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Saltmarsh, Mosquitoes and Insectivorous Bats: Seeking a Balance



Leroy Gonsalves B. Env. Sc. (HonsI) (2008), Australian Catholic University

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

School of Arts & Sciences (NSW/ACT)
Australian Catholic University, North Sydney NSW

May 2012

Statement of originality

This thesis contains no material published elsewhere or extracted in whole or in part from a thesis by which I have qualified for or been awarded another degree or diploma. No parts of this thesis have been submitted towards the award of any other degree or diploma in any other tertiary institution. No other person's work has been used without due acknowledgment in the main text of the thesis. All research procedures reported in the thesis received the approval of the relevant Ethics/Safety Committees. Animal ethics approval was received from the NSW Department of Primary Industries Director-General's Animal Care and Ethics Committee to harp-trap, light-tag and radio-track bats (TRIM 09/6902 (6) and TRIM 09/7861 (7). All work was carried out under scientific licence S12771 issued by the NSW National Parks and Wildlife Service.

Leroy Gonsalves, May 2012

Dedication

This thesis is dedicated to the memory of my dear friend, Julia.

Abstract

Coastal saltmarsh provides suitable breeding habitat for a number of estuarine mosquito species. The saltmarsh mosquito (*Aedes vigilax* Skuse) can be locally abundant throughout summer, representing a potentially important prey resource for insectivorous bat species. However, *Ae. vigilax* has been identified as an important vector of mosquito-borne viruses such as Ross River virus and Barmah Forest virus and is a known nuisance biting pest. Coastal residential areas adjacent to Empire Bay (e.g., Killcare, Pretty Beach and Hardys Bay) on the Central Coast of New South Wales, Australia are prone to nuisance biting from *Ae. vigilax* and other estuarine mosquito species, particularly in late summer each year. Residents have requested use of a broadscale mosquito spraying regime (using *Bacillus thuringiensis israelensis, Bti*) to control numbers of *Ae. vigilax.* While it has been suggested that *Ae. vigilax* may be an important dietary item for insectivorous bats foraging within saltmarsh, no study to date has specifically investigated the importance of the mosquito in the diet of these bats. In this thesis, I investigated the importance of *Ae. vigilax* to insectivorous bats on the NSW Central Coast by examining relationships between bat activity, habitat use by bats, bat diet and the availability of *Ae. vigilax* and non-mosquito prey in three major habitats (saltmarsh, urban and forest) within the area. In all, 15 bat species and two species groups were recorded, of which eight are listed as threatened under the NSW *Threatened Species Conservation Act* (1995). Bats were most active in forest habitat, however, proportional feeding activity was greatest in saltmarsh. Positive relationships between prey abundance and total bat activity were only detected in the less cluttered saltmarsh habitat. Activity of bats in saltmarsh habitat was greatest along vegetation interfaces between saltmarsh and neighbouring landward habitat (coastal swamp forest) and the seaward habitat (mangrove forest). Of the species able to be identified acoustically, five species were trapped and an assessment was made of their diet using molecular analysis of faeces. The diets of five bat species trapped in forest adjacent to saltmarsh consisted of a diverse range of prey, dominated by moths. Only two bat species (eastern forest bat *Vespadelus pumilus* Gray and little forest bat *Vespadelus vulturnus* Thomas) consumed *Ae. vigilax*. Neither species is considered threatened in this area. I radio-tracked *V. vulturnus* to investigate habitat use during two extremes of *Ae. vigilax* abundance. *Vespadelus vulturnus* shifted from preferential use of saltmarsh to neighbouring coastal swamp forest, corresponding to a shift in the distribution and abundance of *Ae. vigilax* and no other prey taxa. The findings of this study are used to

make an assessment of the importance of *Ae. vigilax* to insectivorous bat diet. I conclude that *Ae. vigilax*, along with moths, is an important prey resource for bats of the *Vespadelus* genus. It is beyond the scope of this project to infer potential impacts of broadscale mosquito control on all insectivorous bat species. However, in the absence of a before-after-control-impact (BACI) designed study, a precautionary approach is recommended whereby any application of *Bti* is restricted to later in summer in order to avoid bat lactation periods (Nov-Jan), when energetic demands are greatest. I also propose an adaptive management approach, whereby careful monitoring of insectivorous bat populations before and after the application of broadscale control measures is required.

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Chapter 1

General Introduction

Recent decades have seen severe declines in threatened coastal saltmarsh in eastern Australia due, in part, to housing pressures. As housing densities adjacent to saltmarsh have increased so has pressure to control insects due to nuisance biting and potential health risks. Coastal saltmarsh provides suitable breeding habitat for a number of estuarine mosquito species including the saltmarsh mosquito (*Aedes vigilax* Skuse) that can be locally abundant throughout summer, representing a potentially important prey resource for insectivorous bat species. However, *Ae. vigilax* has been identified as an important vector of important mosquito-borne viruses such as Ross River virus (RRV) and Barmah Forest virus (BFV). In response to the health and social impacts of *Ae. vigilax*, broadscale mosquito control has been implemented in many coastal areas (Bell 1989; Russell and Kay 2008), with *Bacillus thuringiensis israelensis* (*Bti*) the most common microbial larvicide used in reduction of larval mosquito populations (Poulin *et al*. 2010).

NSW Central Coast residential areas adjacent to Empire Bay (e.g., Killcare, Pretty Beach and Hardys Bay) are prone to nuisance biting from *Ae. vigilax* and other estuarine mosquito species, particularly in late summer each year. Residents have requested use of a broadscale mosquito spraying regime (*Bti*) to control the numbers of *Ae. vigilax*. While it has been suggested that *Ae. vigilax* may be an important dietary item for insectivorous bats foraging within saltmarsh (Hoye 2002; Laegdsgaard *et al*. 2004; Belbasé 2005), no study has specifically investigated the importance of the mosquito to the diet of these bats.

The aim of this thesis is to address this knowledge gap. I firstly identify those bat taxa most likely to consume *Ae. vigilax* based on the strength of relationships between the activity of these taxa and the abundance of *Ae. vigilax* and non-mosquito prey. These taxa are then the focus of follow up work investigating relationships between *Ae. vigilax* abundance and geographical home ranges of bats and the presence of *Ae. vigilax* in the diets of these bats. I then conclude by discussing the importance of *Ae. vigilax* as a prey resource for local insectivorous bats and the implications for broadscale mosquito control. The following introduction provides a review of previous work conducted in the fields of insectivorous bat and *Ae. vigilax* ecology. Due to the large breadth of work conducted in these fields, this introduction does not seek to be exhaustive, but rather representative.

1.1 Coastal saltmarsh as foraging habitat for insectivorous bats

Coastal saltmarshes are diverse and vulnerable ecosystems of high ecological value that are increasingly under threat from a range of natural and anthropogenic processes (Adam 2009). This includes, but is not limited to, predicted sea level rise (Boorman 1992), particularly in those areas where saltmarshes are abutted by urban infrastructure or agricultural development (Adam 2009). Changing rainfall regimes are also likely to influence the patterns of soil salinity in saltmarshes, while increased storm frequency and intensity also threaten to erode saltmarsh vegetation, crucial in accreting sediment from within an estuary (Boorman 1999; Adam 2009). The impacts of natural processes on saltmarshes are influenced by anthropogenic modifications to saltmarsh and adjacent habitats, both directly and indirectly. One such example of this principle is that of mangrove encroachment in addition to advancing fringe vegetation, influenced by changes to the catchment due to urbanisation along the landward periphery of saltmarshes, resulting in significant declines in the size of coastal saltmarshes in south-eastern Australia (Saintilan and Williams 1999). Declines in coastal saltmarsh in south-eastern Australia have been recognised, with coastal saltmarsh listed as an endangered ecological community in three bioregions in New South Wales (NSW *Threatened Species Conservation Act* 1995). Declines in saltmarsh have also been identified in other parts of the world including south-east England (Pye 2000; Adam 2002) and along the Louisiana coast, USA (Viles and Spencer 1995; Adam 2002).

The importance of Australian coastal saltmarsh to vertebrate fauna has not been well studied (Spencer *et al*. 2009). Only in the last decade has use of coastal saltmarsh by foraging insectivorous bats been investigated in Australia (Hoye 2002; Laegsdgaard *et al*. 2004; Belbasé 2005; Lamb 2009). In these studies, ultrasonic bat detectors were used to record the echolocation calls of bats flying in coastal saltmarsh. A diverse group of bats from three families (Miniopteridae, Molossidae and Vespertilionidae) was positively identified, including six threatened species and an additional two that could not be positively identified (Hoye 2002; Laegsdgaard *et al*. 2004; Belbasé 2005). The studies of Hoye (2002) and Belbasé (2005) undertaken in the same area (Kooragang Island and surrounds, Newcastle, NSW) but three years apart, recorded similar bat assemblages, indicating that saltmarsh may be used regularly by the same bat taxa within a geographical area. Laegsdgaard *et al*. (2004) investigated bat activity in six saltmarsh sites across New

South Wales and Victoria and revealed that activity was variable across sites, ranging from no activity to relatively low levels of activity (~50 passes night^{-1}). Additionally, variability in the diversity of bat species recorded across study sites was reported with only *Vespadelus darlingtoni* (Allen) recorded in multiple sites. However, it is unclear whether sampling was undertaken at similar times of the year at each site. Additionally, greater sampling effort (more than one detector night site^{-1}) may have also recorded additional species.

Despite the variability in activity and species diversity of bats recorded in saltmarsh, the detection of bats within this habitat is of importance given the declines in saltmarsh across eastern Australia (Saintilan and Williams 1999), warranting the need for further studies to investigate the importance of saltmarsh as a foraging habitat for insectivorous bats. While the studies of Hoye (2002) and Laegsdgaard *et al*. (2004) did not report on feeding activity, Belbasé (2005) recorded moderate levels of feeding, indicating that bats do forage within saltmarsh. However, it is unclear what insects are targeted by bats in saltmarsh and to what extent feeding takes place. While arthropod populations in saltmarsh have not been well studied, available information suggests that saltmarsh supports diverse and abundant insect assemblages (Davis and Gray 1966; Hutchings and Recher 1974; Clarke and Miller 1983; Laegsdgaard *et al*. 2004). One particular insect, *Ae*. *vigilax* (saltmarsh mosquito), is periodically abundant throughout summer and has been suggested as a potentially important prey item for insectivorous bats (Hoye 2002; Laegsdgaard *et al*. 2004).

1.2 Saltmarsh mosquito (*Aedes vigilax*) ecology

1.2.1 Biology

Aedes vigilax is an estuarine mosquito species distributed along the Australian coast (Russell 1993). This mosquito breeds predominantly in saltmarsh habitats by laying its eggs on the ground surface (e.g., damp soil or plant stems) (Dale and Breitfuss 2009). Eggs conditioned by drying, hatch during rainfall or tidal inundation of the saltmarsh and larvae then develop through four larval stages (instars) before pupating and finally emerging as adults (Dale and Breitfuss 2009). The development from egg through to adult requires the presence of water and development time varies with environmental variables such as temperature, with more rapid development during warmer months (Russell 1998). Upon emergence, female *Ae. vigilax* individuals mate before dispersing to obtain a blood meal to

provide protein required for egg development. However, if appropriate nutrient levels are present in the emerging adult, *Ae. vigilax* females can develop one batch of eggs without a blood meal (a characteristic known as autogeny) (Sinclair 1976). The average life of *Ae. vigilax* in the field is approximately two weeks (Dale and Breitfuss 2009), with females generally laying three batches of eggs during that period. The life cycle of *Ae. vigilax* is closely associated with flooding of estuarine habitats by tides and/or rainfall. This association allows general predictions to be made about when large population abundances of *Ae. vigilax* are likely to occur. Generally, abundant adult populations tend to be present two weeks after a spring tide (associated with tidal inundation of the saltmarsh) (Kokkinn *et al*. 2009).

1.2.2 Vector capacity, human health and social impacts

Aedes vigilax has been identified as an important vector of arboviruses including RRV and BFV (Russell and Kay 2004). While not fatal, the disease caused by these two mosquito-borne viruses are debilitating, characterised by polyarthritic symptoms as well as rash, myalgia and fatigue with approximately 5,000 cases of human illness reported every year in Australia (Curran *et al*. 1997; MacKenzie *et al*. 1998; Russell and Kay 2004; Dale and Breitfuss 2009). Transmission of these viruses to humans occurs when an infected host-seeking female mosquito takes a human blood meal with the virus injected into the host within mosquito saliva. Infection of the mosquito generally occurs when a blood meal is taken from an intermediate vertebrate host (also known as a reservoir host) such as a macropod (Harley *et al*. 2001). The virus then replicates over a period of approximately one week and is able to be transmitted to a human host if a human blood meal is taken following infection (Dale and Breitfuss 2009). Vertical transmission of both arboviruses may occur when the eggs of an infected female hatch and produce infected adults. In addition to being a vector of arboviruses, *Ae. vigilax* is also an aggressive nuisance biting pest (Lee *et al*. 1984), generating social impacts for residents living in close proximity to saltmarshes.

1.2.3 Broadscale mosquito control

Given the nuisance biting impacts and health risks associated with *Ae. vigilax* populations, broadscale mosquito control has been implemented in many coastal areas (Russell and Kay

2008). Many methods have been employed to reduce mosquito population abundances (Floore 2006). These vary depending on the nature of the habitat (e.g., saline, freshwater or irrigated wetlands) and the target mosquito species. In the case of *Ae. vigilax*, methods available for mosquito control include habitat removal and/or modification, and the use of biological control or chemical control agents (Dale *et al*. 1993; Dale and Knight 2006). Mosquito control through habitat modification most commonly involves the reduction in available larval (breeding) habitat by improving drainage in the wetland. Improved drainage may be achieved in some cases by maintaining or renovating tidal channels to ensure maximum tidal flushing (Turner and Streever 1999). Runnelling, in which shallow ditches are constructed between isolated pools and the natural tidal channels, is a technique used to improve tidal flushing of isolated pools (Hulsman *et al*. 1989). While runnelling can be quite effective in reducing the abundances of *Aedes* spp. (Dale 2008), by facilitating the increased penetration of tidal water into saltmarsh environments, runnels can also assist in the colonisation of the upper marsh by mangroves and consequently adversely impact saltmarsh ecology (Breitfuss *et al*. 2003).

There is some evidence that insects such as dragonfly nymphs and beetles may represent biological control agents for mosquitoes in some circumstances (Russell 1999; Quiroz-Martinez and Rodríguez-Castro 2007). Additionally, bats and birds have also been observed feeding on adult mosquitoes at constructed wetlands in the USA (Mitsch and Gosselink 1993; Russell 1999). While few studies have specifically investigated the effectiveness of birds or bats as biological control agents for adult mosquito populations, Reiskind and Wund (2009) demonstrated that through predation of adult female mosquitoes (*Culex* spp.), high densities of bats are capable of reducing oviposition in experimental enclosures.

In Australia, biological control is generally limited to predatory fish (Morton *et al*. 1988; Russell 1999). The introduction of non-native larvivorous fish such as *Gambusia* spp. as mosquito control agents is considered to generate undesirable impacts on native fish fauna (Howe *et al*. 1997) and native anurans (Morgan and Buttemer 1996; Komak and Crossland 2000) and the effectiveness of the fish as a biological mosquito control agent is unclear (Rupp 1996; Russell 1999).

In Australia, mosquito control activities are primarily directed towards the immature mosquito populations and not adult mosquito control (Russell and Kay 2008). Chemical control of mosquitoes is advantageous as most pesticides can be rapidly applied, providing quick results (Russell 1998). However prolonged use may lead to development of resistance to the pesticide (Russell 1999). The toxicity of chemical agents such as Temephos (an organophosphate) to vertebrate taxa has precluded its use in estuarine wetlands (Russell 1999), restricting chemical control to two agents in Australia (*Bti* and *s*-methoprene). A commercially derived product from a naturally occurring soil bacterium, *Bti* is used as a larvicide which kills larvae when ingested. Given the general specificity of the *Bti* formulation, non-target effects are generally restricted to chironomids and usually when application rates exceed the registered rate (Kondo *et al*. 1992; Liber *et al*. 1998), promoting its use as a chemical control agent in estuarine wetlands. Although the persistence of *Bti* is limited to 2-3 days (Russell and Kay 2008; de Little *et al*. 2012), the use of *Bti* has been attributed to a decline in fledgling success of insectivorous passerines (Poulin *et al*. 2010). Methoprene, an insect hormone analogue, retards the growth of mosquito larvae preventing emergence of adults from the pupal stage (Russell and Kay 2008). While methoprene is also generally mosquito-specific, it can persist for longer periods than *Bti* if applied as a slow-release formulation, providing control over months (Kramer and Beesley 1991; Kramer *et al*. 1993; Russell and Kay 2008). The effectiveness and suitability of each of the mosquito control options described will be influenced by site conditions and associated logistics, environmental variables, the target mosquito species and the desired outcome from the control measures.

1.2.4 Ecological importance of mosquitoes

Although mosquitoes may be associated with negative or detrimental impacts to humans and other animals (e.g., as disease vectors and nuisance pests), they may also play important roles within many ecosystems. While mosquitoes may be important prey for aquatic invertebrates (Kumar and Rao 2003; Lundkvist *et al*. 2003; Kumar and Hwang 2006) and terrestrial insectivores, including bats (Poole 1932; Buchler 1976; Rydell 1990; Whitaker Jr. and Lawhead 1992; Long *et al.* 1998; Pavey *et al.* 2001b), some mosquito species are also important plant pollinators (Thien 1969).

1.3 Microchiropteran bats

1.3.1 Taxonomy

Bats belong to the Order Chiroptera and are the only mammals capable of self-powered flight (Teeling *et al*. 2005). There are currently 1116 recognised bat species worldwide (Simmons 2005). Traditionally, bats have been classified into two sub-orders, Megachiroptera (189 species of Old World fruit bats) and Microchiroptera (927 species) (Simmons 2005). The classification of bats into these two sub-orders has been based on paleontological and morphological data, with the most well-known morphological tree derived from the classification system of Miller (1907). In this classification system, the distinction between the two sub-orders was based on a bat's ability to produce echolocation calls in the larynx, with those capable of echolocation falling into the sub-order Microchiroptera and all others falling into the sub-order Megachiroptera. Recent advances in molecular biology have facilitated further investigation of taxonomic relationships of bats based on genetic differences. Studies by Teeling *et al*. (2000) and Springer *et al*. (2001) reported that rhinolophoid microbats are most closely related to the megabats and therefore proposed a revised classification system for bats in which the two sub-orders have been renamed as Yinpterochiroptera (echolocating rhinolophoids and the megabats) and Yangochiroptera (all other echolocating bats).

The traditional microchiropteran bats (Yangochiropterans and Rhinolophoids) are comprised of four superfamilies (Rhinolophoidea, Emballonuroidea, Noctilionoidea and Vespertilionoidea) representing 18 families. In Australia, six of these families (Rhinolophidae, Hipposideridae, Vespertillionidae, Miniopteridae, Molossidae, Emballonuridae) are represented. The classification of bats into each of these families is primarily based on the existence of certain morphological characteristics. The emballonurids are characterised by the presence of a sheathtail while hipposiderids possess a leaf-nose (Churchill 2009). Molossids all have a free tail, while miniopterids are equipped with bent wings (Churchill 2009). The rhinolophids have a horseshoe-shaped nose-leaf structure while vespertilionids possess a terminal phalanx of the third digit of the wing that is a similar length to the second phalanx (Churchill 2009).

1.3.2 Roosting and foraging habitat

An animal's habitat, in the most general sense, is where it lives (Morrison *et al.* 1992). This broad definition of habitat encompasses areas in which animals roost, forage, and reproduce. Microchiropteran bats are widespread, occurring worldwide except for the Arctic, Antarctic and some isolated oceanic islands (Hutson *et al.* 2001). Consequently, they utilise a diverse range of habitats.

A bat roost is an important site where a bat can sleep, hibernate, mate, rear young, digest food, interact socially and have the benefit of safety (Kunz and Lumsden 2003). There are a variety of different roost types, with some used permanently by some species, and others used ephemerally (Kunz and Lumsden 2003). Roosting habits of a number of microchiropteran bat species have been reported. Generally, bats can be classified into three broad categories based on their roosting habits: tree roosting, cave and crevice roosting, and bats that roost in artificial structures (e.g., stormwater culverts, house attics). Each of these roost categories vary in their permanency and availability (Kunz and Lumsden 2003). Generally, cave roosts and artificial structures, such as mine tunnels, will afford bats thermal stability and relative permanency of roost site (Kunz and Lumsden 2003). However, the distribution and availability of these types of roosts is patchy. In Australia, more than half of all bats roost in tree hollows (Tidemann and Flavel 1987). Radio-tracking studies have provided valuable information about roosting habitats (Lunney *et al*. 1988; Taylor and Savva 1988; Law and Anderson 2000; Lumsden *et al*. 2002a; Rhodes and Wardell-Johnson 2006; Webala *et al*. 2010), roost types (Tidemann and Flavel 1987; Taylor and Savva 1988; Lunney *et al*. 1995; Schulz 1998; Law and Anderson 2000; Lumsden *et al*. 2002b; Campbell *et al*. 2005; Rhodes 2007; Campbell 2009; Webala *et al*. 2010), colony sizes (Tidemann and Flavel 1987; Churchill 1991; Law *et al*. 2005; Rhodes and Wardell-Johnson 2006; Campbell 2009) and microclimates of roosts (Churchill 1991; Baudinette *et al*. 2000; Law and Chidel 2002).

Flight allows bats to be highly mobile (Law and Lean 1999; Cryan *et al.* 2000; O'Donnell 2001). This mobility facilitates access to a diverse range of habitats (Fenton 1997). Bats have been detected in rainforests (Lloyd *et al*. 2006), temperate forests (O'Donnell 2000), agricultural paddocks (Gerht and Chelsvig 2003; Wickramasinghe *et al*. 2003; Lumsden and Bennett 2005), deserts (Bell 1980; Kuenzi and Morrison 2003), wetlands (Menzel *et*

al. 2005; Lookingbill *et al*. 2010) and urban areas (Avila-Flores and Fenton 2005; Hourigan *et al*. 2006; Threlfall *et al*. 2011). However, not all habitats sustain similar bat assemblages. Habitat use by bats is influenced by a range of factors including vegetation clutter (Bradshaw 1996; Brigham *et al*. 1997; Law and Chidel 2002; Lloyd *et al*. 2006), bat wing morphology (Norberg and Rayner 1987), echolocation call design (Neuweiler 1984), prey availability (Rydell 1989; Fenton 1990; Rautenbach *et al*. 1996; Meyer *et al*. 2004; Fukui *et al*. 2006), predation (Speakman 1991; Verboom and Huitema 1997), interspecific competition (Arlettaz *et al*. 1997) and environmental variables such as temperature (Negraeff and Brigham 1995; O'Donnell 2000), humidity (Lacki 1984) and lunar phobia (Fenton *et al*. 1977).

1.3.3 Diet, energetic requirements and prey selection of bats

Accounts of insectivorous bat diets have traditionally been based on morphological identification of prey remains within bat faeces (Belwood and Fenton 1976; Anthony and Kunz 1977; Whitaker Jr. 1988) or inside the stomachs of bats (Whitaker Jr. 1972, 1988; Buchler 1976) as well as the identification of culled prey remains near bat roosts (Rabinowitz and Tuttle 1982; Whitaker Jr. 1988; Fenton *et al*. 1990). These techniques generally enable identification of prey to family or order level and allow for quantification of prey, usually represented as percent volume (i.e., percentage of faecal pellet or stomach contents that consists of particular prey taxa), percent frequency (i.e., percentage of faecal pellets or animals that contain a particular prey taxa) or density (i.e., number of culled prey in a defined area: Whitaker Jr. 1988; Razgour *et al*. 2011). The degree to which these analyses are able to provide data representative of all prey consumed by bats has been examined (Rabinowitz and Tuttle 1982; Kunz and Whitaker Jr. 1983; Dickman and Huang 1988; Robinson and Stebbings 1993). While some studies indicate that these techniques provide an adequate assessment of the prey consumed by bats (Kunz and Whitaker Jr. 1983), others have identified biases and limitations (Rabinowitz and Tuttle 1982; Dickman and Huang 1988; Robinson and Stebbings 1993). Biases associated with digestibility of prey have been discussed (Belwood and Fenton 1976; Dickman and Huang 1988). It is suggested that soft-bodied prey are more susceptible to the processes of mastication and digestion and hence are less likely to be recognisable in bat faeces (Dickman and Huang 1988). Additionally, larger more sclerotised prey items may occur in multiple faecal

pellets of bats, potentially leading to an overestimation of these items (Robinson and Stebbings 1993). In turn, this is likely to result in smaller prey being underestimated.

More recently, novel molecular techniques have been employed to study bat diet (McCracken *et al*. 2005; Clare *et al*. 2009; Brown 2010; Bohmann *et al*. 2011; Zeale *et al*. 2011). These techniques have provided greater resolution than more traditional techniques, providing identification of consumed prey to genus or species level. While the pathways employed in these studies varied, they were all based on the same fundamental principle: DNA from consumed prey is separated from non-target DNA, compared to reference DNA libraries of potential prey and then assigned to a certain taxonomic level based on the degree to which the prey DNA matches a reference DNA sequence. Unlike early molecular studies of bat diet that targeted specific prey items (McCracken *et al*. 2005; Brown *et al*. 2010), ensuing studies employed pathways that did not require *a priori* knowledge of bat prey (Clare *et al*. 2009; Bohmann *et al*. 2011; Zeale *et al*. 2011). Instead, these studies analysed subsamples of prey fragments (chitinous remains of prey found within bat faeces) using universal DNA barcoding primers in PCR followed by direct sanger sequencing (Clare *et al*. 2009) or analysed the entire faecal pellet using arthropod-specific primers in PCR followed by cloning and subsequent Sanger sequencing of DNA from a subsample of clones (Zeale *et al*. 2011). More recently, molecular studies have employed next-generation DNA sequencing platforms that circumvent the physical separation of prey fragments or the need for cloning, providing greater coverage of prey within bat faeces (Bohmann *et al*. 2011; Razgour *et al*. 2011). In addition to molecular approaches, measurement of carbon and nitrogen stable isotopes in bat faeces has been applied to analyse bat diets, providing dietary data over multiple temporal scales (Painter *et al*. 2009; Dunlop and Bullen 2011).

Both traditional and more modern techniques for the analysis of bat diets have revealed that bats consume a diverse range of prey (Anthony and Kunz 1977; Agosta 2002; Painter *et al*. 2009; Zeale *et al*. 2011) overwhelmingly represented by lepidopterans, coleopterans, homopterans, hemipterans and trichopterans (Jones *et al*. 2009). Dietary studies have indicated that while some bats opportunistically feed on items when they become abundant (Belwood and Fenton 1976; Rydell 1986; O'Neill and Taylor 1989), others are more selective and target particular prey taxa (Anthony and Kunz 1977; Sample and Whitmore 1993; Whitaker Jr. *et al*. 1994; Hurst and Lacki 1997; Schulz 2000). Diets of other bats

suggest that they are flexible in their selectivity of prey, taking particular prey when abundant (opportunistic) but at other times, specialising on particular prey taxa (Churchill 1994; Agosta 2002). While prey taxa taken by bat species vary, so too do prey sizes. There is conjecture as to the mechanism driving the lower-limit of prey size selection by bats. Barclay and Brigham (1991) suggested that differences observed in the sizes of prey consumed by bats were based on constraints imposed on prey detection by echolocation design – with higher frequency echolocation more suited for detection of small prey (Møhl 1988). Since echolocation frequency negatively scales with bat size (Jones 1999), smaller bats are thought to be more able to detect small prey given high frequencies have short wavelengths that return strong echoes when shorter than the insect wingspan (Houston *et al*. 2004). Although this hypothesis is supported by the diets of a number of bat species (Barclay 1985; O'Neill and Taylor 1989), the diets of many European medium-large size bats consist predominantly of small dipterans (Chironomidae and Culicidae: Rydell 1989; Waters *et al*. 1995; Jones 1999). Waters *et al*. (1995) proposed that detection ranges of prey size are generally independent of echolocation frequency, suggesting small prey are available to large bats that employ low frequency echolocation. It has been hypothesised that the upper limit of prey sizes selected by bats is dictated by bat jaw morphology (length and strength: Freeman 1979, 1981; Freeman and Lemen 2007) and prey hardness (Aguirre *et al*. 2003; Freeman and Lemen 2007). Diets of several bat species support this hypothesis, although the applicability of these two factors as predictors of the upper limit of prey sizes selected is not consistent for all bats (Freeman and Lemen 2007). For instance, while dentary length (index of jaw size) closely corresponds to the lengths of the largest prey consumed by small bats, the lengths of the largest consumed insects tend to be greater than the dentary length of larger bats (Freeman and Lemen 2007). Other factors likely to influence what prey (taxa and size class) bats select include digestibility of prey (Barclay *et al*. 1991), relative ease of capture and palatability (Buchler 1976) and intra-specific and inter-specific competition (Arlettaz *et al*. 1997). Together, these factors are likely to interact and influence the energetic profitability of particular prey.

Daily activities undertaken by bats are constrained by what is energetically feasible (Butler *et al*. 2004). The minimum energy required to undertake these activities is termed field metabolic rate (FMR). Body mass is the single most important factor influencing FMR, with FMR positively scaling with body mass. Consequently it is possible to estimate the FMR of bats (Speakman and Thomas 2003). Ultimately, FMR of bats will influence what

prey and what quantity of prey bats are required to consume. The energy that prey provide for bats can be measured using bomb calorimetry and is termed calorific or caloric value. Not all energy from prey is absorbed or assimilated by bats (Barclay *et al*. 1991; Webb *et al*. 1993), making it difficult to assess the quantity of prey that bats need to consume in order to meet FMR requirements. Additionally, FMR varies seasonally when bats undertake energetically more demanding activities (e.g., spermatogenesis, lactation) (Racey and Speakman 1987). For instance, the energy requirements of small mammals during late lactation while in captivity are 2.5-5 times greater than those of non-lactating females (McLean and Speakman 1999).

Few studies have investigated the diets or the energetic requirements of Australian bats. Much of what is known about the diets of Australian bats stems from two studies. Vestjens and Hall (1977) provided qualitative descriptions of prey items found in the stomach contents of 29 Australian bat species, while O'Neill and Taylor (1989) presented qualitative and quantitative (percent volume) data about prey consumed by eight species in Tasmania. Other assessments of Australian bat diets have focused on groups of species within a foraging guild (Pavey and Burwell 1998) or individual species (Churchill 1994; Pavey and Burwell 1997; Schulz and Wainer 1997; Law and Urquhart 2000; Campbell 2007). While no FMR data are available for any Australian insectivorous bats, FMR data have been reported for a single nectar-feeding species (*Syconycteris australis* Peters) (Geiser and Coburn 1999). However, outside Australia, FMR data have been reported for a number of insectivorous bat species (Kurta *et al*. 1989, 1990; Kunz *et al*. 1995). Basal metabolic rate (BMR) (minimum energy required to maintain vital body functions excluding energy costs of thermoregulation, movement, growth and digestion – Geiser 2006) data are available for six Australian insectivorous bat species representing three families (Megadermatidae, Hipposideridae and Vespertilionidae: Hosken 1997; Hosken and Withers 1997; Baudinette *et al*. 2000; Geiser and Brigham 2000).

1.3.4 Mosquitoes as bat prey

Mosquitoes have been identified as prey of bats in Australia and elsewhere, present in bat stomach contents (Poole 1932; Buchler 1976) and faeces (Rydell 1990; Whitaker Jr. and Lawhead 1992; Churchill 1994; Kurta and Whitaker Jr. 1998; Long *et al.* 1998; Shiel *et al.* 1998; Law and Urquhart 2000; Pavey *et al.* 2001b; Campbell 2007; Sophia 2010), in some

cases representing large proportions of the diet (Rydell 1992; Jones 1995). Additionally, Reiskind and Wund (2009) demonstrated that via predation of adult *Culex* spp., high densities of bats are capable of reducing mosquito egg-laying. While mosquitoes are a recognised prey resource for many bats, they are not considered an important prey for most bat species (Whitaker Jr. and Lawhead 1992; Kurta and Whitaker Jr. 1998). However, no study has specifically investigated the importance of mosquitoes relative to other flying insects, to insectivorous bat diet in a targeted approach accounting for the ecology of the insect.

1.4 Thesis aims and structure

In this thesis, I aim to investigate the importance of *Ae. vigilax* as a potential prey resource for foraging insectivorous bats on the Central Coast of New South Wales, Australia by examining relationships between *Ae. vigilax* availability and the activity, habitat use and diets of local insectivorous bats.

Chapter 2 compares diversity and activity (commuting and feeding) of bats over spring-neap tidal cycles in three major habitats (saltmarsh, urban, and forest) within the study area that represent potential larval and refuge habitats of mosquitoes. Prey (*Ae. vigilax* and other non-mosquito prey) are also surveyed and their abundances compared across habitats and tidal cycles. Using data collected from ultrasonic bat detectors, I examine relationships between the activity of bats and the abundance of mosquito and non-mosquito prey in the three major habitats to assess whether relationships are consistent across habitats that represent a clutter gradient, ranging from low to high (saltmarsh-forest), with clutter considered to be negatively associated with prey detectability (Rainho *et al*. 2010). I discuss relationships observed between total bat activity (and feeding), the activity of individual species and prey abundance in each habitat and for each tidal cycle. Furthermore, based on these relationships, I discuss the potential importance of *Ae*. *vigilax* as a prey resource for the local bat fauna.

In Chapter 3, I focus in more detail on how bats use coastal saltmarsh, the major larval habitat of *Ae. vigilax* that is in decline across south-east Australia (Saintilan and Williams 1999). Coastal saltmarsh, often situated on the landward side of mangrove communities but on the seaward side of coastal swamp forest, may be impacted by management actions

pertaining to these adjoining habitats. Vegetation communities that adjoin coastal saltmarsh represent interfaces that may be used by foraging bats. Given that vegetation interfaces are known to be areas of high bat activity in other habitats, I investigate whether the same applies to saltmarsh. Using data collected with ultrasonic bat detectors, I investigate and compare species richness, bat activity and the activity of individual species across two vegetation interfaces within saltmarsh and the saltmarsh interior (no interface). I discuss the potential implications of management of saltmarsh and adjoining habitats for foraging bats.

In Chapter 4, I investigate diets of bats and examine whether there is an upper limit on the size of bat that consumes *Ae. vigilax*. I employ novel molecular techniques to identify prey remains within faeces obtained from bats trapped throughout the austral summer. I also sampled the abundance of prey (*Ae. vigilax* and other taxa) at time of trapping using CO_2-baited EVS traps and blacklight traps. I discuss relationships between bat species size and *Ae. vigilax* consumption inferred from dietary analyses and discuss potential implications of broadscale mosquito control for those bats identified as predators of *Ae. vigilax*.

In Chapter 5, I examine habitat use of a target bat species (*Vespadelus vulturnus* Thomas) that I found to consume *Ae. vigilax* regularly throughout summer. I assess whether *Ae. vigilax* availability in two adjoining habitats influences which of these are used for foraging by *V. vulturnus*. Using radio-tracking, I track the movements of *V. vulturnus* individuals over a spring-neap tidal cycle predicted to sustain differing population abundances of *Ae. vigilax* while also sampling prey (mosquito and non-mosquito) abundance. I then discuss relationships between *Ae. vigilax* availability and habitat use of *V. vulturnus* and use these relationships to infer importance of *Ae. vigilax* as a prey resource.

Finally, in Chapter 6, I discuss the major findings of each of Chapters 2-5, the implications of these findings for broadscale mosquito control and future directions for research. Chapters 2-5 of this thesis are stand-alone chapters that are *in press* as research papers in journals (Chapters 2 and 3) or have been submitted for publication (Chapters 4 and 5). As a result, there is a small degree of repetition in the introduction and methods sections of Chapters 2-5, necessary to make these stand-alone publications. The relevant publication details are given at the start of these chapters. Details of secondary authors are also given

at the start of each chapter. During this project I designed and carried out all the work, in collaboration with my supervisors Associate Professor Vaughan Monamy, Dr Bradley Law and Dr Cameron Webb. Dr Brian Bicknell provided technical advice for the novel molecular analysis of bat diets in Chapter 4, while Associate Professor Neil Saintilan provided guidance during the design of the study. A sub-set of data collected using ultrasonic detectors for Chapter 2 overlaps with data collected and reported by Ms Susan Lamb in her Honours thesis (Lamb 2009). The dataset used by Lamb (2009) was sourced from detectors (away from light traps) separate to the ones used in Chapter 2 of this thesis (in the presence of light traps). Additionally, prior to analysis, data were pooled across sampling fortnights in Lamb's (2009) study, while temporal variability in data was preserved for analyses in Chapter 2 of this thesis that examined temporal variability in bat activity across the three major habitats in the study area while also accounting for variability in the abundance of prey. In addition, no analysis of foraging data (feeding buzzes) was undertaken by Ms Lamb. I collected data, undertook statistical analyses and drafted all chapters. I have acknowledged the assistance of volunteers and collaborators, in the Acknowledgements section of this thesis.

Chapter 2

The Influence of Clutter on Relationships between Bat Activity and the availability of Mosquitoes and Non-mosquito Prey in a Coastal Mosaic, New South Wales, Australia



This chapter is *in press* as Gonsalves, L., Lamb, S., Webb, C., Law, B. and Monamy, V. Do mosquitoes influence bat activity in coastal habitats? *Wildlife Research*

Abstract

Conservation of insectivorous bat populations requires appropriate management of foraging habitats and the prey resources they sustain. Endangered coastal saltmarsh communities support a diverse range of aquatic and terrestrial arthropods, including the saltmarsh mosquito (*Aedes vigilax* Skuse), a potentially important prey resource for insectivorous bats. Prey detectability by bats is considered to be limited with low-frequency echolocation, particularly in cluttered habitats, that may render abundant *Ae. vigilax* populations unavailable to some bat species. However, small dipteran prey have been identified in the diets of a number of low-frequency echolocating bats. To investigate the potential importance of *Ae. vigilax* to foraging insectivorous bats in a coastal mosaic of habitats with varying clutter levels, I measured nightly bat activity and the abundance of prey (mosquito and non-mosquito) concurrently during neap and spring tides in saltmarsh (low clutter), urban (intermediate clutter) and forest (high clutter) habitats in New South Wales, Australia. While prey abundances were generally greatest in saltmarsh and forest habitats, bat activity was greatest in the forest habitat. However, proportional feeding activity was greatest in saltmarsh. Additionally, prey abundance was positively correlated with total bat activity only in the saltmarsh, suggesting that clutter negatively influences prey detectability. Positive correlations between *Ae. vigilax* abundance and bat activity were restricted to bats of the *Vespadelus* genus, which are small sized bats that employ high frequency echolocation suitable for detection of small prey along edges. These findings suggest that *Ae. vigilax* may be an important prey resource for small, high-frequency echolocating bats capable of discerning small prey within cluttered forest as well as exploiting abundant prey in the open saltmarsh.

2.1 Introduction

The conservation of insectivorous bat fauna requires an understanding of species-specific biology and ecology. An important requirement is a detailed understanding of the associations between bats and their foraging habitats (Fenton 1997). Given bats can be highly mobile (Law and Lean 1999; Cryan *et al.* 2000; O'Donnell 2001), they may able to access a diverse range of habitats (Fenton 1997). However, not all habitats sustain similar bat assemblages.

Patterns of bat activity have been documented for a wide range of habitats, including riparian forests (Lloyd *et al*. 2006), temperate forests (O'Donnell 2000), agricultural paddocks (Gerht and Chelsvig 2003; Wickramasinghe *et al*. 2003; Lumsden and Bennett 2005), deserts (Fenton and Morris 1976; Bell 1980; Kuenzi and Morrison 2003), wetlands (Menzel *et al*. 2005; Lookingbill *et al*. 2010) and urban areas (Avila-Flores and Fenton 2005; Hourigan *et al*. 2006; Threlfall *et al*. 2011). Only recently has use of coastal saltmarsh by insectivorous bats been documented (Hoye 2002; Laegdsgaard *et al*. 2004; Belbasé 2005; Lamb 2009). The use of this productive but endangered and declining ecological community (Saintilan and Williams 1999) by a number of threatened bat species highlights the need for further investigation of bats using this habitat.

Coastal saltmarsh represents suitable breeding habitat for a number of estuarine mosquito species. The saltmarsh mosquito (*Aedes vigilax* Skuse) can be locally abundant throughout summer (Webb and Russell 2009a), representing a potentially important prey resource for insectivorous bats foraging within coastal saltmarsh habitats. However, *Ae*. *vigilax* has been identified as an important vector of mosquito-borne viruses, such as Ross River virus and Barmah Forest virus. Notwithstanding the health risks associated with *Ae*. *vigilax*, the mosquito also generates nuisance biting impacts. In response to the health and social impacts of *Ae*. *vigilax*, broadscale mosquito control has been implemented in many coastal areas (Bell 1989; Russell and Kay 2008) with *Bacillus thuringiensis israelensis* (*Bti*) the most commonly used microbial larvicide used in the reduction of larval mosquito populations (Russell and Kay 2008; Poulin *et al*. 2010). Concern has been raised as to the potential impact broadscale mosquito control activities may have on local bat species (Laegdsgaard *et al*. 2004), but while there is evidence that mosquitoes may be an important dietary item for insectivorous bats foraging within saltmarsh (Hoye 2002;

Laegdsgaard *et al*. 2004; Belbasé 2005; Lamb 2009), no study has specifically investigated relationships between the abundance of *Ae*. *vigilax* and bat activity.

Although the immature aquatic stage of *Ae*. *vigilax* is generally limited to estuarine habitats, the adults can disperse widely with biting impacts experienced at a distance of over 5 km from estuarine habitats (Webb and Russell 2009a). Forested habitats are considered refuge habitats that provide sheltered areas for mosquitoes while also supporting sources of blood meals that female *Ae*. *vigilax* individuals require for egg development. Consequently, any investigation of relationships between bat activity and *Ae*. *vigilax* abundance should also seek to investigate other habitats to which *Ae*. *vigilax* may disperse upon emergence from coastal saltmarsh habitat.

Along the eastern Australian coast, large areas of saltmarsh habitat can produce abundant *Ae. vigilax* populations and adjacent forest and woodland habitats may provide suitable refuge habitat for adult mosquitoes (Webb and Russell 2009a). These habitats also are known to sustain hollow- and cave-roosting insectivorous bat species (Payne 2006). Located between the larval habitat and potential refuge habitat of *Ae*. *vigilax* lies built-up urban habitat that may also provide suitable larval habitat for many container-breeding mosquitoes and also refuge habitat for other mosquitoes as well as *Ae*. *vigilax*. An investigation of the patterns of *Ae*. *vigilax* abundance and bat activity in these three habitat types may elucidate any relationships between *Ae*. *vigilax* abundance and bat activity.

Foraging activity of bats can be influenced by a number of factors. Prey abundance has been found to influence activity of bats in different habitat types (Rydell 1989; Fenton 1990; Rautenbach *et al*. 1996; Fukui *et al*. 2006; Adams *et al*. 2009), as has vegetation clutter (i.e., structures that produce non-target echoes) (O'Neill and Taylor 1986; Bradshaw 1996; Brigham *et al.* 1997; Law and Chidel 2002; Lloyd *et al.* 2006). Vegetation clutter can also influence bat mobility so that bats with a particular wing morphology and echolocation design display differential habitat use (Neuweiler 1984; Norberg and Rayner 1987; Fenton 1990; Saunders and Barclay 1992; Brigham *et al.* 1997). Fast-flying bats that are less manoeuvrable generally have a high wing aspect ratio (Rhodes 2002), with low frequency echolocation calls that make it difficult to navigate or detect prey in high levels of clutter (Møhl 1988; Barclay and Brigham 1991). These clutter-sensitive bats have been grouped together to form an open space foraging guild (Schnitzler and Kalko 2001; Adams

et al. 2009). The sensitivity to clutter of this guild of bats would suggest that they would use more open areas for foraging.

Although prey abundance and vegetation clutter on their own are known to influence the habitats in which bats forage, the effects of these two factors may also interact with each other. It has been demonstrated that prey abundance does not necessarily equate to its availability to bats as clutter rendered prey less accessible (Boonman *et al*. 1998; Adams *et al*. 2009; Rainho *et al*. 2010). Furthermore, it is unclear whether echolocation design, particularly echolocation frequency, limits the size of prey that can be located by foraging bats. Longer wavelengths associated with low frequency echolocation, are thought to be unsuited to detection of small prey at distances sufficient for interception by bats (Barclay and Brigham 1991). Since bat size is negatively correlated with echolocation frequency (Jones 1999), larger (less-manoeuvrable) bats are thought to be restricted to larger prey, while smaller bats able to detect large prey are restricted to smaller prey due to morphological constraints (e.g., prey hardness and jaw size – Freeman and Lemen 2005). However, many studies investigating diets of medium-large size bats have reported the presence of many small dipterans (chironomids and mosquitoes). Waters *et al*. (1995) challenged the assumption that small prey are unavailable to large bats that employ low frequency echolocation and proposed that detection ranges of prey size are generally independent of echolocation frequency.

If *Ae*. *vigilax* is an important prey resource for insectivorous bats, one would predict relationships between bat activity and *Ae*. *vigilax* abundance to be positively associated, with the strength of this association dependent on the importance of *Ae*. *vigilax* to the diets of individual species. However, given limitations imposed on foraging bats by vegetation clutter, as well as the restrictions in prey size associated with particular echolocation designs, it is unclear whether relationships between *Ae*. *vigilax* abundance and bat activity as well as activity of individual species are consistent across habitats with varying levels of clutter. In order to elucidate relationships between *Ae*. *vigilax* abundance and bat activity that may be inconsistent among habitats or bat species, I surveyed bat activity and activity of individual species as well as prey (*Ae*. *vigilax* and non-mosquito) abundance in saltmarsh, urban and forest habitats, which vary broadly in acoustic complexity (least-to-greatest). I predict: a) Relationships between *Ae*. *vigilax* abundance and bat activity will not be consistent across habitats or species. b) Stronger associations between prey (*Ae*.

vigilax and non-mosquito) abundance will occur in less cluttered habitats (saltmarsh), though not restricted to these, particularly for small sized bats that employ high-frequency echolocation. c) Positive associations will occur between *Ae*. *vigilax* abundance and those species that utilise *Ae*. *vigilax* as a prey resource, while the activity of other bat species will be correlated with the abundance of non-mosquito prey.

2.2 Methods

2.2.1 Study site

The study area was located in the Empire Bay region (33°29'57"S, 151°21'40"E) of the Central Coast of New South Wales, Australia (Fig. 2.1). This region is approximately 50 km north of Sydney and experiences a warm sub-tropical climate.

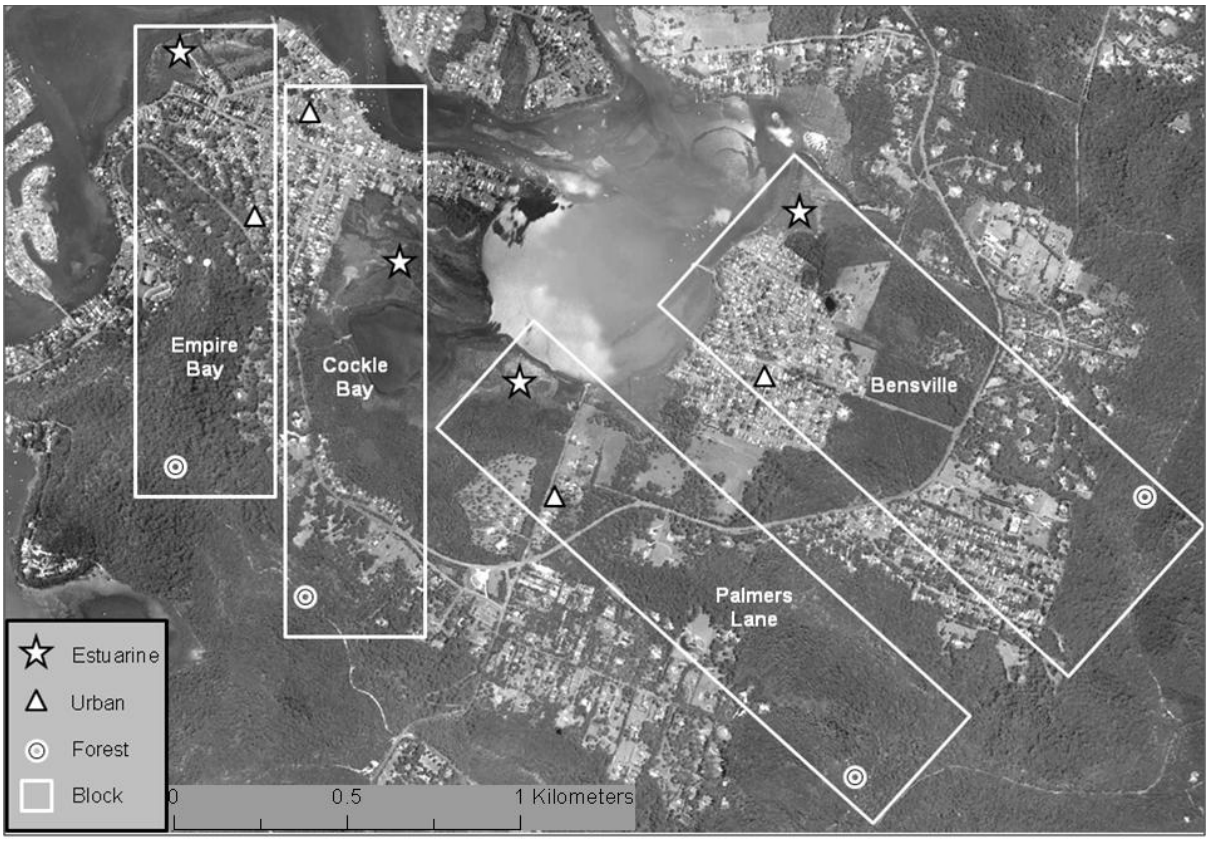


Figure 2.1: Satellite image of study area (adapted from Google Earth). Sampling location depicted by stars = saltmarsh; triangle = urban; doughnut = forest in each blocked site.

The study area is characterised by more than 40 vegetation communities, many of which occur within a large national park (Bouddi National Park, 1 189 ha) and five smaller nature reserves (Cockle Bay Nature Reserve, 68.5 ha; Rileys Island Nature Reserve, 45.7 ha;

Pelican Island Nature Reserve, 40 ha; Saratoga Island Nature Reserve, 2 ha). The national park and nature reserves support populations of hollow- and cave-roosting insectivorous bats, including six threatened species listed under the NSW *Threatened Species Conservation Act* 1995 (Payne 2006). Coastal saltmarsh, urban areas and forests represent three major habitats in the study area. These habitats are grossly different to one another and characteristics of each of these habitats are described below.

Coastal saltmarsh habitat is characterised by low-growing salt-tolerant succulent herbs such as samphire (*Sarcocornia quinqueflora* (Bunge ex Ung.-Sternb.) A.J.Scott) and creeping brookweed (*Samolus repens* (J.R.Forst. & G.Forst.) Pers). While saltmarsh lacks trees, a small number of grey mangrove shrubs (*Avicennia marina* (Forsk.) Vierh.) (< 3m high) occurred in patches due to the landward transgression of mangroves (Saintilan and Williams 1999). This vegetation community represents important larval habitats for many estuarine mosquito species, including *Ae. vigilax*, *Aedes alternans* Westwood and *Culex sitiens* Wiedemann (Webb and Russell 2009a). Additionally, coastal saltmarsh communities have been found to support moderately high levels of bat activity (Lamb 2009) and represent important foraging habitats for bats (Belbasé 2005).

Urban habitat is characterised primarily by residential areas that include dwellings and structures associated with residential areas, such as schools, roads and street lights. Urban habitats provide a range of suitable larval habitats for urban mosquito species, such as *Aedes notoscriptus* Skuse, *Culex quiquefasciatus* Say, and *Culex molestus* Forskal (Webb and Russell 2009a). Urban areas also sustain a diverse range of bat species and typically support lower levels of bat activity (Avila-Flores and Fenton 2005; Scanlon and Petit 2008; Hourigan *et al*. 2010; Threlfall *et al*. 2011, 2012). However, species diversity and activity levels within urban habitats vary with the degree of urbanisation (Hourigan *et al*. 2006; Threlfall *et al*. 2011, 2012). Although no attempt has been made to define the urban habitat based on degree of urbanisation, residential density in the overall study area was relatively low (2.5 dwellings ha^{-1}) and could be classified as suburban. The close proximity of urban areas to saltmarsh and forest habitats meant that within the urban habitat, patches of moderately vegetated areas were present. While attempts were made to avoid sampling near these patches, this was not always possible given the small extent of urban space within the study area as well as limitations imposed by landowners. All sampling was

undertaken in a manner that attempted to minimise the influence of these vegetated areas (i.e., detectors were oriented away from vegetation and arthropod sampling was conducted at the furthest distance away from vegetation as practically possible: ~30-420 m).

The forest habitat is situated on an escarpment that straddles the landward side of the urban habitat. Sampling of this habitat was undertaken in Narrabeen Coastal Blackbutt Forest within Bouddi National Park. Dominated by *Eucalyptus pilularis* Smith, *Syncarpia glomulifera* subsp. *glomulifera* Smith and *Allocasuarina torulosa* (Aiton) L.A.S. Johnson, this vegetation community has a typical canopy height of 20 m, occurring on Narrabeen sandstone that supports a sparse-to-moderate understorey of shrubs and a well developed grass layer (Bell 2009). With ~40 % canopy cover, this vegetation community can be described as an open forest (Specht 1970). The forest habitat contains areas that provide suitable larval habitats for many floodwater species including *Aedes multiplex* Theobald, *Aedes procax* Skuse and *Verrallina funerea* Theobald. Additionally, forest communities are known to sustain a number of hollow- and cave-roosting bats (Payne 2006) and represent an important habitat for foraging bats in the study area (Lamb 2009).

2.2.2 Data collection

I surveyed insectivorous bat activity and the abundances of mosquito fauna and other volant insect fauna at four sites (Empire Bay, Cockle Bay, Palmers Lane and Bensville) within the study area. Each site was considered a block, containing a corresponding saltmarsh, urban and forest habitat that was sampled concurrently (Fig. 2.1). Sites were surveyed from dusk to dawn over three consecutive nights each fortnight from December 2008 to April 2009 as this represented a period in which bats are most active and mosquitoes are most abundant. The start of each fortnight coincided with a spring or a neap tide, which is considered to have a strong influence on *Ae. vigilax* abundance (de Little *et al*. 2009; Kokkin *et al*. 2009). Data were collected over five spring tides and four neap tides, with two sites surveyed concurrently each fortnight.

Bat survey

In each habitat, one Anabat SD1 detector (Titley Electronics, Ballina NSW) was set at a height of one metre and at a 45° angle to the ground, facing open areas (interior of saltmarsh, away from structures in urban areas and along fire trails within forested areas).

Detectors recorded commuting and feeding echolocation calls of microchiropteran bats that vary from species to species and can be used to differentiate between most species. All recordings were stored on a compact flash card before being uploaded to a laptop for analysis. Recorded bat calls were identified to species where possible using the automated call identification software, AnaScheme (Gibson and Lumsden 2003), in association with a key for the lower north-eastern NSW coastal plain (Adams *et al.* 2010). Testing of this key revealed 0.5 % of all calls (n=191) were incorrectly identified, with misidentifications restricted to calls from a single species (*Vespadelus darlingtoni* Allen) (Adams *et al.* 2010). Bat calls with fewer than three valid pulses (i.e., minimum of six data points and model quality of ≥0.9) were not analysed by AnaScheme. Since multiple bat species may call simultaneously, calls were only assigned to a species if >50 % of pulses within the sequence were attributed to that species and only passes with a minimum of three pulses classified to the same species were identified. All calls that could not be assigned to a bat taxon were included in counts of total bat activity but were labelled as 'unidentified'.

Echolocation calls of certain species overlap to such a degree that it is not possible to differentiate between them. Consequently, the identification key grouped certain species together (e.g., *Nyctophilus gouldi* Tomes and *Nyctophilus geoffroyi* Leach = *Nyctophilus* spp.; *Vespadelus pumilus* Gray, *Vespadelus troughtoni* Kitchener, Jones and Caputi, *Vespadelus vulturnus* Thomas = *Vespadelus* spp.). While *V. troughtoni* calls could contribute to the activity of the *Vespadelus* species group, intensive harp trapping in the area (throughout a field season) failed to catch any *V. troughtoni* individuals, suggesting that the activity of *Vespadelus* spp. is likely to represent activity of *V. pumilus* and *V. vulturnus* only, both of which were commonly captured in harp traps (see Chapter 4). All identified calls were screened manually for feeding buzzes – a rapid increase in pulse repetition rate, slope, frequency and speed (associated with pursuit and capture of prey: Griffin *et al*. 1960; Pennay *et al*. 2004). For each detector and each night, the number of bat passes and number of feeding buzzes for each species was tabulated.

Prey survey

In each habitat, mosquito abundance was surveyed nightly using two CO_2–baited encephalitis virus surveillance (EVS) traps (Rohe and Fall 1979) (Australian Entomological Supplies, Bangalow, NSW, Australia), while other aerial insect fauna were

sampled using one light trap (Australian Entomological Supplies, Bangalow, NSW, Australia). All traps were set at a minimum distance of 10 m from bat detectors. Traps were set near structures (isolated mangroves within coastal saltmarsh, garden shrubs in urban areas and vegetation along the edge of a fire trail in forested areas) in each habitat that allowed EVS traps to be suspended. Mosquito collections were identified to species according to keys (Russell 1996) and abundance of each species was recorded. All light trap specimens were sorted into three insect orders (Lepidoptera, Coleoptera, Diptera), with any other specimens pooled into an 'other' category. These insect groups were then further sorted by size, into four size classes (tip of head to tip of abdomen) (<5 mm, 5-9 mm, 10-14 mm and >14 mm). Sorting into these four size classes was undertaken to provide prey availablity data that accounted for sizes of prey that bat species are known to consume (O'Neill and Taylor 1989). Counts of all insects then were carried out and recorded for each night of trapping in each habitat. Insects were oven dried at 60° C for a minimum of 48 h until a constant weight could be recorded (to nearest 1 x 10^{-5} g). For samples with more than 100 insects in a <5 mm-size class, the mass of the total sample was used along with regression equations (calculated for each taxa using count and dry-weight data obtained for all other samples of insects <5 mm) to estimate the abundance of the sample.

2.2.3 Data analyses

Prior to analysis, all collected data were log-transformed and averaged across consecutive nights for each site during each fortnight. While dry-weight data were available for all insect taxa and size classes, only abundance values were used in analyses to allow for comparison with *Ae*. *vigilax*, for which only abundance values were available. A repeated measures mixed-model ANOVA was used to test for effects of habitat (saltmarsh/urban/forest), tidal cycle (neap/spring) and all interactions on mean nightly bat activity, the activity of the commonly recorded species (representing >0.50 % of total bat activity), *Ae. vigilax* abundance and the abundance of other volant insects. An autoregressive order one covariance structure was used for the repeated factor (tidal cycle) in each analysis since this covariance structure assumes that measurements taken close to one another in time will be more closely correlated than measurements taken at greater intervals in time. Comparisons of main effects were Bonferonni corrected for multiple

comparisons. For each dependent variable, if data were not normally distributed, a Kruskal Wallis test was instead used to examine the effect of each independent variable.

A goodness of fit chi-square test was used to compare proportional feeding activity (proportion of bat calls containing feeding buzzes) and the proportional feeding activity of individual species between habitats and tidal cycle, while best subsets regression analysis was used to examine relationships between the abundances of non-mosquito prey (lepidopterans, coleopterans, dipterans, other insects, all taxa pooled, insects <5 mm, insects, 5-9 mm, insects, 10-14 mm and insects >14 mm), *Ae*. *vigilax* abundance and bat activity as well as the activity of the commonly recorded bat species (representing >0.50 % of total bat activity). Selection of models for each dependent variable was based on Akaikes Information Criteria scores corrected for small sample sizes (AICc). Since vegetation clutter influences prey detectability (Adams *et al*. 2009; Rainho *et al*. 2010), analyses were conducted for each habitat separately. Given insect abundances vary with height within forests (Adams *et al*. 2009), it was considered that prey trapped in light traps set on the forest floor would not provide data representative of insect assemblages in the forest canopy. For this reason, *Tadarida australis* Gray (a high-flying bat) was excluded from all regression analyses.

2.3 Results

2.3.1 Bat fauna

In all, 17,025 bat calls were recorded across all habitats, representing 13 species and two species groups, of which six are currently listed as threatened under the NSW *Threatened Species Conservation Act* 1995. The forest habitat sustained all recorded taxa, while 12 and 11 taxa were present in saltmarsh and urban habitats, respectively (Table 2.1). In all, 13 190 (77.5 %), 2 237 (13.1 %) and 1 598 (9.4 %) bat passes were recorded in forest, urban and saltmarsh habitats, respectively.

Four taxa (*Chalinolobus gouldii* Gray, *Mormopterus* sp. 2 Peters, *T. australis* and *Vespadelus* spp.) represented ~67 % and ~56 % of all activity recorded in saltmarsh and urban habitats, respectively (Table 2.1). In the forest habitat, three taxa (*C. gouldii*,

Chalinolobus morio Gray and *Vespadelus* spp.) contributed ~77 % of all recorded activity (Table 2.1).

Mean nightly species diversity (measured as species richness) was significantly different between habitats (df=45, F=5.699, P=0.009) and tides (df=45, F=7.246, P=0.012), with greater species diversity recorded in forest when compared with urban habitat (P=0.007) and greater species diversity recorded during neap tides (Table 2.1).

Nightly bat activity (number of bat passes night^{-1}) was significantly different between habitats (df=45, F=20.141, P<0.001), with significantly greater activity recorded in forest compared to saltmarsh and urban habitats respectively (P<0.001 respectively; Fig. 2.2).

Table 2.1: Mean ± SE nightly bat activity (untransformed data) of individual species in each habitat during neap and spring tides. Means followed by different letters within the same row were significantly different from one another.

Taxa	Saltmarsh			Urban			Forest			Total		
	Neap	Spring	Total	Neap	Spring	Total	Neap	Spring	Total	Neap	Spring	Total
Chalinolobus dwyeri	0.1 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.1 ± 0.1		0.1 ± 0.1	0.2 ± 0.2		0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
C. gouldii	7.2 ± 2.0	6.0 ± 1.7	6.6 ± 1.3	7.0 ± 2.2	11.2 ± 4.7	9.2 ± 2.6	17.2 ± 8.9	9.1 ± 5.6	12.9 ± 5.1	10.5 ± 3.1	8.8 ± 2.4	9.6 ± 2.0
C. morio		0.1 ± 0.1	0.1 ± 0.1				13.6 ± 13	7.7 ± 7.1	10.5 ± 7	4.5 ± 4.3	2.6 ± 2.4	3.5 ± 2.4
Falsistrellus tasmaniensis							0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1		
Kerivoula papuensis							0.1 ± 0.1		<0.1 ± <0.1			
Miniopterus australis		0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.2		0.1 ± 0.1	1.7 ± 0.7	2.2 ± 1.7	2.0 ± 0.9	0.7 ± 0.3	0.8 ± 0.6	0.7 ± 0.3
Mi. schreibersii oceanensis	0.3 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Mormopterus sp. 2	6.9 ± 1.2	3.3 ± 0.8	5.0 ± 0.8	4.4 ± 1.5	2.8 ± 1.1	3.6 ± 0.9	1.5 ± 0.8	0.7 ± 0.5	1.0 ± 0.4	4.3 ± 0.8	2.3 ±0.5	3.2 ± 0.5
Nyctophilus spp.		0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1		0.1 ± 0	1.0 ± 0.2	0.1 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
Rhinolophus megaphylus					0.1 ± 0.1	0.1 ± 0	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1		0.1 ± 0.1	0.1 ± 0.1
Scoteanax rueppellii	0.2 ± 0.1		0.1 ± 0.0	0.8 ± 0.7		0.4 ± 0.3	0.2 ± 0.1		0.1 ± 0.1	0.4 ± 0.2		0.2 ± 0.1
Scotorepens orion	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ±0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Tadarida australis	2.3 ± 0.6	1.2 ± 0.4	1.7 ± 0.4	2.2 ± 0.9	1.1 ± 0.3	1.6 ± 0.5	1.0 ± 0.5	0.4 ± 0.2	0.7 ± 0.2	1.9 ± 0.4	0.9 ± 0.2	1.3 ± 0.2
Vespadelus darlingtoni	0.1 ± 0.1		0.1 ± 0.0				0.4 ± 0.3	0.1 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Vespadelus spp.	11.7 ± 8.6	3.1 ± 1.6	7.1 ± 4.1	2.5 ± 0.8	1.7 ± 0.6	2.1 ± 0.5	211.2 ± 85.2	147.7 ± 67.6	177.6 ± 52.6	75.2 ± 33.9	50.8 ± 25.5	62.3 ± 20.7
Total	**38.5 ± 14.6**	**17.6 ± 4.4**	**27.4 ± 7.5**	**46.5 ± 19.9**	**35.8 ± 10.7**	**40.9 ± 10.7**	**338.7 ± 88.8**	**208.8 ± 81.3**	**269.9 ± 60.3**	**141.2 ± 41.4**	**87.4 ± 31.3**	**112.7 ± 25.6**
Species richness	**6.0 ± 0.3**	**5.4 ± 0.7**	**5.7ab ± 0.4**	**5.0 ± 0.6**	**4.1 ± 0.5**	**4.5a ± 0.4**	**8.0 ± 0.7**	**5.3 ± 0.6**	**6.6b ± 0.6**	**6.3 ± 0.4**	**5.0 ± 0.4**	**5.6 ± 0.3**

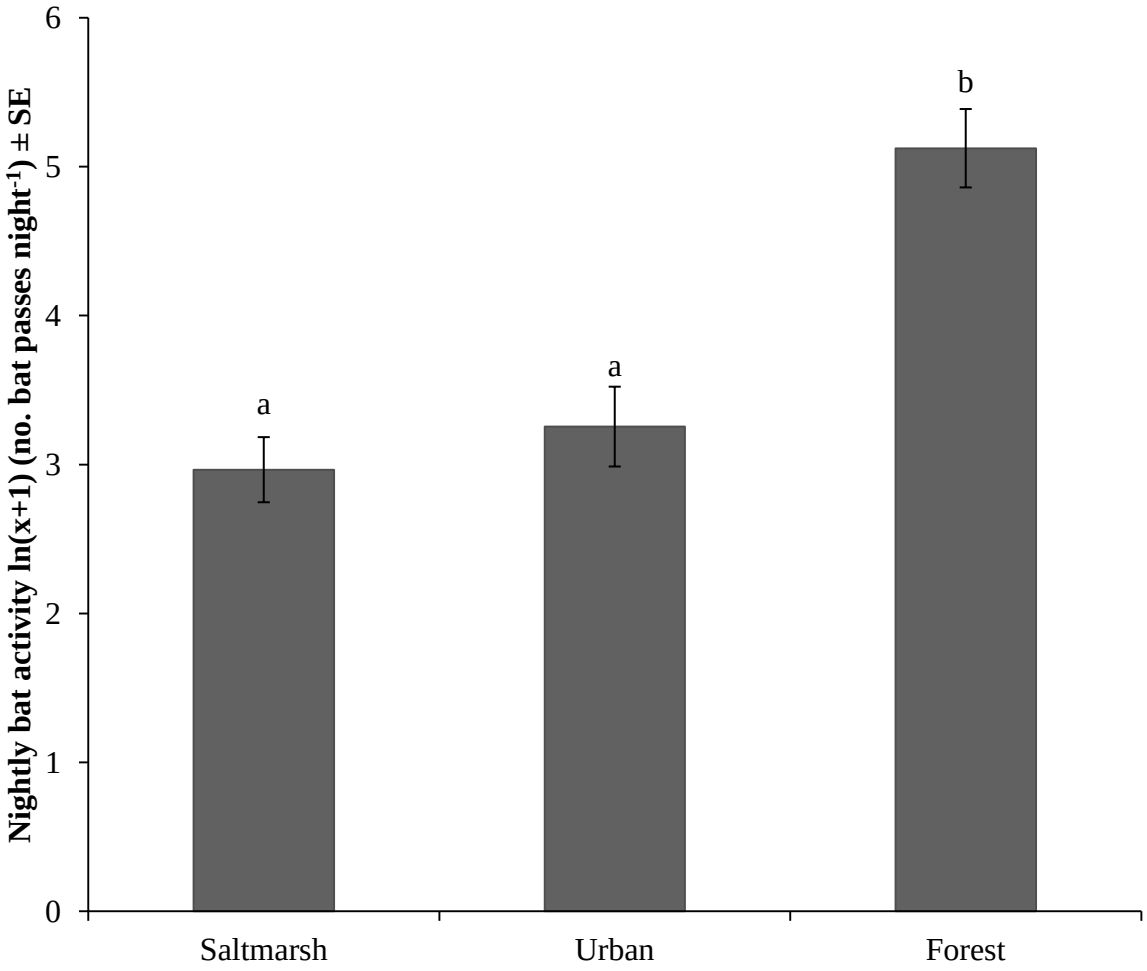


Figure 2.2: Nightly bat activity recorded in each habitat. Means denoted by different letters are significantly different from one another.

The activity of *Mo.* sp. 2 (df=45, F=14.108, P<0.001), *T. australis* (df=45, F=3.636, P=0.040) and *Vespadelus* spp. (df=45, F=70.180, P<0.001) differed significantly between habitats. Kruskal Wallis ANOVA revealed the activity of *C. morio* (df=2, *H*=24.162, P<0.001) and *Miniopterus australis* Tommes (df=2, *H*=18.160, P<0.001) differed significantly between habitats. Bonferroni comparisons indicated *C. morio*, *Mi. australis* and *Vespadelus* spp. were significantly more active in forest when compared to saltmarsh (P=0.05, P=0.011, P<0.001; Fig. 2.3a, 2.3b & 2.3e) and urban habitats (P=0.002, P=0.001, P<0.001; Fig. 2.3a, 2.3b & 2.3e). *Tadarida australis* was significantly more active in saltmarsh when compared with forest habitat (P=0.050; Fig. 2.3d), while *Mo.* sp. 2 was significantly more active in saltmarsh (P<0.001) and urban (P=0.001) habitats than in the forest habitat (Fig. 2.3c).

Although nightly bat activity did not differ between tidal cycles, the activity of individual species did, with significantly greater activity recorded for *Mo*. sp. 2 (df=45, F=6.339, P=0.018) and *T*. *australis* (df=45, F=5.068, P=0.033) during neap tides (Figs. 2.4a & 2.4b).

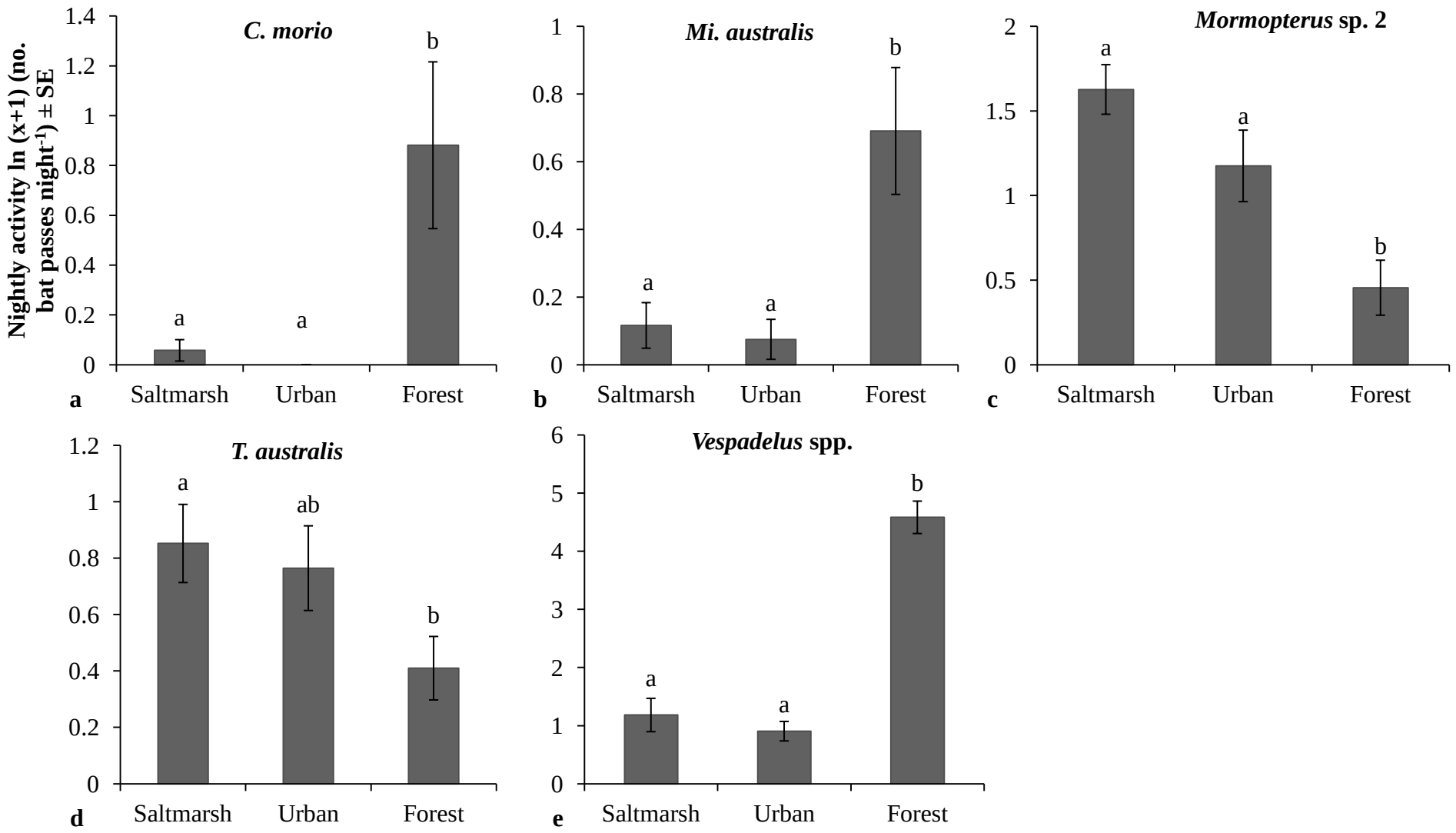


Figure 2.3: Mean nightly bat activity in each habitat: a) *Chalinolobus morio*; b) *Miniopterus australis*; c) *Mormopterus* sp. 2; d) *Tadarida australis*; e) *Vespadelus* spp.. Means denoted by different letters are significantly different from one another.

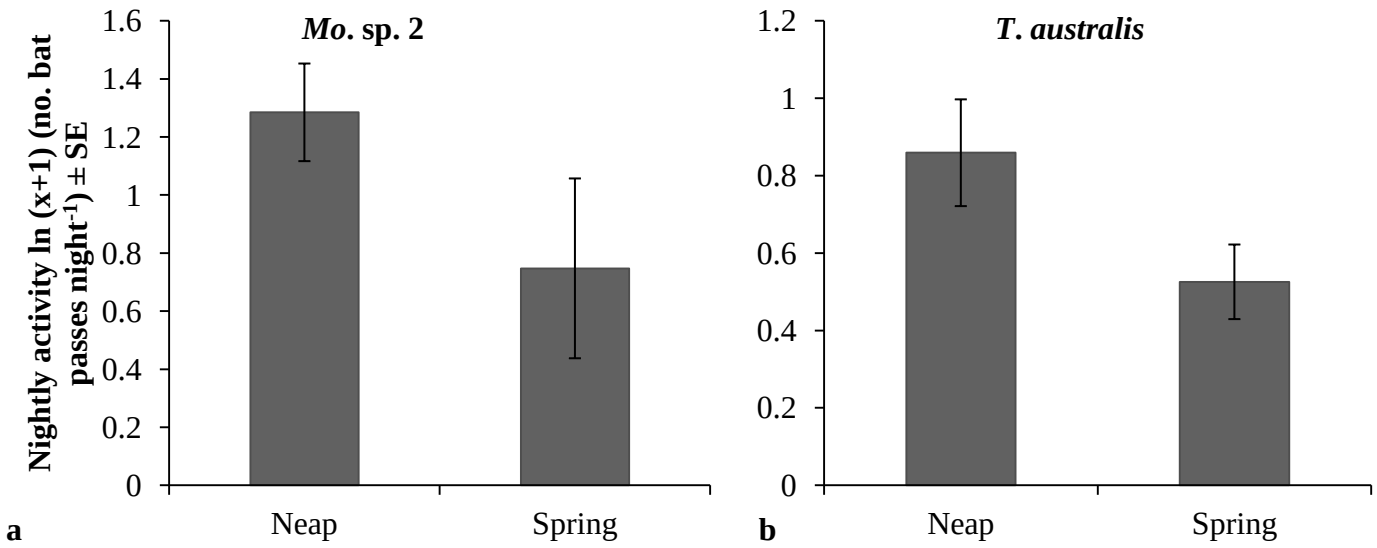


Figure 2.4: Mean nightly bat activity during neap and spring tides: a) *Mormopterus* sp. 2; b) *Tadarida australis*.

2.3.2 Bat foraging activity

In all, 268 feeding buzzes (1.7 % of all calls) were recorded across all habitats, with 55, 8 and 205 buzzes detected in saltmarsh, urban and forest habitats, respectively. Proportional feeding activity differed significantly between habitats (df=2, χ^2=33.600, P<0.001), representing 4.0 %, 0.4 % and 1.6 % of calls recorded in saltmarsh, urban and forest habitats, respectively. While the number of feeding buzzes recorded during neap tides (159) was greater than spring tides (109), proportional feeding activity did not differ between tidal cycles.

Feeding buzzes were recorded for three species (*C. gouldii* - 15, *Mi. australis* - 3, *Mo.* sp. 2 - 9) and one species group (*Vespadelus* spp. - 241). Proportional feeding activity of *C. gouldii*, and *Vespadelus* spp. was significantly different between habitats (df=2, χ^2=32.976, P<0.001; df=2, χ^2=6.500, P=0.039), with most feeding activity recorded in saltmarsh (3.13 % and 10.19 %), followed by urban (0.47 % and 3.88 %) and forest (0.46 % and 2.25 %) habitats. Proportional feeding activity of all taxa combined did not differ between tidal cycles, irrespective of habitat.

2.3.3 Mosquito fauna

In all, 70 364 mosquitoes were sampled across all habitats during the study, representing 27 species (Table 2.2). The forest habitat supported 25 species, while 16 species and 14 species were present in saltmarsh and urban habitats, respectively (Table 2.2). In all, 33 125 (46.9 %), 3 560 (5.1 %) and 33 679 (47.9 %) mosquitoes were recorded in saltmarsh, urban and forest habitats, respectively.

Aedes vigilax was the most abundant species in each habitat, representing 91.6 %, 41.6 % and 91.1 % of all individuals trapped in saltmarsh, urban and forest habitats, respectively (Table 2.2). The other commonly collected species were *Ae. alternans* and *Cx. sitiens*, two species closely associated with estuarine habitats, *Culex annulirostris* Skuse, a species closely associated with freshwater habitats, and *Cx. molestus*, a species associated with waste-water habitats (Table 2.2).

While the abundance of *Ae. vigilax* differed between habitats (df=2, *H*=23.966, P<0.001; Fig. 2.5), there was no significant difference between tidal cycle (df=1, *H*=0.142,

P=0.706). Bonferroni comparisons revealed *Aedes vigilax* abundance was significantly lower in urban habitat when compared to saltmarsh (P=0.001) and forest habitats (P<0.001).

Table 2.2: Mean ± SE nightly abundance of mosquito taxa in each habitat during neap and spring tides.

Taxa	Saltmarsh			Urban			Forest			Total		
	Neap	Spring	Total	Neap	Spring	Total	Neap	Spring	Total	Neap	Spring	Total
Aedes alboannulatus							0.2 ± 0.8	0.2 ± 0.8	0.2 ± 0.5	0.7 ± 0.3	0.5 ± 0.3	0.6 ± 0.2
Ae.alternans	12.0 ± 4.7	5.9 ± 2.9	8.8 ± 2.7	0.9 ± 0.6	0.2 ± 0.2	0.6 ± 0.3	2.6 ± 2.7	0.5 ± 0.4	1.5 ± 1.0	5.2 ± 1.9	2.3 ± 1.7	3.6 ± 1.8
Ae.australis		0.2 ± 0.2	0.1 ± 0.1	0.3 ± 0.2	0.5 ± 0.3	0.2 ± 1.0				0.9 ± 0.7	0.2 ± 0.1	0.5 ± 0.3
Ae.camptorhynchus	0.3 ± 0.3		1.0 ± 1.0				0.3 ± 0.3		0.1 ± 0.1	0.2 ± 0.2		0.7 ± 0.6
Ae.flavifrons							0.3 ± 0.3	0.4 ± 0.4	0.2 ± 0.2	0.7 ± 0.7	0.1 ± 0.1	0.7 ± 0.6
Ae.mallochi							0.3 ± 0.3		1.0 ± 1.0	0.7 ± 0.7		0.3 ± 0.3
Ae.marks#52							0.3 ± 0.3		0.1 ± 1.0	0.8 ± 0.7		0.4 ± 0.3
Ae.multiplex	0.1 ± 0.8	0.4 ± 0.3	0.3 ± 0.2	0.1 ± 0.8	0.8 ± 0.4	0.5 ± 0.2	1.3 ± 1.8	7.8 ± 5.2	4.7 ± 2.9	0.5 ± 0.4	3.9 ± 1.9	1.8 ± 1.0
Ae.notoscriptus	0.2 ± 0.8	0.3 ± 0.1	0.2 ± 0.7	4.9 ± 2.3	2.4 ± 1.2	3.5 ± 1.3	1.4 ± 0.3	1.0 ± 0.2	1.1 ± 0.2	2.2 ± 0.8	1.2 ± 0.4	1.6 ± 0.5
Ae.palmarum			0.0 ± 0.0				0.1 ± 0.6	0.2 ± 0.2	0.2 ± 1.0	0.4 ± 0.2	0.7 ± 0.6	0.6 ± 0.3
Ae.procax		0.2 ± 0.2	0.1 ± 0.2		0.2 ± 0.2	0.1 ± 1.0	1.4 ± 0.7	12.0 ± 9.2	7.0 ± 4.9	0.5 ± 0.3	4.1 ± 3.1	2.4 ± 1.7
Ae.quasirubrithorax							0.3 ± 0.3	0.2 ± 0.2	0.1 ± 0.1	0.7 ± 0.7	0.6 ± 0.6	0.3 ± 0.3
Ae.rubrithorax							0.3 ± 0.3	0.4 ± 0.3	0.3 ± 0.2	0.9 ± 0.8	0.1 ± 0.9	0.2 ± 0.6
Ae.tremulus							0.3 ± 0.3		1.0 ± 1.0	0.7 ± 0.7		0.3 ± 0.3
Ae.vigilax	528.2 ± 347.9	261.1 ± 127.5	386.8 ± 174.2	13.4 ± 5.0	15.9 ± 7.6	14.7 ± 4.5	339.4 ± 113.8	329.3.0 ± 134.3	334.0 ± 86.3	293.6 ± 124.8	202.1 ± 65.0	245.2 ± 67.6
Anopheles annulipes				0.4 ± 0.3	0.6 ± 0.4	0.5 ± 0.2	0.1 ± 0.1	0.6 ± 0.3	0.8 ± 0.6	0.5 ± 0.4	0.4 ± 0.2	0.4 ± 0.2
An.atratipes								0.2 ± 0.2	1.0 ± 1.0		0.6 ± 0.6	0.3 ± 0.3
Coquillettidia linealis	0.5 ± 0.3	0.2 ± 0.2	0.3 ± 0.2	0.3 ± 0.3		1.0 ± 1.0	0.8 ± 0.4	0.1 ± 0.6	0.4 ± 0.2	0.3 ± 0.2	0.5 ± 0.2	0.2 ± 0.7
Culex.annulirostris	1.4 ± 0.5	2.6 ± 1.2	2.3 ± 0.7	0.2 ± 0.9	0.6 ± 0.4	0.5 ± 0.2	9.2 ± 7.6	8.7 ± 4.6	8.6 ± 4.2	3.6 ± 2.6	3.8 ± 1.6	3.7 ± 1.5
Cx.australicus		0.4 ± 0.3	0.2 ± 0.2	0.4 ± 0.3	0.2 ± 0.2	0.1 ± 0.6	0.1 ± 0.8	0.1 ± 0.8	0.1 ± 0.5	0.5 ± 0.3	0.2 ± 0.2	0.2 ± 0.6
Cx.molestus	0.2 ± 0.8	0.4 ± 0.1	0.3 ± 0.8	3.3 ± 1.7	18.6 ± 9.9	11.4 ± 5.5	0.4 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	1.3 ± 0.7	6.5 ± 3.6	4.6 ± 1.9
Cx.orbostiensis	0.5 ± 0.3	0.2 ± 0.2	0.3 ± 0.2	0.1 ± 0.1	0.9 ± 0.4	0.1 ± 0.6	0.8 ± 0.8	0.1 ± 0.6	0.1 ± 0.6	0.9 ± 0.5	0.9 ± 0.3	0.9 ± 0.3
Cx.qinquefasciatus	0.8 ± 0.2	0.9 ± 0.1	0.8 ± 0.1	3.3 ± 1.8	3.2 ± 1.3	3.3 ± 0.7	0.8 ± 0.2	0.6 ± 0.3	0.7 ± 0.2	1.7 ± 0.4	1.6 ± 0.4	1.6 ± 0.3
Cx.sitiens	4.7 ± 3.4	24.7 ± 11.3	15.4 ± 6.5	0.2 ± 0.1	1.6 ± 1.0	0.9 ± 0.5	0.5 ± 0.5	0.8 ± 0.5	0.7 ± 0.3	1.8 ± 1.7	9.6 ± 4.2	5.6 ± 2.3
Mansonia uniformis								0.2 ± 0.2	1.0 ± 1.0		0.6 ± 0.6	0.3 ± 0.3
Tripteroides atripes							0.3 ± 0.3		1.0 ± 1.0	0.7 ± 0.7		0.3 ± 0.3
Verrallina funerea	0.3 ± 0.3		0.1 ± 0.1							0.8 ± 0.8		0.4 ± 0.4
Total	396.5 ± 220.0	300.4 ± 139.1	345.6 ± 123.4	26.9 ± 6.3	44.0 ± 17.5	36.0 ± 9.7	340.4 ± 114.0	362.9 ± 136.3	352.3 ± 87.2	254.6 ± 85.9	235.8 ± 68.2	244.6 ± 53.7

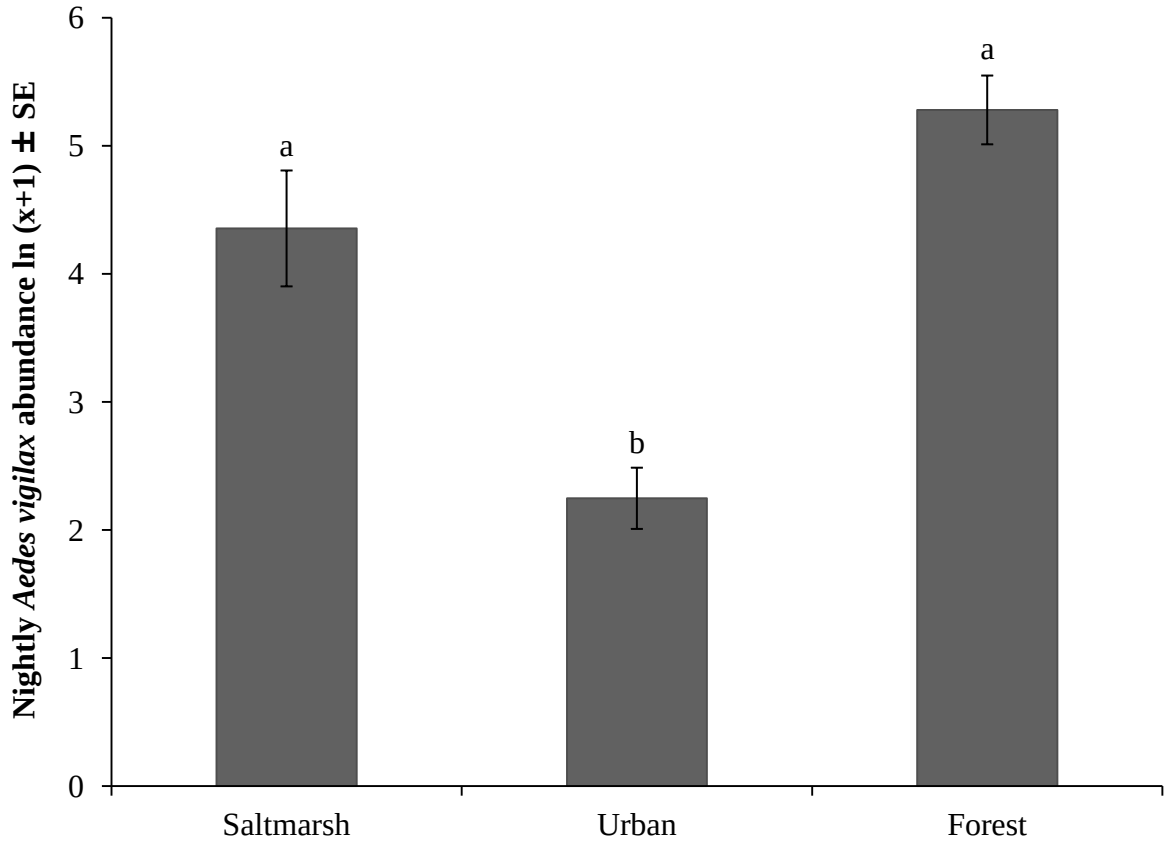


Figure 2.5: Mean nightly *Aedes vigilax* abundance recorded in each habitat. Means denoted by different letters are significantly different from one another.

2.3.4 Non-mosquito fauna

Over 45 000 insects were sampled across all habitats during the study, with 27.6 %, 29.5 % and 42.9 % of all insects collected in saltmarsh, urban and forest habitats, respectively. More than half of all sampled insects (67.7 %) were trapped during neap tides. Lepidopterans, coleopterans, dipterans and 'other' insects (Blattodea, Hemiptera, Hymenoptera, Isoptera, Odonata, and Orthoptera) represented 24.9 %, 11.7 %, 18.5 % and 45.7 % of the sampled insects, respectively.

The abundance of all insects (all taxa pooled together) in the <5-mm size class was significantly different between habitats (df=38, F=11.394, P=0.001) with fewer of these insects recorded in urban habitat when compared with saltmarsh (P=0.015) and forest (P=0.001) habitats (Table 2.3). The abundance of all insects in the 10-14-mm size class was also significantly different between habitats (df=38, F=9.490, P=0.001), with greater abundances recorded in forest habitat when compared with saltmarsh (P=0.002) and urban (P=0.005) habitats (Table 2.3). The abundances of all insects in the 5-9-mm and >14-mm

size classes also differed between habitats (df=38, F=4.215, P=0.043; df=38, F=5.049, P=0.030), with greater abundances recorded in the forest habitat when compared with saltmarsh habitat (P=0.044; P=0.036) (Table 2.3). The abundance of all insects (all size classes of all taxa pooled together) differed significantly between habitats (df=38, F=9.126, P=0.003), with greater abundances recorded in forest habitat when compared with urban habitat (P=0.003) (Table 2.3).

Table 2.3: Mean ± SE nightly abundances of insect taxa and insect size classes in each habitat during neap and spring tides. Means followed by different letters (lower case for habitats and upper case for neap and spring tides) within the same row were significantly different from one another.

Taxa	Saltmarsh			Urban			Forest			Total		
	Neap	Spring	Total	Neap	Spring	Total	Neap	Spring	Total	Neap	Spring	Total
Lepidoptera												
<5 mm	52.5 ± 11.5	29.9 ± 10.2	40.5 ± 8.0	47.4 ± 10.1	22.2 ± 5.0	33.3 ± 6.0	200.3 ± 62.7	200.3 ± 64.7	200.3 ± 47.3	83.4 ± 20.4	86.2 ± 27.4	85.1 ± 18.0
5-9 mm	13.5 ± 4.2	13.7 ± 5.2	13.6 ± 3.3	22.0 ± 7.0	13.6 ± 2.7	17.3 ± 3.5	54.5 ± 17.7	30.6 ± 8.6	37.9 ± 8.3	25.9 ± 6.0	19.5 ± 3.7	22.1 ± 3.3
10-14mm	6.8 ± 3.6	1.6 ± 0.6	4.0 ± 1.8	8.4 ± 5.4	2.1 ± 0.7	4.9 ± 2.4	16.0 ± 9.0	8.9 ± 2.1	11.1 ± 3.0	9.5 ± 3.1	4.3 ± 1.0	6.4 ± 1.4
>14 mm	1.2 ± 0.8	0.4 ± 0.2	0.7 ± 0.4	2.7 ± 1.3	0.3 ± 0.2	1.4 ± 0.6	3.5 ± 1.3	3.7 ± 1.3	3.6 ± 1.0	2.3 ± 0.7	1.5 ± 0.6	1.8 ± 0.4
All sizes	74.0 ± 16.7	45.6 ± 10.8	58.8 ± 10.1	80.6 ± 20.8	37.7 ± 6.8	56.5 ± 10.9	274.0 ± 80.9	243.0 ± 73.3	252.5 ± 54.7	121.0 ± 27.3	111.2 ± 31.3	115.2 ± 21.4
Coleoptera												
<5 mm	57.9 ± 44.5	15.9 ± 6.8	35.5 ± 21.0	33.3 ± 11.2	14.7 ± 6.5	22.8 ± 6.3	94.3 ± 49.4	52.0 ± 20.0	65.0 ± 20.1	56.4 ± 20.4	28.0 ± 8.1	39.6 ± 9.7
5-9 mm	3.3 ± 2.2	0.5 ± 0.4	1.8a ± 1.1	19.4 ± 7.2	5.4 ± 1.5	11.6b ± 3.6	40.8 ± 22.5	19.1 ± 7.4	25.8b ± 8.5	17.9 ± 6.3	8.6 ± 3.0	12.4 ± 3.1
10-14mm	0.1 ± 0.1	0.6 ± 0.5	0.4 ± 0.3	2.3 ± 1.0	1.0 ± 0.4	1.6 ± 0.5	12.0 ± 9.4	4.1 ± 1.9	6.5 ± 3.1	3.6 ± 2.2	2.0 ± 0.7	2.6 ± 1.0
>14 mm	0.6 ± 0.3	0.0 ± 0.0	0.3 ± 0.2	0.1 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	1.3 ± 0.8	2.4 ± 1.5	2.1 ± 1.1	0.6 ± 0.2	1.0 ± 0.5	0.8 ± 0.3
All sizes	61.8 ± 46.7	17.0 ± 7.0	37.9 ± 22.0	55.1 ± 17.1	21.1 ± 8.3	36.0 ± 9.5	148.2 ± 81.9	77.3 ± 30.2	99.1 ± 32.0	78.4 ± 26.2	39.3 ± 12.0	55.3 ± 13.0
Diptera												
<5 mm	337.6 ± 142.3	65.2 ± 12.5	192.3a ± 73.5	26.4 ± 10.3	23.7 ± 5.1	24.9b ± 5.1	87.8 ± 29.3	82.0 ± 25.2	83.8ab ± 19.0	161.1 ± 63.4	56.7 ± 10.6	99.4 ± 27.4
5-9 mm	10.7 ± 4.4	3.8 ± 1.2	7.1 ± 2.3	4.9 ± 2.7	1.1 ± 0.6	2.8 ± 1.3	4.5 ± 1.9	17.9 ± 14.4	13.8 ± 10.0	7.1 ± 2.1	7.8 ± 5.0	7.5 ± 3.1
10-14mm	2.5 ± 2.2	0.0 ± 0.0	1.1 ± 1.0	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.8 ± 0.5	0.9 ± 0.7	0.8 ± 0.5	1.1 ± 0.9	0.3 ± 0.2	0.7 ± 0.4
>14 mm	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.0
All sizes	350.9 ± 143.0	69.0 ± 13.0	200.6 ± 74.5	31.3 ± 10.8	24.7 ± 5.1	27.6 ± 5.4	92.7 ± 29.2	100.6 ± 23.6	98.2 ± 18.0	169.2 ± 64.2	64.6 ± 10.9	107.4 ± 27.7
'Other'												
<5 mm	15.0 ± 8.4	9.6 ± 4.9	12.1 ± 4.6	10.6 ± 5.3	6.7 ± 2.6	8.4 ± 2.7	22.5 ± 7.7	25.8 ± 10.5	24.8 ± 7.4	14.9 ± 4.1	14.2 ± 4.2	14.5 ± 3.0
5-9 mm	1.8 ± 0.9	1.5 ± 0.4	1.6 ± 0.5	1.0 ± 0.8	2.7 ± 1.2	1.9 ± 0.8	7.5 ± 5.0	5.1 ± 2.1	5.8 ± 2.0	2.7 ± 1.3	3.2 ± 0.9	3.0 ± 0.7
10-14mm	0.3 ± 0.3	0.2 ± 0.1	0.3a ± 0.1	1.1 ± 0.6	0.1 ± 0.1	0.6a ± 0.3	3.8 ± 1.8	3.7 ± 1.4	3.7b ± 1.1	1.4 ± 0.5	1.4 ± 0.6	1.4 ± 0.4
>14 mm	0.9 ± 0.7	0.2 ± 0.1	0.5 ± 0.3	0.9 ± 0.6	0.2 ± 0.1	0.5 ± 0.3	3.8 ± 2.3	1.2 ± 0.5	2.0 ± 0.8	1.5 ± 0.6	0.6 ± 0.2	0.9 ± 0.3
All sizes	17.9 ± 8.5	11.5 ± 4.9	14.5 ± 4.6	13.5 ± 5.9	9.4 ± 3.2	11.2 ± 3.1	37.3 ± 16.1	35.7 ± 11.0	36.2 ± 8.7	20.5 ± 5.5	19.1 ± 4.7	19.7 ± 3.5
All insects												
<5 mm	462.9 ± 126.6	120.7 ± 25.4	280.4a ± 73.9	117.7 ± 27.0	66.9 ± 17.2	89.2b ± 16.1	443.7 ± 97.3	360.0 ± 88.2	389.9a ± 65.1	330.7A ± 63.9	184.9B ± 40.2	246.5 ± 36.8
5-9 mm	29.2 ± 8.3	19.5 ± 5.6	24.1a ± 4.9a	47.3 ± 15.5	22.1 ± 3.9	33.1ab ± 7.6	101.5 ±35.4	72.1 ± 25.5	82.6b ± 20.3	54.9 ± 12.6	38.6 ± 10.0	45.5 ± 7.9
10-14mm	9.7 ± 3.8	2.4 ± 0.6	5.8a ± 2.0a	11.9 ± 5.7	2.9 ± 0.8	6.8a ± 2.7	30.1 ±14.9	17.3 ± 4.0	21.9b ± 5.8	15.9 ± 4.8	7.7 ± 2.0	11.2 ± 2.4
>14 mm	2.7 ± 1.2	0.6 ± 0.2	1.6a ± 0.6a	3.6 ± 1.2	0.9 ± 0.3	2.1ab ± 0.6	7.9 ±2.6	7.2 ± 2.8	7.4b ± 2.0	4.4 ± 1.0	3.0 ± 1.1	3.6 ± 0.8
All sizes	504.6 ± 124.4	143.1 ± 27.3	311.8ab ± 74.9	180.5 ± 42.2	92.9 ± 20.5	131.2b ± 23.7	583.2 ±134.4	456.5 ± 109.9	501.8a ± 83.9	405.9A ± 69.8	234.2B ± 50.1	306.7 ± 42.8

The abundances of certain insect taxa of particular size class also were found to differ between habitats. While the abundance of lepidopterans in the <5-mm size class was found to differ between habitats (df=38, F=4.090, P=0.034), pairwise comparisons revealed that no single habitat differed from another. A significant difference between habitats was observed for the abundance of coleopterans in the 5-9-mm size class (df=38, F=7.996, P=0.004) with higher abundances recorded in forest (P=0.006) and urban (P=0.014) habitats when compared to saltmarsh habitat (Table 2.3). The abundance of dipterans in the <5-mm size class was also significantly different between habitats (df=2, χ^2=16.466, P=0.001), with significantly higher abundances in saltmarsh when compared with urban habitat (P=0.001) (Table 2.3). The abundance of other insects in the 10-14-mm size class was found to differ between habitats (df=2, χ^2=10.668, P=0.005) with greater abundances recorded in forest habitat when compared with saltmarsh (P=0.032) and urban (P=0.009) habitats (Table 2.3).

A significant interaction effect was observed for the abundance of all dipterans (all size classes pooled) (df=38, F=3.655, P=0.047), with significantly more dipterans in the saltmarsh habitat during neap tides (Fig. 2.6).

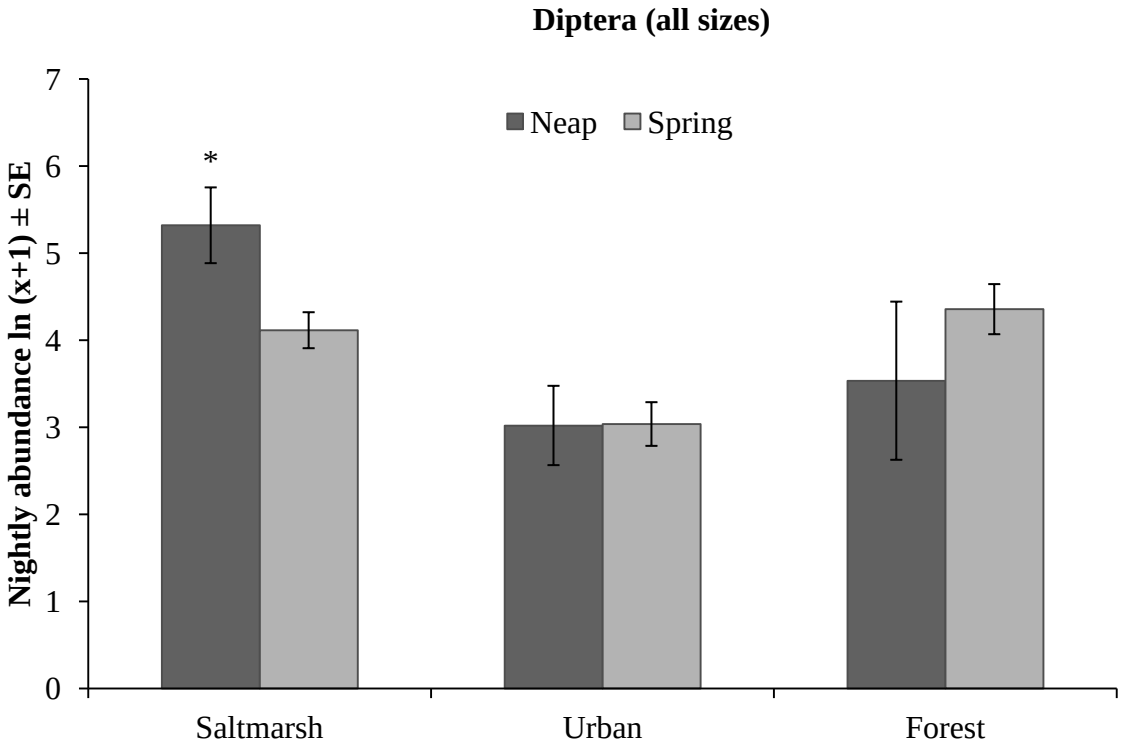


Figure 2.6: Nightly abundance of dipterans (all sizes) in each habitat during neap and spring tides. Asterisk denotes significant interaction effect.

The abundances of all insects in the <5-mm size class and all insects (all size classes of all taxa pooled together) were significantly greater during neap tides (df=38, F=5.233, P=0.038; df=38, F=5.717, P=0.031; Table 2.3).

2.3.5 Relationships between bat activity and the abundance of mosquito and non-mosquito prey

Relationships between response variables (nightly bat activity and the activity of individual species) and predictor variables were not consistent across habitats. In saltmarsh, the abundance of lepidopterans, dipterans and all insects <5 mm in size together accounted for 90.0 % of variability in nightly bat activity (df=3, F=43.20, P<0.001; Table 2.4). The abundance of lepidopterans was positively correlated with the activity of *C. gouldii* (df=1, R^2=0.306, F=5.73, P=0.032; Table 2.4). The activity of *Mo.* sp. 2 was positively correlated with nightly insect abundance (df=1, R^2=0.520, F=14.08, P=0.002; Table 2.4). *Vespadelus* spp. activity was positively correlated with the abundances of coleopterans, large insects (>14 mm) and *Ae. vigilax* (df=1, R^2=0.882, F=35.98, P<0.001; Table 2.4).

In the urban habitat, the activity of *Mi. australis* was negatively correlated with the abundance of 'other' insects and positively correlated with the abundance of dipterans (df=2, R^2=0.319, F=4.51, P=0.033; Table 2.4). A significant relationship between prey abundance variables and the activity of bats was observed for *Vespadelus* spp., with activity positively correlated with the abundance of dipterans (df=1, R^2=0.413, F=9.84, P=0.007; Table 2.4).

In the forest habitat, activity of *C. gouldii* was negatively correlated with dipteran abundance (df=1, R^2=0.318, F=7.05, P=0.021; Table 2.4). The activity of *Vespadelus* spp. was positively correlated with the abundance of *Ae. vigilax* (df=1, R^2=0.511, F=15.66, P=0.001; Table 2.4).

Table 2.4. Final statistical models for relationships between prey variables and bat activity in each habitat based on Akaike Information Criterion (AICc) score (corrected for small sample size) ranking of models. Lep = All lepidopterans; Col = All coleopterans; Dip = All dipterans; Other = All other insects; <5 mm = All insects <5 mm; >14mm = All insects >14mm.

Habitat	Species	AICc	Variables in model	Coefficient	T	P	Model		
							R^2	F	P
Saltmarsh	Nightly activity	-1.15347	Lep	0.25990	2.51	0.029	0.900	43.20	<0.001
			Dip	-0.85650	-5.68	<0.001			
			<5 mm	1.27240	7.15	<0.001			
	Chalinolobus gouldii	-4.57533	Lep	0.50590	2.39	0.032	0.306	5.73	0.032
	Mormopterus sp. 2	-5.48820	All	0.44260	3.75	0.002	0.520	14.08	0.002
	Vespadelus spp.	-1.11306	*Ae. vigilax*	0.20474	3.46	0.005	0.882	35.98	<0.001
			Col	0.69998	8.34	<0.001			
			>14 mm	0.59770	3.65	0.004			
Urban	*Miniopterus australis*	-2.23056	Dip	0.15644	2.50	0.027	0.319	4.51	0.033
			Other	-0.11871	-2.56	0.024			
	Vespadelus spp.	-4.96057	Dip	0.45730	3.14	0.007	0.413	9.84	0.007
Forest	*Chalinolobus gouldii*	-4.65227	Dip	-0.6466	-2.65	0.021	0.318	7.05	0.021
	Vespadelus spp.	-5.45900	*Ae. vigilax*	0.72090	3.96	0.001	0.511	15.66	0.001

2.4 Discussion

This is the first study to comprehensively investigate relationships between mosquito prey and other insects, and bat activity across a range of coastal habitats. The results of this study indicate that complex spatial and temporal relationships exist between bats, prey and the local environment. Although prey (*Ae. vigilax* and all non-mosquito prey) were generally most abundant in saltmarsh and forest habitats, relationships between prey abundances and total bat activity were only identified in the less-cluttered saltmarsh habitat, suggesting a potential interaction of the effects of clutter and prey abundance on prey detectability. Such an interaction is further highlighted by greater proportional feeding activity recorded in saltmarsh. However, relationships between prey abundance and the activity of particular species were identified in all habitats. Only the activity of bats of the *Vespadelus* genus was positively correlated with *Ae. vigilax* abundance in both saltmarsh and forest habitats, suggesting that any interacting effects of clutter and prey abundance on prey detectability may not apply equally to all bat taxa, particularly those with greater manoeuvrability and an echolocation design suited for detection of small prey along edges of cluttered vegetation (Barclay and Brigham 1991). While positive relationships between the activity of bats of the *Vespadelus* genus and *Ae. vigilax* abundance support suggestions that *Ae. vigilax* may be an important prey resource for these bats (Laegdsgaard *et al*. 2004), detailed dietary investigations are required to confirm these suggestions.

2.4.1 Bat commuting and feeding activity

Activity of individual species differed between habitats, but these differences were in line with predictions based on echolocation design and wing morphology. *Chalinolobus morio*, *Mi. australis* and *Vespadelus* spp. were significantly more active in the forest when compared to saltmarsh and urban habitats. These three taxa employ high frequency echolocation considered to be appropriate for foraging close to edges of cluttered environments. Activity of *Mo.* sp. 2 was significantly higher in saltmarsh and urban habitats when compared with forest habitat, while activity of *T. australis* was significantly greater in saltmarsh when compared with forest habitat. It is possible that calls from the high-flying *T. australis* may have been attenuated by vegetation in the forest canopy, deflating the actual level of activity of this bat. However, both *T. australis* and *Mo.* sp. 2

are clutter-sensitive, fast-flying bats that are adapted to foraging in more open areas (Fullard *et al.* 1991; Law and Chidel 2002; Adams *et al.* 2009).

Nightly bat activity was significantly higher in forest when compared to saltmarsh and urban habitats. While some studies have reported similar findings when comparing the activity of bats in forested and urban habitats (Legakis *et al*. 2000; Avila-Flores and Fenton 2005; Hourigan *et al*. 2006), other studies have failed to detect significant differences in bat activity between landscape categories (e.g., urban vs. vegetated) but have detected significant differences between landscape elements within these categories (e.g., bushland vs. Backyard: Threlfall *et al*. 2011). In other studies, wetlands have been found to be productive habitats for bats, with significantly greater bat activity recorded in this habitat when compared to adjacent upland forested areas (Brosset *et al*. 1995; Menzel *et al*. 2005). Although bat activity was significantly higher in the forest habitat when compared to both saltmarsh and urban habitats, the proportion of activity that represented feeding was significantly greater in saltmarsh habitat. Studies investigating the activity of bats in saltmarsh have suggested that the habitat may be productive for foraging bats, given insects form a major component of terrestrial fauna within the habitat (Laegdsgaard *et al*. 2004). While neighbouring habitats such as mangrove forests are known to be productive foraging habitats for bats elsewhere (McKenzie and Rolfe 1986; Hoye 2002), only one study has documented feeding activity of bats in saltmarsh (Belbasé 2005). The higher proportional feeding activity recorded in saltmarsh relative to urban and forest habitats in my study indicates that it may be more efficient to detect and capture prey in the less-cluttered habitat. Fenton (1990) suggested that it may be energetically less demanding and perhaps more efficient to locate prey in an open habitat such as saltmarsh than in a cluttered forest environment and this hypothesis may serve as a reasonable explanation for the discrepancy in proportional feeding activity detected between saltmarsh and forest habitats, particularly since the overall abundances of prey (mosquito and non-mosquito) were similar in both habitats.

The proportional feeding activity of two bat taxa (*C. gouldii* and *Vespadelus* spp.) was also significantly higher in the more open saltmarsh habitat than the urban and forest habitats. While similar levels of overall activity were detected for *C. gouldii* in each habitat, the higher level of feeding activity in the more open saltmarsh may reflect the influence of clutter on the foraging activity of this bat species. *Chalinolobus gouldii* is an edge-adapted,

clutter-sensitive bat species (Fullard *et al.* 1991; Law and Chidel 2002; Adams *et al.* 2009) capable of foraging in open spaces and edge environments. While *C. gouldii* may be able to negotiate openings in forest habitats, foraging (involving detection, pursuit, and capture of prey as well as collision avoidance) is likely to be more difficult in the cluttered forest habitat than in the more open saltmarsh habitat. *Vespadelus* spp., however, are agile bats, adapted to fly close to edges of cluttered vegetation (O'Neill and Taylor 1986; Rhodes 2002). Although these bats were significantly more active in forest when compared to saltmarsh and urban habitats, greater proportional feeding activity recorded in saltmarsh by these taxa may reflect improved efficiency of prey detection and capture in a less-cluttered environment. Clutter-tolerant bats are known to forage in less-cluttered habitats when prey abundances are high (Pavey *et al.* 2001a). Radio-tracking of *V. vulturnus* in the study area also indicated that this species spent proportionately more time in forest than in saltmarsh (see Chapter 5). However, it should be noted that saltmarsh constitutes a very small proportion (18 ha) of the total habitat available to bats in the study area.

2.4.2 Prey abundance

Aedes vigilax was the most abundant mosquito species in each habitat. However, the abundance of *Ae. vigilax* was significantly higher in saltmarsh and forest habitats when compared with the urban habitat. Saltmarsh habitat represents a major larval habitat of *Ae. vigilax*, supporting abundant populations of adult *Ae. vigilax*, particularly in the days immediately following emergence from their immature stages. Forest habitat is likely to provide adult *Ae. vigilax* populations a humid refuge and sources of blood meals, sustaining population abundances for longer periods than exposed saltmarsh environments.

General patterns such as peaks in population abundances of *Ae. vigilax* can be predicted (Kokkinn *et al.* 2009), with larger populations tending to be present two weeks after inundation of saltmarshes by spring tides and/or heavy rainfall. This trend, though not significant, was observed in the saltmarsh habitat with nightly *Ae. vigilax* abundances recorded during neap tides doubling those recorded during spring tides. When *Ae. vigilax* abundances were pooled across habitats, abundances did not differ significantly between neap and spring tides. It is likely that a wide range of factors (other than rainfall and tidal inundation) including evaporation, frequency of saltmarsh inundation and abundance of

adults in the preceding month also influence abundances of *Ae. vigilax* (de Little *et al.* 2009).

Nightly abundance of each insect taxon across different habitats decreased with body size, a phenomenon reported in a study investigating relationships between arthropod abundance and body size in an Indonesian rainforest (Stork and Blackburn 1993). In my study, average nightly insect abundance in forest habitat was 3.8 times greater than insect abundance recorded in the urban habitat. While this trend has been observed previously in some studies (Avila-Flores and Fenton 2005), other studies have found suburban habitats with sandstone geology support similar levels of insect biomass as bushland habitats of the same geology (Threlfall *et al*. 2011). Given the geology of my study area is dominated by Hawkesbury sandstone that overlies Narrabeen shales and sandstone (NSW National Parks and Wildlife Service 1999), it is unclear why the forest habitat supported greater abundances of insects than the urban habitat.

Nightly insect abundance was not consistent between habitats and tidal cycle for all taxa and all size classes of insects. Generally, small insects (<5 mm) were similarly abundant in saltmarsh and forest habitats, and were significantly less abundant in urban habitats, while large insects (≥10 mm) were significantly more abundant in the forest habitat. In this study, nightly insect abundance and the abundance of small insects (<5 mm) was significantly greater during neap tides. Since tidal and lunar cycles are related, it is difficult to identify whether the observed difference in insect abundance was due to the tide, lunar illumination or both. Generally, neap tides are associated with new moons while spring tides tend to coincide with full moons. While some studies have reported a decrease in light trap captures with increased lunar illumination (Taylor 1986; McGeachie 1989; Nag and Nath 1991), other studies have found insects to be more abundant in light traps during the full moon period (Ito *et al*. 1993) or that other factors such as cloud cover also influence the abundance of insects collected in light traps (Nowinszky *et al*. 2010).

2.4.3 Relationships between prey abundance and bat activity

In this study, failure to detect a relationship between *Ae. vigilax* abundance and nightly bat activity (all bat species pooled together) in any of the habitats investigated was not unexpected given *Ae. vigilax* is not likely to be available to all bat species due to

constraints imposed on prey detectability by echolocation frequency (Møhl 1988; Barclay and Brigham 1991). However, activity of *Vespadelus* spp. was positively correlated with *Ae. vigilax* abundance. Members of this genus utilise high-frequency echolocation considered to be suited to detection of small-sized prey like mosquitoes (Barclay and Brigham 1991). Additionally, members of this genus are small in size (*V. vulturnus*, 4 g; *V. pumilus*, 4.5 g), a characteristic associated with consumption of small-sized prey given constraints imposed by morphology (e.g., jaw structure). Given low sample sizes of many bat taxa considered capable of detecting small prey such as *Ae. vigilax* (high-frequency echolocating bats), it was not possible to examine relationships between *Ae. vigilax* abundance and activity of these bat taxa. No relationship was observed between *Ae. vigilax* abundance and the activity of *Mi. australis*. Although *Ae. vigilax* represents an abundant prey resource for this small-sized species, increased energetic requirements of this bat (compared to smaller bats of the *Vespadelus* genus) in association with the lower profitability of mosquitoes (20.6 kJg^{-1} – Cummins and Wuycheck 1971; Kunz 1988) relative to other abundant prey taxa (e.g., moths: 27.2 kJg^{-1} – McLean and Speakman 1999; beetles 24.48 kJg^{-1} – Cummins and Wuycheck 1971; Kunz 1988) may diminish the importance of *Ae. vigilax* as a prey resource to this bat species.

Many studies have found bat activity to be positively correlated with the abundance of insects (de Jong and Ahlén 1991; O'Donnell 2000; Adams *et al*. 2009). In this study, prey abundance tended to be positively correlated with bat activity, though relationships varied between habitats. Abundances of lepidopterans, dipterans and insects <5 mm together were significant predictors of nightly bat activity in the saltmarsh habitat, accounting for 90.0 % of variability observed in this habitat. Studies investigating influence of vegetation clutter on access to prey by bats have demonstrated that prey abundance does not necessarily equate to prey availability (Boonman *et al*. 1998; Adams *et al*. 2009; Rainho *et al*. 2010). In this study, failure to detect any significant relationships between prey abundance and overall bat activity in the urban and forest habitats may reflect a negative influence of clutter on prey detectability. It has been suggested that it may be energetically more efficient to forage in habitats with less clutter, given prey detection and pursuit is enhanced in habitats with less acoustic noise and structural barriers (Fenton 1990). While no direct measurements of clutter were made during this study, it is likely that forest (with understorey, mid-storey and canopy) and urban habitats (with retained natural and domestic vegetation as well as urban structures, e.g., telegraph poles) were acoustically

more complex than the generally very open saltmarsh habitat. However, it should be acknowledged that the patchy distribution of juvenile mangroves (height <3 m) transgressing into saltmarsh habitat is likely to generate moderate levels of clutter that may impact upon the detection of prey by low-flying foraging bats in saltmarsh habitat.

Given most insects in each habitat were small (<5 mm) it was expected that positive relationships between prey abundance and activity of clutter-sensitive bats were more likely to be detected in the more open (less cluttered) saltmarsh habitat. In this study, positive relationships between prey abundance and activity of bats were observed in saltmarsh for three taxa (*C. gouldii*, *Mormopterus* sp. 2, and *Vespadelus* spp.). Two of these taxa are clutter-sensitive, low-frequency echolocating bats (Fullard *et al*. 1991). *Chalinolobus gouldii* was positively correlated with the abundance of lepidopterans, while *Mo*. sp. 2 was positively correlated with nightly insect abundance. Given the restrictions imposed on prey detection by echolocation frequency, it is surprising that the activity levels of *C. gouldii* and *Mo*. sp. 2 were positively correlated with abundance of small prey and not larger prey (Table 2.4) that are considered to be more detectable by these taxa. However, predictions of minimum target sizes that can be detected by bats (based on target strengths of objects such as spheres or disks) may be different for real insects (Waters *et al*. 1995). Wing movements of prey may enhance prey detection, particularly when wings are perpendicular to the echolocation call, producing the greatest target strength (glint). In support of this hypothesis, small dipterans (chironomids and mosquitoes) are present in the diets of many medium-large sized European bats that employ low-frequency echolocation (Rydell 1989; Waters *et al.* 1995). Additionally, small prey have been identified as a dominant size class in the diets of both *C. gouldii* and *Mo*. sp. 2 in other areas (unpublished data - Lumsden and Wainer in Lumsden 2004).

The strongest positive relationship between prey abundance and bat activity in the more open saltmarsh habitat was observed for *Vespadelus* spp. While the bats that make up this species group are small, agile bats that utilise high-frequency echolocation (>50 kHz) suited to flying close to edges of high clutter vegetation as well as the detection of small prey, they are not restricted to foraging in cluttered habitats. Coleopteran abundance explained most of the variability in the activity of this taxa, while large insects (>14 mm) and the abundance of *Ae*. *vigilax* accounted for a smaller amount of the variability. Given morphological constraints on this taxa associated with jaw size and prey hardness

(Freeman and Lemen 2007), it is unlikely that the relationship between *Vespadelus* spp. activity and large insects (>14 mm) reflects an ecological response of *Vespadelus* spp. to the abundance of this prey resource. The positive relationship between coleopteran abundance (dominated by small beetles (<5 mm)) and the abundance of *Ae. vigilax* (typically <5 mm) with the activity of *Vespadelus* spp. is more likely to reflect an ecologically relevant response.

Positive relationships between prey abundance and the activity of clutter-sensitive bats (*C. gouldii* and *Vespadelus* spp.) in the more cluttered forest habitat also were identified. *Chalinolobus gouldii* employs broadband calls with a narrowband tail, typical of edge-space foraging bats (Adams *et al*. 2009). Additionally, the use of alternating frequencies in successive pulses may allow for detection of near targets in a cluttered forest (Jones and Corben 1993). *Vespadelus* spp. are suited to foraging close to the edges of cluttered vegetation, as well as detecting small prey such as mosquitoes. While it is not surprising that a strong positive relationship was observed between the activity of this taxa and *Ae. vigilax*, small lepidopterans (<5 mm) were also abundant in the forest habitat, yet did not account significantly for variability in the activity of this bat taxa. One possible explanation for this may be the lower profitability of tympanate moths, able to detect and avoid echolocating bats, making their capture by bats more difficult than other non-tympanate taxa.

While prey (*Ae. vigilax* and all non-mosquito prey) were generally most abundant in saltmarsh and forest habitats, positive associations between total bat activity and prey abundance as well as greater proportional feeding activity occurred in the less cluttered saltmarsh habitat, indicating a likely interaction of the effects of clutter and prey abundance on bat activity. *Aedes vigilax* abundance was positively correlated with the activity of bats of the *Vespadelus* genus supporting the view that *Ae. vigilax* may be an important prey resource for these bats. Open habitats that occur in close juxtaposition to forested habitats providing roosts are likely to be profitable foraging areas for bats.

In the next Chapter, I investigate the importance of vegetation interfaces to bats foraging in saltmarsh. Given coastal saltmarsh is threatened by encroaching mangroves as well as urban development on the landward fringe, appropriate management of these edge habitats is critical to the conservation of remnant saltmarsh. In many studies of non-estuarine

habitats, linear elements such as vegetation interfaces have been found to be areas of high bat foraging activity. To guide management for habitats adjacent to saltmarsh that provide linear edges for foraging bats, I investigate whether bat activity along landward and seaward edges are areas of higher bat activity (commuting and foraging). Based on the findings of this investigation, I provide recommendations for management of saltmarsh and adjoining habitats.

Chapter 3

Are Vegetation Interfaces important to foraging insectivorous Bats in endangered Coastal Saltmarsh on the Central Coast of New South Wales?



This chapter is *in press* as Gonsalves, L., Law, B., Webb, C. and Monamy, V. Are vegetation interfaces important to foraging insectivorous bats in endangered coastal saltmarsh on the Central Coast of New South Wales? *Pacific Conservation Biology*

Abstract

Conservation of insectivorous bats and their habitats is of increasing concern in Australia and linear elements in the landscape form important foraging habitats for many species. Only recently has use of endangered coastal saltmarsh habitat by bats been documented. Vegetation adjoining saltmarsh provides echolocating bats with linear elements that may be used while foraging and commuting to patches of high insect abundance. I used acoustic detectors to investigate whether individual species and total bat activity along seaward and landward saltmarsh edges was different to the interior of the saltmarsh. Four taxa accounted for >80 % of all bat activity in each zone with similar taxa recorded in both edge and interior zones. However, significantly more bat activity was recorded in edge zones. While differences in microhabitat use by individual species were also found, bat morphology did not account for the observed differences. Conservation managers of saltmarsh and adjoining habitats should consider potential impacts of management actions on foraging bats and their prey. Retention of strips of edge vegetation may help to balance the conservation requirements of endangered coastal saltmarsh habitats and the foraging insectivorous bat populations they sustain.

3.1 Introduction

Australia supports a diverse insectivorous bat fauna and the conservation of many threatened species is of growing concern. Effective conservation strategies require an understanding of many aspects of bat biology and autecology. One important requirement is a detailed understanding of the associations between bats and their foraging habitats (Fenton 1997). However, bats can be highly mobile (Law and Lean 1999; Cryan *et al.* 2000; O'Donnell 2001), facilitating access to a wide range of foraging habitats (Fenton 1997). While bats are able to access and move between different habitats, not all habitats sustain similar bat assemblages.

Vegetation clutter (i.e., non-target structures that produce echoes) has been shown to influence bat mobility and foraging activity (O'Neill and Taylor 1986; Bradshaw 1996; Brigham *et al.* 1997; Law and Chidel 2002; Lloyd *et al.* 2006) so that bats with a particular wing morphology and echolocation display differential habitat use (Neuweiler 1984; Norberg and Rayner 1987; Fenton 1990; Saunders and Barclay 1992; Brigham *et al.* 1997). For example, fast-flying bats that are less manoeuvrable generally have a high wing aspect ratio with low frequency echolocation calls that make it difficult to commute within or detect prey in high levels of clutter. This sensitivity to clutter would suggest that less manoeuvrable bats may use more open areas for foraging. Conversely, bats with adaptations such as constant frequency echolocation are able to discriminate prey items in high clutter habitats, allowing them to exploit resources in these more cluttered environments, though not exclusively as these bats can also be more active in open areas when prey abundances are high (Pavey *et al.* 2001a).

In many landscape mosaics, vegetation interfaces (i.e., edges) are areas of high bat activity (Ekman and de Jong 1996; Grindal and Brigham 1998, 1999; Grindal *et al.* 1999; Verboom and Spoelstra 1999; Law and Chidel 2002; Menzel *et al.* 2002; Estrada *et al.* 2004; Lumsden and Bennett 2005; Lloyd *et al.* 2006). Edges provide bats with a number of benefits. Bats may use edge vegetation as a navigational aid while foraging (Limpens *et al.* 1989; Limpens and Kapteyn 1991; Verboom and Huitema 1997). Edges also may support high concentrations of small swarming prey for foraging bats (Verboom 1998; Pavey *et al.* 2001a). Another potential benefit of edges to foraging bats is protection from predation. Bats have been found to be preyed upon while foraging in open pastures and

fields adjacent to forests (Estrada *et al.* 2004). The greatest risk of bat predation by avian predators occurs while bats commute or forage in open habitats (Speakman 1991; Baxter *et al.* 2006).

Coastal saltmarshes are diverse and vulnerable ecosystems, dominated by flowering plants, principally low-growing succulent herbs and shrubs, of high ecological value that are increasingly under threat from a range of processes (Adam 2009). The landward transgression of mangroves, advancing fringe vegetation and increased levels of urbanisation along the landward periphery of coastal saltmarshes, have resulted in significant declines in the size of coastal saltmarshes in south-eastern Australia (Saintilan and Williams 1999). While the conservation of coastal saltmarsh is a growing concern (Laegdsgaard *et al.* 2004), these habitats provide suitable conditions for pest and vector mosquitoes, with mosquito control programs in place in some areas to suppress adult mosquito populations and reduce the public health risks associated with the transmission of disease-causing pathogens such as Ross River virus (Russell and Kay 2004). A balance is needed between the strategies of coastal saltmarsh conservation and management of potential public health risks.

A diverse group of bats has been recorded from coastal saltmarsh in Australia, including at least nine threatened species (Laegdsgaard *et al.* 2004; Belbasé 2005; Lamb 2009). While the activity of bats in saltmarsh habitats has been investigated (Laegdsgaard *et al.* 2004; Belbasé 2005; Lamb 2009), the relationship between bats and specific insect groups has not been well studied in these environments. However, mosquito populations can be abundant during the warmer months and in coastal areas of south-eastern Australia, the large population sizes of the saltmarsh mosquito *Aedes vigilax* Skuse has led to speculation that this species may represent a potentially important prey resource for local bat species (Laegdsgaard *et al.* 2004; Lamb 2009).

Conservation efforts being implemented to rehabilitate coastal saltmarshes have modified the vegetation structure within and surrounding degraded habitats (Laegdsgaard 2006). Removal of mangroves in response to mangrove encroachment into saltmarshes, along with the implementation of environmental buffers and/or wildlife corridors between

coastal saltmarshes and urban development are likely to result in changes to the structure and diversity of fringing vegetation communities.

In order to understand what potential impacts modification of vegetation structure will have on the activity of foraging insectivorous bats, an investigation of saltmarsh microhabitat use by bats is first required. This aim of this study was to investigate the importance of edge vegetation to bats that forage in coastal saltmarsh. A comparison of bat activity and species diversity between three saltmarsh zones: 'landward edge', 'saltmarsh interior' and 'seaward edge' was undertaken. I predicted that bat activity would be higher along edge vegetation and that species diversity would differ across zones, with more clutter-sensitive species in the open 'saltmarsh interior'.

3.2 Methods

3.2.1 Study locations

Three study sites (Empire Bay, Cockle Bay, and Palmers Lane) within Brisbane Water, a tidal arm of Broken Bay, NSW (33°29'57"S, 151°21'40"E) were selected. The Empire Bay site was located one kilometre from the Cockle Bay site, and a further 0.75 kilometres from the Palmers Lane site. The Cockle Bay and Palmers Lane sites are part of Cockle Bay Nature Reserve, originally gazetted as a 26-ha Nature Reserve in 1992, which now comprises 68.5 ha of land due to additions to the Nature Reserve in the last two decades (NSW National Parks and Wildlife Service 2009). The Empire Bay site is not part of the same Nature Reserve but instead is a component of the Empire Bay Wetland, managed by Gosford City Council. All sites are situated in close proximity to Bouddi National Park (1 189 ha) and a number of smaller island Nature Reserves (Pelican Island, Rileys Island and Saratoga Island) known to sustain populations of insectivorous bats (Payne 2006).

The common vegetation composition and structural characteristics of each site allow for saltmarsh habitat to be separated into three distinct zones – landward edge, saltmarsh interior, and seaward edge (Fig. 3.1). All three study sites are generally characterised by the same vegetation structure, bordered by stands of casuarinas (*Casuarina glauca* Sieb.) >10 m in height on the landward side, a seaward side bordered by stands of the grey mangrove (*Avicennia marina* (Forssk.) Vierh) <5 m in height and an interior primarily

composed of characteristic saltmarsh species (eg. *Sarcocornia quinqueflora* (Bunge ex Ung.-Sternb.) A.J.Scott, *Sporobolus virginicus* (L.) Kunth). Throughout the saltmarsh interior there is a small number of juvenile mangroves that are transgressing into the saltmarsh as is evident in many saltmarshes in eastern Australia (Saintilan and Williams 1999).

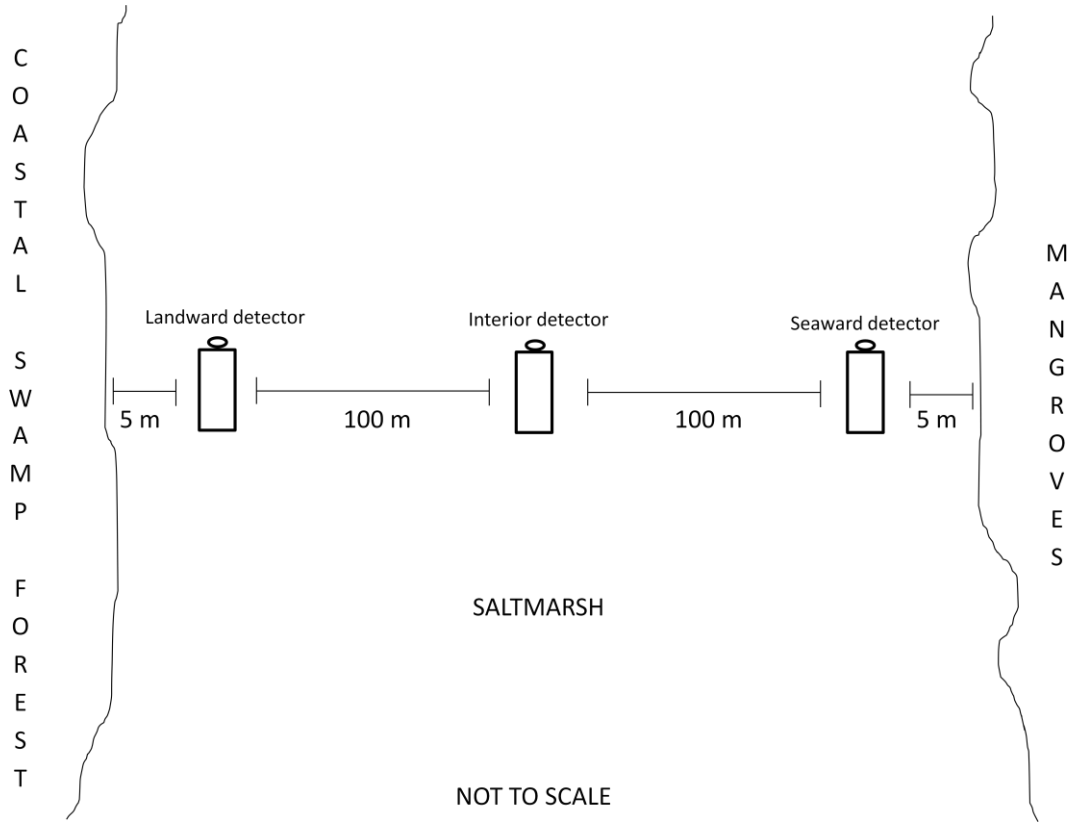


Figure 3.1. Schematic of detector set-up at each saltmarsh site.

3.2.2 Data collection

Nine sampling locations (representing each saltmarsh zone at each site) were surveyed for insectivorous bat activity using ultrasonic bat detectors. Sampling locations were surveyed concurrently from dusk to dawn over four consecutive nights in March, 2010 with a waxing crescent moon phase. Ambient conditions were favourable for bats, with minimum temperatures experienced during the sampling period ranging from 14.7-15.5° C and maximum temperatures ranging from 28.1-33.7° C (Bureau of Meteorology – Narara weather station). No rainfall was recorded during the survey.

At each sampling location, one Anabat SD1 detector (Titley Electronics, Ballina NSW) was secured above incoming tides, with the microphone set at a height of one metre from the marsh surface and at a 45° angle to the ground, facing parallel to both vegetation edges. At each study site, the maximum distance that could be maintained between detectors positioned in adjacent saltmarsh zones was 100 m (Fig. 3.1). Detectors recorded commuting and feeding echolocation calls of insectivorous bats that vary from species to species and can be used to differentiate between most bat species. All recordings were stored on a compact flash card before being uploaded to a laptop for analysis.

3.2.3 Call identification

Recorded bat calls were identified to species where possible using the automated call identification software, Anascheme (Gibson and Lumsden 2003), in association with a key for the lower north-eastern NSW coastal plain (Adams *et al.* 2010). Bat calls with fewer than three valid pulses (i.e., minimum of six data points and model quality of ≥0.9) were not analysed by Anascheme. Since multiple bat species may call simultaneously, calls were only assigned to a species if more than 50 % of pulses within the sequence were attributed to that species and only passes with a minimum of three pulses classified to the same species were identified.

Echolocation calls of certain species overlap to such a degree that it is not possible to differentiate between them. Consequently, the identification key grouped certain species together (e.g., *Nyctophilus gouldi* Tomes and *Nyctophilus geoffroyi* Leach = *Nyctophilus* spp.; *Vespadelus pumilus* Gray, *Vespadelus troughtoni* Kitchener, Jones and Caputi, *Vespadelus vulturnus* Thomas = *Vespadelus* spp.). All identified calls then were screened manually for feeding buzzes – a rapid increase in pulse repetition rate, slope, frequency and speed (associated with pursuit and capture of prey: Griffin *et al*. 1960; Pennay *et al*. 2004). For each detector and each night, the number of bat passes and number of feeding buzzes for each species were tabulated.

3.2.4 Data analyses

Given the potential for spatial dependence of data collected from zones separated by 100 m, two-factor repeated measures analysis of variance (ANOVA) was used to test for

differences in species diversity (expressed as richness), mean nightly bat activity and the activity of individual species between zones and sites. For each analysis, 'zone' and 'site' were assigned as the within-subjects factors (random). When data failed to meet assumption of homogeneity of variance of the differences between samples (sphericity), a Greenhouse-Geisser correction (Geisser and Greenhouse 1958) was adopted. Wilcoxon-pairwise comparison was used to identify which zones or sites differed from each other. Bonferroni correction was not applied to Wilcoxon-pairwise comparisons as this correction is a highly conservative approach (Moran 2003).

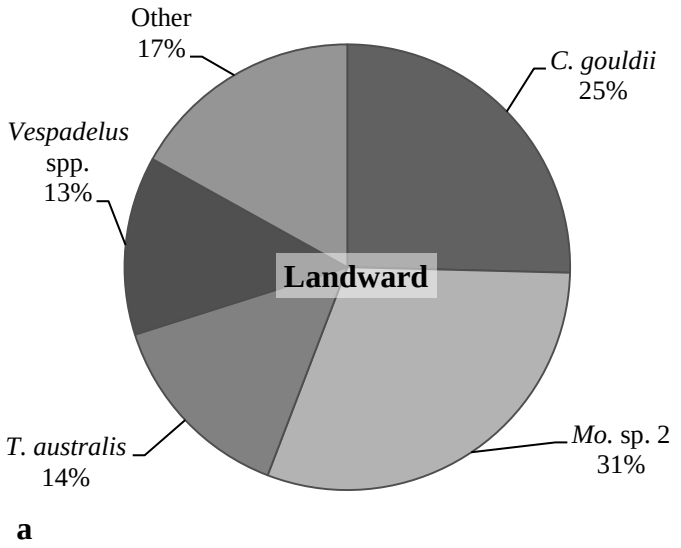
3.3 Results

A total of 1 162 bat calls was recorded, representing 12 species and one species group (*Vespadelus* spp.). The landward and seaward edges supported eight species and one species group, while seven species and one species group were detected within the saltmarsh interior (Table 3.1). In all, 378 (32.5 %), 208 (17.9 %) and 576 (49.6 %) bat passes were recorded along the landward edge, saltmarsh interior and seaward edge, respectively. Feeding buzzes were recorded for two species and one species group (Table 3.1). A total of 17 feeding buzzes was recorded, with 53 %, 18 % and 29 % of feeding buzzes recorded along the landward edge, saltmarsh interior and seaward edge, respectively. Given the limited sample size of feeding buzzes, no statistical comparison of feeding activity could be made between zones.

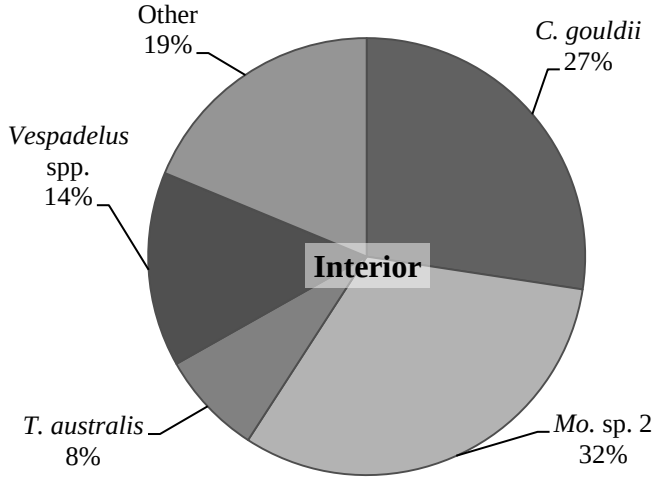
Table 3.1: Mean nightly activity (bat passes night^{-1}) ± SE of bat species detected in each saltmarsh zone. Passes of <3 pulses are not included. NB. Species in bold are listed as threatened species under the NSW *Threatened Species Conservation Act* 1995; * represents taxa detected feeding in saltmarsh (number of buzzes recorded).

Species	**Landward**	**Interior**	**Seaward**
Chalinolobus dwyeri	0.7 ± 0.3	-	-
*C. gouldii** (5)	8.0 ± 1.0	5.0 ± 0.5	15.0 ± 3.0
Miniopterus australis	0.8 ± 0.3	0.5 ± 0.2	2.0 ± 0.4
Mi. schreibersii oceanensis	0.6 ± 0.3	0.4 ± 0.2	-
Mormopterus norfolkensis	-	-	0.5 ± 0.2
Mo. sp. 2* (8)	9.0 ± 1.0	6.0 ± 0.6	13.0 ± 3.0
Rhinolophus megaphyllus	-	-	0.3 ± 0.2
Saccolaimus flaviventris	0.1 ± 0.1	-	-
Scoteanax rueppellii	-	0.3 ± 0.2	-
Scotorepens orion	-	0.3 ± 0.2	1.0 ± 0.4
Tadarida australis	5.0 ± 1.0	1.0 ± 0.4	5.0 ± 1.0
Vespadelus darlingtoni	0.6 ± 0.2	-	2.0 ± 0.6
Vespadelus spp.*(4) *(V. pumilus, V. troughtoni, V. vulturnus)*	4.0 ± 0.8	3.0 ± 0.3	6.0 ± 2.0
Total	**32.0 ± 5.0**	**17.0 ± 2.0**	**48.0 ± 11.0**

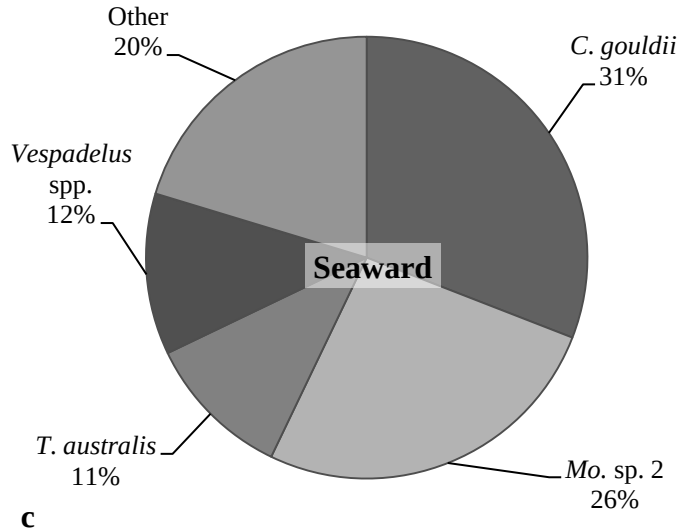
In all three saltmarsh zones, *Chalinolobus gouldii* Gray, *Mormopterus* sp. 2 Peters, *Tadarida australis* Gray and *Vespadelus* spp. accounted for ≥ 80 % of all bat activity (Fig. 3.2a-c).



a



b



c

Figure 3.2: Percentage of total bat activity contributed by each species in a) Landward edge; b) Interior; c) Seaward edge. "**Other" represents activity from all other species recorded and any calls that could not be assigned to a single taxon.**

There was no significant difference ($F_{2, 6}$=2.899; P=0.132) in mean species richness between the three zones (5.4±1.9; landward edge, 5.4±1.6; saltmarsh interior, 5.1±2.3; seaward edge). However, there was a significant difference in mean species richness between sites ($F_{2, 6}$=5.828; P=0.039). Mean nightly species richness did not differ between Empire Bay (5.9±0.5) and Cockle Bay (5.9±0.4). Cockle Bay supported greater species richness than the Palmers Lane (4.8±0.5) (Z=-2.221; P=0.026; Fig. 3.3).

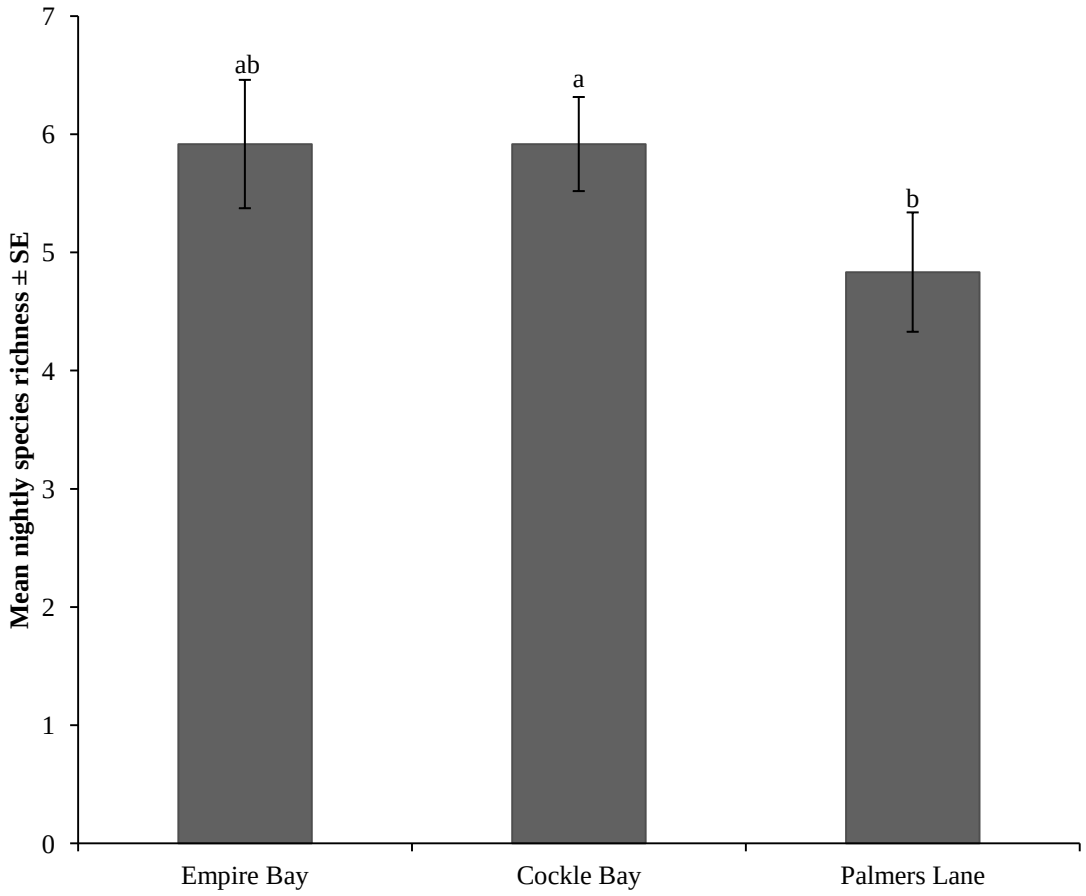


Figure 3.3: Mean nightly species richness of insectivorous bats detected at three coastal saltmarsh sites. Means denoted by different letters are significantly different from one another.

There was a significant difference in mean nightly bat activity (number of bat passes night^{-1}) between saltmarsh zones ($F_{2, 6}$=5.575; P=0.043). While the level of bat activity along the landward (32±5 bat passes) and seaward (48±11 bat passes) edges did not differ significantly from each other (Z=-0.982; P=0.326), the level of activity recorded in the saltmarsh interior (17±2 bat passes) was significantly lower than activity recorded along landward (Z=-2.316; P=0.021) and seaward (Z=-2.432; P=0.015) edges (Fig. 3.4).

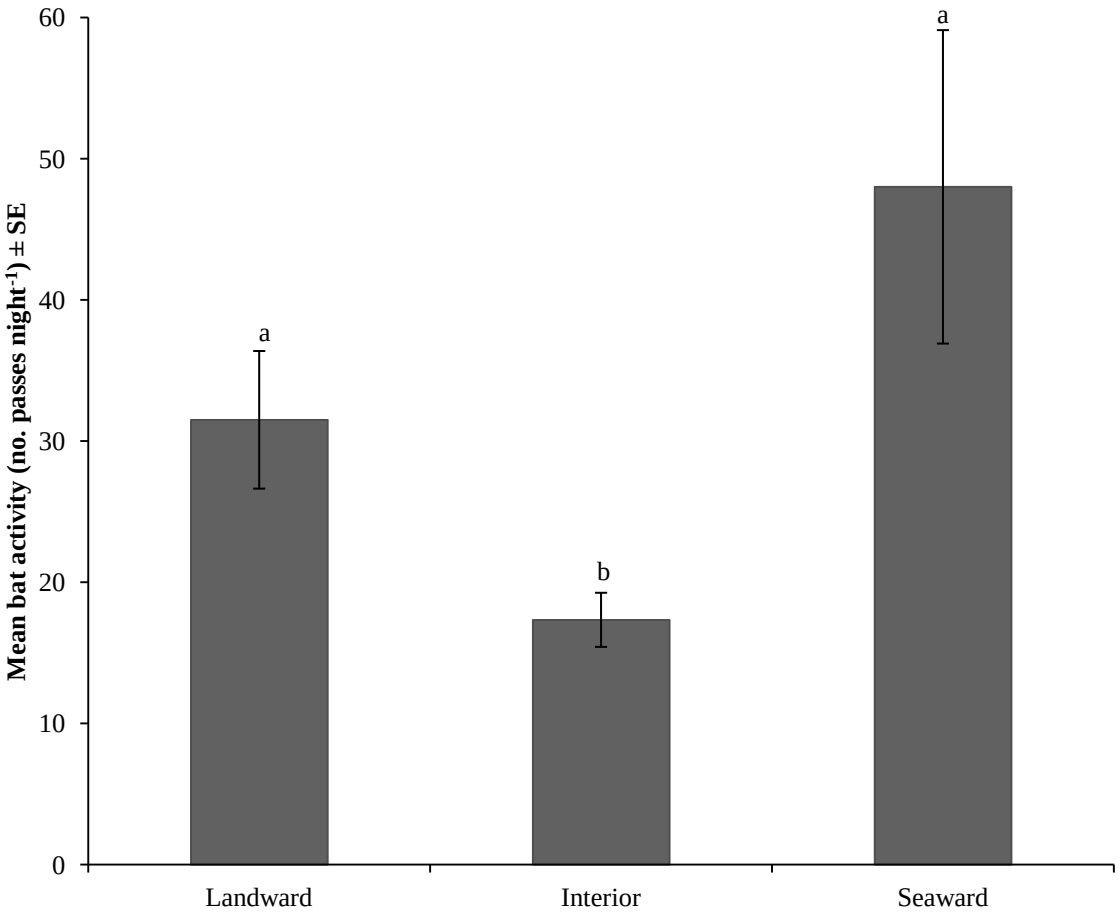


Figure 3.4: Mean nightly bat activity detected in each saltmarsh zone. Means denoted by different letters are significantly different from one another.

There was also a significant difference in mean nightly bat activity (number of bat passes night^{-1}) between sites ($F_{2, 6}$ = 5.635; P=0.043). The level of activity at the Cockle Bay site (46±9 bat passes) was not significantly greater than the Empire Bay site (32±4 bat passes), but it was significantly greater than activity recorded in the Palmers Lane site (19±8 bat passes) (Fig. 3.5).

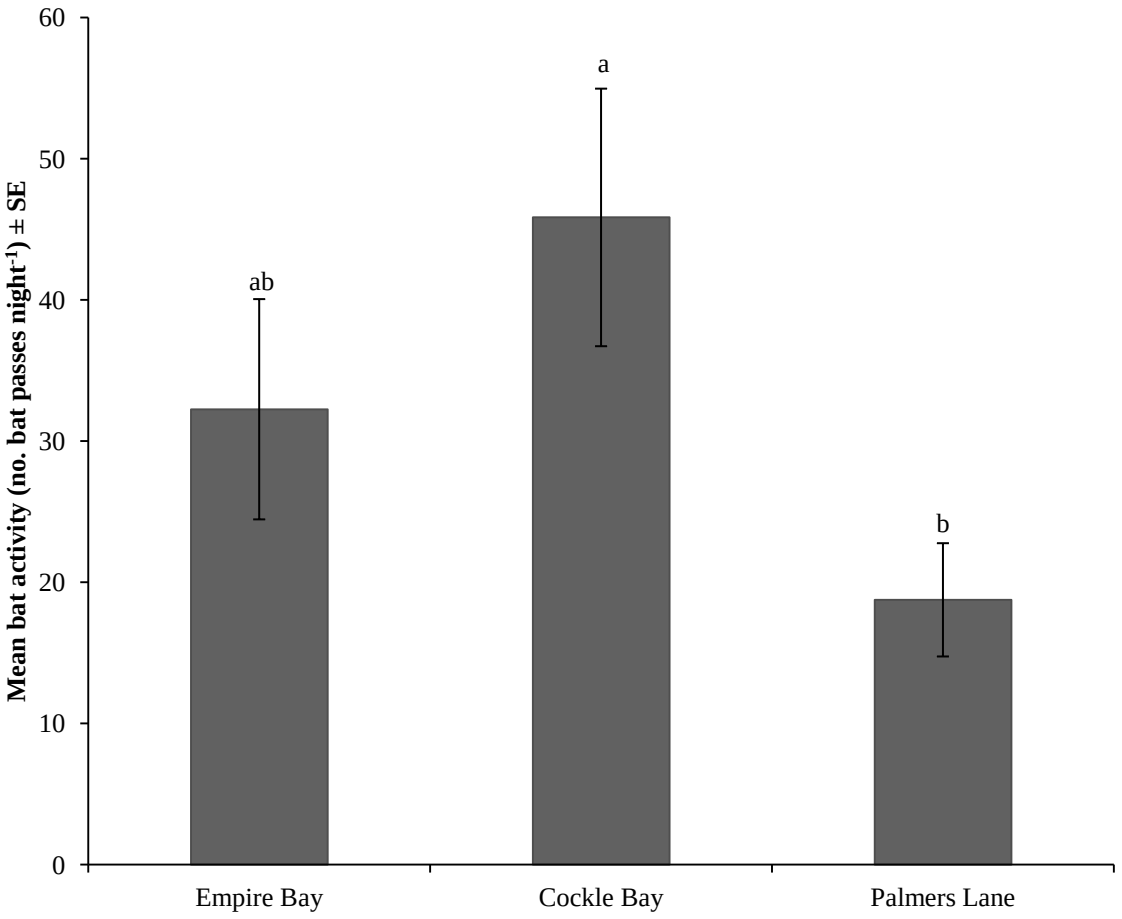


Figure 3.5: Mean nightly bat activity detected at each site. Means denoted by different letters are significantly different from one another.

The activity (number of bat passes night^{-1}) of individual species was found to differ between saltmarsh zones and also between sites. Activity of *Chalinolobus dwyeri* Ryan, *C. gouldii*, *Scotorepens orion* Troughton, *T. australis* and *Vespadelus darlingtoni* Allen differed between zones (Table 3.2, Fig. 3.6a, 3.6b, 3.6d-f), while the difference in activity between zones for *Mo*. sp. 2 was near significant (Table 3.2, Fig. 3.6c). *Chalinolobus dwyeri* was significantly more active along the landward edge than along the seaward edge (Z=-2.070; P=0.038) or in the saltmarsh interior (Z=-2.070; P=0.038) (Fig. 3.6a), while the activity of *C. gouldii*, *T. australis* and *V. darlingtoni* was significantly greater along the landward (Z=-2.020; P=0.028, Z=-2.298; P=0.022 & Z=-2.070; P=0.038) and seaward edges (Z=-2.277; P=0.023, Z=-2.281; P=0.023 & Z=-2.699; P=0.007) when compared to the saltmarsh interior (Fig. 3.6b, 3.6d & 3.6f). Pairwise comparisons also indicated that *S. orion* was significantly more active along the seaward edge when compared to the landward edge (Z=-2.264; P=0.024) (Fig. 3.6e).

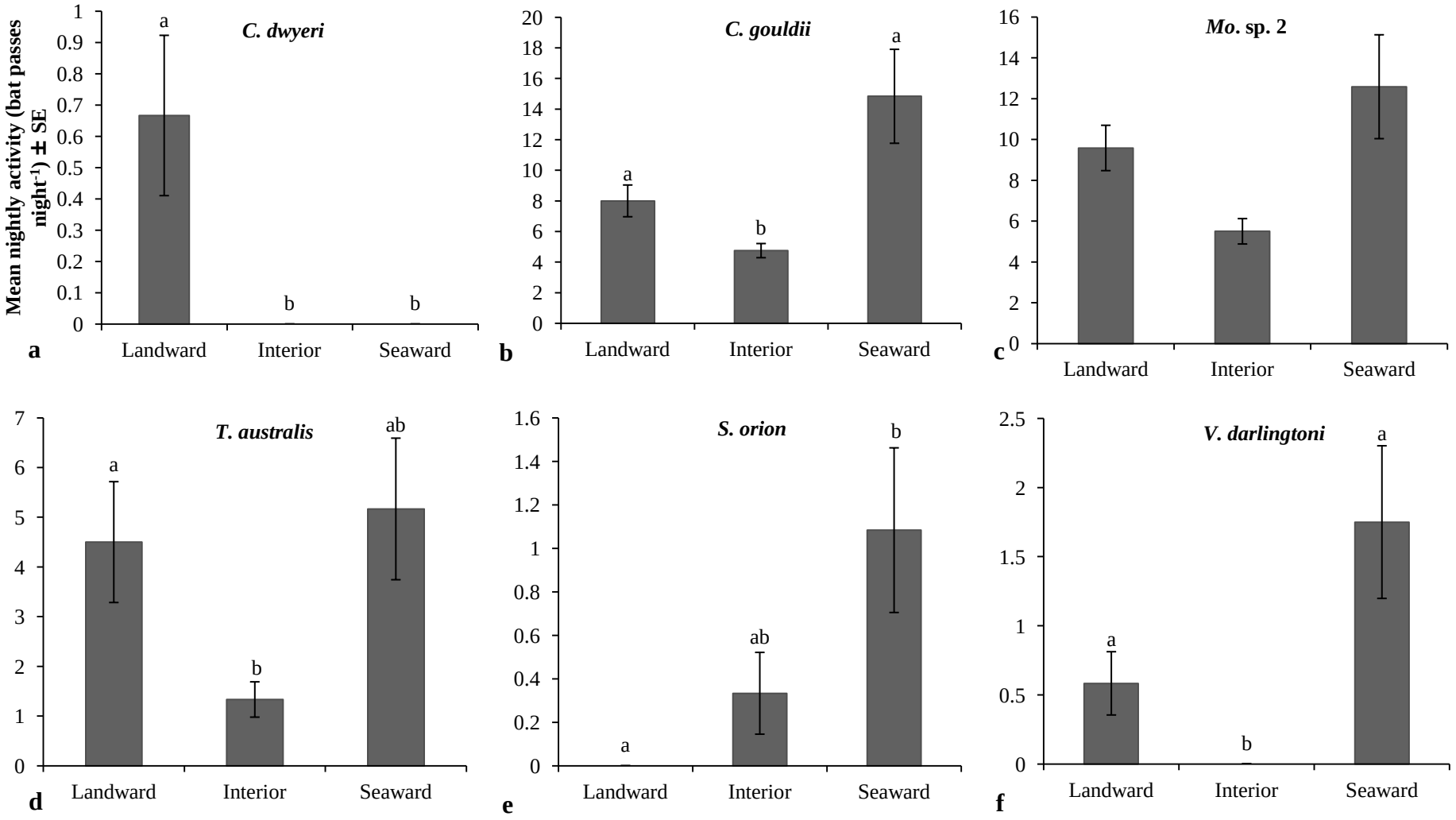


Figure 3.6. Mean nightly activity in each saltmarsh zone: a) *Chalinolobus dwyeri;* b) *Chalinolobus gouldii;* c) *Mormopterus* sp. 2*;* d) *Scotorepens orion;* e) *Tadarida australis;* f) *Vespadelus darlingtoni.* Means denoted by different letters are significantly different from one another.

The activity of *C. gouldii*, *Mo*. sp. 2 and *T. australis* differed significantly between sites (Table 3.2, Fig. 3.7a-c). The activity of these species was significantly higher in the Cockle Bay site when compared to the Palmers Lane site (Z=-2.812; P=0.005, Z=-2.671; P=0.008, Z=-2.938; P=0.003).

A significant interaction effect of site and zone was detected for *Miniopterus australis* Tommes, with greater activity detected along the seaward edge of the Empire Bay site (Table 3.2, Fig. 3.8).

Table 3.2: Two-factor repeated measures ANOVA P-values obtained for each bat species. NB. Bold formatting indicates that P-value is significant at 0.05.

Species	$F_{x,y\ df}$ = *P*-value		
	Site	**Zone**	**Site*Zone**
Chalinolobus dwyeri	$1.114_{2,6\ df} = 0.388$	$\mathbf{24.000_{1,\ 3\ df} = 0.016}$	$1.114_{1.923,\ 5.769\ df} = 0.387$
C. gouldii	$\mathbf{6.750_{2,6\ df} = 0.029}$	$\mathbf{7.982_{2,6\ df} = 0.020}$	$2.582_{1.219,\ 3.657\ df} = 0.194$
Miniopterus australis	$1.690_{2,6\ df} = 0.262$	$4.094_{2,6\ df} = 0.076$	$5.091_{4.000,\ 12.000\ df} = \mathbf{0.012}$
Mi. schreibersii oceanensis	$1.800_{2,6\ df} = 0.244$	$1.800_{2,6\ df} = 0.244$	$1.636_{3.521,\ 10.563\ df} = 0.238$
Mormopterus norfolkensis	$2.455_{2,6\ df} = 0.166$	$5.400_{2,6\ df} = 0.103$	$2.455_{2.343,\ 7.030\ df} = 0.153$
Mo. sp. 2	$\mathbf{7.194_{2,6\ df} = 0.025}$	$5.015_{2,6\ df} = 0.052$	$1.992_{1.888,\ 5.665\ df} = 0.221$
Rhinolophus megaphyllus	$2.333_{2,6\ df} = 0.178$	$2.000_{1,3\ df} = 0.216$	$2.333_{1.476,\ 4.429\ df} = 0.115$
Saccolaimus flaviventris	$1.000_{1,3\ df} = 0.391$	$1.000_{1,3\ df} = 0.391$	$1.000_{1,\ 3\ df} = 0.391$
Scoteanax rueppellii	$0.429_{2,6\ df} = 0.670$	$3.000_{1,3\ df} = 0.182$	$0.429_{1.968,\ 5.903\ df} = 0.667$
Scotorepens orion	$1.780_{1.003,\ 3.010\ df} = 0.247$	$\mathbf{14.778_{2,6\ df} = 0.005}$	$2.714_{2.038,\ 6.113\ df} = 0.143$
Tadarida australis	$\mathbf{12.019_{2,6\ df} = 0.008}$	$\mathbf{11.420_{2,6\ df} = 0.009}$	$2.464_{2.355,\ 7.064\ df} = 0.151$
Vespadelus darlingtoni	$0.866_{2,6\ df} = 0.467$	$\mathbf{9.106_{2,6\ df} = 0.015}$	$1.657_{2.243,\ 6.728\ df} = 0.262$
Vespadelus spp.	$2.375_{2,6\ df} = 0.174$	$3.272_{2,6\ df} = 0.109$	$2.091_{1.297,\ 3.890\ df} = 0.232$
Total	$5.635_{2,6\ df} = 0.042$	$5.575_{2,6\ df} = 0.043$	$2.130_{1.396,\ 4.189\ df} = 0.224$

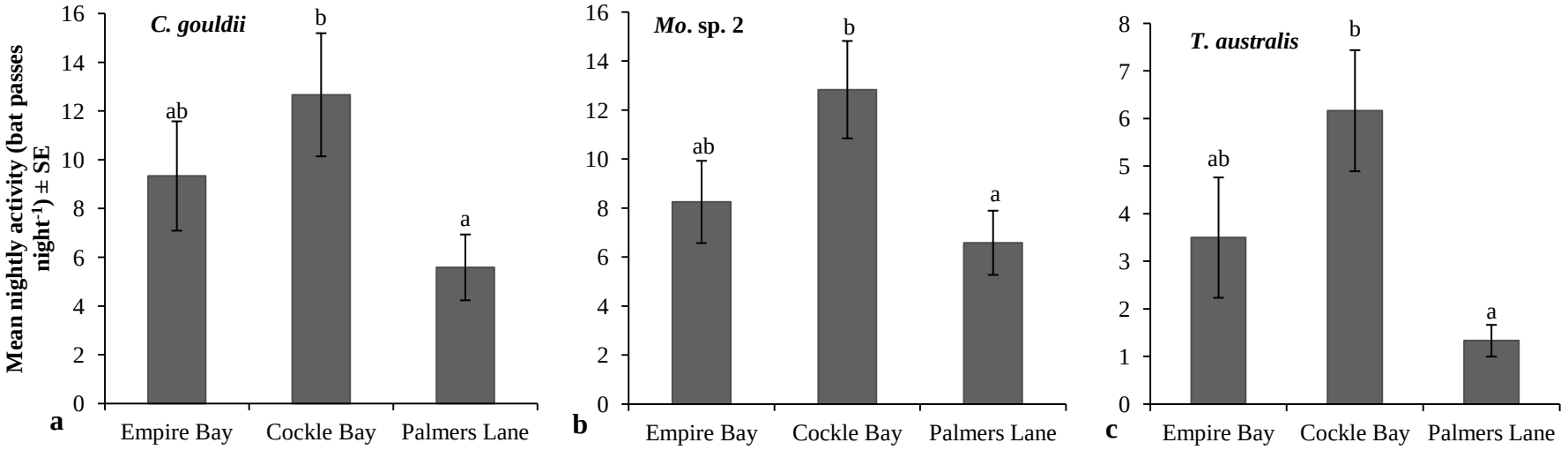


Figure 3.7: Mean nightly activity at each site: a) *Chalinolobus gouldii*; b) *Mormopterus* sp. 2; c) *Tadarida australis*. Means denoted by different letters are significantly different from one another.

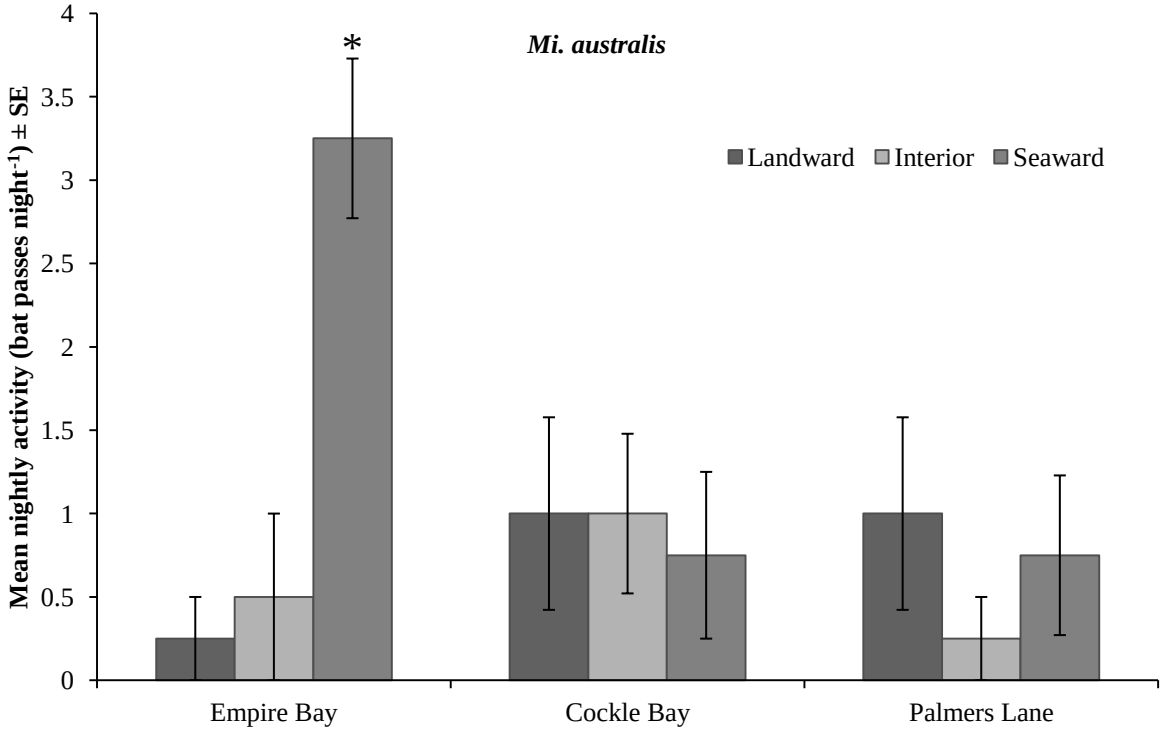


Figure 3.8: Column graph illustrating mean nightly activity of *Miniopterus australis* in each saltmarsh zone at each site. Asterisk indicates a significant interaction effect of zone and site on activity.

3.4 Discussion

The results of this study revealed that total bat activity and the activity of individual species were significantly different between saltmarsh zones, with higher levels of activity along saltmarsh edges compared to the interior, highlighting the importance of vegetation interfaces to foraging bats. This has implications for the management and rehabilitation of saltmarsh and adjoining vegetation communities that are likely to undergo further modification and experience rapid rates of change as urbanisation in coastal areas intensifies and the predicted elevations in sea level alter the intertidal dynamic (Nicholls *et al.* 1999).

The activity of individual bat species differed between saltmarsh zones and between sites. This is quite interesting for species like *C. dwyeri* and *S. orion* for which little to no detail about foraging activity and behaviour is known (Churchill 2009). However, with such low levels of activity recorded for these species (0.7 and 1.3 passes night^{-1}), it is possible that more intensive sampling would fail to detect a significant difference between saltmarsh zones. *Chalinolobus gouldii*, *T. australis* and *V. darlingtoni* were more active along both edges than in the interior of the saltmarsh. *Chalinolobus gouldii* is a ubiquitous bat species, widely distributed throughout Australia (Churchill 2009) indicating it has adapted to foraging in a wide variety of habitats. However, as a member of the edge-space foraging guild, the significantly higher activity levels recorded for this bat along both saltmarsh edges is not unexpected.

The activity of *Vespadelus* spp. did not differ significantly among zones. It is possible that detectors in the saltmarsh interior may have recorded commuting activity as individuals moved between vegetation edges. Given the small area of saltmarsh available at each site, the spatial dependence of data collected along vegetation edges and the saltmarsh interior (separated by ≤100 m) may potentially mask any differences between the bat activity recorded along edges and in the saltmarsh interior. Light-tagged *V. vulturnus* individuals (n=5) released in saltmarsh interior have been observed flying close to the marsh surface whilst crossing the saltmarsh interior before flying along edge vegetation at a height of 3-5 m above the marsh surface (unpublished data - L. Gonsalves). However, the detection of a feeding buzz from this species group in the saltmarsh interior suggests that at least some of

the activity recorded in the interior represents foraging activity and not just commuting activity as individuals move between edges.

Bat activity recorded in saltmarsh habitats during this study was similar to that found in a more extensive long-term investigation of bat activity within the local area (Chapter 2). An average of 27±8 passes night^{-1} was recorded in the saltmarsh interior during surveys in 2008-2009 (Chapter 2) compared to an average of 17±2 passes night^{-1} recorded during this study. The level of bat activity recorded in saltmarsh in both studies was low relative to the level of activity reported for forest habitat (270±60 passes night^{-1}) in the study area (Chapter 2). Given insect abundance has been shown to be positively correlated with primary productivity (Haddad *et al.* 2000), one might expect highly productive habitats such as mangroves (Komiyama *et al.* 2008) and saltmarshes (Simas *et al.* 2001) to sustain higher levels of bat activity.

Over 34 nights of data collection, nine species and two species groups of insectivorous bat fauna were recorded in the saltmarsh interior (Chapter 2), while only seven species and one species group were recorded during this study, probably due to the relatively short duration (i.e., four nights) of bat detection as well as seasonal differences between the two studies. However, two of the three taxa (*C. dwyeri* and *V. darlingtoni*) that weren't recorded in the saltmarsh interior during this study, were rarely recorded in the more extensive survey (0.014 % of total bat activity: Chapter 2), but were detected in the saltmarsh edge microhabitats during this study. Furthermore, three additional bat species (*Mormopterus norfolkensis* Gray, *Rhinolophus megaphyllus* Gray and *Saccolaimus flaviventris* Peters) that were not detected during the more extensive study were recorded during this short study. The overall similarity in the diversity of bats and the levels of bat activity recorded in both studies, suggest that this short-term study provided a reliable snap-shot of bat activity in the saltmarsh.

Species richness did not differ significantly between zones and ≥80 % of all activity in each zone was provided by four taxa (*C. gouldii, Mo*. sp. 2, *T. australis* and *Vespadelus* spp. (species complex including *V. pumilus, V. troughtoni* and *V. vulturnus*)). Three of these taxa (*C. gouldii, Mo*. sp. 2 and *T. australis*) are clutter-sensitive (Fullard *et al.* 1991; Law and Chidel 2002; Adams *et al.* 2009), so it is not unexpected that these bats were

more active than other bats in an open habitat such as saltmarsh. However, of these species, only *T. australis* is an open-space forager while *C. gouldii* and *Mo.* sp. 2 represent the edge-space foraging guild. The activity recorded for these two species in the saltmarsh interior may also represent commuting activity as bats move from one edge to the other.

The forest bats that comprise the *Vespadelus* spp. complex are small, agile bats, adapted to flying close to edges of cluttered vegetation (O'Neill and Taylor 1986; Rhodes 2002). Comprised of small succulent shrubs and herbs (Adams *et al.* 2009), saltmarsh is an acoustically less complex environment than adjacent forested areas. It is possible that forest bats are active in saltmarsh as it may be energetically less demanding and perhaps more efficient to locate prey than in a cluttered environment. While adapted to fly in cluttered environments, clutter-tolerant bats are not restricted to these environments (Fenton 1990; Brigham *et al.* 1997) and are known to forage in less-cluttered habitats when prey abundances are high (Pavey *et al.* 2001a). *Vespadelus* spp. have high frequency echolocation calls (i.e., end frequency range 50-53 kHz) that make these small bats well suited to detecting small prey. Two species of the *Vespadelus* spp. complex (*V. pumilus* and *V. vulturnus*) consume saltmarsh mosquitoes (*Ae. vigilax*) in the study area, while *C. gouldii* does not (see Chapter 4).

Nightly bat activity was significantly different between saltmarsh zones, with higher levels of bat activity along the seaward and landward edges than in the saltmarsh interior. The seaward edge supported more than twice the bat activity of the interior and no species were more active in the interior. The seaward edge in this study was composed of stands of mangroves. Mangroves have been found to be productive foraging habitat for insectivorous bats in Western Australia (McKenzie and Rolfe 1986). Additionally, to the north of the study site (in the Hunter Estuary, NSW) mangroves provide roosting habitat for insectivorous bats (A. McConville pers. comm. 2010).

Greater use of edge habitat by bats has been reported in non-estuarine studies (e.g., Ekman and de Jong 1996; Verboom and Spoelstra 1999; Kusch *et al.* 2004). For example, within forests of British Columbia, Canada, the activity of foraging bats was reported to be greatest along the edges of cut blocks (Grindal and Brigham 1999). During the same study, insect availability followed the same trend and was greatest along edges. In a study on the central-western slopes of New South Wales, it was also found that insect abundance was

higher on the edge of remnant forest than within the interior of the remnants (Major *et al.* 2003).

While the association between differences in bat activity and vegetation structure have been documented, there is some uncertainty as to whether the structural differences have a direct or indirect influence on bat activity. Wind speed affects the distribution of nocturnal insects (Peng *et al.* 1992) and vegetation adjacent to open areas, not unlike saltmarsh, acts as a windbreak, influencing local insect distributions (Lewis 1969). Swarms of small insects are more likely to occur low to the ground around remnant forests and along strips of vegetation adjacent to open areas (Verboom 1998; Pavey *et al.* 2001a). Bats that feed on these insects will be expected to favour edge habitat, including areas above water, as hunting sites (Pavey *et al.* 2001a). Since no insect availability data were collected during this study, it is only possible to speculate that the greater bat activity recorded along both saltmarsh edges reflected higher concentrations of insects along these edges. While this may be a reasonable hypothesis, most of the bat species detected in saltmarsh are edge-adapted species and are therefore more likely to be active along the edge microhabitats than in the interior, providing an alternative explanation for the difference in activity between zones.

Given the low number of feeding buzzes recorded in this study, it was not possible to compare foraging activity between saltmarsh zones. However, other studies have detected strong positive correlations between overall bat activity and feeding activity (Law *et al.* 1998; O'Donnell 2000; Borkin and Parsons 2009), suggesting that bat activity is a reasonable proxy for foraging activity. Lower levels of bat activity in the saltmarsh interior could reflect more profitable foraging conditions. Due to the wind-sheltering effect of vegetation, small insect swarms may be distributed along edge vegetation adjacent to open areas (Verboom and Huitema 1997), while larger insects, not as dependent on the wind-sheltering effect may be available in more open habitats (Taylor 1974; Verboom and Huitema 1997). Additionally, these larger prey items may be more easily detected in a less cluttered environment, resulting in shorter foraging bouts in this microhabitat. However, the availability of these larger insects will be restricted to the larger bat species that are morphologically capable of consuming these prey items. Since the activity recorded in the saltmarsh interior was not typically restricted to larger bats, it is unlikely that the lower levels of bat activity in the saltmarsh interior reflect more profitable foraging conditions.

Although edges are important, I recorded moderately high activity within the interior of saltmarshes. Due to landward transgression of mangroves, occurring in the saltmarshes of south-east Australia (Saintilan and Williams 1999), patches of juvenile mangroves occurring in parts of the interior of all sites may act as an edge for bats to forage around. It has been found that bats are significantly more active around single paddock trees (Lumsden and Bennett 2005) and have also been found to be 10 times more active in the presence of at least two trees (Fischer *et al.* 2010). While the presence of juvenile mangrove clumps within parts of the saltmarsh may inflate the level of activity that would normally occur in the interior, all interior detectors were positioned 15-20 m away from these patches in order to survey the areas of saltmarsh that lacked these mangrove shrubs.

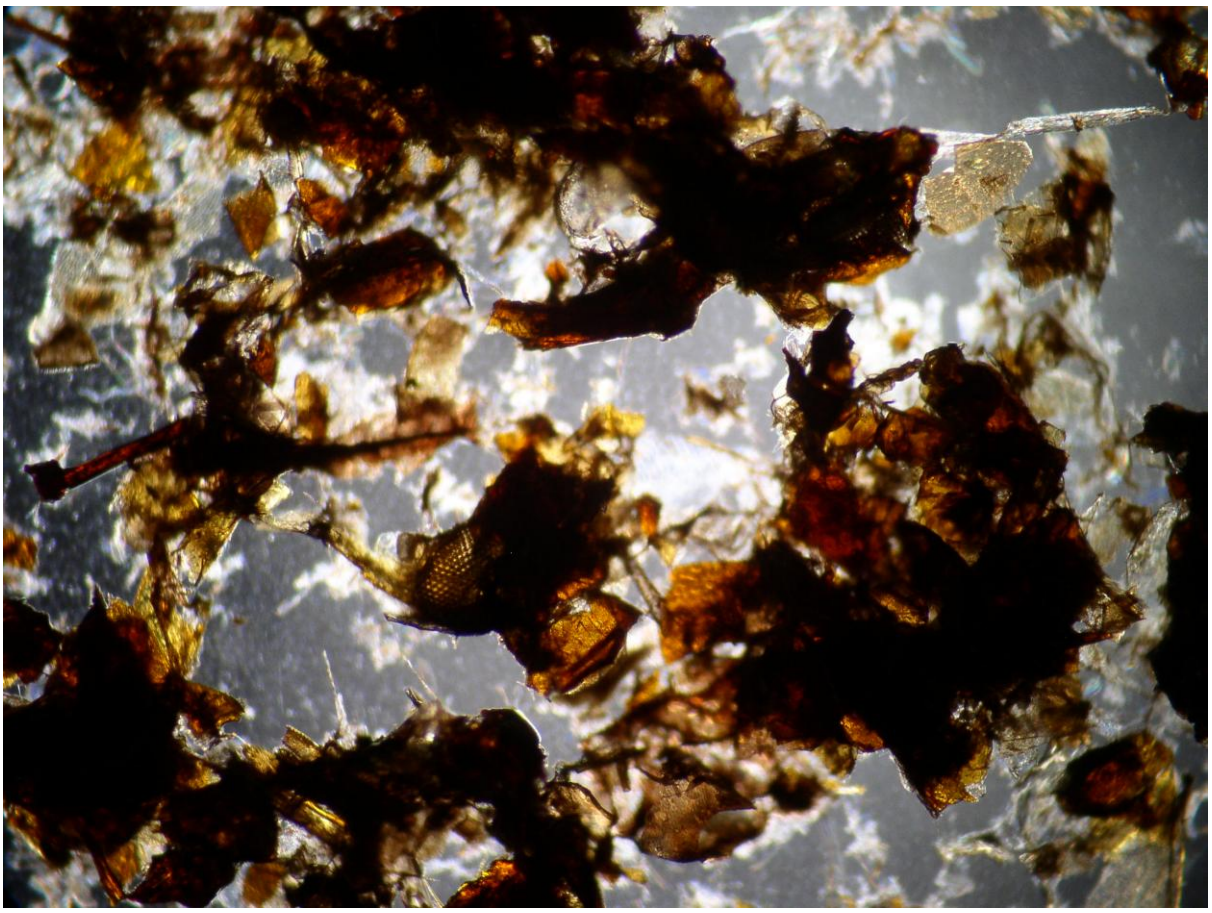
As the threats to coastal saltmarsh communities in south-eastern Australia have become better understood, greater emphasis has been placed on conserving existing areas of saltmarsh and rehabilitating degraded saltmarsh habitats. The removal of mangroves transgressing into saltmarshes along with the implementation of buffer zones between saltmarsh and urban areas are likely to influence the activity of insectivorous bats and other fauna that require connectivity between saltmarsh and adjacent habitats. While some individual species may benefit from the habitat modification, others may be excluded from saltmarsh as a result. *Rhinolophus megaphyllus*, *V. darlingtoni* and three threatened species, *C. dwyeri*, *Mo. norfolkensis*, and *S. flaviventris* were recorded exclusively in saltmarsh edge microhabitats. While the levels of activity recorded for these species were low, loss or modification of saltmarsh edge microhabitats may exclude these species from saltmarsh, further highlighting the need for appropriate management of saltmarsh and adjoining habitats.

My study indicates that saltmarsh is a habitat actively used by insectivorous bats, many of which are threatened species. Management of saltmarsh and adjoining habitats should take into consideration potential impacts of management practices on both insectivorous bats and their prey. Higher bat activity along saltmarsh edges suggests that these microhabitats are more optimal for foraging than the interior of the saltmarsh. Consequently, removal of vegetation neighbouring saltmarsh (e.g., mangroves, or coastal swamp forest) has the potential to reduce the foraging habitat available to bats. Should the clearing of vegetation adjacent to saltmarsh be required, the retention of strips of mangroves and casuarinas would be beneficial to bats, providing an edge for foraging bats.

In the next chapter, based on the findings of Chapters 2 & 3, I examine the diets of a suite of bat species (high-frequency echolocating bats and those recorded foraging in saltmarsh) predicted to utilise *Ae. vigilax* as a prey resource to establish whether bat size constrains the use of *Ae. vigilax* as a prey resource given prey detectability and manoeuvrability is negatively associated with mass. Based on the findings of Chapter 4, I make predictions about which bat species are likely to be sensitive to broadscale mosquito control.

Chapter 4

The Importance of Mosquitoes to Insectivorous Bat Diet: Does Size Matter?



This chapter has been prepared for publication as Gonsalves, L., Bicknell, B., Law, B., Webb, C. and Monamy, V. The importance of mosquitoes to insectivorous bat diet: Does size matter? *PloS*

Abstract

Mosquitoes may represent a highly abundant prey resource for insectivorous bats, particularly smaller sized bats that employ high frequency echolocation suited to the detection of small prey. Few field studies, however, have specifically investigated predation of mosquitoes by bats. Many mosquito species have been identified as vectors of disease-causing pathogens worldwide and may pose a significant threat to public health. The saltmarsh mosquito (*Aedes vigilax* Skuse) is an estuarine mosquito species that can be highly abundant throughout summer in coastal habitats, is a known nuisance-biting pest and has been identified as a vector of Ross River virus (RRV) and Barmah Forest virus (BFV). No studies have investigated the potential indirect impacts of broadscale mosquito control on insectivorous bats that feed on mosquitoes. However, the first step is an understanding of the extent to which bats consume mosquitoes in the wild. To assess this, I investigated the diets of five eastern Australian bat species (*Vespadelus vulturnus* Thomas, *V. pumilus* Gray, *Miniopterus australis* Tomes, *Nyctophilus gouldi* Tomes and *Chalinolobus gouldii* Gray) ranging in size from 4-14 g, using molecular analysis of faecal DNA. The abundances of potential mosquito and non-mosquito prey were also concurrently measured at my forest sites. *Aedes vigilax* was locally the most abundant mosquito species, while Lepidoptera the most abundant insect order. A diverse range of prey was detected in bat faeces, including coleopterans, dipterans, hemipterans and blattodeans, although members of Lepidoptera dominated, reflecting the proportional abundance of each insect order in the environment. Consumption of mosquito prey was restricted to *V. vulturnus* and *V. pumilus*, two smaller sized bats (4 and 4.5 g mean mass). No correlation was observed between the abundance of non-dipteran prey and the frequency in which mosquitoes were detected in bat faeces, suggesting that mosquitoes may not simply represent an alternative prey that small bats use when larger prey become less available. The findings of this study demonstrate that mosquitoes, particularly *Ae. vigilax*, are a prey resource utilised by smaller sized bats during summer and that these bats are likely to be the most sensitive to broadscale mosquito control.

4.1 Introduction

Balancing environmental conservation and human health can be difficult. The emergence of zoonotic diseases (Jones *et al*. 2008) have highlighted the human health risks associated with close contact between vertebrates and humans. However, conservation is of critical concern as urbanisation provides direct and indirect threats to wildlife and their habitats (Davis 1976). The conservation of coastal insectivorous bat populations is of increasing concern as urbanisation threatens local insectivorous bat communities (Kurta and Teramino 1992; Threlfall *et al*. 2012). However, mosquito control programs prompted by public health concerns may indirectly threaten bat populations by reducing the availability of potential prey items. To balance these conflicting objectives, an understanding of the role mosquitoes may play as a food source for bats is required.

The ability of echolocating insectivorous bats to detect and capture prey is influenced by a range of factors. Clutter (i.e. non-target structures that produce echoes) can restrict access of bats to prey, both directly and indirectly. It has been demonstrated that clutter reduces the ability of bats to detect prey (Boonman *et al.* 1998; Rainho *et al.* 2010), primarily by masking the echoes of potential prey (Arlettaz *et al.* 2001), rendering them unavailable. Clutter also influences the foraging activity and mobility of bats (Bradshaw 1996; Brigham *et al.* 1997; Schnitzler and Kalko 1998; Law and Chidel 2002; Lloyd *et al.* 2006; Adams *et al*. 2009), limiting the habitats that bats are able to successfully negotiate, and ultimately restricting access to prey within the habitats. However, the influence of clutter on prey detection and bat mobility is not the same for all bat species.

Wing morphology and echolocation call design influence the speed and manoeuvrability of bats (Neuweiler 1984; Norberg and Rayner 1987; Fenton 1990; Saunders and Barclay 1992; Brigham *et al.* 1997), two characteristics closely associated with tolerance to clutter. Echolocation design was found to be the major cause of differences observed in the prey capture success of five sympatric congeneric European species of *Myotis* (Siemers and Schnitzler 2004). Since echolocation call frequencies are negatively associated with bat size (Jones 1999), bats with low-frequency echolocation are generally relatively large, fast-flying and less manoeuvrable. Conversely, bats with high frequency echolocation are usually smaller, more manoeuvrable and therefore more suited to foraging within clutter than larger

bats. Based on this sensitivity, the limitations imposed by clutter on bats to successfully detect and capture prey are more applicable to larger bat species.

Although echolocation call design influences the ability of bats to forage within habitats of varying clutter levels, it is also thought to influence the size of prey that bats are able to successfully locate (Barclay and Brigham 1991). Since larger bats use low-frequency echolocation to detect prey, the longer wavelength associated with this echolocation is considered to be unsuitable for detecting small prey (Barclay and Brigham 1991). However, the diet of a number of medium to large sized European bats does not support this theory in that small prey were frequently consumed (Rydell 1989; Waters *et al.* 1995; Jones 1999). While bats that use high-frequency echolocation are thought to be more suited to detecting small prey, they are still able to detect larger prey items. However, morphological constraints associated with jaw structure and prey hardness impose limitations on the size of prey that can be consumed by these bats (Freeman and Lemen 2007).

Mosquitoes represent a small sized (~5.2 mm) prey resource for insectivorous bats, particularly during summer months when they can be highly abundant. Though only one field study has specifically investigated predation of mosquitoes by microchiropteran bats (Reiskind and Wund 2009), bats of various sizes (small to large) have been observed consuming mosquitoes (Griffin *et al.* 1960; Rydell 1990), sometimes up to 10 per minute (Griffin *et al.* 1960). Additionally, mosquitoes have been identified in the stomach contents of bats (Poole 1932; Buchler 1976) as well as in bat faeces (Rydell 1990; Whitaker Jr. and Lawhead 1992; Kurta and Whitaker Jr. 1998; Long *et al.* 1998; Shiel *et al.* 1998; Pavey *et al.* 2001; Sophia 2010). Furthermore, mosquitoes are common components of the diets of some bats (Anthony and Kunz 1977; Kurta and Whitaker Jr. 1998; Pavey *et al*. 2001).

Mosquitoes also serve as vectors of many pathogens worldwide that are of particular concern to public health. The saltmarsh mosquito, *Aedes vigilax* Skuse, is an estuarine species that is a serious nuisance biting pest and has been identified as an important vector of mosquito-borne pathogens such as Ross River virus (RRV) and Barmah Forest virus (BFV) (Russell 1998). In some coastal areas of Australia, broadscale mosquito control programs have been implemented to mitigate the risk of eruptions in the number of cases of mosquito-borne arbovirus infections and nuisance biting (Bell 1989; Russell and Kay 2008). The most commonly used microbial larvicide during aerial spraying of mosquito larval habitats is

Bacillus thuringiensis var. israelensis, serotype H14 (*Bti*) (Poulin *et al.* 2010). Given the general specificity of the *Bti* formulation, non target effects are generally restricted to chironomids (Kondo *et al*. 1992; Liber *et al*. 1998). However, *Bti* has been found to adversely impact breeding house-martens (Poulin *et al.* 2010). There is very little known about the potential indirect impacts of broadscale mosquito control on insectivorous bats that feed on mosquitoes.

Some microchiropteran bats have been found to reduce arthropod densities via predation (Kalka *et al.* 2008; Williams-Guillén *et al.* 2008) and may be economically important in agricultural systems (Boyles *et al*. 2011). Tuttle (2005) suggested that bats may serve as an alternative approach to broadscale mosquito control, and proposed that a single bat is capable of consuming up to 600 mosquitoes per hour. Additionally Reiskind and Wund (2009) also suggested a possible role for bats in the reduction of disease vectors after observing a 32 % reduction in oviposition by *Culex* spp. associated with bat predation. However, the suggestion by Tuttle (2005) was based on an extrapolation from the laboratory study of Griffin *et al*. (1960), that like the study of Reiskind and Wund (2009), did not account for a range of other factors such as satiation of bats, the abundance of mosquitoes relative to other prey, the ability of bats to detect mosquitoes amongst various levels of clutter, the digestibility of mosquitoes as well as the calorific requirements of bats, all of which will presumably influence the degree to which bats consume mosquitoes.

In order to assess the potential impacts of broadscale mosquito control on insectivorous bat diets, an understanding of the extent to which bats consume mosquitoes in the wild is first required. However, one obstacle to this is the bias associated with techniques available to study bat diet (Belwood and Fenton 1976; Kunz and Whitaker Jr. 1983; Dickman and Huang 1988). Many dietary studies have used microscopic analysis of prey fragments within bat faeces or stomach contents to describe bat diets (Whitaker Jr. 1972; Buchler 1976; Vestjens and Hall 1977; O'Neill and Taylor 1989; Rydell 1989; Brigham and Fenton 1991; Jones 1999). Due to the greater susceptibility of soft bodied prey to the processes involved in mastication and digestion (Rabinowitz and Tuttle 1982; Dickman and Huang 1988) often rendering these prey unidentifiable, this technique is likely to underestimate the importance of small prey such as mosquitoes. In addition, single large prey items can be represented in multiple faecal pellets, potentially leading to an overestimation of these taxa, thus

contributing further to an underestimation of smaller prey taxa (Robinson and Stebbings 1993).

Recent advances in molecular techniques have allowed greater resolution of animal diets, particularly for cryptic animals that are difficult to observe foraging. Since these techniques rely on DNA for the identification of prey, either in gut contents or in faeces, detection of soft-bodied prey may be improved. However, like traditional microscopic analysis, prey-specific biases associated with differential digestibility may translate into varied levels of prey DNA degradation (Deagle and Tollit 2007), limiting the degree to which prey can be quantified. Nevertheless, in a dietary study of *Barbastellus barbastellus* (Schreber) using this technique, soft-bodied micro-moths were detected among a range of larger, more sclerotised insects which suggests that the technique is robust enough to detect those prey items most susceptible to digestion (Rabinowitz and Tuttle 1982; Dickman and Huang 1988; Zeale *et al*. 2011). The molecular approach has been applied in at least five studies investigating bat diets (McCracken *et al*. 2005; Clare *et al*. 2009; Brown 2010; Zeale *et al*. 2011; Bohmann *et al*. 2011) and offers an alternative approach to investigate the presence of mosquitoes in bat faeces.

Of 19 insectivorous bat taxa that occur on the central coast of New South Wales, Australia, five species representing a range of sizes (small – large) were sampled and their diets investigated using molecular techniques to: 1) examine the extent to which they consume *Ae. vigilax* and 2) identify which species are more vulnerable to reductions in prey resulting from broadscale mosquito control. Four of the five bat species employ high-frequency echolocation (>50 kHz) thought to be more suited for detection of small prey (Barclay and Brigham 1991). I concurrently measured the abundance of mosquitoes and other insects through the course of one field season to provide data on the availability of prey and predicted that the frequency of consumption of *Ae. vigilax* would be greatest for bats in the small size class.

4.2 Methods

4.2.1 Study site & design

The study area was located in the Empire Bay region (33°29'57"S, 151°21'40"E) of the central coast of New South Wales, Australia (Fig. 4.1). This region is approximately 50 km to the north of Sydney and experiences a warm sub-tropical climate. Within the study area, a large national park (Bouddi National Park) sustains populations of hollow and cave roosting insectivorous bats, including six threatened species listed under the NSW *Threatened Species Conservation Act* 1995 (Payne 2006).



Figure 4.2: Satellite image of study area (adapted from Google Earth). Donuts represent harp trapping locations along Daleys Point and Strohms fire trails in Bouddi National Park. Star represents location of sea cave in which *Miniopterus australis* individuals were trapped in 2011.

Within the study area, large areas of estuarine habitats (primarily coastal saltmarsh and mangrove forests) support highly abundant populations of *Ae*. *vigilax* throughout the austral summer. The population abundance of *Ae. vigilax* is heavily driven by tidal and rainfall inundation of larval habitats (i.e., coastal saltmarsh and mangrove communities). General patterns such as peaks in population abundances can be predicted (Kokkinn *et al.* 2009), with larger populations tending to be present two weeks after inundation of saltmarshes by spring tides and/or heavy rainfall.

To encompass the variation in *Ae*. *vigilax* population abundances, sampling was undertaken over two consecutive nights in each of eight fortnights during spring and neap tides during the austral summer of 2009/10, at times that coincided with either predicted large or predicted smaller mosquito population abundances. Faecal samples for dietary analysis were obtained from five insectivorous bat species. The selection of bat species for analysis was based on predicted minimum detectable prey size and trapping frequency (Table 4.1). These species were the Gould's wattled bat (*Chalinolobus gouldii* Gray), little bent-wing bat (*Miniopterus australis* Tommes), Gould's long-eared bat (*Nyctophilus gouldi* Tomes), eastern forest bat (*Vespadelus pumilus* Gray) and little forest bat (*Vespadelus vulturnus* Thomas). The five species range in mass from 4-14 g (*C. gouldii*, 14 g, forearm=43.7 mm; *Mi. australis*, 6.7 g, 39.3 mm; *N. gouldi*, 12 g, 44 mm; *V. pumilus*, 4.4 g, 30.9 mm; *V. vulturnus*, 4 g, 28.4 mm). With the exception of *C. gouldii* (25-34 kHz), all bats employ frequency modulated echolocation calls with terminal frequencies >50 kHz. Additionally, *N. gouldi* is often considered to be a gleaning bat because of the steep linear nature of its calls and its use of passive listening as a hunting strategy (see Grant 1991). The terminal portion of its calls is often <50 kHz (see Reinhold *et al*. 2001).

Bats (*C. gouldii, N. gouldi, V. pumilus and V. vulturnus*) were trapped at three sites (Daleys F1, Daleys F2 and Strohms) within Bouddi National Park (Figure 4.1). Trapping was undertaken in Narrabeen Coastal Blackbutt Forest. This vegetation community has a typical canopy height of 20 m, occurring on Narrabeen sandstone that supports a sparse-to-moderate understorey of shrubs and a well developed grass layer (Bell, 2009). Canopy cover in this vegetation community is approximately 40 %. Each site was situated 1-2 km from saltmarsh (major larval habitat of *Ae*. *vigilax*).

Prey abundance data were obtained concurrently with the collection of faecal samples. This involved surveying the mosquito populations at each site, as well as all other aerial insect fauna. Faecal samples from *Mi. australis* were collected during a one-off trapping event in a sea cave within the national park in February of 2011, during which no prey abundance data were collected (Figure 4.1).

Table 4.1: Minimum detectable prey size and energetic requirements of each bat taxa recorded in this study. Species in bold represent bats whose diets were analysed for this study.

Species	No. trapped individuals	Mass (g)	Echolocation frequency (kHz)	Min detectable size[a] (mm)	Detectability of mosquito (5.2mm)	FMR[b] (kjd^{-1})	No. mosquitoes required[c]	No. moths required[c]	Foraging time[d] (hrs)	
									Mosquito	Moth
Chalinolobus dwyeri	0	7.7	26	10.5		28.91	1395	380	6.6	3.2
C. gouldii	**5**	**13.8**	**34**	**8.1**		**44.31**	**2139**	**582**	**10.2**	**4.8**
C. morio	4	8.9	53	5.2	✓	32.14	1551	422	7.4	3.5
Falsistrellus tasmaniensis	0	20.5	39	7		59.2	2857	777	13.6	6.5
Kerivoula papuensis	0	6.9	160	1.7	✓	26.68	1288	350	6.1	2.9
Miniopterus australis	**7**	**6.7**	**65**	**4.2**	✓	**26.11**	**1260**	**343**	**6.0**	**2.9**
Mi. schreibersii oceanensis	0	14.1	48	5.7		45.02	2173	591	10.3	4.9
Mormopterus sp. 2	0	9	31	8.8		32.41	1564	426	7.4	3.5
***Nyctophilus* spp.**	**10**	**12.3**	**80**	**3.4**	✓	**40.73**	**1966**	**535**	**9.4**	**4.5**
Rhinolophus megaphylus	0	11.5	70	3.9	✓	38.78	1872	509	8.9	4.2
Scoteanax rueppellii	0	25.4	37	7.4		69.26	3343	909	15.9	7.6
Scotorepens orion	1	9.3	37	7.4		33.19	1602	436	7.6	3.6
Tadarida australis	0	37.6	15	18.3		92.29	4454	1212	21.2	10.1
Vespadelus darlingtoni	0	7.2	44	6.2		27.52	1328	361	6.3	3.0
V. pumilus	**10**	**4.4**	**53**	**5.2**	✓	**19.51**	**942**	**256**	**4.5**	**2.1**
V. vulturnus	**20**	**4**	**53**	**5.2**	✓	**17.89**	**863**	**235**	**4.1**	**2.0**

[a] Predicted minimum detectable prey size using equation of Møhl (1988).

[b] Field metabolic rate using equation of Speakman and Thomas (2003).

[c] Number of prey required to meet FMR assuming energy absorption of 70 % (Barclay *et al*. 1991); calorific value of mosquito (0.002 g) = 14.8 kJg^{-1} (Foster 1995), moth (0.004 g) = 27.2 kJg^{-1} (McLean and Speakman 1999).

[d] Foraging time required to obtain enough mosquitoes or moths (5–10 mm) to meet FMR requirements assuming attack rate of 5 min^{-1} for both prey and 70 % success for mosquitoes and 40 % success for moths (Kalko 1995).

4.2.2 Collection of bat faeces

Bat trapping was conducted using harp-traps (Tidemann and Woodside 1978) set along flyways on two fire trails within Bouddi National Park. Traps were not set in salt marsh habitats where *Ae. vigilax* emerges because of the difficulty in trapping bats in open habitats where trees were absent. Each trap was checked and cleared at midnight, as well as at first light. Captured bats were removed from traps, placed into individual calico holding bags and processed (including identification and collection of morphometric data). All individuals were held for one hour to defecate if trapped during the first half of the night, or until dusk the following night, at which time they were released at the point-of-capture. In February 2011, harp traps were set in front of the entrance to a sea cave used by three species of cave roosting bats *Mi. australis*, eastern bent wing bat, *Miniopterus schreibersii oceanensis* Maeda and the eastern horseshoe bat, *Rhinolophus megaphyllus* Gray). Captured bats (*Mi. australis* individuals) were immediately removed from traps, placed into calico bags and transported out of the cave to be processed (as above). Individuals were held overnight to defecate before release from the point-of-capture. Faeces produced by bats in calico bags were transferred to 1.5 mL microcentrifuge tubes and were frozen immediately to reduce the degradation of faecal DNA. Bat faeces were collected from 52 individuals (five *C. gouldii*, seven *Mi. australis*, ten *N. gouldi*, ten *V. pumilus*, 20 *V. vulturnus* – Table 4.1).

4.2.3 Collection of prey abundance data

The mosquito fauna at each site was surveyed concurrently with bat trapping using one CO_2–baited encephalitis virus surveillance (EVS) trap (Rohe and Fall 1979) (Australian Entomological Supplies, Bangalow, NSW, Australia), while other aerial insect fauna was sampled using one standard light trap (Australian Entomological Supplies, Bangalow, NSW, Australia). All specimens were killed by being placed into dry-ice, stored dry and frozen. Mosquito collections were identified to species according to keys (Russell 1996) and the nightly abundance of each species was recorded. Light trap collections were sorted into three Orders (Lepidoptera, Coleoptera and Diptera), with all other specimens pooled into an ‘other’ category. The nightly abundance of each insect order was recorded.

4.2.4 Analysis of bat faeces

Genomic DNA was extracted from a pooled sample of five faecal pellets for each trapped individual using a commercial DNA extraction kit (Appendix 1). A 157bp section of the DNA barcoding region, cytochrome oxidase I was amplified using taxon-specific primers, ZBJ-ArtF1c and ZBJ-ArtR2c (Zeale *et al*. 2011), purified and cloned. DNA from a sub-sample of 16 clones from each clone library was then sequenced at the Australian Genome Research Facility (Westmead Millennium Institute, Sydney) (Appendix 1). DNA sequences were trimmed of flanking vector and entered into the identification engine on the barcoding of life database (BOLD). The nearest sequence match and percent similarity of each sequence was recorded, with a taxonomic assignment to order, family, genus or species using taxonomic assignment thresholds (Zeale *et al*. 2011). When sequence similarity was greater for a taxon not currently known to exist in Australia, the sequence was assigned to the nearest match known to occur in Australia.

4.2.5 Calibration of technique sensitivity for detection of mosquito DNA

While Zeale *et al*. (2011) reported no bias in detectability of various taxa, the tested taxa did not include mosquitoes. To provide a baseline for detectability of mosquito DNA amongst DNA of other taxa, artificial bat faeces were manufactured with two prey items: mealworms (*Tenebrio molitor* L.) and mosquitoes (*Aedes aegypti* L.). Artificial bat faeces consisting of 0 %, 5 %, 10 %, 15 %, 20 % and 100 % of mosquito (by volume - representing 0, ~6, ~11, ~17, ~22, ~110 mosquitoes, respectively) were made by adding an appropriate volume of mosquito slurry for each concentration and then adding a volume of mealworm mixture that accounted for the lower concentration of mosquito material relative mealworm material in the stock solutions (0.18 g mL^{-1} and 0.30 g mL^{-1}). Each solution was vortexed vigorously for 1 min to mix the mosquito and mealworm material. Once mixed, 1 mL of the mixture was used to extract DNA for use in PCR as described above for bat faeces, except there was no cloning step. Each mosquito concentration was treated in triplicate. If after sequencing, a sequence appeared to be mixed, it was inferred that both the mosquito and mealworm DNA had been amplified. To confirm this, one PCR product from each mosquito concentration was cloned and ten clones were sequenced from each clone library.

4.2.6 Data analysis

Repeated measures-analysis of variance (RM-ANOVA) was used to test the significance of differences in mean nightly *Ae. vigilax* population abundances between spring and neap tides. Additionally, RM-ANOVA was used to test the significance of differences in mean nightly abundance of all insects and each insect order between spring and neap tides. For each bat species, the relative importance of each insect order to bat diet was based on the frequency of occurrence of the insect order (percentage of sample size that contained a DNA sequence matching a taxon within that order). A chi-square goodness of fit test was used to investigate whether consumption of each prey taxa reflected prey availability. For each bat species (except *Mi. australis* for which no prey abundance data were collected), the percentage of identified prey items belonging to each insect order was compared with the percentage of each insect order represented in light trap collections. Pearson correlation analysis related prey abundance and detection of *Ae. vigilax* in bat faeces.

4.3 Results

4.3.1 Prey abundance

A total of 12 898 mosquitoes was collected during the study representing 14 mosquito species (Table 4.2). Of these, 12 were recorded during spring and neap tides, respectively. The most abundant species irrespective of tidal cycle was *Ae. vigilax*, representing 77.81 % of all mosquitoes recorded during spring tides, and 83.9 % of all mosquitoes sampled during neap tides. The other commonly collected species were *Aedes multiplex* Theobald, *Aedes notoscriptus* Skuse and *Culex sitiens* Wiedemann.

As predicted, nightly *Ae. vigilax* abundance was greater during neap tides, however it was not significantly different from spring tides ($F(1,14)=2.125$. $P=0.152$; Fig. 4.2).

Table 4.2: Nightly abundances (averaged across sites) ± SE of mosquito species trapped during spring and neap tides. Values in brackets represent percent of total mosquito abundance in each habitat.

Species	Spring	Neap
Aedes alternans	3.40 ± 0.53 (1.15)	7.12 ± 1.24 (1.64)
Ae. multiplex	15.12 ± 3.12 (5.14)	11.19 ± 4.72 (2.58)
Ae. notoscriptus	12.77 ± 3.50 (4.34)	8.24 ± 0.68 (1.90)
Ae. procax	4.10 ± 0.87 (1.39)	5.27 ± 0.87 (1.21)
Ae. rubrithorax	0.10 ± 0.10 (0.03)	
Ae. vigilax	229.10 ± 60.00 (77.81)	364.67 ± 86.96 (83.93)
Anopheles annulipes		0.05 ± 0.05 (0.01)
Coquillettidia linealis	0.05 ± 0.05 (0.02)	0.05 ± 0.05 (0.01)
Culex annulirostris	12.20 ± 5.8 (4.14)	15.31 ± 4.99 (3.52)
Cx. australicus		0.1 ± 0.07 (0.02)
Cx. molestus	4.10 ± 0.77 (1.39)	2.13 ± 0.11 (0.49)
Cx. qinquefasciatus	2.71 ± 0.47 (0.92)	2.15 ± 0.18 (0.49)
Cx. sitiens	10.72 ± 4.12 (3.64)	18.23 ± 7.43(4.20)
Tripteroides atripes	0.05 ± 0.05 (0.02)	
Total	294.42 ± 99.97	434.80 ± 100.00

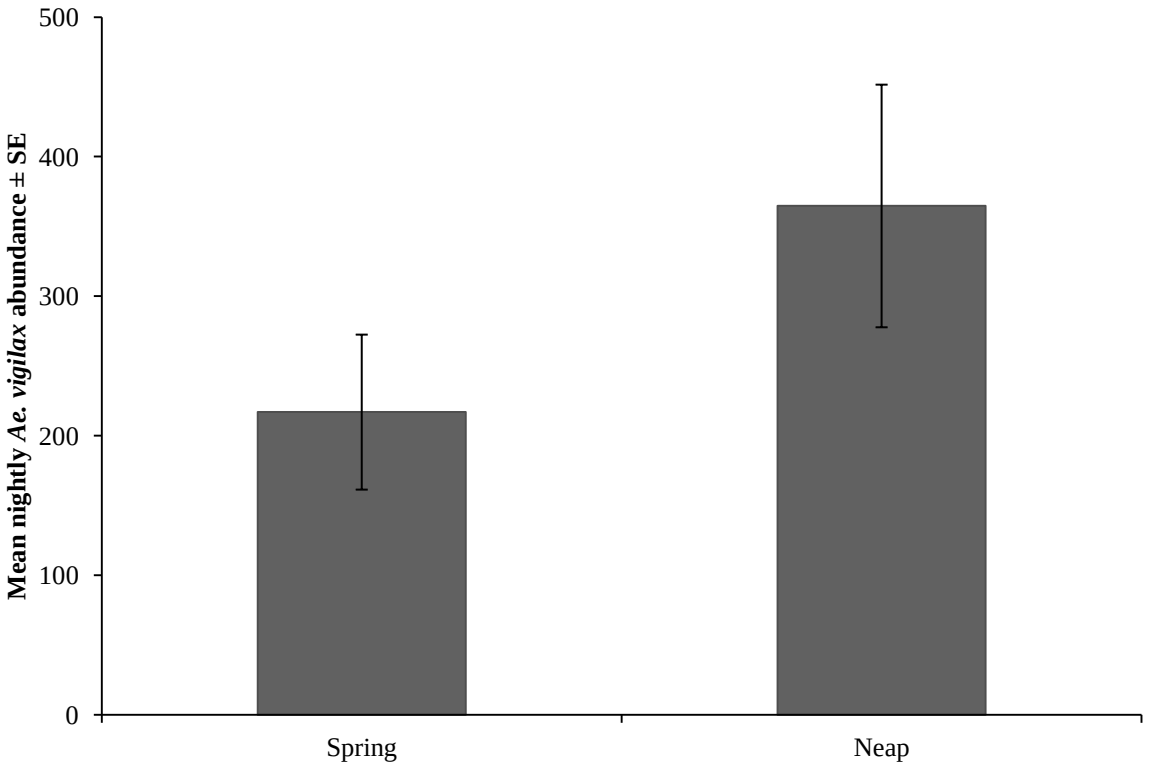


Figure 4.3: Nightly *Aedes vigilax* abundance during spring and neap tides.

Total nightly insect abundance during spring tides (267.29±9.69) was not significantly different to total nightly insect abundance during neap tides (286.00±8.90) ($F(1,14)=1.982$, $P=0.166$; Fig. 4.3). Lepidopterans, coleopterans, dipterans and 'other' taxa, consisting of representatives of Blattodea, Hemiptera, Hymenoptera, Isoptera, Odonata and Orthoptera,

were recorded in light trap collections. Irrespective of tide height, lepidopterans were the most abundant taxa in light trap collections, representing 45.5 % and 48.6 % of all insects trapped during spring and neap tides, respectively. Coleopterans were the second most abundant taxa, representing 23.8 % of all insects trapped during both spring and neap tides, while dipterans were less abundant, representing 13 % and 12.8 % of all insects during spring and neap tides, respectively. All other taxa represented 17.7 % and 14.8 % of insect collections during spring and neap tides, respectively. The abundance of lepidopterans, coleopterans, dipterans and all other taxa did not differ between spring and neap tides ($F(1,14)=3.632$, $P=0.063$; $F(1,14)=0.491$, $P=0.487$; $F(1,14)=0.462$, $P=0.500$; $F(1,14)=3.463$, $P=0.070$; Fig. 4.3).

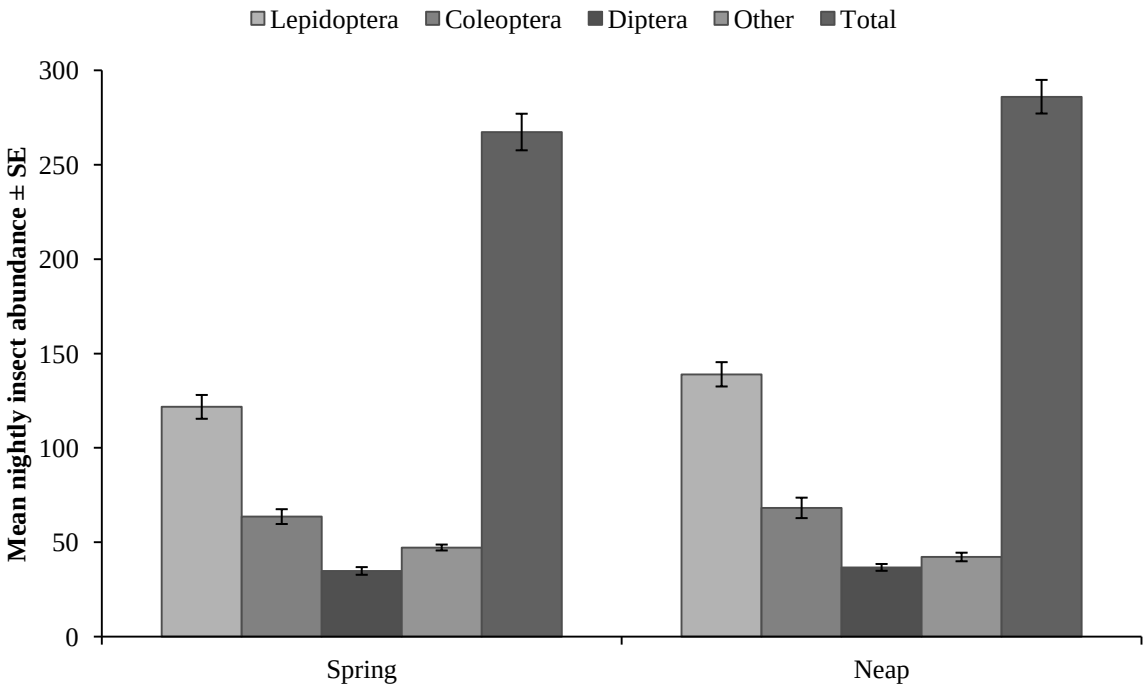


Figure 4.4: Nightly insect abundance during spring and neap tides.

4.3.2 Bat diet

Calibration of technique sensitivity for detection of mosquito DNA

All mosquito-mealworm mixtures produced visible PCR products after amplification. Mosquito-mealworm mixtures containing 0 % and 100 % *Ae. aegypti* produced readable sequences that provided species-level matches to either *T. molitor* (0 % mosquito) or *Ae. aegypti* (100 % mosquito) (Table 3). For each of the other mosquito-mealworm mixes (i.e. 5 %, 10 %, 15 %, 20 % and 25 % mosquito), direct sequencing (without cloning) provided mixed DNA sequences that could not be interpreted (i.e. erroneous sequences) (Table 4.3). Sequences from clone libraries of each of the different mosquito-mealworm mixtures revealed the presence of both *Ae. aegypti* and *T. molitor*, though the ratio of sequences belonging to *Ae*. *aegypti* and *T. molitor* from each clone library did not appear to be related to the ratios of mosquito-mealworm in the various mixtures (Table 4.3). These results indicate that while mosquitoes can be detected when present in low concentrations relative to other prey (*T. molitor*), the proportion of sequences corresponding to mosquito DNA in clone libraries do not correspond to ratios of mosquito-mealworm in the original samples (artificial bat faeces).

Table 4.3: Detectability of mosquito DNA in artificial bat faeces with increasing concentrations of *Ae. aegypti* (by volume; 0-100 %). ✖ represents successful PCR amplification with a corresponding DNA sequence matching *Ae. aegypti*; ✓ represents successful PCR amplification with a corresponding DNA sequence matching *T. molitor*; ✓✖ represents mixed DNA sequence; ° represents a non-readable sequence due to excessive loss of PCR products during purification prior to sequencing.

% *Aedes aegypti* (by volume)	0	5	10	15	20	25	100
Replicate 1	✓	✓✖	✓✖	✓✖	✓✖	✓✖	✖
Replicate 2	✓	✓✖	✓✖	✓✖	✓✖	°	✖
Replicate 3	✓	✓✖	°	✓✖	✓✖	✓✖	✖
% of mosquito sequences in clone library	n/a	20	30	10	30	30	n/a

Diets of wild trapped bats

A total of 41 prey taxa was identified from the faeces of 52 insectivorous bats representing five bat species. For all bat species, lepidopterans were the most frequently detected prey, present in the faeces of 71-100 % of all individuals (Table 4.4, Fig. 4.4). Coleopterans were only detected in the faeces of *C. gouldii*, *V. pumilus* and *V. vulturnus*, but were not commonly present in faecal samples (5-40 % frequency of occurrence) (Table 4.4, Fig. 4.4). Dipterans and 'other' taxa were detected in the faeces of four species (Table 4.4, Fig. 4.4). *Aedes vigilax* and an unidentifiable mosquito of the *Aedes* genus were detected only in the faeces of two bat species (*V. pumilus* and *V. vulturnus*). Mosquitoes were present in two (20 %) individuals of *V. pumilus*, both of which were trapped during neap tides (Jan and Mar). Mosquitoes were detected in the faecal samples of eleven (55 %) individuals of *V. vulturnus*, trapped during spring and neap tides (Dec-Feb). A representative of the non-insect order, Aranae was detected in the faecal samples of one *V. pumilus* individual (Table 4.4, Fig. 4.4).

Table 4.4: Nearest matches and percentage similarity of DNA sequences obtained from the faeces of each insectivorous bat species. 'Unknown' labels are provided if percent similarity to nearest match was not sufficient to assign the match to a particular taxa, or if reference sequences were not designated a taxon label. * Represents cases where sequences showed greater similarity to taxa not known to occur in Australia, but were assigned to the nearest match currently known to occur in Australia. [+] Represents lepidopteran families with tympanal organs.

Order	Family	Genus	Species	% similarity to nearest match on BOLD
C. gouldii				
Blattodea	Unknown	Unknown	Unknown sp.	95.24
Coleoptera	Unknown	Unknown	Unknown sp.	97.51
Lepidoptera	Geometridae[+]	*Dysbatus*	*singularis*	99.49
	Geometridae[+]	*Nisista*	Unknown sp.	98.72
	Xyloryctidae	*Cryptophasa*	Unknown sp.	97.38
Mi. australis				
Blattodea	Unknown	Unknown	Unknown sp.	94.05
Diptera	Drosophilidae	*Drosophila*	Unknown sp.	97.53
	Hippoboscidae	Unknown	Unknown sp.	97.51
Lepidoptera	Oecophoridae	*Antipterna*	Unknown sp.	97.51*
	Geometridae[+]	*Dysbatus*	*singularis*	99.49
N. gouldi				
Blattodea	Unknown	Unknown	Unknown sp.	94.05
Diptera	Drosophilidae	*Drosophila*	Unknown sp.	97.53
	Pipunculidae	*Tomosvaryella*	Unknown sp.	91.43
Hemiptera	Cicadidae	*Psaltoda*	Unknown sp.	98.51
Lepidoptera	Crambidae[+]	*Orthospila*	Unknown sp.	97.44*
	Noctuidae[+]	*Lysimelia*	*lenis*	100
V. pumilus				
Araneae	Unknown	Unknown	Unknown sp.	87.18
Coleoptera	Unknown	Unknown	Unknown sp.	97.51
Diptera	Tabanidae	Unknown	Unknown sp.	99.50
	Culicidae	*Aedes*	*vigilax*	99.00
		Aedes	Unknown sp.	97.00
Lepidoptera	Geometridae[+]	*Scioglyptis*	*lyciaria*	99.49
	Limacodidae	*Pseudanapaea*	*denotata*PS1	99.49
	Noctuidae[+]	*Mythimna*	*convecta*	99.49
	Oecophoridae	*Antipterna*	Unknown sp.	98.00
	Pyralidae[+]	*Spectrotrota*	*fimbrialis*	99.49
	Xyloryctidae	*Thymiatris*	Unknown sp.	96.43

V. vulturnus				
Coleoptera	Unknown	Unknown	Unknown sp.	95.20
Diptera	Culicidae	*Aedes*	*vigilax*	99.49
		Aedes	Unknown sp.	98.48
Lepidoptera	Choreutidae	*Brenthia*	Unknown sp.	97.53*
	Crambidae[+]	*Maruca*	Unknown sp.	97.33*
		Notarcha	Unknown sp.	98.89
	Geometridae[+]	*Nearcha*	Unknown sp.	97.37*
	Noctuidae[+]	*Achaea*	Unknown sp.	94.87*
		Characoma	Unknown sp.	98.04*
		Ericeia	Unknown sp.	96.74*
	Nymphalidae	*Acraea*	Unknown sp.	98.13
	Oecophoridae	*Oecophorine*	Unknown sp.	96.02
		Oligoloba	Unknown sp.	97.06*
	Unknown	Unknown	Unknown sp.	97.79

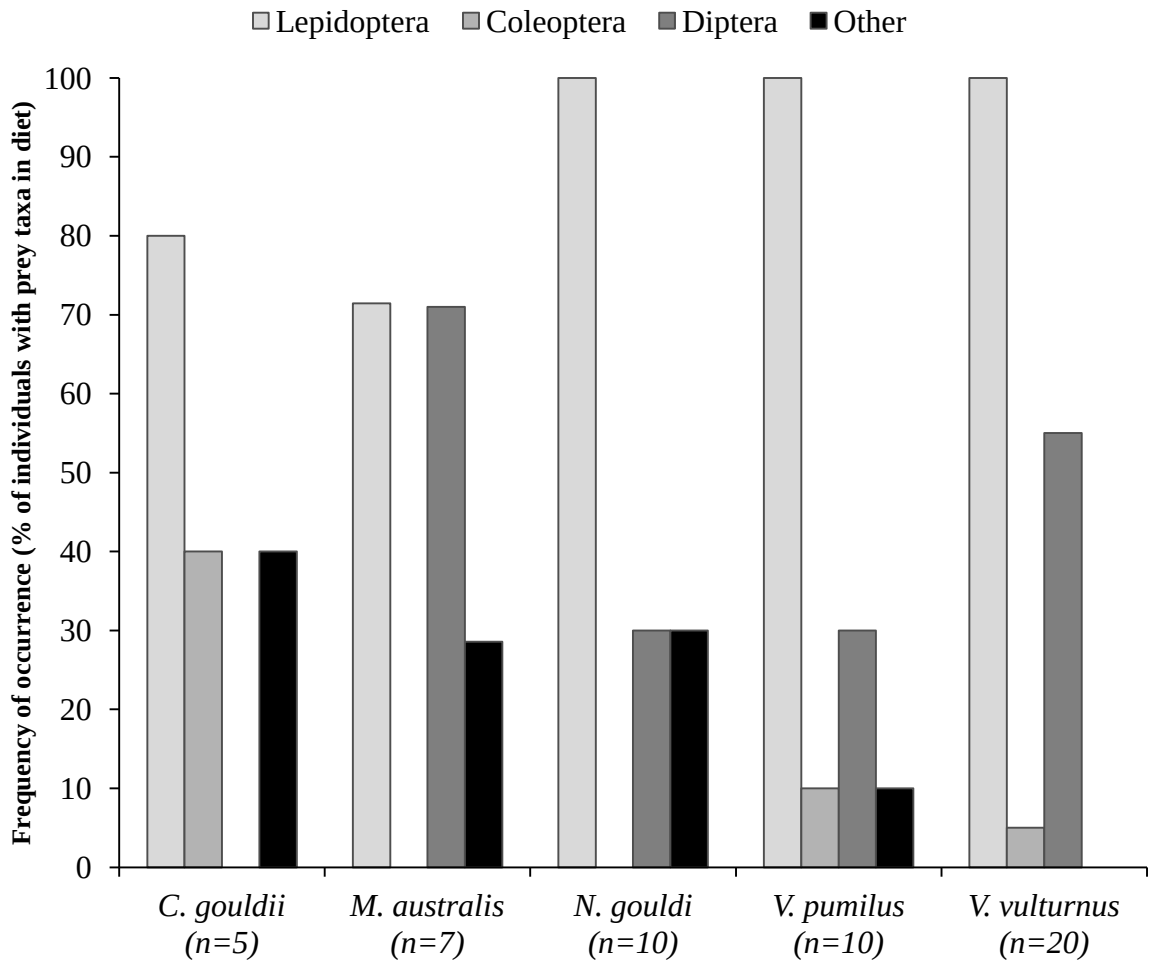


Figure 5.4: Frequency of occurrence of each insect taxa in the diets of the five insectivorous bats (i.e., percentage of individuals of a species that consumed each insect taxa).

Chalinolobus gouldii, *N. gouldi*, *V. pumilus* and *V. vulturnus* all consumed prey in proportion to the abundance of arthropods in light trap collections (χ^2=0.324, P=0.955; χ^2=2.773, P=0.428; χ^2=1.637, P=0.651; χ^2=1.637, P=0.651).

Correlation analyses revealed no significant relationships between *Ae. vigilax* consumption and abundance of lepidopterans, coleopterans, other insects or all insects combined (r(7)=0.018, P=0.969; r(7)=0.274, P=0.552; r(7)=-0.226, P=0.627; r(7)=0.178, P=0.703). A significant positive relationship between *Ae. vigilax* consumption and dipteran abundance was observed (r(7)=0.889, P=0.007). While not significant, a positive correlation was identified between *Ae. vigilax* consumption and *Ae. vigilax* abundance (r(7)=0.702, P=0.079).

4.4 Discussion

This study successfully used prey DNA within bat faeces to characterise the diets of five Australian insectivorous bat species, providing greater taxonomic resolution of prey items than previously described for these species using traditional microscopic analysis (Vestjens and Hall 1977; O'Neill and Taylor 1989). Although the diets of all bat species reflected the abundance of prey taxa in light trap collections, mosquitoes were only detected in the faeces of the two smaller bat species (*V. pumilus* and *V. vulturnus*). While a positive correlation was observed between the abundance of dipterans and the frequency in which mosquitoes were detected in bat faeces, no such relationship was observed for any other bat prey, suggesting that mosquitoes may not simply represent an alternative prey that small bats utilise when other more larger prey become less available. These findings suggest that smaller sized bats are likely to be sensitive to broadscale mosquito control in environments with highly abundant vector mosquito populations that pose a threat to public health.

4.4.1 Limitations of dietary study

Although the molecular identification of prey within bat faeces was successful in this study, it remains difficult to quantify the amount of prey consumed. While at least two studies have discussed the potential use of clone library proportions to infer quantitative information about consumed prey (Sutherland 2000; Zeale *et al*. 2011), given the variable percentage of prey sequences identified from clone libraries developed for artificial bat faeces in this study as well as the variability associated with DNA degradation rates of different prey taxa (Deagle and Tollit 2007), this information is likely to be unreliable and ambiguous.

The molecular technique provided great resolution about consumed prey, however, the low number of identified prey for each individual bat (1-3 prey bat^{-1}) is only likely to represent a subset of all prey consumed by an individual bat. While this limitation was avoided in a previous study (Clare *et al*. 2009) by separating out insect fragments within bat faeces prior to the application of molecular techniques (e.g. PCR), it is possible that many soft-bodied prey without chitinous body parts may be overlooked using this technique. The use of next-generation sequencing applications (e.g. pyrosequencing) in studies of bat diet (e.g.

Bohmann *et al*. 2011) may also allow for the detection of more taxa than traditional DNA techniques (cloning and Sanger sequencing). However, given the variable degradation of DNA of different taxa as well as the variability associated with mtDNA copy-numbers, it is likely that any quantification of consumed prey will still be limited.

4.4.2 Prey abundance

While fourteen mosquito species were represented in CO_2-baited EVS traps during the study, *Ae. vigilax* was consistently the most abundant, irrespective of tidal cycle. This trend has been observed during long-term mosquito surveillance in the study area, in which *Ae. vigilax* represented 41.2 % of all mosquitoes trapped over nine consecutive trapping seasons (unpublished data – L. Gonsalves and C. Webb). The consistent presence of highly abundant populations of this species in the study area provides bats, particularly small sized species, with a consistent prey resource during summer. Nightly abundance of *Ae*. *vigilax* populations recorded in the forest habitat during this study (364.67±86.96 and 229.10±60.00 during neap and spring tides) was comparable to nightly abundance of *Ae*. *vigilax* populations recorded in saltmarsh habitats over the same period in 2008-09 (528.2±347.9 and 261.1±127.5 during neap and spring tides: Chapter 2).

The population abundance of *Ae. vigilax* is heavily driven by tidal and rainfall inundation of larval habitats (i.e. coastal saltmarsh and mangrove communities). General patterns such as peaks in population abundances can be predicted (Kokkinn *et al.* 2009), with larger populations present two weeks after inundation of saltmarshes by spring tides and/or heavy rainfall. In my study, abundance of *Ae. vigilax* populations in forest habitat did not reflect predicted peaks and troughs in *Ae*. *vigilax* abundance. This finding suggests that forests may provide a refuge habitat, sustaining *Ae*. *vigilax* populations for longer periods between tidal cycles.

The most abundant taxa present in light trap collections were lepidopterans. While it is acknowledged that certain insect taxa may be more attracted to particular attractant traps (Kunz 1988) and therefore the relative abundance of these taxa can be overestimated, light trapping is commonly used to measure insect abundance and can be used to investigate temporal trends in local insect abundances (Lowman 1982). Coleopterans, dipterans and 'other' insects were also present in light trap collections, but were significantly less

abundant than lepidopterans. Similar trends in insect abundances have been observed in other habitats (coastal swamp forest) within the study area, with lepidopterans representing the greatest amount of biomass in light traps (see Chapter 5). In bushland of the nearby Sydney region, lepidopteran and coleopteran biomass represented most of the insects collected using the same trapping technique (Threlfall 2011). The abundance of all insects and particular insect taxa was not found to differ between tidal cycles (spring and neap). Neap tides are generally associated with new moons while spring tides tend to coincide with full moons. There is no consensus in the literature regarding the influence of tides or lunar illumination on the abundance of insects recorded in light traps. Though some studies have reported a decrease in light trap captures due to increased lunar illumination (Taylor 1986; McGeachie 1989; Nag and Nath 1991), others have found insects to be more abundant in light traps during the full moon period (Ito *et al*. 1993). It is also likely that other factors such as cloud cover will also influence the abundance of insects collected in light traps (Nowinszky *et al*. 2010).

Since two different trapping techniques were used to survey mosquito populations and aerial insect fauna, it is not possible to directly compare the abundance of mosquitoes to the abundance of insects in light trap collections. However, the abundance of *Ae. vigilax* (229±60 during spring tides and 365±87 during neap tides) suggests that mosquitoes, like lepidopterans, represent a highly abundant prey resource. Discounting any limitations of detectability thought to be imposed by echolocation call design (Barclay and Brigham 1991), if the bat species investigated were opportunistic in nature, they would be expected to consume both *Ae. vigilax* and lepidopterans.

4.4.3 Relationships between bat size, diet and mosquito consumption

The diets of the five insectivorous bat species investigated during this study consisted of a diverse range of prey. Previous dietary studies of the five bat species also report a diverse range of prey (Vestjens and Hall 1977; O'Neill and Taylor 1989; Churchill 2009). In this study, lepidopterans were the most frequently detected insects in the faeces of all bat species, ranging from 71 to 100 % frequency of occurrence with only six of 52 bats not having lepidopteran DNA in their faeces. Lepidopterans were also the most abundant insect taxa in light trap collections at each site. It is assumed that the bats investigated during this study were foraging within the habitats in which they were captured, as

echolocation calls of all trapped bat species have previously been detected in the same study sites (Lamb 2009; Chapter 2), with three of the five species producing feeding buzzes.

The prey detected in the faeces of all bats reflected the locally abundant prey taxa. However, *Ae. vigilax* was only detected in the faeces of *V. pumilus* and *V. vulturnus*. These two congeneric species are morphologically very similar with echolocation designs that overlap to such a degree that it is not possible to differentiate between the calls of the two species in the study area (Reinhold *et al*. 2001). Both bats are small in size (4 and 4.5 g) and employ high frequency echolocation (51-55 kHz). Mosquitoes have been found to be common components of the diets of other smaller bats elsewhere (*Myotis sodalist* Miller and Allen and *Myotis lucifigus* Le Conte: Anthony and Kunz 1977; Kurta and Whitaker Jr. 1998). The absence of mosquitoes from the diets of larger bats in this study suggests that there may be a negative relationship between bat size and the importance of *Ae. vigilax* as a prey resource. However, similar to findings from studies of diets of many medium-large sized European bats (Rydell 1989; Jones 1995; Waters *et al*. 1995), two of the medium-large sized bats in my study (*N. gouldi* and *Mi. australis*) on occasion consumed prey that were smaller than *Ae. vigilax* (*Tomosvaryella* sp., 3-6.5 mm; *Drosophila* sp., 2-4 mm).

It has been suggested (Kunz 1988) and demonstrated (Boonman *et al*. 1998; Rainho *et al*. 2010) that prey abundance does not necessarily equate to prey availability. This may be due to restrictions imposed by variables associated with the habitats in which bats forage (e.g., clutter) as well as morphological constraints (e.g., echolocation design, jaw structure, wing design). Generally, smaller predators acquire small prey, while larger predators are capable of consuming both, small and large prey (Rosenzweig 1968). However, this generalisation may not be appropriate for echolocating aerial foraging bats restricted to prey of a certain size due to detectability constraints imposed by echolocation design (Barclay and Brigham 1991). It is thought that bats that employ high-frequency echolocation (with corresponding short wavelengths) are more suited to detecting small prey (Barclay and Brigham 1991) such as mosquitoes, than bats that utilise low-frequency echolocation. Given echolocation call frequencies are negatively associated with bat size (Jones 1999), any reduction in the ability of larger bats to detect small prey may result in the absence of small prey in the diets of these bats. In my study, the presence of small dipterans in the faeces of all high-frequency echolocating bats (and not the low-frequency

echolocating *C. gouldii*; 25-34 kHz) supports the suggestions of Barclay and Brigham (1991). Additionally, moths consumed by all bat species were larger than *Ae. vigilax*, and may have been more easily detected than *Ae. vigilax*. However, small prey such as mosquitoes may be more common in the diets of larger bats that forage in less-cluttered habitats since these prey are likely to be more detectable in these habitats than in more-cluttered forest.

The absence of mosquitoes from the faeces *N. gouldi* and *Mi. australis*, given mosquito DNA was still detectable when mosquitoes were present as 5 % of insect material in artificial bat faeces (approximately equivalent to the mass of 5 mosquitoes), suggests that it is unlikely that larger (> 6 g) bats actively seek mosquitoes as prey in forest. Additionally, *N. gouldi* employs a gleaning foraging strategy, with a diet predominantly consisting of moths in other areas (Churchill 2009). Gleaning bats tend to use prey-generated sounds as cues for detection of prey on substrates. Consequently it is unlikely that *Ae. vigilax* represents an important prey resource for these bats. It is acknowledged, however, that the level of sensitivity of the molecular technique for the detection of mosquito DNA in artificial faeces may be an over-estimate of the detectability of mosquito DNA in the faeces of wild caught bats, since no attempts were made to incorporate the effects of enzymatic degradation of prey known to occur in the guts of bat species (Barclay *et al*. 1991). Additionally, the complexity and diversity of prey in the faeces of wild bats is greater than what was used in the artificial faeces in this study.

How many mosquitoes would *Vespadelus* need to consume to satisfy energy requirements?

Using the equation of Speakman and Thomas (2003), the minimum energy required to maintain day-to-day activity (field metabolic rate) for the two bat species found to consume mosquitoes is 17.89 kJ d^{-1} (*V. vulturnus*) and 19.51 kJ d^{-1} (*V. pumilus*). If it is assumed that the two bat species were specialist foragers and consumed only mosquitoes, *V. vulturnus* and *V. pumilus* would be required to consume ~604 and ~659 mosquitoes, respectively, each night just to maintain day-to-day activity (assuming a mosquito weighs 0.002 g and provides 14.8 kJ $g^{-1}$of energy – Foster 1995). Conversely, if the two bats consumed only lepidopterans of similar size, *V. vulturnus* and *V. pumilus* would need to consume ~164 and ~179 moths, respectively (assuming a moth weighs 0.004 g and provides 27.2 kJ g^{-1} of energy - McLean and Speakman 1999). Assuming both prey items

are equally available, the disparity in foraging time required to meet FMR requirements for *V. vulturnus* and *V. pumilus* under these two scenarios is small (~2 hours) relative to other larger bats (~5 hours) (Table 4.1). Consequently, the profitability of mosquitoes as prey items would be especially low for larger bats with greater energetic demands.
Despite the low energetic profitability of mosquitoes relative to other prey, bats may still pursue them in the study area if: 1) they are extremely abundant, 2) handling time for mosquitoes is lower than for larger prey and 3) mosquitoes are more easily captured than larger prey.

In this study, the abundance of mosquitoes was comparable to or greater than (during neap tides) the abundance of all insect taxa combined. A similar phenomenon has previously been observed in the area, with recorded *Ae. vigilax* biomass comparable to the combined biomass of all other nocturnal insect fauna (see Chapter 5). Bats are known to switch habitats to select alternative, more abundant prey despite no apparent shortage of profitable prey (Arlettaz 1996). Similarly, in my study area, bats have been found to shift habitat use from larval to refuge habitats of *Ae. vigilax* in association with fluctuations in the abundance of the mosquito in the two habitats (see Chapter 5).

The rate at which bats forage is a factor likely to influence the profitability of different prey items and ultimately influence which prey bats pursue. Encarnação and Dietz (2006) reported an attack rate of 8.3 prey attacks min^{-1} for *Myotis daubentonii* (Kuhl) (a medium-sized bat; 7 g), while Rydell (1986) reported a maximum average attack rate of 14 min^{-1} for *Eptesicus nilsonii* (Keyserling and Blasius) (a large bat; 16 g), though this rate probably reflected an attack rate for small prey (bats continuously foraged) and not larger prey (>10 mm) that require greater handling time (bats ceased foraging for 41±29 s from time of prey capture; n=19), presumably for chewing and swallowing (Rydell 1986). In the presence of swarming insects, attack rates of up to 28 min^{-1} have been reported (Kalko 1995). The higher rate of attack and reduced handling time associated with small prey may encourage foraging on mosquitoes by bats, despite the lower energy intake that they provide relative to larger, more energy-rich prey. However, this is less likely to be the case for low-frequency echolocating bats in cluttered habitats where attack rates are likely to be lower due to background echoes preventing the detection of small prey (Aldridge and Rautenbach 1987, Schnitzler and Kalko 2001; Chapter 2) as highlighted by attack rates of

0.6 min^{-1} for *E. nilsonii* hawking lekking ghost swift moths (*Hepialus humuli*) (L.) that employed acoustic crypsis (flying <0.5 m from vegetation) (Rydell 1998).

Coupled with the rate of attacks on prey, the success rate of attacks (rate at which bats successfully capture prey) is known to vary for prey of different sizes, with greater success rates recorded for small mosquito-like prey (60-70%) than larger moths (30-40%) (Kalko 1995). This may be related to the flight speed of these insects, with smaller mosquito-sized prey flight-velocities ranging from 0.5-1 ms^{-1} and larger moths approaching velocities of 3-4 ms^{-1} (Kalko 1995). Tympanate moths are also effective at avoiding many bat species (Acharya and Fenton 1999, Fullard *et al*. 2003, Waters 2003). The improved success rate for capture of small prey may serve to mitigate the lower energetic gain provided by mosquitoes relative to other more energy-rich prey.

Notwithstanding the incentives for bats to pursue mosquitoes, mosquitoes were not detected in the faeces of all *V. pumilus* and *V. vulturnus* individuals. Bat diets can vary between sexes and between breeding and non-breeding individuals (Anthony and Kunz 1977). This variability may provide an explanation for the absence of mosquitoes in the diets of some bats in my study. Additionally, since mosquitoes tend to swarm at dusk, and are likely to be more detectable under these conditions (Waters *et al*. 1995; Shiel *et al*. 1998), time of trapping may provide an explanation for the absence of mosquitoes in the diets of some bats. However, no clear relationship is evident between mosquito consumption and the sex or reproductive condition of bats, or time of trapping (data not shown). Perhaps with larger sample sizes for the two species that consumed mosquitoes, relationships between mosquito consumption and the variables described above may have been apparent.

4.4.4 Implications for broadscale mosquito control

In Australia, while broadscale control of mosquito populations is generally only undertaken during periods of epidemic disease activity, early season treatment to suppress irruptions of mosquito populations later in the season is becoming increasingly applied (Webb *et al.* 2011). Although mosquito control activities are designed to reduce the abundance of mosquitoes, not complete eradication, reductions in larval mosquito populations can be as high as 98.2 % (Webb *et al*. 2011), substantially diminishing the

availability of mosquitoes to small foraging bats. While it is beyond the scope of this study to infer potential impacts of broadscale mosquito control and the associated reductions in larval mosquito populations on insectivorous bat diet and health, declines in bat populations have previously been attributed to deteriorating feeding conditions (Gerell and Gerell Lunderg 1993).

Although reductions in the abundance of a particular prey resource may be associated with reduced incidence of the prey item within the diets of bats, it is also possible that invertebrate predators of these prey (e.g., dragonfly nymphs and beetles), occupying higher trophic levels, will be less available to bats. The use of other physiological, behavioural or life-history indicators to assess the impacts of broadscale mosquito control on insectivorous bats may help to elucidate any impacts that dietary analysis on its own is unable to identify. Clutch size was used as an indicator in the assessment of the impact of broadscale mosquito control on breeding house martens (Poulin *et al*. 2010). In that study, lower numbers of eggs as well as reduced fledgling survival at sites treated with *Bti* were attributed to scarcity of food at those sites (Poulin *et al*. 2010). The implication of findings from my study for mosquito control strategies is that the timing of control activities should avoid the peak breeding season of local bats (November-February) known to utilise mosquitoes as a prey resource and that bat populations should be carefully monitored.

In the next chapter, based on the findings of dietary analyses, I examine whether habitat selection by *V. vulturnus* is associated with the distribution and abundance of *Ae. vigilax* in two focal habitats for the mosquito (saltmarsh and coastal swamp forest) within the study area. Relationships between the habitats selected by *V. vulturnus* and *Ae. vigilax* distribution and abundance are then used to infer importance of *Ae. vigilax* as a prey resource. Following from this, I discuss the implications of these findings for the use of broadscale mosquito control within the study area.

Chapter 5

Short-term shift in Habitat Selection by the Little Forest Bat (*Vespadelus vulturnus*) associated with fluctuating Prey Resources



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Abstract

The need to develop effective management strategies for insectivorous bat populations requires an understanding of the factors influencing their spatial and temporal habitat use. Availability of prey, such as mosquitoes, is one factor that may influence habitat use. The saltmarsh mosquito (*Aedes vigilax* Skuse) represents a prey resource that fluctuates in abundance, both spatially and temporally, and is an important dietary item for the little forest bat (*Vespadelus vulturnus* Thomas). Other insects, such as moths and beetles also represent a potential food resource. To assess whether habitat use by *V. vulturnus* is influenced by prey abundance, I radio-tracked *V. vulturnus* individuals to document habitat use during periods of relatively high and low *Ae. vigilax* population abundances, while also measuring the abundance of non-mosquito prey items, in saltmarsh and neighbouring coastal swamp forest. There was no significant difference in the non-mosquito insect biomass between sampling periods or habitats. When mosquito abundance in the low-clutter saltmarsh was similar to the more cluttered coastal swamp forest, use of saltmarsh by *V. vulturnus* was disproportionately greater than its availability, with saltmarsh preferentially selected over coastal swamp forest for foraging. However, when *Ae. vigilax* abundance was lower in the saltmarsh, use of saltmarsh by *V. vulturnus* was reduced and all habitats were used in proportion to availability in the study area. This is the first radio-tracking study to demonstrate a shift in habitat selection by insectivorous bats in association with fluctuations in the abundance and distribution of a particular prey resource. The shift in habitat use by *V. vulturnus*, corresponding with the spatio-temporal variation in the abundance of *Ae. vigilax,* further highlights the importance of the mosquito as a dietary item. Broadscale control of *Ae. vigilax* populations is likely to have implications for foraging *V. vulturnus*. An adaptive management approach is proposed, whereby careful monitoring of insectivorous bat populations is required before and after the application of broadscale mosquito control measures.

5.1 Introduction

Conservation of insectivorous bats requires appropriate management of the habitats in which bats forage as well as the prey resources these habitats sustain. To assist policy-makers, greater knowledge about factors influencing habitat use by foraging bats is required. While vegetation clutter is known to influence the mobility and foraging activity of many bat species (O'Neill and Taylor 1986; Bradshaw 1996; Brigham *et al*. 1997; Schnitzler and Kalko 1998; Law and Chidel 2002), the availability of prey is also a key factor (Rydell 1989; Fenton 1990; Rautenbach *et al*. 1996; Fukui *et al*. 2006; Adams *et al*. 2009). Many studies have investigated the influence of prey availability on the activity of bats, using prey abundance as a proxy for prey availability. However, it has been shown that vegetation clutter can also reduce access by bats to these prey resources (Adams *et al*. 2009; Rainho *et al*. 2010), suggesting that habitats with high insect abundances may not necessarily be suitable foraging habitats for bats if the acoustic complexity of these habitats restricts access to abundant prey resources within.

Mosquitoes can be locally abundant and have been identified as prey items for many bat species world-wide (Poole 1932; Griffin *et al*. 1960; Buchler 1976; Rydel 1990; Whitaker Jr. and Lawhead 1992; Long *et al*. 1998; Shiel *et al*. 1998; Pavey *et al*. 2001b; Sophia 2010). The saltmarsh mosquito, *Aedes vigilax* Skuse, is an estuarine species that is a serious nuisance biting pest and has been identified as an important vector of mosquito-borne pathogens such as Ross River virus (RRV) and Barmah Forest virus (BFV) (Russell 1998). Notwithstanding the potentially significant public health impacts, this species can cause substantial nuisance-biting impacts and broadscale mosquito control programs have been implemented in many coastal regions of Australia (Bell 1989; Russell and Kay 2008).

The population abundance of *Ae. vigilax* is heavily driven by tidal and rainfall inundation of larval habitats (i.e., coastal saltmarsh and mangrove communities), and as a consequence, can be highly variable, both spatially and temporally. However, general patterns such as peaks in abundances can be predicted (Kokkinn *et al*. 2009). Generally, more abundant populations tend to be present approximately two weeks after inundation of saltmarshes by spring tides and/or heavy rainfall, representing a more plentiful prey resource for insectivorous bats. Given that *Ae. vigilax* can disperse more than 5 km from larval habitats (Webb 2002), neighbouring coastal swamp forests are likely to provide

refuge for this mosquito species as well as providing sources of blood meals, sustaining population abundances for longer periods.

The little forest bat, *Vespadelus vulturnus* Thomas is a small insectivorous bat closely associated with forested and woodland habitats in both coastal and inland regions of south-eastern Australia (Churchill 2009). This species feeds opportunistically on flying insects with a diet composed of locally abundant prey items including moths and beetles, (Churchill 2009). With a high frequency modulated echolocation call (end frequency 50-53 kHz) capable of detecting small prey items, *V. vulturnus* has been observed hawking (hunting) mosquitoes in estuarine habitats (Hoye 2002). It has also been suggested that mosquitoes are likely to represent a major portion of the dipterans present in *V. vulturnus* diet, when they are locally abundant (Herr 1998). DNA belonging to *Ae. vigilax* has been detected in the faeces of 55 % of *V. vulturnus* individuals studied (n=20) in an area where *Ae. vigilax* can be highly abundant throughout summer (Chapter 4).

Although having an apparently opportunistic diet, I predicted that habitat use by *V. vulturnus* would closely correlate with the spatio-temporal variation in *Ae. vigilax* population abundances in acoustically simple saltmarsh habitat. To evaluate this prediction, I investigated habitat use by *V. vulturnus* over two periods predicted to sustain relatively large and small *Ae. vigilax* abundances, respectively. I assessed habitat use by radio-tracking in the larval saltmarsh habitats where *Ae. vigilax* emerge and the neighbouring, but acoustically more complex, coastal swamp forest that provides a sheltered habitat for host-seeking adult mosquitoes. To assess whether both tracking periods sustained differing mosquito abundances, mosquito populations were surveyed nightly during both tracking periods in saltmarsh and coastal swamp forest. In addition, other aerial insect fauna were also surveyed nightly to determine if insects other than mosquitoes were influencing habitat use by *V. vulturnus*.

5.2 Methods

5.2.1 Study site

The study area was located in the Empire Bay region (33°29'57"S, 151°21'40"E) of the Central Coast of New South Wales, Australia (Fig. 5.1). This region is approximately 50 km north of Sydney and experiences a warm sub-tropical climate.

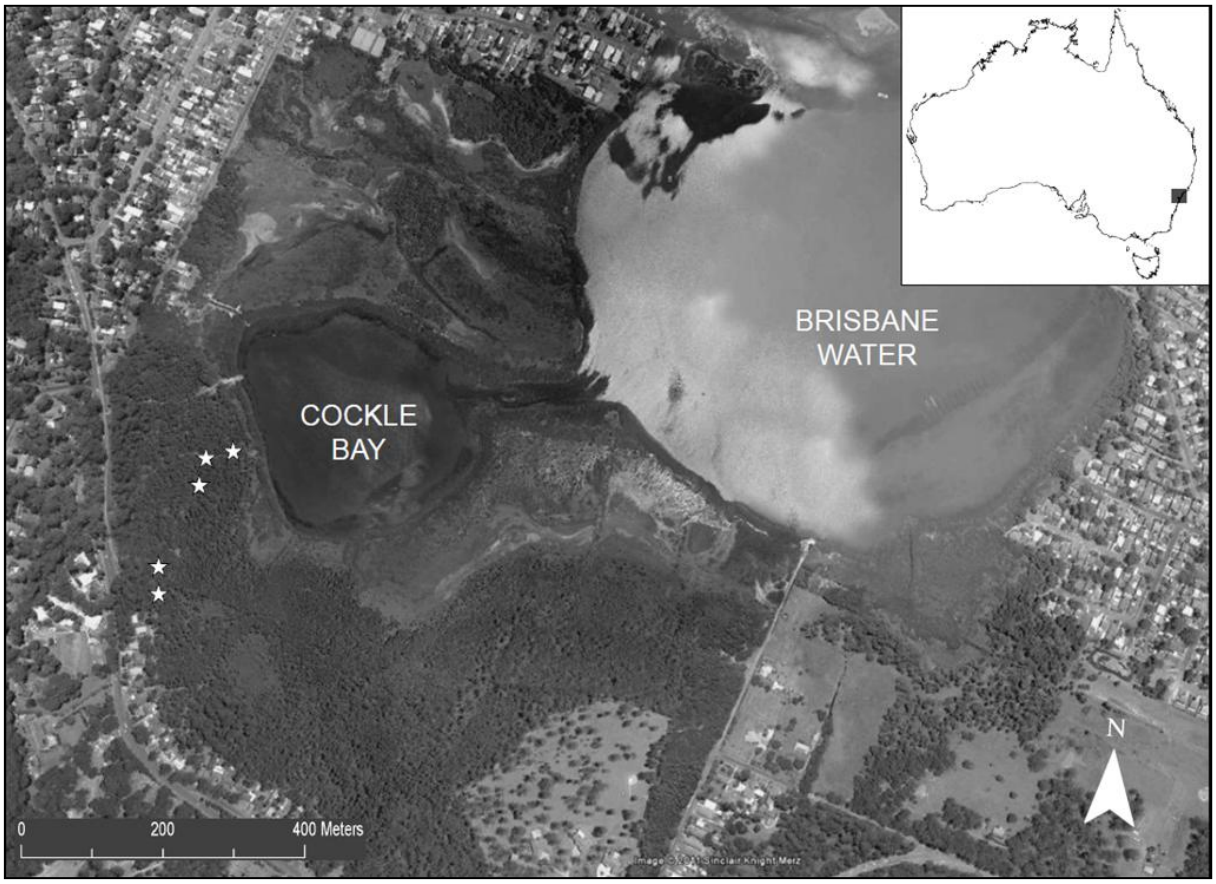


Figure 5.1: Satellite image of study area (adapted from Google Earth). Stars represent harp trapping locations within coastal swamp forest habitat. Saltmarsh areas are visible around Cockle Bay between the strip of mangroves and coastal swamp forest

Within the study area, a large national park and a number of smaller nature reserves sustain populations of hollow- and cave-roosting insectivorous bats, including six threatened species listed under the NSW *Threatened Species Conservation Act* 1995 (Payne 2006). The most commonly recorded species in ultrasonic bat detection surveys included the Gould's wattled bat, *Chalinolobus gouldii* Gray, eastern freetail bat, *Mormopterus* sp. 2 Peters, and the little forest bat, *V. vulturnus*. The latter was selected for this study as it is a small bat (~4 g) capable of discerning small prey items with its high frequency echolocation call (end frequency 50-53 kHz) and has been found to consume mosquitoes within the study area (Chapter 4).

Coastal saltmarshes and coastal swamp forests are two threatened vegetation communities (NSW *Threatened Species Conservation Act* 1995) that occur in the area and provide important larval and refuge habitat for many estuarine and freshwater mosquito species, including *Ae. vigilax*. Occurring at lower elevations than coastal swamp forest but higher than mangroves (Saintilan and Williams 1999), saltmarshes are periodically inundated by tides and are dominated by flowering plants, principally low-growing salt-tolerant succulent herbs (Adam 2009), including samphire (*Sarcocornia quinqueflora* (Bunge ex Ung.-Sternb.) A.J.Scott) and creeping brookweed (*Samolus repens* (J.R.Forst. and G.Forst.) Pers). Trees and shrubs are mostly absent in saltmarshes. Dominated by broad-leaved paperbark (*Melaleuca quinquenervia* (Cav.) Blake), fringing swamp mahogany (*Eucalyptus robusta* Anon) and swamp oak (*Casuarina glauca* Sieber ex Spreng.), coastal swamp forest has a typical canopy height of 13 m and occurs in poorly drained depressions that support a dense understorey of wetland or mesic shrubs (Bell 2009).

The small nature reserve (Cockle Bay) contains approximately 18 ha of coastal saltmarsh and 20 ha of coastal swamp forest. All arthropod sampling was conducted in these two vegetation communities within the nature reserve, while bat trapping was confined to flyways in coastal swamp forest. Approximately 300 m south of the nature reserve is an adjacent up-sloping forest that lies behind a local school and a number of small residential dwellings. Higher density residential areas are located 200 m to the east and 1 km west of the nature reserve.

5.2.2 Harp trapping, attachment of radio-transmitters and tracking methods

Habitat use by *V. vulturnus* was investigated by radio-tracking in two periods during the austral late summer (February 2010) and early autumn (March 2010). Bats were trapped in harp-traps (Tidemann and Woodside 1978) along flyways in coastal swamp forest habitat neighbouring saltmarsh. In February 2010, 10 *V. vulturnus* individuals were tracked while six were tracked three weeks later in March 2010, with three individuals tracked in both periods. The sex ratio of bats tracked during February 2010 was 3:7 (M:F), while in March 2010 there was an equal number of male and female bats. The reproductive condition of all female bats was post lactating while male bats were in a stage of sperm production. Although individuals were not marked to differentiate between them, physical characteristics such as forearm length, reproductive condition and sex were used to

determine the likely identities of three bats trapped in March 2010 that were tracked in the preceding month (as determined by the presence of a bare patch in the fur where a transmitter had been removed). In February 2010, up to nine bats were tracked simultaneously, while in March 2010 up to five bats were tracked at the same time.

Each trapped individual was fitted with a LB-2N radio-transmitter (Holohil, Carp, Canada), attached between the shoulder blades with Vetbond (3M, Pymble NSW). Each transmitter weighed 0.31 g and represented 7.95 % of *V. vulturnus* mean mass, with an aerial length of 10 cm. While the radio-transmitters exceeded the guideline of 5 % of body mass suggested by Aldridge and Brigham (1988), heavier transmitters have been used to study the same species as well as other similar sized bats and have not been reported to significantly restrict the mobility of individual bats (Campbell 2000, Law and Anderson 2000). Additionally, pregnant females can weigh 6.5 g and are therefore capable of carrying at least 42 % extra body mass.

Signals from bats with attached radio-transmitters were located with Australis 26k receivers (Titley Electronics, Ballina NSW) in conjunction with three element AY/C yagi antennae (Titley Electronics, Ballina NSW) each night. The locations of tagged bats were recorded on foot throughout the night, usually separated by at least 10 min for each individual bat. Estimated locations for each bat were obtained by either bisecting or triangulating the signal direction. Simultaneous bearings were taken using Suunto sighting compasses (Prospectors Earth Sciences, Baulkham Hills NSW) from stations for which GPS locations were recorded. To co-ordinate the taking of simultaneous bearings, tracking teams were in radio contact and one team instructed the other(s) when a bearing was to be taken. Bearings were recorded along with other observations regarding the strength and the consistency of the signal (an indicator as to whether or not the bat was in flight or stationary).

Since bats have been found to be more active in the hours immediately after dusk (Law *et al.* 1998), most survey effort was employed in the 4-5 hours after dusk each night during both tracking periods. However, all individuals were also tracked during the second half of the night (the hours preceding dawn) for at least two nights each tracking period. Location data were only obtained over a maximum of 10 consecutive nights each tracking period as it was predicted that mosquito population abundances after the 10th night, would not be

consistent with the preceding 10 nights due to the time lag from the inundation of the saltmarsh and the resulting egg hatches.

In addition to documenting the foraging range of *V. vulturnus*, and in order to provide data about distances travelled by bats to foraging sites, day roosts of tagged bats were located by homing in on signals of stationary bats. When it was not possible to locate a roosting structure (if on private land or in difficult terrain) a series of bearings was taken around the general roost location and later triangulated to provide coordinates for the general roost location.

5.2.3 Analysis of radio-tracking data

Simultaneous bearings recorded in the field were entered into LocateIII (Nams 2006) along with their corresponding GPS coordinates and were triangulated or bisected. The coordinates for the point at which a triangulation or bisection was successful were plotted in ArcMap 9.0 (ESRI, Redlands, California, USA) and overlayed onto a vegetation layer of the study sites (Bell 2009). Prior to the start of radio-tracking, transmitters were placed in known locations in saltmarsh and coastal swamp forest to determine a minimum range and error associated with transmitters in each habitat. The distance between triangulated and true locations of transmitters was 78±11.1 m (n=2) in saltmarsh and 94±10.9 m (n=3) in coastal swamp forest. The error ellipses of all triangulations (347±175 m^2; n=15) were smaller than the size of both saltmarsh and coastal swamp forest habitats and were included in all analyses. To determine whether locations calculated using bisects were consistent with those obtained through triangulation, and therefore appropriate to include in foraging analyses, the distance between each triangulated location and the bisected location that resulted from the removal of one bearing from the corresponding triangulation, was measured. This was repeated so that each bearing in a triangulation had been removed once. On nine occasions, the removal of a bearing resulted in an unsuccessful bisection. The average distance between bisected locations and the triangulated location was 73±37 m (n=36). Since this value was less than the calculated transmitter error in each habitat, all bisects were also included in all analyses.

To determine if sufficient location data were collected to calculate foraging ranges for each individual bat, bootstrap analysis was conducted using the animal movements' extension (Hooge and Eichenlaub 1997) in ArcView 3.2 (ESRI, Redlands, California, USA). A

minimum of 15 foraging locations was deemed a sufficient sample size to estimate foraging range since individuals required 17.7±1.5 foraging locations (range = 15-25) before asymptotes for home ranges were observed. Foraging ranges were not calculated for individuals with fewer than 15 foraging locations.

For each tracking period, foraging ranges were calculated for each individual bat using a 95-% fixed kernel density estimator (KDE) (a non-parametric density estimator used to calculate spatial use based on a series of location data) in ArcMap 9.0 using the HRT extension (Rodgers *et al*. 2005). For four individuals in February 2010 with location data that were not deemed to be independent (Schoener's index <1.6 or >2.4 and/or Swihart and Slade index >0.6), location data (3±0.7 locations) were randomly deleted until the indices were no longer significant, as suggested by Ackerman *et al*. (1990). The Least Squares Cross Validation (LSCV) smoothing parameter (h) was used to determine the spread of the kernel centred over each observation.

Compositional analysis (Aebischer *et al*. 1993) was used for both periods of tracking to evaluate if bats were using habitats according to their availability or whether they preferentially selected certain habitats, and whether this changed between tracking periods. While the analysis can be undertaken at two scales (population and the individual), the roost locations of two individuals tracked in February 2010 were located >1.8 km from other tracked individuals, suggesting that foraging data were likely to be representative of more than one population/social group. Use of foraging data collected from individuals of multiple social groups has the potential to confound foraging preferences at the population scale, since habitats available to members of one social group may not necessarily be available to members of another social group. For this reason, compositional analysis was only undertaken at the individual scale. For the analysis, the proportion of bisected/triangulated locations within each habitat was treated as a measure of habitat use, while available habitats were considered to be those that made up an individual's foraging range (95 % KDE) (Fig. 5.2).

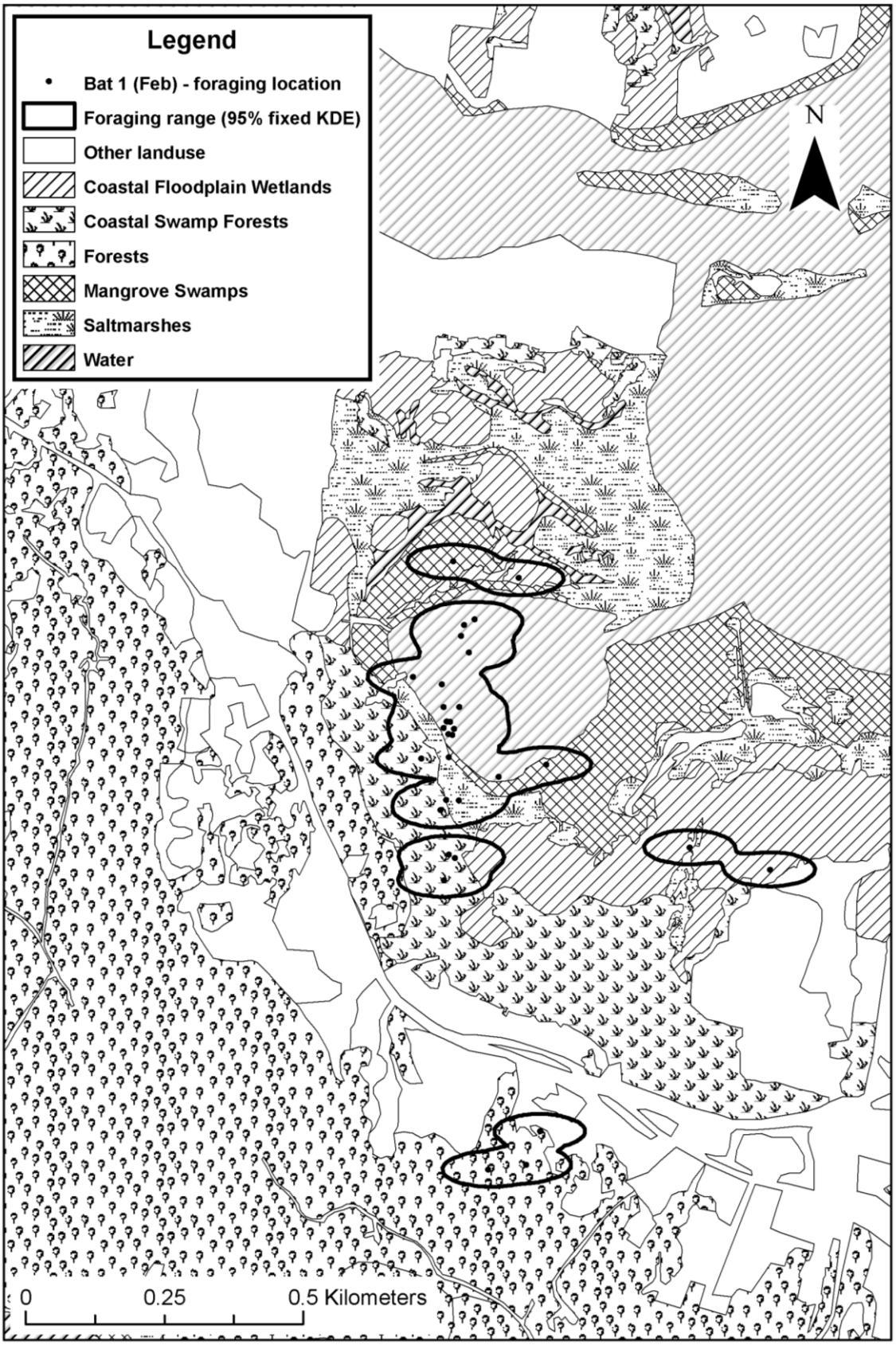


Figure 5.2: Typical GIS output illustrating the spread of habitats in the study area and the foraging locations ('used habitat') used to construct foraging ranges ('available habitat').

The "join" application in ArcMap 9.0 combined the vegetation layer with the foraging range and the bisected/triangulated locations of each individual bat. The areas represented by used and available habitats were calculated using queries that tallied the area of each habitat within a foraging range and the number of locations in each habitat. Habitats assessed in the compositional analyses were coastal floodplain wetlands, coastal swamp forests, mangroves, saltmarshes, water and a forest complex (consisting of wet sclerophyll forest, dry sclerophyll forest, and subtropical rainforest). Given the calculated transmitter error and average error ellipse, it was deemed appropriate to pool forest habitats together.

A chi-square goodness-of-fit test was used to assess whether habitat selection was non-random and whether each habitat was used in a similar proportion to its availability. The differences between log-transformed relative proportions of both used and available habitats were used to rank habitats according to whether they were being used more than other habitats after accounting for each habitat's availability. A Wilcoxon-pairwise comparison was used to ascertain the significance of these ranks.

5.2.4 Surveillance of available prey

In each habitat, mosquito abundance was surveyed nightly using two CO_2–baited encephalitis virus surveillance (EVS) traps (Rohe and Fall 1979) (Australian Entomological Supplies, Bangalow, NSW, Australia), while other aerial insect fauna was sampled using one light trap (Australian Entomological Supplies, Bangalow, NSW, Australia). All traps were set in forest gaps within the coastal swamp forest, while in coastal saltmarsh, traps were set along the interface of the saltmarsh habitat and a stand of encroaching mangroves. Mosquito collections were identified to species according to keys (Russell 1996) and the abundance of each species was recorded. All light trap specimens <2 mm in size were pooled together while all other specimens were sorted into three insect orders (Lepidoptera, Coleoptera, Diptera), with any other specimens pooled into an 'other' category. Insects were then oven dried at 60° C for a minimum of 48 h and until a constant weight could be recorded. All weights were recorded to the nearest 1×10^{-5} g and used as a measure of biomass. A log-linear analysis was used to compare *Ae. vigilax* abundance and nightly insect biomass between habitats (saltmarsh/coastal swamp forest) and tracking period (February/March 2010).

5.3 Results

5.3.1 Prey abundance

In all, 13 243 mosquitoes representing 13 species were collected over both tracking periods (Table 5.1). *Aedes vigilax* was the most abundant species in each habitat irrespective of tracking period, representing ≈74 % of specimens trapped in both habitats during February 2010 and ≈56 % and ≈85 % of specimens trapped in saltmarsh and coastal swamp forest habitats, respectively during March 2010. The other commonly collected species were *Aedes alternans* Westwood and *Culex sitiens* Wiedemann, two species closely associated with estuarine habitats, and *Culex annulirostris* Skuse, a species closely associated with freshwater habitats.

During February 2010, 4 387 mosquitoes representing all 13 species were trapped in the coastal swamp forest, while 5 481 mosquitoes representing 12 species were collected in the saltmarsh. In March 2010, 2 391 and 984 mosquitoes representing 10 species were sampled in coastal swamp forest and saltmarsh habitats, respectively. Log-linear analysis found that nightly *Ae. vigilax* abundance was significantly lower in saltmarsh habitat during March 2010 (L.R. $\chi^2(1)=892.440$, $P<0.001$; Fig. 5.3).

Table 5.1: Mosquito species and their total abundances in saltmarsh and coastal swamp forest during February 2010 and March 2010. Values in brackets represent percent of total mosquito abundance in each habitat.

Species	FEBRUARY 2010 (n=10)		MARCH 2010 (n=8)	
	Saltmarsh	**Coastal swamp forest**	**Saltmarsh**	**Coastal swamp forest**
Aedes alternans	564 (10.29)	56 (1.28)	243 (24.70)	23 (0.96)
Ae .imperfectus	16 (0.29)	7 (0.16)		
Ae. multiplex	49 (0.89)	219 (4.99)	7 (0.71)	92 (3.83)
Ae. notoscriptus	68 (1.24)	49 (1.12)	18 (1.83)	50 (2.08)
Ae. procax	46 (0.84)	499 (11.37)	3 (0.30)	24 (1.00)
Ae. vigilax	4051 (73.91)	3243 (73.92)	555 (56.40)	2035 (84.83)
Anopheles annulipes	6 (0.11)	20 (0.46)	2 (0.20)	12 (0.50)
Coquillettidia linealis		2 (0.05)		
Culex. annulirostris	291 (5.31)	155 (3.53)	48 (4.88)	67 (2.79)
Cx. molestus	1 (0.02)	3 (0.07)	2 (0.20)	42 (1.75)
Cx. qinquefasciatus	15 (0.27)	8 (0.18)	8 (0.81)	41 (1.71)
Cx. sitiens	350 (6.39)	116 (2.64)	98 (9.96)	13 (0.54)
Verrallina funerea	24 (0.44)	10 (0.23)		
Total	5481	4387	984	2391

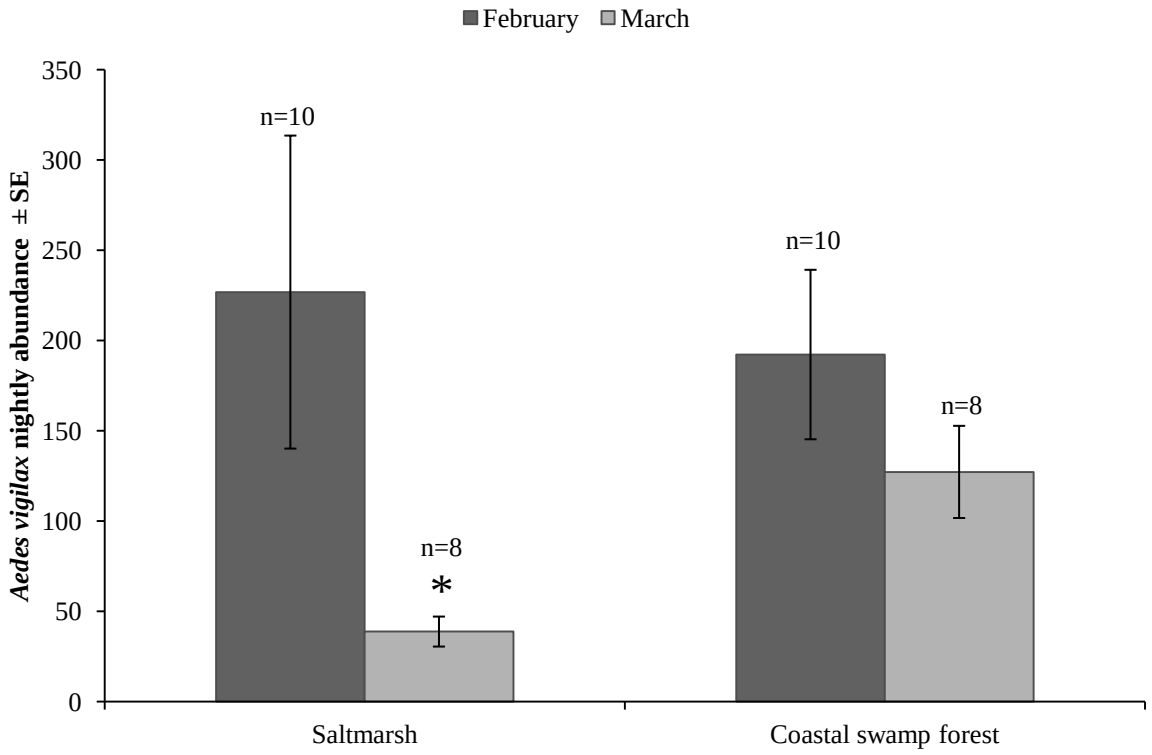


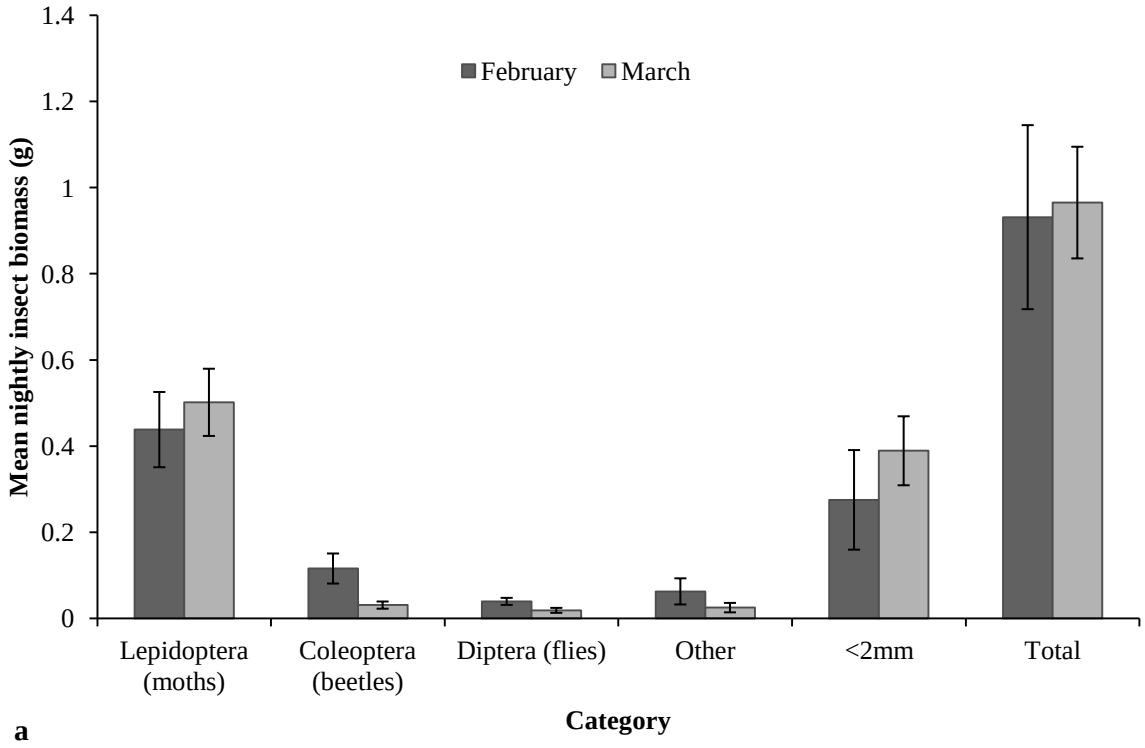
Figure 5.3: Mean nightly abundance of *Aedes vigilax* in saltmarsh and coastal swamp forest during February 2010 and March 2010. Error bars represent ±1 standard error from the mean across trapping nights. * Indicates interaction effect.

A total of 37.27 g of insect biomass was collected in light traps over both tracking periods (Table 5.2). During February 2010, 10.24 g and 11.15 g of insect biomass was collected in light traps in saltmarsh and coastal swamp forest habitats, respectively. In March 2010, 7.72 g and 8.14 g of insect biomass was sampled in saltmarsh and coastal swamp forest habitats, respectively. During both tracking periods, lepidopterans and insects <2 mm in size were the two classes that contributed the greatest amount of biomass to saltmarsh light trap collections, while coleopterans and lepidopterans provided the greatest amount of biomass to coastal swamp forest light trap collections.

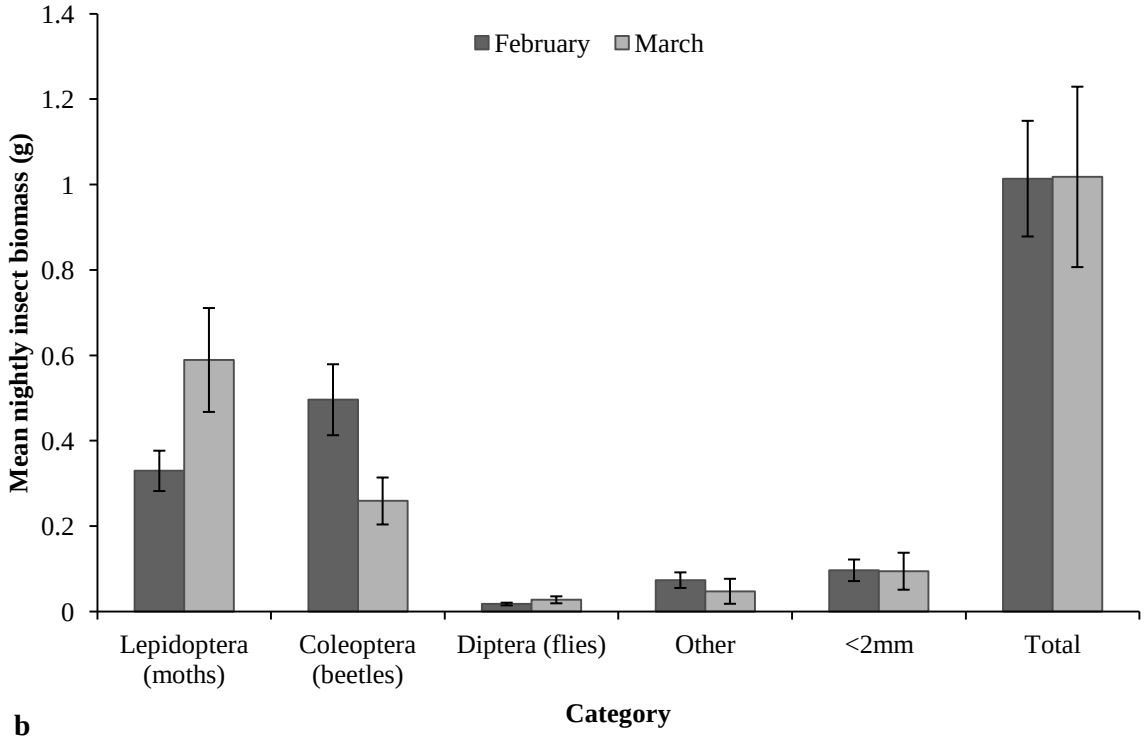
Table 5.2. Total insect biomass (g) collected in light traps in saltmarsh and coastal swamp forest habitats during February 2010 and March 2010. Values in brackets represent percent of total insect biomass in each habitat.

Taxa/class	FEBRUARY 2010		MARCH 2010	
	Saltmarsh	**Coastal swamp forest**	**Saltmarsh**	**Coastal swamp forest**
Lepidoptera (moths)	4.82 (47.05)	3.63 (32.51)	4.01 (51.96)	4.71 (57.88)
Coleoptera (beetles)	1.27 (12.43)	5.46 (48.94)	0.25 (3.20)	2.07 (25.44)
Diptera (flies)	0.43 (4.24)	0.19 (1.75)	0.15 (1.94)	0.22 (2.71)
Other	0.69 (6.73)	0.81 (7.26)	0.20 (2.59)	0.38 (4.67)
<2 mm	3.03 (29.54)	1.06 (9.55)	3.11 (40.31)	0.76 (9.29)
Total	10.24	11.15	7.72	8.14

Mean nightly insect biomass did not differ significantly between habitats or tracking periods (L.R. $\chi^2(3)=0.874$, P=0.832), ranging from 0.93 g and 1.01 g in saltmarsh and coastal swamp forest habitats, respectively in February 2010, and 0.97 g and 1.02 g in March 2010 (Figs. 5.4a & 5.4b). The biomass of lepidopterans, dipterans, other insects and insects <2 mm in size did not differ significantly between tracking periods or habitats (L.R. $\chi^2(1)=0.019$, P=0.991; L.R. $\chi^2(1)=0.097$, P=0.953; L.R. $\chi^2(1)=0.463$, P=0.793; L.R. $\chi^2=2.479$, P=0.290; Figs. 5.4a & 5.4b). Coleopteran biomass, however, was significantly greater in coastal swamp forest habitat (L.R. $\chi^2(1)=6.597$, P=0.037; Figs. 5.4a & 5.4b).



a



b

Figure 5.4: Mean nightly insect biomass and insect biomass separated by taxa or size during both tracking sessions in: a) saltmarsh and b) coastal swamp forest.

5.3.2 Radio-tracking of, and habitat selection by, *Vespadelus vulturnus*

In all, 422 triangulation attempts were undertaken for all 10 bats during February 2010. Of these, 188 (45 %) were successful (at least two triangulated bearings intersected one another). Bats were tracked for a mean of 6.5±2.95 (± 1 SE) nights per bat, with 42±7 triangulation attempts, of which 45±7 % were successful. Bat #2 and Bat #10 were only tracked for one and two nights, respectively. An active signal from Bat #2 was only detected up to one hour after the release of this bat suggesting that the radio-transmitter had probably been removed by the bat, while an active signal for Bat # 10 was still present on the last night of the tracking session, but only two nights of foraging data were collected since this bat was trapped on the penultimate night of the tracking session. Foraging ranges were not calculated for these two individuals.

During March 2010, 327 triangulation attempts were made with 149 of these successful (46 %). Bats were tracked for a mean of 5.67±2.25 nights per bat, with 55±11 triangulation attempts, of which 46±5 % were successful. Bat #6 was trapped on the penultimate night of the tracking session and was only tracked for two nights. A foraging range was not calculated for this individual.

With the exception of one night in March 2010 in which an individual was roosting within the coastal swamp forest, all radio-tagged bats roosted outside the coastal swamp forest. Most individuals roosted in eucalypt vegetation on an escarpment 300-400 m away adjacent to the coastal swamp forest. Additionally, triangulated bearings for these individuals found that roosts were located <200 m from each other. Three individuals (two in February 2010 and one in March 2010) roosted beneath the metal cap of telegraph poles in urban areas. The two individuals in February 2010 (male and female) only roosted in telegraph poles, roosting in three poles separated by a maximum distance of 670 m. These roost locations were 1.82±0.16 km from the trap site, while the roost location for all other bats was no greater than 600 m from the trap site.

Foraging ranges (95 % KDE) of *V. vulturnus* individuals were larger in February 2010 (35±4 ha) than in March (14±7 ha). Habitat use of *V. vulturnus* individuals was non-random in both months (February 2010 - $\chi^2(5)=28.802$, $P<0.001$; March 2010 - $\chi^2(5)=56.480$, $P<0.001$). In February 2010, the use of saltmarshes was significantly greater

than the availability of the habitat ($\chi^2(1)=3.846$, P=0.05), while all other habitats were used in similar proportions to their availability (Fig. 5.5). Compositional analysis revealed that saltmarshes ranked highest of all habitats followed by coastal swamp forest, coastal floodplain wetlands, water, forests and mangrove forests (Table 5.3). The use of saltmarshes was significantly greater than the use of coastal floodplain wetlands (Z=-2.380, P=0.017), mangrove forests (Z=-1.960, P=0.050) and forests (Z=-2.380, P=0.017).

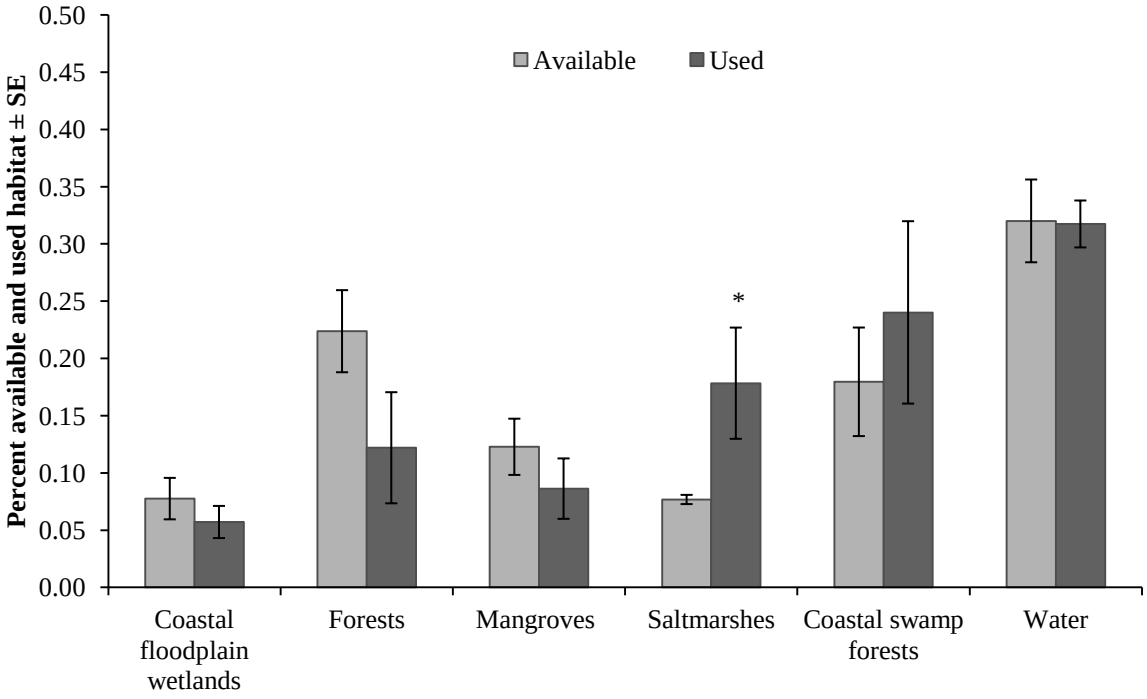


Figure 5.5: Percentage of available and used habitat in February 2010.

In March 2010, all habitats were used in similar proportions to their availability (Fig. 5.6). Compositional analysis revealed that water ranked highest of all habitats used by *V. vulturnus* individuals, followed by coastal swamp forests, mangrove forests, saltmarshes, forests and coastal floodplain wetlands (Table 5.3). The use of water was significantly higher than the use of forests (Z=-2.023, P=0.043).

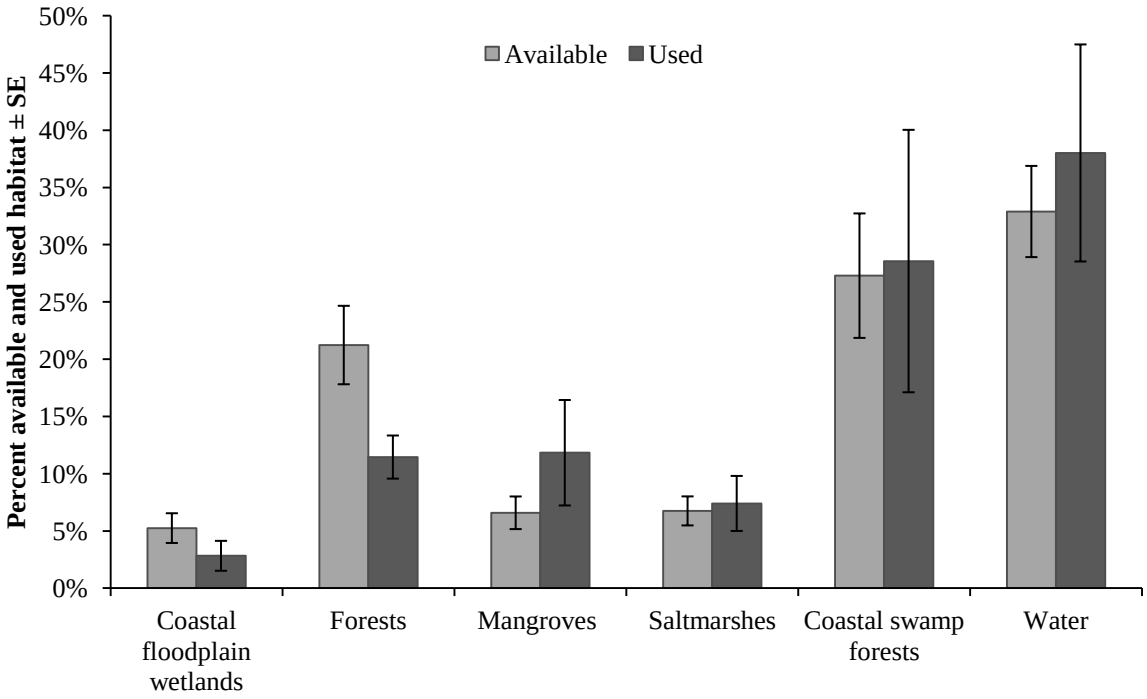


Figure 5.6: Percentage of available and used habitat in March 2010.

Table 5.3: Ranking matrices for *V. vulturnus* in February and March 2010, based on comparisons of the proportions of locations for each bat in each habitat type with the proportion of each habitat type available within the bat's foraging range (95 % KDE).

Habitat	Coastal floodplain wetlands	Coastal swamp forests	Forests	Mangrove forests	Saltmarshes	Water	Rank
February 2010							
Coastal floodplain wetlands	0	-1.06	0.21	0.39	-1.38	-0.17	**3**
Coastal swamp forests	1.06	0	1.27	1.45	-0.32	0.90	**2**
Forests	-0.21	-1.27	0	0.18	-1.59	-0.37	**5**
Mangrove forests	-0.39	-1.45	-0.18	0	-1.77	-0.56	**6**
Saltmarshes	1.38	0.32	1.59	1.77	0	1.21	**1**
Water	0.17	-0.90	0.37	0.56	-1.21	0	**4**
March 2010							
Coastal floodplain wetlands	0	-1.43	-0.96	-1.37	-0.96	-1.59	**6**
Coastal swamp forests	1.43	0	0.47	0.06	0.46	-0.16	**2**
Forests	0.96	-0.47	0	-0.41	0.00	-0.63	**5**
Mangrove forests	1.37	-0.06	0.41	0	0.40	-0.22	**3**
Saltmarshes	0.96	-0.46	0.00	-0.40	0	-0.63	**4**
Water	1.59	0.16	0.63	0.22	0.63	0	**1**

5.4 Discussion

This is the first study to identify a short-term shift in habitat use by insectivorous bats in response to fluctuations in the abundance and distribution of a particular prey resource. The change in habitat use by *V. vulturnus* corresponded to a shift in the abundance and distribution of *Ae. vigilax*, an abundant prey resource for small insectivorous bats. This has implications for the use of broadscale mosquito control to manage the abundance of *Ae. vigilax*, a recognised vector of mosquito-borne pathogens and a nuisance biting pest (Russell 1998; Poulin *et al*. 2010).

5.4.1 Prey biomass

Aedes vigilax was the most abundant mosquito species in saltmarsh and coastal swamp forest habitats during both tracking periods (February 2010 and March 2010). This trend has been observed during long-term mosquito surveillance in the study area with *Ae. vigilax* representing 41.2 % of all mosquitoes trapped over nine consecutive trapping seasons (unpublished data – L. Gonsalves and C. Webb). While population abundances of this mosquito species can be variable, the consistent presence of this species in the study area provides *V. vulturnus* and other similarly small-sized bats, with a reliable prey resource during summer.

As expected, the abundance of *Ae. vigilax* during March 2010 was significantly lower in saltmarsh habitats than during February 2010. This result was in line with expectations as the lack of extensive tidal flooding of the saltmarsh habitats in the weeks preceding the March 2010 tracking did not provide suitable conditions for immature mosquito populations (de Little *et al*. 2009). However, there was no significant difference in the mean abundance of *Ae. vigilax* in the coastal swamp forest between the two tracking periods. Mark-release-recapture experiments have shown that *Ae. vigilax* can disperse more than 5 km from larval habitats (Webb 2002) and the coastal swamp forest is likely to provide a humid refuge and sources of blood meals for this species, sustaining population abundances for longer periods than exposed saltmarsh environments.

Since the trapping techniques used to survey mosquito and other insect fauna were not the same, any comparison of mosquito abundance with the abundance of other insect fauna

must be interpreted with caution. The mosquito traps collect a subset of the extant mosquito population as they specifically target host-seeking female mosquitoes as those mosquitoes are most attracted to the carbon dioxide-baited traps. A comparison of *Ae. vigilax* biomass with the biomass of other insect fauna revealed that in February 2010, *Ae. vigilax* biomass in saltmarsh (8.02 g) and coastal swamp forest (6.42 g) was similar to the biomass of all other insect taxa combined (assuming one adult mosquito weighs 0.00198 g). In March 2010, *Ae. vigilax* biomass in saltmarsh (1.10 g) and coastal swamp forest (4.02 g) was within the range of biomass contributed by other aerial nocturnal insect fauna. This emphasises the potentially important contribution of the biomass of *Ae. vigilax* to bats.

While the average nightly insect biomass did not differ between habitats or tracking periods, the biomass of particular taxa did. Coleopteran biomass was significantly greater in the coastal swamp forest than in the saltmarsh during both tracking periods. While few studies investigating the distribution of beetles in saltmarshes (Wyatt and Foster 1988; Irmler *et al*. 2002; Finch *et al*. 2007) have identified elevation and associated saltmarsh vegetation gradients as variables closely associated with beetle distribution, no studies have specifically investigated beetle distribution along a saltmarsh-adjacent forest gradient. Other studies investigating beetle distribution and abundance in forested areas and more open habitats have reported higher abundances of coleopterans in forests and forest fragments than in adjacent clear cuts (Klein 1989), forest clearings (Howden and Nealis 1975; Driscoll and Weir 2005), and open pastures (Major *et al*. 2003; Andresen 2008). With relatively few studies on the differing abundance of beetles between different structural vegetation classes, it is difficult to compare my results directly to previous investigations given the unknown influence of species-specific ecological or behavioural factors.

5.4.2 Habitat selection by *Vespadelus vulturnus*

Given the error associated with radio-tracking, it is often difficult to elucidate habitat use at fine spatial scales and this may result in the use of particular habitats being underestimated. Many of the foraging locations classified as water in this study were located close to mangroves that fringe saltmarsh on the seaward side. While some individuals were recorded to commute (>1.8 km) across open water from roosts to

foraging areas, it is quite possible that some of these locations were in fact in saltmarsh edge zones (saltmarsh-mangrove interface), where ultrasonic detectors have found bats to be more active than in the interior of the saltmarsh (Chapter 3). Additionally, light-tagged *V. vulturnus* individuals released in the saltmarsh interior (n=4) were observed commuting to edge vegetation before leaving the saltmarsh (unpublished data – L. Gonsalves), further supporting the view that these vegetation interfaces provide an edge for bats to forage along. Despite the potential for underestimation of small portions of habitat, compositional analysis revealed that after accounting for the availability of habitats within the foraging range, saltmarsh was the most preferred habitat for foraging *V. vulturnus* individuals in February 2010. While *V. vulturnus* has been recorded echolocating and feeding in saltmarsh previously (Chapters 2 and 3), this is the first study to identify the preferential use of saltmarsh for foraging by insectivorous bats. *Chalinolobus gouldii* was the species most commonly recorded by ultrasonic detectors in saltmarsh (Chapter 2), yet radio-tracking of this medium-sized bat (14 g) with a low echolocation frequency (29 kHz), revealed that saltmarsh was used in proportion to its availability (Lamb 2009). In March 2010, *V. vulturnus* preferred to forage over water and in coastal swamp forest, with reduced use of saltmarsh for foraging. However, the continued use of both threatened vegetation communities reaffirms that they are important foraging patches for *V. vulturnus*.

5.4.3 Relationships between prey biomass and habitat selection

If prey abundance is influencing habitat use by foraging *V. vulturnus* individuals, one would expect that a change in prey abundance in one habitat from February 2010 to March 2010 would also be reflected in a change in habitat use over this time. During this study, while *Ae. vigilax* populations were highly abundant in both saltmarsh and coastal swamp forest habitats in February 2010, *V. vulturnus* preferentially foraged in saltmarsh. However, when *Ae. vigilax* populations were more abundant in coastal swamp forest, habitat use by foraging *V. vulturnus* individuals shifted with preferential use of this habitat. This trend was not observed for any other prey taxa measured in this study.

While prey may be abundant in a given habitat, the ability of bats to access these resources can be inhibited by other physical characteristics of the habitat such as clutter, indicating that prey abundance does not necessarily equate to availability (Adams *et al*. 2009; Rainho *et al*. 2010). The preferential use of saltmarsh for foraging in February 2010 may reflect

this principle – it is energetically less demanding and perhaps more efficient to locate prey in an open habitat such as saltmarsh than in a cluttered forest environment (Fenton 1990).

The shift to foraging in coastal swamp forest in March 2010 when *Ae. vigilax* was more abundant in this habitat than the neighbouring saltmarsh may indicate that *V. vulturnus* preferentially seeks *Ae. vigilax* as a dietary resource. Alternatively, the use of coastal swamp forest by *V. vulturnus* suggests that this species chooses to forage in a habitat that, whilst being energetically more demanding due to clutter, sustains prey items that are energetically more profitable, mitigating the cost of foraging in this clutter. Lepidopterans and coleopterans contributed the greatest amount of biomass in coastal swamp forests in March 2010. Lepidopterans and coleopterans provide about 25.5 kJg^{-1} (De Foliart 1992) and 21.3 kJg^{-1} (Chena *et al*. 2004) of energy, respectively. Mosquitoes, however, provide lower levels of energy to predators, representing 6.3-14.8 kJg^{-1} (Foster 1995). However, the 'hardness' of prey items will also influence the net energy gained from 'energy rich' prey items that may require more extensive processing times (mastication and digestion) and thus increased energy expenditure (De Gueldre and De Vree 1984, 1988; Aguirre *et al*. 2003). Handling time (time taken to capture prey) associated with each prey item, presumably, will also influence which habitats bats choose to forage in (Bell 1982).

It is possible that other factors may be influencing which habitats *V. vulturnus* selects for foraging. An artefact of the design of this study, based around tidal activity, is the potential influence of lunar illumination on the habitat use by *V. vulturnus*. During the March 2010 tracking period (commencing with a waxing crescent moon phase and concluding on a full moon), the level of lunar illumination was greater than the February 2010 tracking period (commencing on the night of a new moon and concluding on the night following the first quarter moon phase). It is possible that *V. vulturnus* foraged in the more sheltered coastal swamp forest during March 2010 to mitigate the risk of predation associated with foraging in open habitats (Speakman 1991; Baxter *et al*. 2006). However, I did not observe any nocturnal predators (i.e., owls) while radio-tracking (L. Gonsalves pers. obs.) and cloud cover may act to mitigate the influence of lunar illumination on habitat use by bats. At least two studies have found no influence of moonlight on the activity of insectivorous bats (Negraeff and Brigham 1995; Karlsson *et al*. 2002).

With the exception of one roost, bats were roosting outside the confines of the coastal swamp forest, sometimes >1.8 km away and separated by a water body. Despite this, all tracked individuals were trapped in the coastal swamp forest and foraged there or in the neighbouring saltmarsh each night of the study, further highlighting that these two threatened vegetation communities are important foraging patches for *V. vulturnus* in the study area. Individuals travelled distances greater than previously reported for this species (1370 m from trapping location to roost: Campbell 2000), with some individuals travelling >1.8 km from roosts to foraging habitats. Foraging ranges observed during this study were also greater than predicted in a bat banding study by Lunney *et al*. (1988) and foraging ranges in forest estimated for the similar-sized *V. pumilus* (Law and Anderson 2000).

5.4.4 Implications for broadscale mosquito control

Appropriate management of *Ae. vigilax* populations requires consideration of the potential impacts of broadscale mosquito control on the diets of insectivorous bats. A threatening process for bats worldwide is the loss or reduction of prey items due to pesticide use (Hutson *et al*. 2001). However, the impact of pesticides on local insect populations is dependent on the type and delivery method of those insecticides. The most commonly used mosquito control agents in Australia (e.g., *s*-methoprene and *Bacillus thuringiensis israelensis*) are generally mosquito-specific and target the aquatic immature stages (Russell and Kay 2008). While broadscale insecticide use against mosquito populations in Australia is generally only undertaken during periods of epidemic disease activity, the use of early season treatment to assist in the suppression of irruptions of mosquito populations later in the season is gaining acceptance within authorities undertaking control programs (Webb *et al*. 2011). Given such control programs can substantially diminish larval mosquito populations, in some cases reducing larval populations by up to 98.2 % (Webb *et al*. 2011), the use of broadscale mosquito control will diminish the important prey resource that *Ae. vigilax* represents to *V. vulturnus* (Chapter 4), and may potentially lead to prey switching. The use of such mosquito control measures has been linked to bird declines (Poulin *et al*. 2010), while declines in bat populations have previously been attributed to deteriorating feeding conditions (Gerell and Gerell Lunderg 1993).

In conclusion, my study demonstrates a short-term shift in habitat use by *V. vulturnus* in association with fluctuations in *Ae. vigilax* population abundances and distributions,

indicating that this prey item is an important dietary resource for this bat species. Consequently, appropriate management of *Ae. vigilax* populations requires consideration of the potential impacts of broadscale mosquito control on the diets of insectivorous bats. To more adequately assess the impact of mosquito control on insectivorous bats, an adaptive management process should be followed whereby careful monitoring of bats before and after the application of broadscale mosquito is required. However, in the interim, control programs should avoid the lactation period of bats, when energetic demands are greatest.

Chapter 6

General Discussion

This study adopted a multidisciplinary approach to investigate the importance of the saltmarsh mosquito, *Aedes vigilax* Skuse as a potential prey resource for foraging insectivorous bats on the Central Coast of NSW. Using a range of ecological techniques in conjunction with novel molecular approaches, my findings have extended current knowledge of the use of coastal saltmarsh by foraging insectivorous bats and confirmed that *Ae. vigilax* is a prey resource for at least two local bat species. This general discussion will provide a synthesis of the major findings of this study, identify the significance of these findings and provide scope for future research.

6.1 Synthesis of findings

In order to identify those bat taxa most likely to utilise *Ae. vigilax* as a prey resource, I initially investigated and compared the diversity and activity (including foraging) of bats between spring and neap tidal cycles and across three major habitats (saltmarsh, urban, and forest) that represent potential larval and refuge habitats of mosquitoes within the study area (Chapter 2). While no differences in total bat activity and proportional feeding activity were observed between tidal cycles, differences were found between habitat types, supporting the hypothesis of Fenton (1990) that low clutter habitats may represent more efficient foraging habitats than more cluttered habitats. This research also demonstrates that relationships between bat activity and the abundance of prey (mosquito and non-mosquito) are constrained by clutter, whereby areas with high clutter levels may be linked to a reduction in the detectability and hence availability of particular prey sizes.

To guide management of saltmarsh and encroaching adjoining habitats, this study also investigated whether interfaces between coastal saltmarsh and adjacent habitats were areas of greater bat activity when compared with the saltmarsh interior (Chapter 3). This investigation revealed that bats were more active along saltmarsh edges than the interior of the saltmarsh and highlights the importance of vegetation interfaces to foraging bats. The removal of vegetation adjoining saltmarsh (e.g., mangroves or coastal swamp forest) has the potential to reduce the foraging habitat available to bats. It is recommended that retention of vegetation (strips of mangroves and casuarinas) would be beneficial to bats, providing an edge for foraging.

Those bat species identified foraging in saltmarsh (Chapters 2 and 3) and positively correlated with the abundance of *Ae. vigilax* (Chapter 2) were then the focus of follow-up dietary investigations that utilised novel molecular techniques to examine bat diet. The diets of five bat species that ranged in size from 4–14 g were investigated to examine the extent to which *Ae. vigilax* comprised their diets and to identify whether consumption of *Ae. vigilax* was restricted to bats of a particular size, given minimum energy intake (field metabolic rate) positively scales with mass (Chapter 4). Those taxa that employ high-frequency echolocation were targeted since these bats are considered to be more suited for detection of small prey (Barclay and Brigham 1991). Additionally, a low frequency echolocating bat (*Chalinolobus gouldii* Gray) recorded feeding in all habitats in the study area was also included in dietary analyses. A diverse range of prey, dominated by Lepidoptera, was detected in the faeces of all bats. However, *Ae. vigilax* was only detected in the faeces of two smaller-sized bats of the *Vespadelus* genus (*Vespadelus pumilus* Gray and *Vespadelus vulturnus* Thomas), indicating that mosquitoes may be particularly important for small-sized bats.

I next investigated whether habitat use by one of these bats (*V. vulturnus*) was associated with the distribution and abundance of the mosquito over a spring and neap tidal cycle in the larval (saltmarsh) and adjoining refuge (coastal swamp forest) habitats (Chapter 5). *Vespadelus vulturnus* preferentially selected saltmarsh for foraging when *Ae. vigilax* was similarly abundant in saltmarsh and coastal swamp forest habitats, but shifted to preferentially forage in coastal swamp forest when *Ae. vigilax* abundance was significantly greater in this habitat. The close association between habitat use by *V. vulturnus* and the distribution and abundance of *Ae. vigilax* highlights the importance of *Ae. vigilax* as a prey resource for *V. vulturnus*. Although radio-tracking studies for other fauna (e.g., spotted owls) have demonstrated shifts in habitat use corresponding to shifts in distribution of particular prey (Zabel *et al*. 1995), no radio-tracking studies prior to my study had identified such a shift by bats.

6.2 Significance of findings

Findings from this thesis provide valuable and novel information about the importance of saltmarsh and other habitats on the Central Coast of New South Wales to local foraging

insectivorous bats (Chapters 2 and 3). Additionally, this research provides unprecedented resolution of diets of insectivorous bats in Australia and confirms that *Ae. vigilax* is a prey resource for local bats (Chapter 4). This research also is the first to report a short-term shift in preferential habitat use by an insectivorous bat corresponding to a shift in the distribution and abundance of a known prey resource (*Ae. vigilax*) (Chapter 5). Together, these findings form the basis from which an assessment of the importance of *Ae. vigilax* to insectivorous bat diet can be made.

Only in the last decade has use of coastal saltmarsh by foraging insectivorous bats been investigated in Australia (Hoye 2002; Laegsdgaard *et al*. 2004; Belbasé 2005; Lamb 2009). A diverse group of bats representing three families (Miniopteridae, Molossidae and Vespertilionidae) has been positively identified, including six threatened species and an additional two species that could not be positively identified (Hoye 2002; Laegsdgaard *et al*. 2004; Belbasé 2005). All studies of bats in saltmarsh were conducted at different times of the year and were relatively short in duration (sampling intensity). The studies of Hoye (2002) and Belbasé (2005) undertaken in the same area (Kooragang Island and surrounds, Newcastle, NSW) three years apart, recorded similar bat assemblages, indicating that saltmarsh may be regularly used by the same bat taxa within a small geographical area. This pattern was also observed during my research, with similar taxa recorded in the same saltmarsh habitats during two surveys over two seasons (Chapters 2 and 3). The overall similarity in the diversity and activity of bats recorded in both surveys, suggests that short-term studies of bats in saltmarsh provide a reliable snap-shot of bat activity in this habitat. However, the duration of any survey should accommodate the aims of the survey and sampling should be undertaken over multiple nights (Fischer *et al*. 2009).

Radio-tracking studies have demonstrated that bats exhibit fidelity to local foraging areas over time (Duchamp *et al*. 2004; Hillen *et al*. 2009). However, while bats may show fidelity to a local area over some temporal scale, one would expect that bats would shift foraging ranges within this area in response to prey fluctuations. Radio-tracking of *V. vulturnus* over a single tidal cycle (Chapter 5) demonstrated that although bats exhibit fidelity to a local foraging area, they display patchy use of habitats within this area, shifting between patches in response to prey fluctuations. These findings also demonstrate

that foraging ranges are in fact comprised of discrete patches rather than a continuous foraging area.

Mosquitoes represent a small-sized prey resource for insectivorous bats, particularly during summer months when they can be abundant. Bats have been observed consuming mosquitoes (Griffin *et al*. 1960; Rydell 1990) and mosquitoes have been identified in stomach contents of bats (Poole 1932; Buchler 1976) as well as in bat faeces (Rydell 1990; Whitaker Jr. and Lawhead 1992; Kurta and Whitaker Jr. 1998; Long *et al*. 1998; Shiel *et al*. 1998; Pavey *et al*. 2001b; Sophia 2010). When assessing the relative importance of *Ae. vigilax* to local bat populations, some consideration must be given to the highly variable population abundances of this mosquito, and other species associated with nearby non-estuarine habitats. The abundance of mosquitoes is generally dependent on the availability of suitable environmental conditions provided by local rainfall and tidal inundation of habitats. Abundance of *Ae. vigilax* can vary within and between seasons and understanding how local bat populations respond to this temporally variable resource through shifts in prey preference and foraging habitats is fundamental. Although low-frequency echolocation is likely to limit the detectability of small prey (like mosquitoes) by bats (Barclay and Brigham 1991), high-frequency echolocating bats of varying size may be constrained by the relative profitability of *Ae.vigilax* (given increased energetic demands of larger bats). Additionally, given that it has been demonstrated that clutter reduces availability of abundant prey (Rainho *et al*. 2010), relationships associated with echolocation frequency and bat size are likely to be further influenced by the effects of clutter. Relationships between prey abundance and overall bat activity in my research were restricted to the low-clutter saltmarsh habitat. Proportional feeding activity was also greatest in this habitat, indicating that low-clutter, open habitats may represent the most profitable foraging grounds for bats when juxtaposed with forest habitats that provide roosting habitat. Supporting this hypothesis, habitat use by *V. vulturnus* indicated that this high-frequency echolocating bat selected habitats in which *Ae. vigilax* was most available. Additionally, my research has demonstrated that mosquitoes make up an important component of the diet of local small-sized, high frequency echolocating bats, including *V. vulturnus*. However, these bats also consumed a diverse range of prey, dominated by lepidopterans, with only 55 % of individuals found to consume *Ae. vigilax*. It should be noted however, that the diets documented in this thesis are representative of individuals

trapped in the forest habitats in the study area and not the more open saltmarsh habitat. It is expected that the frequency of occurrence of *Ae. vigilax* in the diets of these bats would be higher for those individuals trapped in open saltmarsh where it may be more efficient for foraging for small prey such as mosquitoes.

Outcomes from this research are likely to be applicable to other habitats and geographical areas around the world. While information about the use of saltmarsh by insectivorous bats outside of Australia is scant, many studies overseas have investigated the use of similar marshland (Barclay 1984; Popa-Lisseanu *et al*. 2009) and wetland habitats by bats (Menzel *et al*. 2005). These habitat types, like saltmarsh, are inherently open, usually bounded by structurally favourable habitats for bats (e.g., vegetation edges) and represent sources of prey (Fukui *et al*. 2006). Findings from this thesis may be extrapolated to make predictions about the types of bat taxa that are likely to frequent these open habitats. It is expected that bats with a morphology suited for fast-flying in open spaces (e.g., long and narrow wings) utilise these profitable foraging habitats, while those species capable of navigating background clutter may also utilise these habitats, particularly when emerging insect abundances are high (Fukui *et al*. 2006). It is also expected that edge vegetation in these habitats would represent linear elements for foraging bats and therefore appropriate management of these adjoining habitats is likely to be crucial. Abundant prey resources that emerge from these habitats (e.g., Chironomidae and Culicidae) are likely to represent important prey items for bats that forage in these habitats, particularly since it should be more efficient to forage for small prey in these open habitats. Predictions may also be made about what bat species are likely to be consuming particular prey that emerge from these habitats. My research suggests that small prey items are likely to be restricted to small, high-frequency echolocating bats, although larger bats may utilise these prey if they are energetically worthwhile. Although small chironomids and mosquitoes have been detected in the diets of medium-large size European bats, I predict that mosquitoes are only likely to constitute part of the diet of larger bats during periods of extreme abundance in open habitats, where small prey may be more easily detected and efficiently captured. The reduced detectability of small prey as well as the energetic costs of foraging for small prey in structurally complex habitats is likely to render the mosquito an unprofitable prey for large bats (with greater energetic demands) in cluttered habitats.

6.3 Management implications

The findings of this thesis together with data from the literature provide a basis that can be used to assess the importance of *Ae. vigilax* to local foraging insectivorous bats and guide management of saltmarsh and encroaching adjoining habitats. Although bat activity was significantly greater in forest habitat, proportional feeding activity was significantly higher in the open saltmarsh, highlighting the importance of this endangered ecological community to foraging insectivorous bats. Despite the presence of juvenile mangroves transgressing into saltmarshes in south-east Australia (Saintilan and Williams 1999), saltmarsh is a more open habitat than forest and may represent a more efficient foraging habitat for local insectivorous bats, especially as it tends to occur in small patches close to vegetated edges. Consequently, it is important to continue to conserve coastal saltmarsh and adopt appropriate management strategies that address the many threats that coastal saltmarshes face at both, local and catchment levels.

This research confirmed that, along with moths, *Ae. vigilax* is an important prey resource for local bats of the *Vespadelus* genus. Therefore management of *Ae. vigilax* populations will have direct implications for these bats. While it is beyond the scope of this study to infer potential impacts of broadscale mosquito control on insectivorous bat diet and health, declines in bat populations have previously been attributed to deteriorating feeding conditions (Gerell and Gerell Lunderg 1993). A threatening process for bats worldwide is the loss or reduction of prey items due to pesticide use (Hutson *et al*. 2001). While the broadscale use of insecticides in agriculture can impact a wide range of insects, the most commonly used mosquito control agents in Australia (e.g., *s*-methoprene and *Bacillus thuringiensis israelensis*) are generally mosquito-specific and target the aquatic immature stages of mosquitoes (Russell and Kay 2008). Reductions in larval mosquito populations can be as high as 98.2 % (Webb *et al*. 2011), substantially diminishing the availability of mosquitoes to small foraging bats, but will not impact the abundance of non-mosquito prey or mosquito populations associated with non-estuarine habitats. Consequently, any mosquito management plan should consider potential impacts of substantial reductions in immature or adult mosquitoes on their predators. For insectivorous bats, a key consideration is the timing of mosquito control. In Europe, consumption of mosquitoes by *Myotis sodalis* Miller and Allen increased during pregnancy (Kurta and Whitaker Jr.

1998), a period when energetic demands are high for insectivorous bats (Studier *et al*. 1973). Although in my study, *Ae*. *vigilax* was only detected in the faeces of those bats trapped from December to mid-February (the lactation period of the two bat species found to consume *Ae*. *vigilax*), failure to detect the presence of mosquitoes in bat diet late in the Austral summer may be influenced by a limited sample size of bats trapped during this time. Further research is needed to assess whether consumption of mosquitoes is greater during the lactation period of the two *Vespadelus* species found to consume *Ae*. *vigilax*.

Given *Ae*. *vigilax* was detected in the faeces of two of the more common bats in the study area, broadscale mosquito control may be able to proceed with an adaptive management process whereby careful monitoring of bats before and after the application of broadscale mosquito control is required. Monitoring may be undertaken in saltmarsh and forest habitats in the study area, using ultrasonic detectors to examine the activity of bats prior to, during and after the application of broadscale mosquito control. If significant declines in the activity of particular bat species are found to be associated with broadscale mosquito control activities, all control measures should cease immediately while monitoring continues in the area. A precautionary approach is recommended whereby broadscale control of mosquitoes is restricted to periods outside lactation of bats (mid-February onwards), given the period of lactation is when energetic demands are greatest for bats (Studier *et al*. 1973). The time following the usual lactation period of bats is also when risk of contracting mosquito-borne diseases such as Ross River virus and Barmah Forest virus is greatest (Russell 1995). Alternatively, the use of a mosquito and arbovirus surveillance program can provide data on mosquito abundance and activity of mosquito-borne viruses that can be used to trigger mosquito control activities only when there is an elevated risk of public health concerns. Current mosquito control activities, although resulting in high mortality in larval mosquito abundance, do not completely eradicate adult mosquito populations. Even during periods of effective mosquito control, populations of adult *Ae. vigilax* will still be present in the local area. It may be argued that when mosquito control activities are directed towards reducing the exceptional peaks in mosquito abundance, sufficient mosquito populations will be present to not adversely impact local bat populations. However, mosquito control can reduce the abundance of *Ae*. *vigilax* larvae by up to 98 % and consequently is likely to diminish the availability of the mosquito to bats. This is particularly important since small prey may be more detectable when in swarms, as

together they represent a larger target for bats (Waters *et al*. 1995; Shiel *et al*. 1998). In the absence of mosquito control, nuisance biting may be experienced by local residents in the early summer months. This impact may be mitigated by behavioural practises, physical barriers and the use of personal protective measures including the use of topical repellents containing the active ingredients diethyltoluamide (DEET) and picaridin (Barnard and Xue 2004; Webb and Russell 2009b, 2011).

6.4 Scope for future research

The research undertaken for this thesis has covered a wide range of aspects pertaining to the importance of coastal saltmarsh and *Ae. vigilax* to foraging insectivorous bats. There are however, a number of information gaps that have been identified which may be the focus of future research. These are briefly listed below.

1. While the use of molecular techniques has improved resolution of bat diets and increased the detectability of smaller, more soft-bodied prey, it does not allow for quantification of prey items since variable DNA degradation rates may exist for different taxa. Consequently, development of new analytical techniques that can be used to quantify prey without sacrificing the resolution achieved using molecular approaches would greatly improve characterisation of insectivorous bat diets.

2. Dietary analyses provided in this thesis are for those bats that were trapped in forest habitats. While *Ae. vigilax* was detected in the faeces of two small-sized bat species, it is possible that *Ae. vigilax* is more available to larger, less manoeuvrable bats in more open habitats, such as saltmarsh. Additionally, it is still unknown what impacts broadscale mosquito control will have on the diets of insectivorous bats (in low or high clutter habitats). Data obtained during this research provide a baseline from which comparisons can be made after a trial *Bti* spraying application is undertaken. However, any future assessment of bat diet should also seek to investigate those bats foraging directly in saltmarsh where *Ae. vigilax* is likely to be more available. Harp trapping and mist netting in saltmarsh near known roosts of insectivorous bats in adjacent mangroves (outside the study area used for this research) may provide faecal samples for dietary analysis.

3. Although data collected to measure irruptions in *Ae. vigilax* abundance indicated that saltmarsh and forest habitats support similar abundances of *Ae. vigilax*, it remains unknown which local saltmarsh habitats contribute the greatest number of *Ae. vigilax* individuals to forest habitats. These data are critical to any future assessment of *Ae. vigilax* importance to bat diet post-*Bti* application, particularly if *Ae. vigilax* in forest habitats disperse from multiple saltmarsh habitats in the study area (a possibility given *Ae. vigilax* can disperse at least 5 km from larval habitats (Webb 2002)). Additionally, the extent to which *Ae. vigilax* disperses throughout the study area is unknown. A mark-release-recapture experiment may be used to assess dispersal patterns of *Ae. vigilax* and identify which local saltmarsh habitats contribute the greatest number of *Ae. vigilax* individuals to forest habitats in the study area.

4. Although a shift in preferential habitat use by *V. vulturnus* corresponding to the distribution and abundance of *Ae. vigilax* was identified, it remains unknown whether a similar shift in habitat use by *V. pumilus* (the other species found to prey upon *Ae. vigilax*) would be observed. A radio-tracking study of this species following a similar design employed in this thesis would assist in elucidating any relationship between *Ae. vigilax* abundance and distribution and the habitat use of this bat species. Given the similar morphology, echolocation design and energetic requirements of these congeners, an investigation of potential competition between these two species is warranted.

5. Patterns of *Ae. vigilax* distribution and abundance identified in this research indicate that forest habitats adjacent to saltmarsh support similar abundances of *Ae. vigilax* as saltmarsh. Consequently, *Ae. vigilax* may represent a prey resource for insectivorous bird species in both saltmarsh and forest habitats. While reduced fledgling survival of insectivorous birds in Europe has been attributed to broadscale mosquito control (Poulin *et al*. 2010), no such data are available to make a similar assessment for insectivorous birds (transient and local species) in the study area.

Conference presentations and publications

Conference presentations

Work conducted as part of this project has been presented at international and domestic conferences. These are listed below.

Gonsalves, L., Webb, C. and Monamy, V. (2009). Temporal variation in saltmarsh mosquito, *Aedes vigilax*, populations on the central coast of NSW, Australia. *Poster presentation: Poster 894, 10th International Congress of Ecology, 16-21 August 2009, Brisbane, Australia.*

Gonsalves, L., Lamb, S., Monamy, V., Law, B. and Webb, C. (2009). An investigation of the importance of declining coastal saltmarsh communities to insectivorous bat species. *Spoken presentation: Paper 893, 10th International Congress of Ecology, 16-21 August 2009, Brisbane, Australia.*

Gonsalves, L., Law, B., Webb, C. and Monamy, V. (2010). Habitat use of the little forest bat in response to changes in saltmarsh mosquito population abundances. *Poster presentation, 50th Ecological Society of Australia Conference, 6-10 December 2010, Canberra, Australia.*

Gonsalves, L., Webb, C., Law, B. and Monamy, V. (2010). Aspects of the dietary ecology of forest bat species, Central Coast, NSW: molecular analysis of prey DNA in guano. *Spoken presentation: Paper 3, 14th Australasian Bat Society Conference, 12-14 July 2010, Darwin, Australia.*

Lamb, S., Gonsalves, L., Law, B. and Monamy, V. (2010). Estuarine macrohabitat use by *Chalinolobus gouldii* as revealed by acoustic bat detection and radiotelemetry, Central Coast, NSW. *Spoken presentation: Paper 2, 14th Australasian Bat Society Conference, 12-14 July 2010, Darwin, Australia.*

Gonsalves, L., Law, B., Webb, C. and Monamy, V. (2011). Unravelling the ecological importance of mosquitoes to bats: can radiotracking and molecular scatology help? *Spoken presentation, 51st Ecological Society of Australia Conference, 21-25 November 2011, Hobart, Australia*.

Webb, C. and Gonsalves, L. (2011). What is the ecological role of mosquitoes and what are the environmental risks associated with mosquito control? *Spoken presentation: Paper 9, Living with mosquitoes around wetlands, Wetland Education Training meeting, 26-27 October 2011, Sydney Olympic Park, Australia.*

Gonsalves, L., Lamb, S., Webb, C., Law, B. and Monamy, V. (2012). Clutter influences relationships between bat activity and abundance of mosquitoes and non-mosquito prey in a coastal mosaic, New South Wales, Australia. *Spoken presentation: Paper 2, 15th Australasian Bat Society Conference, 11-13 April 2012, Melbourne, Australia.*

Gonsalves, L., Law, B., Webb, C., Monamy, V. and Bicknell, B. (2012). Unravelling the ecological importance of mosquitoes to insectivorous bats: can radiotracking and fecal DNA analysis help? *Spoken presentation: Paper 24, 15th Australasian Bat Society Conference, 11-13 April 2012, Melbourne, Australia.*

Manuscripts submitted and accepted for publication

In addition to conference presentations, manuscripts have been accepted for publication in peer reviewed journals. These are listed below.

Gonsalves, L., Lamb, S., Webb, C., Law, B. and Monamy, V. (in press). Do mosquitoes influence bat activity in coastal habitats? *Wildlife Research*

Gonsalves, L., Law, B., Webb, C. and Monamy, V. (in press). Are vegetation interfaces important to foraging insectivorous bats in endangered coastal saltmarsh on the Central Coast of New South Wales? *Pacific Conservation Biology*

Two other manuscripts have been prepared and submitted for publication in peer reviewed journals. These are listed below.

Gonsalves, L., Bicknell, B., Law, B., Webb, C. and Monamy, V. (submitted). The importance of mosquitoes to insectivorous bat diet: Does size matter? *PloS ONE*

Gonsalves, L., Law, B., Webb, C. and Monamy, V (submitted). Short-term shift in habitat selection of the little forest bat (*Vespadelus vulturnus*) in association with fluctuating prey resources. *Biological Conservation*

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Appendix A

Analysis of faecal samples

Genomic DNA was extracted from a pooled sample of five faecal pellets for each trapped *V. vulturnus* individual using the Ultraclean Fecal DNA kit (Mo Bio Laboratories). The Ultraclean Fecal DNA kit was used according to manufacturer's instructions, after the faecal pellets were broken apart with sterile toothpicks. For all other species, in order to retain prey fragments for future microscopic analysis, the QIAamp DNA Stool Mini Kit (Qiagen) was instead used to extract DNA as described by Zeale *et al*. (2011).

PCRs reactions were based on the Kappa Robust HS PCR kit and consisted of Buffer A (5X concentrate), forward and reverse primers (5 μM), $MgCl_2$ (25 mM), dNTPs mix (10 mM), Kapa2G Robust HS polymerase (0.5 U) and made up to 10 μL reactions using sterile milliQ water. All PCRs were carried out using an Eppendorf EG thermocycler.

Taxon-specific primers, ZBJ-ArtF1c and ZBJ-ArtR2c (Zeale *et al*. 2011) were used to amplify a 157 bp section of the DNA barcoding region, cytochrome oxidase I with the following cycling conditions: initial denaturation at 95° C for 3 minutes, followed by 16 cycles of 95° C for 30 s, 61-53° C (-0.5° C per cycle) for 30 s, 72° C for 30 s, followed by an additional 24 cycles of 95° C for 30 s, 53° C for 30 s, 72° C for 30 s and a final extension of 72° C for 10 m. Gel electrophoresis (1 % agarose) was used to verify the presence of a PCR product of the correct size.

Samples that failed to produce visible PCR products were subjected to a nested PCR, in which samples were initially amplified using a universal mini-barcoding primer, Uni-MinibarF1 (Meusnier *et al.* 2008) and ZBJ-ArtR2c. The cycling conditions consisted of an initial denaturation at 95° C for 3 m, followed by 5 cycles of 95° C for 30 s, 46° C for 30 s and 72° C for 30 s, and a further 35 cycles of 95° C for 30 s, 53° C for 30 s, 72° C for 30 s and a final extension of 72° C for 5 m. One microlitre of PCR product was then used as the template in a second round of amplification using taxon-specific primers.

Duplicate PCR products generated using the taxon-specific primers were pooled, purified using Ultraclean PCR clean-up kit (Mo-Bio laboratories) and cloned using either a pGem-T-easy vector cloning kit and JM109 high competency *E. coli* cells (Promega), or an

Acquire PCR Cloning Kit and high efficiency competent cells (Alchemy Biosciences) according to manufacturers' instructions. A selection of clones were lysed in 50 μL of Sterile MilliQ water at 95° C and 1 μL was subjected to PCR to amplify DNA inserts, using vector primers T7 and SP6 (10 μM), and GoTaq green mastermix (Promega). Gel electrophoresis was used to verify insert size. Sixteen clones from each bat, with an insert of appropriate size (ca.320 bp) were purified using the Ultraclean PCR purification kit (Mo Bio Laboratories) and sequenced at the Australian Genome Research Facility (Westmead Millennium Institute, Sydney) using the ABI Prism Big Dye Terminator reaction on a AB3730xl sequencer. DNA sequences were aligned using Clustal X (Larkin *et al.* 2007) and trimmed of flanking vector bases in BioEdit 7.0.0 (Hall 2004). All spurious sequences (those shorter than ca.157 bp) were excluded from further analysis. All other sequences were entered into the identification engine on the barcoding of life database (BOLD). The nearest sequence match and percent similarity of each sequence was recorded, with a taxonomic assignment to order, family, genus or species using taxonomic assignment thresholds (Zeale *et al*. 2011). When sequence similarity was greater for a taxon not currently known to exist in Australia, the sequence was assigned to the nearest match known to occur in Australia.

Calibration of technique sensitivity for detection of mosquito DNA

While Zeale *et al*. (2011) reported no bias in detectability of various taxa, the tested taxa did not include mosquitoes. To provide a baseline for detectability of mosquito DNA among DNA of other taxa, artificial bat faecal material was manufactured with mealworms and varying concentrations of mosquitoes (by volume). One batch of each prey (mosquito plus mealworm) was made by grinding known numbers of mosquitoes and mealworms in separate 2-mL vials with distilled water. For the mosquito stock mixture, 0.43 g of *Aedes aegypti* individuals was added to 2 mL of distilled water (0.18 g mL^{-1} assuming 1 g of mosquito = 1 mL), while 0.44 g of mealworm individuals was added to 1 mL of distilled water for the stock mealworm mixture (0.30 g mL^{-1} assuming 1 g of mealworm = 1 mL). Five sterile glass balls were added before vigorous shaking in a mosavex machine (Department of Medical Entomology, Westmead Hospital and University of Sydney) for 1 h 15 m. Mealworm and mosquito slurries were then transferred to separate sterile jars using 1-mL pipettes. Using the two stock slurries, 0 %, 5 %, 10 %, 15 %, 20 % and 100 % of mosquito solutions were made.