat filter for *C. morio,* we postulate that this weak detection is a reflection of the high variability, particularly in 5-14 year old restoration, and overall low mean nightly detections of *C. morio* throughout the landscape*.* In contrast, we identified midstorey cover as a dynamic filter to *Nyctophilus* spp. use of restoration, with activity fluctuating with midstorey cover. *Nyctophilus* spp. activity was low in both <5 and 10-14 year old restoration, when midstorey cover was both low and high, respectively, but had higher activity at moderate levels (<20%) of midstorey cover. Our results are consistent with findings in the southern jarrah forest where both *C. morio* and *Nyctophilus* spp. activity was negatively associated with understorey clutter, an indexed variable including midstorey cover (Webala et al. 2011).

Although we did not detect habitat filters to bat use of restoration for *T. australis*, *F. mackenziei* and *C. gouldii,* tree density was the best predictor of their use of restoration. In contrast, we identified a dynamic habitat filter to *M. kitcheneri* use of restoration and canopy height as the best predictor of *M. kitcheneri* activity. We speculate that canopy height itself is not the mechanism filtering *M. kitcheneri* activity but rather that it may be correlated with the true (unmeasured) habitat filter. Similar to the situation for *V. regulus*, our inability to pinpoint a clear habitat filter for *M. kitcheneri* may reflect that a complex amalgam of filters, some unmeasured, drives *M. kitcheneri* activity. While *M. kitcheneri* is considered an open / edge microhabitat user, this species is less manoeuvrable than either *F. mackenziei* or *C. gouldii* (Table 1; Fullard et al. 1991) so we would expect that tree density would also filter *M. kitcheneri* activity. Indeed in eastern Australia *Mormopterus* spp. activity was correlated with tree density (Hanspach et al. 2012) but not with any vegetation structure variable in the southern jarrah forest (Webala et al. 2011). Again, these mixed results underscore that filtering is complex and multiple habitat features may cumulatively influence bat activity. For the remaining large bodied, less manoeuvrable species activity levels were negatively associated with tree density. *T. australis* activity was negatively related to tree density only until densities reached those found in the unmined forest, ~1600 stems ha^{-1}, and then activity plateaued at ~5 calls per night. The strong, negative relationships between activity and either tree density or canopy height may indicate detectability issues, although this is unlikely as Molossidae echolocation calls are of relatively high intensity and can be detected up to 30 m away (Surlykke & Kalko 2008). These large, less manoeuvrable bat species are adapted for fast flight in open and edge environments (Fullard et al. 1991). Research elsewhere suggests that bats suited to open environments vertically stratify their use of the forest (Adams et al. 2009). It is likely that these species either use the upper canopy as an upper edge or forage and commute in the open space above the canopy in all forest types (e.g., Lumsden & Bennett 2005). Consequently, reduced detection in sites with either taller trees or higher tree densities may be an artefact of increased call attenuation as vegetation complexity increases. The preference for young restoration by *T. australis* may well be an artefact of detectability as younger restoration is indicative of open sites that lack trees and thus have reduced potential of call attenuation. However, our findings corroborate other studies where large, less manoeuvrable bats preferred structurally simplified sites over unharvested forest (e.g., McConville et al. 2013; Morris et al. 2010; Patriquin & Barclay 2003), suggesting that our findings are ecologically driven. To disentangle detectability and activity, future research in the northern jarrah forests should follow research elsewhere by vertically stratifying bat echolocation call surveys at multiple heights (Adams et al. 2009; Plank et al. 2012; Scrimgeour et al. 2013).

Tree Density

We predicted that tree density would be the most influential habitat filter to bat use of restoration. Yet we only identified tree density as a strong predictor of bat activity for the three species where filters were absent. Our results are contrary to findings in south-eastern Australia where tree density was related to activity levels of all bat species (Hanspach et al. 2012). The discrepancy between studies may be due to the exceptionally high tree densities within the northern jarrah forests. Tree density averaged 2112 ± 141 stems ha^{-1} in restoration and 1601 ± 152 stems ha^{-1} in unmined forest across our study sites. In variegated landscapes in south-eastern Australia, where tree density changes continuously from low to high, bat activity peaked around 20-50 stems ha^{-1} (Hanspach et al. 2012; Lumsden & Bennett 2005). This density likely allows less manoeuvrable species the room to navigate while still providing adequate vegetation to act as cover for the more agile species. Similarly, in timber harvested landscapes in North America tree densities averaged 180 stems ha^{-1} in sites <25 years old with bats preferring the less dense, unmanaged forest (Morris et al. 2010). In eucalypt plantations of eastern Australia, only *Nyctophilus* spp. had a positive relationship with tree density (Law & Chidel 2006), but at 1000 stems ha^{-1} these plantations were less dense than our study area. While bats may avoid areas of high tree density when possible (Hanspach et al. 2012; Lumsden & Bennett 2005), our results indicate that the same bat species are capable of using landscapes with substantially higher tree densities. Our findings underscore the importance of region specific studies, as bat responses to specific vegetation characteristics can be dependent on the specific environment (Petrites et al. 2009; Sleep & Brigham 2003).

Management Implications

To facilitate / accelerate bat use of restoration, management strategies should ensure tree densities are no greater than 1600 stems ha^{-1} and midstorey cover does not exceed ~15-20%. For sites not yet restored, reducing the number of seeds in the seeding mix will likely be adequate to reduce vegetation densities. For already restored sites, thinning and burning may be an option to reduce the influence of these habitat filters on bat use of restoration. While northern jarrah forest reptile communities responded well to the thinning and burning (Craig et al. 2010), these benefits were relatively short-lived (Smith 2011). However, we postulate that the benefits of thinning, and particularly burning, would be longer lived for bat communities. Research elsewhere suggests that bats are tolerant to landscape-scale fire (Buchalski et al. 2013; Lacki et al. 2009) and that fire may be beneficial to bats in the short term by reducing both midstorey and canopy cover and thus creating additional edge and open spaces for bats to exploit (Inkster-Draper et al. 2013). Thinning and burning of northern jarrah forest restoration temporarily reduced overstorey stem density and canopy cover, but <5 years post burning these reductions were no longer evident (Grigg et al. 2010). The regional prescribed burn management plan burn rotations are typically every 6-10 years for unmined forest, although this depends on site productivity and rainfall (Burrows 2008). Restoration is not integrated with the prescribed burning practices until it is at least eight years old (Grigg et al. 2010). As average midstorey cover did not exceed 15-20% until restoration was >10 years, inclusion of restoration in the regional plan should ensure burns are frequent enough to minimise the influence of midstorey cover as a habitat filter to bat use of restoration.

Conclusion

Habitat filtering is a useful framework to explain bat successional patterns in restoration. Although we were unable to relate the type (i.e., unidirectional or dynamic) of filter to bats based on ecomorphologies, we were able to relate the specific habitat feature identified as a filter to ecomorphology. Midstorey cover generally filtered the use of restoration by small bodied, more manoeuvrable bat species while tree density influenced the activity of large bodied, less manoeuvrable bat species. This suggests that the habitat filtering framework is applicable to restoration beyond the jarrah forest. The lack of a clear connection between vegetation variables as specific habitat filters to bat use of restoration for some species indicates that filtering is complex and may be influenced by the cumulative effects of vegetation structure, rather than one vegetation variable. It also suggests that vegetation structure may not solely be driving bat activity, but rather that other features, such as the roosting suitability of an area, may be equally influential in bat use of a site. As south-western Australia is faced with rapid environmental change, both in terms of climate and land-use (Batini 2007; Bradshaw 2012), our findings have important implications for land managers grappling with restoring fully functioning ecosystems. Increased manipulation and intervention of restored systems, such as thinning (Armitage & Ober 2012; Patriquin & Barclay 2003) and burning (Buchalski et al. 2013; Inkster-Draper et al.

2013), will likely be necessary to ensure similar bat community composition within restored and unmined forest.

Chapter Three

Who's' for dinner? High-throughput sequencing reveals bat dietary differentiation in a biodiversity hotspot where prey taxonomy is largely undescribed.

This chapter has been published and is presented here in full. Supplementary materials have been deposited to DRYAD and are accessible under doi:10.5061/dryad.km6ph.

Burgar, J. M., D. C. Murray, M. D. Craig, J. Haile, J. Houston, V. Stokes, and M. Bunce. 2014. Who's for dinner? High-throughput sequencing reveals bat dietary differentiation in a biodiversity hotspot where prey taxonomy is largely undescribed. Molecular Ecology **23**:3605-3617.

Abstract

Effective management and conservation of biodiversity requires understanding of predator-prey relationships to ensure the continued existence of both predator and prey populations. Gathering dietary data from predatory species, such as insectivorous bats, often presents logistical challenges, further exacerbated in biodiversity hotspots because prey items are highly speciose yet their taxonomy is largely undescribed. We used high-throughput sequencing (HTS) and bioinformatics analyses to phylogenetically group DNA sequences into molecular operational taxonomic units (MOTUs) to examine predator-prey dynamics of three sympatric insectivorous bat species in the biodiversity hotspot of south-western Australia. We could only assign between 4-20% of MOTUs to known genera or species, depending on the method used, underscoring the importance of examining dietary diversity irrespective of taxonomic knowledge in areas lacking a comprehensive genetic reference database. MOTU analysis confirmed that resource partitioning occurred, with dietary divergence positively related to the ecomorphological divergence of the three bat species. We predicted bat species' diets would converge during times of high energetic requirements, i.e., the maternity season for females and the mating season for males. There was an interactive effect of season on female, but not male, bat species' diets, although small sample sizes may have limited our findings. Contrary to our predictions, females of two ecomorphologically similar species showed dietary convergence during the mating season rather than the maternity season. HTS-based approaches can help elucidate complex predator-prey relationships in highly speciose regions, which should facilitate the conservation of biodiversity in genetically uncharacterised areas, such as biodiversity hotspots.

Introduction

To effectively manage and conserve biodiversity, it is critical to understand predator-prey relationships so that both predator and prey populations can be conserved. This is becoming increasingly important as continuing habitat loss and degradation may lead to trophic collapse (Dobson et al. 2006). Accurate dietary studies can contribute greatly to understanding predator-prey relationships and can also provide integral knowledge concerning food webs and trophic interactions, which in turn influence ecological processes such as niche partitioning and inter-specific competition (Amarasekare 2008; Pompanon et al. 2012; Roughgarden 1983). Determining the dietary requirements of species through direct field observations is often difficult and time-consuming (Williams et al. 2012), particularly in regions where prey are highly speciose and undersampled. Tropical and southern temperate biodiversity hotspots, which support the highest number of globally threatened species (Bohm et al. 2013; Myers et al. 2000), typify regions where most prey species are taxonomically undescribed and their DNA sequences unknown (Bohmann et al. 2011; Fonseca 2009). Biodiversity hotspots are estimated to harbour over 40% of the world's insects, most of which are undescribed, and conservative estimates suggest at least 22% are threatened (Fonseca 2009). The poor taxonomic knowledge of prey items, such as invertebrates, may hamper conservation efforts by limiting identification of important prey items for many species.

Recent advances in molecular technologies have enabled dietary analysis of DNA food remains in gut or faecal samples while precluding the need for prey items to be taxonomically described (e.g., Brown et al. 2013). One technique, high-throughput sequencing (HTS), increases the breadth of prey items identified, as HTS involves sequencing many pooled amplicons in parallel often using universal primers, such as sequencing the preys' mitochondrial DNA (mtDNA) cytochrome *c* oxidase I (COI) gene, or DNA barcode (Hebert et al. 2003a; Mitchell 2008). Apart from negating the need for physically dissecting individual prey remains, HTS allows complex, heterogeneous DNA mixtures to be analysed, thus examining the prey base in its entirety and at a finer taxonomic resolution than morphological methods, without adding to the cost of analysis (Boyer et al. 2012; Pompanon et al. 2012; Razgour et al. 2011; Shokralla et al. 2012). Molecular studies, such as HTS, can still be limited in that prey DNA sequences from genetically uncharacterised areas may not be confidently matched to reference databases (e.g., Brown et al.

2013). However, DNA sequences derived from molecular studies can be phylogenetically grouped into molecular operational taxonomic units (MOTUs; Floyd et al. 2002), which, irrespective of taxonomic assignment, can then be used to compare diets within, and between, predatory species (Caporaso et al. 2010), elucidating complex predator-prey relationships in highly biodiverse ecosystems.

Insectivorous bats are important, yet often overlooked, top predators that consume a variety of prey (Kalka & Kalko 2006; Morrison & Lindell 2012). Differences in manoeuvrability, size, and foraging strategy influence the prey base of individual bat species (Fenton 1990; Fullard et al. 1991). In addition, prey availability and accessibility may vary sexually and seasonally, leading to both intra and interspecific differentiation in bat diets (Andreas et al. 2012; Clare et al. 2011). While bats can minimise energy expenditure behaviourally (e.g. torpor and hibernation) when prey are limited (Dietz & Horig 2011; Hope & Jones 2012), forced fasting can cause metabolic deterioration within relatively short timeframes (Freitas et al. 2010). Thus, regular prey consumption is necessary, with bats often consuming over a quarter of their body weight in invertebrates each night and even greater amounts during energetically demanding periods (Kunz et al. 2011); the maternity season for females and mating season for males (Dietz & Kalko 2007). During these times, bats may forage less selectively to ensure adequate energetic intake (Whitaker 2004). Overall prey consumption by bats is typically diverse and even bat species conventionally considered specialists consume many prey species within a single order (Clare et al. 2011). Being nocturnal, cryptic and typically generalist predators, bat diet studies embody some of the most challenging aspects of studying predator-prey interactions (Andrew et al. 2013).

However, the fine taxonomic resolution of molecular technologies, such as HTS, is enabling factors that influence dietary variation to be elucidated, leading to an improved understanding of predator-prey relationships and resource partitioning between sympatric bat species (Bohmann et al. 2011; Razgour et al. 2011).

Previous studies have compared MOTU diversity in bat diets (e.g., Alberdi et al. 2012; Clare et al. 2011; Clare et al. 2009; Zeale et al. 2011), two utilising HTS (Bohmann et al. 2011; Razgour et al. 2011), but this is the first study to use HTS approaches to investigate both intra and interspecific dietary differentiation in multiple bat species. Significantly, the target species are sampled within the biodiversity (Myers et al. 2000) and invertebrate diversity (Cooper et al. 2011) hotspot of south-western Australia, a region with high levels of habitat loss (Bradshaw 2012) and a rapidly drying climate that both pose a threat to biodiversity (Klausmeyer & Shaw 2009; Wardell-Johnson et al. 2011). Within this hotspot, the jarrah (*Eucalyptus marginata*) forest supports nine species of insectivorous bat and a highly speciose invertebrate fauna, estimated between 15,000 and 20,000 species, of which only 10% have been formally described (Abbott 1995). We examined intra and interspecific dietary differentiation between three sympatric jarrah forest insectivorous bat species to identify sexual and seasonal variations in diets and to determine if diets converge during times of resource limitation.

Of the three species we studied Gould's wattled bat (*Chalinolobus gouldii*, Gray 1841) is the largest and is capable of fast, agile flight (Bullen & McKenzie 2001). Compared to the other two species *C. gouldii* has a high aspect ratio and wing loading and low echolocation call frequency (Table S1, Supplementary Info); in the jarrah forest *C. gouldii* likely forages in open habitat adjacent to the forest edge (Bullen & McKenzie 2001; Fullard et al. 1991). The southern forest bat (*Vespadelus regulus*, Thomas 1906) and Gould's long-eared bat (*Nyctophilus gouldi*, Tomes 1858) have similar, comparatively low, aspect ratio and wing loading (Fullard et al. 1991). While both are agile, *V. regulus* is capable of medium to fast flying, in contrast to *N. gouldi* which flies at slower speeds but is more manoeuvrable (Brigham et al. 1997; Bullen & McKenzie 2001; O'Neill & Taylor 1986). In the jarrah forest, *N. gouldi* and *V. regulus* are likely to exploit vegetated and edge habitat (Fullard et al. 1991). *N. gouldi* employs both aerial hawking and gleaning, in contrast to the other two bat species who primarily take prey aerially (Brigham et al. 1997; Fullard et al. 1991). We hypothesized that dietary partitioning would occur between species and that dietary divergence would be related to ecomorphological divergence. Specifically, we predicted that the most ecomorphologically divergent species, *C. gouldii* and *N. gouldi*, would have the most divergent diets. As *N. gouldi* exhibits multiple foraging strategies and is capable of exploiting multiple microhabitats, we also predicted *N. gouldi* would have the most diverse diet whilst *C. gouldii*, would have the least diverse diet, with *V. regulus* having an intermediate level of dietary diversity. Lastly, we predicted that there would be dietary differentiation, both intra and interspecifically, based on the individual and combined influences of season and sex. Intraspecfically, we expected convergent diets during the mating season when females were not as constrained by roosting requirements. Interspecifically, we expected diets to converge when energy demands were high; i.e., during the maternity season for females and mating season for males.

Methods & Materials

Study site

The study was conducted at Huntly minesite (32°36'S, 116°07'E), operated by Alcoa of Australia, located 10 km N of Dwellingup in the northern jarrah forest of south-western Australia. The area has a Mediterranean climate with cool, wet winters and warm, dry summers. Rainfall at Dwellingup averages 1222 mm annually, with >75% falling between May and September. The minesite is a mosaic of unmined and restored forest, both with a canopy dominated by two eucalypt species, jarrah and marri (*Corymbia calophylla*). All bat faecal sample collection locations occurred adjacent to waterholes within unmined forest, although bats are known to forage in both forest types (J. Burgar, unpublished data).

Sample collection

Bats were trapped at eight locations over 14 nights between October 2010 and March 2011, in both the maternity (15 October to 1 December) and mating (3 February to 30 March) seasons. All bats were captured in harp traps (Two-Bank 4.2 square metres; Ausbat Research Equipment, Victoria), removed almost immediately, placed in individual, clean bags and held for ~30 to 60 mins to obtain faecal samples. We collected 209 faecal samples from three species (24 from *C. gouldii*, 50 from *N. gouldi*, and 135 from *V. regulus*), which were placed in labelled sterile vials and frozen as soon after collection as possible.

DNA extraction and amplification

DNA was extracted from all *C. gouldii* faecal samples (24), and from subsets of *N. gouldi* (30) and *V. regulus* (27) faecal samples, randomly stratified by site, date and sex. Faecal samples remained frozen until processed for DNA extraction, which occurred in four batches alongside extraction controls. For the first two batches of DNA extraction, each pellet per faecal sample was cut in half using a sterile scalpel blade. On average, faecal samples contained 6.2 pellets (range: 1 to 19) per individual bat, with DNA extracted from approximately half of each pellet (average 230 mg extraction^{-1}). For the remaining batches, pellets were ground together prior to obtaining 100 mg from each sample, which was then placed into a 2 ml tube. Extractions were performed using QIAamp DNA Stool Mini Kit (QIAGEN) according to manufacturer's instructions with the modifications noted in the supporting information (Appendix S1). A short section (157 bp) of the mtDNA COI gene was amplified via qPCR using generic arthropod primers (ZBJ-ArtF1c and ZBJ-ArtR2c; Zeale et al. 2011). All extracts deemed successful in yielding DNA, free of inhibition as determined via qPCR curves across dilutions, were selected for HTS library preparation.

HTS library preparation and sequencing

The generic arthropod forward primers were modified into fusion primers with the addition of Roche Genome Sequencer (GS) Junior FLX compatible A and B primers and a series of 30 unique DNA-based Multiplex Identifiers (MID). Each successful extract was assigned a unique MID tag and subsequent fusion tagged qPCR was carried out. See supporting information for detailed HTS sequencing methods (Appendix S1). HTS was carried out on the Roche GS Junior FLX system at Murdoch University, Australia, following the Lib-A amplicon sequencing protocols.

MOTU selection

Amplicon sequences obtained from the GS Junior FLX were separated into sample batches based on MID tags; tags and sequencing adapters were subsequently trimmed using Geneious v.5.6.5 (Drummond et al. 2012). In each case, an exact match in base composition and length was required. Sequences not meeting these criteria were discarded, as were sequences of short length that resulted from primer dimer. Each set of batched sequences was then compared against the National Centre for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) database through YABI, a bioinformatics workflow software system (Hunter et al. 2012). Sequences were searched without a low complexity filter, with a gap penalties existence of five and extension of two, expected alignment value <0.1 and a word count of seven. BLAST output files obtained from YABI were then imported into MEtaGenome ANalyzer (MEGAN) version 4.70.4 (Huson et al. 2011) with the following Lowest Common Ancestor (LCA) assignment algorithm parameters: minimum support 1, minimum score 35, top percent 5, win-score 0 and no minimum complexity filter. For each sample, sequences assigned by MEGAN to the Arthropod phylum were extracted for further analysis.

Extracted Arthropod sequences were processed in Quantitative Insights in Microbial Ecology (QIIME) version 1.5.0 (Caporaso et al. 2010). All sequences were checked to ensure they were >95 bp in length, then grouped into MOTUs using the

USEARCH method, with a specified 98% sequence similarity threshold. Potential chimeric sequences were removed, as were singleton sequences. MOTUs were aligned using the MUSCLE alignment (Edgar 2004). Representative MOTUs selected for taxonomic alignment and assignment were ~157 bp in length. Phylogenetic trees were constructed using the fasttree method.

MOTU sequences were queried through the Biodiversity of Life Database (BOLD) version 3 (Ratnasingham & Hebert 2007) on 29 May 2013, as the online BOLD engine enables sequence identification using both private and public records. Examination of intra versus interspecific variation of the COI gene suggests that arthropod sequence divergence ranges from 6% in Lepidoptera to over 11% in Coleoptera and Hymenoptera (Hebert et al. 2003b; Waugh 2007). Bat dietary studies have used a variety of percentage similarity cut-off criteria, for 157 bp fragments, to obtain taxonomic level thresholds, ranging from 99.3% (Zeale et al. 2011) to 98.5% (Razgour et al. 2011) for species and 98% (Razgour et al. 2011) to 94.9% (Zeale et al. 2011) for genus, with some researchers suggesting assignments below 97.4% are inaccurate and potentially erroneous (Alberdi et al. 2012). Intraspecific variation is known to increase with geographic distance (Bergsten et al. 2012), which may have implications for low matching success in areas with limited genetic reference databases. This is particularly relevant due to the paucity of Australian records and as <6% of Australian invertebrate genetic records come from Western Australia. When species matches are unavailable the accuracy of higher taxon assignment is questionable, particularly where reference libraries are incomplete (Wilson et al. 2011). Thus, a conservative matching system was employed where sequences were filtered to ensure those examined had a minimum 98% sequence similarity to a potential taxonomic assignment. Taxonomy was assigned to MOTUs matched against the BOLD database using the online batch identification engine, following slightly modified 'strict' and 'best match' methods (Ross et al. 2008). The 'strict' method refers to matching based on phylogenetic tree placement where the query sequence must be nested within a clade comprising members of a single taxon to be considered a match (Ross et al. 2008). The 'best match' method simply assigns taxonomy based on percent similarity. While this method may have similar true positive identification rates as the 'strict' method, it also has much higher false positive rates. Thus, only when the 'best match' was for a taxon sampled within Australia was the MOTU considered a match. MOTUs were considered a "species match" if sequences had ≥99% similarity to a single species and were considered a "genus match" if sequences had ≥98% similarity to one or more species within the same genus. The matching method was recorded for each MOTU taxonomic assignment.

Dietary diversity

Dietary diversity for each bat species was assessed using MOTUs, irrespective of taxonomic assignment. Two types of diversity were assessed: α-diversity for diversity within each individual bat and β-diversity for diversity within each bat species. To determine α-diversity independent of sample size, 10 rarefactions were performed at a minimum depth of five and maximum depth of 95 sequences per sample, with a step increase of 10. Rarefaction plots were derived from collated α-diversity metrics generated from two diversity indices: Chao1 (Chao 1984) and Faith's phylogenetic diversity (PD) (Faith 1992). Chao1 provides a relatively unbiased and conservative estimate of species richness (Bunge & Fitzpatrick

1993) while Faith's PD reflects evolutionary history with higher values indicating greater taxonomic distinctiveness (Faith & Baker 2006). Rarefaction curves not only provide information on α-diversity, irrespective of sample size, but also act as a check to ensure sufficient sequence sample depth in subsequent analyses. Examination of α-diversity values was set at an even depth of 65 sequences per sample, which was selected based on the relative levelling off of rarefaction curves at this depth while considering sample sizes (*C. gouldii* $n = 21$; *N. gouldi* $n = 15$; *V. regulus* $n = 19$) for further analyses.

β-diversity was computed as a function of jackknifed β-diversity using the previously created phylogenetic tree and rarefaction was set at an even depth of 60 sequences per sample. A rarefaction level of 60 ensured β-diversity analyses were not influenced by sequencing effort, but instead reflected the underlying biology; bat samples with fewer than 60 sequences were removed from β-diversity analyses. β-diversity was not limited by the size of the step increase (as were α-diversity values), so the slight difference in rarefaction depths was to ensure similar depths while maximising the number of sequences per sample. Distance matrices, generated from unweighted Unifrac statistics, formed the basis of the principal coordinate analysis (PCoA) plots and further analyses. Unifrac is an ecological distance measure based on phylogenetic information and is able to deal with undersampled environments (Lozupone & Knight 2005). As HTS is frequency based, treating each prey item equally, HTS can overestimate the presence of rare prey while underestimating common prey, potentially biasing HTS towards the detection of resource partitioning. Consequently we analysed dietary diversity twice, once including all MOTUs (in 64 samples: *C. gouldii* $n = 23$; *N. gouldi* $n = 19$;

V. regulus n = 22) and again after removing MOTUs only present in one bat sample (e.g., Bohmann et al. 2011; Brown et al. 2013). Diversity values and subsequent distance matrices were generated in QIIME, using the default parameters unless otherwise stated.

MOTU diversity was compared using unweighted Unifrac distance matrices, permuted 9999 times, in the R Vegan package – function Adonis (Oksanen et al. 2012). Diversity was compared between bat species, seasons (maternity and mating) and sexes, as well as combinations of these factors depending on sample sizes. We also examined the interactive effects of season and sex both intra and interspecifically. Bat species, season and sex were considered fixed factors while site was included as a random factor to account for any spatial-variation in invertebrate communities. Tukey's HSD post-hoc tests were run to determine homogeneity of group variances, pooled across sites, using the R Vegan package – function betadisper (Oksanen et al. 2012). These analyses were performed in R version 2.15.0 (R Core Team 2013).

Results

Of 81 bat faecal samples processed, 64 yielded DNA of sufficient quality for deep sequencing, resulting in 14,673 amplicon sequences representing 579 MOTUs (deposited in DRYAD doi:10.5061/dryad.0gq63). Bat samples contained between one and 44 MOTUs (median of 11 and mean of 15 MOTUs sample^{-1}), with 23 *C. gouldii* samples yielding 193 MOTUs, 218 MOTUs in 19 *N. gouldi* samples and 267 MOTUs in 22 *V. regulus* samples. Removing MOTUs found in only one bat sample resulted in 190 MOTUs (33%), excluding one *V. regulus* sample for a total of 63 samples: 95 MOTUs in *C. gouldii* samples; 83 MOTUs in *N. gouldi* samples; and 111 MOTUs in *V. regulus* samples. The significance of tests did not differ when dietary diversity was analysed with all MOTUs, or the subset, so we only present results derived from all MOTUs to ensure potentially important prey items were not removed, considering MOTUs occurring in low abundance were removed earlier in the analysis. Refer to Table S2 (Supplementary Info) for the results derived from analyses with the subset of MOTUs.

MOTU taxonomic assignment

Matching all MOTUs against BOLD databases resulted in MOTUs being assigned taxonomically in ~4% (19 of 579) of cases using the 'strict' method and ~20% (121 of 579) of cases using the 'best match' method (Table 1). Using the 'strict' method, prey DNA was detected solely from Lepidoptera, comprising seven families and 11 genera. The 'best match' method detected prey DNA from five insect orders: Diptera, Hemiptera, Lepidoptera, Mantodea and Neuroptera, with most (51 genera within 19 families) assigned to Lepidoptera. *C. gouldii* consumed the most assigned MOTUs (43), compared to *V. regulus* (32) and *N. gouldi* (17). More assigned MOTUs were consumed during the maternity season (54) than the mating season (18), with only eight taxa consumed during both seasons.

Table 1: Taxonomic assignment of MOTUs through the BOLD online identification engine using two different methods: the neighbour-joining hierarchical tree-based ‘strict’ (S) and the sequence similarity ‘best match’ (BM) approach (Ross et al. 2008). Only sequences with >98% similarity were considered as a possible match: >98% for a “genus” match and >99% for a “species” match. For the BM approach, only matches with sampling sites in Australia were considered; * indicates sampling sites in south-western WA while ** indicates sampling sites in WA but outside of the south-west. Species highlighted in grey are thought to use hearing based defences against the echolocation calls of bats.

Order	Family	Genus	Species	Season	*C. gouldii*	*N. gouldi*	*V. regulus*
Diptera	Syrphidae	*Simosyrphus*	*sp.*	maternity		BM	
			grandicornis	maternity		BM	
	Tipulidae	*TipulidGC*	*sp.*	maternity			BM
Hemiptera	Cicadellidae	*Balcutha*	*incisa*	maternity		BM	
	Lygaeidae	*Nysius*	*sp.*	maternity	BM	BM	
	Pentatomidae	*Oechalia*	*Schellenbergii**	mating	BM		
Lepidoptera	Blastobasidae	*Blastobasis*	*sp.*	maternity	BM		BM
	Carposinidae	*Carposina*	*sp. ***	maternity			BM
			*autologa**	maternity			BM
		Sosineura	*mimica*	maternity			BM
	Cossidae	*Endoxyla*	*sp. **	maternity	BM		
			*sp.ANIC4**	maternity	BM		BM
	Crambidae	*Achyra*	*sp.*	maternity	BM		
			affinitalis	maternity	BM & S		
		Hellula	*hydralis*	both	BM & S	BM & S	
		Nacoleia	*sp. **	mating	BM		
			*rhoeoalis**	mating	BM & S		
	Elachistidae	*Elachista*	*sp.**	mating			BM & S
		Thalamarchella	*alveola**	maternity	BM		
	Gelechiidae	*Anarsia*	*sp. **	maternity			BM
		Aproaerema	*sp.*	maternity			BM
		Ardozyga	*amblopis**	both	BM		BM
			catarrhacta	maternity	BM		BM
		Dichomeris	*sp. **	mating		BM	BM
	Geometridae	*Dinophalus*	*idiocrana***	mating	BM		
		Ectropis	*sp. **	mating	BM		
		Idaea	*sp.*	both	BM	BM	

	Oenochroma	*ochripennata**	maternity	BM		
	Scopula	*sp. **	maternity	BM		
Lasiocampidae	*Pernattia*	*sp. ***	maternity			BM
Limacodidae	*Pseudanapaea*	*sp. ***	maternity	BM		
Noctuidae	*Argotis*	*sp.*	maternity	BM & S		
		*munda***	maternity	BM & S	BM & S	
	Dasypodia	*selenophora**	maternity		BM	
	Helicoverpa	*sp. **	maternity	BM & S		
	Leucania	*diatrecta**	maternity	BM		
	Persectania	*ewingii**	maternity	BM		BM & S
	Proteuxoa	*sp.*	maternity	BM		
Nolidae	*Nola*	*sp. **	maternity		BM	
Notodontidae	*Destolmia*	*sp. **	mating	BM	BM	
Nymphalidae	*Melinaea*	*sp.*	maternity		BM & S	BM & S
Oecophoridae	*Conobrosis*	*acervata***	maternity	BM		
	Crepidosceles	*sp.*	maternity	BM		
	Euchaetis	*sp. **	maternity			BM
	Hesperenoeca	*leucostemma**	both	BM		BM
	Pachyceraia	*sp.*	maternity			BM
	Philobota	*sp. **	maternity			BM
		*xanthastis**	maternity	BM		BM
	Placocosma	*resumptella**	maternity	BM		
	Prodelaca	*sp.*	mating	BM		
	Telanepsia	*sp. **	mating			BM
	Wingia	*sp. **	maternity			BM
	Zonopetala	*sp. **	mating			BM
Plutellidae	*Plutella*	*sp.*	maternity			S
		xylostella	maternity			BM & S
Pyralidae	*Endotricha*	*sp. **	mating	BM		
		*pyrosalis**	mating	BM		
	Meyiccia	*latro**	maternity		BM	
	Meyrickiella	*sp. **	maternity	BM		
	Mimaglossa	*sp. **	maternity	BM		
	Spectrotrota	*sp. **	maternity	BM		
		*fimbrialis**	mating	BM & S		
	Vinicia	*sp. ***	maternity	BM		

	Roeslerstammiidae	*Macarangela*	*sp. **	maternity			BM
			*pyracma**	maternity			BM
	Tineidae	*Moerarchis*	*sp. **	maternity		BM	
			*clathrata**	maternity	BM	BM	
		Opogona	*sp.*	both			BM
	Tortricidae	*Ancylis*	*sp. **	maternity			BM
		Holocola	*sp. **	maternity	BM		
		Strepsicrates	*macropetana*	both	BM		BM
	Xylorctidae	*Crypophasa*	*sp. ***	maternity	BM		
		Lichenaula	*sp.*	both	BM		
		Maroga	*melanostigma**	maternity	BM & S		
		Xylorycta	*sp. **	mating			BM
			*sp.ANIC71**	maternity			BM
Mantodea	Mantidae	*Orthodera*	*sp.*	both	BM		
Neuroptera	Chrysopidae	*Mallada*	*signata*	mating	BM	BM	
	Hemerobiidae	*Micromus*	*tasmaniae***	mating	BM	BM	BM

Interspecific dietary diversity

Our results show that while sequencing breadth was not sufficient to capture the entire prey base within any of the species (Figure 1), sequencing depth was sufficient to capture the prey base within an individual bat (Figure 2). *V. regulus* consumed the most MOTUs, although prey accumulation curves did not approach asymptotes for either all species combined, or individual species. There were no differences in prey α-diversity levels, for either Chao1 or Faith's PD, between bat species (Figure 3). Seasonal differences were apparent, though, with bat species having similar α-diversity values during the maternity season, but significant differences during the mating season (Chao1: $F_{2,18} = 4.20$, $P = 0.032$; Faith's PD: $F_{2,18} = 5.20$, $P = 0.017$); post-hoc tests indicated *N. gouldi* had higher α-diversity than *C. gouldii* in the mating season (Chao1: $P = 0.025$; Faith's PD: $P = 0.014$).

β-diversity differed significantly between species overall (Figure 4; $F_{2,52} = 2.40$; $P < 0.001$) and between pairs of species (*C. gouldii – N. gouldi*, $F_{1,41} = 2.37$, $P < 0.001$; *C. gouldii – V. regulus*, $F_{1,44} = 1.78$, $P < 0.001$; *N. gouldi – V. regulus*, $F_{1,40} = 2.94$,, $P < 0.001$). Multivariate dispersion was heterogeneous across species ($F_{2,61}=4.44$, $P = 0.014$), being significantly different between *C. gouldii* and *N. gouldi* ($P = 0.014$) but not between *V. regulus* and either *C. gouldii* ($P = 0.086$) or *N. gouldi* ($P = 0.113$).

Examination of interspecific β-diversity revealed an interactive effect between species and season on bat species' diets ($F_{3,52} = 1.93$, $P < 0.001$), with differences between species in both the maternity ($F_{2,31} = 2.03$,, $P < 0.001$) and mating ($F_{2,21} = 2.40$, $P < 0.001$) seasons. In the maternity season, β-diversity differed between all species (*C. gouldii – N. gouldi*, $F_{1,22} = 1.69$, $P = 0.012$; *C. gouldii – V. regulus*, $F_{1,25} =$

2.12, P = 0.123; *N. gouldi* – *V. regulus*, $F_{1,21}$ = 2.29, P = 0.004). In the mating season, β-diversity differed between *N. gouldi* and both *C. gouldii* ($F_{1,16}$ = 2.81, P = 0.002) and *V. regulus* ($F_{1,16}$ = 2.76, P = 0.014) but was marginally non-significant between *C. gouldii* and *V. regulus* ($F_{1,16}$ = 1.70, P = 0.066). Multivariate dispersion was homogeneous across all three species in both seasons (maternity, $F_{2,34}$ = 1.51, P = 0.237; mating. $F_{2,24}$ = 1.78, P = 0.191).

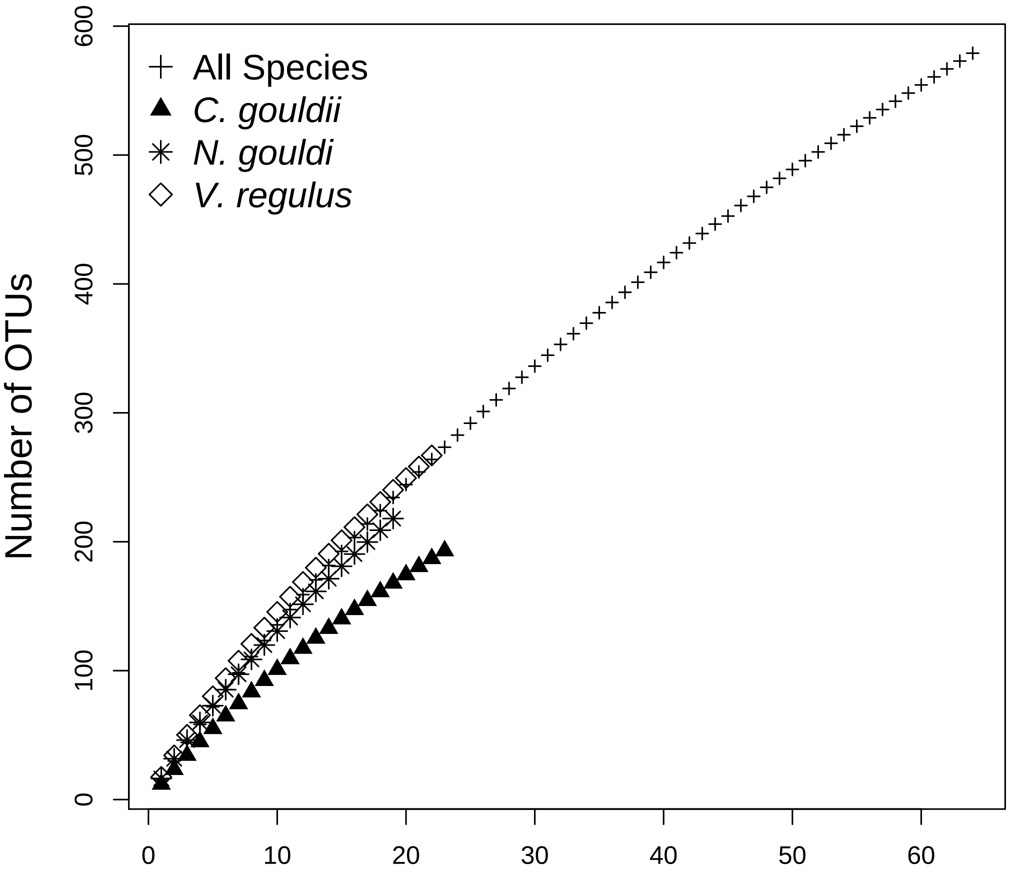


Figure 1: Prey accumulation curves for all species combined and individual bat species.

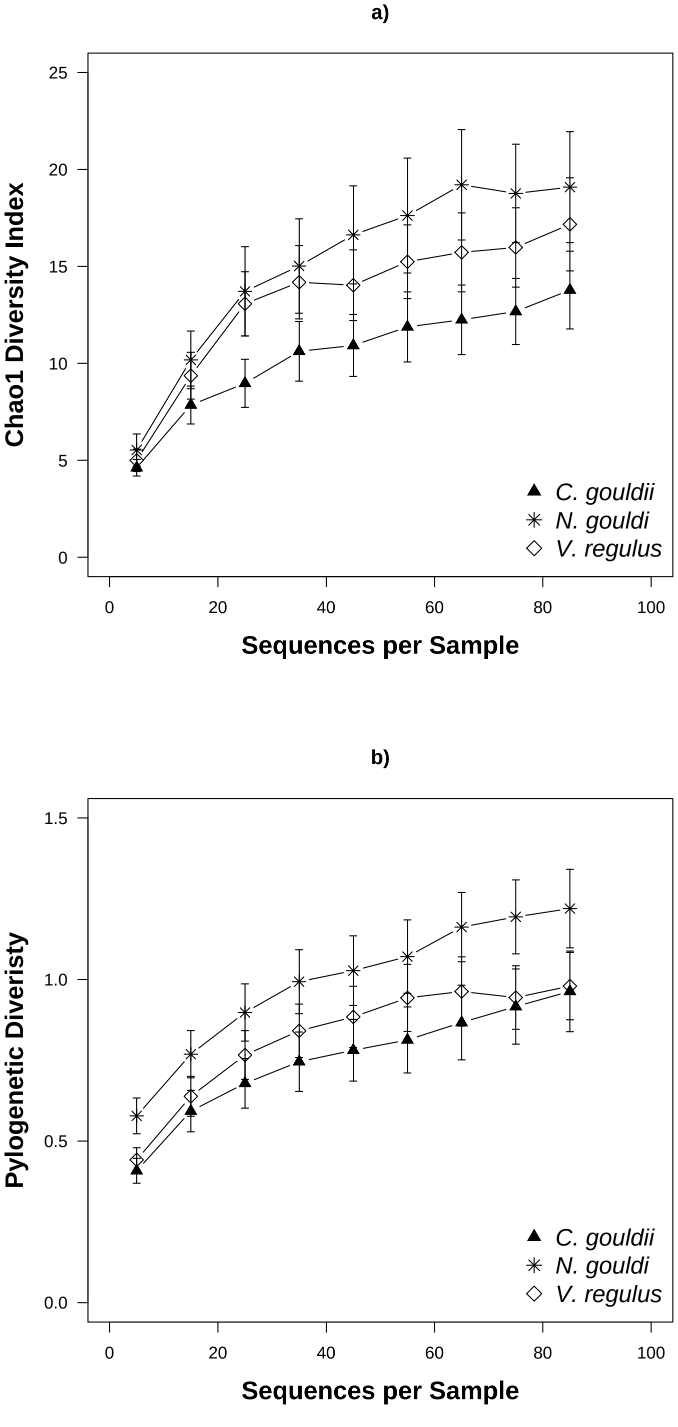


Figure 2: Rarefaction curves for three bat species using two diversity indices: (a) Chao1 and (b) Faith's phylogenetic diversity. Ten rarefactions were performed at a minimum sequence depth of five and maximum of 95, with a step of 10 between. Error bars denote standard error.

Interspecific β-diversity differed for females during both the maternity ($F_{2,23}$ = 1.66, P = 0.004) and mating ($F_{2,14}$ = 1.79, P = 0.003) seasons. Female *C. gouldii* and *N. gouldi* had significantly different β-diversity in both seasons (maternity, $F_{1,17}$ = 1.63, P = 0.019; mating. $F_{1,9}$ = 2.04, P = 0.028) whilst there were no differences in β-diversity between female *C. gouldii* and *V. regulus* in either season (maternity, $F_{1,15}$ = 1.50, P = 0.116; mating. $F_{1,8}$ = 1.88, P = 0.331). Female *N. gouldi* had similar β-diversity to *V. regulus* in the mating ($F_{1,10}$ = 2.06, P = 0.167) but not the maternity ($F_{1,14}$= 1.88, P = 0.006) season. There was no difference in β-diversity between male bat species' diets overall ($F_{2,15}$= 1.27, P = 0.166) or when analysed by season ($F_{2,15}$= 1.32, P = 0.310). Examination of interspecific β-diversity revealed no effect of sex ($F_{3,52}$ = 0.84, P = 0.866) or an interactive effect of sex and season ($F_{3,52}$ = 0.85, P = 0.903) on bat species' diets.

Intraspecific dietary diversity

Examination of intraspecific gender and seasonal differences found no gender differences in dietary β-diversity for any bat species (*C. gouldii*, $F_{1,21}$ = 0.98. P = 0.624; *N. gouldi*, $F_{1,17}$ = 0.74, P = 0.861; *V. regulus*, $F_{1,20}$ = 0.82, P = 0.617) and this effect was independent of season (*C. gouldii*, $F_{1,19}$ = 1.88, P = 0.814; *N. gouldi*, $F_{1,15}$ = 2.04, P = 0.641; *V. regulus*, $F_{1,18}$ = 1.91, P = 0.737). However, even our limited sample sizes revealed seasonal dietary differences for *C. gouldii* ($F_{1,21}$ = 1.90, P = 0.002) and *N. gouldi* ($F_{1,41}$ = 2.08, P = 0.025), but not *V. regulus* ($F_{1,20}$ = 1.95, P = 0.127) (Figure 3). Only *C. gouldii* showed seasonal intraspecific dietary differences in prey α-diversity, with higher Chao1 values during the maternity than mating season (t_{14} = 3.27, P = 0.006). We were unable to compare dietary diversity between sexes within a season due to inadequate samples sizes.

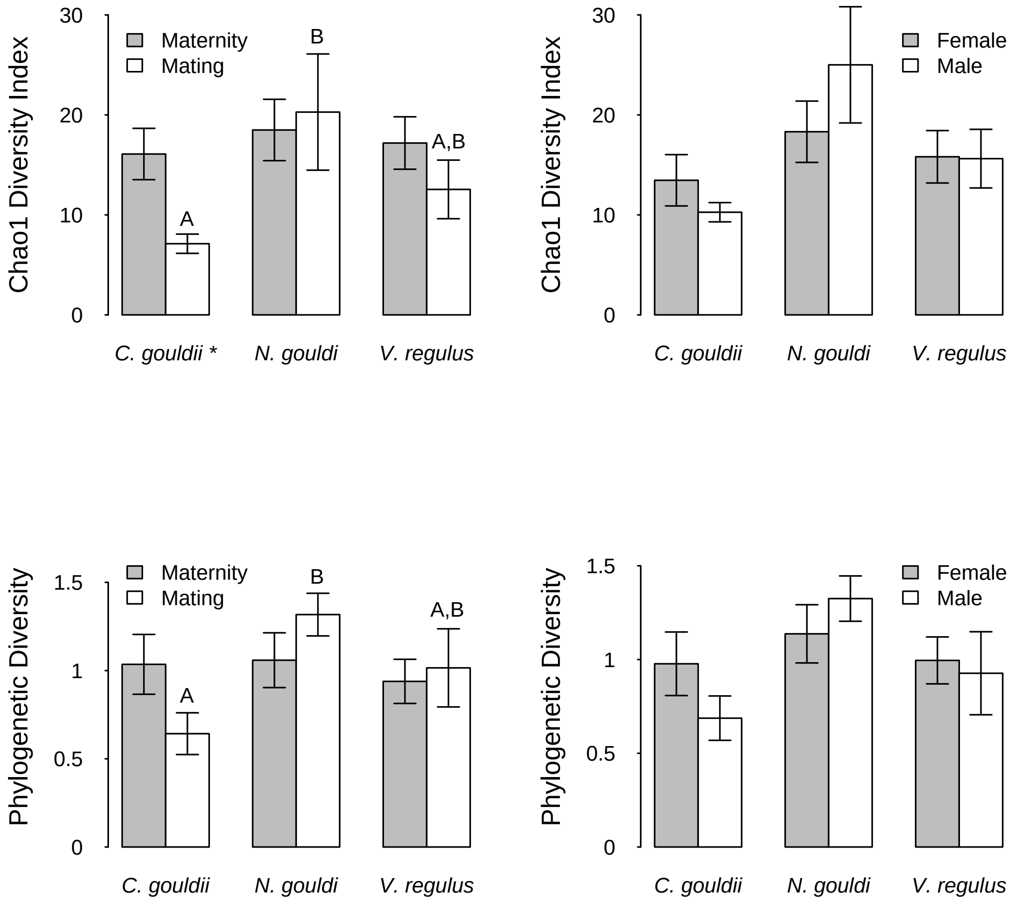


Figure 3: Diversity indices for three species of bat, by sex and season: species richness as estimated by Chao1 for each bat species by season (top left) and sex (top right); Faith's phylogenetic diversity for each bat species by season (bottom left) and sex (bottom right). Statistical differences in α-diversity within a species are indicated by * and between species by letters. Error bars denote standard error.

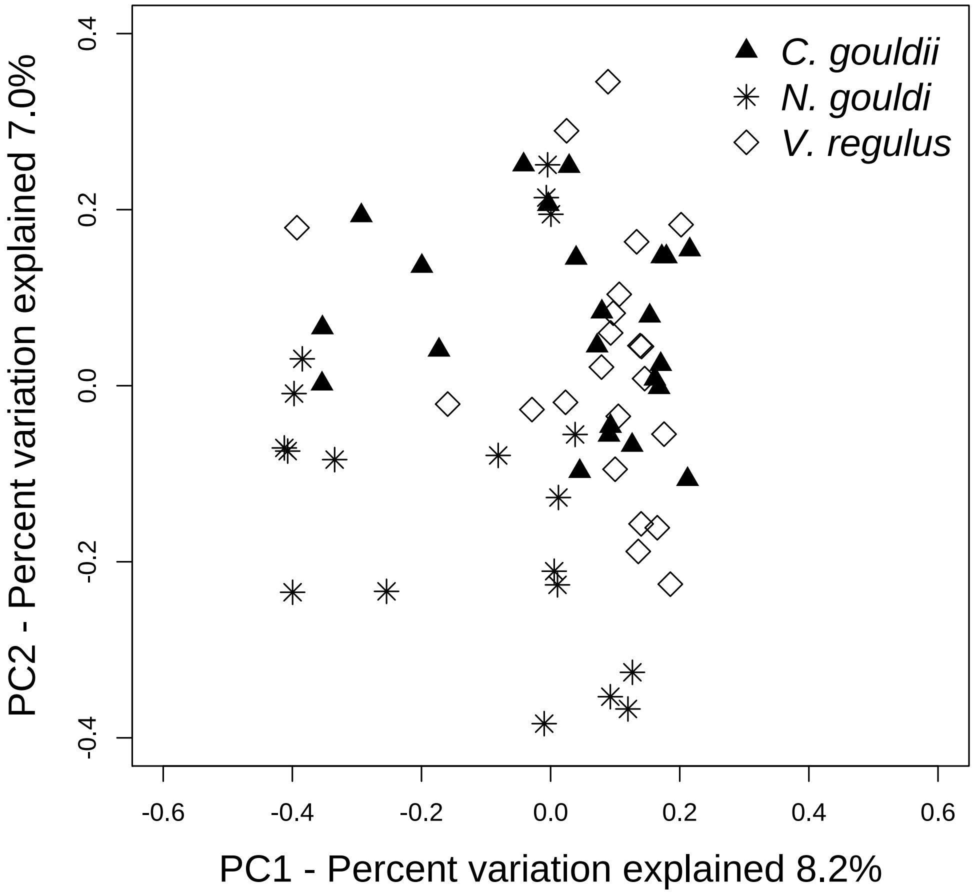


Figure 4: Principal coordinates analysis (PCoA) of MOTUs for three bat species, based on UniFrac distances unweighted by MOTU abundances. Each symbol corresponds to one faecal sample, i.e., individual bat. The first two principal coordinate (PC) axes are shown, explaining 15.4 % of total variation.

Discussion

We were successful in identifying bat dietary differences and niche partitioning in three sympatric bat species in a biodiversity hotspot where prey is largely undescribed. As predicted, dietary divergence was positively related to ecomorphological divergence but dietary convergence did not occur when resources were limited. Our study demonstrates that the fine resolution of HTS, and MOTU analysis, provides important insight into complex predator-prey relationships; we elucidated seasonal intra and interspecific differences in prey consumption in a genetically uncharacterised area that is increasingly fragmented and experiencing a drying climate (Batini 2007; Klausmeyer & Shaw 2009).

MOTU taxonomic assignment

Using the BOLD reference database we detected between one and five prey orders (Diptera, Hemiptera, Lepidoptera, Mantodea and Neuroptera) for each bat species, depending on the assignment method. As the 'best match' method has both higher true and false positive rates than the 'strict' method (Ross et al. 2008), our 'best match' assignments should be considered cautiously, as a working list of prey items. As we only accepted 'best match' assignments within Australian sampled taxa, often from south-western Australian, we are fairly confident in these assignments. Based on this, our study shows similar results to morphological studies that identified between three and six prey orders for the same species, although prey orders differed. A morphological study elsewhere in the jarrah forest detected three prey orders (Coleoptera, Hymenoptera and Lepidoptera) for these three bat species (Fullard et al. 1991). In Tasmania *C. gouldii* foraged primarily on caterpillars, as well as adult Lepidoptera and Coleoptera while *V. regulus* primarily consumed Lepidoptera, in addition to Coleoptera, Hemiptera, Isoptera, Neuroptera and Trichoptera (O'Neill & Taylor 1989). In south-eastern Australia, *C. gouldii* consumed Hemiptera and Lepidoptera, *N. gouldi* consumed Coleoptera and Lepidoptera and *V. regulus* consumed Coleoptera, Diptera and Lepidoptera (Lumsden & Bennett 2005). The lack of Coleoptera, Hymenoptera, Isoptera and Trichoptera in any of our bat samples is most likely a reflection of their poor taxonomic representation in the genetic reference database, as all orders occur within the jarrah forest (Bunn 1983; Farr et al. 2011).

The majority of assigned MOTUs were Lepidoptera and most were consumed by *C. gouldii*. As predicted, species richness estimates suggest individual *N. gouldi* preyed on more species than *C. gouldii* individuals and prey accumulation curves suggest *C. gouldii* also had the least diverse prey base for all three bat species. These somewhat contradictory results may be indicative of *C. gouldii* consuming larger and/or more ubiquitous prey species that are also invertebrate species most often sampled (Farr et al. 2011) and represented within global reference databases (Dodd et al. 2012a), as opposed to an actual greater α-diversity of Lepidoptera prey within *C. gouldii*'s diet. While one-third of known Lepidoptera species DNA sequences are available in global genetic reference databases, other taxa have substantially lower proportions of described species represented by DNA sequences, e.g., 6% for Formicidae and 18% for Trichoptera (Jinbo et al. 2011). Whilst our study only assigned between 4 and 20% of MOTUs taxonomically, depending on the method, dietary HTS studies in other biodiverse regions did not yield any matches of invertebrate prey to online reference databases (Brown et al. 2013). These results reinforce how limiting molecular approaches can be in identifying specific prey items in areas lacking a comprehensive genetic reference database and underscore the necessity of examining dietary diversity irrespective of taxonomy. Even in regions where taxonomy is relatively well described, prey can be highly speciose and/or underrepresented in genetic reference databases, limiting taxonomic assignment (Clare et al. 2011).

Interspecific dietary diversity

As expected, our study found significant differences in β-diversity, suggesting niche partitioning, between the three bat species over the entire sampling period, likely influenced by ecomorphology and foraging strategy. The relatively large *C. gouldii* forages in edge and open habitat whereas both *N. gouldi* and *V. regulus* exploit closed and edge habitat, navigating through small openings in vegetation (Fullard et al. 1991; O'Neill & Taylor 1986). Differences between *N. gouldi* and *V. regulus* are likely explained by *N. gouldi* employing two foraging strategies, aerial hawking and gleaning from vegetation, increasing accessibility to a diversity of prey. Unsurprisingly, the more ecomorphologically divergent species, *C. gouldii* and *N. gouldi*, showed the greatest divergence in diet, suggesting niche partitioning is greatest between these two species.

Interspecific dietary differentiation also occurred seasonally for α and β-diversity. *N. gouldi* individuals consumed more prey taxa than *C. gouldii* individuals during the mating season. Marginal non-significance of β-diversity between *C. gouldii* and *V. regulus* during the mating season suggests an overall greater degree of dietary overlap between these two aerial hawkers than between either species and the facultative gleaner, *N. gouldi.* During the maternity season, dietary composition differed between all three bat species but not the number of prey taxa consumed per individual bat. Contrary to predictions, we found β-diversity dietary differentiation between bat species depended on season for females, but not for males, and within females *N. gouldi* and *V. regulus* diets converged during the mating, rather than the maternity, season. Our results, instead, suggested that ecomorphologically distinctiveness was the best predictor of β-diversity dietary differentiation in females. Females of the more ecomorphologically divergent, *C. gouldii* and *N. gouldi*, had divergent diets regardless of season whilst females of the more ecomorphologically similar species, *C. gouldii* and *V. regulus*, had similar diets in both seasons. Our findings may reflect how constrained female bats are by roosting sites during the maternity season (Lumsden et al. 2002b; Taylor & Savva 1988) and, while able to commute relatively large distances between roosting and foraging sites (e.g., Lumsden et al. 2002a), the associated energetic costs may influence selective foraging, or niche partitioning, during the maternity season. As energetic requirements and dietary diversity vary for lactating and pregnant females (Dietz & Kalko 2007; Leelapaibul et al. 2005), pooling females of various reproductive stages may be masking dietary differentiation. Alternatively, some bat species contend with increased energetic demands by employing metabolic compensation, rather than increasing prey consumption (Becker et al. 2013), which may explain the inconsistent patterns between bat studies examining dietary differences between sexes (e.g., Carter et al. 1998; Whitaker 2004). Our study occurred during a very dry year (rainfall ~50% of long-term average) and further research into bat diets would benefit from longitudinal surveys, as determining clear mechanistic processes is best achieved by collecting multi-year data to disentangle seasonal and environmental influences.

Intraspecific dietary diversity

Our study corroborated other bat dietary studies that have shown intraspecific seasonal differences in some species (e.g., Andreas et al. 2012) but not others (e.g., Johnson et al. 2012). *C gouldii* individuals consumed fewer prey taxa during the maternity season than the mating season. In addition, *C. gouldii* and *N. gouldi* showed intraspecific differences in dietary β-diversity between seasons whilst there was no difference for *V. regulus*. This suggests that *C. gouldii* and *N. gouldi* are more opportunistic foragers, consuming available prey, in comparison to *V. regulus,* who appears to be tracking the same prey species over time. This is in contrast to a Tasmanian study where *C. gouldii* was considered a specialist, and *V. regulus* a generalist, forager (O'Neill & Taylor 1989). The difference between these two studies may reflect a difference in foraging ecology of two geographically distinct populations or be reflective of the taxonomic resolution of each study as the Tasmanian study relied on ordinal level analyses.

We expected dietary differentiation for male and female bats in both the maternity season, when energetic requirements for females are higher (Kurta et al. 1989), and the mating season, when energetic demands for males are higher (Dietz & Kalko 2007). However, small sample sizes limited our ability to meaningfully compare intraspecific sexual dietary differences within a season. Studies with increased sample sizes are required to address sexual dietary differentiation of jarrah forest bats between seasons.

Conclusion

This study shows the value of HTS as a technique for determining dietary differentiation in three sympatric insectivorous bat species consuming a speciose prey base lacking representation in genetic reference databases. Niche partitioning likely facilitates the co-existence of bat species and while ecomorphologically divergent species show the strongest dietary divergence future studies are needed to determine causation between bat species co-existence and dietary overlap. Understanding the extent of niche partitioning is particularly important in disturbed systems where predator species evolved adaptations to minimise dietary overlap but changing environments now interfere with traditional predator-prey relationships and species co-existence. HTS-based approaches clearly have the power to elucidate complex predator-prey relationships, including dietary differentiation between sympatric predatory species, and will facilitate best-practice management and conservation of biodiversity in a rapidly changing environment.

Acknowledgements

JMB was a recipient of a Murdoch University International Postgraduate Scholarship during this study, and was funded by Alcoa of Australia Limited, Holsworth Wildlife Research Endowment the Ecological Society of Australia and the Australian Research Council (LP0882677). All work was carried out under Murdoch University Animal Ethics Committee permit W2347/10 and Department of Environment and Conservation permits CE002999 and SF007648. We thank Susan Campbell, Sian Contarino, Tina Jorgensen, Rod McGregor, Rebecca Parsons, Paul Mitrovski and Jesse Young for assistance in the field, and Nicole White, Frances Brigg and Matthew Bellgard and the iVEC supercomputing resources for assistance and advice regarding data generation and analysis. MB is funded by the Australian Research Council (FT0991741).

Author Contributions Box:

J.M. Burgar, D.C. Murray, M.D., Craig, J. Haile, V. Stokes and M. Bunce designed and supervised the experiments; J.M. Burgar contributed samples; J. Houston, D.C. Murray and J.M. Burgar performed the experiments; J.M. Burgar and D.C. Murray analysed the results; J.M. Burgar and M.D. Craig drafted the article; all authors revised the article.

Data accessibility:

All additional supporting materials have been deposited to DRYAD and are accessible under doi:10.5061/dryad.km6ph.

Table 1: Table 1 with associated MOTUs.

Table S1: Table listing bat species traits.

Table S2: β-diversity results for all MOTUs and subset of MOTUs.

Table S3: Table with geographic coordinates of data collection locations.

Table S4: Input table used for analyses within R – unweighted unifrac distance matrix.

Table S5: Input table used for analyses within R – prey (species) accumulation data.

Table S6: Input table used for analyses within R – Chao1 and PD rarefaction.

Appendix S1: Molecular methods.

Appendix S2 Alignment of representative sequences generated for this study.

Appendix S3: Scripts used for analyses in R.

Appendix S4: Scripts used for analyses in QIIME.

Appendix S5: Mapping file for QIIME and R analyses.

Appendix S6: List of MOTUs found in only one sample.

AppendixS7: DNA Sequences.

Chapter Four

Prey occurrence does not necessarily equate to prey accessibility: the effect of vegetation structure on bats and their insect prey across a restored landscape.

Introduction

Understanding trophic interactions is imperative for the effective management and conservation of biodiversity, particularly in disturbed systems where continued habitat loss can lead to trophic collapse (Dobson et al. 2006). Within human modified landscapes restoration is a key tool in achieving conservation goals and ameliorating habitat loss (Dobson et al. 1997; Young 2000), but to be effective restoration needs to consider all of the needs of recolonising species, including foraging requirements. Vegetation structure can markedly differ between restored and remnant sites (Munro et al. 2009; Norman et al. 2006), and this difference may remain from decades to over a century (e.g., Vesk et al. 2008). Where recolonising species are animalivorous, rather than herbivorous, the vegetation structure of restoration must not only provide suitable habitat for both predator and prey, but also ensure prey populations are accessible to predators. Restored systems have the ability to improve food-web structures that may have declined in degraded landscapes (e.g., Albrecht et al. 2007). Insectivorous bats are an integral, yet often underestimated, part of the food web (Kalka & Kalko 2006; Kunz et al. 2011; Morrison & Lindell 2012). Foraging bats capture between 100-500 insects per hour (Gould 1955), consuming between 30-80% of their body mass in insects each night (Anthony & Kunz 1977; Kalka & Kalko 2006). Lactating females, at peak energy expenditure, consume between 70% to over 100% of their body mass in insects nightly (Barclay et al. 1991; Kunz & Stern 1995; Kurta et al. 1989). While food is not generally considered a limiting resource for insectivorous bat populations (Ciechanowski et al. 2007; Fenton 1990), the sheer volume of prey consumed necessitates consideration of both predator and prey populations within disturbed systems to ensure effective conservation of both predators and their prey base.

Most bats are generally considered relatively resilient to disturbance (Arnett 2003). Many bats forage in disturbed systems (Dodd et al. 2012b; Lumsden & Bennett 2005; Morris et al. 2010; Webala et al. 2011) and fragmented landscapes can provide commuting corridors between foraging and roosting sites (Ethier & Fahrig 2011). In disturbed systems, many bats species show higher activity along tracks, or at the edge, than within regrowth (Jantzen & Fenton 2013; Lloyd et al. 2006; Monadjem et al. 2010; Webala et al. 2011). Vegetation structure is often attributed as the reason for reduced bat activity within regrowth (Chapter 2); vegetation structure poses both a mechanical and perceptual challenge to bats (Fenton 1990). Mechanically, bats must be able to manoeuvre amongst vegetation in addition to the perceptual task of differentiating echoes between their target prey and vegetation. To a foraging bat, where echoes rebounding from vegetation are extraneous to those from the prey target, vegetation is viewed as “clutter” (Fenton 1990; Schnitzler et al. 2003). Bats adapted for fast, agile flight (i.e., both high wing loading and aspect ratio) generally emit low, narrowband echolocation calls and are suited for foraging within open environments (Fenton 1990; Schnitzler & Kalko 2001). In contrast, more manoeuvrable bats are adapted for slow flight, emit short, high frequency echolocation calls and are adept at navigating cluttered or closed environments (Fenton 1990; Schnitzler & Kalko 2001). Some bat species have incredible plasticity in their echolocation call structure, varying their calls to suit the microhabitat (Jakobsen & Surlykke 2010; Siemers & Schnitzler 2004), but this flexibility is unidirectional. Bats adapted to navigating cluttered environments are also able to exploit more open environments but not vice versa (Fenton 1990; Schnitzler et al. 2003). Thus, while some bats may be capable of foraging in regrowth, most bat species respond negatively to vegetation clutter (Adams et al. 2009) and avoid cluttered environments unless they provide an energetic benefit (Sleep & Brigham 2003). The occurrence of prey within regrowth may well dictate the extent that bats use restoration, at least for the more manoeuvrable bat species that can navigate cluttered environments (Petrites et al. 2009).

Similar to bats, invertebrates exhibit differential responses to disturbance with changes in abundance, species richness and community composition dependent on the disturbance regime (Dodd et al. 2012b; Koch et al. 2010; Majer et al. 2013; Summerville 2010). At the ordinal level Lepidoptera abundance is typically lower (Dodd et al. 2012b; Summerville 2010; Webala et al. 2011) while other taxa, such as Coleoptera and Diptera, tend to have similar or greater abundance in degraded, compared to remnant, sites (Dodd et al. 2012b; Koch et al. 2010). Some studies show invertebrate community composition of restored sites converging to remnant sites (Gibb & Cunningham 2010; Strehlow et al. 2002) while others suggest continued separation between remnant sites and sites restored nearly four decades previously (e.g., Majer et al. 2013). Differences in findings are often taxa dependent. For Lepidoptera in managed forest systems, undisturbed areas tend to have higher species richness and more diverse species assemblages than degraded areas, with dominant species in restoration typically converging on dominant species in remnant areas (Dodd et al. 2012b; Summerville & Crist 2008). In contrast, Coleoptera and Diptera diversity tends to be similar between degraded and remnant sites with differences in diversity only arising between the sites most distinct from remnant sites in terms of vegetation structure (Dodd et al. 2012b; Koch et al. 2010). Even where invertebrate species richness is comparable between degraded and remnant sites at the local scale, degraded sites are generally more homogenous than remnant sites at the landscape scale (Farr et al. 2011). Invertebrate recolonisation of restored sites is often linked to vegetation structure (Davis et al. 2002; Gibb & Cunningham 2010; Taillefer & Wheeler 2012). Low levels of vegetation clutter may be advantageous to invertebrates as it provides more opportunity for navigation and predator evasion (Dodd et al. 2012b). The high spatial and temporal variation of invertebrate abundance and communities across multiple scales (e.g., Farr et al. 2011; Werner & Raffa 2000) underscores the importance of studying prey populations within restoration as prey occurrence cannot be assumed and prey occurrence will influence the suitability of restoration as foraging habitat for predators.

Bat studies explicitly examining the effect of disturbance on both predator and prey have found contrasting results, with some showing an effect of disturbance on trophic linkages (e.g., Dodd et al. 2012b) and others not (e.g., Webala et al. 2010). The explicit relationship between predatory bats and their invertebrate prey is rarely explored in restored systems (but see Morrison & Lindell 2012) so we investigated the ability of restoration to provide suitable foraging habitat for bats, both in terms of prey occurrence and accessibility. We focused on restored northern jarrah (*Eucalyptus marginata*) forests of south-western Australia. Within this region ~600 ha of forest is cleared, mined and restored annually, with the aim of restoring a fully functioning ecosystem (Koch 2007a). The northern jarrah forest is home to between 15 000 and 20 000 invertebrate species (Abbott 1995) and nine species of insectivorous tree-dwelling bats (Churchill 2008) that range in microhabitat use from open to edge to closed environment adapted species (Fullard et al. 1991). This biodiversity (Myers et al. 2000) and invertebrate diversity (Cooper et al. 2011) hotspot has undergone a substantial amount of habitat loss (Bradshaw 2012), increasing the importance that current human-induced activities minimise any negative effects on biodiversity and trophic interactions.

Our study aim was to investigate the dynamics between predatory bats and their insect prey within both restored and unmined northern jarrah forest. Specifically, we were interested in determining if there were differences between >10 year old restored and unmined forest in terms of (i) vegetation structure; (ii) insect prey; (iii) the accessibility of insect prey to predatory bats. Previous vegetation studies within the restored jarrah forest suggest that while floral density and cover of restored sites does not become more similar to unmined forest as restoration ages (Norman et al. 2006), vegetation structure of restoration does (Craig et al. 2012; Norman et al. 2006). Thus, we predicted that restored vegetation structure would become more similar to unmined forest vegetation structure as restoration aged. We also predicted that insect orders would show differential responses to restoration in terms of biomass and diversity: Lepidoptera would be the most sensitive to restoration while Coleoptera and Diptera would either show no, or a positive, effect of restoration on biomass and diversity. While bat dietary diversity has been shown to differ across species in the restored jarrah forests, dietary overlap in consumed prey species does occur (Chapter 3), particularly when examining prey at the ordinal level (Fullard et al. 1991). As bats tend to be opportunistic foragers (Fenton 1990) we speculated that bat activity would be positively correlated with prey occurrence if prey was accessible (e.g., Lumsden & Bennett 2005). We predicted that restoration would limit the ability of edge foragers to access their prey but not affect those adapted for either open or closed environments. The increased vegetation clutter of restoration, compared to unmined forest (Craig et al. 2012), potentially reduces the energetic benefits of foraging within restoration for edge adapted bat species, reducing the accessibility of their insect prey. In contrast, closed environment adapted bats are more tolerant of cluttered vegetation, thus vegetation structure is less likely to limit their ability to access prey (e.g., Petrites et al. 2009). At the other end of the spectrum, open environment adapted bat species fly above the canopy and thus are unlikely to forage within either restored or unmined forest, instead foraging for prey above either forest type (e.g., Fenton & Griffin 1997).

Methods & Materials

Study area and experimental design

The study was conducted at Huntly minesite (32°36'S, 116°07'E), operated by Alcoa of Australia (hereafter Alcoa) and located~80-100 km SSE of Perth in south-western Australia. The region is characterised by a Mediterranean climate with cool, wet winters and warm, dry summers. Since 1990, Huntly has averaged 1180 mm annually with >75% falling between May and September, although the year preceding our study (2010) was exceptionally dry with only 630 mm rainfall. The northern jarrah forest (~700,000 ha) is a multiple-use forest with mineral extraction, conservation, timber production, water supply, and recreation values (Grant 2006). Alcoa has been mining in the area for more than 40 years and, within Huntly minesite (~15 000 ha), Alcoa mines, clears and restores 40-50% of the forest (Koch 2007a). The resulting landscape is a patchwork mosaic of unmined and restored forest. Vegetation in the study area is dry sclerophyll eucalypt forest; the two dominant canopy species are jarrah and marri (*Corymbia calophylla*), the second storey is commonly populated by bull banksia (*Banksia grandis*), sheoak (*Allocasuarina fraseriana*), snottygobble (*Persoonia longifolia*) and woody pear (*Xylomelum occidentale*) and the undergrowth is a variety of sclerophyllous shrubs up to ~3 m high (Koch 2007b).

Since 1989, Alcoa has restored sites by seeding with native, local overstorey species to reflect species composition within the unmined forest (Grant 2006). To determine if the restored forest was suitable for foraging bats we compared post 1989 restoration to unmined forest. We identified three forest types for this study: unmined forest; 10-14 year old restoration (R 10-14); and >15 year old restoration (R >15). Both unmined forest and >15 year old restoration have been burnt in recent years as part of a regional prescribed burn management regime (Grant 2006). Thus, we included both 10-14 and >15 year old restoration to ensure that any effects could be attributed to restoration rather than fire. We selected restored sites that met the criteria of size (>4 ha), pit shape (at least one edge bordered by unmined forest); and maximum density of eucalypts other than jarrah and marri (<5 stems ha^{-1}). We surveyed 24 sites within the restored landscape: eight sites each within unmined forest, R 10-14 and R >15 (Figure 1).

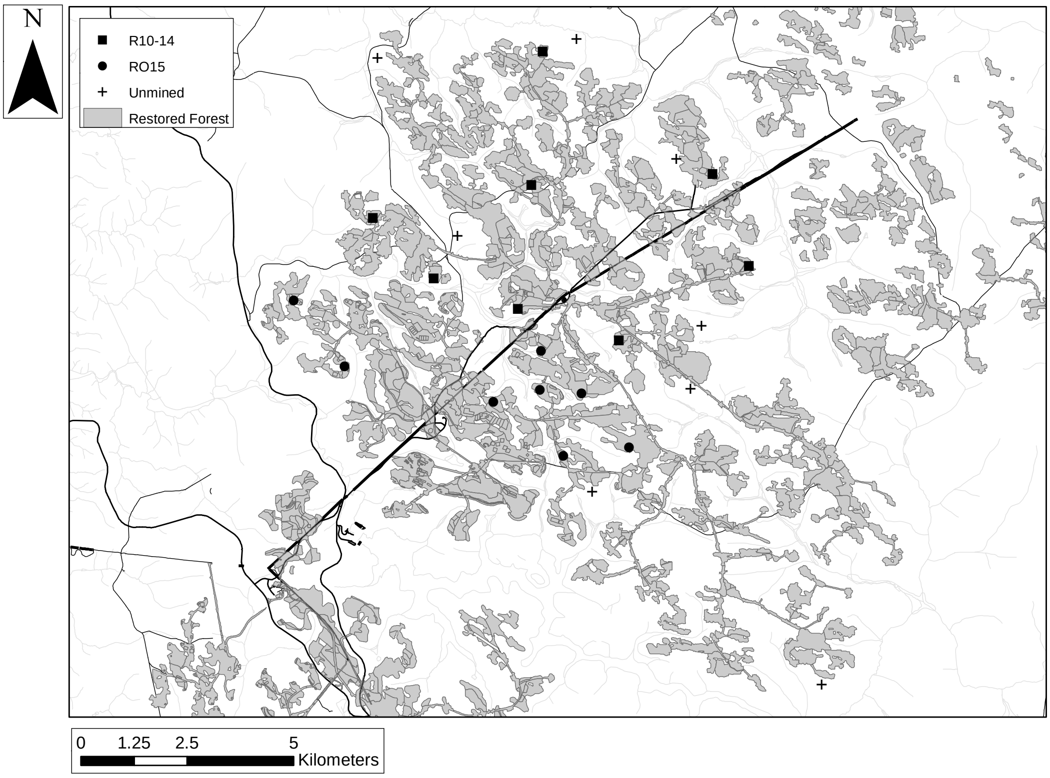


Figure 1: Map showing the spatial arrangement of 24 bat and invertebrate survey sites at Alcoa's Huntly minesite in south-western Australia. White denotes unmined forest, light grey lines denote streams and black lines denote sealed roads.

Vegetation surveys

Vegetation surveys were conducted after bat and invertebrate surveys from April to July 2011. We collected vegetation characteristics from five 5 x 5 m quadrats at each site, one at the bat and invertebrate survey location and four at 30 m in each of the four cardinal directions from the survey location. To estimate vertical vegetation structure we assessed four levels of vegetation strata: overstorey (>15 m), midstorey (5 to 15 m), shrub (0.75 to 5 m) and ground (<0.75 m). We measured the maximum vegetation height using a tape measure for the ground and shrub strata and a tree vertex for the overstorey and midstorey strata. Within each 5 x 5 m quadrat we visually estimated percent vegetation cover in each strata. Canopy cover was calculated from canopy photographs taken at the centre of each quadrat (Macfarlane 2011) and was recorded as the proportion of cover within each photograph. Canopy height was the average of the five tallest overstorey plants within 10 m of the centre of each quadrat. We averaged values across the five quadrats to obtain an average value for each vegetation variable per site.

Prey surveys

We assessed invertebrate prey occurrence using standard funnel and bucket (diameter 26 cm) light traps (Australian Entomological Supplies, Australia) with a 12 V 8 W battery powered ultraviolet light on a timing switch; lights functioned from 30 mins before sunset to 30 mins after sunrise. We placed commercial pest strips containing Dichlorvos, the killing agent, within the trap bucket to quickly immobilise trapped invertebrates, minimising specimen damage (Farr et al. 2011). We surveyed invertebrates in February and March 2011, concurrently with the bat activity surveys, although we rotated sites so that invertebrate and bat surveys did not occur at the same site during the same night to avoid confounding bat activity with increased invertebrate activity around light traps (Adams et al. 2005). As collection method can influence the direction and magnitude of change in estimated species richness across disturbance types (Farr et al. 2011), we also employed 110 cm^3 malaise traps (Standard SLAM Trap EM508; Australian Entomological Supplies, Australia) at each site, with ethylene glycol as the killing agent, to passively capture invertebrates (Dodd et al. 2012b). Invertebrate traps and bat detectors were placed at the same point in each site. We surveyed invertebrates twice at each site, surveying one site in each forest type each survey night to minimise potential variation between nights. Due to the logistics of moving field equipment, we surveyed invertebrates with light traps on two concurrent nights and we deployed malaise traps for seven consecutive nights at each site. Minimum night air temperatures were recorded near each trap at each site using digital thermometers. Invertebrate samples were frozen as soon after collection as possible to minimise sample degradation.

We collected so few invertebrates using malaise traps that we did not analyse the malaise trap data. Invertebrates were sorted to order and wingspan size class (5 mm increments for all orders other than Lepidoptera, which we sorted <3 mm, 3-6 mm, 6-10 mm and then 5 mm increments for >10 mm) and counted. Dry biomass can be derived from the length of individuals (Rogers et al. 1976) although we found the generalised equation to be somewhat inaccurate so we derived our own equations. We dried (in ovens for 48 hours at 40°C) and weighed a subset (19 658 individuals, 42%) to derive equations. For each invertebrate order we fitted three different functions (linear, exponential, and power) and selected the one with the highest R^2 value as the final equation. Using this final equation we then calculated dry biomass values for each order and size class per sample (Table 1). From diet studies in the study area and elsewhere in Australia (Chapter 3; Fullard et al. 1991; Lumsden & Bennett 2005; O'Neill & Taylor 1989), we identified eight insect orders as jarrah forest bat dominant prey: Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Mantodea, Neuroptera and Trichoptera. We removed orders that occurred in less than 25% of the samples (Mantodea and Neuroptera) for a total of six insect orders considered as potential prey. Biomass of all retained orders was not correlated (all <0.38, Spearman correlation). Considering the constraints of echolocation call wavelength on prey detection (Gould 1955), the energetic costs of handling certain sized prey items (Barclay et al. 1991) and observations from studies in eastern Australia (Lumsden & Bennett 2005), we analysed all insects from each potential prey order as well as all insects <15 mm in size from each potential prey order.

Table 1: Equations for calculating dry biomass where y is biomass in mg and x is length in mm. We derived calculations by measuring and weighing dried biomass of a subset of individuals, denoted by N (subset). The biomass value is the calculated dry biomass, for all individuals (Total N). The fit of each equation is provided (R^2). For Mantodea we used the equation derived from all orders.

Order	Equation	R²	N (subset)	Total N	Biomass (g)
All Orders	$y = 0.0487x^{2.0369}$	0.83	19 658	46 977	203.18
Arachnid	$y = 0.1172e^{0.4198x}$	0.80	38	63	0.05
Blattodea	$y = 0.3189e^{0.2612x}$	0.81	28	76	12.39
Coleoptera	$y = 0.8207e^{0.2215x}$	0.82	760	1832	75.12
Diptera	$y = 0.085e^{0.3383x}$	0.76	3722	5898	23.06
Hemiptera	$y = 0.1265e^{0.4568x}$	0.88	1311	2622	4.78
Hymenoptera	$y = 0.3e^{0.2151x}$	0.85	4407	6046	11.50
Lepidoptera	$y = 0.2308e^{0.2202x}$	0.90	9226	29 946	69.16
Mantodea*	$y = 0.0487x^{2.0369}$	--	4	28	1.06
Neuroptera	$y = 0.2464x + 0.1$	0.90	34	156	0.52
Orthoptera	$y = 0.193e^{0.2052x}$	0.89	9	38	4.99
Trichoptera	$y = 0.1461e^{0.2812x}$	0.74	119	272	0.55

Bat surveys

We surveyed bats, at each site, throughout February and March 2011 by recording echolocation calls using ultrasonic detectors (Anabat, Titley Electronics, Australia), set to record from 30 mins before sunset until 30 mins after sunrise. Detector microphones were placed on PVC poles 1.5 m above the ground and at a 45° angle, facing a gap in the vegetation to minimise vegetation attenuation (Law & Chidel

2002), and oriented towards the closest unmined forest edge. We acknowledge that positioning detectors towards the canopy reduced our ability to detect bats foraging along the ground. However, as we were limited by the number of detectors we chose to focus on bats foraging >1.5 m above the ground as we anticipated that this is where the majority of bats would be foraging (Adams et al. 2009). Detectors were calibrated at the start of the season to ensure sensitivity was consistent between detectors (Larson & Hayes 2000). Detectors were placed ≥100 m from the nearest edge of different aged restoration and ≥80 m from the nearest unmined forest edge to minimise edge effects (Jantzen & Fenton 2013). Sites were not surveyed during the full moon or on nights with high winds or rain. Each site was surveyed twice, surveying all sites once before re-surveying. We deployed three detectors each survey night, one in each forest type, to minimise potential variation between nights. Temperatures at each site were recorded every 15 mins by attaching a Thermochron iButton (model DS1921G-F5; MAXIM, Ireland) to the PVC poles.

Analyses

Vegetation structure

To reduce the complexity of the analyses we excluded correlated (>0.65, Spearman correlations) vegetation variables, retaining only canopy cover, midstorey cover, shrub cover and ground cover. We visually checked the final variables to ensure they were normally distributed and transformed those that were not (natural log transformation for log cover; square root transformation for shrub cover and ground cover). We took a model-based approach to test the effect of forest type on vegetation structure, using the function manyglm in the R package mvabund

(Wang et al. 2012). This approach uses a multivariate generalised linear model (GLM) framework to make community level and vegetation structure specific inferences by fitting separate GLMs to each variable, with a common set of explanatory variables, and testing significance through resampling-based hypothesis testing (Wang et al. 2012). We ran negative binomial GLMs with a two-dimensional matrix of the vegetation cover variables as the dependent variable and forest type as the independent variable. Wald test statistics were constructed assuming correlation (matrix shrunk by parameter 0.66) and *P*-values were calculated using 999 resampling iterations via PIT trap resampling.

Prey occurrence

As bats consume a relatively large proportion of prey each night and are only constrained in prey size by mechanical limitations (Fenton 1990) and echolocation call structure (Jung et al. 2014), we used biomass, rather than abundance, to measure potential prey (six dominant orders) occurrence. Similar to the vegetation structure multivariate analysis, we again took a model-based approach to test the effect of forest type on prey diversity, using the function manyglm in the R package mvabund (Wang et al. 2012). We ran negative binomial GLMs with a two-dimensional matrix of the insect biomass variables as the dependent variable and forest type as the independent variable. We assumed that there was no correlation of biomasses across the insect orders so constructed log likelihood ratio test statistics and calculated *P*-values using 999 resampling iterations via PIT trap resampling. We ran models with biomass for each insect order averaged over the two survey nights at each site. In consideration of the ability of different sized bats to handle different sized prey we ran models including the biomass of all potential prey order individuals and also for individuals of all potential prey orders that were <15 mm in size.

Bat activity

We quantified bat activity as the number of search-phase bat call files per night; call files contained a group of echolocation pulses recorded within a 15 second span, which we assumed to belong to one individual bat (Fenton 1999; O'Farrell et al. 1999b). Call files were downloaded using CFCRead© software (C. Corben/Titley Electronics) and processed using Analook version 3.8 (C. Corben; http://hoarybat.com). All downloaded files were run through a filter (see Appendix 1 for filter details) to remove extraneous noise, ensure objective quality of calls and to target search-phase navigation calls (Britzke et al. 2011). Nine species of bat occur in the study area (J. Burgar, unpublished data): white-striped free-tailed bat *Tadarida australis* (Gray 1838), southern free-tailed bat *Mormopterus kitcheneri* (Reardon et al. 2014), western false pipistrelle *Falsistrellus mackenziei* (Caputi and Jones 1986), Gould's wattled bat *Chalinolobus gouldii* (Gray 1841), chocolate wattled bat *C. morio* (Gray 1841), lesser long-eared bat *Nyctophilus geoffroyi* (Leach 1821), Gould's long-eared bat *N. gouldi* (Tomes 1858), western greater long-eared bat *N. major* (Gray 1844), and southern forest bat *Vespadelus regulus* (Thomas 1906). The echolocation calls of the three *Nyctophilus* spp. are indistinguishable when recorded using the Anabat system (Adams et al. 2010) and thus were grouped as *Nyctophilus* spp. Bat pulses were automatically identified to species / species grouping using a random forest model from the R caret package (Kuhn 2008). Call files were assigned to the species / species group with the highest number of pulses per call file. When there was a tie the call file was classified as “unknown”. The random forest model is a supervised learning machine, which we trained using a regional call library (see Chapter 2 and Appendix 1 for details on the automation process).

When attempting prey capture, bats typically emit a characteristic terminal feeding buzz, i.e. a quick succession of short duration pulses at the end of the echolocation call (Jakobsen & Surlykke 2010). Emission of a feeding buzz does not necessarily equate to foraging success (Britton & Jones 1999), although it does signify foraging behaviour. Thus, we manually checked each call file for feeding buzzes. This study was part of a larger concurrent study examining bat activity across the restored landscape (64 sites including the 24 used in this study; see Chapter 2). To determine the validity of using bat activity as a surrogate for foraging activity (quantified as the number of feeding buzzes per night) we also manually checked all 9 304 call files recorded at the 64 sites for feeding buzzes (Table 2).

We excluded call files that weren’t identified to a species / species grouping and those identified as *F. mackenziei* and *M. kitcheneri*, due to small sample sizes. Of the remaining 8 848 call files only 151 (1.7%) contained feeding buzzes. At the species level the number of feeding buzzes was highly correlated to the number of search-phase call files (0.98 Spearman, $P < 0.001$). However, similar to other studies (Law et al. 2011) we did not find a strong correlation at the treatment type level (0.57 Spearman, $P = 0.108$); only *C. gouldii* and *V. regulus* feeding buzzes were correlated with search-phase call files (*T. australis* 0.11 Spearman, $P = 0.797$; *C. gouldii* 0.93 Spearman, $P = 0.001$; *C. morio* 0.17 Spearman, $P = 0.689$; *Nyctophilus* spp. 0.58

Table 2. Total number (fb) and percentage of feeding buzzes (% fb) recorded throughout the restored landscape of south-western Australia. The percentage of feeding buzzes was quantified as the number of feeding buzzes divided by the total number of call files (both search phase and feeding) for each treatment type (N=8, sites were surveyed 4 times for a total of 32 surveys per treatment type). Desired and dense refer to eucalypt stem densities; where 500-2500 stems ha-1 is categorised as desired and >2500 stems ha-1 is categorised as dense. Forest types examined in this study are shaded in grey.

	R 0-4		R 5-9				R 10-14				R >15				Unmined			
	Desired		Desired		Dense		Desired		Dense		Desired		Dense				Total	
	fb	% fb	fb	% fb	fb	% fb	fb	% fb	fb	% fb	fb	% fb	fb	% fb	fb	% fb	fb	% fb
T. australis	3	0.7%	0	0.0%	0	0.0%	0	0.0%	1	2.9%	0	0.0%	0	0.0%	0	0.0%	4	0.4%
C. gouldii	2	2.0%	1	1.2%	0	0.0%	0	0.0%	1	1.4%	0	0.0%	0	0.0%	2	1.3%	6	0.9%
C. morio	0	0.0%	1	2.9%	1	2.7%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	1	2.6%	3	0.9%
Nyctophilus spp.	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	1	0.9%	1	0.3%
V. regulus	4	1.4%	12	2.0%	59	5.1%	1	0.2%	8	2.8%	6	1.2%	20	2.5%	27	1.1%	137	2.1%
Total	**9**	**1.1%**	**14**	**1.6%**	**60**	**4.3%**	**1**	**0.2%**	**10**	**1.8%**	**6**	**0.8%**	**20**	**2.0%**	**31**	**1.1%**	**151**	**1.7%**

Spearman, *P* = 0.134; *V. regulus* 0.84 Spearman, *P* = 0.009). However, the lack of a correlation for *T. australis*, *C. morio* and *Nyctophilus* spp. may be due to the very limited number of feeding buzzes recorded for each of these species. The feeding buzzes of gleaning bats, such as *Nyctophilus* spp., are rarely recorded. Researchers suggest that this is either because gleaning bats emit terminal buzzes in half, or fewer, of their foraging attempts (Faure et al. 1990; Grant 1991) or because the feeding buzz is of such low amplitude that it is unlikely to be detected by recording equipment (Schnitzler et al. 2003). While we acknowledge that other studies quantify feeding buzzes as a proxy for foraging activity (e.g., Law et al. 2011; Webala et al. 2011) we believe that we can cautiously use bat activity as a surrogate for foraging activity, similar to other foraging studies (e.g., Lumsden & Bennett 2005). We feel justified in using bat activity rather than feeding buzzes for *C. gouldii* and *V. regulus* and while less so for *T. australis*, *C. morio* and *Nyctophilus* spp. we decided to include these species with the caveat that their results be interpreted cautiously.

To compare bat activity between each forest type, we ran generalised linear mixed models (GLMMs) with bat activity as the dependent variable, forest type as the explanatory variable, and site as a random factor, in the R glmmADMB package (Fournier et al. 2012; Skaug et al. 2013). Models used the Laplace likelihood approximation (Raudenbush et al. 2000), type III sum of squares and were fitted with a negative binomial distribution and a logit function to account for overdispersed count data. To determine goodness of fit we compared each model with significant variables against a null model and tested the likelihood ratio using χ^2 (Zuur et al. 2009).

Prey accessibility

To ascertain if the vegetation structure was limiting the ability of bats to access their prey, we employed an information-theoretic approach to model selection (Burnham & Anderson 2002). For each bat species / species group we constructed 40 separate models with bat activity regressed against insect order biomass (individual models for each of the six insect orders: Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Trichoptera, and biomass of the six orders combined; 7 models), vegetation cover (canopy, midstorey, shrub, and ground cover; 4 models), and then each combination of insect order biomass and vegetation cover (28 models), in addition to a null model. For *T. australis* and *C. gouldii* we used the biomass of all insects within each order while for *C. morio*, *Nyctophilus* spp. and *V. regulus* we only used the biomass of insects <15 mm in size, as these species are only likely to aerially forage for insects below this size (Fenton 1990; Lumsden & Bennett 2005). We ran GLMMs with site as a random factor, a negative binomial regression structure and a log-link function using the R package glmmADMB (Fournier et al. 2012; Skaug et al. 2013). Models used the Laplace likelihood approximation (Raudenbush et al. 2000) and type III sum of squares. Explanatory variables were checked for collinearity prior to model creation using the vif function in the R package HH (Heiberger 2013); there was no evidence of collinearity as all variables had variance inflation factor values less than three (Zuur et al. 2009). We standardised, by centring on the mean, explanatory variables prior to modelling. Models were ranked based on Akaike's information criterion (AIC) score and AIC weights using the R package MuMIn (Barton 2013). From the global set we only considered models with AIC weights higher than the null model that contained both an insect and vegetation variable as potential models describing the influence of vegetation structure on a bat species' ability to access prey. We used model averaging, across the full suite of models, to calculate parameter estimates and retained models with significant interaction terms (Burnham & Anderson 2002).

For retained models we examined the influence of vegetation structure on the impact of insect biomass on bat activity. We did this by plotting the marginal effect of insect biomass on bat activity along a gradient of vegetation measurements. Marginal effects for continuous variables measure instantaneous rates of change, thus provided an approximation of the magnitude of the effect that insect biomass exerted on bat activity as vegetation structure changed. All analyses were performed in the statistical program R (R Core Team 2013).

Results

Vegetation structure

Multivariate vegetation structure was significantly different between 10-14 year old restoration and unmined forest but not between >15 year old restoration and unmined forest (unmined – R 10-14 $P = 0.009$, unmined – R >15 $P = 0.207$). Multivariate, compared to univariate, tests were more powerful in detecting differences in vegetation structure between restored and unmined forest. Vegetation structure specific tests revealed that only shrub cover was significantly higher in 10-14 year old restoration than unmined forest ($P = 0.013$; Figure 2). While mean canopy cover of 10-14 year old restoration was about half that of either unmined forest or >15 year old restoration, high variability between restored sites resulted in a marginally non-significant difference (P = 0.051; Figure 2).

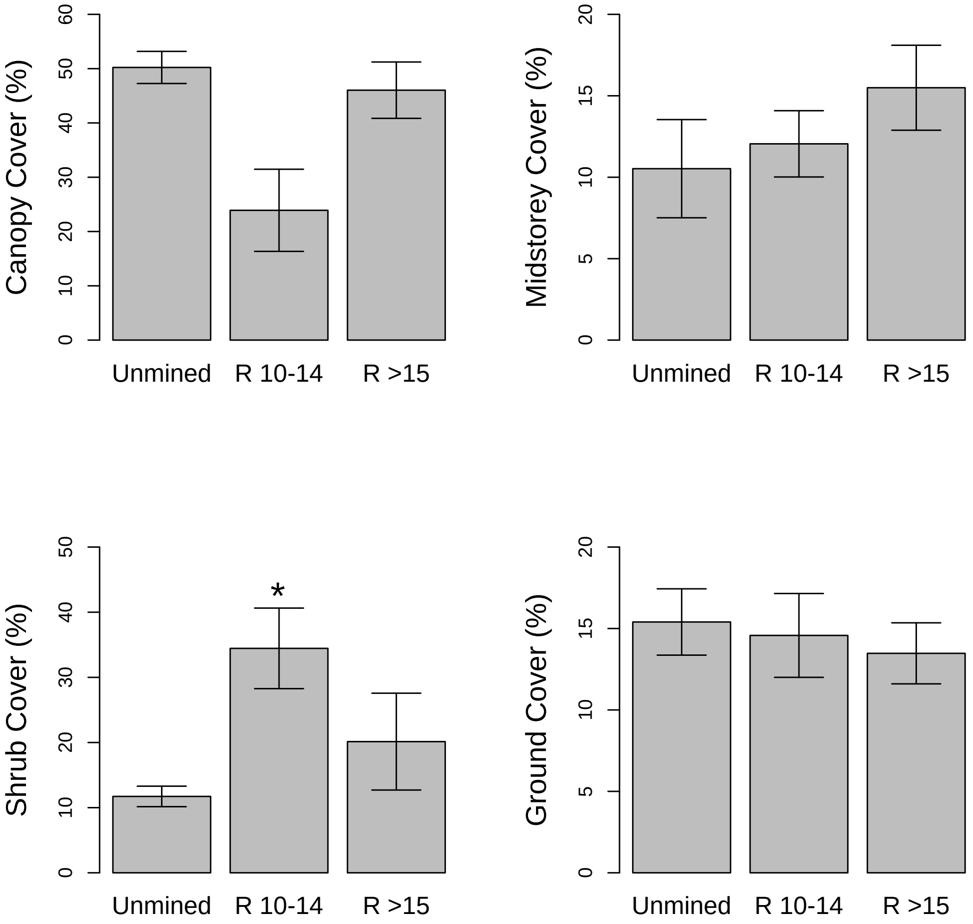


Figure 2: Mean (± SE) vegetation structure values per forest type for canopy, midstorey, shrub and ground cover. * denotes a significant difference in vegetation structure values compared to unmined forest.

Prey occurrence

We trapped 46 977 individual invertebrates over 48 light trap nights, collecting 10 insect orders and a small number (63) of arachnids. Lepidoptera comprised nearly two-thirds (64%) of all individuals, followed by Hymenoptera (13%), Diptera (13%), Hemiptera (6%) and Coleoptera (4%) with the remaining orders making up less than 2% of total individuals. Five orders constituted over 90% of the biomass: Coleoptera (37%), Lepidoptera (34%), Diptera (11%), Hymenoptera (6%) and Blattodea (6%). Insects from the six orders considered as potential prey comprised 46 616 individuals, weighing 184.2 g; potential prey <15 mm in size accounted for 45 335 individuals and 59.5 g (97% of all individuals but only 32% of the biomass).

Community differences in potential prey biomass diversity between forest types depended on the size of the insects. When all insects were considered both restored forest types had significantly different communities compared to unmined forest (unmined – R 10-14 *P* = 0.009, unmined – R >15 *P* = 0.002). In contrast, community biomass did not differ between both restored forest types and unmined forest when only looking at potential prey <15 mm in size (unmined – R 10-14 *P* = 0.391, unmined – R >15 *P* = 0.445; Table 4). Univariate taxon-specific tests revealed that unmined forest had significantly higher Coleoptera biomass than either type of restored forest (unmined – R 10-14 *P* = 0.020, unmined – R >15 *P* = 0.006; Figure 3) and 10-14 year old restoration had significantly higher Diptera biomass than unmined forest (*P* = 0.020). There were no differences across forest types for biomass of any order when examining insects <15 mm (Figure 4).

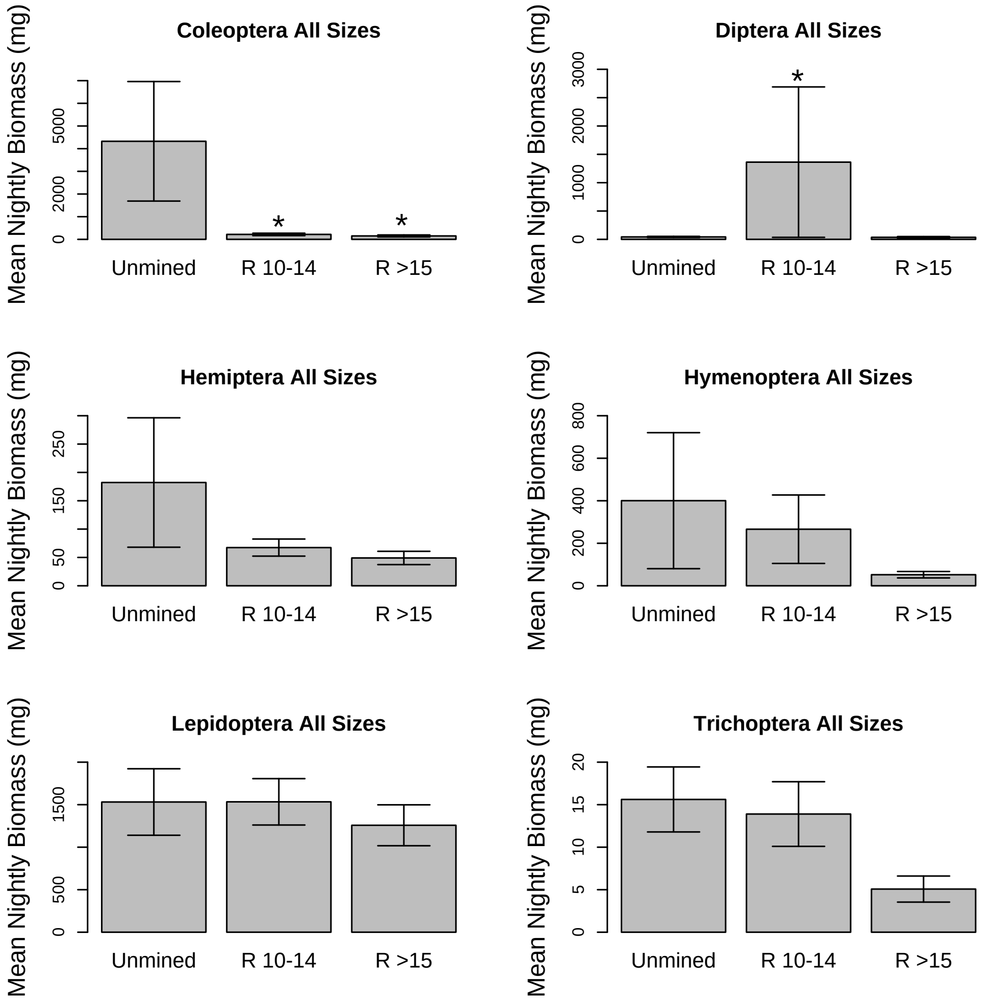


Figure 3: Mean (± SE) biomass (g) of insects less than 15 mm per forest type for all potential prey, Coleoptera, Diptera, Hemiptera, Hymenoptera, and Lepidoptera. * denotes a significant difference in vegetation structure values compared to unmined forest. Note the different values on the y-axes.

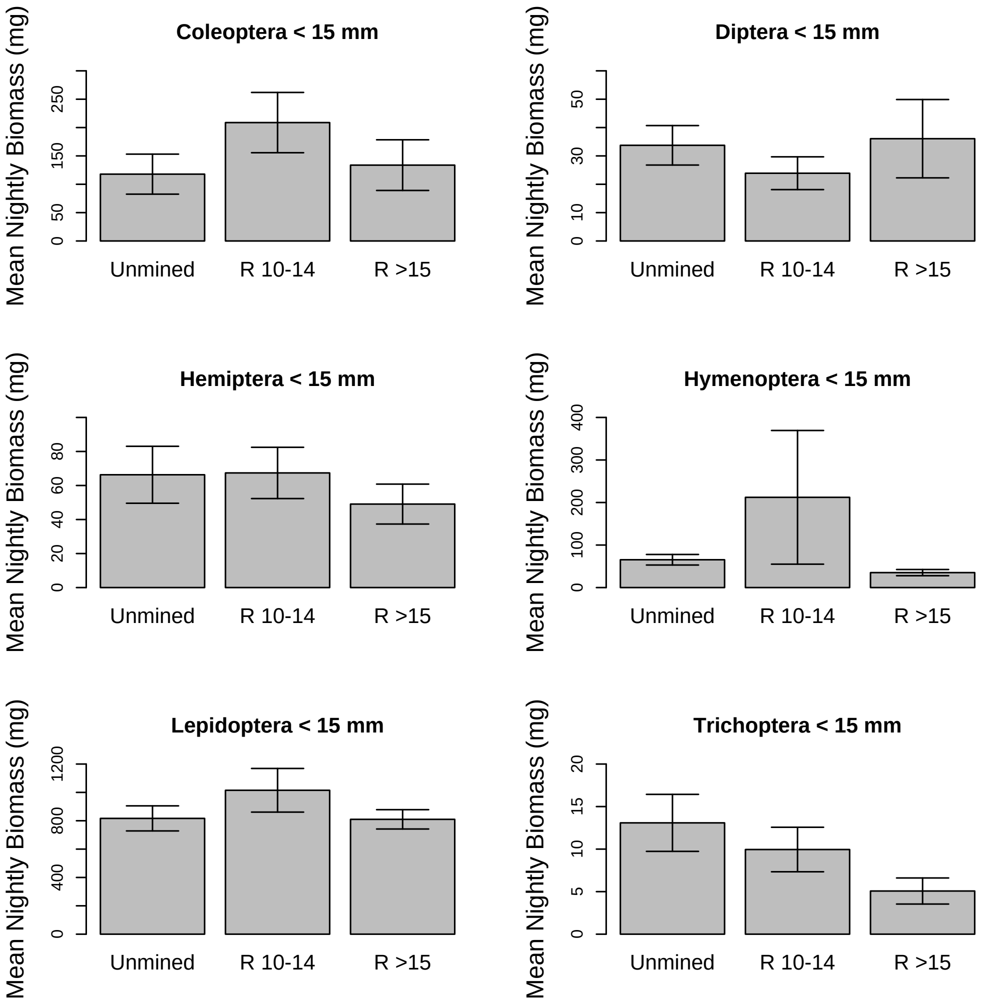


Figure 4: Mean (± SE) biomass (g) of insects less than 15 mm per forest type for all potential prey, Coleoptera, Diptera, Hemiptera, Hymenoptera, and Lepidoptera. There were no significant difference in insect biomass between unmined and either type of restored forest. Note the different values on the y-axes.

Bat activity

We recorded 2 493 bat call files, of which 2 416 were identified to species / species group: *C. gouldii* (194), *C. morio* (47), *F. mackenziei* (7), *Nyctophilus* spp. (72), *M. kitcheneri* (37), *T. australis* (168) and *V. regulus* (1 891). Due to small sample sizes we excluded *F. mackenziei* and *M. kitcheneri* from further analyses. Bat activity was highly variable between forest types, with significantly more call files recorded in unmined forest (N = 1 561) than either restored forest type (R 10-14 N = 364, R

>15 N = 447). Only the closed environment adapted *V. regulus* showed a clear preference for unmined forest with increased activity in unmined forest compared to either type of restored forest (unmined – R 10-14 $P < 0.001$; unmined – R >15 $P = 0.011$; Figure 5). Edge environment adapted *Nyctophilus* spp. had higher activity in unmined forest compared to 10-14 year old restoration ($P < 0.001$) but not >15 year old restoration ($P = 0.112$; Figure 5). There were no differences in activity levels between unmined and restored forest types for the other bat species.

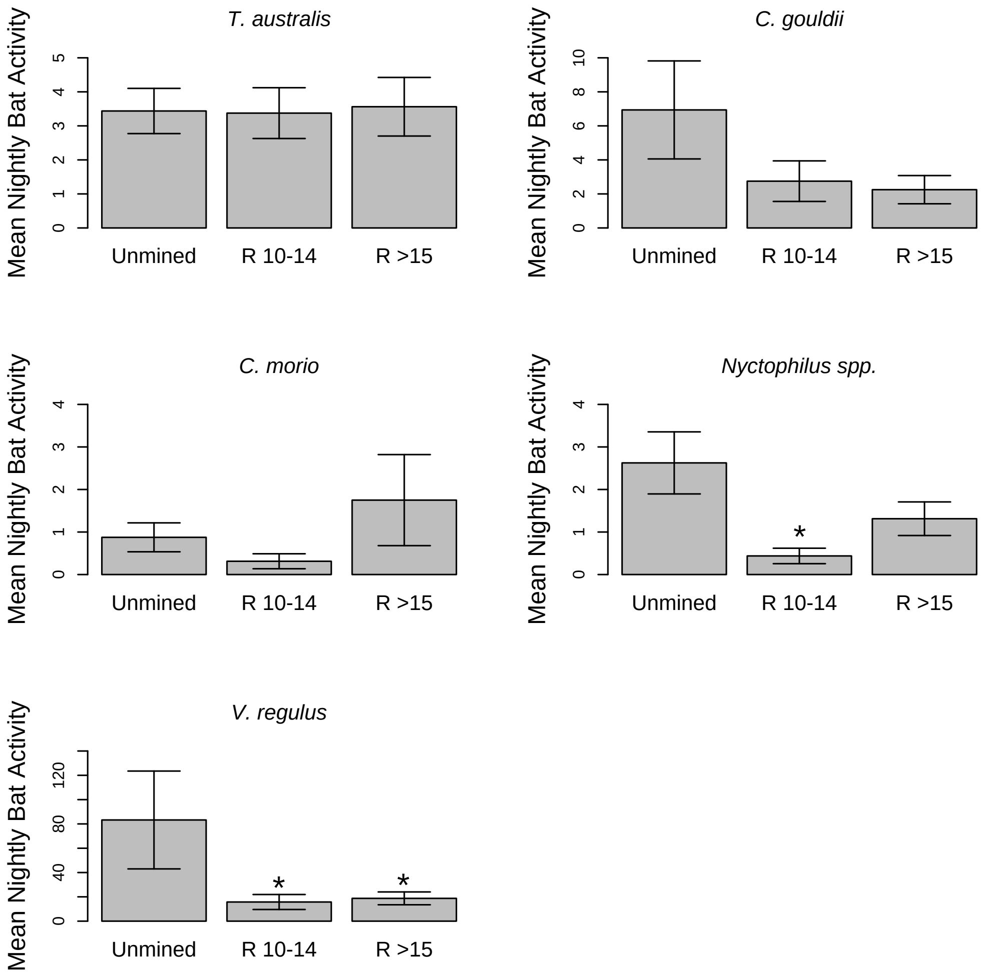


Figure 5: Mean (± SE) bat activity per forest type for *T. australis*, *C. gouldii*, *C. morio*, *Nyctophilus* spp., and *V. regulus*. Bat activity was quantified as the number of bat call files recorded per night. * denotes a significant difference in activity levels compared to unmined forest. Note the different values on the y-axes.

Prey accessibility

We identified nine models where there was support for a significant interaction of vegetation structure and insect biomass on bat activity, relating to the edge adapted *C. gouldii*, *C. morio* and *Nyctophilus* spp. (Table 3; Table 4). Plotting the marginal effects of insect biomass on vegetation cover for six of the nine models revealed large confidence intervals that made interpretation of the results meaningless (Appendix 2, Figure S1). Of the remaining three models, an increase in vegetation cover corresponded to a decreasing influence of insect biomass for *C. gouldii* and *C. morio*. Specifically, as canopy cover increased the influence of Hymenoptera biomass on *C. gouldii* activity decreased while increasing shrub cover reduced the influence of Hemiptera (<15 mm) biomass on *C. morio* (Figure 6). In contrast, increasing ground cover increased the influence of potential prey (<15 mm) on *Nyctophilus* spp. activity (Figure 6).

Table 3: Vegetation structure, insect biomass and their interactive effects on bat activity across the restored northern jarrah forests of south-western Australia. AICc scores for the top models are as follows: *T. australis* 224.24, *C. gouldii* 224.42; *C. morio* 118.56; *Nyctophilus* spp. 158.69; and *V. regulus* 416.79. For *T. australis* and *C. gouldii* all insects were included in the biomass whereas only specimens <15 mm were included in the biomass for *C. morio*, *Nyctophilus* spp. and *V. regulus*. Insect biomass or vegetation structure single variable models had df=4, and the interactive models had df=6. Models ranked higher than null models are highlighted in bold. Vegetation structure variables refer to percent cover for each strata.

	T. australis		*C. gouldii*		*C. morio*		*Nyctophilus* spp.		*V. regulus*	
Model Name	ΔAIC	Wgt	ΔAIC	Wgt	ΔAIC	Wgt	ΔAIC	Wgt	ΔAIC	Wgt
Canopy	**0.04**	**0.10**	**1.94**	**0.10**	7.25	0.01	6.38	0.01	**6.58**	**0.01**
Midstorey	1.91	0.04	4.72	0.03	7.35	0.01	4.82	0.02	**0.00**	**0.30**
Shrub	**0.00**	**0.10**	3.89	0.04	**2.80**	**0.07**	4.61	0.02	**5.83**	**0.02**
Ground	2.71	0.03	4.85	0.02	**4.08**	**0.04**	**1.42**	**0.09**	**5.78**	**0.02**
Potential Prey	2.58	0.03	5.64	0.02	7.19	0.01	6.37	0.01	8.34	0.00
Coleoptera	1.38	0.05	4.10	0.04	7.32	0.01	5.87	0.01	**5.10**	**0.02**
Diptera	1.64	0.04	5.27	0.02	7.30	0.01	**2.10**	**0.07**	8.82	0.00
Hemiptera	2.32	0.03	4.95	0.02	6.95	0.01	5.94	0.01	8.68	0.00
Hymenoptera	2.64	0.03	5.87	0.01	7.23	0.01	5.99	0.01	7.94	0.01
Lepidoptera	2.78	0.02	5.86	0.01	7.38	0.01	6.32	0.01	**6.10**	**0.01**
Trichoptera	1.76	0.04	3.92	0.04	5.88	0.02	6.38	0.01	8.88	0.00
Canopy * Potential Prey	3.73	0.02	4.94	0.02	12.24	0.00	11.46	0.00	9.04	0.00
Canopy * Coleoptera	1.90	0.04	5.48	0.02	11.79	0.00	10.27	0.00	8.64	0.00
Canopy * Diptera	0.82	0.07	5.54	0.02	12.03	0.00	6.98	0.01	10.35	0.00
Canopy * Hemiptera	3.57	0.02	**3.46**	**0.05**	10.89	0.00	10.87	0.00	11.51	0.00

Midstorey * Potential Prey	6.71	0.00	9.62	0.00	9.31	0.00	8.62	0.00	**2.03**	**0.11**
Midstorey * Coleoptera	3.85	0.01	9.28	0.00	12.31	0.00	4.30	0.02	**4.65**	**0.03**
Midstorey * Diptera	4.59	0.01	7.35	0.01	11.92	0.00	5.69	0.01	**2.69**	**0.08**
Midstorey * Hemiptera	6.42	0.00	7.23	0.01	11.63	0.00	8.95	0.00	**3.92**	**0.04**
Midstorey * Hymenoptera	6.82	0.00	7.29	0.01	10.67	0.00	8.63	0.00	**4.63**	**0.03**
Midstorey * Lepidoptera	6.91	0.00	9.19	0.00	9.41	0.00	**3.84**	**0.03**	**2.63**	**0.08**
Midstorey * Trichoptera	4.93	0.01	6.38	0.01	9.91	0.00	9.93	0.00	**2.10**	**0.10**
Shrub * Potential prey	2.51	0.03	7.91	0.01	7.36	0.01	8.86	0.00	9.84	0.00
Shrub * Coleoptera	3.04	0.02	4.38	0.03	5.93	0.02	8.70	0.00	**5.09**	**0.02**
Shrub * Diptera	4.23	0.01	8.34	0.00	**0.00**	**0.30**	**3.70**	**0.03**	10.47	0.00
Shrub * Hemiptera	4.53	0.01	6.86	0.01	**1.64**	**0.13**	7.38	0.00	8.61	0.00
Shrub * Hymenoptera	5.01	0.01	6.45	0.01	6.61	0.01	7.77	0.00	8.62	0.00
Shrub * Lepidoptera	4.05	0.01	6.46	0.01	7.41	0.01	9.40	0.00	7.28	0.01
Shrub * Trichoptera	3.70	0.02	6.88	0.01	**1.04**	**0.18**	6.53	0.01	10.38	0.00
Ground * Potential Prey	5.19	0.01	8.78	0.00	8.56	0.00	**1.38**	**0.10**	9.51	0.00
Ground * Coleoptera	3.09	0.02	6.97	0.01	5.18	0.02	**1.17**	**0.11**	**5.92**	**0.02**
Ground * Diptera	4.84	0.01	9.28	0.00	7.18	0.01	**2.06**	**0.07**	10.12	0.00
Ground * Hemiptera	4.78	0.01	8.35	0.00	**4.83**	**0.03**	**0.00**	**0.19**	9.25	0.00
Ground * Hymenoptera	7.68	0.00	9.67	0.00	8.06	0.01	5.68	0.01	7.39	0.01
Ground * Lepidoptera	7.05	0.00	8.86	0.00	8.91	0.00	**2.28**	**0.06**	**4.94**	**0.02**
Ground * Trichoptera	6.77	0.00	7.86	0.01	**4.23**	**0.04**	**3.06**	**0.04**	10.72	0.00
Null Model	0.40	0.08	3.56	0.05	5.00	0.02	4.00	0.03	6.69	0.01

Table 4: Model-averaged parameters (coefficients ± adjusted SE and Z-values) for terms from all models regressing bat activity against insect biomass and/or vegetation structure at 24 sites across the restored jarrah forests of south-western Australia. Model terms were checked for significance only if they had a higher AIC weight than the null model. Significance is indicated by * ≤0.05, ** ≤0.01, and * ≤0.001 and are highlighted in bold. Vegetation structure variables refer to percent cover for each strata.**

	T. australis		*C. gouldii*		*C. morio*		*Nyctophilus* spp.		*V. regulus*	
Model Term	Coeff±SE	Zvalue	Coeff±SE	Zvalue	Coeff±SE	Zvalue	Coeff±SE	Zvalue	Coeff±SE	Zvalue
Intercept	1.07±0.17	6.36	0.75±0.27	2.76**	-0.8±0.58	1.37	0.06±0.29	0.21	2.96±0.30	9.83***
Canopy	-0.26±0.16	1.69	-0.31±0.27	1.14	-0.13±0.47	0.28	-0.13±0.30	0.44	-0.44±0.31	1.41
Midstorey	-0.14±0.16	0.88	-0.28±0.25	1.10	0.08±0.62	0.14	-0.30±0.28	1.07	**-0.75±0.29**	**2.63****
Shrub	0.24±0.17	1.40	-0.40±0.28	1.41	-1.25±0.71	1.76	-0.39±0.34	1.16	-0.60±0.36	1.67
Ground	0.03±0.16	0.16	-0.28±0.26	1.09	-0.99±0.67	1.48	**-0.63±0.30**	**2.11***	-0.43±0.47	0.91
Potential Prey	0.05±0.14	0.37	-0.1±0.22	0.47	0.19±0.42	0.45	-0.17±0.22	0.75	-0.14±0.24	0.58
Coleoptera	0.17±0.15	1.18	-0.28±0.21	1.29	-0.37±0.44	0.84	-0.35±0.26	1.36	-0.44±0.30	1.46
Diptera	0.15±0.13	1.09	-0.14±0.22	0.65	-0.43±0.41	1.04	**0.39±0.20**	**2.00***	0.15±0.26	0.58
Hemiptera	-0.08±0.13	0.57	-0.11±0.23	0.48	-0.37±0.42	0.87	-0.32±0.28	1.13	-0.22±0.27	0.81
Hymenoptera	0.04±0.14	0.30	-0.26±0.21	1.22	-1.61±4.01	0.40	0.66±1.90	0.35	-0.94±3.04	0.31
Lepidoptera	-0.05±0.15	0.36	-0.02±0.24	0.10	-0.05±0.41	0.11	0.04±0.27	0.14	-0.27±0.32	0.84
Trichoptera	0.09±0.12	0.78	-0.28±0.19	1.45	0.28±0.32	0.86	-0.01±0.21	0.05	0.06±0.25	0.23
Canopy * Potential Prey	0.12±0.14	0.88	-0.31±0.23	1.37	-0.01±0.35	0.03	-0.03±0.2	0.16	-0.16±0.21	0.77
Canopy * Coleoptera	-0.08±0.12	0.72	0.15±0.18	0.79	0.37±0.53	0.70	-0.24±0.29	0.82	0.15±0.30	0.49
Canopy * Diptera	-0.19±0.12	1.64	0.19±0.18	1.08	0.21±0.47	0.44	-0.10±0.26	0.39	0.27±0.32	0.84
Canopy * Hemiptera	-0.12±0.10	1.21	0.28±0.15	1.93	0.43±0.53	0.81	-0.10±0.31	0.32	0.14±0.37	0.37
Canopy * Hymenoptera	0.11±0.10	1.12	**-0.34±0.13**	**2.68****	-2.50±2.05	1.22	1.03±0.96	1.07	0.91±1.18	0.77
Canopy * Lepidoptera	-0.23±0.14	1.62	0.26±0.22	1.17	0.07±0.39	0.17	**-0.54±0.26**	**2.10***	-0.35±0.25	1.40
Canopy * Trichoptera	-0.08±0.08	0.94	0.11±0.12	0.98	0.09±0.51	0.17	-0.34±0.34	0.99	-0.70±0.38	1.83

Midstorey * Potential Prey	0.01±0.13	0.07	-0.01±0.21	0.03	-0.59±0.36	1.67	-0.26±0.23	1.09	-0.33±0.20	1.62
Midstorey * Coleoptera	-0.20±0.21	0.98	0.26±0.31	0.84	-0.24±0.24	1.02	-0.66±0.46	1.43	-0.10±0.19	0.55
Midstorey * Diptera	-0.14±0.11	1.29	0.00±0.18	0.02	0.14±0.57	0.25	-0.38±0.38	0.99	-0.06±0.37	0.17
Midstorey * Hemiptera	0.07±0.13	0.49	0.29±0.21	1.36	0.38±0.64	0.60	-0.20±0.35	0.56	-0.30±0.31	0.96
Midstorey * Hymenoptera	-0.05±0.15	0.37	-0.30±0.20	1.51	-6.05±4.88	1.24	1.86±2.96	0.63	-1.64±2.96	0.56
Midstorey * Lepidoptera	0.01±0.13	0.11	0.17±0.22	0.79	-0.57±0.29	1.95	**-0.51±0.23**	**2.24***	-0.28±0.20	1.38
Midstorey * Trichoptera	-0.11±0.10	1.07	0.16±0.13	1.20	0.39±0.42	0.92	-0.02±0.27	0.07	-0.51±0.30	1.68
Shrub * Potential prey	-0.35±0.25	1.40	0.35±0.40	0.88	-0.09±0.54	0.18	-0.33±0.37	0.90	0.33±0.40	0.81
Shrub * Coleoptera	-0.07±0.21	0.31	-0.44±0.29	1.54	-0.86±0.75	1.15	-0.30±0.50	0.61	-0.45±0.49	0.92
Shrub * Diptera	-0.04±0.20	0.21	-0.10±0.30	0.35	**-2.44±0.97**	**2.51***	-0.17±0.19	0.91	0.07±0.28	0.24
Shrub * Hemiptera	0.09±0.17	0.51	-0.25±0.27	0.95	**-1.42±0.61**	**2.33***	-0.69±0.50	1.39	-0.56±0.52	1.08
Shrub * Hymenoptera	-0.01±0.14	0.06	0.33±0.21	1.56	3.37±4.70	0.72	-3.43±2.63	1.30	-0.72±3.13	0.23
Shrub * Lepidoptera	-0.22±0.22	1.01	-0.53±0.34	1.56	-0.47±0.69	0.68	-0.17±0.40	0.43	-0.34±0.43	0.79
Shrub * Trichoptera	0.09±0.15	0.61	0.14±0.24	0.58	**-0.67±0.34**	**1.97***	-0.45±0.27	1.68	-0.21±0.29	0.73
Ground * Potential Prey	-0.28±0.19	1.53	-0.26±0.31	0.86	-0.34±0.48	0.72	**-0.50±0.22**	**2.24***	-0.20±0.32	0.62
Ground * Coleoptera	-0.26±0.14	1.81	-0.23±0.24	0.97	-1.29±0.76	1.69	**-0.70±0.33**	**2.14***	-0.11±0.40	0.29
Ground * Diptera	-0.26±0.19	1.32	0.06±0.31	0.21	-0.44±0.37	1.18	-0.02±0.12	0.14	0.13±0.18	0.72
Ground * Hemiptera	-0.22±0.14	1.53	-0.19±0.23	0.82	-1.81±1.08	1.68	**-1.10±0.47**	**2.36***	-0.35±0.46	0.76
Ground * Hymenoptera	0.00±0.16	0.02	-0.09±0.21	0.45	-5.09±5.85	0.87	-1.71±2.63	0.65	4.82±3.37	1.43
Ground * Lepidoptera	-0.11±0.13	0.85	-0.18±0.20	0.91	-0.19±0.48	0.41	**-0.44±0.22**	**2.01***	-0.39±0.28	1.37
Ground * Trichoptera	0.05±0.24	0.21	-0.14±0.32	0.44	-0.96±0.56	1.71	-0.41±0.24	1.76	-0.12±0.34	0.36

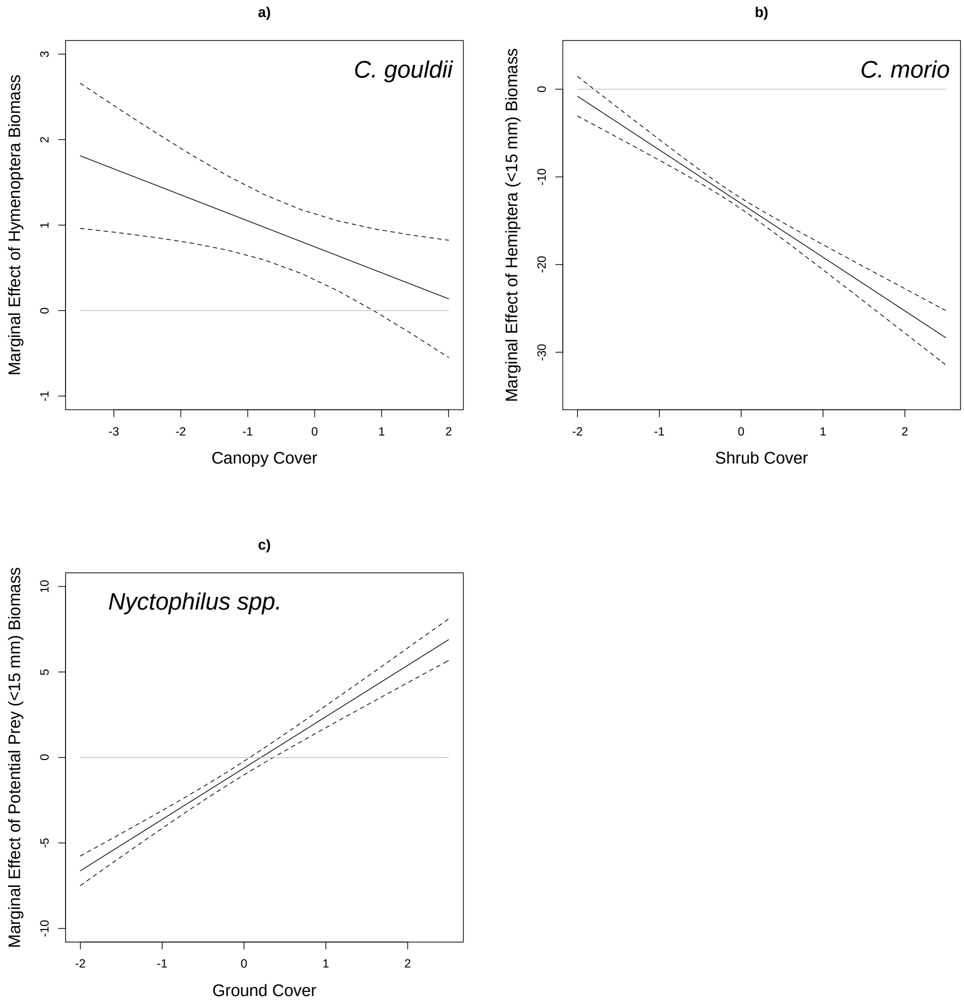


Figure 6: The marginal effect of insect biomass on bat activity, for a range of vegetation structure values. Explanatory variables (insect biomass and vegetation structure) were standardised prior to modelling so 0 on graphs represent mean values. 95% confidence limits are represented by dotted lines. The marginal effect of a) Hymenoptera biomass on *C. gouldii* activity as influenced by canopy cover; b) Hemiptera <15 mm biomass on *C. morio* activity as influenced by shrub cover; and c) potential prey <15 mm biomass on *Nyctophilus* spp. activity.

Discussion

Our results demonstrate that occurrence does not necessarily equate to accessibility for predatory bats and their insect prey in the restored jarrah forests of south-western Australia. At the ordinal level, there was no difference in potential prey availability between restored and unmined forests for smaller bat species (*C. morio*, *Nyctophilus* spp. and *V. regulus*) but potential prey availability for larger bat species (*T. australis* and *C. gouldii*) differed between both restored forest types and unmined forest. Despite the availability of food resources and the increasing similarity of vegetation cover in restored, compared to unmined, forest as restoration aged, not all bat species were equally active in restored and unmined forest. Bat activity was influenced by the synergistic effects of insect biomass and vegetation structure for some edge environment foragers, suggesting that vegetation structure may limit prey accessibility.

Prey occurrence

Contradictory to our predictions, ordinal level differences in potential prey biomass between restored and unmined forest was driven by the presence of a few large Coleoptera and Diptera specimens in unmined forest and 10-14 year old restoration, respectively. Our findings are also somewhat inconsistent with research in the southern jarrah forest where only Lepidoptera was found to be sensitive to disturbance and had higher biomass in old regrowth compared to sites with more recently disturbed logging histories (Webala et al. 2011). The different findings may reflect the seasonal fluctuations of insect populations as the southern jarrah forest study occurred during the maternity season and ours during that mating season. In the northern jarrah forest Lepidoptera biomass was substantially higher in unmined forest, compared to 10-14 year restoration, during the maternity season (J. Burgar, unpublished data), reflecting the findings of the southern jarrah forest and supporting our prediction that Lepidoptera would be sensitive to restoration. In North America seasonal patterns of adult Lepidoptera emergence resulted in differences in Lepidoptera abundance between forest types late, but not early, in the summer season (Summerville & Crist 2008). There are mixed results in the literature when comparing insect abundance across disturbance regimes; at the ordinal level or higher some studies found no difference in invertebrate abundance (e.g., Fenton 1998) or biomass (e.g., Adams et al. 2009; Lentini et al. 2012) between treatments while others did (e.g., Dodd et al. 2012b; Lumsden & Bennett 2005). The fact that potential prey <15 mm biomass and diversity did not differ between forest types suggests that, at the ordinal level, prey is not limiting for smaller bats in the restored forest. Despite differences in potential prey biomass and diversity when examining all sizes of potential prey, we also suggest that prey does not limit larger bats, particularly *C. gouldii*. During the mating season in the northern jarrah forest, *C. gouldii*, *N. gouldi* and *V. regulus* show significant dietary overlap (Chapter 3). In addition, in Tasmania *C. gouldii* forages heavily on Lepidoptera (O'Neill & Taylor 1989), an order equally available in restored and unmined forest regardless of Lepidoptera size.

Similar to the conclusions of other studies (e.g., Lumsden & Bennett 2005), we suggest that the ordinal level may be too coarse a taxonomic resolution to effectively examine trophic interactions, particularly as jarrah forest bats consume a great diversity of prey (Chapter 3). Molecular studies show that even with diverse diets, bats may specialise in their diets, either within an order (e.g., Clare et al. 2009) or between orders (e.g., Clare et al. 2011). In southern England sympatric, cryptic bat species differentiated their diets in terms of specialist prey, while consuming similar common species (Razgour et al. 2011). Even if the volume of prey is the same between forest types at the ordinal level, examination of prey diversity at a finer taxonomic resolution may be necessary to ensure sufficient biomass of certain prey species. This is particularly relevant within modified landscapes as insects within an order may have differential responses to forest management (e.g., Albrecht et al. 2007; Farr et al. 2011; Summerville 2010; Werner & Raffa 2000). In the restored jarrah forest, ant communities were still distinct from unmined forest communities after nearly four decades (Majer et al. 2013). While the diversity of other insect taxa, such as Diptera and Coleoptera, within restoration may converge on the insect diversity within unmined forests more readily (Koch et al. 2010), the differential insect taxa responses suggests that restoration may take decades, or longer, to provide foraging bats with all potential prey species. Thus it may be necessary to assess the occurrence, and volume, of potential prey at a finer taxonomic resolution than order to elucidate the ability of restoration to provide the same prey resources as found in remnant sites.

Prey Accessibility

To maintain trophic interactions, restoration must ensure that prey is both available and accessible to predatory bats. Our study found that prey is readily available in restoration but that vegetation structure directly influenced the ability of edge environment foraging bats to access their prey. Contradictory to findings from eastern Australia (Lumsden & Bennett 2005), we only found support for one instance where bat activity was solely influenced by insect biomass and two instances where bat activity was solely influenced by vegetation structure. However, we found that bat activity responded to the interactive effects of vegetation structure and insect biomass for three edge adapted bat species. While most bat foraging studies relate bat activity, prey occurrence and vegetation structure (e.g., Armitage & Ober 2012; Dodd et al. 2012b; Lumsden & Bennett

2005), ours is one of the few explicitly examining the interactive influences of vegetation structure and prey occurrence on bat activity (but see Adams et al. 2009; Webala et al. 2011).

Similar to findings in the southern jarrah forest we did not find a relationship between vegetation clutter and either overall insect or Lepidoptera biomass for *C. gouldii*, *C. morio* or *V. regulus* (Webala et al. 2011). However, consistent with our predictions we did find a synergistic effect of vegetation structure and insect biomass for some edge environment foragers: canopy cover and Hymenoptera biomass on *C. gouldii* activity, shrub cover and Hemiptera biomass on *C. morio* activity, and ground cover and all potential prey <15 mm biomass on *Nyctophilus* spp. activity. In eastern Australia, increasingly open forest structure, in combination with increasing insect biomass, was related to an increase in bat activity overall and for edge adapted species with high echolocation calls, such as *C. morio* (Adams et al. 2009). Similarly, we found that insect biomass had a greater influence on bat activity at low levels of vegetation cover for both *Chalinolobus* species. Inline with previous research categorising *C. gouldii* as an edge environment forager, capable of using open environments and *C. morio* capable of exploiting closed environments (Fullard et al. 1991), we found canopy cover to moderate *C. gouldii* activity and shrub cover to moderate *C. morio* activity, in conjunction with insect biomass. The unmined jarrah forest is typified by tall trees (~25 m) with open canopy structure, a second storey of smaller trees and an undergrowth shrub layer up to ~3 m (Koch 2007b). In contrast, the restored jarrah forest comprises a multi-tiered vegetation structure: a short canopy (~15 m tall) of relatively dense trees and either a thick *Acacia* understorey (~4 m tall) prior to being burnt or a reduced *Acacia* understorey (~3 m tall) after being burnt (J. Burgar, unpublished data; Grant 2006). Thus, while both forest types contain an open sub-canopy, the foraging area available in the sub-canopy and canopy of restoration is greatly limited, compared to the unmined forest. It is somewhat surprising that neither of these bat species showed a preference for any forest type, despite the higher levels of shrub cover, and potentially lower canopy cover, in 10-14 year old restoration, compared to unmined forest. However we speculate that this may be due to the highly variable vegetation cover between sites coupled with highly variable mean nightly bat activity for both species and generally low activity levels for *C. morio* (<50 call files recorded during the entire study). However, our study corroborates research in the southern jarrah forest where neither *Chalinolobus* species had a preference for forest types, for either bat activity or feeding buzzes occurrences (Webala et al. 2011). Edge environment foragers are typically more active at sub-canopy and canopy heights, where vegetation is less cluttered, than in the forest understorey, where vegetation is more cluttered (Adams et al. 2009). The lack of a forest type preference by either species may then reflect the ability of both *C. gouldii* and *C. morio* to partially stratify their use of the restored forest by foraging in the sub-canopy and canopy (e.g., Adams et al. 2009). This may suggest that even with the reduced foraging space within restoration, restored forest may be sufficient to meet bat foraging needs at a landscape level.

In contrast, we found that the most manoeuvrable bats (*Nyctophilus* spp. and *V. regulus*), adapted for navigating edge and closed environments (Fullard et al. 1991), were the only species who showed a preference for unmined forest compared to one, or both, types of restored forest. We predicted no relationship between bat activity and insect biomass and/or vegetation structure for *V. regulus*. Correspondingly we found evidence to suggest that *V. regulus* activity was influenced by midstorey cover, but no support for a synergistic effect of vegetation cover and insect biomass on activity. Our findings are consistent with the southern jarrah forest where significantly more *V. regulus* feeding buzzes were recorded in old regrowth (>30 years post logging) compared to young regrowth (12-30 years post logging) and there was no relationship between *V. regulus* activity and insect biomass and/or vegetation structure (Webala et al. 2011). The lack of an interactive effect for *V. regulus* may indicate that this species is able to access prey even in cluttered environments, corroborating earlier studies placing *V. regulus* as a closed environment forager (Fullard et al. 1991). The positive association in this study between *Nyctophilus* spp. activity and Diptera (<15 mm) biomass, coupled with previous research finding high dietary diversity of *N. gouldi* (Chapter 3), suggests that *Nyctophilus* spp. aren't limited by foraging. Instead, the avoidance of restoration by *V. regulus* and *Nyctophilus* spp. implies that other factors are limiting their use of restoration. In both the southern and northern jarrah forest *N. gouldi* and *V. regulus* were found to be selective in roost site selection (Webala et al. 2010; Chapter 5); thus the increased activity in unmined forest may be due to roosting preferences rather than foraging preferences.

Consistent with our predictions and similar to findings in both the southern jarrah forest (Webala et al. 2011) and elsewhere in Australia (Lumsden & Bennett 2005), we found that the open environment adapted species was not constrained by restoration. The lack of a relationship between activity and vegetation structure and/or insect biomass is consistent with consideration of *T. australis* as an obligate open environment species (Fullard et al. 1991). *T. australis* flies high above the canopy with a low frequency echolocation call, adapted to cover large distances (Jung et al. 2014; Schnitzler & Kalko 2001). In Zimbabwe the feeding buzzes of *Tadarida* conspecifics of similar size and echolocation call frequency were recorded at altitudes up to 550 m (Fenton & Griffin 1997) with a range of detection estimated to be between 90-600 m (for frequencies between 20-10 kHz, respectively; Fenton et al. 1998). Thus, it is highly likely that we detected *T. australis* commuting (and foraging) well above the canopy, rather than within the forest, on insects that are out of range of light traps (Lumsden & Bennett 2005).

Conclusion

While ordinal level prey biomass, particularly for insects <15 mm, may be similar between forest types, it is unlikely that restoration is suitable foraging habitat for all bat species. Similar to other studies, bat species with similar ecomorphology exhibited similar responses to vegetation clutter (Adams et al. 2009; Jung et al. 2012). Edge environment foragers were affected by prey availability but the influence of prey changed as vegetation cover changed. In contrast, open and closed environment foragers were minimally affected by restoration and we speculate that this is because they foraged above and within the canopy, respectively. Integrating restoration into regional prescribed burn practices may improve restoration as foraging habitat; *Nyctophilus* spp. had similar activity levels in unmined forest and burnt, rather than unburnt, restoration. In addition, *C. morio* foraging was negatively associated with shrub cover, which is reduced in recently burnt restoration. Fire periodicity is important as both insect prey and predatory bat community assemblages may follow successional patterns after fire (Armitage & Ober 2012). Seasonal timing of fires is also important as autumn burning may lead to high legume density and dense understorey vegetation (Grigg et al. 2010), minimising some of the positive effects of fire on foraging habitat for bats. Another management option to accelerate the suitability of restoration as foraging habitat may be to use ecological thinning to isolate individual trees by clearing adjacent trees. Restoration >10 year old has relatively high tree density, compared to unmined forest (Grant 2006), and jarrah saplings / trees have low mortality (Norman et al. 2006; Stoneman et al. 1997), thus there is little capacity for restoration to self-thin. Management practices to promote the growth of individual trees would increase both canopy height and canopy structure of the emerging trees, attributes beneficial to foraging bats, particularly those suited for the edge environment.

The consistency between our findings and research in the southern jarrah forest where analyses used feeding buzzes, rather than bat activity, strengthens our use of bat activity as a surrogate for foraging activity (Webala et al. 2011). We still advise cautious interpretation of our findings as only *C. gouldii* and *V. regulus* activity was highly correlated with feeding buzz occurrences across the forest types. Future studies should examine feeding buzzes at various altitudes (e.g., Fenton & Griffin 1997) and vertical stratifications (e.g., Adams et al. 2009) of the restored forest to explicitly elucidate the vertical spatial extent of jarrah forest bat foraging behaviours. Our ability to detect significant interactions between insect biomass and ground cover for *Nyctophilus* spp. lends justification to our placement of bat detectors 1.5 m above the ground at a 45° angle. For a more comprehensive landscape perspective, future studies should also examine the accessibility of prey in restoration of various ages and vegetation densities. We assumed higher prey availability in older restoration (e.g., Summerville & Crist 2008) and thus limited the scope of our study to >10 year old desired restoration as these sites are on a vegetation successional trajectory towards the restoration objective of a self-sustaining jarrah forest ecosystem (Grant 2006). However, results from the concurrent larger bat activity study found proportionally more feeding buzzes in dense (>2500 stems ha^{-1}) than desired (500-2500 stems ha^{-1}) restoration, particularly in 5-9 year old sites. This suggests that younger restoration may be contributing more to foraging bats than we anticipated. As the nature of mining leaves a patchwork mosaic of various ages of restoration, interspersed amongst unmined forest (Koch 2007a), future studies should examine both prey availability and accessibility within a wider range of restoration ages and stem densities to fully explore the foraging potential of the entire restored landscape. Restoration needs to provide adequate foraging space across a landscape and at multiple vertical levels to ensure it is suitable foraging habitat for all bat species, thus maintaining trophic interactions and effectively conserving bat populations.

Chapter Five

Location location location: the importance of mature forest as bat roosting habitat within a restored landscape.

Introduction

Restoration, an interventionist activity towards the management or repair of degraded ecosystems (Hobbs & Cramer 2008), is increasingly being used as a means of managing and conserving biodiversity (Suding 2011; Young 2000). Within this context, restoration aims to re-establish functioning ecosystems to a particular reference system, with a complement of species similar to those historically present (Hallett et al. 2013; Hobbs & Cramer 2008). But restoration predominantly focuses on the re-establishment of plants (Brudvig 2011; Ruiz-Jaen & Aide 2005), assuming that fauna will naturally recolonise once vegetation is established (Palmer et al. 1997). While some fauna do passively recolonise other faunal groups may avoid restoration or initially recolonise but then fail to persist in restored sites (e.g., Craig et al. 2012; Cristescu et al. 2012). Bats are one group we may expect to naturally recolonise due to their mobility and ecological adaptability exemplified by their dietary diversity (e.g., Chapter 3, Whitaker 2004), exploitation of artificial structures for roosting (e.g., Boughey et al. 2011; Lumsden et al. 2002b), and colonisation of urban environments (e.g., Neubaum et al. 2007; Rhodes 2007). The ability of restoration to provide roosting habitat is especially critical as roosts buffer daily and long-term microclimates, reducing the energetic costs of thermoregulation, (e.g., Sedgeley 2001), facilitate predator evasion (e.g., Fenton et al. 1994) and support social relationships (Lewis 1995), necessary for rearing non-volant young (e.g., Law & Chidel 2007). For forest-dwelling bats, roosting habitat typically comprises multiple roosting structures within a given area as many bat species exhibit roost site fidelity, switching between a pool of suitable roosts in close proximity to one another (Lewis 1995; Threlfall et al. 2013; Webala et al. 2010). To be effective in conserving bat populations restoration must provide suitable roosting habitat, including ample roosting structures.

Forest-dwelling bats typically roost in large, mature trees but exhibit intra and interspecific variations in roosting preferences (Goldingay 2009; Kalcounis-Ruppell et al. 2005; Threlfall et al. 2013; Vonhof & Gwilliam 2007). Roosting preferences can differ at multiple spatial scales: 'roost', a roosting structure such as a tree (Threlfall et al. 2013; Vonhof & Gwilliam 2007); 'site', the vegetation structure immediately surrounding the roost (Broders & Forbes 2004; Lumsden et al. 2002b; Perry & Thill 2007); and 'landscape', the habitat type surrounding the roost (Broders et al. 2006; Lumsden et al. 2002a). Males and non-breeding female forest bats are generally less selective in their roosting requirements than reproductive females at all three spatial scales. Reproductive females tend to select larger roost trees than non-reproductive females (Lumsden et al. 2002b; Threlfall et al. 2013) and maternity roosts are typically farther from foraging sites than male roosts (e.g., Lumsden et al. 2002a). Bat species exhibiting flexibility in their choice of roosting structures may roost under decorticating bark or within fissures in the trunk while more conservative species may be restricted to roosting in hollows (e.g., Law et al. 2011; Turbill 2006; Webala et al. 2010). Understanding roost requirements at multiple spatial scales and across seasons within a restored landscape is imperative for ensuring effective management and conservation of habitat for bat populations.

Considerable research has focused on the roosting preferences of forest-dwelling bats in timber-managed landscapes and those revegetated after agricultural use (e.g., Elmore et al. 2004; Law et al. 2011; Lumsden et al. 2002a; O'Keefe et al. 2009; Perry et al. 2007) but no published studies have specifically examined the suitability of post-mining restoration as roosting habitat. Timber managed forests are generally on a rotational harvesting system with silvicultural treatments ranging from the retention of some to none of the trees within a site, which are surrounded by forests of varying age. Mature tree retention within timber-harvested landscapes provides roosting resources for hollow-dependent bats (Perry et al. 2007; Webala et al. 2010). In contrast, the extensive clearing of agricultural lands results in little retention of native vegetation; revegetated sites are typically surrounded by an expanse of tree-less paddocks and farmland mosaic landscapes provide few roosting resources (e.g., Lumsden et al. 2002a). Restored landscapes, comprising a patchwork of restored sites embedded within a habitat of reference site vegetation, differ from timber-harvesting landscapes in that restored sites rarely retain remnant vegetation (Koch 2007a). Restored landscapes differ from revegetated agricultural landscapes in terms of the surrounding habitat and the aim of restoration to actively re-establish a full suite of historical species, compared to revegetated lands that assume passive ecosystem succession once overstorey trees are planted (Hobbs & Cramer 2008; Munro et al. 2009). The lack of large, mature trees in newly restored sites likely limits the suitability of restoration as bat roosting habitat.

To determine if restoration provides roosting habitat, we radio-tracked two bat species (Gould's long-eared bat *Nyctophilus gouldi*, Tomes 1858; and the southern forest bat *Vespadelus regulus*, Thomas 1906) within a restored landscape in the northern jarrah (*Eucalyptus marginata*) forest in south-western Australia. Parts of the northern jarrah forest have been mined for bauxite for over forty years with >15 000 ha of the 700,000 ha of forest already mined and ~600 ha of forest still annually cleared, mined, and restored (Koch 2007a). Mine restoration aims to return a fully-functioning jarrah forest ecosystem while maintaining multiple-use values, including conservation, water catchment, timber production, and recreation (Bell & Hobbs 2007). Restored sites are similar to unmined forest in terms of floristic composition, but lack large, mature trees (Koch & Hobbs 2007). This is a concern for hollow-dependent fauna, including bats, particularly as the dominant canopy tree species are unlikely to produce hollows suitable for fauna for more than a century (Whitford 2002). Very little is known about the roosting requirements of jarrah forest bats, with only one study examining mating season roosting preferences in a timber-harvested landscape of the southern jarrah forest (Webala et al. 2010).

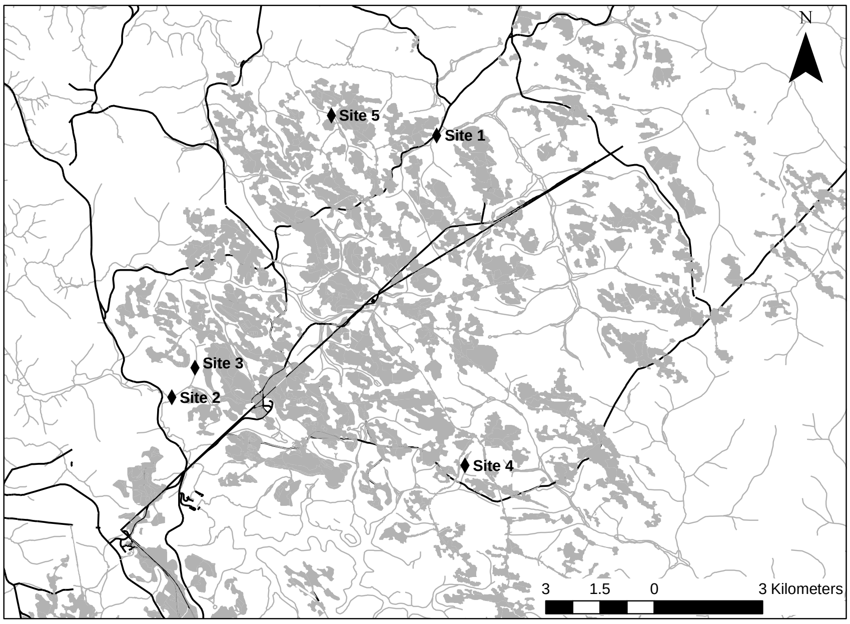
We aimed to assess whether restoration provided bat roosting habitat by determining (i) species specific bat roosting preferences at three spatial scales (tree, site and landscape) and two temporal scales (mating and maternity seasons) and (ii) the relative availability of suitable roosting trees and sites in restoration and adjacent unmined forest. We predicted that bats would preferentially roost in large, mature trees (Kalcounis-Ruppell et al. 2005; Webala et al. 2010) that were in intermediate stages of decay (Vonhof & Gwilliam 2007) and situated in relatively open sites with low canopy cover (Elmore et al. 2004; Vonhof & Barclay 1996). We predicted that roosting sites would be lacking within the restored forest due to the absence of large, mature trees (Law et al. 2011; Taylor & Savva 1988). Drawing from roosting studies of the same species or congenerics elsewhere in Australia we predicted that *N. gouldi* would be more flexible in roost, site, and landscape selection than *V. regulus* (Lunney et al. 1988; Webala et al. 2010). We also predicted that males and non-reproductive females would be less selective in their roost preferences than reproductive females, who are constrained by roosting in maternity colonies (Law & Anderson 2000; Lumsden et al. 2002a; Threlfall et al. 2013). Indeed, we anticipated that male and female roost preferences would converge during the mating season (Lumsden et al. 2002b).

Methods & Materials

Study area

The study was conducted at Huntly minesite (32°36’S, 116°07’E), operated by Alcoa of Australia (hereafter Alcoa), located between 80-100 km SSE of Perth, Western Australia. The study area is characterised by a Mediterranean climate with cool, wet winters and warm, dry summers. Annual rainfall for Dwellingup, ~10 km S of Huntly, is 1237 mm, with >75% falling between May and September. Mean maximum temperature ranges from 15°C in July to 30°C in January and February while mean minimum temperature ranges from 5°C in July to 15°C in February. The original vegetation at Huntly was jarrah forest, a dry sclerophyll forest where the overstorey is dominated by two eucalypt species, jarrah and marri (*Corymbia calophylla*), but with small components of blackbutt (*E. patens*) and bullich (*E. megacarpa*) in the gullies. The midstorey typically comprises sheoak (*Allocasuarina fraseriana*), bull banksia (*Banksia grandis*) and snottygobble (*Persoonia longifolia*) while common understorey species are *Bossiaea aquifolium*, *Lasiopetalum floribundum*, *Macrozamia riedlei, Xanthorrhoea gracilis* and *X. preissii* (Koch 2007b). Post-mining, Huntly minesite is a mosaic of unmined and restored forest of various ages (Figure 1). Of the 300-400 plant species found in the unmined forest, >75% are returned to the restored forest, although restored sites are more homogenous floristically across the landscape than unmined forest (Koch 2007b). Young unburnt restored forest (<15 years) typically has a two-tiered vegetation structure with an overstorey of jarrah and marri and a thick senescent *Acacia* shrub understorey and moderate species richness compared to the unmined forest (Grant 2006). For further details on the mining and restoration process, see Gardner and Bell (2007) and Koch (2007a).

a)



b)

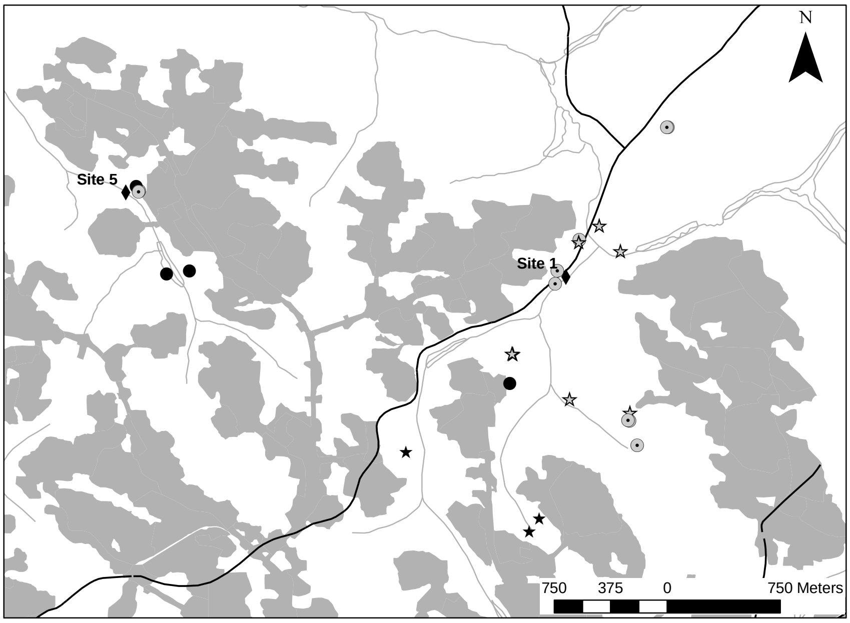


Figure 1a-b: a) The location of 5 bat trapping sites (diamonds), adjacent to waterholes within Huntly minesite. b) Detailed view of Sites 1 and 5 with roost trees selected by *N. gouldi* females (black stars), *N. gouldi* males (grey stars), *V. regulus* females (black circles) and *V. regulus* males (grey circles). Restored forest is denoted by grey while unmined forest is white. Black lines denote roads while grey lines denote streams.

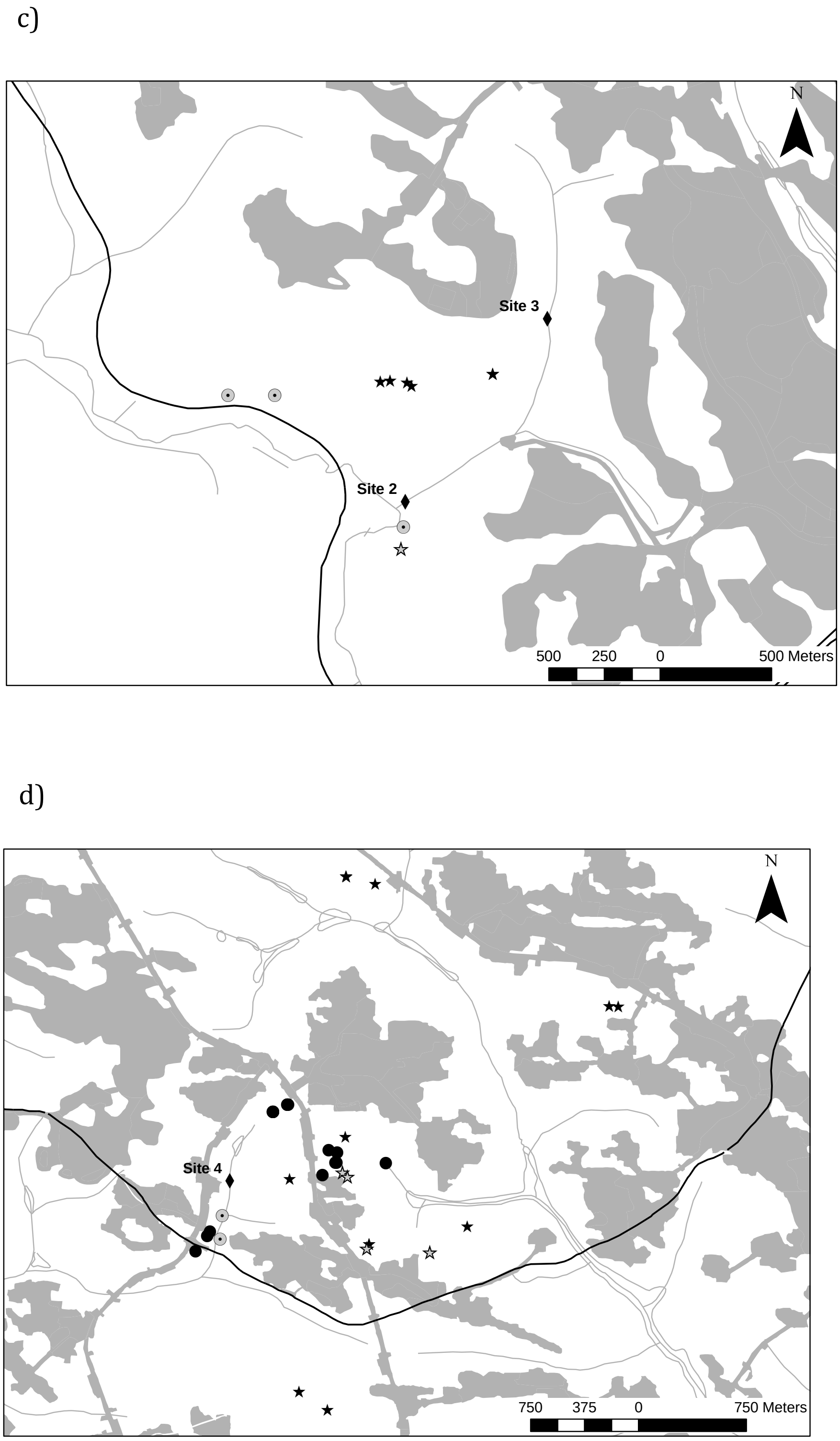


Figure 1c-d: c) Detailed view of Sites 3 and 4 and d) Site 5 with roost trees selected by *N. gouldi* females (black stars), *N. gouldi* males (grey stars), *V. regulus* females (black circles) and *V. regulus* males (grey circles). Restored forest is denoted by grey while unmined forest is white. Black lines denote roads while grey lines denote streams.

Field methods

Bats were trapped and tracked during the maternity (31 October to 9 December 2011) and mating (30 January to 17 March 2012) seasons. Bats were trapped a maximum of five hours after sunset using harp traps (Two-Bank 4.2 square metres; Ausbat Research Equipment, Victoria) at five separate waterholes within the unmined forest (Figure 1). Relatively close proximity of two sets of waterholes effectively meant that we surveyed from three general trapping areas (trapping area 1: Figure 1b; trapping area 2: Figure 1c; trapping area 3: Figure 1d). Preliminary trapping attempts within the restored forest were ineffective in capturing bats, requiring a great deal of survey effort before any bats were captured, so we trapped bats at waterholes to capture sufficient numbers of bats for meaningful analyses. Miniature position-sensitive single-stage radio transmitters with 12-14 cm antenna (0.27 or 0.31 g for *N. gouldi* and 0.22 g for *V. regulus*; model LB2X, Holohil Systems Ltd., Canada) were attached dorsally to *N. gouldi* (*N* = 21: 9 female and 12 male) and ventrally (Bullen & McKenzie 2001) to *V. regulus* (*N* = 22: 11 female and 11 male). In all but one case transmitters weighed less than 5% (range 1.3 -5.5%) of the body mass of the bat (Aldridge & Brigham 1988). Diurnal roost sites were located by tracking individual bats, on foot, from the day following capture until transmitters dropped off or the battery failed (*N. gouldi* range 1-6 days, mean 2.6; *V. regulus* range 1-5, mean 3.1), using three element hand-held Yagi antennas and R-1000 Telemetry Receivers (Communications Specialists). Due to logistic constraints we were only able to track between 4-6 bats simultaneously. Transmitter signals may bounce off surrounding trees making it difficult to pinpoint the exact signal location. However, we spent considerable time at each potential roost tree, varying signal frequency and intensity from multiple locations around the tree, locating fallen transmitters, and visually detected two bats affixed with transmitters roosting in low structures. Thus, we are quite confident we correctly identified roost trees. Roost tree location (using a GPS) was recorded for each diurnal roost. We only generally estimated roost height as jarrah and marri hollows are difficult to detect from the ground and the number of hollows observed is poorly correlated with actual numbers of tree hollows (Stojanovic et al. 2012; Whitford 2002).

To determine bat roosting preferences at the tree scale we compared roost trees with available trees. We identified one available tree for every roost tree by randomly selecting the nearest tree (≥20 cm DBH) to a random point between 50 and 100 m in a random direction from each roost tree (adapted from Vonhof & Gwilliam 2007; Webala et al. 2010). As all bats roosted in unmined forest, we ensured each available tree was also in unmined forest. For each roost and available tree we recorded tree species and measured the diameter at breast height over bark (DBH) and height of the tree. We assessed the health of each tree using five ordinal scale variables (Whitford 2002): snag class; dead branch order (DBO); crown senescence; bark cover; and the presence/extent of a fire scar. See Table 1 for full details of tree variable measurements.

To determine bat roosting preferences at the site scale we compared the vegetation structure surrounding roost trees with the vegetation structure surrounding available trees by centring a 5x5 m plot on each tree (Table 1). We measured canopy height (average of the five tallest overstorey plants within 10 m of the plot), height difference (the difference between the roost/available tree

Table 1: Roost tree and site characteristics measured within the unmined and restored northern jarrah forest of south-western Australia. Roost tree ordinal variables* were adapted from Whitford (2002). Vegetation clutter variables (overstorey and shrub clutter) were adapted from Webala et al. (2010). Height measurements were taken using a digital clinometer (Haglof Electronic Clinometer). Landscape variables were derived from GIS (ArcGIS 10.1).

Variable	Category/Measurement (units)	Multivariate Analyses
Roost Tree		
DBH	Diameter at breast height (1.3 m) of tree (cm), measured over bark	Tree
Height	Height of tree (m)	
Snag class*	Snag class (decay stage): 1 = all live tree; 2 = <30% dead; 3 = >30% dead; and 4 = 100% dead	Tree
DBO*	Dead branch order: scale of assessing DBO (DB1-DB9) where DB9 is a tree trunk more deteriorated than DB8	
Crown senescence*	Crown senescence: scale of assessing where a value of 1 is a crown with no or very little senescence to 9 where there is no crown remaining	Tree
Bark cover*	Bark cover class: 1 = none; 2 = <10%; 3 = 10-25%; 4 = >25%	Tree
Fire scar*	Presence of fire scar: 1 = no visible scar; 2 = small scar; 3 = large scar	Tree
Roost Site		
Canopy height	Height of canopy (m)	Site – both
Nearest tree height	Average height of five nearest trees (≥20 cm DBH)	Site – roost
Nearest tree distance	Average distance of five nearest trees (≥20 cm DBH)	Site – roost
Height difference	Difference between roost/available tree height and canopy height	Site – roost
Canopy cover	Proportion of canopy cover, derived from photographs	Site – both
Shrub cover	% Shrub cover of roost / available plot – estimated	Site – both
Ground cover	% Ground cover of roost / available plot – estimated	Site – both
Log cover	% Log cover of roost / available plot – estimated	Site – both
Litter cover	% Litter cover of roost / available plot – estimated	Site – both
Roost Landscape		
Elevation	Elevation (m)	Landscape
Slope Code	Slope categories: 1=<3°, 2 =3-5°, 3 =6-7°, 4=8-9°, 5=10-11°, 6=12-14°,7=15-17°, and 8=≥18°	Landscape
Time Since Fire	Time since last fire (years)	Landscape
Distance to restoration	Distance to closest edge of restoration (m)	
Distance to stream	Distance to closest stream (m)	Landscape
Distance to track	Distance to closest track or road (m)	Landscape
Unmined 250 m	Proportion of unmined forest within 250 m of roost (ha)	Landscape
Unmined 1000 m	Proportion of unmined forest within 1000 m of roost (ha)	Landscape
Unmined 3000 m	Proportion of unmined forest within 3000 m of roost (ha)	Landscape
Edge perimeter 250 m	Length of restoration perimeter edge within 250 m (ha)	Landscape
Edge perimeter 1000 m	Length of restoration perimeter edge within 1000 m (ha)	Landscape
Edge perimeter 3000 m	Length of restoration perimeter edge within 3000 m (ha)	Landscape

height and the canopy height) and the average height and distance of the five overstorey plants (≥20 cm DBH) nearest to the roost/available tree. Overstorey plant heights were measured with a tree vertex. We calculated canopy cover for each plot using digital photography (Macfarlane et al. 2007) and averaged the values from the four corners of the plot. We also visually estimated the percent cover of litter, logs, ground vegetation (<0.75 m) and shrub vegetation (0.75-5 m) within each plot. To investigate bat roosting behaviour and selection preferences at the landscape scale we randomly identified an equal number of locations (65) as roost locations within unmined forest (no more than ~3 km from each trapping area) using GIS (esri ArcMap v10.1, USA). For roost and random locations we used GIS to calculate commuting capabilities, site fidelity, and 12 variables derived from GIS spatial layers: elevation, slope, time since last fire, distance to nearest restored mine-pit edge, distance to nearest stream, distance to nearest track/road, and the proportion of unmined forest and length of restored mine-pit edge within three radii (250 m, 1000 m, and 3000 m).

To determine the suitability of restoration as bat roosting habitat we compared vegetation structure at roost sites with vegetation structure within 56 restored sites, from a concurrent bat study (Chapter 2). Alcoa has adapted their seeding mix and fertilising practices to reduce eucalypt densities in recent years, categorising restored sites as desirable (500-2500 eucalypt stems ha^{-1}) or dense (>2500 eucalypt stems ha^{-1}) based on nine-month monitoring data (Grant 2006). To capture the differences in eucalypt densities over time we sampled eight sites each from the following restored forest types: 0-4 years desirable, 5-9 years desirable, 5-9 years dense, 10-14 years desirable, 10-14 years dense, >15 years desirable, and >15 years dense. We measured vegetation structure in five 5x5 m plots within each site. We followed the same methodology as above for quantifying the vegetation structure of plots surrounding roost/available trees. We also measured the same variables with the exception of height difference and the average height and distance of the five nearest overstorey plants (Table 1). For canopy cover, we used digital photography but took one picture at the centre of each plot. We averaged measurements over the five plots for an overall site value.

We determined straight-line bat flight distances and travel paths between trapping sites and the first roosting site (with the exception of three cases in the mating season this was the roost recorded the day immediately following capture). We counted the number of time(s) the straight-line travel path crossed over restoration to quantify the proportion of potential instances when bats commuted through/above restoration on their way from foraging to roosting. To determine roost site fidelity we calculated the distance between roost trees for each individual bat. See Table 1 for full details of landscape variable measurements.

Statistical analyses

To determine if bats chose specific trees for roosting we compared the collective characteristics of roost to available trees. We removed non-eucalypt trees from the analyses, retaining jarrah (N = 91), marri (N = 17), bullich (N = 10), and blackbutt (N = 9). We removed highly correlated (>0.80 Spearman test) variables, excluding DBO, before constructing a Euclidean resemblance matrix of the remaining scaled tree variables (DBH, height, snag class, crown senescence, bark cover and fire scar) for each bat species. As there was no difference in the overall tree characteristics by tree species, for either *N. gouldi* ($F_{3,126}$ = 1.40, *P* = 0.179) or *V. regulus* ($F_{3,65}$ = 0.92, *P* = 0.4978), we pooled eucalypts for all analyses. To determine bat roosting preferences at the site and landscape scales we compared the collective vegetation structure and landscape variables, respectively, of roost to available/random sites, including all roost and available/random sites in the analyses. We removed highly correlated (>0.80 Spearman test) variables, difference in height between roost/available tree, the five nearest trees at the site scale and distance to restoration at the landscape scale. We constructed a Euclidean resemblance matrix of the eight remaining scaled site variables and another for the 11 remaining scaled landscape variables. We used the three resemblance matrices to test for differences between tree, site, or landscape type (roost and available/random), bat species, and the interaction between the two (fixed factors) with individual bat as a random factor for the tree and site analyses and trapping area as a random factor for the landscape analysis. We used the Adonis function, over 9 999 permutations, in the R vegan package (Oksanen et al. 2012).

To identify whether individual variables were related to bat roost preferences at the tree, site and landscape scale we ran Gaussian generalized linear mixed models (GLMM) using the R lmerTest package (Kuznetsova et al. 2014) for each bat species separately. As we were interested in the influence of each variable (Table 1) on intraspecific bat roosting preferences we ran separate models for each of the seven tree, nine site, and 12 landscape variables for each bat species separately. Although we measured a “paired” available tree/site and random location for each roost we had no reason to assume that individual bats would not be associated with a non-paired available tree/site or random location so we tested each bat group (male, female, maternity and mating) against all available trees/sites and random locations. Thus, each tree, site or landscape variable was the dependent variable with a three category ordinal fixed factor of sex (male, female, and available/random) or season (maternity, mating, and available/random), and both individual bat and trapping area as random factors. We specified available/random as the reference level so model parameters for each bat category are in relation to the available/random category. Small sample sizes prohibited us from dissecting the data further. i.e., into sex by season or season by sex. Due to the number of tests conducted only those with a significance of $P < 0.01$ are graphically presented or discussed, although all with $P < 0.05$ are noted in the results.

To determine the suitability of restoration as roosting habitat we compared the vegetation structure at roost sites with vegetation structure at restored sites. We constructed a Euclidean resemblance matrix of the six site vegetation variables (scaled) collected at both roost and restored sites: canopy height, canopy cover, shrub cover, ground cover, litter cover and log cover (Table 1). Tree density was highly variable across restored sites and there were no significant differences in tree density between Alcoa's desirable and dense categories (R5-9 $t_{14} = 1.40$, $P = 0.184$; R10-14 $t_{14} = -0.35$, $P = 0.786$; R>15 $t_{14} = -0.84$, $P = 0.416$). Consequently, we grouped desirable and dense sites within each restored forest age group and considered forest type as a fixed factor with five categories: roost (N = 36) and restored forest of ages 0-4 (N = 8), 5-9 (N = 16), 10-14 (N = 16), and >15 (N = 16). We tested for differences between forest types using the Adonis function, over 9 999 permutations, in the R vegan package (Oksanen et al. 2012). We ran a principal coordinate analysis (PCoA) to visually represent the site vegetation composition between forest types, using the R ape package (Paradis et al. 2004). To identify how the vegetation structure differed between roost and restored sites we ran separate generalized linear models for each of the seven vegetation structure variables with forest type as the explanatory variable. To account for the lack of independence of site data (i.e., individual bats with multiple roost sites and five plots per restored site) we averaged vegetation structure variable values by individual bat for roost sites and by plots for restored sites to give a single value for each individual bat or site.

For intra and interspecific comparisons of straight-line bat flight distance and site fidelity we ran Welch's two sample *t*-tests to compare between bat species and two-factor ANVOAs to compare within species (i.e., between sexes, seasons and the interaction of the two), testing significant interactions with Tukey's post-hoc tests. All statistical analyses were performed in R (R Core Team 2013).

Results

Radio-tracking

Of the 43 bats affixed with transmitters three transmitters attached to *N. gouldi* and four attached to *V. regulus* either failed, or bats could not be relocated, while the remaining 36 bats were tracked to 59 different roost trees for a total of 101 fixes (46 fixes for *N. gouldi* and 55 for *V. regulus*; Table 2 and Appendix 2, Tables S1 and S2). Due to logistical issues we were unable to observe bat emergence from roosts at dusk. However, we did opportunistically encounter communal roosting on three occasions, all during the maternity season.

Table 2: Radio-tracking results for *N. gouldi* and *V. regulus*, by season and sex, tracked between October 2011 and March 2012 in south-western Australia. Roost tree species are jarrah (J), marri (M) and other (O), comprising sheoak and banksia for *N. gouldi* and bullich for *V. regulus.*

Species	Season	Sex	No. fitted	No. never located	Total no. of roosts located	Dist. to first roost (m) ± SE	Dist. between roosts (m) ± SE	Roost tree species		
								J	M	O
N. gouldi	Maternity	F	5	1	7	931±182	341±86	7		
		M	3	0	3	1232±138	--*	3		
	Mating	F	7	0	11	1831±290	200±77	8	1	2
		M	7	2	9	705±135	83±59	8		1
V. regulus	Maternity	F	6	1	8	526±115	83±25	5	1	2
		M	4	1	4	198±55	6*		2	2
	Mating	F	5	1	6	628±91	113±80	5		1
		M	7	1	11	685±225	100±42	7	2	2

*During the maternity season only one male *V. regulus* was tracked to more than one roost and no male *N. gouldi* were tracked to subsequent roosts

We tracked two female *N. gouldi* to the same tree and two female *V. regulus* to the same two trees. While marking a large *N. gouldi* jarrah roost tree (83 cm DBH) we observed 15-20 bats (two known to be *N. gouldi*) exiting the tree via a burned out cavity (from the base to ~3 m above the ground; Figure 2). All emerging bats quickly roosted in nearby trees and we found the tagged individual roosting on subsequent days indicating none of the disturbed bats were harmed. No tracked bats were observed roosting together during the mating season.

Tree scale roost preferences

All bats roosted in trees in the unmined forest (Figure 1). Bats were tracked predominantly to jarrah (43) but also to marri (6), bullich (7), sheoak (2) and one bull banksia. Both *N. gouldi* and *V. regulus* were tracked to jarrah and marri but only *N. gouldi* was tracked to sheoak and banksia while only *V. regulus* was tracked to bullich (Table 2). While we were unable to pinpoint exact roost locations within a tree, we made general observations, surmising that most roosts were hollows (54 of the 62 roosts) in the top half of the tree (≥10 m above the ground). Known exceptions to hollows were observed during the mating season (Figure 2): we audibly observed one *V. regulus* male roosting 1.5 m above the ground in a fissure in the trunk of a dead jarrah stag (12.4 cm DBH and 8.4 m tall) and tracked one *N. gouldi* male to the foliage/canopy leaves of a bull banksia (10.5 cm DBH and 7.1 m tall), roosting 5.7 m above the ground. We also tracked one female *V. regulus* to a hollow within a fallen branch, caught at the base of the tree, and roosting 0.8 m above the ground (Figure 2).

Figure 2: a) Jarrah tree (83.0 cm DBH and 25.5 m tall) used as a roost during the maternity season with a burnt out cavity at the base to ~3 m where 15-20 bats were observed exiting after being disturbed; b) Bull banksia where one male *N. gouldi* was tracked during the mating season; we presumed he was roosting in the foliage, 5.7 m above the ground; c) Dead jarrah stag where we tracked and had audible confirmation of one male *V. regulus* roosting, ~1.5 m above the ground, in a trunk fissure, during the mating season; and d) Fallen coarse woody debris where one female *V. regulus* was recorded roosting in a hollow 0.8 cm above the ground, during the mating season.

Overall, eucalypt tree characteristics differed between tree types ($F_{1,126}$ = 11.27, $P <$ 0.001) and bat species ($F_{1,126}$ = 3.19, $P < 0.001$), but not the interaction between the two ($F_{3,126}$ = 0.74, P = 0.553). *N. gouldi* preferred roost trees that were in greater stages of decay (snag class P = 0.003, DBO $P < 0.001$, and crown senescence $P <$ 0.001) than available trees (Table 3, Table 4). Female *N. gouldi* and all *N. gouldi* during the maternity season preferred roost trees that had a significantly larger DBH (both $P < 0.001$) and were taller (female P = 0.027, maternity P = 0.040) than available trees (Table 4). Male *N. gouldi* preferred roost trees with less bark cover than available trees (P = 0.014, Table 4). *V. regulus* roost tree preferences varied by season and sex. Female *V. regulus* and *V. regulus* during that mating season preferred roost trees that had a larger DBH (female P = 0.049, mating $P < 0.001$), were taller (female P = 0.037, mating P = 0.012), and were in greater stages of decay (female: snag class P = 0.002, DBO P = 0.006, and crown senescence $P <$ 0.001; mating: snag class P = 0.012, DBO $P < 0.001$, and crown senescence $P <$ 0.001) than available trees (Table 5). Male *V. regulus* also preferred trees that had a larger DBH (P = 0.013) and higher DBO (P = 0.033) than available trees (Table 5). During the maternity season *V. regulus* roosted in trees with larger fire scars than those present on available trees (P = 0.011; Table 5). See Appendix 2 for graphical representation of roost tree preferences for *N. gouldi* (Figure S2) and *V. regulus* (Figure S3).

Site scale roost preferences

At the site scale, overall vegetation structure differed between roost and available sites ($F_{1,127}$ = 2.25, P = 0.015), but not for bat species ($F_{1,127}$ = 3.31, P = 0.051) or the interaction between the two ($F_{1,127}$ = 0.81, P = 0.525). *N. gouldi* females and all

Table 3: Mean (± SE) tree, site and landscape variables for *N. gouldi* and *V. regulus* roosts and available/random.

	N. gouldi	*V. regulus*	Available / Random
Roost Tree			
DBH (cm)	69.4±7.1	60.8±4.5	44.0±2.7
Height (m)	23.4±1.0	23.3±1.0	20.9±0.6
Snag class	2.7±0.2	2.2±0.1	1.9±0.1
DBO	2.9±0.3	1.9±0.2	1.3±0.1
Crown senescence	5.0±0.6	3.0±0.4	1.9±0.2
Bark cover	3.6±0.2	3.7±0.2	3.9±0.1
Fire scar	2.1±0.1	2.3±0.2	2.2±0.1
Roost Site			
Canopy height (m)	23.3±0.6	25.7±0.8	24.3±0.5
Nearest tree height (m)	18.1±0.4	19.6±0.4	19.6±0.4
Nearest tree distance (m)	6.4±0.3	6.5±0.3	6.5±0.3
Height difference (m)	-0.93±0.8	-2.41±1.0	-3.39±0.5
Canopy cover (%)	64±3	61±2	67±2
Shrub cover (%)	14±2	17±2	20±2
Ground cover (%)	22±3	22±3	24±3
Log cover (%)	12±2	12±1	9±1
Litter cover (%)	97±1	95±2	96±1
Landscape			
Elevation (m)	287±4	277±4	283±3
Slope code	2.3±0.3	2.8±0.3	3.0±0.2
Time since fire (yr)	10.8±1.2	9.9±0.9	12.7±0.8
Distance to restoration (m)	182±22	161±35	268±41
Distance to stream (m)	264±32	168±26	217±22
Distance to track (m)	161±22	133±25	117±13
Unmined 250 m (ha)	16.8±0.5	16.4±0.4	16.4±0.5
Unmined 1000 m (ha)	224±5	211±7	241±5
Unmined 3000 m (ha)	1994±21	2022±20	2105±29
Edge perimeter 250 m (m)	953±148	1326±154	865±116
Edge perimeter 1000 m (km)	14.7±0.8	15.7±1.0	12.4±0.8
Edge perimeter 3000 m (km)	117.9±3.3	116.3±3.5	100.0±4.1

N. gouldi during the maternity season preferred roost sites where the roost tree was at or above canopy height compared to available roost trees, which were below canopy height (female $P = 0.003$, maternity $P = 0.016$, Table 4). During the maternity season *N. gouldi* also preferred roost sites with more ground cover than available sites ($P = 0.013$, Table 4). Male *N. gouldi* preferred roost sites with more log cover than available sites ($P = 0.004$; Table 4).

Table 4: We ran Gaussian GLMMs for each *N. gouldi* roost landscape variable as the dependent variable and either sex or season as a three categorical response variable (i.e., random as one category n = 32). Significant relationships are indicated by * for *P*<0.05, ** for *P*<0.01, and * for *P*<0.001 and are highlighted in bold.**

	Roost*Male		Roost*Female		Roost*Maternity		Roost*Mating	
N. gouldi	Estimate ± SE	*t*-value	Estimate ± SE	*t*-value	Estimate ± SE	*t*-value	Estimate ± SE	*t*-value
Roost Tree								
DBH (cm)	16.9±10.3	1.64	30.1±8.6	**3.50*** **	49.6±9.6	**5.20*** **	10.0±1.8	1.26
Height (m)	0.6±1.6	0.36	3.1±1.4	**2.27***	3.4±1.3	**2.09***	1.3±1.4	0.98
Snag class	0.8±0.3	**2.67****	0.7±0.2	**3.06****	0.7±0.3	**2.48***	0.7±0.2	**3.10****
DBO	1.8±0.5	**3.64*** **	1.3±0.4	**3.22****	2.0±0.5	**3.98*** **	1.2±0.4	**3.01****
Crown senescence	3.3±1.0	**3.40****	2.5±0.8	**3.21****	2.7±1.0	**2.82****	2.9±0.8	**3.64*** **
Bark cover	-0.7±0.3	**-2.54***	-0.0±0.2	-0.05	-0.0±0.3	-0.15	-0.4±0.2	-1.73
Fire scar	0.1±0.3	0.33	-0.2±0.2	-0.78	-0.3±0.3	-1.11	0.1±0.2	0.27
Roost Site	n = 11		n = 18		n = 11		n = 18	
Canopy height (m)	-0.1±1.1	-0.11	-0.4±0.9	-0.46	0.2±1.1	0.18	-0.6±0.9	-0.66
Nearest tree height (m)	0.1±0.8	0.06	-1.3±0.7	-1.91	-1.3±0.9	-1.48	-0.5±0.7	-0.68
Nearest tree distance (m)	-0.2±0.8	0.30	0.2±0.7	0.75	0.6±0.8	0.73	-0.0±0.7	-0.00
Height difference (m)	1.0±1.3	0.74	3.4±1.1	**3.08****	3.2±1.3	**2.48***	1.9±1.1	1.76
Canopy cover (%)	-0.5±5.5	-0.10	-3.6±4.5	-0.80	-3.0±5.5	-0.55	-2.1±4.5	-0.46
Shrub cover (%)	-1.8±3.5	-0.51	-1.5±2.9	-0.52	-1.6±3.7	-0.43	-1.6±2.9	-0.57
Ground cover (%)	6.4±5.2	1.22	1.9±4.4	0.44	12.6±5.0	**2.55***	-2.1±4.2	-0.52
Log cover (%)	7.8±2.6	**3.00****	0.6±2.2	0.29	2.8±2.7	1.04	3.7±2.3	1.62
Litter cover (%)	1.6±1.7	0.95	-0.3±1.4	-0.19	-0.2±1.7	-0.14	0.9±1.5	0.59
Landscape								
Elevation	-12.2±7.1	-1.72	15.8±5.7	**2.77****	1.5±8.1	0.20	7.7±6.3	1.23
Slope code	-0.3±0.6	-0.54	-1.32±0.5	**-2.80****	-2.3±0.6	**-4.19*** **	-0.2±0.4	-0.42

Distance to restoration	-49.6±70.9	-0.70	-43.6±58.1	-0.75	-61.2±75.3	-0.81	-38.0±58.0	-0.66
Distance to stream	-39.9±57.4	-0.70	147.6±45.9	**3.22****	79.8±63.7	1.25	78.0±49.0	1.59
Distance to track	75.4±33.9	**2.22***	78.4±26.9	**2.92****	76.2±35.4	**2.15***	77.8±26.6	**2.93****
Unmined 250 m (ha)	0.8±1.1	0.73	0.5±0.9	0.57	2.3±1.1	**2.05***	-0.2±0.9	-0.28
Unmined 1000 m (ha)	-8.3±12.4	-0.68	-0.63±10.4	-0.06	14.5±12.4	1.17	-13.0±9.9	-1.31
Unmined 3000 m (ha)	-15.0±62.5	-0.24	-64.4±52.3	0.23	-34.7±64.7	-0.54	-51.7±52.0	-1.00
Edge perimeter 250 m	-66±294	-0.22	76±239	0.32	-524±288	-1.82	311±223	1.40
Edge perimeter 1000 m	-17±1684	-0.01	1596±1383	1.15	804±1808	0.45	1115±1399	0.80
Edge perimeter 3000 m	8058±7210	1.12	13825±6000	**2.30***	19540±7622	**2.56***	7538±5913	1.28

Table 5: We ran Gaussian GLMMs for each *V. regulus* roost landscape variable as the dependent variable and either sex or season as a three categorical response variable (i.e., random as one category n = 33). Significant relationships are indicated by * for *P*<0.05, ** for *P*<0.01, and * for *P*<0.001 and are highlighted in bold.**

	Roost*Male (n = 15)		Roost*Female (n = 18)		Roost*Maternity (n = 14)		Roost*Mating (n = 19)	
V. regulus	Estimate ± SE	*t*-value	Estimate ± SE	*t*-value	Estimate ± SE	*t*-value	Estimate ± SE	*t*-value
Roost Tree								
DBH (cm)	19.9±7.8	**2.55***	14.8±7.4	**2.01***	5.6±7.7	0.72	25.6±7.0	**3.69*****
Height (m)	2.0±1.6	1.20	3.3±1.6	**2.13***	1.1±1.7	0.66	3.9±1.5	**2.58***
Snag class	0.3±0.2	1.35	0.6±0.2	**3.19****	0.4±0.2	1.95	0.5±0.2	**2.60***
DBO	0.7±0.3	**2.18***	0.9±0.3	**2.83****	0.4±0.3	1.19	1.1±0.3	**3.77*****
Crown senescence	0.6±0.6	0.96	2.1±0.5	**3.85*****	0.5±0.6	0.83	2.1±0.5	**3.83*****
Bark cover	-0.2±0.2	-1.07	-0.3±0.2	-1.78	-0.2±0.2	-1.09	-0.3±0.2	-1.78
Fire scar	0.2±0.3	0.85	-0.0±0.3	-0.10	0.7±0.3	**2.62***	-0.3±0.2	-1.46
Roost Site								
Canopy height (m)	1.2±1.4	0.87	0.9±1.3	0.69	-0.4±1.4	-0.30	2.2±1.3	1.68
Nearest tree height (m)	-1.4±1.0	-1.40	-0.1±0.9	-0.06	-0.6±1.0	-0.57	-0.7±0.9	-0.79
Nearest tree distance (m)	-0.7±0.6	-1.20	-0.1±0.6	-0.17	-0.1±0.6	-0.15	-0.6±0.6	-1.09
Height difference (m)	0.5±1.5	0.35	2.5±1.4	1.75	1.5±1.6	0.93	1.7±1.4	1.21
Canopy cover (%)	-4.7±4.1	-1.14	-8.6±3.8	**-2.25***	-3.3±4.3	-0.78	-9.5±3.8	**-2.49***
Shrub cover (%)	-4.6±4.0	-1.16	-10.5±3.8	**-2.80****	-11.4±4.1	**-2.78****	-5.2±3.7	-1.41
Ground cover (%)	-2.2±6.2	-0.35	-11.2±5.8	-1.92	-2.7±6.4	-0.42	-10.3±5.6	-1.86
Log cover (%)	0.4±2.1	0.19	6.0±1.9	**3.09****	1.2±2.2	0.54	5.1±1.9	**2.68****
Litter cover (%)	0.5±3.3	0.15	-1.9±3.1	-0.61	-0.4±3.4	-0.11	-1.1±3.1	-0.37
Landscape								
Elevation	-17.2±4.9	**-3.48*****	0.8±4.6	0.18	-0.81±5.1	-0.16	-12.20±2.6	**-2.68****
Slope code	0.07±0.5	0.15	-0.1±0.5	-0.25	0.11±0.5	0.23	-0.1±0.5	-0.31
Time since fire	-3.0±1.6	-1.88	-3.8±1.5	**-2.52***	-4.4±1.7	**-2.67****	-2.7±1.4	-1.91

Distance to restoration	-147.2±83.4	-1.77	-138.1±77.7	-1.78	-171.9±82.0	**-2.10***	-120.4±73.8	-1.63
Distance to stream	-116.9±46.4	**-2.52***	-54.9±43.5	-1.26	-92.3±48.5	-1.90	-76.7±42.8	-1.79
Distance to track	-16.37±38.1	-0.43	-10.5±35.7	-0.30	-49.7±39.4	-1.26	12.9±34.6	0.37
Unmined 250 m (ha)	0.4±0.9	0.44	-0.7±0.9	-0.77	-1.8±0.9	**-2.00***	1.0±0.8	1.29
Unmined 1000 m (ha)	-30.8±11.3	**-2.72****	-53.7±10.6	**-5.09*****	-66.9±10.7	**-6.23*****	-26.0±9.4	**-2.76****
Unmined 3000 m (ha)	-122.4±52.5	**-2.33***	-170.2±49.0	**-3.48*****	-200.0±51.9	**-3.85*****	-112.4±45.8	**-2.45***
Edge perimeter 250 m	183±250	0.73	750±234	**3.21****	1048±241	**4.34*****	81±209	0.39
Edge perimeter 1000 m	2889±1380	**2.09***	6023±1290	**4.67*****	7075±1371	**5.16*****	2794±1183	**2.36***
Edge perimeter 3000 m	23631±6218	**3.80*****	21661±5811	**3.73*****	25380±6361	**3.99*****	20557±5535	**3.71*****

Female *V. regulus* and all *V. regulus* during the mating season preferred roost sites that had less canopy cover (female $P = 0.028$, mating $P = 0.016$, Table 5) and more log cover (female $P = 0.003$, mating $P = 0.010$) than available sites. Female *V. regulus* and all *V. regulus* during the maternity season preferred roost sites with less shrub cover than available sites (both $P = 0.007$, Table 5, Figure 5). See Appendix 2 for graphical representation of roost site preferences for *N. gouldi* and *V. regulus* (Figure S4).

Landscape scale roost preferences

Overall landscape scale characteristics differed between roost and random locations ($F_{1,126} = 5.34$, $P < 0.001$), the interaction between roost type and bat species ($F_{1,126} = 3.17$, $P = 0.007$) but not between bat species ($F_{1,126} = 0.99$, $P = 0.358$). Univariate analyses found sexual and seasonal landscape differences between roosts and random locations for both *N. gouldi*, (Table 3, Table 4) and *V. regulus* (Table 3, Table 5). All *N. gouldi* preferred roosts located farther from tracks than random locations (female $P = 0.005$; male $P = 0.030$; maternity $P = 0.036$; mating $P = 0.005$). In addition, female *N. gouldi s*elected roosts that were at a higher elevation ($P = 0.008$), on ground with a flatter slope ($P = 0.007$), farther from streams ($P = 0.002$), and had more restoration edge within 3000 m ($P = 0.025$) than random locations. During the maternity season, *N. gouldi* selected roosts that were on ground with a flatter slope ($P < 0.001$), had a greater proportion of unmined forest within 250 m ($P = 0.045$), and had more restoration edge perimeter within 3000 m ($P = 0.013$) than random locations. See Appendix 2 for graphical representation of roost landscape preferences for *N. gouldi* (Figure S5).

All *V. regulus* preferred roosts surrounded by a lower proportion of unmined forest within 1000 m (female P < 0.001; male *P* = 0.009; maternity *P* < 0.001; mating *P* = 0.008) and 3000 m (female P = 0.001; male *P* = 0.023; maternity *P* < 0.001; mating *P* = 0.017), and a greater restoration edge within 1000 m (female *P* < 0.001; male *P* = 0.041; maternity *P* < 0.001; mating *P* = 0.022) and 3000 m (all *P* < 0.001; Figure 9) of roosts, compared to the random locations. In addition, *V. regulus* males preferred roosts located at lower elevations (*P* < 0.001) and closer to streams (*P* = 0.014), while *V. regulus* females preferred roosts located in areas that had been burnt more recently (*P* = 0.015) and had greater amounts of restoration edge perimeter within 250 m (*P* = 0.002), compared to random locations. During the maternity season *V. regulus* preferred roosts located in forests that had been burnt more recently (*P* = 0.010), were closer to the restored forest edge (*P* = 0.040), had a lower proportion of unmined forest within 250 m (*P* = 0.050), and had more restoration edge within 250 m (*P* < 0.001), compared to random locations. During the mating season *V. regulus* showed a preference for roosts situated at lower elevations compared to random locations (*P* = 0.010). See Appendix 2 for graphical representation of roost landscape preferences for *V. regulus* (Figures S6 and S7).

Suitability of the restored forest as roosting habitat

Multivariate analyses indicated that vegetation structure differed significantly between roost sites and restored sites ($F_{1,90}$ = 46.18, *P* < 0.001) although the vegetation structure in restoration became more similar to the vegetation structure surrounding roosts as restoration matured (Figure 3). Univariate analyses revealed that vegetation structure was significantly different between roost sites and each age of restored forest for all site vegetation structure variables, except for shrub clutter where the model was marginally non-significant (Appendix 2 Figure S8; canopy height $F_{4,87} = 22.65$, $P < 0.001$, adj $R^2 = 0.91$; canopy cover $F_{4,87} = 58.07$, $P < 0.001$, adj $R^2 = 0.72$; shrub cover $F_{4,87} = 8.23$, $P < 0.001$, adj $R^2 = 0.24$; ground cover $F_{4,87} = 6.47$, $P = 0.001$, adj $R^2 = 0.19$; log cover $F_{4,87} = 18.93$, $P < 0.001$, adj $R^2 = 0.44$; and litter cover $F_{4,87} = 95.14$, $P < 0.001$, adj $R^2 = 0.81$).

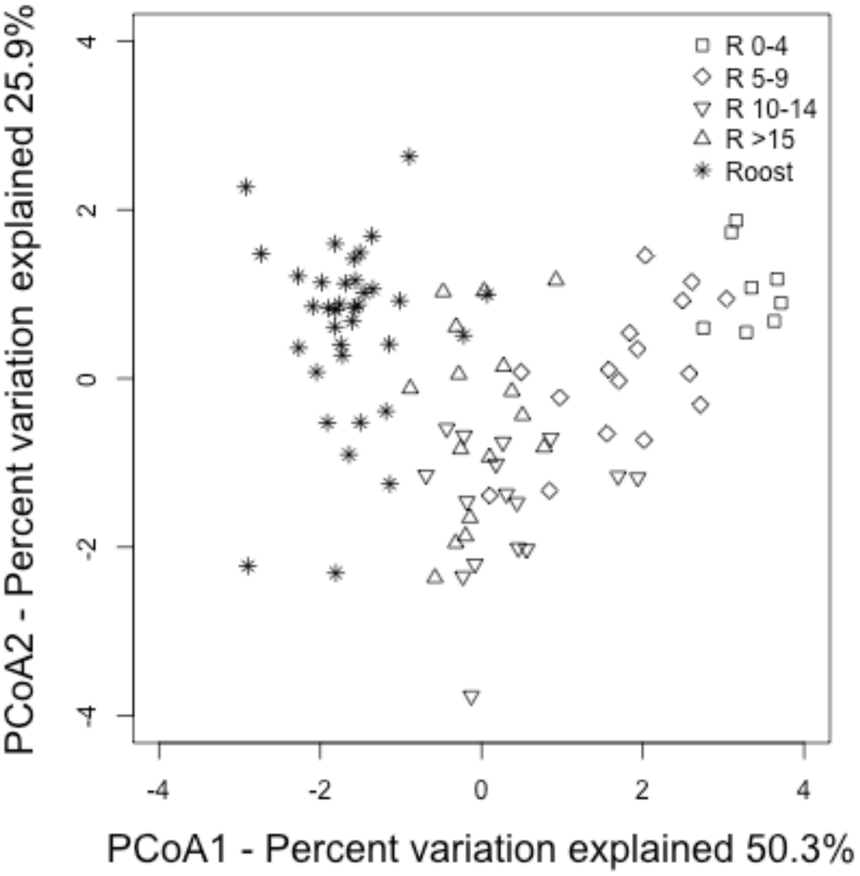


Figure 3: Principal coordinate analysis (PCoA) of vegetation structure between roost sites and restored forest sites (R 0-4 N = 8, R 5-9 N = 16, R 10-14 N = 16, R >15 N = 16, Roost N = 36). Roost sites include both *N. gouldi* and *V. regulus* roosts, and all were located in the unmined forest.

Roost site fidelity

Of the 36 bats tracked eight were only tracked to one diurnal roost for one day; on subsequent days we either found the transmitter on the ground or were unable to locate the bat. Of those bats tracked more than one day, 70% switched roosts after the first day. During the maternity season, all female *N. gouldi* ($N = 3$) and all but one female *V. regulus* ($N = 5$) switched roosts between the first and second day, compared to only one of the three male *V. regulus*. During the mating season, all male and all but one female *N. gouldi* ($N = 3$ and $N = 6$, respectively) switched roosts between the first and second day. In contrast, only one female *V. regulus* ($N = 4$) switched roosts between the first and second day; two females did not change roosts during the tracking period (four and five days) while one female switched roosts between the second and third day. Only one male *V. regulus* was tracked for more than one day during the mating season and this individual did not change roosts. There was no difference in distance between roost sites between the sexes or seasons for either *N. gouldi* or *V. regulus* (*N. gouldi* sex $F_{1,9} = 2.88$, $P = 0.124$; season $F_{1,9} = 1.75$, $P = 0.218$, *V. regulus* sex $F_{1,7} = 0.07$, $P = 0.804$; season $F_{1,7} = 1.07$, $P = 0.336$) or the interaction between the two for *V. regulus* ($F_{1,7} = 0.34$, $P = 0.578$). Small sample sizes meant we were unable to test the interaction for *N. gouldi*. *N. gouldi* travelled farther from roost to roost than *V. regulus* (218 ± 51 m and 88 ± 21 m, respectively; $t_{15} = 2.35$, P=0.033; Table 2). Considering straight-line distances from capture site to first roosting site, 13 bats (36%) potentially travelled through restoration to reach their first diurnal roost after capture.

Discussion

This is the first study to examine the roosting preferences of bats across a restored landscape; our results underscore the importance of unmined forest as roosting habitat in the northern jarrah forest. Absence of roosts, suitable roost trees and suitable roost sites in restored jarrah forest indicates that restoration to date provides poor, or unsuitable, roosting habitat for both *N. gouldi* and *V. regulus*.

Hollows drive roost preferences

Our study agrees with other bat roosting research; forest-dwelling bats generally prefer roosting in large, mature trees with some intra and interspecific preferences (Kalcounis-Ruppell et al. 2005; Lumsden et al. 2002b; Vonhof & Gwilliam 2007). In concordance with predictions, and similar to previous findings (Threlfall et al. 2013; Webala et al. 2010), both *N. gouldi* and *V. regulus* selected eucalypt roost trees based on tree size and decay stage, preferring roost trees that were older and more senescent than available trees. Contrary to our predictions, *V. regulus* was slightly more flexible in roost tree selection than *N. gouldi*, particularly during the maternity season. Hollow occurrence and abundance increases in eucalypts as DBH and senescence increases (Rayner et al. 2013). In jarrah and marri hollow abundance peaks in trees with intermediate levels of DBO and crown senescence (Whitford 2002). The tree characteristics preferred by both *N. gouldi* and *V. regulus* likely correspond to eucalypt trees with the greatest number of hollows.

N. gouldi and *V. regulus* selected eucalypt trees that were ~60 and 80 cm DBH, respectively, slightly smaller than mating season roost trees in the southern jarrah forest (Webala et al. 2010) but substantially larger than trees in restored forest (~24 cm DBH in 15 year old restoration, J. Burgar unpublished data). Trees selected for roosting are estimated to be ~150-200 years old and to contain one or more hollows (Whitford 2002). By ~60 years old both jarrah and marri trees are estimated to contain at least one hollow with a slit entrance of 20 mm (Whitford 2002). As bats roost in hollows with slit entrances only slighter larger than themselves (Campbell 2009; Goldingay 2009; Tidemann & Flavel 1987) they may not be as restricted by hollow size, and subsequently tree age, as many other hollow-dependent fauna, at least during the mating season. Small hollows are less likely to be used during the maternity season as maternity colonies may number ≥50 females, in addition to their young (Law & Anderson 2000; Vonhof & Gwilliam 2007). Published accounts of maternity colony sizes for jarrah forest bat species are limited but suggest colonies of 10-19 *N. gouldi* adults (Lunney et al. 1988; Threlfall et al. 2013; Tidemann & Flavel 1987) and 25-66 *V. regulus* adults (Taylor & Savva 1988; Tidemann & Flavel 1987). *N. gouldi*'s preference for roost trees almost twice as large in the maternity season, compared to the mating season (~90 cm compared to ~50 cm DBH), is consistent with roosting studies of *N. gouldi* in suburban eastern Australia (Threlfall et al. 2013) and emphasizes the importance of moderate sized hollows for bats during the maternity season.

Hollow formation is dependent on stem density as well as age. Revegetated sites in south-eastern Australia show delayed hollow development where stem densities were over 1 000 stems ha^{-1} (Vesk et al. 2008). In the jarrah forest, hollows typically form from limb breakage or shedding of a limb at the joint of a larger branch in large trees with moderately senescent crowns of intermediate DBO (Whitford

2002). Hollow formation will likely be delayed in restored jarrah forest as stem densities are higher than in unmined forest, particularly in >10 year old restoration where tree densities exceed 2 500 stems ha^{-1}, compared to 1601 ± 152 stems ha^{-1} in unmined forest (J. Burgar, unpublished data). Changes to Alcoa's seeding rates and fertilisation practices mean that eucalypt stem densities in younger restoration are more comparable to unmined forest stem densities (1 484 ± 259 stems ha^{-1} in forest restored 5-9 years previously). High stem densities, coupled with the low mortality of jarrah trees (Koch & Ward 2005; Stoneman et al. 1997) imply that restored jarrah forest, particularly older sites, may not produce hollows suitable for bats for well over a century. Management actions should be taken to thin restoration to unmined forest stem densities, where necessary, and then shed and/or break limbs from jarrah and marri trees to accelerate hollow formation. As thinning is expensive and labour intensive (Stoneman et al. 1997), management actions to accelerate hollow formation could include thinning around specific trees to increase growth thereby adding spatial heterogeneity to restored mine-pits.

Emulating unmined forest tree densities and spatial patterns in restored forest is beneficial for more than just accelerating hollow formation. Bats generally prefer roost sites that are slightly open, with lower levels of surrounding vegetation than non-roost sites (e.g., Elmore et al. 2004; Vonhof & Barclay 1996; Webala et al. 2010). We predicted that bats would roost in relatively open sites with low canopy cover, and these predictions were partially met. Contrary to predictions, but similar to bats in the southern jarrah forest, neither *N. gouldi* nor *V. regulus* selected roost sites with different levels of canopy cover, compared to available sites (Webala et al. 2010). While *N. gouldi* females selected roost trees that were at, or above, canopy height, compared to available trees, which were typically below canopy height, we were unable to pinpoint exact roost entrances. Thus it is unclear if *N. gouldi* females' preferences were related to open forest structure or perhaps are an artefact of older, more mature trees being taller than the surrounding canopy. The lack of roosting preferences for overstorey cover and relatively weak preferences for canopy height may be related to the flexibility shown by bats in roost emergence time (Russo et al. 2007). Both *N. gouldi* and *V. regulus* are manoeuvrable fliers (Brigham et al. 1997; Fullard et al. 1991) and may exploit their ability to navigate in amongst dense vegetation to roost in trees sheltered from predators (Russo et al. 2007). In contrast, more open sites may allow an energetic advantage of sun-exposed roosts; both *N. gouldi* and *V. regulus* easily enter torpor and can take advantage of poorly insulated sites and sun-exposed tree roosts (Turbill 2006, 2009). Restoration (≤20 years) has substantially higher shrub cover and lower log cover than unmined forest; thus the preference for less shrub cover and higher log cover at roost, compared to available sites reinforces the importance of considering all facets of vegetation structure in restoration efforts. While coarse woody debris is manually returned to restored sites, our findings are consistent with other faunal research that suggests that the current rate of coarse woody debris in restoration may not be adequate for fauna (Christie et al. 2011; Craig et al. 2014; Koch et al. 2010), at least until restoration matures enough to produce coarse woody debris. Although burning immediately reduces the shrub layer in restoration, it also stimulates growth (Smith et al. 2004), and within five years previously burnt restored sites can be at the same vegetation density as unburnt sites (Grigg et al. 2010). Reducing shrub cover may require the active management of repeated prescribed burns, in addition to reducing the seed load of dominant shrub species in future restoration seeding mixes. Restoration should emulate the variability of vegetation surrounding roost trees in unmined forests, particularly as bats require both three-dimensional heterogeneity in vegetation structure (Adams et al. 2009).

Suitability of the restored landscape as roosting habitat

We trapped bats at five locations throughout the study area, during both the maternity and mating season, and not one bat was observed roosting in restored forest. This was despite over a third of bats presumably travelling adjacent to, or through, restored forest to reach roosting sites from trapping locations. In concordance with our predictions, roosting sites were lacking within the restoration. In eastern Australia *Nyctophilus* spp. roost under decorticating bark (Law et al. 2011; Threlfall et al. 2013) and in the southern jarrah forest *N. gouldi* was observed roosting under the dry leaves of a balga tree, in addition to tree hollows (Webala et al. 2010). As eucalypt species in the jarrah forest don't have decorticating bark, balga trees are slow-growing, and hollows can take decades to form, the absence of roosting structures in restoration was likely driving roost preferences at the landscape level. Indeed, a concurrent study investigating bat box use in the northern jarrah forest suggests that some bat species may be capable of roosting in restored forest once roost structures are available (J. Burgar, unpublished data). *N. gouldi* was recorded in bat boxes in both restored and unmined forest (*N* = 6 and *N* = 3, respectively) while *V. regulus* was only recorded in bat boxes in unmined forest (*N* = 2). As we recorded at least two *V. regulus* roosting close to the ground in unmined forest and have detected *V. regulus* echolocation calls within restored forest (J. Burgar, unpublished data) we speculate that the lack of *V. regulus* recorded in bat boxes in restoration was due to the relatively short time between box installation and monitoring (~18 months) as bat box occupancy typically increases with time since installation (Goldingay & Stevens 2009). Further monitoring of the bat boxes is necessary to definitively determine the ability of both species to roost in restoration, given the presence of suitable roosting structures.

In timber-harvested landscapes, *N. gouldi* roosted in forests logged within 10-25 years, albeit in large eucalypt trees retained during harvesting (Webala et al. 2010). In contrast, *V. regulus* avoided roosting in regrowth, preferring mature forest and/or unlogged buffers (Taylor & Savva 1988; Webala et al. 2010). In south-eastern Australia the similar sized, congeneric *V. pumilus* preferred roosting in undisturbed forest when available, but was capable of roosting in remnant, regrowth and eucalypt plantation forest when mature forest was absent (Law & Anderson 2000). Similarly, eastern *N. gouldi* and *V. pumilus* individuals roosted in *Acacia* regrowth when mature forest was absent (Law & Anderson 2000; Lunney et al. 1988). Although unburnt >15 year old restoration contains a thick senescent *Acacia* understorey (Grant 2006) it is unlikely that *Acacia* in restored jarrah forest will provide suitable roosting habitat for bats as the two predominant species used in restoration (*A. pulchella* and *A. urophylla*) are small (<4 m) pioneer species that typically live to 13 years and reach a maximum of 3 cm DBH (Maslin 2001). The *Acacias* in eastern Australia likely contained small hollows (Law & Anderson 2000) and may have been up to 30 cm and 50 cm DBH (Law & Anderson 2000; Lunney et al. 1988). The lack of roosting in restoration likely reflects the lack of roosting structures within restoration, particularly as the presence of bats in bat boxes indicates that bats are capable of roosting in restoration when suitable roosting structures are present.

Roost site fidelity

Roost switching, by both *N. gouldi* and *V. regulus*, while maintaining fidelity to a roosting area may suggest that roost availability in the northern jarrah forest is not limiting in the unmined forest. Many species of bat frequently switch roosts while maintaining fidelity to an area (Lacki et al. 2009; Law et al. 2011; Lumsden et al. 2002a; O'Keefe et al. 2009), a beneficial behaviour that increases familiarity with several roosts of potentially different microclimates and lowers both predation risk and ectoparasite loads (Lewis 1995). Within a mine-site between 40-50% of the forest is cleared (Koch 2007a), typically as 10-20 ha mine-pits (Grant 2006). Mine-pits potentially encompass entire roosting areas, particularly given that *N. gouldi* and *V. regulus* travelled an average of 218 m and 88 m between roosts, respectively. Assuming that bats travelled within a circular area, distance travelled might equate to a 15 ha and 2 ha roosting area for *N. gouldi* and *V. regulus*, respectively. These distances were substantially shorter than in the southern jarrah forest (Webala et al. 2010), but similar to distances in south-eastern Australia for *N. gouldi* (Lunney et al. 1988; Threlfall et al. 2013; Turbill 2006). Future studies are needed to accurately elucidate minimum roosting areas for bats within restored landscapes. Retention of mature forest should aim to capture enough roost trees to ensure roost area fidelity is maintained.

In addition to retaining patches of mature forest that are large enough to encompass entire roosting areas, mature forest patches should be interspersed across the landscape to reflect both intra and interspecific landscape scale roost requirements. Similar to the southern jarrah forest (Webala et al. 2010), retention of roosting habitat at lower elevations will benefit *V. regulus*, particularly males during both seasons and females during the mating season. Retaining roosting habitat at higher elevations and on relatively flat ground will benefit *N. gouldi*, particularly both sexes during the maternity season and females in both seasons. *N. gouldi* was less flexible in roosting location than *V. regulus*. *N. gouldi* prefers to roost in patches of contiguous mature forest in eastern Australia (Threlfall et al. 2013); similarly we found *N. gouldi* to prefer roosts that were farther from tracks and streams. In contrast, *V. regulus* was more tolerant of roosting near restoration, preferring roosting sites surrounded by lower proportions of unmined forest and consistently higher amounts of restoration edge. These findings may suggest that foraging resources are equally as important as roosting resources for *V. regulus*, even during the maternity season. Although we did not explicitly explore foraging resources in this study, research from the southern jarrah forest found that *V. regulus* had significantly higher feeding activity on forest tracks than off tracks (Webala et al. 2011). In addition to forest tracks, bat use of edge habitat is typically greater than use within the habitat (Jantzen & Fenton 2013; Jung & Kalko 2011). Edges may provide foraging opportunities, orientation clues and established routes that decrease commuting time to foraging grounds, and provide shelter from wind and/or predators (Verboom & Huitema 1997). While it is encouraging that *V. regulus* exploited roosting structures surrounded by restoration, the proportion of unmined forest surrounding roosts or available sites never fell below 60%, and it is unclear what either bat species can tolerate as the minimum proportion of unmined forest surrounding roosts. When retaining mature forest for conserving bat populations a landscape perspective is necessary to ensure mature forest patches are most effectively dispersed across a landscape.

Roosting and fire

The preference for *V. regulus* to use maternity roosts in more recently burnt forests, compared to random locations, and the fact that all bats roosted in unmined forest where trees are routinely subjected to fires, suggests that bats are tolerant of roosting in a fire managed landscape. One of the few studies to explicitly examine bat roosting preferences across a burned landscape found that bats were generally tolerant to fire and preferentially chose roosts in burnt areas after a prescribed fire, selecting cavities, rather than exfoliating bark (Lacki et al. 2009). Both the unmined and restored forest (>15 year old restoration) are scheduled for prescribed burns every 6-10 years as part of a regional prescribed burn management regime (Burrows 2008). While fires are only likely to cause formation of 10% of hollows (Whitford 2002), fires may assist in the formation of hollows where limbs have already been broken (Lacki et al. 2009; Whitford 2002). Our findings are consistent with research in North America, where forest-dwelling bat communities are generally resilient to fires (Buchalski et al. 2013; Lacki et al. 2009), and suggests that, at least in the jarrah forest, bats are resilient to current burning frequencies.

Conclusion

The lack of roosting in restoration underscores the importance of mature forest patches in conserving and maintaining bat populations across restored landscapes. Bats' resilience and adaptability generally make them tolerant to disturbance (Arnett 2003) and, while affected by habitat destruction, such as the loss of canopy trees, their vagility reduces the immediate impact of habitat loss for many bat species (Fenton 1998). During our study neither species was found roosting in restoration of any age (up to 35 years at Huntly minesite), despite having somewhat flexible roosting preferences and the occurrence of *N. gouldi* in bat boxes within the restored forest. The ability of restoration to provide natural roosting resources and support maternity colonies within the next few decades is unclear and requires further study, although reproductive females have been observed within plantation forests (Borkin & Parsons 2011), suggesting that reproductive females of at least some bat species may be capable of using restoration. Thus, while records of bats roosting in regrowth in other studies are encouraging (Law & Anderson 2000; Lumsden et al. 2002a), the general avoidance of restoration as roosting habitat reinforces the importance of retaining mature forest and/or large habitat trees. Equally important is the location of retained mature forest patches, as certain landscape features may benefit one bat species over another. Effective conservation of bat populations within a restored landscape requires retaining mature forest patches interspersed across the landscape.

Chapter Six: General Discussion

Bat use across a restored landscape and management implications

This thesis contributes knowledge to the understanding of habitat requirements of forest-dwelling insectivorous bats and their use of a restored landscape. It provides seminal information on how vegetation structure functions as both unidirectional and dynamic habitat filters to bat use of restoration at the community and species level. This thesis provides insight on the dietary diversity of jarrah forest bats and the role of ecomorphology in resource partitioning. It adds to the knowledge base with respect to bat foraging, explicitly examining the links between bat activity, vegetation structure and prey occurrence. Lastly, this thesis provides an understanding of the roosting requirements of two vespertilionid bats, Gould's long-eared bat *Nyctophilus gouldi* and the southern forest bat *Vespadelus regulus*, and examines the ability of restoration to provide bat roosting habitat. Overall, this thesis shows that restoration is clearly not meeting the foraging or roosting needs of all jarrah forest bats.

To improve bat use of restoration, management can employ strategies at three levels: (i) pre-mining, where there is an opportunity to identify, map and protect mature trees, both individually and in patches, at suitable spatial scales across the landscape; (ii) current restoration practices, altering practices to ensure a more representative forest structure at the outset, particularly with respect to tree densities; and (iii) post-restoration treatments, involving manipulations (i.e., thinning, burning, promoting growth of individual trees, and installation of artificial roosting structures) to existing restoration vegetation structure to increase heterogeneity and habitat diversity. This final chapter summarises and integrates the mains findings from chapters 2 through 5, focusing on the implications for maintaining and conserving bat populations in a restored landscape.

Findings from Chapter 2 corroborated earlier studies (Law et al. 1999; Webala et al. 2011), indicating that restoration was more likely to meet the habitat requirements of disturbance tolerant species (*Tadarida australis, Mormopterus kitcheneri* and *Chalinolobus gouldii*) than those considered disturbance sensitive (*C. morio*, *Falsistrellus mackenziei*, *Nyctophilus* spp. and *V. regulus*). Over the past thirty years, south-western Australia has been undergoing a drying climate (Batini 2007). This study occurred during two quite different years in terms of local climatic conditions, enabling a unique opportunity to investigate the potential for temporal fluctuations in bat use of restoration. The first field year (2010/2011) was exceptionally dry and warm, being the driest year on record (630 mm rainfall) and experiencing higher than average maximum temperatures, especially during bat survey periods (Brouwers et al. 2013). In contrast, the second field year (2011/2012) experienced average temperature and rainfall (1205 mm) conditions. Bat activity was higher in unmined forest, compared to all ages of restoration combined, for *F. mackenziei*, *Nyctophilus* spp. and *V. regulus*, and this difference was particularly pronounced for *F. mackenziei* and *V. regulus* during the first field year. All bat species, other than *C. morio*, had higher activity during the second field year, compared to the first. While difficult to disentangle the direct and indirect effect of local climatic conditions over two years, this study suggests that, overall, bats used restoration less in the dry, compared to the average, year. As south-western Australia is faced with a rapidly drying climate (Batini 2007) there is the potential that bats, particularly the disturbance sensitive species., will avoid use of restoration.

While the influence of habitat and climate on community composition is often considered separately, recent research suggests that habitat and climate work synergistically in shaping species' distributions at the macroecology scale (Barnagaud et al. 2012). This synergistic interaction affects the ability of species possessing particular traits to utilise certain habitats, effectively reducing the suitability of habitat as climate changes (Boucek & Rehage 2014; Poff et al. 2010). Assessment of undisturbed benthic communities suggest that habitat filters may vary in either magnitude or intensity depending on the influence of external factors, such as climate, leading to the expression of various community compositions (Poff et al. 2010). The interplay of habitat filters and climate in disturbed systems has not been studied despite the potential implications for conserving biodiversity at the local scale. This is of particular concern for more vulnerable species, such as *F. mackenziei,* which is endemic to the jarrah forest and listed as near threatened on the IUCN Red List of Threatened Species. Future studies are required to fully elucidate how climate may influence bat use of disturbed and restored landscapes.

I tested the hypothesis that bat succession would be congruent with vegetation succession within restoration. Contrary to predictions bat activity and community composition did not become more similar to the unmined forest state as restoration matured, despite the increasing similarity in vegetation structure between restoration and unmined forest. Although there was some support for the prediction that bat communities in densely vegetated restoration were distinct from unmined forest communities, this was not due to a reduction in activity of less manoeuvrable bat species, but rather avoidance of restoration by the most manoeuvrable bat species. Findings from Chapter 2 revealed that habitat filters to bat use of restoration exist for some, but not all, jarrah forest bat species. Similar to other fauna studies in the restored jarrah forest (Craig et al. 2012), filters were both unidirectional and dynamic. Novel to this study, dynamic filters fluctuated over both shorter (between two years at the same site) and longer (chronosequence ~10 years) timeframes. Similar to studies elsewhere, the least manoeuvrable bat species had highest activity in <5 year old restoration, i.e., structurally simple sites (Morris et al. 2010; Patriquin & Barclay 2003) and had similar activity in >5 year old restoration as unmined forest. These findings suggest an absence of habitat filters to use of restoration by large bodied, less manoeuvrable species, although tree density was the best predictor of bat activity for bat species adapted to open and open/edge environments.

Unidirectional filters were identified for the more manoeuvrable species; consistent with other studies finding that these species prefer unmined forest sites over more densely vegetated regrowth sites (Law & Chidel 2006; Webala et al. 2011). Short-term dynamic filters were apparent at the community level as bat community composition differed between years in young (<5 year old) and older (>15 year old) restoration. However, dynamic filters were apparent only for two species and both at longer-term timescales. *Nyctophilus* spp. avoided <5 and 10-14 year old restoration while *M. kitcheneri* avoided >15 year old restoration. Despite clearly confirming the presence of filters, identification of specific habitat filters for individual species was less clear. Filters were complex and the cumulative effects of multiple habitat features were likely more influential on bat activity than one specific habitat feature. The temporal shift in some species use of restoration, i.e., the short-term dynamism of habitat filters, reinforces the importance of multi-year surveys, particularly years experiencing different climatic conditions, and agrees with previous research suggesting that habitat filters may intensify during stressful climatic conditions (Poff 1997). Future, preferably multi-year, studies are required to elucidate specific habitat filters to bat use of restoration and better understand both shorter and longer timeframe dynamic filters.

Chapters 3 through 5 continued to support the hypothesis that restoration would meet some, but not all, jarrah forest bat habitat requirements. In particular, Chapter 3 showed that dietary partitioning did occur in three sympatric bat species (*C. gouldii, N. gouldi,* and *V. regulus*) and this divergence was related to ecomorphology. *C. gouldii* has similar aspect ratio but higher wing loading, greater mass and longer forearms than either *N. gouldi* or *V. regulus* and thus is the least manoeuvrable of the three bat species, foraging in open and edge environments (Fullard et al. 1991). While *N. gouldi* and *V. regulus* share similar aspect ratio and wing loading values, *V. regulus* is considerably smaller, averaging half the mass and two-thirds the forearm length of *N. gouldi*, the intermediate sized of the three bat species. *N. gouldi* and *V. regulus* both forage within closed and edge environments although *V. regulus* is considered more adept at navigating the closed environment (Fullard et al. 1991). But wing morphology and manoeuvrability are not the only factors driving dietary partitioning (Aldridge & Rautenbach 1987). Echolocation call parameters and echo-processing abilities influence bat species' habitat use and foraging abilities (Siemers & Schnitzler 2004). The incredible diversity and plasticity of echolocation call structure suggests that habitat has been extremely influential in shaping call structure (Jones & Teeling 2006). Typical *C. gouldii* echolocation calls are of relatively long duration (>5 ms), low characteristic frequency (29 kHz) and shallow slope (52). In contrast both *N. gouldi* and *V. regulus* have calls of shorter duration, higher characteristic frequency, and steeper slopes (*N. gouldi* 3 ms, 48 kHz, 223; and *V. regulus* 4 ms, 43 kHz, 59). Similar to other species (Broders et al. 2004; Petrites et al. 2009), *V. regulus* alters echolocation call structure depending on the habitat, with steeper calls that begin to emulate *N. gouldi* calls in more densely vegetated environments, such as dense restoration (J. Burgar, pers obs.). Unsurprisingly, the more ecomorphologically distinct species (*C. gouldii* and *N. gouldi*) had the greatest divergence in diet diversity whereas *V. regulus* had an intermediate level of divergence in diet diversity, compared to the other two species. All three bat species are aerial foragers (Fullard et al. 1991), taking prey while in flight but *N. gouldi* also gleans prey, taking stationary prey from surfaces of vegetation or the ground (Schnitzler & Kalko 2001). Thus *C. gouldii* and *V. regulus* are more constrained by the size of their prey than *N. gouldi*, as bats typically can only take small (<5 % of bat's mass) airborne prey but much larger (>10% of bat's mass) non-airborne prey (Fenton 1990). The greater breadth of prey diversity seen in *N. gouldi* is consistent with the ability of this bat to employ the two foraging strategies. *N. gouldi* may have an advantage foraging in densely vegetated restoration, compared to *C. gouldii*, which lacks the ability to manoeuvre through dense vegetation, and *V. regulus,* which is constrained by prey size and limited to aerial foraging.

Chapter 4 built on this knowledge by explicitly examining the link between bat activity (as a proxy for foraging activity), vegetation structure and prey occurrence. The highly speciose (Abbott 1995), yet genetically and taxonomically uncharacterised, invertebrate fauna limited full integration of findings between Chapters 3 and 4. Only a small handful of the 21,000 Australian moths have been barcoded (Zborowski & Storey 2010), despite a worldwide Lepidoptera barcoding campaign (Jinbo et al. 2011). As genetic reference databases improve, and prey items become better known taxonomically, molecular diet studies will become more powerful. In situations where the aim is to elucidate the consumption of specific species, comprehensive genetic reference databases are essential (e.g., Alberdi et al. 2012). Until then, future molecular bat diet studies should genetically analyse representatives of invertebrates from taxonomically known species (or genus and order) caught within the jarrah forest to compare DNA sequences from the representative invertebrates with those found within bat diet. Despite being severely limited in the ability to identify invertebrate specimens to species, findings from Chapter 4 found that prey occurrence did not equate to prey accessibility for all bat species. Similar to studies elsewhere on ecomorphology and stratification of bat forest use (e.g., Adams et al. 2009), activity of edge foraging bat species was related to the synergistic effect of vegetation structure and insect biomass. The influence of prey biomass was less influential on *C. gouldii* activity as canopy cover increased, supporting previous findings that this bat species preferentially forages in edge and open environments (Fullard et al. 1991).

Nyctophilus spp. activity was positively associated with the combined influence of ground cover and prey biomass, consistent with behavioural studies observing this gleaning bat taking prey from the ground (Brigham et al. 1997; Grant 1991).

Results from the two chapters corroborated one another and clearly indicate niche partitioning across a restored landscape. The fact that there was some overlap in diet is consistent with all bats opportunistically foraging in the edge environment and being aerial foragers (Fullard et al. 1991). *C. gouldii* likely forages on large prey (up to 0.75 g) in both edge (the interface between different ages of restoration as well as between restored and unmined forest) and open (<5 year old restoration and above the canopy of both restored and unmined forest) environments. *V. regulus* likely forages on small prey (up to 0.25 g) in both the edge and closed (unmined and restored forest, particularly dense 5-9 and >15 year old restoration) environment. Whispering bats, such as *Nyctophilus* spp. emit such low amplitude feeding buzzes that they are rarely recorded (Schnitzler et al. 2003), which may explain the lack of *Nyctophilus spp.* feeding buzzes detected. However, as *N. gouldi* generally flies between 2-5 m above the ground in amongst vegetation (Brigham et al. 1997), it is plausible to assume that in the jarrah forest *N. gouldi* forages on medium sized airborne prey (up to 0.45 g) and gleans large prey (over 1.0 g) in both edge and closed environments. Results from Chapter 4 suggest that although the more manoeuvrable bat species are capable of foraging within restoration, they avoid restoration in favour of unmined forest. Similar to bats elsewhere, jarrah forest bats generally avoid foraging within densely vegetated restoration unless it provides an energetic benefit (Sleep & Brigham 2003).

Bats adapted for navigating densely vegetated environments are capable of using less vegetated environments but the move from one habitat type to another is unidirectional (Schnitzler et al. 2003). Unsurprisingly, overall bat use of edge environments is higher than use of either open or closed environments, regardless of ecomorphology (Jantzen & Fenton 2013). Increased edge use may ameliorate the loss of foraging habitat as unmined forest is cleared and replaced with restored forest. This study was particularly interested in the impact of restoration on bat habitat use so pointedly avoided echolocation call surveys along edge habitat, instead focusing on areas within restoration and unmined forest >80 m from the edge. However, studies in the southern jarrah forest that explicitly examined the influence of edge habitat, in the way of forest tracks, found consistently higher activity levels on tracks than within regrowth for all bat species (Webala et al. 2011). Between 40-50% of the forest within a mine-site is cleared and restored (Koch 2007a) and, due to the demand for particular bauxite ore grades, this results in a patchwork mosaic of various aged restored mine-pits (~2-30 ha) interspersed across the landscape (Grant 2006). The restored landscape provides ample edge habitat for foraging bats. In addition, the jarrah forest contains numerous forest tracks, some maintained as part of the mining process but others maintained by the state government as part of their management for timber harvesting, wildfire risk, recreation and potable water (Gardner & Bell 2007). Thus, while jarrah forest bats generally avoid foraging within restoration, they likely exploit restoration edges and forest tracks, potentially ameliorating some of the negative impact of mining and restoration on foraging bats. Indeed, results from Chapter 5 found that both *N. gouldi* and *V. regulus* preferentially select roosts with more surrounding edge habitat within a 1000 m and 3000 m area than random locations, suggesting that some restoration edge may be beneficial to jarrah forest bats. Future studies examining bat use of forest tracks and restoration edge will assist managers tasked with ensuring restored landscapes provide connectivity and foraging resources for bats.

While some restoration edge may be beneficial, current restoration provides unsuitable roosting habitat for at least two jarrah forest bat species. The final part of this study examined the roosting preferences of *N. gouldi* and *V. regulus*, during both the maternity and mating season. Similar to other studies, jarrah forest bats preferred roosting in mature, tall trees in intermediate to late stages of decay (Kalcounis-Ruppell et al. 2005; Lumsden et al. 2002b; Vonhof & Gwilliam 2007). These attributes generally coincide with peak hollow abundance in eucalypts (Rayner et al. 2013; Whitford 2002) and likely explain why bats solely roosted in unmined forest during this study. The straight-line distances and paths travelled by both species from capture site to first roosting site suggest that bats were presumably traveling through, or adjacent to, restoration but preferentially chose to roost in unmined forest. A concurrent bat box study within Huntly minesite found that *N. gouldi* roosted in bat boxes in both unmined and restored forest (J. Burgar, unpublished data), supporting the conclusion that lack of roosting structures (i.e., hollows) in restoration is limiting its suitability as roosting habitat. Roost fidelity to an area for both species necessitates the retention of patches of mature forest, preferably encompassing known roost trees, to ensure roosting habitat is available across the restored landscape, while waiting for restoration to provide roosting habitat. Average mine-pits (10-20 ha; Grant 2006) likely encompass entire roosting areas. Given that *V. regulus* and *N. gouldi* travel an average of 88 m and 218 m between roosts, and assuming that bats travel between roosts in a circular area, this roughly equates to 2 ha and 15 ha roosting areas, respectively. To ensure suitable roosting habitat remains within the northern jarrah forest, mining practices should maintain current practices of only clearing 40-50% of the landscape within a minesite (Koch 2007a). This is of particular importance as the proportion of unmined forest within 250 m of roosts never fell below 60% for either species and natural hollow formation in the restored jarrah forest is likely to take close to a century (Whitford 2002).

Spatial distribution of unmined forest within the landscape is also important for roosting bats. Results from Chapter 2 confirm that bats travel through restoration and research from the southern jarrah forest indicates that bats will use forest tracks for commuting (Webala et al. 2011). In this study *N. gouldi* and *V. regulus* travelled an average of 1218 m and 547 m, respectively, from foraging (i.e., capture) to roosting sites. If a greater proportion of unmined forest is cleared and restored, bat boxes might be a possible solution to accelerate bat use of restoration for roosting. Bat preferences for wood, rather than pipe, bat boxes (J. Burgar, unpublished data) suggests that use of bat boxes should be a last resort as wood boxes will likely need to be replaced every 10-15 years. To ensure all jarrah forest bats are able to access adequate foraging and roosting habitat, small patches (2 ha) of unmined forest should not be separated by more than 500 m from other unmined forest patches unless connected by small (<6 m) forest tracks. Future studies tracking individual bats over a longer period and during nightly foraging bouts are needed to provide a more definitive understanding of use of a restored landscape by both roosting and foraging bats (e.g., Law et al. 2011).

Retention of as few as one mature tree ha^{-1} may increase bat use of young restoration (<5 years old) as foraging habitat and provide roosting opportunities for large bodied, less manoeuvrable bats. Studies from agricultural landscapes in eastern Australia found that there was significantly higher bat activity in open paddocks that had retained at least one mature tree, compared to treeless paddocks, nearly reaching activity levels within remnant forest (Fischer et al. 2010; Lumsden & Bennett 2005). Furthermore, in eucalypt plantations within an agricultural landscape, total bat activity was positively correlated to the number of remnant trees within a site (Law et al. 2011). In addition to providing foraging habitat, retaining mature tree(s) in mine-pits may also provide roosting habitat for species adapted for fast flight in open environments, such as *T. australis*. This species appeared unaffected by restoration in this study, although this may be a consequence of the patchwork mosaic of the restored landscape and the ability of this species to travel long distances, above the canopy, from foraging to roosting sites. Few forest roosting studies exist for *T. australis*, but research from suburban Brisbane suggests that *T. australis* adapted to urban life by commuting over 6000 m from diurnal roosts to nightly foraging grounds above a floodplain (Rhodes 2008) and routinely travelled ~500-5000 m between roost sites (Rhodes 2007). *T. australis* roosted in eucalypt trees in later stages of decay, averaging ~80 cm DBH, although this size was no different from available trees (Rhodes & Wardell-Johnson 2006). These findings suggest that *T. australis* is unlikely to roost in restoration but retention of mature trees, even at intervals of one per mine-pit or perhaps on the edge of mine-pits, may provide *T. australis* with adequate roosting opportunities. However, it may not be practical to retain only one tree within a mine-pit due to the depth of mining and the need to landscape restored areas into the surrounding forest (Koch 2007a), as well as the potentially reduced survivorship of such an isolated tree in the long-term. In addition, it is unknown whether *T. australis* would use restoration as roosting habitat as this species was not tracked to diurnal roosting sites as part of this study. Thus, it is crucial that unmined forest patches are retained, encompassing a minimum of one and, mature, hollow-bearing tree.

There are currently >15,000 ha of restored jarrah forest and within a minesite~40-50% of the forest is been cleared (Koch 2007a), thus management interventions are necessary to accelerate bat use of existing restoration. Thinning and burning are two strategies that have been employed within restored jarrah forests, successfully accelerating the use of restoration by reptiles, at least within the first few years following fire (Craig et al. 2010). Within the timber-harvested landscape, thinning of natural forests typically accelerates use by bats adapted for open and edge environments (Morris et al. 2010; Patriquin & Barclay 2003). However, in the southern jarrah forest logging reduced foliage gleaning insectivore bird species richness (Craig & Roberts 2005). Thus, thinning may be detrimental to gleaning bats, such as *Nyctophilus* spp. in the northern jarrah forest. In North America, thinning reduced both the canopy and shrub vegetation and resulted in comparable bat activity between thinned and remnant forest sites, both of which had higher activity than unthinned forest (Humes et al. 1999). Thinning can be both expensive and labour intensive thus, while beneficial for bats if both strategies are employed, burning may be practiced in conjunction with, or in place of, thinning.

Low-intensity fires promote vertical stratification of vegetation structure by reducing the shrub and ground vegetation within restoration (Grant 2006), particularly important for improving the edge space for foraging bats (Adams et al. 2009). Northern jarrah forest bird communities show a temporary response to fire in both unmined and restored jarrah forest, initially showing a reduction in species richness and abundance but rebounding to pre-burn levels six years post-burn (Nichols & Grant 2007). This temporary response parallels the temporary influence of thinning and burning on vegetation structure. Within five years of burning 10-13 year old restoration fuel loads were nearly comparable to pre-burn levels, primarily due to dense understorey regrowth (Grigg et al. 2010). Restoration is not incorporated into regional prescribed burn practices until it is at least eight years old (Grigg et al. 2010). The intervals between fires will also influence the ability of bats to forage within restoration as the removal of dense shrub vegetation can alter insect prey successional patterns (Armitage & Ober 2012). Management plan burn rotations aim to burn sites every 6-10 years (Burrows 2008), while avoiding burns during hot, dry conditions. Thus the time between fires is dependent on local weather conditions and site productivity (Burrows 2008); recent hot, dry conditions have extended the time between fires in recent years. Rotations longer than 12-15 years are not recommended in restoration as a thick *Acacia* understorey substantially builds fuel loads by 15 years (Grant et al. 1997). Burning 1-2 years pre-thinning might be the most beneficial strategy to accelerate bat use of restoration as this technique minimises the ability of reseeder understorey establishment (Grigg et al. 2010).

Not only can fire temporarily increase foraging and/or commuting space available to bats within restoration, fire may also add to roost availability by enhancing hollow development. Although only ~10% of jarrah and marri hollows are caused by fire, trees with a visible fire scar contained twice as many hollows as trees without a fire scar (Whitford 2002), suggesting that fires enhance the development of tree hollows. The effects of thinning and/or low-severity burning may be positive at both the local and landscape scale, particularly as thinning and burning of many different areas results in a patchy landscape. By increasing the structural heterogeneity of restoration, these actions are likely to benefit both bat occurrence and activity levels (Jung et al. 2012). From studies of bats elsewhere (Lacki et al. 2009; Loeb & Waldrop 2008; Morris et al. 2010; Patriquin & Barclay 2003), and fauna within the jarrah forest (Craig et al. 2010; Nichols & Grant 2007), it is likely that thinning, in combination with low-severity fires on a 12-15 year rotational burn will benefit jarrah forest fauna, including bats. However it is important to ensure adequate roosting structures remain post-burning. In savanna-woodland systems, thinning and burning was a positive management strategy for aerial insectivorous birds but limited use of the landscape by cavity-nesting birds (Mabry et al. 2010). Future studies examining bat use of restoration pre and post thinning and/or burning will clarify the usefulness of these management strategies in conserving bat populations.

Another management option to accelerate the suitability of restoration as bat habitat is to isolate individual trees by clearing adjacent trees. Removing adjacent trees allows increased water uptake and growth of the remaining tree (Stoneman et al. 1997). This may be particularly useful in restoration with relatively high tree density, compared to unmined forest (Grant 2006), particularly as jarrah has low mortality and thus low capacity to self-thin (Norman et al. 2006; Stoneman et al. 1997). Improving growth rates of individual trees would result in structural heterogeneity in vegetation structure and vertical stratification at the local scale, attributes beneficial to bats by increasing available commuting/foraging space (Adams et al. 2009; Jung et al. 2012). Revegetated landscapes with high tree densities generally delay the provision of habitat resources such as large boughs, tree hollows and fallen timber (Vesk et al. 2008). Hollows are important for bat roosting while fallen timber may provide foraging resources. Isolating and increasing the growth of individual trees could ameliorate some of these negative effects and potentially accelerate the use of restoration as roosting and/or foraging habitat for bats. Once jarrah and marri trees reach ~18 m in height and have a DBH of ~50 cm, they are estimated to contain at least one hollow (Whitford 2002). This study found that trees in >15 year old restoration were ~15 m tall and ~24 cm DBH (J. Burgar, unpublished data), and thus are unlikely to contain hollows. However, management manipulations may be employed to accelerate hollow formation by breaking limbs from trees, the leading cause of hollow formation in jarrah and marri trees (Whitford 2002). Promoting the growth of individual trees within restoration, by clearing adjacent trees, and then returning to break limbs once the trees are ~ 15 years old has the potential to improve both the foraging and roosting habitat of restoration by increasing structural heterogeneity and accelerating hollow development. However, accessing >15 year old restored forest can be difficult and expensive so may only be practical in some situations. An more cost-effective and feasible strategy could be employed at the edge of each new pit where mining equipment used in pits during clearing could be taken advantage of to break the limbs of trees (that lack hollows) that are left along the mined forest edge.

This study clearly shows that restoration does not meet the needs of all bat species, either in terms of foraging or roosting habitat. The lack of roosting in restoration underscores the importance of mature forest patches for conserving and maintaining bat populations across restored landscapes. This study did not explicitly explore the spatial distribution of restoration across the landscape and so it is not clear if the current patchwork of restored and unmined forest is sufficient for maintaining bat populations. Future bat roosting preference research is needed, particularly on the requirements of the endemic jarrah forest bat species. It is unlikely that restoration will provide roosting habitat for decades, or even a century. Older (>10 years) restoration is already providing foraging habitat for some species and may be suitable for all species within much shorter time frames than required for roosting. While management strategies may accelerate bat use of restoration as both foraging and roosting habitat, retention of mature forest patches is imperative to ensure roosting habitat is maintained in the interim. Across the restored landscape, management recommendations are to (i) retain mature patches of forest, interspersed across the landscape at ~500 m to 1000 m intervals; (ii) conduct roosting surveys prior to clearing to ensure retained mature patches contain roosting habitat, i.e., mature hollow-bearing trees; (iii) continue current practices of integrating >8 year old restoration with the prescribed burn schedule, on a 6-10 year rotation; (iv) promote growth of individual trees within existing restoration by clearing adjacent trees, preferably at ~100 m spacing if the aim is to accelerate roosting habitat; and (v) break limbs of mature trees along the edge of mine-pits during the clearing process. Even when restoration is considered a success in terms of vegetation, faunal use of restoration may be limiting; if restoration is to effectively conserve biodiversity, it must meet the needs of all species, including bats.

Appendix One: Bat Call Automation

A comparison of automated techniques and manual identification: the importance of validating classification models using field generated data.

Introduction

Effective conservation and management of wildlife requires monitoring population trends, both spatially and temporally, but this can be especially difficult for cryptic or nocturnal species. Bioacoustics surveys enable nocturnal, cryptic species that emit sounds to be more effectively monitored (Mellinger et al. 2007; Stahlschmidt & Bruhl 2012) and, increasingly, passive bioacoustics surveys are used to estimate animal population abundances, particularly for cetaceans, birds and bats (e.g., Dawson & Efford 2009; Marques et al. 2013). In contrast to cryptic vocal birds, where point-count field based surveys may provide similar findings to automated methods of recording bioacoustics signals (Digby et al. 2013), the nocturnal nature, and ultrasonic echolocation calls, of most bat species essentially limit surveys to automated acoustic monitoring, particularly for populations not roosting in caves. Passive automated acoustic surveys are currently the main data collection approach to answer fundamental ecological questions related to echolocating bats (e.g., Buchalski et al. 2013; Frey-Ehrenbold et al. 2013; Hanspach et al. 2012; Luck et al. 2013).

Recently, Stahlschmidt and Bruhl (2012) recommended standardized acoustic bat surveys comprise several automated, stationary sampling systems. The benefit of such an approach is the ability to repeatedly, and objectively, survey bats at the landscape scale (Jones et al. 2000; Skowronski & Harris 2006), which is excellent for gathering extensive datasets through long-term monitoring of bat populations. The quantitative use of statistics for automated bat echolocation call analysis has been challenged in the past (e.g., O'Farrell et al. 1999b) as factors, such as geography and habitat type, can produce intraspecific variation (e.g., Chen et al. 2009; Petrites et al. 2009; Veselka et al. 2013). The enormous datasets currently being generated from acoustic surveys is necessitating automation of bat echolocation call analyses (Adams et al. 2010). Indeed automation of the analysis of data from bat acoustic surveys, through modelling to classify bat calls to species, has been increasing with a number of papers devoted to different modelling techniques (e.g., Adams et al. 2010; Britzke et al. 2011; Ross & Allen 2014).

Parametric models are the conventional method for automating the analysis of bioacoustics bat survey data but non-parametric models are increasingly popular (e.g., Parsons & Jones 2000; Preatoni et al. 2005; Ross & Allen 2014; Vaughan et al. 1997). There has been a recent spate of studies comparing parametric and non-parametric model performance, particularly discriminant function analysis with artificial neural networks (Armitage & Ober 2010; Britzke et al. 2011; Jennings et al. 2008; Parsons 2001; Parsons & Jones 2000; Preatoni et al. 2005; Redgwell et al. 2009), support vector machines (Armitage & Ober 2010; Redgwell et al. 2009), *k*-Nearest neighbour (Britzke et al. 2011), classification trees (Adams et al. 2010; Britzke et al. 2011; Preatoni et al. 2005) and machine learning (Skowronski & Harris 2006). These studies have generally concluded that non-parametric models are superior to parametric models because they are flexible, able to deal with nonlinear boundaries between classes, and are relatively robust to both overfitting and a large set of parameters (Cortes & Vapnik 1995; McLachlan 2012). Despite the apparent advantages of non-parametric models, there is no consensus on which model, or set of models, perform best. This may be due to variations in model performance between different species; performance is often species dependent with models capable of correctly classifying >90% of most species but then dropping to 80% or even <50% for other species (e.g., Britzke et al. 2011; Obrist & Boesch 2004). Researchers often deal with this by training and testing models using reference libraries that group calls at both the species and genus level; the more “problematic” species within a genus, such as *Myotis* spp. whose echolocation call characteristics typically overlap (Britzke et al. 2013), are often grouped (e.g., Adams et al. 2010; Armitage & Ober 2010; Redgwell et al. 2009). There is also the concern of type I errors in models that classify each call to a species without the ability to designate calls classified with low certainty as unknown (but see Adams et al. 2010).

While there may not be a general consensus on a single best model for automated acoustic identification, studies routinely advocate the use of models to automate bat call identification in large scale acoustic surveys while acknowledging that field recordings produce calls of much lower quality than calls used in model development (e.g., Adams et al. 2010; Redgwell et al. 2009). There is a general dearth of literature comparing species identification using automated techniques compared to manual identification of bat calls, particularly for calls recording from passive field surveys (but see Jennings et al. 2008). Comparisons are more common in other bioacoustics fields; cetacean research tends to use complimentary visual and acoustic survey methods (e.g., Akamatsu et al. 2008; Barlow & Taylor 2005) and one recent nocturnal bird study compared field-listening surveys to automated methods (Digby et al. 2013). The cetacean and bird research suggests that automation techniques are consistent with manual identification. However, prior to widespread use of automated models for bat call classification of acoustic survey data, there needs to be a comparison of model performance with manual identification using field data collected from acoustic surveys. This is particularly important for studies comparing bat calls across habitat types as bats can alter their echolocation calls depending on their environment (Schnitzler et al. 2003) and reference call libraries rarely contain the fragmentary calls typically associated with dense habitats (Broders et al. 2004; O'Farrell et al. 1999a). Without model validation beyond the training library the accuracy of predictions on field data is likely much lower than commonly reported for acoustic surveys (Clement et al. 2014).

The objectives of this study were to (i) train six classification models to automate bat call species identification using acoustic parameters extracted from recording software with minimal user input; and to compare the accuracy of the six models using (ii) a test set of calls from a regional call reference library; and (iii) manually identified calls from a passive acoustic monitoring study covering various habitat types.

Materials and methods

Data collection

From 2007 to 2011 the echolocation calls of nine bat species (Table 1) were recorded in the jarrah (*Eucalyptus marginata*) and karri (*E. diversicolor*) forests of south-western Australia. Calls were either collected by the authors or donated by colleagues (see Acknowledgements) and were recorded using frequency-division Anabat SDI bat echolocation call detectors (Titley Electronics, NSW Australia). Frequency division detectors record all frequencies of echolocation calls and use zero-crossing analysis where every *n*th sound wave is sampled, preserving the structure of the sound wave but not capturing harmonic or amplitude information (Armitage & Ober 2010; Britzke et al. 2013).

Table 1. Total number of calls, call sequences, and mean (± standard error) number of calls per call sequence for each class. Reference calls were gathered from a variety of locations over multiple nights so we assumed one call sequence represents one individual. Nomenclature follows Armstrong and Reardon (2006), except for *Nyctophilus major* (Parnaby 2009) and *Mormopterus kitcheneri* (Reardon et al. 2014). The grey line provides the grouped data for *Nyctophilus* spp.

Class	Total Call Sequences	Total Calls	Calls per Call Sequence Mean ± SE
Chalinolobus gouldii	20	1722	86.1 ± 8.9
C. morio	18	993	55.2 ± 8.0
Falsistrellus mackenziei	19	1207	63.5 ± 5.4
Mormopterus kitcheneri	6	202	33.7 ± 12.2
Nyctophilus geoffroyi	6	126	21 ± 5.6
N. gouldi	15	429	28.6 ± 6.1
N. major	3	98	49.0 ± 23.2
Nyctophilus spp.	24	653	27.2 ± 4.4
Tadarida australis	8	249	31.1 ± 2.0
Vespadelus regulus	37	2046	55.3 ± 4.5
Noise	10	413	75.1 ± 38.7

We defined a call as a single sound emission, or pulse, and a call sequence as comprising a set of calls emitted by an individual bat (Fenton 1999; O'Farrell et al. 1999b). We conservatively considered a call sequence contained within an individual 15 second Anabat file as belonging to the same individual, similar to other bat call automation studies (e.g., Adams et al. 2010). After calls were recorded they were downloaded and processed in AnaLook 3.8 software. We targeted search-phase navigation calls by running all downloaded call files through a filter in AnaLook, which was also set to remove extraneous noise and ensure objective call quality. As different filters can vary in their ability to exclude non-bat noises (Clement et al. 2014) we tested the performance of a variety of filter parameters before selecting the final parameters: smoothness of 50; body over of 1000 ms; characteristic frequency between eight and 100 kHz; and call duration between 1.5 and 100 ms. For each filtered call we used the built-in capabilities of AnaLook to automatically extract 13 parameters (Figure 1; Table 2): the time between two successive calls (TBC) and 12 features describing the shape of each call: characteristic frequency (Fc), maximum frequency (Fmax), minimum frequency (Fmin), mean frequency (Fmean), frequency at the knee of the call (Fk), call duration (Dur), time until the end of the characteristic slope (Tc), time until knee (Tk), duration of the body of the call (Dc; Dc = Tc-Tk), characteristic slope (Sc), slope at initial part of call (S1), and quality of the knee (Qk).

We created a reference library of calls recorded primarily during hand release but also through visual identification, particularly for *Tadarida australis*. Echolocation calls are highly variable, both within and between individuals (Broders et al. 2004; Fornůsková et al. 2014; O'Farrell et al. 1999a), so we maximised call variability within the library by including calls from males, females, and juveniles (Britzke et al. 2011). In addition, we included all filtered pulses within a

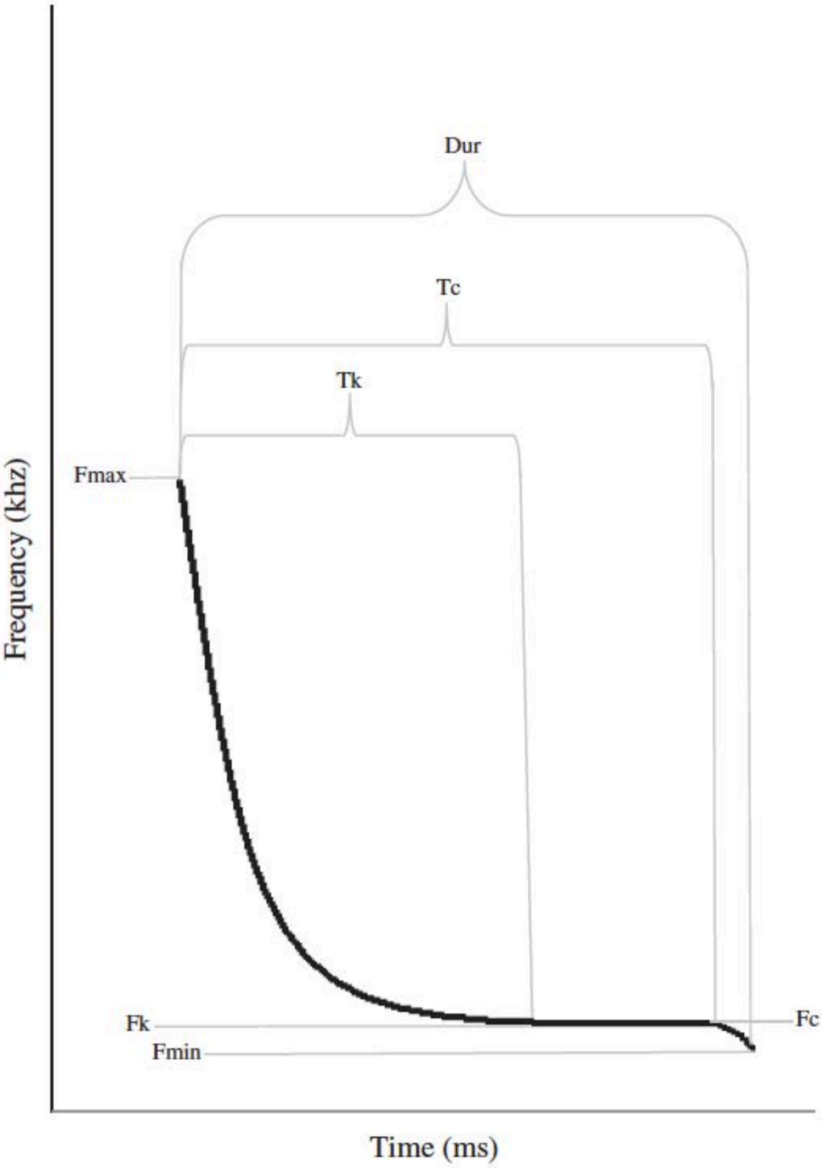


Figure 1. The call features (i.e., parameters) that comprise a bat call (thick black line) (Armitage & Ober 2010). Frequency parameters (Fc: characteristic frequency; Fmax: maximum frequency; Fmin: minimum frequency; Fk: frequency at knee) are measured in kilohertz (kHz) and duration/time parameters (Dc: duration of body of call; Dur: duration; Tc: time until end of characteristic slope; Tk: time until knee; TBC: time between successive calls) are measured in milliseconds (ms).

Table 2. Call parameter values (mean ± SE) for each class, derived from the reference call library. See Figure 1 for description of frequency parameters (measured in kilohertz, kHz) and duration/time parameters (measured in milliseconds, ms). TBC is the time between successive calls, measured in ms. The remaining parameters (Sc: characteristic slope; S1: initial slope; Qk: quality at the knee) are calculated and have no units.

	C. gouldii	*C. morio*	*F. mackenziei*	*M. kitcheneri*	*N. geoffroyi*	*N. gouldi*	*N. major*	*T. australis*	*V. regulus*
Fc	29.06±0.11	49.11±0.05	33.60±0.13	26.90±0.14	41.32±0.36	48.25±0.48	40.58±0.68	12.48±0.08	42.87±0.05
Fmax	41.04±0.28	66.50±0.44	49.39±0.39	34.04±0.34	61.36±0.75	71.01±0.79	65.47±1.23	18.81±0.14	62.83±0.29
Fmin	28.14±0.11	48.22±0.05	32.61±0.13	26.47±0.13	40.28±0.34	43.61±0.35	37.89±0.63	12.25±0.08	41.93±0.06
Fmean	31.12±0.13	53.21±0.13	37.28±0.16	29.08±0.19	48.09±0.39	55.13±0.50	48.73±0.66	14.02±0.08	46.90±0.08
Fk	30.52±0.12	52.63±0.07	36.09±0.15	29.52±0.19	48.43±0.42	55.51±0.57	46.89±0.71	13.78±0.09	45.49±0.07
Dc	2.59±0.04	2.02±0.02	2.40±0.04	2.66±0.19	1.40±0.06	1.20±0.04	1.29±0.09	4.53±0.11	1.84±0.02
Dur	5.46±0.07	3.53±0.04	5.91±0.09	4.15±0.21	2.80±0.09	3.00±0.07	3.41±0.17	10.64±0.23	3.97±0.04
Tc	4.71±0.06	3.28±0.03	5.15±0.08	3.88±0.21	2.58±0.09	2.51±0.07	3.06±0.17	9.49±0.21	3.59±0.03
Tk	2.12±0.03	1.26±0.02	2.75±0.06	1.22±0.05	1.18±0.06	1.31±0.05	1.77±0.13	4.96±0.14	1.75±0.02
Sc	51.77±2.66	62.34±2.14	52.24±2.31	95.87±4.83	176.14±4.5	222.73±7.82	206.29±9.98	33.69±1.25	59.24±1.55
S1	367.9±16.0	418.7±14.8	196.1±20.0	218.1±13.8	286.6±56.8	316.1±31.1	346.0±50.5	183.1±3.5	517.4±12.1
Qk	10.55±0.24	7.29±0.19	9.00±0.25	3.37±0.15	4.63±0.25	3.61±0.18	4.96±0.34	9.5±0.28	10.87±0.17
TBC	121.9±3.6	152.0±15.8	110.7±3.9	211.2±19.8	122.4±20.7	184.2±24.2	103.2±21.6	429.5±13.3	133.6±7.0

call sequence, but excluded call sequences with less than five calls from analyses (Britzke et al. 2011). Previous studies suggest there is no reason to limit bat call classification training datasets (e.g., Armitage & Ober 2010; Britzke et al. 2011) so we included all call sequences recorded for each species that fit the above criteria (Table 1). We acknowledge that initial echolocation calls obtained through hand release are typically distress calls and fragmentary, not necessarily indicative of free-flying calls (Britzke et al. 2013; O'Farrell et al. 1999b). However, we feel justified in using hand release calls as this is the standard method for obtaining voucher calls for reference libraries (Parsons & Szewczak 2009) and because we applied a filter that minimised inclusion of fragmentary calls. In addition, these initial high bandwidth calls (Britzke et al. 2013) may be similar to how bats alter the structure of their calls in densely vegetated habitats (Broders et al. 2004), often recorded during passive acoustic monitoring surveys.

Echolocation calls recorded in the passive acoustic survey were part of a larger study assessing bat use of unmined, and post-mining restored, jarrah forest (Chapter 2). We recorded calls in both unmined and restored forest of various ages (restored (R) age class: 0-4, 5-9, 10-14, >15) and tree densities (desirable: 500-2500 eucalypt stems ha^{-1}; dense: >2500 eucalypt stems ha^{-1}). We surveyed eight sites within eight treatment types for a total of 64 sites: R0-4 desirable, R5-9 desirable, R5-9 dense, R10-14 desirable, R10-14 dense, R>15 desirable, R>15 dense, and unmined. Each site was surveyed four times between October 2010 and March 2011 for a total of 256 survey nights. Anabat detectors were placed on PVC poles 1.5 m above the ground, oriented at a 45° angle, and directed towards a gap in the vegetation to minimise call attenuation (Law et al. 2011). One detector was deployed per site with eight sites surveyed each night and detectors were randomly rotated between sites to minimise potential nightly variation between treatment types. Detectors were calibrated at the start of the survey using an ultrasonic signal source, Anabat Chirper I (Titley Electronics, NSW Australia), to standardise detector sensitivity (Larson & Hayes 2000).

Data preparation

For all data preparation and analysis we used the caret package (Kuhn 2008) within R (R Core Team 2013). Within the extracted call parameters we identified two highly correlated subsets ($r > 0.75$, $p < 0.001$), comprising frequency (Fc, Fmax, Fmin, Fmean, and Fk) and duration (Dur, Dc, Tk, and Tc) parameters. We ran two sets of principal component analysis (PCA), derived from the frequency and duration parameters, to transform the respective correlated parameters into orthogonal variables that retained the information held by the original parameters (Britzke et al. 2011). We extracted five parameters from the frequency PCA and four parameters from the duration PCA. To test if the PCA parameters were more useful in classifying bat calls than the original correlated parameters we ran the classification models first using the original 13 parameters and then using the nine orthogonal PCA variables (five frequency and four duration) plus the remaining four original parameters (Sc, S1, Qk, and TBC,). We checked all parameters to ensure near-zero predictors were not present, as these can cause models to fail, particularly when using likelihood-based estimators such as linear discriminant function analysis (Kuhn 2008).

Acoustic identification studies using zero-crossing analysis files typically pre-process the data by centring and scaling the call parameters (e.g., Armitage & Ober 2010; Britzke et al. 2011). However, in our study area, the range of values for some parameters was quite small (Table 2) and subtle differences between species might be missed if parameters were pre-processed. Thus, we determined the effect of pre-processing on model performance by running models with and without pre-processing. Pre-processing involved centring each parameter on zero by subtracting the column mean from each observation and scaling by dividing each centred value by the column's standard deviation (Kuhn 2008). Echolocation calls of three bat species (*Nyctophilus* spp.) in the study area are considered indistinguishable when recorded using frequency division detectors (Adams et al. 2010; Bullen & McKenzie 2002). Consequently, we trained/tested the models using two datasets: (i) all nine species (All Species) and (ii) six species plus the *Nyctophilus* spp. group (All Species / *Nyctophilus* Grouped). Thus for each dataset (All Species and All Species / *Nyctophilus* Grouped) we trained and tested the models using four sets of parameters: 13 original and unprocessed parameters; 13 original but pre-processed parameters; nine PCA and four original and unprocessed parameters and; nine PCA and four original but pre-processed parameters (Figure 2). In total we created 48 models, 24 using the All Species dataset and 24 using the All Species / *Nyctophilus* Grouped dataset (Figure 2).

Even with filtering in AnaLook, some call files from the passive acoustic survey contained purely noise. To increase model performance we created a noise class to ensure pure noise files were not erroneously identified as a bat species. For model training using the reference call library, we considered each species (or species group) as a class, in addition to the noise class. We randomly assigned 75% of calls, stratified by class, to a training set and reserved the remaining 25% as a test set for model validation. When comparing model performance with manual identification, we used the same set of classes as well as an additional unknown class.

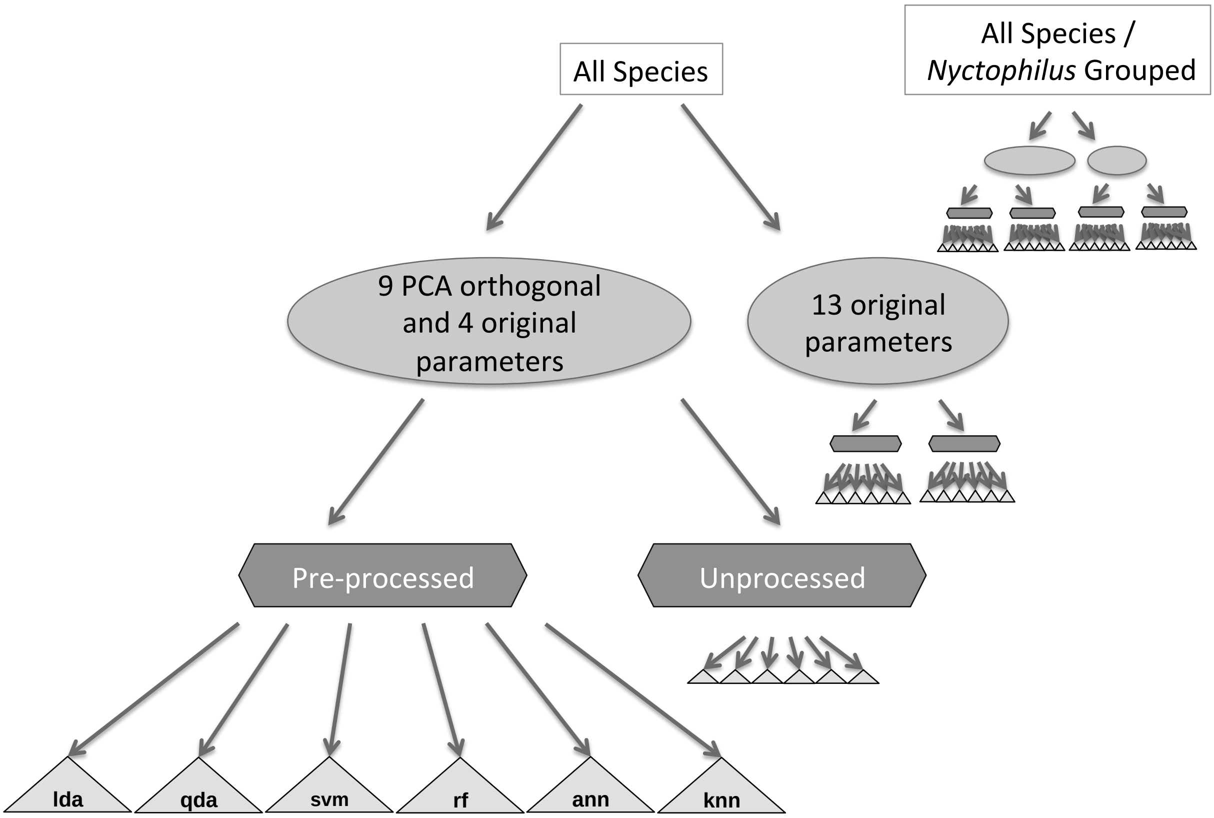


Figure 2. The framework behind the development of the 48 optimal models. We used two datasets (All Species; All Species / *Nyctophilus* Grouped) and two types of parameters (Principal Component Analysis (PCA) orthogonal values for highly correlated frequency and duration parameters plus remaining four original parameters; original 13 parameters) and then either applied pre-processing (centring and scaling) or left as original values to build six types of models (lda = linear discriminant function analysis; qda = quadratic discriminant function analysis; svm = support vector machine; rf = random forest; ann = artificial neural network; and knn = *k*-nearest neighbour).

Model Training

Classification models consisted of six supervised learning models, ranging in flexibility and complexity. We ran both parametric (linear and quadratic discriminant function analysis) and non-parametric (support vector machine, random forest, artificial neural network, and *k*-nearest neighbour) models. The increased complexity of non-parametric models allowed us to evaluate potential models, using five default grids of model-tuning parameters, prior to selecting the optimal model (Kuhn 2008). For all models resampling consisted of bootstrapping (200 iterations) a set of modified datasets created from the reference library model training set. Through resampling we determined the effect of model-tuning parameters on performance and chose the optimal model across these parameters. The apparent error rate of each model can be influenced by sample sizes in each class, in conjunction with the number of parameters (McLachlan 2012), so we selected models with the highest accuracy value, i.e., the overall agreement rate over the 200 bootstrapping iterations. We used the varImp function within the caret package to determine the general effect of each parameter on each model (Kuhn 2008). Parameter importance was characterised using the absolute value of the t-statistic for all models other than the random forest model, where parameter importance was the prediction accuracy of each parameter averaged over all trees and then normalised by the standard error (Kuhn 2008). Parameters were scaled prior to calculating importance so that importance values ranged from 0-100.

Apart from testing the performance of each optimal model against the test set of echolocation reference calls (see below), performance was also measured using sensitivity, specificity, and positive and negative predictive powers (PPP and NPP, respectively). Each measure provides a slightly different evaluation of model performance with values of 1.0 indicating perfect accuracy. Sensitivity and specificity refer to the rates of correctly assigning a positive and negative identification, respectively (Jennings et al. 2008). For example, a sensitivity value of 1.0 would mean that the model correctly assigned all *Vespadelus regulus* calls as *V. regulus* and a specificity value of 1.0 would mean that the model did not assign *V. regulus* calls to any class other than *V. regulus*. Positive and negative predictive powers are the probability that a call is correctly identified and not misidentified, respectively (Jennings et al. 2008).

Models

Discriminant function analysis predicts categorical dependent variables by one or more continuous or binary independent variables. Linear discriminant function analysis uses a linear combination of features to separate two or more objects and is subject to assumptions of normality, homogeneous variance-covariance matrices (Fisher 1936). Quadratic discriminant function analysis can be relatively robust to departures from normality and thus many researchers favour the use of quadratic discriminant function analysis (e.g., Preatoni et al. 2005). Papadatou *et al.* (2008) recommend the use of linear over quadratic discriminant function analysis in bat call identification as they found both methods had equal correct classification rates but linear models provided discriminant function coefficients, potentially enabling the identification of species unknown to the reference call library. Thus we included both linear and quadratic discriminant function analysis models; through the caret package we ran models based on the lda and qda functions within the MASS package (Venables & Ripley 2002).

Support vector machine models build on earlier supervised learning algorithms and are constructed so that the model maps the input parameters, or vectors, into some high dimensional feature space through a non-linear mapping network, chosen a priori (Cortes & Vapnik 1995; Lee et al. 2004). Artificial neural networks, as used in this study, may be limited in that they are not guaranteed to find a global minimum (Rumelhart et al. 1986), compared to support vector machines which are designed to find the global minima while allowing for some misclassification error (McLachlan 2012). Through the caret package we ran support vector machine models based on the svmRadial function within the kernlab package (Karatzoglou et al. 2004). The optimal models were based on a cost value of 2 and a sigma constant of 0.180 and 0.182 for All Species and All Species / *Nyctophilus* Grouped, respectively (Figure S8).

Random forest models use trees to represent classes. Trees are constructed by repeatedly splitting the data using a simple rule based on one of the parameters for each split so that the two subsequent trees are mutually exclusive; the objective is to partition the data into homogenous groups while keeping the number of trees relatively small (De'ath & Fabricius 2000). The final classification is determined by the full collection of tree-structured classifiers; each tree is involved in vote-casting and the most popular vote wins the classification (Breiman 2001). Due to the inherent structuring of the random forest model, they are robust to overfitting and a noisy dataset (Breiman 2001). Through the caret package we ran random forest models based on the rf function within the randomForest package (Liaw & Wiener 2002). The optimal models had mtry values of 4 and 2 for All Species and

All Species / *Nyctophilus* Grouped, respectively. The proportion of trees voting for a particular class produces a class probability or certainty score for each call, which we extracted for the optimal models. The random chance of a type I error (i.e., false positive classifications) for the All Species dataset was 0.100 (1/10) and 0.125 (1/8) for the All Species / *Nyctophilus* Grouped dataset. Thus, we used five certainty score cut-off levels by adding a buffer of 0.30, 0.35, 0.40, 0.45 and 0.50 to the random chance level of 0.100 and 0.125 for All Species and All Species / *Nyctophilus* Grouped, respectively (Figure S8). Certainty scores below these thresholds were relabelled as unknown.

Artificial neural networks are flexible non-linear regression models, emulating biological neural networks in that they consist of an interconnected group of artificial neurons or nodes that adaptively change structure during the learning process (Nickerson et al. 2006). We constructed a three-layered feed-forward, back-propagation neural network with a single hidden layer (Venables & Ripley 2002) and a linear transfer function for the response variable, similar to other bat call automation studies (Armitage & Ober 2010). This type of neural network involves the repeated adjustment of weights, or internal nodes, that are hidden and thus the three layers comprise the input vectors (i.e., call parameters), output vectors (i.e., class) and the hidden units (Bishop 1995). The learning process determines the circumstances in which the hidden units are active and thus it is the interaction of these hidden units that eventually minimises the difference between the actual output vector and the desired output vector (Rumelhart et al. 1986). Artificial neural networks assign calls to a class based on a voting structure; to ensure that there were no ties we created a model with prime numbers from one to 29 nodes in the hidden layer (Redgwell et al. 2009). Through the caret package we ran artificial neural network models based on the nnet function within the nnet package (Venables & Ripley 2002). The optimal models were based on a size of 29 and a decay of 1 (Figure S8).

k-Nearest neighbour is a simple non-parametric classification scheme that was developed to deal with discriminant analysis when there is little or no prior knowledge about the distribution of the data (Fix & Hodges 1951). We ran *k*-nearest neighbour models based on the knn function within the caret package (Kuhn 2008). The optimal models were based on K values of 5 and 7 for All Species and All Species / *Nyctophilus* Grouped, respectively (Figure S8).

Model Testing

Once the 48 optimal models were developed using the training set of reference calls, we used these models to classify the testing set of reference calls (i.e., the 25% of calls set aside for model validation). We then compared each of the 48 model classifications of unknown bat calls recorded during the passive acoustic survey to manually identified calls. To remove the potential of inter-observer bias, the same person (JMB) manually identified all call files. We excluded call files with fewer than two calls per file from model comparison, retaining 30,183 call files recorded in eight treatment types: R0-4 desirable = 4260; R5-9 desirable = 1656; R5-9 dense = 1942; R10-14 desirable = 3476; R10-14 dense = 1611; R>15 desirable = 3105; R>15 dense = 9434; and unmined = 6324. We compared the classification of calls using the optimal models with manual identification for all call files from the passive acoustic survey, determining overall agreement and agreement for each treatment type.

Results

We collected 132 reference call sequences, potentially representing 132 individuals, from nine species of bat in south-western Australia (Table 1). Number of calls and calls sequences varied by bat species, ranging from 37 call sequences and >2000 calls for *V. regulus* to three call sequences and 98 calls for *N. major*. Although there were marginally fewer calls for *N. major* than the recommended 100 minimum (Armitage & Ober 2010), there was a total of 653 calls for the *Nyctophilus* spp. class.

Using the All Species dataset, all models built with the nine PCA orthogonal and four original but pre-processed parameters performed well (~80%-90% accuracy; Figure 3) during model validation, i.e., when we used the models to classify the testing set of the 25% of reference calls left out during model building. Linear and quadratic discriminant function analysis and random forest models built with the nine PCA orthogonal and four original and unprocessed parameters had similar accuracy as the models built with the same, but pre-processed, parameter set. However there was a >30% drop in accuracy for support vector machine, artificial neural network and *k*-nearest neighbour models (Figure 3). Linear and quadratic discriminant function analysis and random forest models also retained similar model validation accuracy (~80-90%) when built with the original parameters, either pre-processed or unprocessed. Model validation accuracy was low for support vector machine (~25%), artificial neural network (~45%) and *k*-nearest neighbour (~65%) models, when looking at models built with either the pre-processed or unprocessed original parameters (Figures 3). Models built using the All Species / *Nyctophilus* Grouped dataset had only marginally higher model validation accuracies, when comparing the modes built using the same parameters but with the All Species dataset (Figure 4).

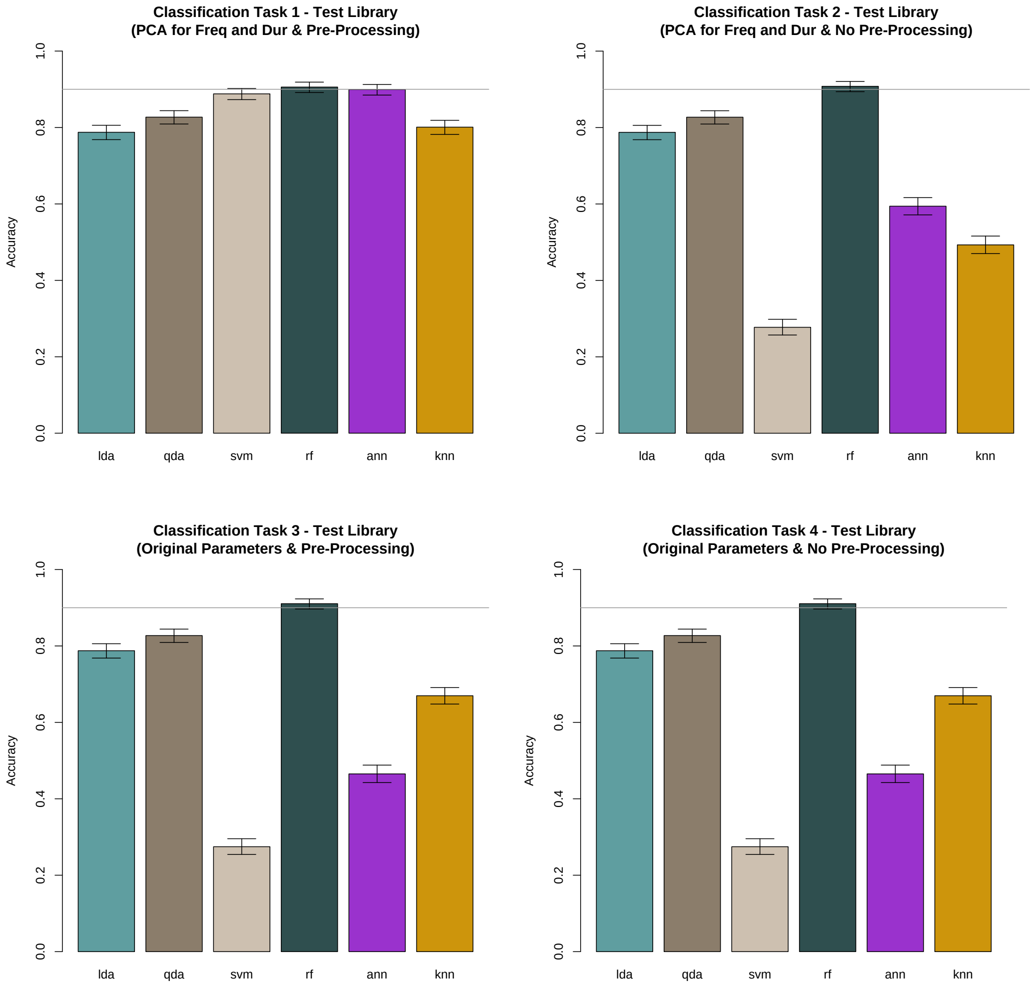


Figure 3. Model comparisons against the test reference call library (25% of calls set aside for validation) using the All Species dataset and for the four sets of parameters: 1 nine PCA plus four originals parameters, pre-processed; 2 nine PCA plus four originals parameters, unprocessed; 3 original parameters, pre-processed; and 4 original parameters, unprocessed. The grey line denotes 90% accuracy, the overall agreement rate. Error bars show upper and lower confidence interval (95%) limits.

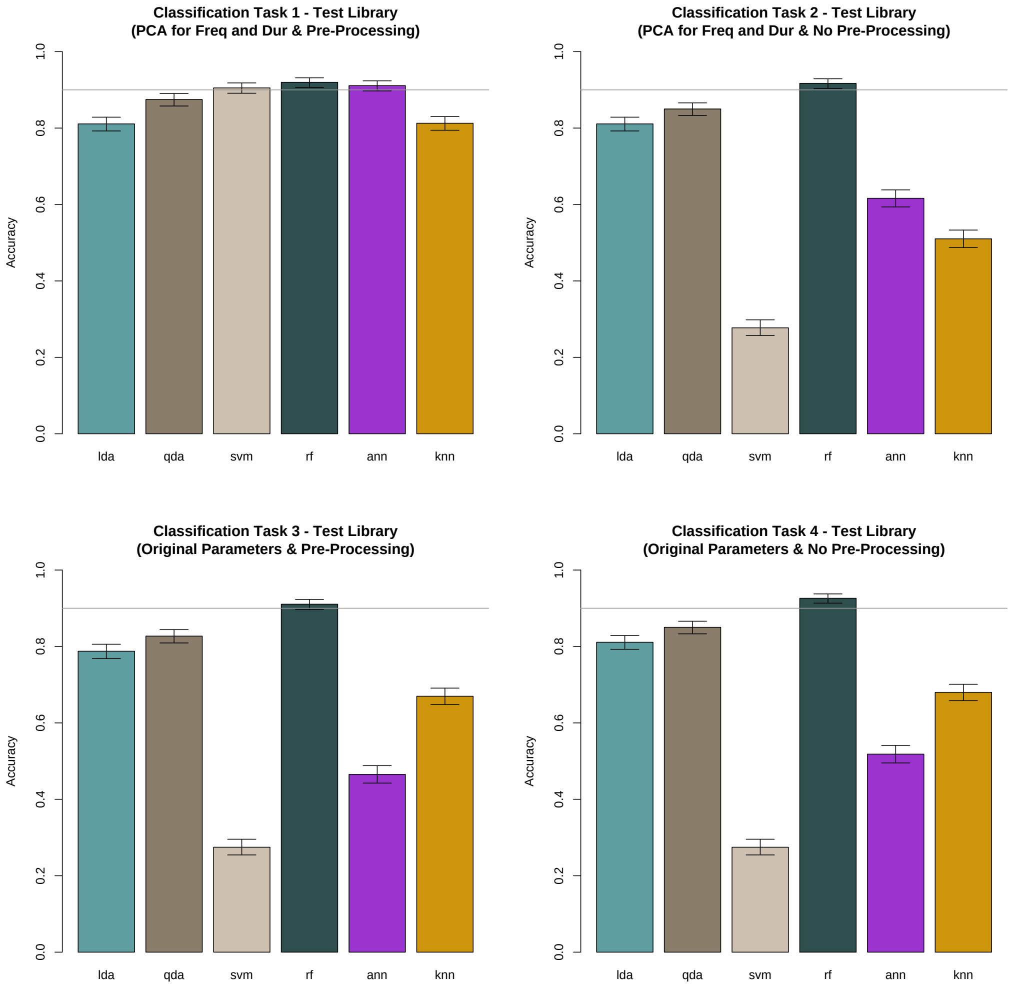


Figure 4. Model comparisons against the test reference call library (25% of calls set aside for validation) using the All Species / *Nyctophilus* Grouped dataset and for the four sets of parameters: 1 nine PCA plus four originals parameters, pre-processed; 2 nine PCA plus four originals parameters, unprocessed; 3 original parameters, pre-processed; and 4 original parameters, unprocessed. The grey line denotes 90% accuracy, the overall agreement rate. Error bars show upper and lower confidence interval (95%) limits.

Using the All Species dataset, classification agreement was extremely low (<15%) between all models built using the nine PCA orthogonal and four original but pre-processed parameters and manual identification (Figure 5). The *k*-nearest neighbour model built using the nine PCA orthogonal and four original unprocessed parameters had higher classification agreement (~65%) with manual identification but all other models built using these parameters had extremely low classification agreement (<10%, Figure 5). No models built using the original pre-processed parameters were able to classify call sequences to each of the nine species and thus we were unable to calculate classification agreement scores for this group of models. Random forest models built using original unprocessed parameters had the highest classification agreement (93%) with manually identified calls (Figure 5). Using the same original unprocessed parameter set linear and quadratic discriminant function analysis and *k*-nearest neighbour models had only slightly lower classification agreement (~80-90%), while support vector machine and artificial neural network models had poor classification agreement (<25%), compared to manually identified calls. Again, models built using the All Species / *Nyctophilus* Grouped dataset had only marginally higher classification agreement scores, where applicable, when comparing the modes built using the same parameters but with the All Species dataset (Figure 6).

We only examined model performance by habitat type for the models that had high classification agreement with manual identification, i.e., those built using the original, unprocessed parameters. For the sake of simplicity we only present the results for models built using the All Species / *Nyctophilus* Grouped dataset. The random forest model had the highest overall classification agreement (94%), compared to manual identification and this agreement level was fairly consistent regardless of habitat type (Figure 7). In contrast, linear and quadratic discriminant function analysis models had lower overall classification agreement (90% and 88%, respectively), compared to manual identification, and this was mainly driven by lower classification agreement for calls recorded in <10 year old restored forest (Figure 7).

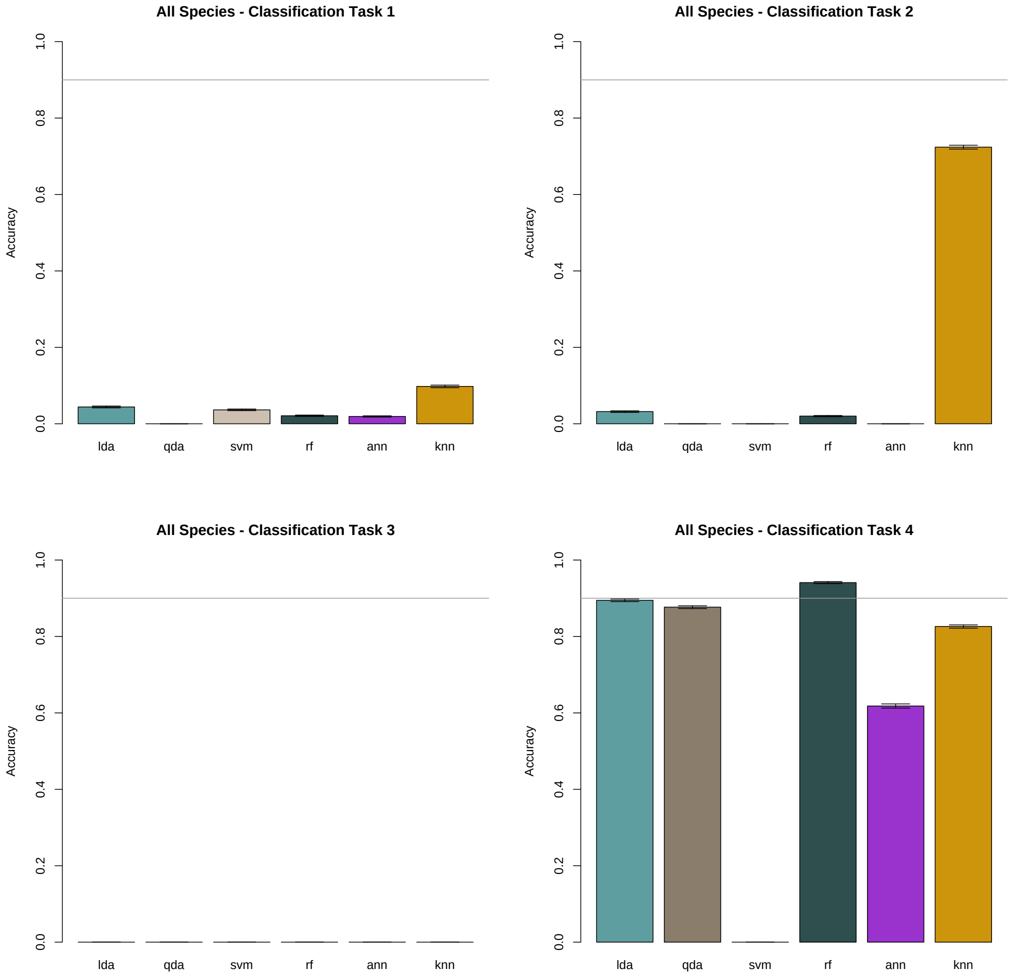


Figure 5. Classification agreement of passive acoustic survey bat calls between manual identification and optimal models using the All Species dataset and for the four sets of parameters: 1 nine PCA plus four originals parameters, pre-processed; 2 nine PCA plus four originals parameters, unprocessed; 3 original parameters, pre-processed; and 4 original parameters, unprocessed. The grey line denotes 90% accuracy, the overall agreement rate. Error bars show upper and lower confidence interval (95%) limits.

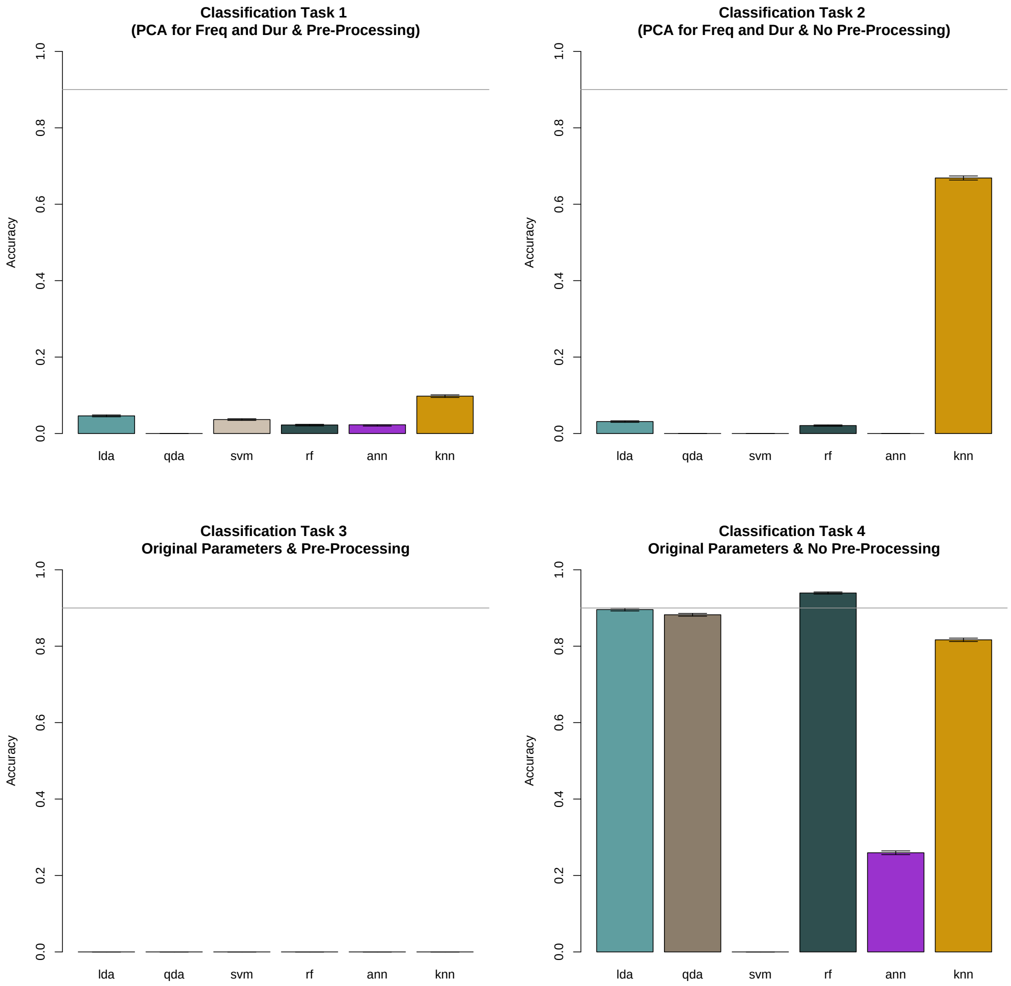


Figure 6. Classification agreement of passive acoustic survey bat calls between manual identification and optimal models using the All Species / *Nyctophilus* Grouped dataset and for the four sets of parameters: 1 nine PCA plus four originals parameters, pre-processed; 2 nine PCA plus four originals parameters, unprocessed; 3 original parameters, pre-processed; and 4 original parameters, unprocessed. The grey line denotes 90% accuracy, the overall agreement rate. Error bars show upper and lower confidence interval (95%) limits.

We considered the random forest model built using original, unprocessed parameters as the overall best model for automating bat call identification. We compared this random forest model with manual identification across varying certainty score cut-off values and found there was no difference in agreement between manual identification and random forest classification when the certainty score was set to 0.30 (Figure 8). Agreement became consistently lower as cut-off scores increased (63% agreement when score was set to 0.50) while the number of calls classified as unknown steadily increased. Manual identification classified 222 calls as unknown compared to 285 by the random forest model without any certainty score cut-off, 539 unknown when the certainty score was set to 0.30 and 10037 unknown classifications for the 0.50 certainty score.

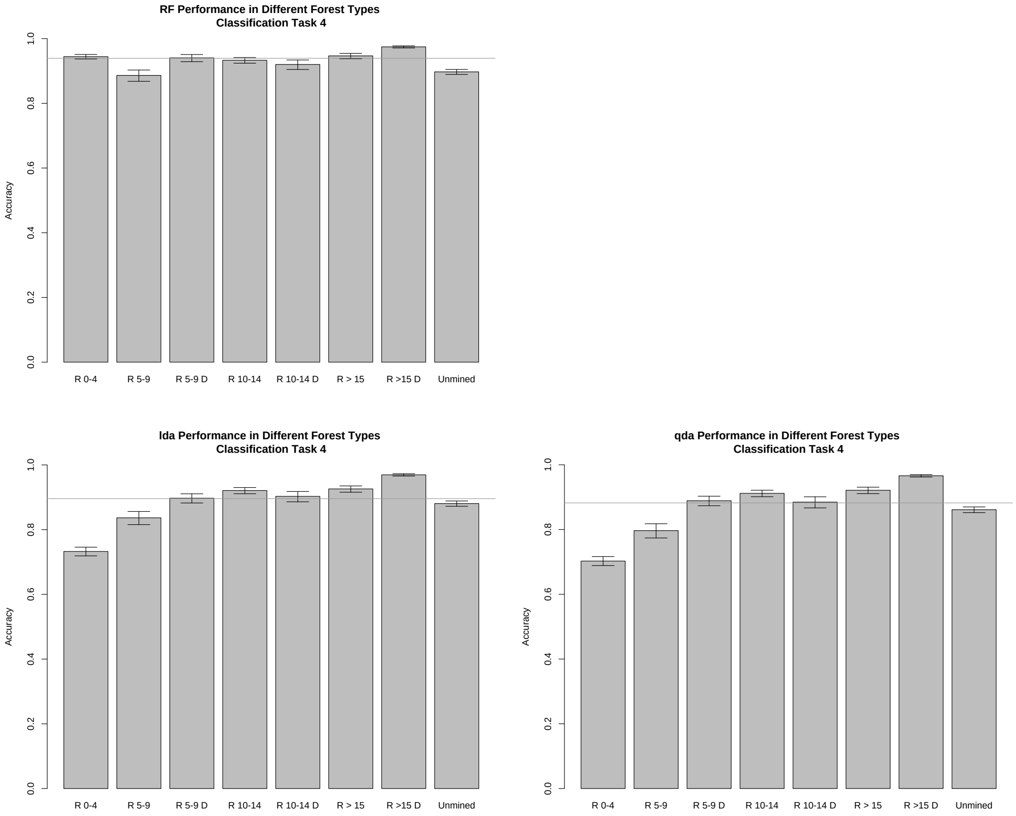


Figure 7. Classification agreement, by habitat type, of passive acoustic survey bat calls between manual identification and the top three optimal models (built using the 13 original, unprocessed call parameters and the All Species / *Nyctophilus* Grouped dataset). The eight treatment types comprised restored forest (R) of various age classes (0-4, 5-9, 10-14, and >15) and unmined forest. Tree density was either desirable (500-2500 stems ha^{-1}) or dense (>2500 stems ha^{-1}; indicated by D) for restored forest ≥5 years. The grey line denotes the overall accuracy of each optimal model: 94% for random forest, 90% for linear and 88% for quadratic discriminant function analysis. Error bars show upper and lower confidence interval (95%) limits.

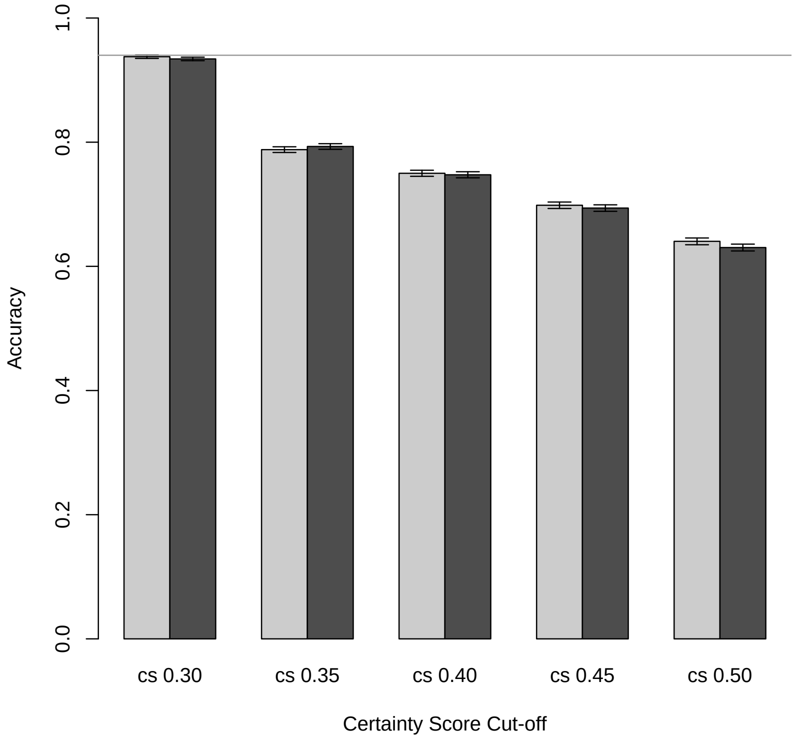


Figure 5. Classification agreement (accuracy) of passive acoustic survey bat calls between manual identification and the random forest model at multiple certainty score buffer thresholds. When the call certainty score buffers were below cut-off values (range 0.3 – 0.5) they were classified as unknown. The grey line denotes 94% accuracy, the random forest model agreement level when not accounting for certainty score buffers. Error bars show upper and lower confidence interval (95%) limits. The random forest model was built using the original, unprocessed parameters; light grey bars denote the All Species dataset while dark grey bars denote the All Species / *Nyctophilus* Grouped dataset.

Species-specific random forest model performance varied greatly, even when using the All Species / *Nyctophilus* Grouped dataset with a certainty score cut-off of 0.30 (Table 3). Sensitivity ranged from 0.28 for *F. mackenziei* to over 0.90 (0.91 for *V. regulus* and 0.95 for *C. gouldi*) while specificity was either 0.99 or 1.00 for all species/species groupings. The probability that the random forest model would classify a species the same as manual identification was highest for *T. australis* (0.97), *V. regulus* (0.95) and *M. kitcheneri* (0.92) and by far the lowest for *F. mackenziei* (0.14). When looking at performance using the All Species dataset, the random forest model performance of the three individual *Nyctophilus* species was best for *N. gouldi* (sensitivity = 0.81, PPP = 0.78), followed by *N. geoffroyi* (sensitivity = 0.55, PPP = 0.53) and *N. major* (sensitivity = 0.50, PPP = 1.00), when validating classification with the “testing” reference library dataset. Misclassification of *N. gouldi* and *N. geoffroyi* was equally likely to be a congeneric as it was to be a species from a different genus while *N. major* misclassifications were predominantly (83%) to a congeneric. The *Nyctophilus* spp. group had high sensitivity and PPP (0.90 and 0.85, respectively), when validating against the “testing” reference library dataset and for the unprocessed parameters. Of the misclassifications, nearly half (47%, 8/17) were classified as *V. regulus*.

Table 3. Class-specific sensitivity, specificity, positive predictive power (PPP), and negative predictive power (NPP), based on classification of passive acoustic survey data using the best random forest model (built using the original, unprocessed parameters, the All Species / *Nyctophilus* Grouped dataset) with a 0.30 certainty score cut-off buffer. Values of 1.00 indicate perfect agreement with manual identification and those greater than 0.90 are highlighted in bold. Greyed lines indicate the unknown and noise classes.

	Sensitivity	Specificity	PPP	NPP
C. gouldii	**0.95**	**0.99**	0.60	**1.00**
C. morio	0.84	**1.00**	0.87	**1.00**
F. mackenziei	0.28	**1.00**	0.14	**1.00**
M. kitcheneri	0.63	**1.00**	**0.92**	**1.00**
Nyctophilus spp.	0.53	**1.00**	0.66	**0.99**
T. australis	0.80	**1.00**	**0.97**	**0.99**
V. regulus	**0.91**	**0.99**	**0.95**	**0.97**
Unknown	0.11	**0.98**	0.05	**0.99**
Noise	**0.97**	**0.94**	**0.97**	**0.94**

A wide range of parameters were important for characterising classification in the random forest model, with importance values generally ranging from ~20-40 (Figure S9). Notable exceptions were characteristic slope, the most important parameter for classifying *Nyctophilus* spp. calls, and characteristic frequency, the most important parameter for classifying *M. kitcheneri* calls (values of 100 and 90, respectively). Frequency parameters were the most important predictors for classification of all species in all other models (Figure S10).

Discussion

Our study underscores the importance of model validation when automating species identification using field generated acoustic survey data. While visual and acoustic studies are often compared and used complimentarily in cetacean research (e.g., Akamatsu et al. 2008; Barlow & Taylor 2005) very few studies compare automated techniques with manual identification in either bat or bird research (but see Digby et al. 2013; Jennings et al. 2008). Our findings strongly suggest that, if automated models are used then they should be validated with manually identified field data to minimise misclassification of calls. We acknowledge that manual identification does not always correctly classify calls to species and, indeed, there can be disagreement between individuals, particularly if their identification experience differs (Jennings et al. 2008). However, to ensure quality data necessary for long-term monitoring research, we need repeatable sampling protocols with objective data interpretation. Using automation to classify the extensive data generated from acoustic surveys will not only ensure repeatability and objectivity of sampling over time, but it will also reduce the cost of conducting studies by reducing manual processing time.

In our study, the random forest model performed best, both when validated with the reference library and compared to manual identification. Random forest models are generally insensitive to the number of parameters used to construct each node between trees and usually the selection of one or two parameters gives near optimum results (Breiman 2001). However, we found that only the classification of *Nyctophilus* spp. and *M. kitcheneri* was highly dependent on a single call parameter, characteristic slope and characteristic frequency respectively. Similar to other studies using random forest models (Armitage & Ober 2010), classification of all other species used several call parameters and these varied in importance, suggesting that no single parameter was best, but rather accurate classification required several parameters. We also found the random forest model was sensitive to pre-processing of the parameters. There was extremely low agreement between the random forest model classification and manual identification when input parameters were pre-processed, both in terms of using orthogonal parameters created from a PCA of correlated parameters and with respect to the centring and scaling of parameters.

In contrast to other studies comparing model performance for classification tasks with all species, or "problematic" congenerics grouped (e.g., Armitage & Ober 2010), we found little difference in model performance when using all species or the grouped *Nyctophilus* spp. dataset. While *Nyctophilus* spp. calls are considered indistinguishable when recorded using the frequency-division Anabat system (Adams et al. 2010; Bullen & McKenzie 2002) we found that the random forest model accurately predicted *N. gouldi* calls ~ 80% of the time. Accuracy was much lower for both *N. geoffroyi* and *N. major*, although this may be due to the lower number of reference calls used to train and test the models, rather than a reflection of the model's performance ability. Artificial neural networks can outperform ~75% of humans when classifying calls of quality similar to those generated by passive acoustic surveys (Jennings et al. 2008). Thus, with an improved reference library, automated techniques may be able to distinguish calls previously considered indistinguishable by manual identification.

Similar to other studies using freely available software (Armitage & Ober 2010; Britzke et al. 2011), the benefit of our approach to automated bat call identification is that researchers can share models, if using the same reference call library, or at the very least R script, to develop models to ensure they are using the optimal model for their region/dataset. While supervised learning machines, particularly artificial neural networks, can be computationally intensive when first determining optimal models (Skowronski & Harris 2006), once the optimal model has been determined the processing of unknown calls is quick, in comparison with manual identification. While complex algorithms that are difficult to determine may hinder use of models (Adams et al. 2010), the benefit of cooperative sharing of R script is that once the underlying concepts of complex models are understood, and set up by an experienced individual, running models can be relatively simple, and the output easily interpreted. Random forest model building took approximately one day of processing time on a laptop (2010 MacBook Pro 2.53 GHz Intel Core i5 with 8G memory), although the use of freely available R packages that build models off-line utilising dual-processing could substantially reduce model building time (Kuhn 2008). Once the model was created it was computationally easy and quick to predict unknown cases. We spent approximately two hours extracting parameters and preparing call files from the passive acoustic survey, approximately one hour running all call files (>30,000) through the model, and approximately one hour of post-processing to aggregate call files and prepare them for further analysis. Manual identification of the same dataset could take one individual anywhere between one and more than two weeks, depending on the experience of the identifier.

When examining the similarity of the random forest model classification with manual identification at the species level, we found that sensitivity and positive predictive power varied widely between species, with more common species more likely to be correctly identified. Thus, we feel justified in recommending our approach for identifying broad patterns and in long-term monitoring where large scale passive acoustic surveys are conducted and reference call libraries have already been constructed. In cases where the aim of the bioacoustics survey is to identify rare or unknown species this approach may not be appropriate, as it requires a reference call library and may misidentify rarer species. Our approach required each model to classify each call and, thus, species not included in the reference library will be misclassified (Adams et al. 2010). One of the benefits of the random forest model is the ability to extract certainty scores for each classification and then apply a buffer or certainty score cut-off where any classification below a certain threshold can be binned as unknown (Kuhn 2008). Thus, using random forest models with certainty score cut-offs instil confidence of low Type I misidentification errors, particularly in regions where rare species might be missing from the reference call library. Indeed, those calls binned as unknown could then be examined and potential similarities in call features identified to determine if a species had been recorded that was missing from the reference library, akin to the use of linear coefficients from discriminant function analysis (Papadatou et al. 2008).

The immense quantity of data generated from bioacoustics surveys warrants development of accurate automation techniques, especially for longitudinal studies where individual researchers may change over time. While our study used bat echolocation calls recorded using frequency-division software, our methodology is widely applicable to bioacoustics surveys of any taxa, including cetaceans and birds. Our approach allowed model tweaking to ensure development of the optimal model for the dataset, requiring only a reference database for model building and recording equipment where acoustic parameters were readily and objectively downloaded. In a time of rapidly changing environments, bioacoustics surveys can play a major role in detecting changes in species habitat use following habitat degradation (Hanspach et al. 2012; Luck et al. 2013) or even population declines (Akamatsu et al. 2008). Automation techniques have the ability to objectively and efficiently translate the enormous amount of data generated into meaningful science, applicable to the conservation and management of wildlife populations.

Appendix Two: Supplementary Figures & Tables

Chapter Four

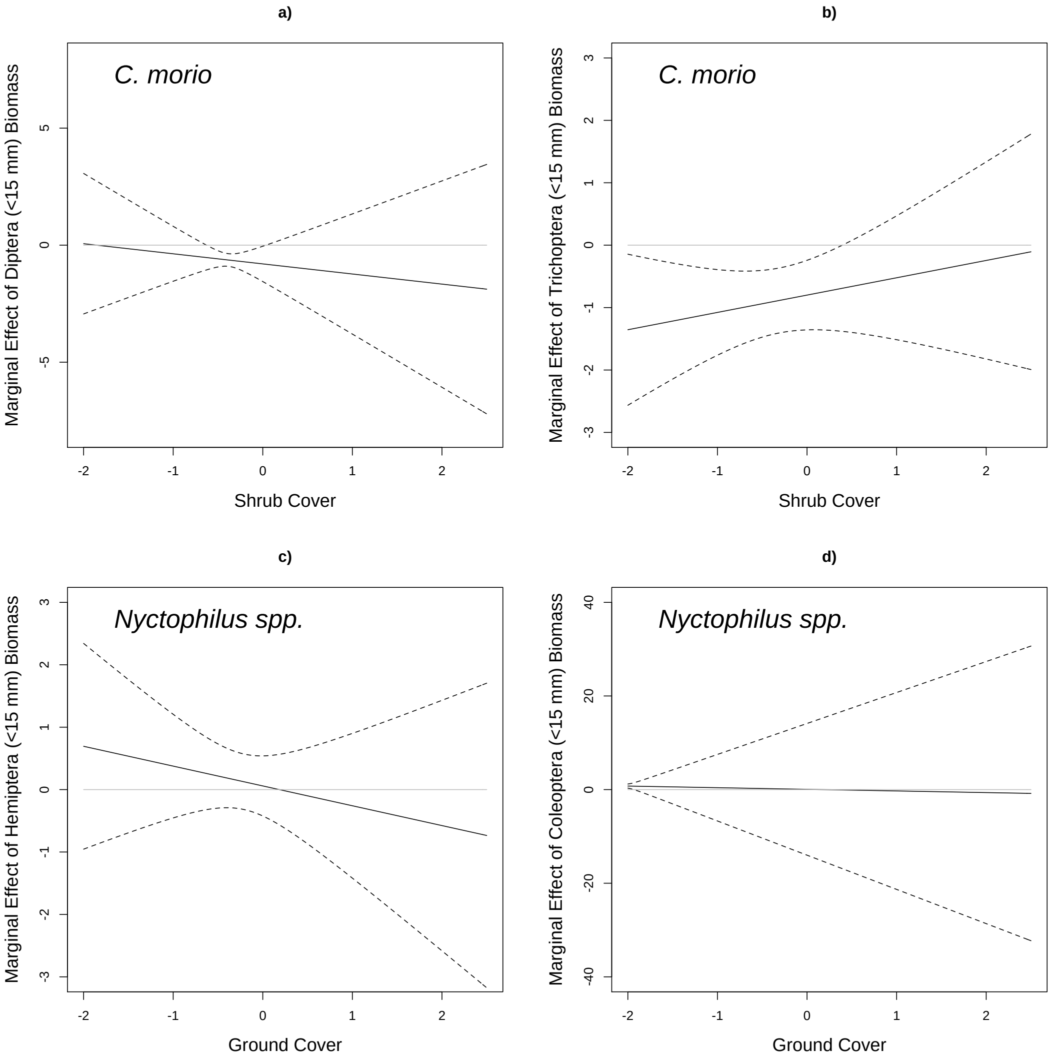


Figure S1a-d: The marginal effect of insect biomass on bat activity, for a range of vegetation structure values. Explanatory variables (insect biomass and vegetation structure) were standardised prior to modelling so 0 on graphs represent mean values. 95% confidence limits are represented by dotted lines. The marginal effect of a) Diptera biomass on *C. morio* activity as influenced by shrub cover; b) Trichoptera <15 mm biomass on *C. morio* activity as influenced by shrub cover; and the marginal effect of insect biomass on *Nyctophilus* spp. activity for c) Hemiptera <15 mm biomass and ground cover; and d) Coleoptera < 15 mm biomass and ground cover.

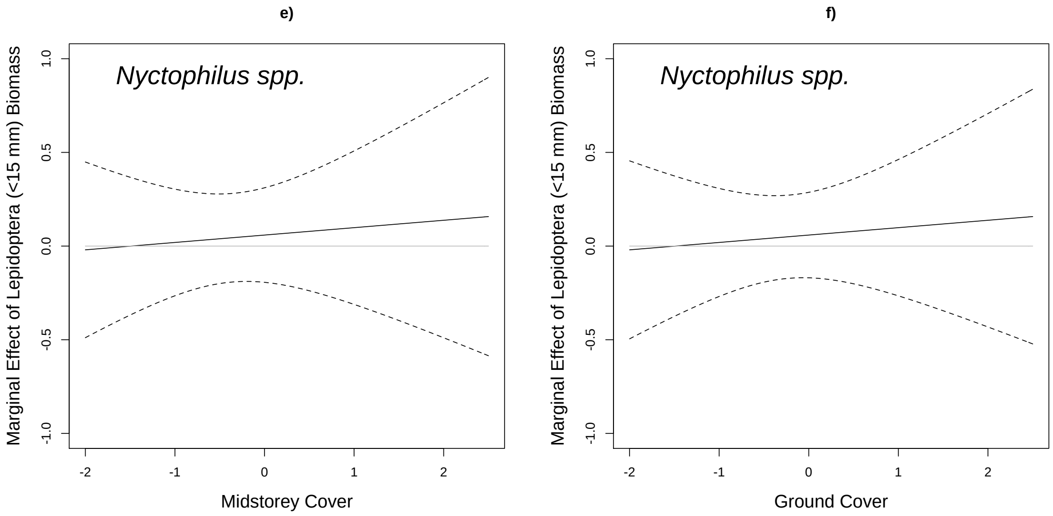


Figure S1e-f: The marginal effect of insect biomass on bat activity, for a range of vegetation structure values. Explanatory variables (insect biomass and vegetation structure) were standardised prior to modelling so 0 on graphs represent mean values. 95% confidence limits are represented by dotted lines. The marginal effect of e) Lepidoptera <15 mm biomass and midstorey cover; and f) Lepidoptera <15 mm biomass and ground cover.

Table S1. *Nyctophilus gouldi* trapping data for all individuals affixed with a transmitter during the roosting survey. Individuals were tracked to diurnal roosts the day immediately following capture and then for consecutive days. All tracked individuals were located on the first day of tracking with the exception of those marked with *. Tracked individuals were located on consecutive days until signals were lost and then were not found again. Thus, number of fixes also indicates number of consecutive days located.

		Bat ID	Date captured	No. fixes	Forearm length (mm)	Mass (g)	Trap Site
Maternity				12			
	Female			9			
		110	15-Nov-11	1	40.1	12.1	Site 3
		270	31-Oct-11	0	41.4	10.2	Site 1
		348	15-Nov-11	2	42.8	11.7	Site 3
		614*	15-Nov-11	2	41.3	9.9	Site 3
		592c*	21-Nov-11	4	41.4	10.5	Site 4
	Male			3			
		694	21-Nov-11	1	39.7	7.1	Site 4
		592a	31-Oct-11	1	37.0	9.6	Site 1
		592b	15-Nov-11	1	39.8	9.0	Site 3
Mating				34			
	Female			22			
		932*	13-Mar-12	2	42.9	9.0	Site 1
		5820*	05-Mar-12	3	41.2	10.9	Site 4
		6510	01-Feb-12	5	40.9	8.2	Site 4
		6945	06-Feb-12	3	41.9	10.5	Site 1
		7334	30-Jan-12	5	41.2	10.6	Site 4
		9885	05-Mar-12	4	41.1	9.5	Site 4
	Male			12			
		2250	05-Mar-12	1	40.6	9.3	Site 4
		3715*	06-Feb-12	2	38.8	8.4	Site 1
		4525	06-Feb-12	2	40.9	8.5	Site 1
		4934	01-Feb-12	4	40.9	8.5	Site 4
		6945b	13-Feb-12	0	38.8	8.9	Site 2
		9085	20-Feb-12	0	39.2	9.0	Site 1
		9735	13-Mar-12	3	40.6	9.0	Site 1

Table S2. *Vespadelus regulus* trapping data for all individuals affixed with a transmitter during the roosting survey. Individuals were tracked to diurnal roosts the day immediately following capture and then for consecutive days. All tracked individuals were located on the first day of tracking and were located on consecutive days until signals were lost and then were not found again. Thus, number of fixes also indicates number of consecutive days located.

		Bat ID	Date captured	No. fixes	Forearm length (mm)	Mass (g)	Trap Site
Maternity				18			
	Female			14			
		308	31-Oct-11	0	34.0	7.3	Site 1
		392	07-Dec-11	2	31.9	6.2	Site 5
		452	23-Nov-11	2	33.2	7.1	Site 4
		531	07-Dec-11	2	31.9	6.0	Site 5
		110b	21-Nov-11	4	32.2	7.2	Site 4
		348b	21-Nov-11	4	32.2	7.2	Site 4
	Male			4			
		147	07-Dec-11	2	32.0	5.2	Site 5
		188	21-Nov-11	1	31.5	5.2	Site 4
		188b	23-Nov-11	0	31.7	5.3	Site 4
		068	31-Oct-11	1	31.3	6.0	Site 1
Mating				37			
	Female			20			
		893	13-Mar-12	4	30.3	5.1	Site 1
		3720	13-Feb-12	0	31.1	6.1	Site 2
		4137	30-Jan-12	6	31.8	6.2	Site 4
		5732	30-Jan-12	6	32.1	6.0	Site 4
		7440	05-Mar-12	4	32.0	5.7	Site 4
	Male			17			
		793	13-Mar-12	2	32.0	5.5	Site 1
		853	13-Mar-12	4	32.1	4.9	Site 1
		1435	13-Feb-12	3	31.2	5.4	Site 2
		2038	30-Jan-12	1	31.6	4.9	Site 4
		3456	05-Mar-12	0	32.0	4.9	Site 4
		4662	14-Feb-12	2	31.0	4.9	Site 2
		0275	06-Feb-12	5	32.1	5.1	Site 1

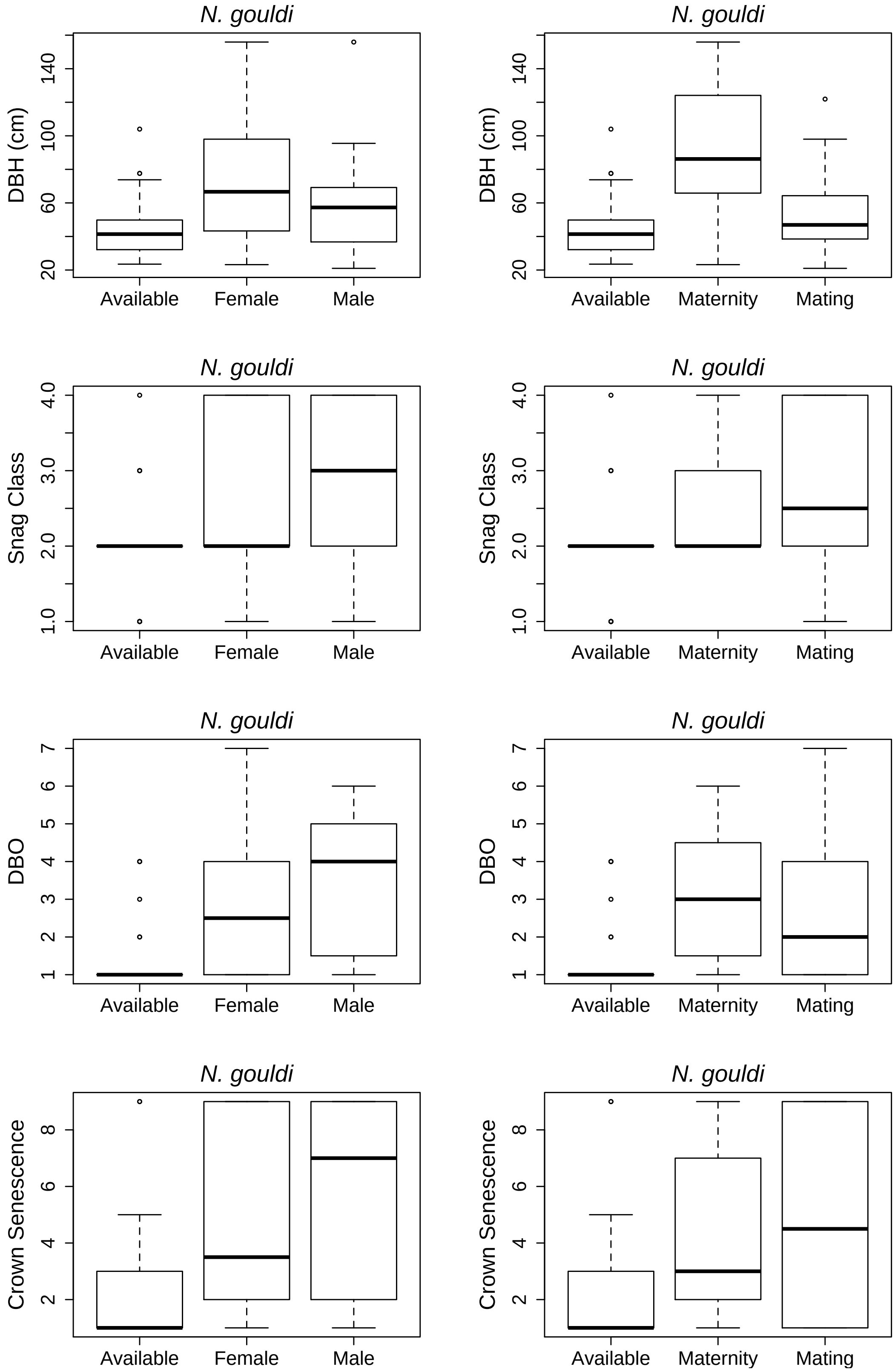


Figure S2: *N. gouldi* roost tree preferences in the restored landscape of south-western Australia. Significant differences ($P < 0.01$) from available roosting trees were apparent for *N. gouldi* females (all variables), males (snag class, DBO and crown senescence), and all *N. gouldi* during the maternity season (DBH, DBO and crown senescence) and mating season (snag class, DBO and crown senescence). Boxplot thick lines represent the median, lighter lines the quartile ranges and circles outliers in this and following graphs.

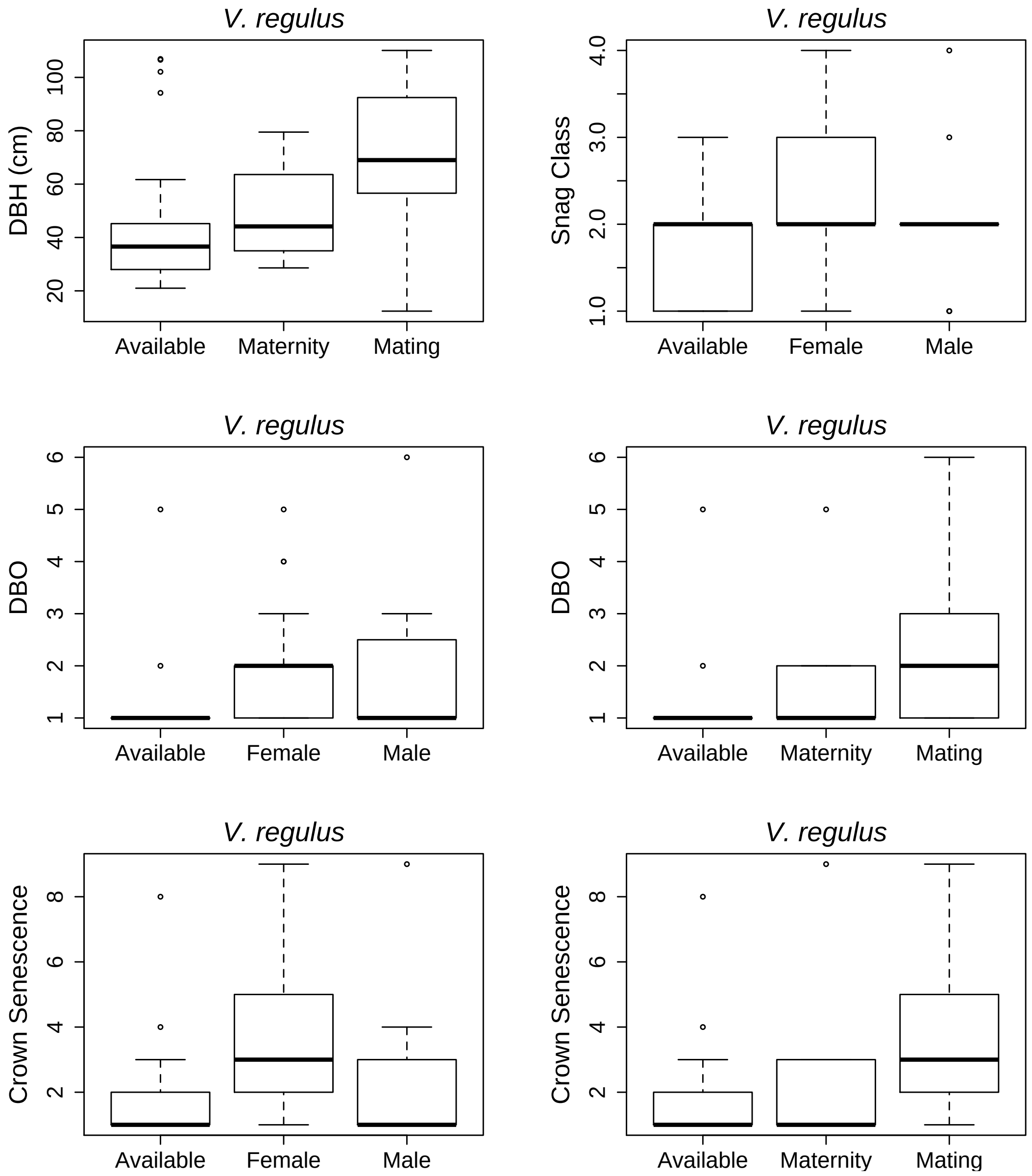


Figure S3: V. regulus roost tree preferences in the restored landscape of south-western Australia. Significant differences ($P < 0.01$) from available roosting trees were apparent for V. regulus females (snag class, DBO and crown senescence), and all V. regulus during the mating season (DBH, DBO and crown senescence).

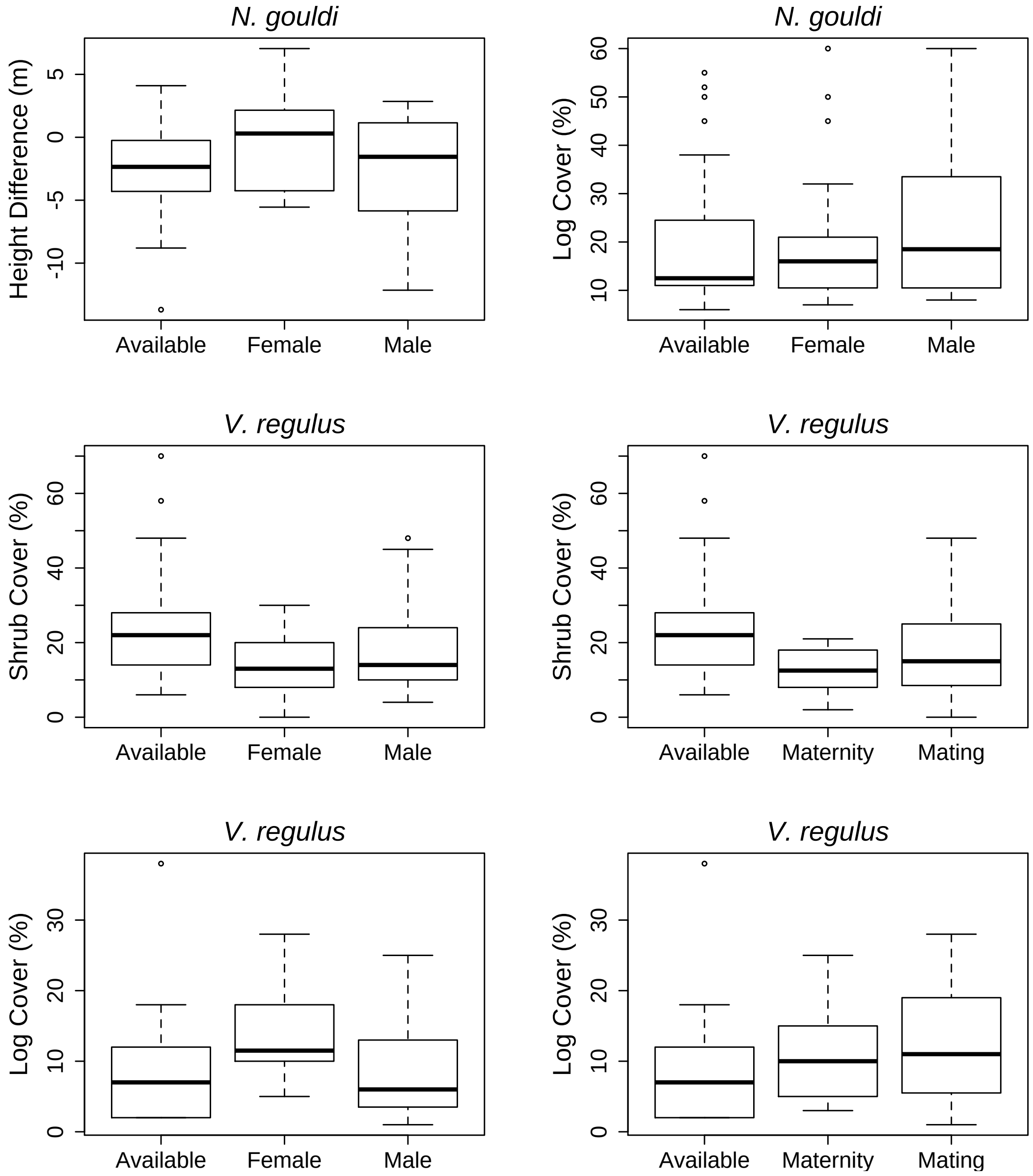


Figure S4: Bat roosting site preferences for N. gouldi and V. regulus in the restored landscape of south-western Australia. Significant differences ($P < 0.01$) from available roosting sites were apparent for N. gouldi females (height difference), N. gouldi males (log cover), V. regulus females (shrub and log cover) and all V. regulus during the maternity season (shrub cover) and the mating season (log cover).

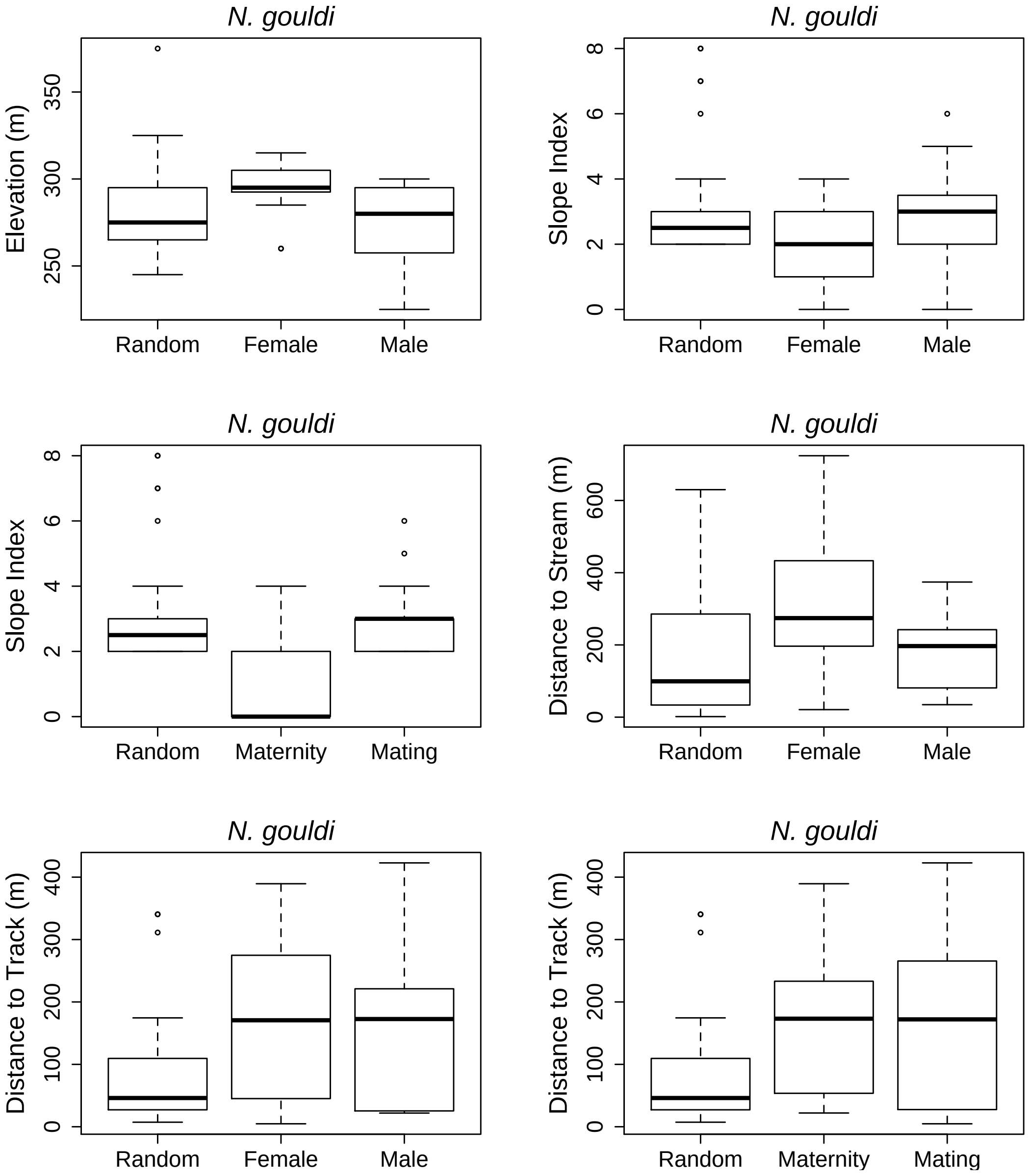


Figure S5: N. gouldi roost landscape preferences in the restored landscape of south-western Australia. Significant differences (P < 0.01) from random locations were apparent for N. gouldi females (all variables), and all N. gouldi during the maternity season (slope) and the mating season (distance to track).

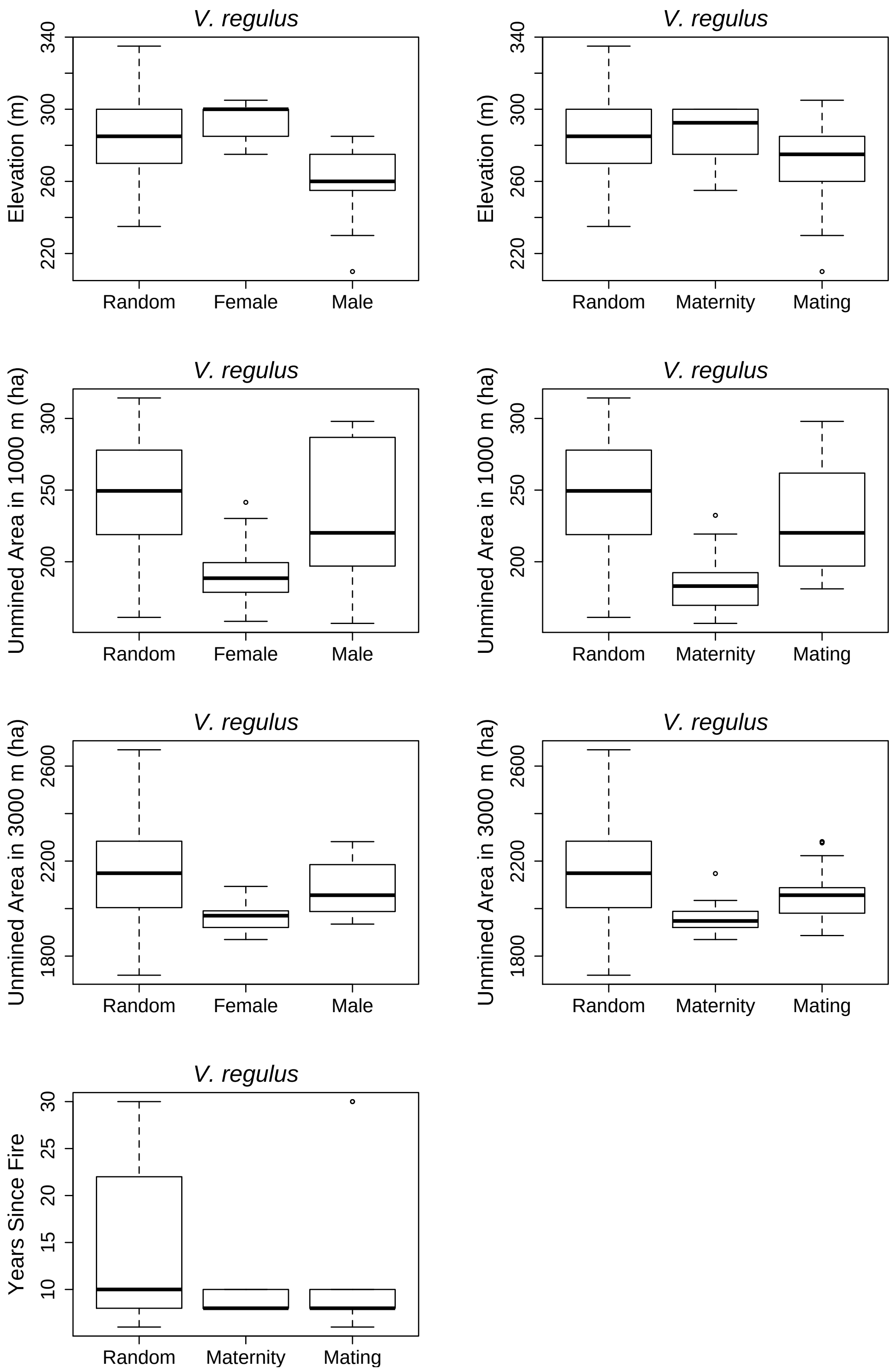


Figure S6: V. regulus roost landscape preferences in the restored landscape of south-western Australia. Significant differences ($P < 0.01$) from random locations were apparent for V. regulus females (unmined area in 1000 m and 3000 m), males (elevation and unmined area in 1000 m) and all V. regulus during the maternity season (unmined area in 1000 m and 3000 m and years since fire) and the mating season (elevation and unmined area in 1000 m).

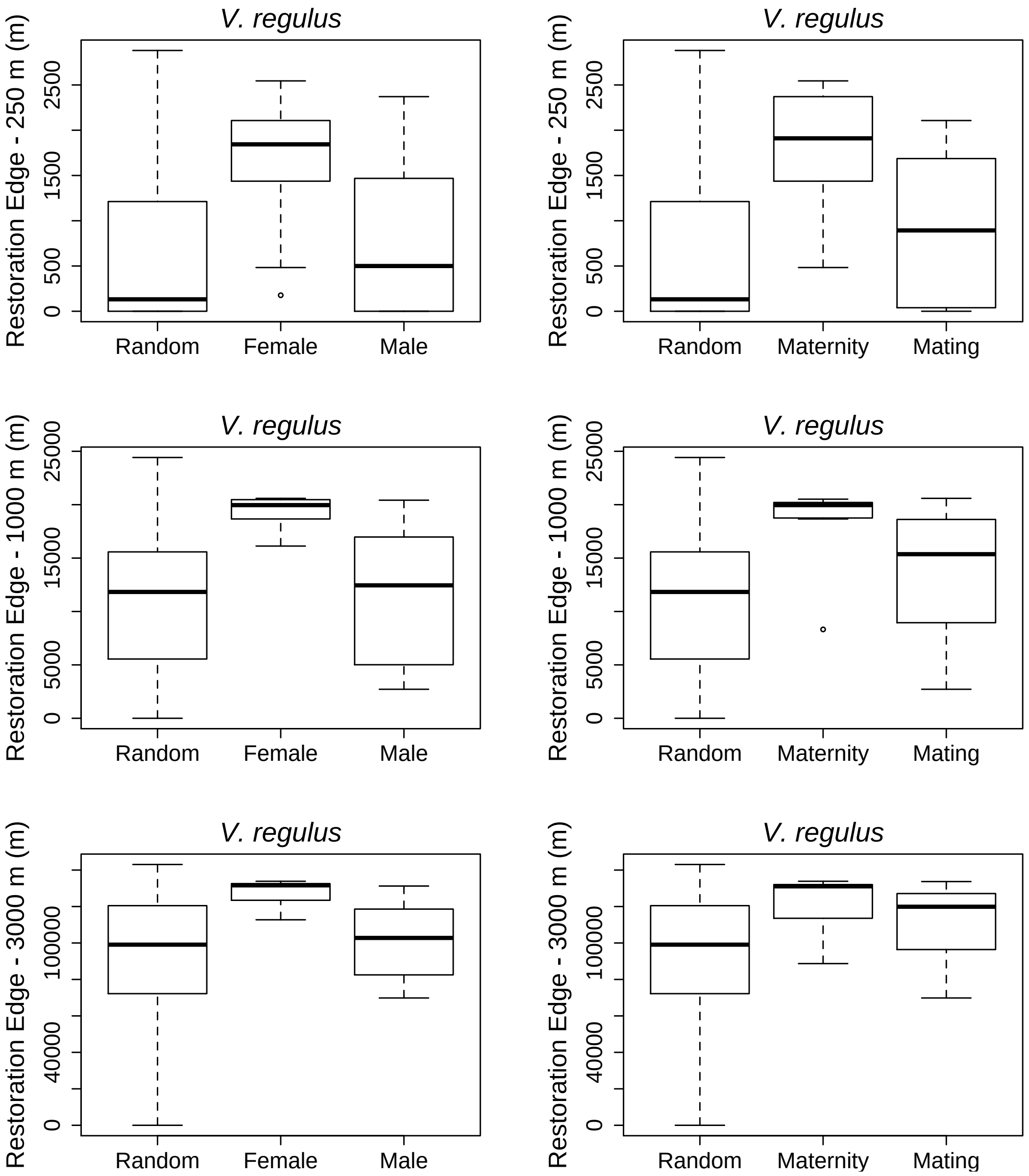


Figure S7: V. regulus roost landscape preferences in the restored landscape of south-western Australia. Significant differences (P < 0.01) from random locations were apparent for V. regulus females (all variables), males (restoration edge within 3000 m) and all V. regulus during the maternity season (all variables) and the mating season (restoration edge within 3000 m).

Appendix One

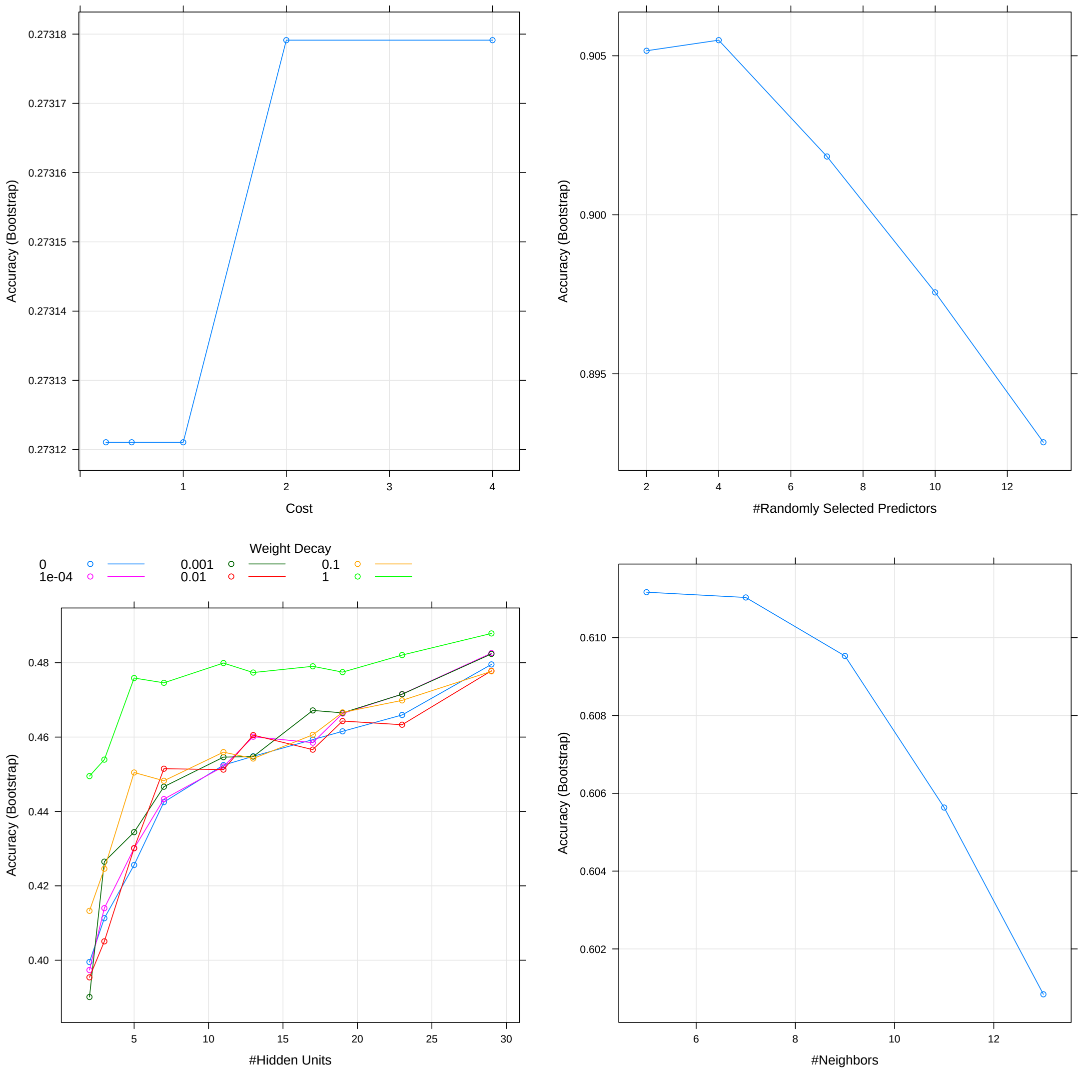


Figure S8a. Tuning parameters used to select the "optimal" classification models using the All Species dataset. There were no tuning parameters for linear or quadratic discriminant function analysis models. From left to right, top to bottom: support vector machine, random forest, artificial neural network, and *k*-nearest neighbour models.

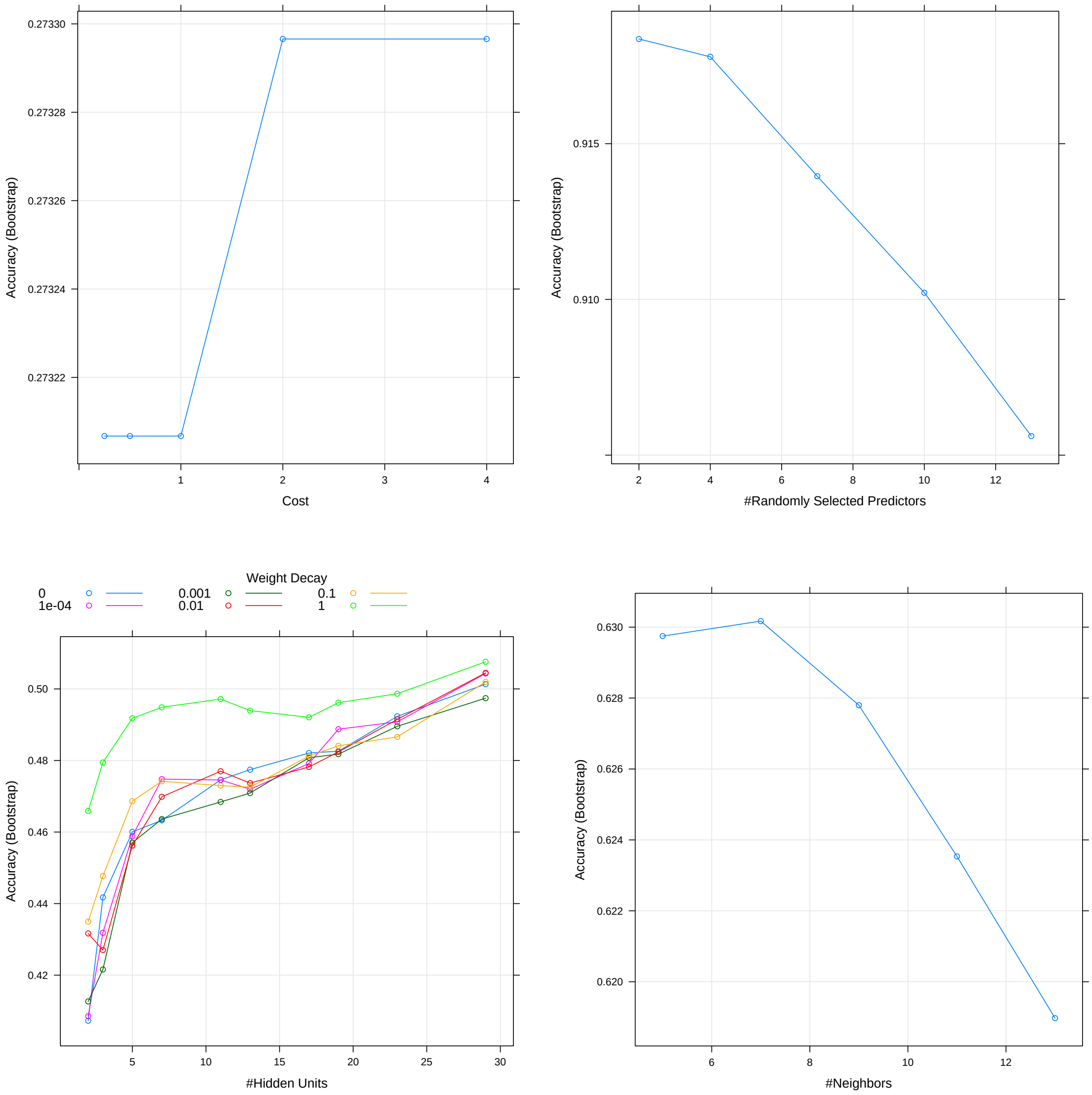


Figure S8b. Tuning parameters used to select the "optimal" classification models using the All Species / Nyctophilus Grouped dataset. There were no tuning parameters for linear or quadratic discriminant function analysis models. From left to right, top to bottom: support vector machine, random forest, artificial neural network, and *k*-nearest neighbour models.

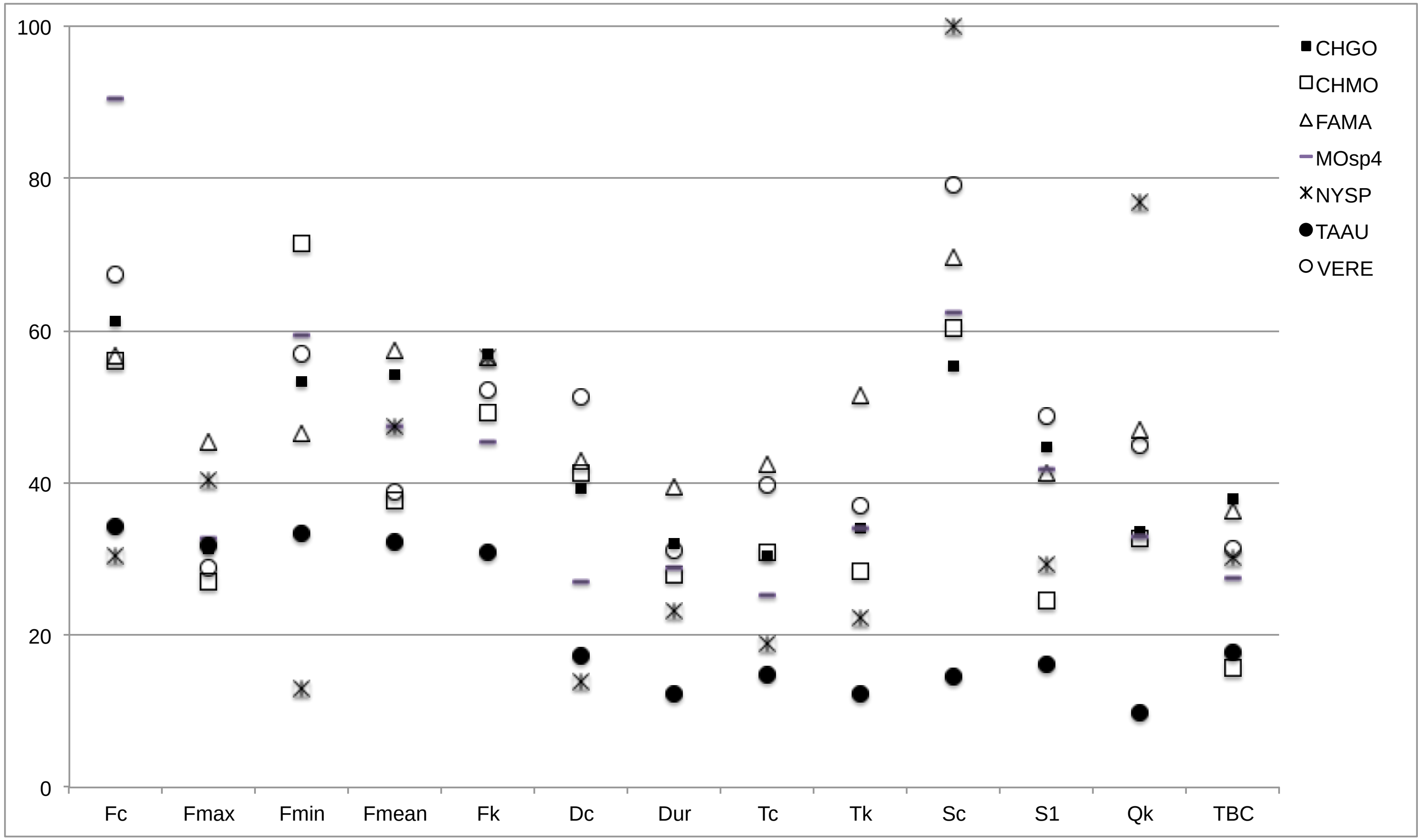


Figure S9. Parameter importance (scaled) values characterising the random forest model built using the original, unprocessed parameters and All Species / *Nyctophilus* Grouped dataset. Values of 100 indicate high importance while values of 0 indicate low importance for model building. Parameter abbreviations: Fc characteristic frequency; Fmax maximum frequency; Fmin minimum frequency; Fk frequency at knee; Dc duration of body of call; Dur duration; Tc time until end of characteristic slope; Tk time until knee; Sc characteristic slope; S1 initial slope; Qk quality at the knee; and TBC time between successive calls. Species abbreviations: CHGO *Chalinolobus gouldii*; CHMO *C. morio*; FAMA *Falsistrellus mackenziei*; MOsp4 *Mormopterus kitcheneri*; NYSP *Nyctophilus* spp.; TAAU *Tadarida australis*; and VERE *Vespadelus regulus*.

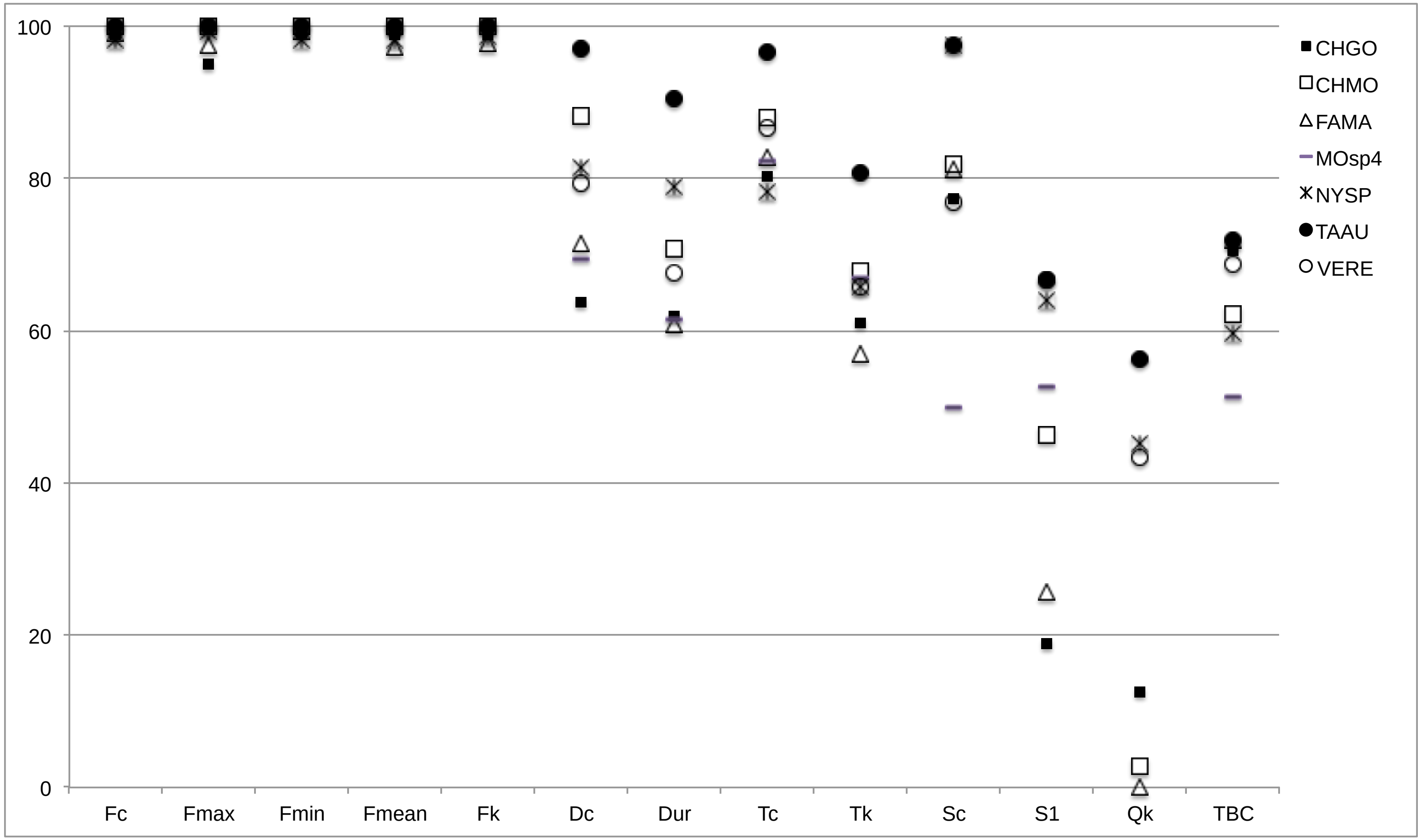


Figure S10. Parameter importance (scaled) values characterising all models, other than random forest, built using the original, unprocessed parameters and All Species / *Nyctophilus* Grouped dataset. Values of 100 indicate high importance while values of 0 indicate low importance for model building. Parameter abbreviations: Fc characteristic frequency; Fmax maximum frequency; Fmin minimum frequency; Fk frequency at knee; Dc duration of body of call; Dur duration; Tc time until end of characteristic slope; Tk time until knee; Sc characteristic slope; S1 initial slope; Qk quality at the knee; and TBC time between successive calls. Species abbreviations: CHGO *Chalinolobus gouldii*; CHMO *C. morio*; FAMA *Falsistrellus mackenziei*; MOsp4 *Mormopterus kitcheneri*; NYSP *Nyctophilus* spp.; TAAU *Tadarida australis*; and VERE *Vespadelus regulus*.

Literature Cited

Abbott, I. 1995. Prodromus of the occurrence and distribution of insect species in the forested part of south-west Western Australia. CALM Science **1**:365-464.

Adams, M. D., B. S. Law, and K. O. French. 2005. Effect of lights on activity levels of forest bats: increasing the efficiency of surveys and species identification. Wildlife Research **32**:173-182.

Adams, M. D., B. S. Law, and K. O. French. 2009. Vegetation structure influences the vertical stratification of open- and edge-space aerial-foraging bats in harvested forests. Forest Ecology and Management **258**:2090-2100.

Adams, M. D., B. S. Law, and M. S. Gibson. 2010. Reliable automation of bat call identification for eastern New South Wales, Australia, using classification trees and AnaScheme software. Acta Chiropterologica **12**:231-245.

Akamatsu, T., D. Wang, K. Wang, S. Li, S. Dong, X. Zhao, J. Barlow, B. S. Stewart, and M. Richlen. 2008. Estimation of the detection probability for Yangtze finless porpoises (*Neophocaena phocaenoides asiaeorientalis*) with a passive acoustic method. The Journal of the Acoustical Society of America **123**:4403-4411.

Alberdi, A., I. Garin, O. Aizpurua, and J. Aihartza. 2012. The Foraging Ecology of the Mountain Long-Eared Bat *Plecotus macrobullaris* Revealed with DNA Mini-Barcodes. PLoS ONE **7**:e35692.

Albrecht, M., P. Duelli, B. Schmid, and C. B. MÜLler. 2007. Interaction diversity within quantified insect food webs in restored and adjacent intensively managed meadows. Journal of Animal Ecology **76**:1015-1025.

Aldridge, H. D. J. N., and R. M. Brigham. 1988. Load Carrying and Maneuverability in an Insectivorous Bat: A Test of The 5% "Rule" of Radio-Telemetry. Journal of Mammalogy **69**:379-382.

Aldridge, H. D. J. N., and I. L. Rautenbach. 1987. Morphology, Echolocation and Resource Partitioning in Insectivorous Bats. Journal of Animal Ecology **56**:763-778.

Amarasekare, P. 2008. Spatial Dynamics of Foodwebs. Annual Review of Ecology, Evolution, and Systematics **39**:479-500.

Anderson, D. R. 2008. Model based inferences in the life sciences: a primer on evidence. Springer, London.

Andreas, M., A. Reiter, and P. Benda. 2012. Prey selection and seasonal diet changes in the western barbastelle bat (*Barbastella barbastellus*). Acta Chiropterologica **14**:81-92.

Andrew, R. L., L. Bernatchez, A. Bonin, C. A. Buerkle, B. C. Carstens, B. C. Emerson, D. Garant, T. Giraud, N. C. Kane, S. M. Rogers, J. Slate, H. Smith, V. L. Sork, G. N. Stone, T. H. Vines, L. Waits, A. Widmer, and L. H. Rieseberg. 2013. A road map for molecular ecology. Molecular Ecology **22**:2605-2626.

Anthony, E., L. P., and T. H. Kunz. 1977. Feeding Strategies of the Little Brown Bat, *Myotis Lucifugus*, in Southern New Hampshire. Ecology **58**:775-786.

Armitage, D. W., and H. K. Ober. 2010. A comparison of supervised learning techniques in the classification of bat echolocation calls. Ecological Informatics **5**:465-473.

Armitage, D. W., and H. K. Ober. 2012. The effects of prescribed fire on bat communities in the longleaf pine sandhills ecosystem. Journal of Mammalogy **93**:102-114.

Armstrong, K. N., and T. R. Reardon. 2006. Standardising the common names of Australian bats - an update. The Australasian Bat Society Newsletter **26**:37-42.

Arnett, E. B. 2003. Advancing science and partnerships for the conservation of bats and their habitats. Wildlife Society Bulletin **31**:2-5.

Barclay, R. M. R. 1999. Bats are not birds - A cautionary note on using echolocation calls to identify bats: A comment. Journal of Mammalogy **80**:290-296.

Barclay, R. M. R., M. A. Dolan, and A. Dyck. 1991. The digestive efficiency of insectivorous bats. Canadian Journal of Zoology-Revue Canadienne De Zoologie **69**:1853-1856.

Barlow, J., and B. L. Taylor. 2005. Estimates of sperm whale abundance in the northeastern temperate pacific from a combined acoustic and visual survey. Marine Mammal Science **21**:429-445.

Barnagaud, J.-Y., V. Devictor, F. Jiguet, M. Barbet-Massin, I. Le Viol, and F. Archaux. 2012. Relating Habitat and Climatic Niches in Birds. PLoS ONE **7**:e32819.

Barton, K. 2013. MuMIn: Multi-model infernce.

Batini, F. 2007. The response of Australian forests to a drying climate: A case study of the jarrah forest. Australian Forestry **70**:213-214.

Becker, N. I., J. A. Encarnacao, M. Tschapka, and E. K. V. Kalko. 2013. Energetics and life-history of bats in comparison to small mammals. Ecological Research **28**:249-258.

Bell, D. T., and E. M. Heddle. 1989. Floristic, morphologic and vegetational diversity. Pages 53-66 in B. Dell, J. J. Havel, and N. Malajczuk, editors. The Jarrah Forest: A complex mediterranean ecosystem. Kluwer Academic Publishers, Dordrect, The Netherlands.

Bell, D. T., and R. J. Hobbs. 2007. Jarrah forest ecosystem restoration: A foreword. Restoration Ecology **15**:S1-S2.

Bergsten, J., D. T. Bilton, T. Fujisawa, M. Elliott, M. T. Monaghan, M. Balke, L. Hendrich, J. Geijer, J. Herrmann, G. N. Foster, I. Ribera, A. N. Nilsson, T. G. Barraclough, and A. P. Vogler. 2012. The Effect of Geographical Scale of Sampling on DNA Barcoding. Systematic Biology **61**:851-869.

Berthinussen, A., and J. Altringham. 2012. The effect of a major road on bat activity and diversity. Journal of Applied Ecology **49**:82-89.

Bishop, C. M. 1995. Neural networks for pattern recognition.

Bjornstad, O. N. 2012. ncf: spatial nonparametric covariance functions.

Bohm, M., B. Collen, J. E. M. Baillie, P. Bowles, J. Chanson, N. Cox, G. Hammerson, M. Hoffmann, S. R. Livingstone, M. Ram, A. G. J. Rhodin, S. N. Stuart, P. P. van Dijk, B. E. Young, L. E. Afuang, A. Aghasyan, A. Garcia, C. Aguilar, R. Ajtic, F. Akarsu, L. R. V. Alencar, A. Allison, N. Ananjeva, S. Anderson, C. Andren, D. Ariano-Sanchez, J. C. Arredondo, M. Auliya, C. C. Austin, A. Avci, P. J. Baker, A. F. Barreto-Lima, C. s. L. Barrio-Amorós, D. Basu, M. F. Bates, et al. 2013. The conservation status of the world's reptiles. Biological Conservation **157**:372-385.

Bohmann, K., A. Monadjem, C. Lehmkuhl Noer, M. Rasmussen, M. R. K. Zeale, E. Clare, G. Jones, E. Willerslev, and M. T. P. Gilbert. 2011. Molecular Diet Analysis of Two African Free-Tailed Bats (Molossidae) Using High Throughput Sequencing. PLoS ONE **6**:e21441.

Borkin, K. M., and S. Parsons. 2011. Home range and habitat selection by a threatened bat in exotic plantation forest. Forest Ecology and Management **262**:845-852.

Boucek, R. E., and J. S. Rehage. 2014. Climate extremes drive changes in functional community structure. Global Change Biology **20**:1821-1831.

Boughey, K. L., I. R. Lake, K. A. Haysom, and P. M. Dolman. 2011. Effects of landscape-scale broadleaved woodland configuration and extent on roost location for six bat species across the UK. Biological Conservation **144**:2300-2310.

Boyer, S., S. D. J. Brown, R. A. Collins, R. H. Cruickshank, M.-C. Lefort, J. Malumbres-Olarte, and S. D. Wratten. 2012. Sliding Window Analyses for Optimal Selection of Mini-Barcodes, and Application to 454-Pyrosequencing for Specimen Identification from Degraded DNA. PLoS ONE **7**:e38215.

Boyles, J. G. 2009. Describing roosts used by forest bats: the importance of microclimate. Acta Chiropterologica **9**:297-303.

Bradshaw, C. J. A. 2012. Little left to lose: Deforestation and forest degradation in Australia since European colonization. Journal of Plant Ecology **5**:109-120.

Breiman, L. 2001. Random Forests. Machine Learning **45**:5-32.

Brigham, R. M., R. L. Francis, and S. Hamdorf. 1997. Microhabitat use by two species of *Nyctophilus* bats: a test of ecomorphology theory. Australian Journal of Zoology **45**:553-560.

Britton, A., and G. Jones. 1999. Echolocation behaviour and prey-capture success in foraging bats: laboratory and field experiments on *Myotis daubentonii*. J. Exp. Biol. **202**:1793-1801.

Britzke, E. R., J. E. Duchamp, K. L. Murray, R. K. Swihart, and L. W. Robbins. 2011. Acoustic Identification of Bats in the Eastern United States: A Comparison of Parametric and Nonparametric Methods. Journal of Wildlife Management **75**:660-667.

Britzke, E. R., E. H. Gillam, and K. L. Murray. 2013. Current state of understanding of ultrasonic detectors for the study of bat ecology. Acta Theriologica **58**:109-117.

Broders, H. G., C. S. Findlay, and L. Zheng. 2004. Effects of Clutter on Echolocation Call Structure of *Myotis septentrionalis* and *M. lucifugus*. Journal of Mammalogy **85**:273-281.

Broders, H. G., and G. J. Forbes. 2004. Interspecific and Intersexual Variation in Roost-Site Selection of Northern Long-Eared and Little Brown Bats in the Greater Fundy National Park Ecosystem. The Journal of Wildlife Management **68**:602-610.

Broders, H. G., G. J. Forbes, S. Woodley, and I. D. Thompson. 2006. Range extent and stand selection for roosting and foraging in forest-dwelling northern long-eared bats and little brown bats in the Greater Fundy Ecosystem, New Brunswick. Journal of Wildlife Management **70**:1174-1184.

Brouwers, N., G. Matusick, K. Ruthrof, T. Lyons, and G. Hardy. 2013. Landscape-scale assessment of tree crown dieback following extreme drought and heat in a Mediterranean eucalypt forest ecosystem. Landscape Ecology **28**:69-80.

Brown, D. S., R. Burger, N. Cole, D. Vencatasamy, C. EL, A. Montazam, and W. O. C. Symondson. 2013. Dietary competition between the alien Asian Musk Shrew (*Suncus murinus*) and a re-introduced population of Telfair's Skink (*Leiolopisma telfairii*). Molecular Ecology.

Brudvig, L. A. 2011. The restoration of biodiversity: where has research been and where does it need to go? American Journal of Botany **98**:549-558.

Buchalski, M. R., J. B. Fontaine, P. A. Heady, III, J. P. Hayes, and W. F. Frick. 2013. Bat Response to Differing Fire Severity in Mixed-Conifer Forest California, USA. PLoS ONE **8**:e57884.

Bullen, R., and N. L. McKenzie. 2001. Bat airframe design: flight performance, stability and control in relation to foraging ecology. Australian Journal of Zoology **49**:235-261.

Bullen, R., and N. L. McKenzie. 2002. Differentiating Western Australian *Nyctophilus* (Chiroptera: Vespertilionidae) echolocation calls. Australian Mammalogy:89-93.

Bunge , J., and M. Fitzpatrick. 1993. Estimating the Number of Species: A Review. Journal of the American Statistical Association **88**:364-373.

Bunn, S. E. 1983. Termite (Isoptera) fauna of jarrah forest in the Wagerup-Willowdale region, Western Australia: Relevance to the rehabilitation of Bauxite minesites. Forest Ecology and Management **6**:169-177.

Burnham, K. P., and D. R. Anderson 2002. Model selection and multi-model inference: a practical information-theoretic approach. Springer, New York.

Burrows, N. D. 2008. Linking fire ecology and fire management in south-west Australian forest landscapes. Forest Ecology and Management **255**:2394-2406.

Campbell, S. 2009. So long as it's near water: variable roosting behaviour of the large-footed myotis (*Myotis macropus*). Australian Journal of Zoology **57**:89-98.

Campbell, S., G. Coulson, and L. F. Lumsden. 2010. Divergent microclimates in artificial and natural roosts of the large-footed myotis (*Myotis macropus*). Acta Chiropterologica **12**:173-185.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Pena, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight. 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods **7**:335-336.

Carter, T. C., M. A. Menzel, D. M. Krishon, and J. Laerm. 1998. Prey selection by five species of vespertilionid bats on Sapelo Island, Georgia. Brimleyana:158-170.

Chao, A. 1984. Nonparametric Estimation of the Number of Classes in a Population. Scandinavian Journal of Statistics **11**:265-270.

Chen, S.-F., G. Jones, and S. J. Rossiter. 2009. Determinants of echolocation call frequency variation in the Formosan lesser horseshoe bat (*Rhinolophus monoceros*). Proceedings of the Royal Society B: Biological Sciences **276**:3901-3909.

Christie, K. 2011. Movement patterns by *Egernia napoleonis* following reintroduction into restored jarrah forest. Wildlife research (East Melbourne) **38**:475.

Christie, K., M. D. Craig, V. L. Stokes, and R. J. Hobbs. 2012. Home Range Size and Micro-habitat Density Requirements of *Egernia napoleonis*: Implications for Restored Jarrah Forest of South Western Australia. Restoration Ecology **20**: 740-746.

Churchill, S. 2008. Australian bats. Allen & Unwin.

Ciechanowski, M., T. Zajac, A. Bitas, and R. Dunajski. 2007. Spatiotemporal variation in activity of bat species differing in hunting tactics: effects of weather, moonlight, food abundance, and structural clutter. Canadian Journal of Zoology-Revue Canadienne De Zoologie **85**:1249-1263.

Clare, E. L., B. R. Barber, B. W. Sweeney, P. D. N. Hebert, and M. B. Fenton. 2011. Eating local: influences of habitat on the diet of little brown bats (*Myotis lucifugus*). Molecular Ecology **20**:1772-1780.

Clare, E. L., E. E. Fraser, H. E. Braid, M. B. Fenton, and P. D. N. Hebert. 2009. Species on the menu of a generalist predator, the eastern red bat (*Lasiurus borealis*): using a molecular approach to detect arthropod prey. Molecular Ecology **18**:2532-2542.

Clement, M. J., K. L. Murray, D. I. Solick, and J. C. Gruver. 2014. The effect of call libraries and acoustic filters on the identification of bat echolocation. Ecology and Evolution **4**:3482-3493.

Cooper, S. J. B., M. S. Harvey, K. M. Saint, and B. Y. Main. 2011. Deep phylogeographic structuring of populations of the trapdoor spider *Moggridgea tingle* (Migidae) from southwestern Australia: evidence for long-term refugia within refugia. Molecular Ecology **20**:3219-3236.

Cortes, C., and V. Vapnik. 1995. Support-vector networks. Machine Learning **20**:273-297.

Craig, M. D., A. H. Grigg, R. J. Hobbs, and G. E. S. J. Hardy. 2014. Does coarse woody debris density and volume influence the terrestrial vertebrate community in restored bauxite mines? Forest Ecology and Management **318**:142-150.

Craig, M. D., G. E. S. J. Hardy, J. B. Fontaine, M. J. Garkakalis, A. H. Grigg, C. D. Grant, P. A. Fleming, and R. J. Hobbs. 2012. Identifying unidirectional and dynamic habitat filters to faunal recolonisation in restored mine-pits. Journal of Applied Ecology **49**:919-928.

Craig, M. D., R. J. Hobbs, A. H. Grigg, M. J. Garkaklis, C. D. Grant, P. A. Fleming, and G. Hardy. 2010. Do Thinning and Burning Sites Revegetated after Bauxite Mining Improve Habitat for Terrestrial Vertebrates? Restoration Ecology **18**:300-310.

Craig, M. D., and J. D. Roberts. 2005. The short-term impacts of logging on the jarrah forest avifauna in south-west Western Australia: implications for the design and analysis of logging experiments. Biological Conservation **124**:177-188.

Cristescu, R. H., C. Frere, and P. B. Banks. 2012. A review of fauna in mine rehabilitation in Australia: Current state and future directions. Biological Conservation **149**:60-72.

Davis, A. L. V., R. J. Van Aarde, C. H. Scholtz, and J. H. Delport. 2002. Increasing representation of localized dung beetles across a chronosequence of regenerating vegetation and natural dune forest in South Africa. Global Ecology and Biogeography **11**:191-209.

Dawson, D. K., and M. G. Efford. 2009. Bird population density estimated from acoustic signals. Journal of Applied Ecology **46**:1201-1209.

De'ath, G., and K. E. Fabricius. 2000. Classification and Regression Trees: A Powerful Yet Simple Technique for Ecological Data Analysis. Ecology **81**:3178-3192.

Dell, B., J. J. Havel, and N. Malajczuk, editors. 1989. The Jarrah Forest: A complex mediterranean ecosystem. Kluwer Academic Publishers, Dordrect, The Netherlands.

Dietz, M., and A. Horig. 2011. Thermoregulation of tree-dwelling temperate bats - a behavioural adaptation to force live history strategy. Folia Zoologica **60**:5-16.

Dietz, M., and E. K. V. Kalko. 2007. Reproduction affects flight activity in female and male Daubenton's bats, *Myotis daubentoni*. Canadian Journal of Zoology **85**:653-664.

Digby, A., M. Towsey, B. D. Bell, and P. D. Teal. 2013. A practical comparison of manual and autonomous methods for acoustic monitoring. Methods in Ecology and Evolution **4**:675-683.

Dixon, K. W. 2009. Pollination and Restoration. Science **325**:571-573.

Dobson, A., D. Lodge, J. Alder, G. S. Cumming, J. Keymer, J. McGlade, H. Mooney, J. A. Rusak, O. Sala, V. Wolters, D. Wall, R. Winfree, and M. A. Xenopoulos. 2006. Habitat loss, trophic collapse, and the decline of ecosystem services. Ecology **87**:1915-1924.

Dobson, A. P., A. D. Bradshaw, and A. J. M. Baker. 1997. Hopes for the Future: Restoration Ecology and Conservation Biology. Science **277**:515-522.

Dodd, L. E., E. G. Chapman, J. D. Harwood, M. J. Lacki, and L. K. Rieske. 2012a. Identification of prey of Myotis septentrionalis using DNA-based techniques. Journal of Mammalogy **93**:1119-1128.

Dodd, L. E., M. J. Lacki, E. R. Britzke, D. A. Buehler, P. D. Keyser, J. L. Larkin, A. D. Rodewald, T. B. Wigley, P. B. Wood, and L. K. Rieske. 2012b. Forest structure affects trophic linkages: How silvicultural disturbance impacts bats and their insect prey. Forest Ecology and Management **267**:262-270.

Drummond, A. J., B. Ashton, S. Buxton, M. Cheung, A. Cooper, C. Duran, J. Heled, M. Kearse, S. Markowitz, R. Moir, S. Stones-Havas, S. Sturrock, F. Swidan, T. Thierer, and A. Wilson. 2012. Geneious v5.6, Available from http://www.geneious.com.

Duchamp, J. E., and R. K. Swihart. 2008. Shifts in bat community structure related to evolved traits and features of human-altered landscapes. Landscape Ecology **23**:849-860.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research **32**:1792-1797.

Elmore, L. W., D. A. Miller, and F. J. Vilella. 2004. Selection of diurnal roosts by red bats (*Lasiurus borealis*) in an intensively managed pine forest in Mississippi. Forest Ecology and Management **199**:11-20.

Ethier, K., and L. Fahrig. 2011. Positive effects of forest fragmentation, independent of forest amount, on bat abundance in eastern Ontario, Canada. Landscape Ecology **26**:865-876.

Faith, D. P. 1992. Conservation evaluation and phylogenetic diversity. Biological Conservation **61**:1-10.

Faith , D. P., and A. M. Baker. 2006. Phylogenetic diversity (PD) and biodiversity conservation: some bioinformatics challenges. Evolutionary Bioinformatics **2**:121-128.

Farr, J. D., A. J. Wills, P. F. Van Heurck, A. E. Mellican, and M. R. Williams. 2011. Forestcheck: the response of macro-invertebrates to silviculture in jarrah (*Eucalyptus marginata*) forest. Australian Forestry **74**:315-327.

Farrow, L. J. F. L. J., and H. G. Broders. 2011. Loss of forest cover impacts the distribution of the forest-dwelling tri-colored bat (*Perimyotis subflavus*). Mammalian Biology **76**:172-179.

Faure, P. A., and R. M. R. Barclay. 1994. Substrate-gleaning versus aerial-hawking: plasticity in the foraging and echolocation behaviour of the long-eared bat, *Myotis evotis*. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology **174**:651-660.

Faure, P. A., J. H. Fullard, and R. M. R. Barclay. 1990. The response of tympanate moths to the echolocation calls of a substrate gleaning bat, *Myotis evotis*. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology **166**:843-849.

Fenton, M. B. 1990. The foraging behavior and ecology of animal-eating bats. Canadian Journal of Zoology-Revue Canadienne De Zoologie **68**:411-422.

Fenton, M. B. 1994. Assessing signal variability and reliability - to thine ownself be true. Animal Behaviour **47**:757-764.

Fenton, M. B. 1999. Describing the echolocation calls and behaviour of bats. Acta Chiropterologica **1**:127-136.

Fenton, M. B. 2003. Science and the conservation of bats: Where to next? Wildlife Society Bulletin **31**:6-15.

Fenton, M. B., Cumming, David H. M., Rautenbach, I. L., Cumming, Graeme S., Cumming, Meg S., Ford, Gavin, Taylor, Russell D., Dunlop, Jenna, Hovorka, Mark D., Johnston, Dave S., Portfors, Christine V., Kalcounis, Matina C., Mahlanga, Zaccheus. 1998. Bats and the Loss of Tree Canopy in African Woodlands. Conservation Biology **12**:399.

Fenton, M. B., and D. R. Griffin. 1997. High-altitude pursuit of insects by echolocating bats. Journal of Mammalogy **78**:247-250.

Fenton, M. B., C. V. Portfors, I. L. Rautenbach, and J. M. Waterman. 1998. Compromises: Sound frequencies used in echolocation by aerial-feeding bats. Canadian Journal of Zoology **76**:1174-1182.

Fenton, M. B., I. L. Rautenbach, S. E. Smith, C. M. Swanepoel, J. Grosell, and J. van Jaarsveld. 1994. Raptors and bats: threats and opportunities. Animal Behaviour **48**:9-18.

Fischer, J., J. Stott, and B. S. Law. 2010. The disproportionate value of scattered trees. Biological Conservation **143**:1564-1567.

Fisher, R. A. 1936. The use of multiple measurements in taxonomic problems. Annals of Human Genetics **7**:179-188.

Fix, E., and J. L. Hodges. 1951. Discriminatory analysis. Nonparametric discrimination: Consistency properties. Pages 238-247. Technical Report 4, Randolph Field, Texas.

Floyd, R., E. Abebe, A. Papert, and M. Blaxter. 2002. Molecular barcodes for soil nematode identification. Molecular Ecology **11**:839-850.

Fonseca, C. R. 2009. The Silent Mass Extinction of Insect Herbivores in Biodiversity Hotspots. Conservation Biology **23**:1507-1515.

Fornůsková, A., E. J. Petit, T. Bartonička, P. Kaňuch, A. Butet, Z. Řehák, and J. Bryja. 2014. Strong matrilineal structure in common pipistrelle bats (*Pipistrellus*

pipistrellus) is associated with variability in echolocation calls. Biological Journal of the Linnean Society:n/a-n/a.

Fournier, D., H. Skaug, J. Ancheta, J. Ianelli, A. Magnusson, M. Maunder, A. Nielsen, and J. Sibert. 2012. AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. Optimum Methods Software **27**:233-249.

Freitas, M. B., L. S. Goulart, M. S. Barros, D. B. Morais, T. S. Amaral, and S. L. P. Matta. 2010. Energy metabolism and fasting in male and female insectivorous bats *Molossus molossus* (Chiroptera: Molossidae). Brazilian Journal of Biology **70**:617-621.

Frey-Ehrenbold, A., F. Bontadina, R. Arlettaz, and M. K. Obrist. 2013. Landscape connectivity, habitat structure and activity of bat guilds in farmland-dominated matrices. Journal of Applied Ecology **50**:252-261.

Fullard, J. H., C. Koehler, A. Surlykke, and N. L. McKenzie. 1991. Echolocation ecology and flight morphology of insectivorous bats (Chiroptera) in south-western Australia. Australian Journal of Zoology **39**:427-438.

Gardner, J. H., and D. T. Bell. 2007. Bauxite mining restoration by alcoa world alumina Australia in Western Australia: Social, political, historical, and environmental contexts. Restoration Ecology **15**:S3-S10.

Gibb, H., and S. A. Cunningham. 2010. Revegetation of farmland restores function and composition of epigaeic beetle assemblages. Biological Conservation **143**:677-687.

Goldingay, R. L. 2009. Characteristics of tree hollows used by Australian birds and bats. Wildlife Research **36**:394-409.

Goldingay, R. L., and J. R. Stevens. 2009. Use of artificial tree hollows by Australian birds and bats. Wildlife Research **36**:81-97.

Gould, E. 1955. The Feeding Efficiency of Insectivorous Bats. Journal of Mammalogy **36**:399-407.

Grant, C. D. 2006. State-and-transition successional model for bauxite mining rehabilitation in the jarrah forest of western Australia. Restoration Ecology **14**:28-37.

Grant, C. D., J. M. Koch, D. T. Bell, and W. A. Loneragan. 1997. Tree species response to prescribed burns in rehabilitated bauxite mines in Western Australia. Australian Forestry **60**:84-89.

Grant, J. D. A. 1991. Prey location by 2 Australian long-eared bats, *Nyctophilus-Gouldi* and *N-Geoffroyi*. Australian Journal of Zoology **39**:45-56.

Greenslade, P., and J. D. Majer. 1993. Recolonization by collembola of rehabilitated bauxite mines in Western Australia. Australian Journal of Ecology **18**:385-394.

Grigg, A. H., M. A. Norman, and C. D. Grant. 2010. Prescribed burning of thinning slash in regrowth stands of jarrah (*Eucalyptus marginata*) following bauxite mining in south-west Australia. International Journal of Wildland Fire **19**:737-745.

Hagen, E. M., and J. L. Sabo. 2011. A landscape perspective on bat foraging ecology along rivers: does channel confinement and insect availability influence the response of bats to aquatic resources in riverine landscapes? Oecologia **166**:751-760.

Hallett, L. M., S. Diver, M. V. Eitzel, J. J. Olson, B. S. Ramage, H. Sardinas, Z. Statman-Weil, and K. N. Suding. 2013. Do We Practice What We Preach? Goal Setting for Ecological Restoration. Restoration Ecology **21**:312-319.

Hanspach, J., J. Fischer, K. Ikin, J. Stott, and B. S. Law. 2012. Using trait-based filtering as a predictive framework for conservation: a case study of bats on farms in southeastern Australia. Journal of Applied Ecology **49**:842-850.

Harris, J. 2009. Soil Microbial Communities and Restoration Ecology: Facilitators or Followers? Science **325**:573-574.

Hebert, P. D. N., A. Cywinska, S. L. Ball, and J. R. deWaard. 2003a. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences **270**:313-321.

Hebert, P. D. N., S. Ratnasingham, and J. R. de Waard. 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London. Series B: Biological Sciences **270**:S96-S99.

Heiberger, R. M. 2013. HH: Statistical Analysis and Data Display: Heiberger and Holland.

Hobbs, R. J., and V. A. Cramer. 2008. Restoration Ecology: Interventionist Approaches for Restoring and Maintaining Ecosystem Function in the Face of Rapid Environmental Change. Pages 39-61. Annual review of environment and resources. Annual Reviews, Palo Alto.

Hobbs, R. J., and J. A. Harris. 2001. Restoration Ecology: Repairing the Earth's Ecosystems in the New Millennium. Restoration Ecology **9**:239-246.

Hope, P. R., and G. Jones. 2012. Warming up for dinner: torpor and arousal in hibernating Natterer's bats (*Myotis nattereri*) studied by radio telemetry. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology **182**:569-578.

Humes, M. L., J. P. Hayes, and M. W. Collopy. 1999. Bat Activity in Thinned, Unthinned, and Old-Growth Forests in Western Oregon. The Journal of Wildlife Management **63**:553-561.

Hunter, A., A. Macgregor, T. Szabo, C. Wellington, and M. Bellgard. 2012. Yabi: An online research environment for grid, high performance and cloud computing. Source Code for Biology and Medicine **7**:1.

Huson, D. H., S. Mitra, H.-J. Ruscheweyh, and S. C. S. N. Weber. 2011. Integrative analysis of environmental sequences using MEGAN 4. Genome Res. **21**:1552-1560.

Inkster-Draper, T. E., M. Sheaves, C. N. Johnson, and S. K. A. Robson. 2013. Prescribed fire in eucalypt woodlands: immediate effects on a microbat community of northern Australia. Wildlife Research:-.

Jakobsen, L., and A. Surlykke. 2010. Vespertilionid bats control the width of their biosonar sound beam dynamically during prey pursuit. Proceedings of the National Academy of Sciences **107**:13930-13935.

Jantzen, M. K., and M. B. Fenton. 2013. The depth of edge influence among insectivorous bats at forest-field interfaces. Canadian Journal of Zoology **91**:287-292.

Jennings, N., S. Parsons, and M. J. O. Pocock. 2008. Human vs. machine: identification of bat species from their echolocation calls by humans and by artificial neural networks. Canadian Journal of Zoology **86**:371-377.

Jinbo, U., T. Kato, and M. Ito. 2011. Current progress in DNA barcoding and future implications for entomology. Entomological Science **14**:107-124.

Johnson, J. S., L. E. Dodd, J. D. Kiser, T. S. Peterson, and K. S. Watrous. 2012. Food Habits of *Myotis leibii* along a Forested Ridgetop in West Virginia. Northeastern Naturalist **19**:665-672.

Jones, G., D. S. Jacobs, T. H. Kunz, M. R. Willig, and P. A. Racey. 2009. *Carpe noctem*: the importance of bats as bioindicators. Endangered Species Research **8**:93-115.

Jones, G., and E. C. Teeling. 2006. The evolution of echolocation in bats. Trends in Ecology & Evolution **21**:149-156.

Jones, G., N. Vaughan, and S. Parsons. 2000. Acoustic identification of bats from directly sampled and time expanded recordings of vocalizations. Acta Chiropterologica **2**:155-170.

Jung, K., S. Kaiser, S. Böhm, J. Nieschulze, and E. K. V. Kalko. 2012. Moving in three dimensions: effects of structural complexity on occurrence and activity of insectivorous bats in managed forest stands. Journal of Applied Ecology **49**:523-531.

Jung, K., and E. K. V. Kalko. 2011. Adaptability and vulnerability of high flying Neotropical aerial insectivorous bats to urbanization. Diversity and Distributions **17**:262-274.

Jung, K., J. s. Molinari, and E. K. V. Kalko. 2014. Driving Factors for the Evolution of Species-Specific Echolocation Call Design in New World Free-Tailed Bats (Molossidae). PLoS ONE **9**:e85279.

Kalcounis-Ruppell, M. C., J. M. Psyllakis, and R. M. Brigham. 2005. Tree roost selection by bats: an empirical synthesis using meta-analysis. Wildlife Society Bulletin **33**:1123-1132.

Kalka , M., and E. K. V. Kalko. 2006. Gleaning bats as underestimated predators of herbivorous insects: diet of *Micronycteris microtis* (Phyllostomidae) in Panama. Journal of Tropical Ecology **22**:1-10.

Karatzoglou, A., A. Smola, K. Hornik, and A. Zeileis. 2004. kernlab - An S4 Package for Kernel Methods in R. Journal of Statistical Software **11**:1-20.

Kingston, T., G. Jones, A. Zubaid, and T. H. Kunz. 2000. Resource partitioning in rhinolophoid bats revisited. Oecologia **124**:332-342.

Klausmeyer, K. R., and M. R. Shaw. 2009. Climate Change, Habitat Loss, Protected Areas and the Climate Adaptation Potential of Species in Mediterranean Ecosystems Worldwide. PLoS ONE **4**:e6392.

Knight, R. 1999. Recalcitrant recolonisers: assessment of rehabilitation success through study of small faunal assemblages. Pages 53-64 in C. J. Asher, and L. C. Bell, editors. Proceedings of the Workshop on Indicators of Ecosystem Rehabilitation Success, Melbourne, Victoria, 23-24 October 1998. Australian Centre for Mining Environmental Research.

Koch, J., A. Grigg, R. Gordon, and J. Majer. 2010. Arthropods in coarse woody debris in jarrah forest and rehabilitated bauxite mines in Western Australia. Annals of Forest Science **67**:106-106.

Koch, J. M. 2007a. Alcoa's mining and restoration process in south Western Australia. Restoration Ecology **15**:S11-S16.

Koch, J. M. 2007b. Restoring a jarrah forest understorey vegetation after bauxite mining in Western Australia. Restoration Ecology **15**:S26-S39.

Koch, J. M., and R. J. Hobbs. 2007. Synthesis: is Alcoa successfully restoring a jarrah forest ecosystem after bauxite mining in Western Australia? Restoration Ecology **15**:S137-S144.

Koch, J. M., and S. C. Ward. 2005. Thirteen-year growth of jarrah (*Eucalyptus marginata*) on rehabilitated bauxite mines in south-western Australia. Australian Forestry **68**:176-185.

Kuhn, M. 2008. Building Predictive Models in R Using the caret Package. Journal of Statistical Software **28**:1-26.

Kunz, T. H., E. B. de Torrez, D. Bauer, T. Lobova, and T. H. Fleming. 2011. Ecosystem services provided by bats. Annals of the New York Academy of Sciences **1223**:1-38.

Kunz, T. H., and A. A. Stern. 1995. Maternal investment and post-natal growth in bats. Pages 123-138 in P. A. Racey, and S. M. Swift, editors. Ecology, Evolution and Behaviour of Bats.

Kurta, A., G. P. Bell, K. A. Nagy, and T. H. Kunz. 1989. Energetics of Pregnancy and Lactation in Freeranging Little Brown Bats (*Myotis lucifugus*). Physiological Zoology **62**:804-818.

Kuznetsova, A., P. Bruun Brockhoff, and R. H. Bojesen Christensen. 2014. lmerTest: Tests for random and fixed effects for linear mixed effect models (lmer objects of lme4 package).

Lacki, M. J., D. R. Cox, L. E. Dodd, and M. B. Dickinson. 2009. Response of northern bats (*Myotis septentrionalis*) to prescribed fires in eastern Kentucky forests. Journal of Mammalogy **90**:1165-1175.

Larson, D. J., and J. P. Hayes. 2000. Variability in sensitivity of Anabat II bat detectors and a method of calibration. Acta Chiropterologica **2**:209-213.

Law, B., and M. Chidel. 2002. Tracks and riparian zones facilitate the use of Australian regrowth forest by insectivorous bats. Journal of Applied Ecology **39**:605-617.

Law, B. S., and J. Anderson. 2000. Roost preferences and foraging ranges of the eastern forest bat Vespadelus pumilus under two disturbance histories in northern New South Wales, Australia. Austral Ecology **25**:352-367.

Law, B. S., J. Anderson, and M. Chidel. 1999. Bat communities in a fragmented forest landscape on the south-west slopes of New South Wales, Australia. Biological Conservation **88**:333-345.

Law, B. S., and M. Chidel. 2001. Bat activity 22 years after first-round intensive logging of alternate coupes near Eden, New South Wales. Australian Forestry **64**:242-247.

Law, B. S., and M. Chidel. 2006. Eucalypt plantings on farms: Use by insectivorous bats in south-eastern Australia. Biological Conservation **133**:236-249.

Law, B. S., and M. Chidel. 2007. Bats under a hot tin roof: comparing the microclimate of eastern cave bat (*Vespadelus troughtoni*) roosts in a shed and cave overhangs. Australian Journal of Zoology **55**:49-55.

Law, B. S., M. Chidel, and T. Penman. 2011. Do young eucalypt plantations benefit bats in an intensive agricultural landscape? Wildlife Research **38**:173-187.

Law, B. S., and C. R. Dickman. 1998. The use of habitat mosaics by terrestrial vertebrate fauna: implications for conservation and management. Biodiversity and Conservation **7**:323-333.

Lee, Y., Y. Lin, and G. Wahba. 2004. Multicategory support vector machines. Journal of the American Statistical Association **99**:67-81.

Leelapaibul, W., S. Bumrungsri, and A. Pattanawiboon. 2005. Diet of wrinkle-lipped free-tailed bat (*Tadarida plicata* Buchannan, 1800) in central Thailand: insectivorous bats potentially act as biological pest control agents. Acta Chiropterologica **7**:111-119.

Lengyel, S., K. Varga, B. Kosztyi, L. Lontay, E. Deri, P. Torok, and B. Tothmeresz. 2012. Grassland restoration to conserve landscape-level biodiversity: a synthesis of early results from a large-scale project. Applied Vegetation Science **15**:264-276.

Lentini, P. E., P. Gibbons, J. Fischer, B. Law, J. Hanspach, and T. G. Martin. 2012. Bats in a Farming Landscape Benefit from Linear Remnants and Unimproved Pastures. PLoS ONE **7**.

Lewis, S. E. 1995. Roost Fidelity of Bats: A Review. Journal of Mammalogy **76**:481-496.

Liaw, A., and M. Wiener. 2002. Classification and Regression by randomForest. R News **2**:18-22.

Lindell, C. A. 2008. The Value of Animal Behavior in Evaluations of Restoration Success. Restoration Ecology **16**:197-203.

Lloyd, A., B. Law, and R. Goldingay. 2006. Bat activity on riparian zones and upper slopes in Australian timber production forests and the effectiveness of riparian buffers. Biological Conservation **129**:207-220.

Loeb, S. C., and T. A. Waldrop. 2008. Bat activity in relation to fire and fire surrogate treatments in southern pine stands. Forest Ecology and Management **255**:3185-3192.

Lozupone, C., and R. Knight. 2005. UniFrac: a New Phylogenetic Method for Comparing Microbial Communities. Applied and Environmental Microbiology **71**:8228-8235.

Luck, G. W., L. Smallbone, C. Threlfall, and B. Law. 2013. Patterns in bat functional guilds across multiple urban centres in south-eastern Australia. Landscape Ecology **28**:455-469.

Lumsden , L. F., and A. F. Bennett. 2005. Scattered trees in rural landscapes: foraging habitat for insectivorous bats in south-eastern Australia. Biological Conservation **122**:205-222.

Lumsden, L. F., A. F. Bennett, and J. E. Silins. 2002a. Location of roosts of the lesser long-eared bat Nyctophilus geoffroyi and Gould's wattled bat *Chalinolobus gouldii* in a fragmented landscape in south-eastern Australia. Biological Conservation **106**:237-249.

Lumsden, L. F., A. F. Bennett, and J. E. Silins. 2002b. Selection of roost sites by the lesser long-eared bat (*Nyctophilus geoffroyi*) and Gould's wattled bat (*Chalinolobus gouldii*) in south-eastern Australia. Journal of Zoology **257**:207-218.

Lunney, D., J. Barker, D. Priddel, and M. Oconnell. 1988. Roost selection by Goulds Long-eared Bat, *Nyctophilus gouldi tomes* (Chiroptera, Vespertilionidae) in logged forest on the south coast of New South-Wales. Australian Wildlife Research **15**:375-384.

Mabry, C. M., L. A. Brudvig, and R. C. Atwell. 2010. The confluence of landscape context and site-level management in determining Midwestern savanna and woodland breeding bird communities. Forest Ecology and Management **260**:42-51.

Macfarlane, C. 2011. Classification method of mixed pixels does not affect canopy metrics from digital images of forest overstorey. Agricultural and Forest Meteorology **151**:833-840.

Macfarlane, C., M. Hoffman, D. Eamus, N. Kerp, S. Higginson, R. McMurtrie, and M. Adams. 2007. Estimation of leaf area index in eucalypt forest using digital photography. Agricultural and Forest Meteorology **143**:176-188.

Majer, J., B. Heterick, T. Gohr, E. Hughes, L. Mounsher, and A. Grigg. 2013. Is thirty-seven years sufficient for full return of the ant biota following restoration? Ecological Processes **2**:19.

Majer, J. D., and O. G. Nichols. 1998. Long-Term Recolonization Patterns of Ants in Western Australian Rehabilitated Bauxite Mines with Reference to Their Use as Indicators of Restoration Success. Journal of Applied Ecology **35**:161-182.

Marques, T. A., L. Thomas, S. W. Martin, D. K. Mellinger, J. A. Ward, D. J. Moretti, D. Harris, and P. L. Tyack. 2013. Estimating animal population density using passive acoustics. Biological Reviews **88**:287-309.

Maslin, B. R. c. 2001. WATTLE: Acacias of Australia. Australian Biologial Resources Study, Canberra, and Department of Conservation and Land Management, Perth.

Matthews, J. W., A. L. Peralta, D. N. Flanagan, P. M. Baldwin, A. Soni, A. D. Kent, and A. G. Endress. 2009. Relative influence of landscape vs. local factors on plant community assembly in restored wetlands. Ecological Applications **19**:2108-2123.

McConville, A., B. Law, T. Penman, and M. Mahony. 2013. Contrasting habitat use of morphologically similar bat species with differing conservation status in south-eastern Australia. Austral Ecology **39**:83-94.

McLachlan, G. J. 2012. Discriminant analysis. Wiley Interdisciplinary Reviews: Computational Statistics **4**:421-431.

Mellinger, D. K., K. M. Stafford, S. Moore, R. P. Dziak, and H. Matsumoto. 2007. An overveiw of fixed passive acoustic observation methods for cetaceans. Oceanography **20**:36.

Mickleburgh, S. P., A. M. Hutson, and P. A. Racey. 2002. A review of the global conservation status of bats. ORYX **36**:18-34.

Mitchell, A. 2008. DNA barcoding demystified. Australian Journal of Entomology **47**:169-173.

Monadjem, A., M. Ellstrom, C. Maldonaldo, and N. Fasel. 2010. The activity of an insectivorous bat Neoromicia nana on tracks in logged and unlogged forest in tropical Africa. African Journal of Ecology **48**:1083-1091.

Morris, A. D., D. A. Miller, and M. C. Kalcounis-Rueppell. 2010. Use of Forest Edges by Bats in a Managed Pine Forest Landscape. Journal of Wildlife Management **74**:26-34.

Morrison, E. B., and C. A. Lindell. 2012. Birds and bats reduce insect biomass and leaf damage in tropical forest restoration sites. Ecological Applications **22**:1526-1534.

Munro, N. T., J. Fischer, J. Wood, and D. B. Lindenmayer. 2009. Revegetation in agricultural areas: the development of structural complexity and floristic diversity. Ecological Applications **19**:1197-1210.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature **403**:853-858.

Neubaum, D. J., K. R. Wilson, and T. J. O'Shea. 2007. Urban Maternity-Roost Selection by Big Brown Bats in Colorado. Journal of Wildlife Management **71**:728-736.

Nichols, O. G., and C. D. Grant. 2007. Vertebrate fauna recolonization of restored bauxite mines - Key findings from almost 30 years of monitoring and research. Restoration Ecology **15**:S116-S126.

Nickerson, C. M., L. L. Bloomfield, M. R. Dawson, and C. B. Sturdy. 2006. Artificial neural network discrimination of black-capped chickadee (*Poecile atricapillus*) call notes. The Journal of the Acoustical Society of America **120**:1111.

Norberg, U. M., and J. M. V. Rayner. 1987. Ecological Morphology and Flight in Bats (Mammalia; Chiroptera): Wing Adaptations, Flight Performance, Foraging Strategy and Echolocation. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences **316**:335-427.

Norman, M. A., J. M. Koch, C. D. Grant, T. K. Morald, and S. C. Ward. 2006. Vegetation Succession After Bauxite Mining in Western Australia. Restoration Ecology **14**:278-288.

O'Farrell, M. J., C. Corben, W. L. Gannon, and B. W. Miller. 1999a. Confronting the Dogma: A Reply. Journal of Mammalogy **80**:297-302.

O'Farrell, M. J., B. W. Miller, and W. L. Gannon. 1999b. Qualitative Identification of Free-Flying Bats Using the Anabat Detector. Journal of Mammalogy **80**:11-23.

O'Keefe, J. M., S. C. Loeb, J. D. Lanham, and H. S. Hill Jr. 2009. Macrohabitat factors affect day roost selection by eastern red bats and eastern pipistrelles in the southern Appalachian Mountains, USA. Forest Ecology and Management **257**:1757-1763.

O'Neill, M., and R. Taylor. 1986. Observations on the Flight Patterns and Foraging Behavior of Tasmanian Bats. Wildlife Research **13**:427-432.

O'Neill , M. G., and R. J. Taylor. 1989. Feeding ecology of tasmanian bat assemblages. Australian Journal of Ecology **14**:19-31.

Ober, H. K., and J. P. Hayes. 2008. Prey selection by bats in forests of western Oregon. Journal of Mammalogy **89**:1191-1200.

Obrist, M. K., and R. Boesch. 2004. Variability in echolocation call design of 26 Swiss bat species: consequences, limits and options for automated field identification with a synergetic pattern recognition approach. Mammalia **68**:307-322.

Obrist, M. K., E. Rathey, F. Bontadina, A. Martinoli, M. Conedera, P. Christe, and M. Moretti. 2011. Response of bat species to sylvo-pastoral abandonment. Forest Ecology and Management **261**:789-798.

Oksanen, J., G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2012. vegan: Community Ecology Package.

Palmer, M. A., R. F. Ambrose, and N. L. Poff. 1997. Ecological Theory and Community Restoration Ecology. Restoration Ecology **5**:291-300.

Papadatou, E., R. K. Butlin, and J. D. Altringham. 2008. Identification of bat species in Greece from their echolocation calls. Acta Chiropterologica **10**:127-143.

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20:289-290.

Parnaby, H. E. 2009. A taxonomic review of Australian Greater Long-eared Bats previously known as *Nyctophilus timoriensis* (Chiroptera: Vespertilionidae) and some associated taxa. Australian Zoologist **35**:39-81.

Parsons, S. 2001. Identification of New Zealand bats (*Chalinolobus tuberculatus* and *Mystacina tuberculata*) in flight from analysis of echolocation calls by artificial neural networks. Journal of Zoology **253**:447-456.

Parsons, S., and G. Jones. 2000. Acoustic identification of twelve species of echolocating bat by discriminant function analysis and artificial neural networks. Journal of Experimental Biology **203**:2641-2656.

Parsons, S., and J. Szewczak. 2009. Detecting, recording, and analyzing the vocalizations of bats. Pages 91-111 in T. H. Kunz, and S. Parsons, editors. Ecological and behavioral methods for the study of bats. John Hopkins University Press, Baltimore, MD.

Patriquin, K. J., and R. M. R. Barclay. 2003. Foraging by bats in cleared, thinned and unharvested boreal forest. Journal of Applied Ecology **40**:646-657.

Pereira, H. M., G. C. Daily, and J. Roughgarden. 2004. A framework for assessing the relative vulnerability of species to land-use change. Ecological Applications **14**:730-742.

Perry, R. W., and R. E. Thill. 2007. Roost selection by male and female northern long-eared bats in a pine-dominated landscape. Forest Ecology and Management **247**:220-226.

Perry, R. W., R. E. Thill, and J. D. M. Leslie. 2007. Selection of roosting habitat by forest bats in a diverse forested landscape. Forest Ecology and Management **238**:156-166.

Petrites, A. E., O. S. Eng, D. S. Mowlds, J. A. Simmons, and C. M. Delong. 2009. Interpulse interval modulation by echolocating big brown bats (*Eptesicus fuscus*) in different densities of obstacle clutter. Journal of Comparative Physiology **195**:603-617.

Plank, M., K. Fiedler, and G. Reiter. 2012. Use of forest strata by bats in temperate forests. Journal of Zoology **286**:154-162.

Poff, N. L. 1997. Landscape Filters and Species Traits: Towards Mechanistic Understanding and Prediction in Stream Ecology. Journal of the North American Benthological Society **16**:391-409.

Poff, N. L., M. I. Pyne, B. P. Bledsoe, C. C. Cuhaciyan, and D. M. Carlisle. 2010. Developing linkages between species traits and multiscaled environmental variation to explore vulnerability of stream benthic communities to climate change. Journal of the North American Benthological Society **29**:1441-1458.

Pompanon, F., B. E. Deagle, W. O. C. Symondson, D. S. Brown, S. N. Jarman, and P. Taberlet. 2012. Who is eating what: diet assessment using next generation sequencing. Molecular Ecology **21**:1931-1950.

Preatoni, D. G., M. Nodari, R. Chirichella, G. Tosi, and et al. 2005. Identifying bats from time-expanded recordings of search calls: comparing classification methods. Journal of Wildlife Management **69**:1601-1614.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ratnasingham , S., and P. D. N. Hebert. 2007. BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). Molecular Ecology Notes **7**:335-364.

Raudenbush, S. W., M.-L. Yang, and M. Yosef. 2000. Maximum Likelihood for Generalized Linear Models with Nested Random Effects via High-Order, Multivariate Laplace Approximation. Journal of Computational and Graphical Statistics **9**:141-157.

Rayner, L., M. Ellis, and J. E. Taylor. 2013. Hollow occurrence and abundance varies with tree characteristics and among species in temperate woodland Eucalyptus. Austral Ecology **39**:145-147.

Razgour, O., E. L. Clare, M. R. K. Zeale, J. Hanmer, I. B. Schnell, M. Rasmussen, T. P. Gilbert, and G. Jones. 2011. High-throughput sequencing offers insight into mechanisms of resource partitioning in cryptic bat species. Ecology and Evolution **1**:556-570.

Reardon, T. B., N. L. McKenzie, S. J. B. Cooper, B. Appleton, S. Carthew, and M. Adams. 2014. A molecular and morphological investigation of species boundaries and phylogenetic relationships in Australian free-tailed bats Mormopterus (Chiroptera Molossidae). Australian Journal of Zoology **62**:109-136.

Redgwell, R., J. Szewczak, G. Jones, and S. Parsons. 2009. Classification of Echolocation Calls from 14 Species of Bat by Support Vector Machines and Ensembles of Neural Networks. Algorithms **2**:907-924.

Rhodes, M. 2007. Roost fidelity and fission-fusion dynamics of white-striped free-tailed bats (*Tadarida australis*). Journal of Mammalogy **88**:1252-1260.

Rhodes, M., Catteral, C. 2008. Spatial foraging behavior and use of an urban landscape by a fast-flying bat, the molossid *Tadarida Australis*. Journal of Mammalogy **89**:34-42.

Rhodes, M., and G. Wardell-Johnson. 2006. Roost tree characteristics determine use by the white-striped freetail bat (*Tadarida australis*, Chiroptera: Molossidae) in suburban subtropical Brisbane, Australia. Austral Ecology **31**:228-239.

Rogers, L. E., W. T. Hinds, and R. L. Buschbom. 1976. A General Weight vs. Length Relationship for Insects. Annals of the Entomological Society of America **69**:387-389.

Ross, H. A., S. Murugan, and L. Wai Lok Sibon. 2008. Testing the Reliability of Genetic Methods of Species Identification via Simulation. Systematic Biology **57**:216-230.

Ross, J. C., and P. E. Allen. 2014. Random Forest for improved analysis efficiency in passive acoustic monitoring. Ecological Informatics **21**:34-39.

Roughgarden, J. 1983. Competition and Theory in Community Ecology. The American Naturalist **122**:583-601.

Ruiz-Jaen, M. C., and T. M. Aide. 2005. Restoration success: How is it being measured? Restoration Ecology **13**:569-577.

Rumelhart, D. E., G. E. Hintont, and R. J. Williams. 1986. Learning representations by back-propagating errors. Nature **323**:533-536.

Russo, D., L. Cistrone, and G. Jones. 2007. Emergence time in forest bats: the influence of canopy closure. Acta Oecologica **31**:119-126.

Sala, O. E., F. S. Chapin, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. M. Lodge, H. A. Mooney, M. Oesterheld, N. L. Poff, M. T. Sykes, B. H. Walker, M. Walker, and D. H. Wall. 2000. Biodiversity - Global biodiversity scenarios for the year 2100. Science **287**:1770-1774.

Schnitzler, H.-U., C. F. Moss, and A. Denzinger. 2003. From spatial orientation to food acquisition in echolocating bats. Trends in ecology & evolution **18**:386-394.

Schnitzler, H. U., and E. K. V. Kalko. 2001. Echolocation by insect-eating bats. Bioscience **51**:557-569.

Scrimgeour, J., L. Molles, and J. R. Waas. 2013. Vertical variation in flight activity of the lesser short-tailed bat in podocarp and beech forests, Central North Island, New Zealand. New Zealand Journal of Ecology **37**:193-198.

Sedgeley, J. A. 2001. Quality of cavity microclimate as a factor influencing selection of maternity roosts by a tree-dwelling bat, *Chalinolobus tuberculatus*, in New Zealand. Journal of Applied Ecology **38**:425-438.

Shokralla, S., J. L. Spall, J. F. Gibson, and M. Hajibabaei. 2012. Next-generation sequencing technologies for environmental DNA research. Molecular Ecology **21**:1794-1805.

Siemers, B. M., and H.-U. Schnitzler. 2004. Echolocation signal reflect niche differentiation in five sympatric congeneric bat species. Nature **429**:657-661.

Skaug, H., D. Fournier, A. Nielsen, A. Magnusson, and B. Bolker. 2013. Generalized Linear Mixed Models using AD Model Builder.

Skowronski, M. D., and J. G. Harris. 2006. Acoustic detection and classification of microchiroptera using machine learning: Lessons learned from automatic speech recognition. The Journal of the Acoustical Society of America **119**:1817.

Sleep, D. J. H., and R. M. Brigham. 2003. An experimental test of clutter tolerance in bats. Journal of Mammalogy **84**:216-224.

Smith, D. A., and S. D. Gehrt. 2010. Bat Response to Woodland Restoration within Urban Forest Fragments. Restoration Ecology **18**:914-923.

Smith, M. A., C. D. Grant, W. A. Loneragan, and J. M. Koch. 2004. Fire management implications of fuel loads and vegetation structure in jarrah forest restoration on bauxite mines in Western Australia. Forest Ecology and Management **187**:247-266.

Smith, M. E. 2011. Long-term effects of thinning and burning minesite restoration on terrestrial vertebrates. Page 111. School of Biological Science. Murdoch University.

Stahlschmidt, P., and C. A. Bruhl. 2012. Bats as bioindicators - the need of a standardized method for acoustic bat activity surveys. Methods in Ecology and Evolution **3**:503-508.

Stevens, R. D., S. B. Cox, R. E. Strauss, and M. R. Willig. 2003. Patterns of functional diversity across an extensive environmental gradient: vertebrate consumers, hidden treatments and latitudinal trends. Ecology Letters **6**:1099-1108.

Stojanovic, D., M. Webb, D. Roshier, D. Saunders, and R. Heinsohn. 2012. Ground-based survey methods both overestimate and underestimate the abundance of suitable tree-cavities for the endangered Swift Parrot. Emu **112**:350-356.

Stoneman, G. L., D. S. Crombie, K. Whitford, F. J. Hingston, R. Giles, C. C. Portlock, J. H. Galbraith, and G. M. Dimmock. 1997. Growth and water relations of *Eucalyptus marginata* (jarrah) stands in response to thinning and fertilization. Tree Physiology **17**:267-274.

Strehlow, K., J. S. Bradley, J. Davis, and G. R. Friend. 2002. Short term impacts of logging on invertebrate communities in jarrah forests in south-west Western Australia. Forest Ecology and Management **162**:165-184.

Suding, K. N. 2011. Toward an Era of Restoration in Ecology: Successes, Failures, and Opportunities Ahead. Pages 465-487 in D. J. Futuyma, H. B. Shaffer, and D. Simberloff, editors. Annual Review of Ecology, Evolution, and Systematics, Vol 42. Annual Reviews, Palo Alto.

Summerville, K. S. 2010. Managing the forest for more than the trees: effects of experimental timber harvest on forest Lepidoptera. Ecological Applications **21**:806-816.

Summerville, K. S., C. J. Conoan, and R. M. Steichen. 2006. Species Traits As Predictors Of Lepidopteran Composition In Restored And Remnant Tallgrass Prairies. Ecological Applications **16**:891-900.

Summerville, K. S., and T. O. Crist. 2008. Structure and conservation of lepidopteran communities in managed forests of northeastern North America: a review. The Canadian Entomologist **140**:475-494.

Surlykke, A., and E. K. V. Kalko. 2008. Echolocating Bats Cry Out Loud to Detect Their Prey. PLoS ONE **3**:e2036.

Taillefer, A. G., and T. A. Wheeler. 2012. Community assembly of Diptera following restoration of mined boreal bogs: taxonomic and functional diversity. Journal of Insect Conservation **16**:165-176.

Taylor, R. J., and N. M. Savva. 1988. Use of roost sites by four species of bats in state forest in south-eastern Tasmania, Australia. Australian Wildlife Research **15**:637-646.

Teeling, E. C. 2009. Hear, hear: the convergent evolution of echolocation in bats? Trends in Ecology & Evolution **24**:351-354.

Threlfall, C. G., B. Law, and P. B. Banks. 2012. Sensitivity of insectivorous bats to urbanization: Implications for suburban conservation planning. Biological Conservation **146**:41-52.

Threlfall, C. G., B. Law, and P. B. Banks. 2013. Roost selection in suburban bushland by the urban sensitive bat *Nyctophilus gouldi*. Journal of Mammalogy **94**:307-319.

Tidemann, C. R., and S. C. Flavel. 1987. Factors affecting choice of diurnal roost site by tree-hole bats (Microchiroptera) in southeastern Australia. Australian Wildlife Research **14**:459-473.

Turbill, C. 2006. Roosting and thermoregulatory behaviour of male Gould's long-eared bats, *Nyctophilus gouldi*: energetic benefits of thermally unstable tree roosts. Australian Journal of Zoology **54**:57-60.

Turbill, C. 2009. Temperature effects on metabolic rate and torpor in southern forest bats (*Vespadelus regulus*). Australian Journal of Zoology **57**:125-127.

Vaughan, N., G. Jones, and S. Harris. 1997. Identification of British bat species by multivariate analysis of echolocation call parameters. Bioacoustics **7**:189-207.

Venables, W. N., and B. D. Ripley 2002. Modern applied statistics with S. Springer.

Verboom, B., and H. Huitema. 1997. The importance of linear landscape elements for the pipistrelle *Pipistrellus pipistrellus* and the serotine bat *Eptesicus serotinus*. Landscape Ecology **12**:117-125.

Veselka, N., L. P. McGuire, Y. A. Dzal, L. A. Hooton, and M. B. Fenton. 2013. Spatial variation in the echolocation calls of the little brown bat (*Myotis lucifugus*). Canadian Journal of Zoology-Revue Canadienne De Zoologie **91**:795-801.

Vesk, P. A., R. Nolan, J. R. Thomson, J. W. Dorrough, and R. M. Nally. 2008. Time lags in provision of habitat resources through revegetation. Biological Conservation **141**:174-186.

Vonhof, M. J., and R. M. R. Barclay. 1996. Roost site selection and roosting ecology of forest dwelling bats in southern British Columbia. Canadian Journal of Zoology-Revue Canadienne De Zoologie **74**:1797-1805.

Vonhof, M. J., and J. C. Gwilliam. 2007. Intra- and interspecific patterns of day roost selection by three species of forest-dwelling bats in Southern British Columbia. Forest Ecology and Management **252**:165-175.

Wallem, P., C. Anderson, G. Martinez-Pastur, and M. V. Lencinas. 2010. Using assembly rules to measure the resilience of riparian plant communities to beaver invasion in subantarctic forests. Biological Invasions **12**:325-335.

Wang, Y., U. Naumann, S. T. Wright, and D. I. Warton. 2012. mvabund– an R package for model-based analysis of multivariate abundance data. Methods in Ecology and Evolution **3**:471-474.

Wardell-Johnson, G. W., G. Keppel, and J. Sander. 2011. Climate change impacts on the terrestrial biodiversity and carbon stocks of Oceania. Pacific Conservation Biology **17**:220-240.

Waugh, J. 2007. DNA barcoding in animal species: progress, potential and pitfalls. BioEssays **29**:188-197.

Webala, P. W., M. D. Craig, B. S. Law, K. N. Armstrong, A. F. Wayne, and J. S. Bradley. 2011. Bat habitat use in logged jarrah eucalypt forests of south-western Australia. Journal of Applied Ecology **48**:398-406.

Webala, P. W., M. D. Craig, B. S. Law, A. F. Wayne, and J. S. Bradley. 2010. Roost site selection by southern forest bat *Vespadelus regulus* and Gould's long-eared bat *Nyctophilus gouldi* in logged jarrah forests; south-western Australia. Forest Ecology and Management **260**:1780-1790.

Werner, S. M., and K. F. Raffa. 2000. Effects of forest management practices on the diversity of ground-occurring beetles in mixed northern hardwood forests of the Great Lakes Region. Forest Ecology and Management **139**:135-155.

Whitaker, J. O. 2004. Prey selection in a temperate zone insectivorous bat community. Journal of Mammalogy **85**:460-469.

Whitford, K. R. 2002. Hollows in jarrah (*Eucalyptus marginata*) and marri (*Corymbia calophylla*) trees: I. Hollow sizes, tree attributes and ages. Forest Ecology and Management **160**:201-214.

Williams, R. L., A. E. Goodenough, and R. Stafford. 2012. Statistical precision of diet diversity from scat and pellet analysis. Ecological Informatics **7**:30-34.

Wilson, J., R. Rougerie, J. Schonfeld, D. Janzen, W. Hallwachs, M. Hajibabaei, I. Kitching, J. Haxaire, and P. Hebert. 2011. When species matches are unavailable are DNA barcodes correctly assigned to higher taxa? An assessment using sphingid moths. BMC Ecology **11**:18.

Wortley, L., J. M. Hero, and M. Howes. 2013. Evaluating Ecological Restoration Success: A Review of the Literature. Restoration Ecology **21**:537-543.

Yates, C. J., and R. J. Hobbs. 1997. Temperate Eucalypt Woodlands: a Review of Their Status, Processes Threatening Their Persistence and Techniques for Restoration. Australian Journal of Botany **45**:949-973.

Young, T. P. 2000. Restoration ecology and conservation biology. Biological Conservation **92**:73-83.

Zborowski, P., and R. Storey 2010. A Field Guide to Insects in Australia. New Holland, Chatswood, NSW.

Zeale, M. R. K., R. K. Butlin, G. L. A. Barker, D. C. Lees, and G. Jones. 2011. Taxon-specific PCR for DNA barcoding arthropod prey in bat faeces. Molecular Ecology Resources **11**:236-244.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith 2009. Mixed effects models and extensions in ecology with R. Springer, New York.