Mosquito control, saltmarsh and insectivorous bats: seeking a balance

Final Report to NSW Environmental Trust

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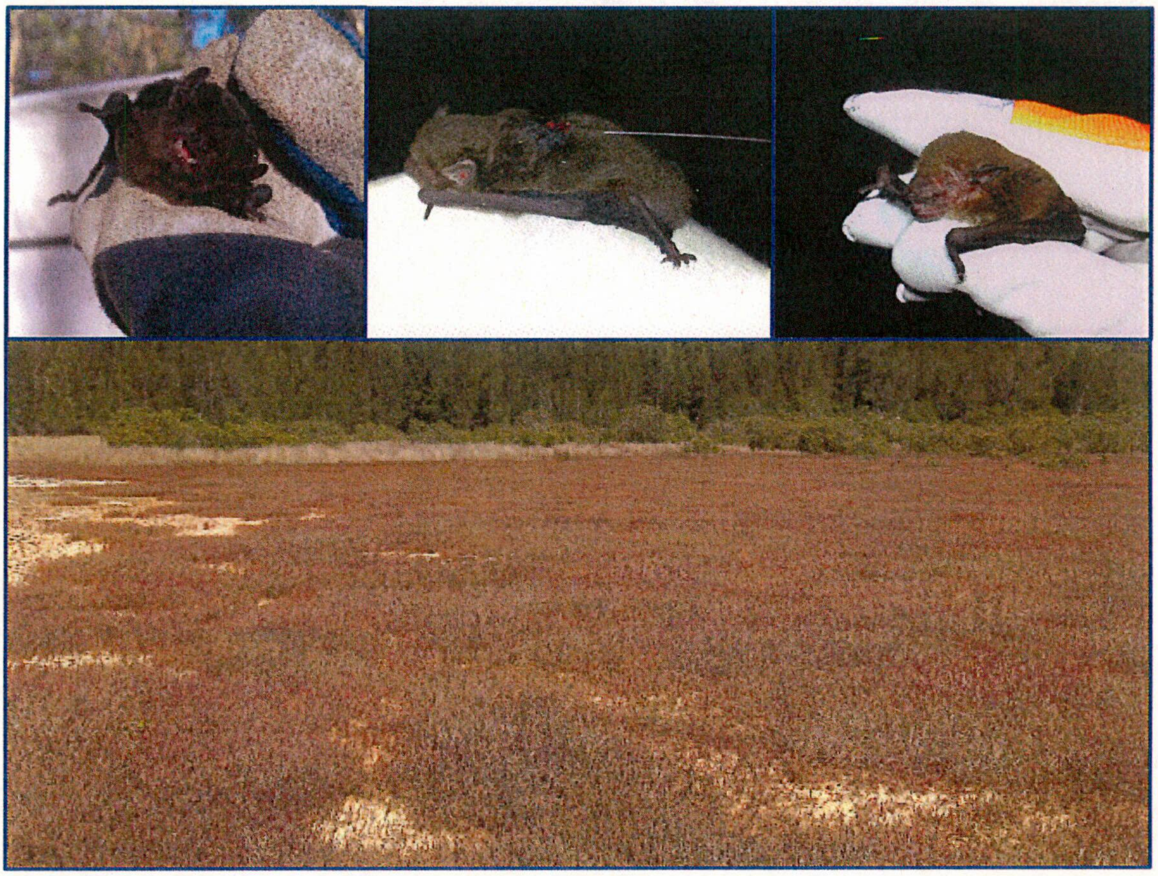

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Executive summary

Coastal saltmarsh provides suitable breeding habitat for a number of estuarine mosquito species. The saltmarsh mosquito (*Aedes vigilax* Skuse) has been identified as an important vector of important mosquito-borne viruses such as Ross River virus and Barmah Forest virus and is a known nuisance biting pest. Coast residential areas adjacent to Empire Bay (e.g., Killcare, Pretty Beach and Hardys Bay) on the Central Coast of NSW are prone to nuisance biting from *Ae. vigilax* and other estuarine mosquito species, particularly in late summer and early autumn each year. Residents have requested use of a broadscale mosquito spraying regime (*Bti*) to control numbers of *Ae. vigilax.* At the same time, it has been suggested that *Ae. vigilax* may be an important dietary item for insectivorous bats foraging within saltmarsh (Hoye 2002; Belbasé 2004; Laegdsgaard *et al.* 2004) and that control of mosquitoes may impact upon foraging bats (Laegdsgaard *et al.* 2004). To date, no study has specifically investigated the importance of the mosquito in the diet of these bats.

The present study 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 only were 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 swamp).

Of the species able to be identified acoustically, five species were able to be trapped and an assessment was made of their diet. The diets of five bat species trapped in forest habitat adjacent to saltmarsh habitat consisted of a diverse range of prey, dominated by moths. Only two bat species (*Vespadelus pumilus* and *V. vulturnus*) consumed *Ae. vigilax*. Neither species is considered threatened in this area. *Aedes vigilax* abundance was positively correlated only with the activity of bats of this genus. Habitat use of *V. 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.

This study provides a baseline to assess the importance of *Ae. vigilax* to insectivorous bat diet. *Aedes vigilax* is an important prey resource for bats of the *Vespadelus* genus but was absent from the diets of all other bat species sampled. The findings of this study can be used as a benchmark for comparison with future research should a pilot *Bti* spraying regime be initiated. A precautionary approach is recommended whereby the application of *Bti* is restricted to later in summer in order to avoid the lactation period of bats (Nov-Jan), when energetic demands are greatest.

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1. Background, objectives of the study and general introduction

1.1 Background

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. The saltmarsh mosquito (*Aedes vigilax* Skuse) can be locally abundant throughout summer and early autumn (early December – late March), 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 and Barmah Forest virus. Notwithstanding the health risks associated with *Ae. vigilax*, the mosquito is also a nuisance biting pest. In response to the health and social impacts of *Ae. vigilax*, broadscale mosquito control has been implemented in many coastal areas (Bell 1989; Russell & 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; Belbasé 2004; Laegdsgaard *et al.* 2004) and that control of mosquitoes may impact upon foraging bats (Laegdsgaard *et al.* 2004), no study has specifically investigated the importance of the mosquito to the diet of these bats.

1.2 Objectives of study

The aims of the present study are to (i) investigate activity of insectivorous bats and quantify the seasonal irruption of *Ae. vigilax* from December to March along a gradient of habitats including saltmarsh (larval habitat of *Ae. vigilax*), urban (highest densities of humans) and forest (refuge habitat of *Ae. vigilax* and a source of insectivorous bat populations); (ii) collect essential ecological information on use of saltmarsh for feeding by insectivorous bats[1]; (iii) determine the frequency of occurrence of mosquitoes in the diet of insectivorous bat species within the study area; (iv) determine the relative proportion each bat species' geographic home range contributed by saltmarsh.

1.3 General introduction

This report collates four studies conducted independently that individually seek to address one or more of the aims of the overall project.

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[1] This aim was changed from the original objectives. Please see Section 6.3(2) for an explanation.

1.3.1 Bat activity and mosquito abundance

In Section 2, a study investigating relationships between bat activity and the abundances of mosquito and non-mosquito prey is presented. Although the immature aquatic stage of *Ae. vigilax* occurs predominantly in coastal saltmarsh habitat, upon emergence, *Ae. vigilax* adults can be abundant in more sheltered habitats adjacent to coastal saltmarsh, with biting impacts experienced at a distance of at least 5 km from estuarine habitats (Webb & Russell 2009a). These habitats are considered refuge habitats that provide sheltered areas for mosquitoes while also supporting sources of bloodmeals 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.

Large areas of coastal saltmarsh habitat can sustain locally abundant *Ae. vigilax* populations throughout summer. However, large forested areas also may provide suitable refuge habitat for host-seeking *Ae. vigilax* individuals. Located between the larval and potential refuge habitat of *Ae. vigilax* is built-up urban habitat that also may provide suitable larval habitat for many container-breeding mosquitoes and also refuge habitat for *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.

While prey abundance has been found to influence the activity of bats in different habitat types (Rydell 1989; Fenton 1990; Rautenbach *et al.* 1996; Fukui *et al.* 2006; Adams *et al.* 2009), other factors also have influenced the habitats in which bats forage. Vegetation clutter (i.e. structures that produce non-target echoes) has been shown to influence bat mobility and foraging activity (O'Neill & Taylor 1986; Bradshaw 1996; Brigham *et al.* 1997; Law & Chidel 2002; Lloyd *et al.* 2006) so that bats with a particular wing morphology and echolocation design display differential habitat use (Neuweiler 1984; Norberg & Rayner 1987; Fenton 1990; Saunders & Barclay 1992; Brigham *et al.* 1997). Fast-flying bats that are less manoeuvrable generally have a high wing-aspect ratio with low frequency echolocation calls that make it difficult to navigate or detect prey in high levels of clutter (Rhodes 2002). This sensitivity to clutter 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 also may interact with each other. It has been demonstrated that prey abundance does not necessarily equate to availability (Boonman *et al.* 1998; Adams *et al.* 2009; Rainho *et al.* 2010). In these studies, clutter rendered prey less available to foraging bats that were unable to locate prey successfully among clutter. 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 & 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 & Lemen 2005). However, many studies investigating diets of medium-large size bats have reported the consumption 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 were 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 restrictions in prey size associated with particular echolocation designs, it is unclear whether relationships between *Ae. vigilax* abundance and bat activity as well as the activity of individual species are consistent across habitats with varying levels of clutter. In order to examine relationships between *Ae. vigilax* abundance and bat activity that may not be consistent in all habitats or for all bat species, we surveyed bat activity and the activity of individual species as well as prey abundance (*Ae. vigilax* and non-mosquito) in saltmarsh, urban and forest habitats, which vary in acoustic complexity (least-to-greatest). We predict: (a) Relationships between *Ae. vigilax* abundance and bat activity will not be consistent across habitats or species. (b) Stronger associations between prey abundance (*Ae. vigilax* and non-mosquito) 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. The methods employed to undertake this study and the findings of the study are presented in Section 2.

1.3.2 Vegetation interfaces and their importance to insectivorous bats

The findings from work presented in Section 2 indicate that coastal saltmarsh is an important habitat for a number of insectivorous bats, many of which are threatened species. The work presented in Section 3 investigates the importance of vegetation interfaces to insectivorous bats that forage within saltmarsh. In many landscape mosaics, vegetation interfaces (i.e. edges) are areas of high bat activity (Ekman & de Jong 1996; Grindal & Brigham 1998, 1999; Grindal *et al.* 1999; Verboom & Spoelstra 1999; Law & Chidel 2002; Menzel *et al.* 2002; Estrada *et al.* 2004; Lumsden & 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 & Kapteyn 1991; Verboom & 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).

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 & Williams 1999). 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 activity of foraging insectivorous bats, an investigation of saltmarsh microhabitat use by bats is first required. This work investigated 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. We 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'. Details of the methods used in this study as well as the findings are provided in Section 3.

1.3.3 Importance of Aedes vigilax in bat diets

Findings reported in Sections 2 and 3 suggest that activity of particular bat species (with particular morphology and echolocation design) is positively correlated with the abundance of *Ae. vigilax*. These correlations generally occurred for those species considered to be able to detect and capture small prey (high frequency echolocating bats, small in size with agile flight).

Mosquitoes represent a small sized 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 insectivorous bats (Reiskind & Wund 2009), bats of various sizes (small-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 stomach contents of bats (Poole 1932; Buchler 1976) as well as in bat guano (Rydell 1990; Whitaker & Lawhead 1992; Kurta & Whitaker 1998; Long *et al.* 1998; Shiel *et al.* 1998; Pavey *et al.* 2001b; Sophia 2010).

Tuttle (2005) hypothesised that bats may serve as an alternative approach to broad-scale mosquito control, with a single bat capable of consuming up to 600 mosquitoes per hour. Additionally Reiskind & Wund (2009) 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 & Wund (2009), did not account for a range of other factors such as satiation of bats, abundance of mosquitoes relative to other prey, ability of bats to detect mosquitoes among various levels of clutter, digestibility of mosquitoes as well as calorific requirements of bats, that will presumably influence the degree to which bats consume mosquitoes.

In order to assess 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 & Fenton 1976; Kunz & Whitaker Jr 1983; Dickman & Huang 1988). Many dietary studies have used microscopic analysis of prey fragments within guano or the stomach contents of bats to describe bat diets (Whitaker Jr 1972; Buchler 1976; Vestjens & Hall 1977; O'Neill & Taylor 1989; Rydell 1989; Brigham & Fenton 1991; Jones 1999) However, due to the greater susceptibility of soft-bodied prey to the processes involved in digestion and mastication (Rabinowitz & Tuttle 1982; Dickman & Huang 1988) often rendering these prey unidentifiable, this technique is likely to underestimate the importance of small prey such as mosquitoes. Additionally, large prey items can be represented in multiple faecal pellets, potentially leading to an overestimation of these taxa, which in turn contributes to further underestimation of smaller prey taxa (Robinson & Stebbings 1993).

Recent advances in molecular techniques however, have allowed greater resolution of animal diets, particularly for cryptic animals that are difficult to observe foraging. Since these techniques rely on DNA for 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 and survival (Deagle & Tollit 2007), limiting the degree to which prey can be quantified. Nevertheless, in a dietary study of the Barbastelle bat (*Barbastella barbastellus*) using this technique, detection of soft-bodied micro-moths among a range of larger, more sclerotised insects suggested that the technique was robust enough to detect those prey items most susceptible to digestion (Rabinowitz & Tuttle 1982; Dickman & Huang 1988; Zeale *et al.* 2011). This molecular approach now has been applied in 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 guano.

In this study molecular techniques are utilised to investigate the extent to which five insectivorous bat species in the study area consume *Ae. vigilax* and whether there is a relationship between bat size and consumption of mosquitoes. The five bat species range in size (4 g – 14 g), and that (with the exception of one species), all employ high-frequency echolocation (>50 kHz) thought to be more suited for detection of small prey (Barclay & Brigham 1991). The methods used during this study are described in Section 4, as are the findings resulting from this work.

1.3.4 Habitat use by Vespadelus vulturnus

Findings from work reported in Section 5 indicated that the consumption of *Ae. vigilax* was restricted to small sized, high frequency echolocating bats of the *Vespadelus* genus. Given *Ae. vigilax* population abundances are driven heavily by tidal and rainfall inundation of larval habitats (i.e. coastal saltmarsh and mangrove communities), they can be highly variable, both spatially and temporally. However, general patterns such as peaks in abundances can be predicted (Kokkinn *et al.* 2009); more abundant populations tending to be present approximately two weeks after inundation of saltmarshes by spring tides and/or heavy rainfall). The ability of *Ae. vigilax* to disperse more than 5 km from larval habitats (Webb 2002) suggests that forested habitats adjacent to coastal saltmarsh are likely to provide refuge for this mosquito species as well as providing sources of blood-meals, sustaining population abundances for longer periods.

The opportunistic nature of bats of the *Vespadelus* genus would suggest that habitat use by these bats would closely correspond to the availability of prey resources. If *Ae. vigilax* is an important prey resource, one may expect that a shift in habitat use will occur in association with shifts in the distribution and abundance of the mosquito. To evaluate this hypothesis, we investigated the habitat use of the Little Forest bat (*Vespadelus vulturnus*), a species known to consume mosquitoes in the study area, over two periods predicted to sustain relatively large and small *Ae. vigilax* population abundances, respectively. Habitat use was assessed by radio-tracking in the larval habitats where *Ae. vigilax* emerge and the neighbouring coastal swamp forest that provides a sheltered habitat for host-seeking adult mosquitoes. Details of all methods employed, as well as findings from the study, are provided in Section 5.

1.3.5 Summary of major findings

Finally, Section 6 provides a summary of the major findings from all studies and a discussion of these findings with reference to the overall aims of the project.

2. Spatial and temporal patterns of bat activity, *Aedes vigilax* abundance and the abundance of volant insects in saltmarsh, urban and forest habitats

2.1 Methods

2.1.1 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. The study area was characterised by more than 40 vegetation communities, many of which occur within a large national park (Bouddi, NP: 1 189 ha) and five smaller nature reserves (Cockle Bay, 68.5 ha; Rileys Island, 45.7 ha; Pelican Island, 40 ha; Saratoga Island, 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.

2.1.1.1 Coastal saltmarsh

The 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 the saltmarsh habitat lacks trees, a small number of Grey Mangrove shrubs (*Avicennia marina*) (< 3m high) are present in patches due to the landward transgression of mangroves, occurring in coastal saltmarsh communities in eastern Australia (Saintilan and Williams 1999). These vegetation communities represent important larval habitats for many estuarine mosquito species, including *Aedes vigilax*, *Ae. alternans* and *Culex sitiens* (Webb & Russell 2009a). Additionally, coastal saltmarsh communities have been found to support moderately high levels of bat activity (Lamb 2009; Gonsalves *et al.* submitted), and represent important foraging habitats for bats (Lamb 2009).

2.1.1.2 Urban

The urban habitat was characterised primarily by residential areas that included 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*, *Culex quiquefasciatus*, and *Cx. molestus* (Webb & Russell 2009a). Urban areas also sustain a diverse range of bats and can be areas of considerable bat activity (Avila-Flores & Fenton 2005; Hourigan *et al.* 2006; Scanlon & Petit 2008; Hourigan *et al.* 2010, Threlfall *et al.* 2011, in press). However, species diversity and activity levels within urban habitats vary with degree of urbanisation (Hourigan *et al.* 2006; Threlfall 2011a, in press).

Although no attempt was made to define the urban habitat based on degree of urbanisation, residential density in the overall study area was approximately 2.5 dwellings ha^{-1}. 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 area 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).

2.1.1.3 Forest

The forest habitat was situated on an escarpment that straddled 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*, *Syncarpia glomulifera* subsp. *glomulifera* and *Allocasuarina torulosa*, this vegetation community has a typical canopy height of 20 m, occurring on Narrabeen sandstone, that support a sparse-to-moderate understorey of shrubs and a well developed grass layer (Bell 2009). With a canopy cover of ~40 %, 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*, *Ae. procax* and *Verrallina funerea*. Additionally, forest

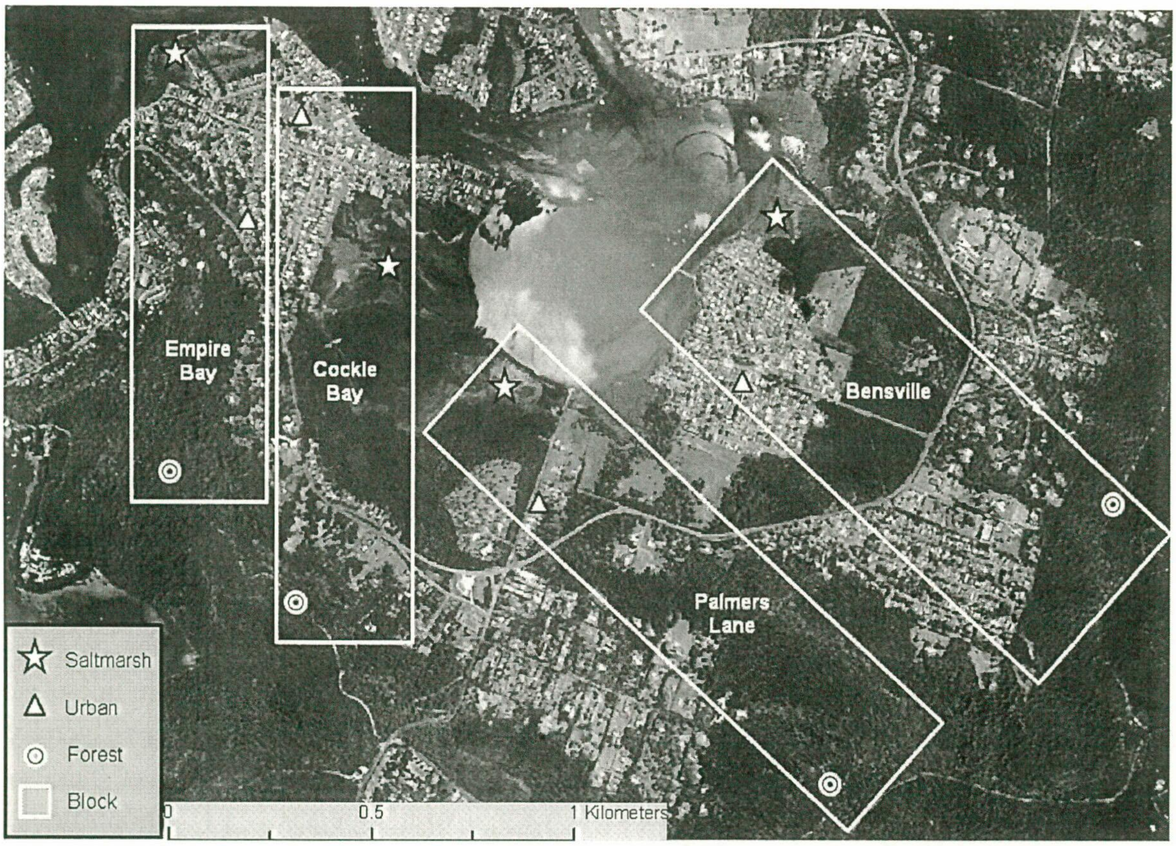

Figure 2.1 Satellite image of study area (adapted from Google Earth). Sampling location depicted by stars = saltmarsh; triangle = urban; donut = forest in each blocked site.

communities are known to sustain a number of hollow- and cave-roosting bats (Payne 2006) and represent important areas for foraging bats in the study area (Lamb 2009).

2.1.2 Data collection

At four sites within the study area, insectivorous bat activity and abundances of mosquito fauna and other volant insect fauna were surveyed. 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. The start of each fortnight coincided with a spring or a neap tide. In all, data were collected over five spring tides and four neap tides, with two sites surveyed concurrently each fortnight.

2.1.2.1 Bat survey

In each habitat, one Anabat SD1 detector (Titley Electronics, Ballina NSW) recorded the navigational 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.

Recorded bat calls were identified to species where possible using the automated call identification software, Anascheme (Gibson & Lumsden 2003), in association with a key for the lower north-eastern NSW coastal plain (Adams *al.* 2010). The 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 *N. geoffroyi* (Leach) = *Nyctophilus* spp.*; Vespadelus pumilus* (Gray), *V. troughtoni* (Kitchener, Jones & Caputi), *V. vulturnus* (Thomas) = *Vespadelus* spp.). While *V. troughtoni* calls could contribute to the activity of the *Vespadelus* species group, harp trapping in the area (throughout a field season) failed to sample any *V. troughtoni* individuals, suggesting that the activity of *Vespadelus* spp. is likely to represent activity of *V. pumilus* and *V. vulturnus*, both of which were commonly captured in harp traps. 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 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.

2.1.2.2 Prey survey

In each habitat, mosquito abundance was surveyed nightly using two CO_2–baited encephalitis virus surveillance (EVS) traps (Rohe & 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). 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-tip of abdomen) (<5 mm, 5-9 mm, 10-14 mm and >14 mm). Counts of all insects then were carried out before insects were oven dried weighed to provide a measure of biomass. All counts and weights were recorded for each night of trapping in each habitat.

2.1.3 Data analyses

Prior to analysis, all collected data were averaged across consecutive nights in each fortnight. A repeated measures mixed-model ANOVA was used to test the effects of habitat (saltmarsh/urban/forest), tidal cycle (neap/spring) and all interactions on mean nightly bat activity, activity of the commonly recorded bat taxa (representing >0.50 % of nightly bat activity), *Ae. vigilax* abundance and abundance of other volant insects. Comparisons of main effects were Bonferonni corrected for multiple comparisons.

A goodness of fit chi-square test was used to compare total feeding activity (proportion of bat calls containing feeding buzzes) and feeding activity of individual species between habitats and tidal cycle, while stepwise linear regressions were 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 nightly bat activity). Since vegetation clutter influences prey detectability (Adams *et al.* 2009, Rainho *et al.* 2010), analyses were conducted for each habitat separately.

2.2 Results

2.2.1 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*, *Mormopterus* sp 2, *Tadarida 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*, *C. morio* and *Vespadelus* spp.) contributed ~77 % of all recorded activity (Table 2.1).

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

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

Taxa	Saltmarsh Neap	Saltmarsh Spring	Saltmarsh Total	Urban Neap	Urban Spring	Urban Total	Forest Neap	Forest Spring	Forest Total	Total Neap	Total Spring	Total 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. sp2	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**	**5.7 ± 0.4^{ab}**	**5.0 ± 0.6**	**4.1 ± 0.5**	**4.5 ± 0.4^{a}**	**8.0 ± 0.7**	**5.3 ± 0.6**	**6.6 ± 0.6^{b}**	**6.3 ± 0.4**	**5.0 ± 0.4**	**5.6 ± 0.3**

Nightly bat activity (number of bat passes night^{-1}) was significantly different between habitats (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).

The activity of *C. morio* (F=12.159, P<0.001), *Mi. australis* (F=11.537, P<0.001), *Mormopterus* sp2 (F=14.108, P<0.001), *T. australis* (F=3.636, P=0.040) and *Vespadelus* spp. (F=70.180, P<0.001) differed significantly between habitats (Figs. 2.3a-e). *Chalinolobus morio*, *Mi. australis* and *Vespadelus* spp. were significantly more active in forest when compared to saltmarsh (P=0.001, P=0.001, P<0.001; Figs. 2.3a, 2.3b & 2.3e) and urban habitats (P=0.001, P=0.001, P<0.001; Figs. 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 *Mormopterus* sp2 was significantly more active in saltmarsh (P<0.001) and urban (P=0.001) habitats when compared with 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 *Mormopterus* sp2 (F=6.339, P=0.018) and *T. australis* (F=5.068, P=0.033) during neap tides (Figs. 2.4a & 2.4b).

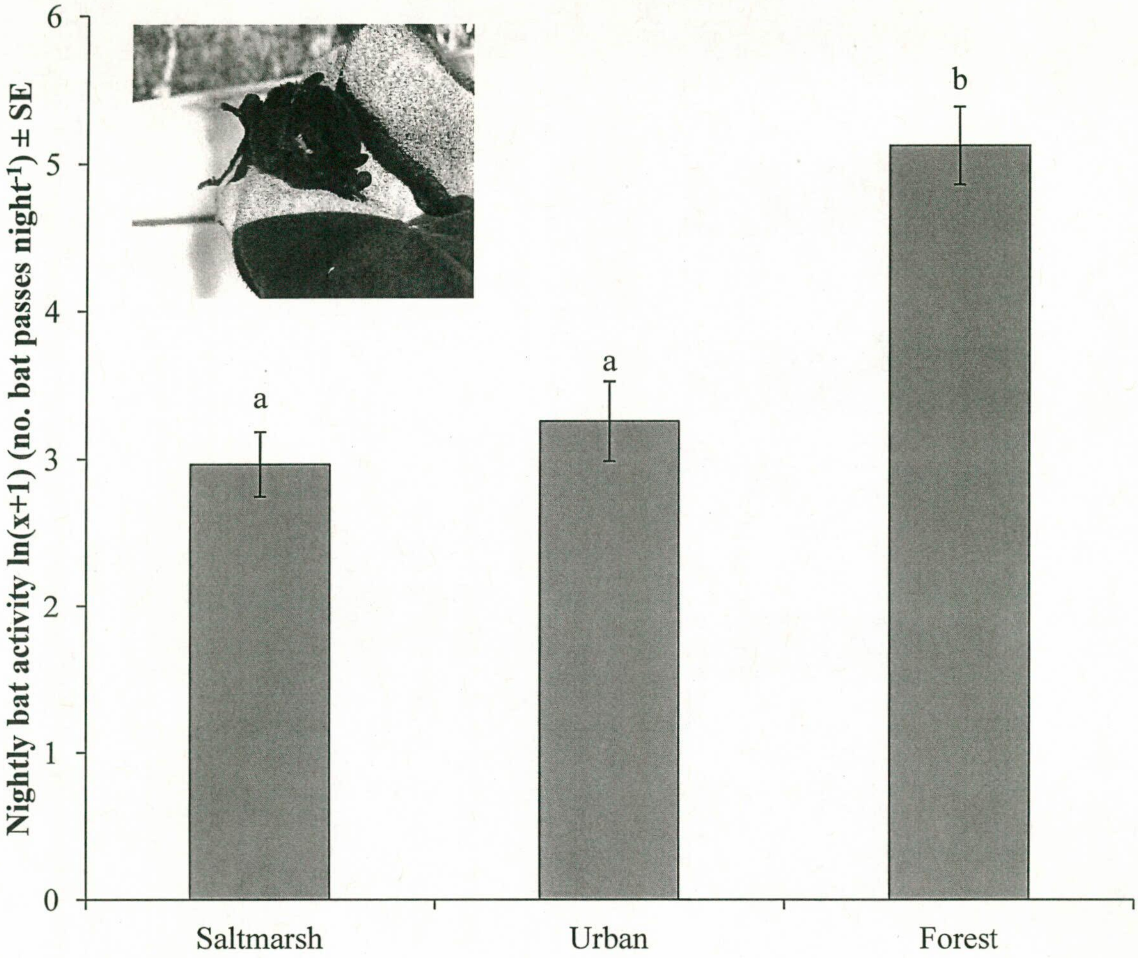

Figure 2.2 Nightly bat activity recorded in each habitat. Means denoted by different letters are significantly different from one another.

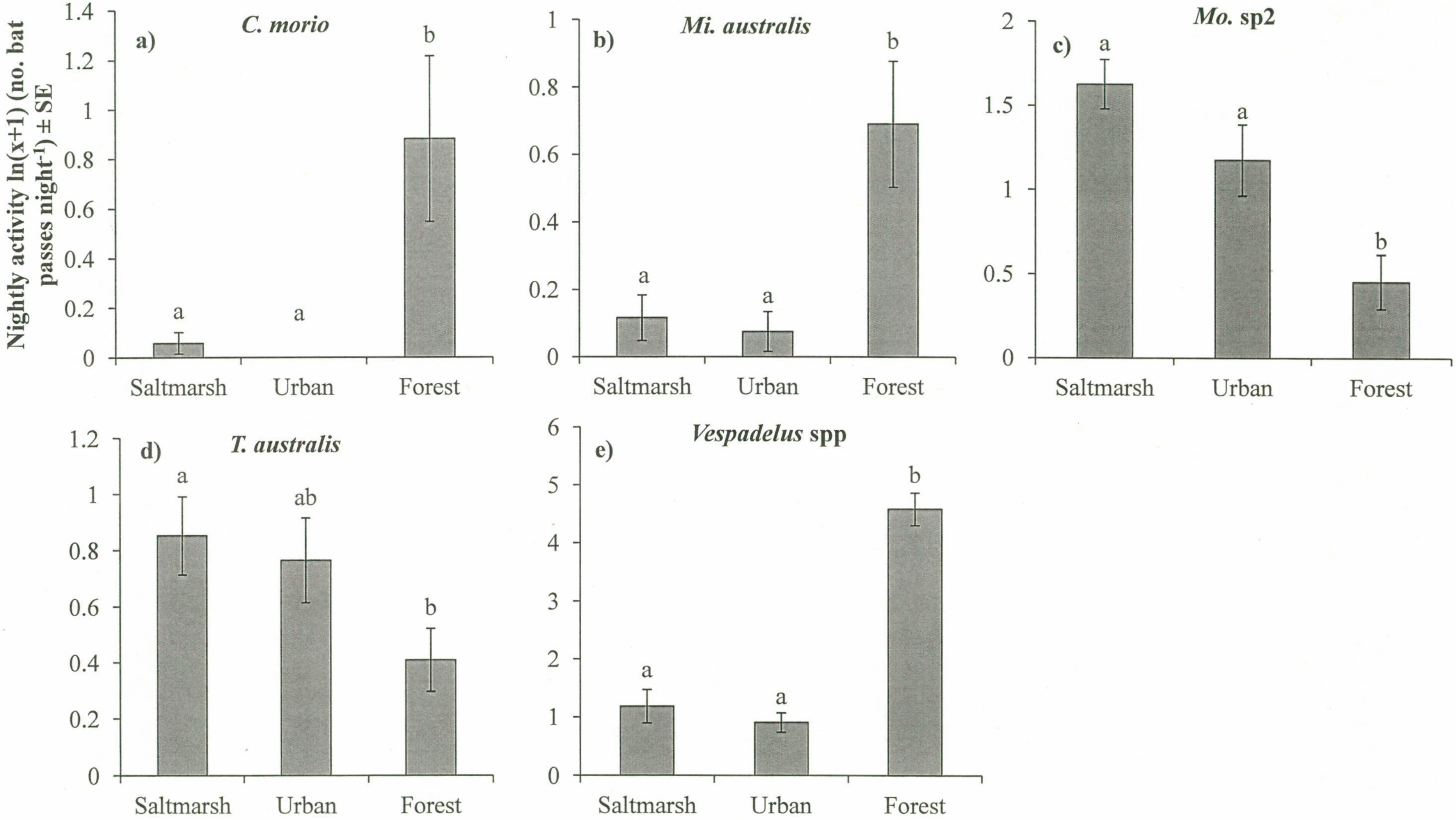

Figure 2.3 Mean nightly bat activity in each habitat: a) *Chalinolobus morio*. b) *Miniopterus australis*. c) *Mormopterus* sp2. d) *Tadarida australis*. e) *Vespadelus* spp. Means denoted by different letters are significantly different from one another.

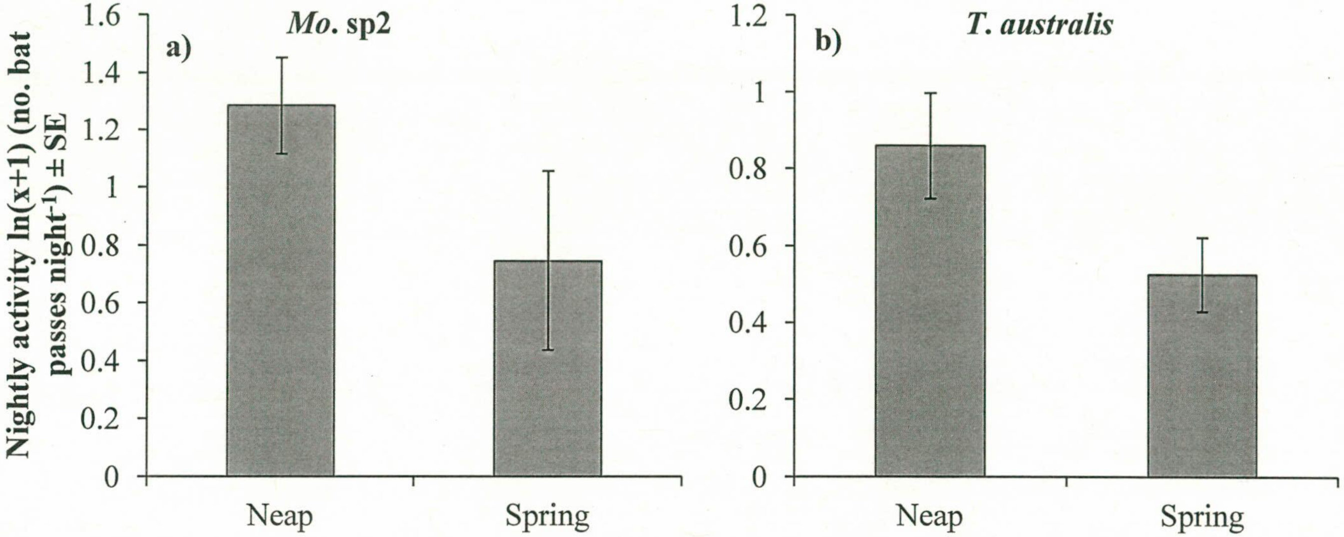

Figure 2.4 Mean nightly bat activity during neap and spring tides: a) *Mormopterus* sp2. b) *Tadarida australis* during neap and spring tides.

2.2.2 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. Feeding activity (proportion of echolocation calls that contained feeding buzzes) differed significantly between habitats (df= 2, χ^2=33.600, P<0.001), with 4.0 %, 0.4 % and 1.6 % of calls recorded in saltmarsh, urban and forest habitats, respectively, containing feeding buzzes. While the number of feeding buzzes recorded during neap tides (159) was greater than spring tides (109), feeding activity did not significantly differ between tidal cycles.

Feeding buzzes were recorded for three species (*C. gouldii* - 15, *M. australis* - 3, *Mormopterus* sp2 - 9) and one species group (*Vespadelus* spp. - 241). The 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. Feeding activity of all taxa combined did not differ between tidal cycles irrespective of habitat.

2.2.3 Mosquito fauna

A total of 70 364 mosquitoes was 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 specimens trapped in saltmarsh, urban and forest habitats, respectively (Table 2.2). The other commonly collected species were *Ae. alternans* (Westwood) and *Culex sitiens* (Wiedemann), two species closely associated with estuarine habitats, *Cx. 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 (F=15.929, P<0.001; Fig 2.5), there was no significant difference between tidal cycle (F=0.413, P=0.526). *Aedes vigilax* abundance was significantly lower in urban habitat when compared to saltmarsh (P=0.003) and forest habitats (P<0.001).

Table 2.2 Mean nightly abundance of mosquito taxa ± SE 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.

2.2.4 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. Two-thirds 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 (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 (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 (F=4.215, P=0.043; 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 (F=9.126, P=0.003), with greater abundances recorded in forest habitat when compared with urban habitat (P=0.003; Table 2.3).

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 (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 (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 also was significantly different between habitats (F=8.420, 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 (F=9.231, P=0.001) with greater abundances recorded in forest habitat when compared with saltmarsh (P=0.001) and urban (P=0.007) habitats (Table 2.3). The abundance of other insects >14-mm in size also was found to differ significantly between habitats (F=4.014, P=0.030), with higher abundances detected in forest habitat when compared with saltmarsh habitat (P=0.049; Table 2.3).

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

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 (F=5.233, P=0.038; F=5.717, P=0.031; Table 2.3).

Table 2.3 Mean 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	1.8**a** ± 1.1	19.4 ± 7.2	5.4 ± 1.5	11.6**b** ± 3.6	40.8 ± 22.5	19.1 ± 7.4	25.8**b** ± 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	192.3**a** ± 73.5	26.4 ± 10.3	23.7 ± 5.1	24.9**b** ± 5.1	87.8 ± 29.3	82.0 ± 25.2	83.8**ab** ± 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	0.3**a** ± 0.1	1.1 ± 0.6	0.1 ± 0.1	0.6**a** ± 0.3	3.8 ± 1.8	3.7 ± 1.4	3.7**b** ± 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**a** ± 0.3	0.9 ± 0.6	0.2 ± 0.1	0.5**ab** ± 0.3	3.8 ± 2.3	1.2 ± 0.5	2.0**b** ± 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	280.4**a** ± 73.9	117.7 ± 27.0	66.9 ± 17.2	89.2**b** ± 16.1	443.7 ± 97.3	360.0 ± 88.2	389.9**a** ± 65.1	330.7**A** ± 63.9	184.9**B** ± 40.2	246.5 ± 36.8
5-9 mm	29.2 ± 8.3	19.5 ± 5.6	24.1**a** ± 4.9**a**	47.3 ± 15.5	22.1 ± 3.9	33.1**ab** ± 7.6	101.5 ±35.4	72.1 ± 25.5	82.6**b** ± 20.3	54.9 ± 12.6	38.6 ± 10.0	45.5 ± 7.9
10-14mm	9.7 ± 3.8	2.4 ± 0.6	5.8**a** ± 2.0**a**	11.9 ± 5.7	2.9 ± 0.8	6.8**a** ± 2.7	30.1 ±14.9	17.3 ± 4.0	21.9**b** ± 5.8	15.9 ± 4.8	7.7 ± 2.0	11.2 ± 2.4
>14 mm	2.7 ± 1.2	0.6 ± 0.2	1.6**a** ± 0.6**a**	3.6 ± 1.2	0.9 ± 0.3	2.1**ab** ± 0.6	7.9 ±2.6	7.2 ± 2.8	7.4**b** ± 2.0	4.4 ± 1.0	3.0 ± 1.1	3.6 ± 0.8
All sizes	504.6 ± 124.4	143.1 ± 27.3	311.8**ab** ± 74.9	180.5 ± 42.2	92.9 ± 20.5	131.2**b** ± 23.7	583.2 ±134.4	456.5 ± 109.9	501.8**a** ± 83.9	405.9**A** ± 69.8	234.2**B** ± 50.1	306.7 ± 42.8

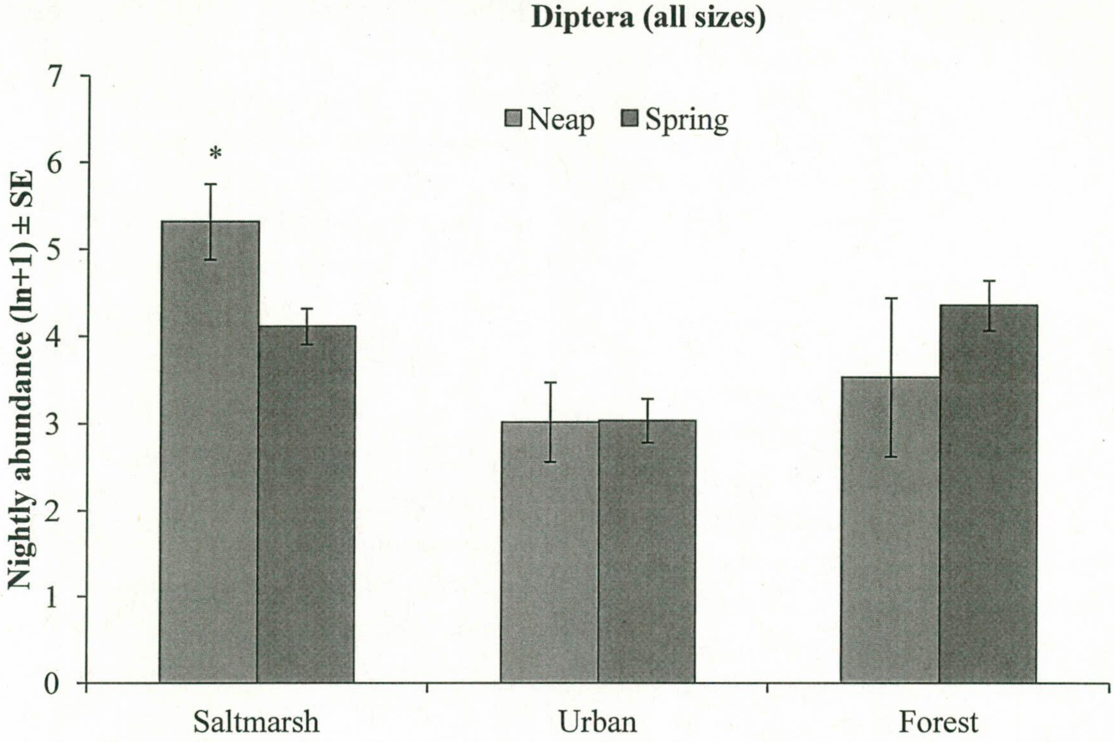

Figure 2.6 Nightly abundance of dipterans (all sizes) in each habitat during neap and spring tides. Asterisk denotes significant interaction effect.

2.2.5 Relationships between bat activity and mosquito and non-mosquito prey abundance

Relationships between response variables (nightly bat activity and the activity of individual species) and predictor variables were not consistent across habitats. In saltmarsh, lepidopteran and coleopteran abundances together accounted for 81.7 % of variability in nightly bat activity (R^2=0.817, F=26.849, P<0.001, df=14).

Abundances of lepidopterans and coleopterans were positively associated with nightly bat activity accounting for 36.9 % and 44.8 % of variability, respectively. The abundance of lepidopterans was positively correlated with the activity of *C. gouldii* (R^2=0.306, F=5.725, P=0.033, df=14) accounting for 30.6 % of variability in the activity of this species. The activity of *Mormopterus* sp2 was positively correlated with nightly insect abundance, accounting for 51.9 % of variability in the activity of this species (R^2=0.519, F=14.017, P=0.002, df=14). *Vespadelus* spp. activity was positively correlated with the abundances of coleopterans, large insects (>14 mm) and *Ae. vigilax*, together accounting for 90.7 % of variability in the activity of this species group (R^2=0.907, F=35.660, P<0.001, df=14). Coleopteran abundance accounted for most of the variability (63.4 %), while the abundance of large insects (>14 mm) and *Ae. vigilax* accounted for 13.8 % and 13.5 % of variability, respectively.

In the urban habitat, a significant relationship between prey abundance variables and the activity of bats only was only observed for *Vespadelus* spp., with activity positively correlated with the abundance of dipterans, accounting for 41.1 % of variability in the activity of this species group (R^2=0.411, F=9.757, P=0.007, df=15).

In the forest habitat, dipteran abundance was positively correlated with the activity of *C. gouldii*, accounting for 37.0 % of variability in the activity of this species (df=14, R^2=0.370, F=7.061, P=0.021). The abundance of *Ae. vigilax* was positively correlated with the activity of *Vespadelus* spp accounting for 51.6 % of variability in the activity of this species group (R^2=0.516, F=12.812, P=0.004, df=14).

2.3 Discussion

The results of this work indicate that although prey (*Ae. vigilax* and all non-mosquito prey) were generally most abundant in saltmarsh and forest habitats when compared with the urban habitat, relationships between prey abundances and 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, further highlighted by greater feeding activity recorded in the less cluttered saltmarsh habitat. However, *Ae. vigilax* abundance was positively correlated with the activity of bats of the *Vespadelus* genus in both saltmarsh and forest habitats, suggesting that any interacting effects of clutter and prey abundance on prey detectability may not equally apply to all bat taxa, particularly those with greater manoeuvrability and an echolocation design suited for detection of small prey among clutter (Barclay & Brigham 1991). While positive relationships between *Ae. vigilax* abundance and the activity of bats of the *Vespadelus* genus support suggestions that *Ae. vigilax* may be an important prey resource for these bats (Laegdsgaard *et al.* 2004), a dietary study is required to confirm these suggestions.

2.3.1 Bat commuting and feeding activity

The activity of individual species differed between habitats, but these differences were in line with predictions based on echolocation design and wing morphology. *Chalinolobus morio*, *M. 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. The activity of *Mormopterus* sp2 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 *Mormopterus* sp2 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 the 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 & Fenton 2005; Hourigan *et al.* 2006), other studies have failed to detect significant differences in bat activity between urban and vegetated habitats (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 (mangrove swamps) of saltmarsh are known to be productive foraging habitats for bats elsewhere (McKenzie & Rolfe 1986; Hoye 2002), only one study has documented feeding activity of bats in saltmarsh (Belbasé 2005). The higher feeding activity recorded in saltmarsh in this study indicates that it may be more efficient to detect and capture prey in the more open (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. This hypothesis may serve as a reasonable explanation for the discrepancy in feeding activity detected between saltmarsh and forest habitats, particularly since the overall abundances of prey (mosquito and non-mosquito) were similar in both habitats.

Feeding activity of two bat taxa (*C. gouldii* and *Vespadelus* spp.) also was significantly higher in the more open saltmarsh habitat than the urban and forest habitats. While similar levels of commuting 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 more-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 & Taylor 1986; Rhodes 2002). Although these bats were significantly more active in forest when compared with both saltmarsh and urban habitats, the greater feeding activity recorded in saltmarsh by this 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).

2.3.2 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 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 larval pools. Forest habitat likely provides adult *Ae. vigilax* populations with 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 insect abundance of each taxa decreased with body size in each habitat. This trend has been reported previously in a study investigating relationships between arthropod abundance and body size in an Indonesian rainforest (Stork & Blackburn 1993). Average nightly insect abundance was significantly higher in forest habitat when compared with the urban habitat. While this trend has been observed previously in other studies (Avila-Flores and Fenton 2005), it 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.

Taxa that comprised ‘other insects’ included hemipterans, blattodeans, hymenopterans, isopterans, orthopterans and odonatans. While presence of most of these taxa in light trap collections was rare throughout the sampling period, abundances of these taxa were high on occasions. This is particularly true for isopterans (represented mainly by winged alates) that were noticeably abundant during one fortnight of sampling that coincided with nuptial flights of this taxa. Given the high protein, fat and water content of this prey taxa, isopterans may represent energy rich prey for bats (Nutting 1969). However, there was no noticeable increase in bat activity in any of the habitats during the isopteran swarms.

In this study, nightly insect abundance and 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 or lunar illumination. 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 & 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).

2.3.3 Relationships between prey abundance and bat activity

Relationships between the activity of bats and *Ae. vigilax* abundance are likely to be influenced by the availability of the small prey resource to all bats. Echolocation frequency is thought to restrict the lower limit of prey sizes that bats are able to detect (Møhl 1988), with longer wavelengths associated with low frequency echolocation, considered to be unsuited to detection of small prey at distances sufficient for interception by bats (Barclay & Brigham 1991). Minimum detectable prey size of bats in this study (based on predictions from Møhl 1988) would suggest that positive relationships between *Ae. vigilax* abundance and bat activity would be restricted to five high-frequency echolocating bat taxa (*Mi. australis*, *K. papuensis*, *Nyctophilus* spp., *R. megaphyllus* and *Vespadelus* spp.; Table 2.4).

In this study, the 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 given minimum detection sizes of prey for each bat species (Table 2.4). However, the activity of bats in the *Vespadelus* genus (*V. pumilus* and *V. vulturnus*) 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. 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). No relationship between *Ae. vigilax* abundance and any of the other four taxa considered to be able to detect *Ae. vigilax* was identified. Although *Ae. vigilax* represents an abundant prey resource for these bats, increased energetic requirements of these larger bats in association with the lower profitability of *Ae. vigilax* relative to other similarly-sized prey taxa may diminish the importance of *Ae. vigilax* as a prey resource to these bats (Table 2.4).

Many studies have found bat activity to be positively correlated with the abundance of insects (de Jong & 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 and coleopterans together were significant predictors of nightly bat activity in the saltmarsh habitat, accounting for 81.7% of variability observed in this habitat. Studies investigating the 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, the failure to detect any significant relationships between prey abundance and nightly bat activity in the urban and forest habitats may reflect a negative influence of clutter on prey detectability. 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 vegetation and domestic vegetation as well as urban structures. e.g.. telegraph poles) were acoustically more complex than more open saltmarsh habitat. 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).

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 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* sp2, and *Vespadelus* spp.). Two of these taxa are clutter-sensitive, low-frequency echolocating bats (Fullard *et al.* 1991). *Chalinolobus gouldii* was positively correlated with abundance of lepidopterans, while *Mormopterus* sp2 was positively correlated with nightly insect abundance. Given the restrictions imposed on prey detection by echolocation frequency, it is surprising that activity levels of *C. gouldii* and *Mormopterus* sp2 were positively correlated with the abundance of small prey and not larger prey (data not presented) 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 greatest target strength (glint). For both *C. gouldii* and *Mormopterus* sp2, under 'glint' conditions, abundant smaller prey taxa are predicted be detectable by both species (Table 2.4). In support of this hypothesis is the presence of small dipterans (chironomids and mosquitoes) in the diets of many medium-large sized bats in Europe 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 *Mormopterus* sp2 in other areas (unpublished data – L. Lumsden and J. Wainer).

The strongest positive relationship between prey abundance and bat activity in 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 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 abundances of large insects (>14 mm) and *Ae. vigilax* accounted for a smaller amount of the variability. Given morphological constraints on this taxa associated with jaw size (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 abundance of *Ae. vigilax* (typically < 5 mm) with the activity of *Vespadelus* spp. is more likely to reflect an ecologically-relevant response.

Only one relationship was observed between prey abundance and activity of a clutter-sensitive bat (*C. gouldii*) in the more cluttered forest habitat. *Chalinolobus gouldii* employs broadband frequency-modulated quasi-constant frequency (FM-QCF) echolocation calls, typical of edge-space foraging bats (Adams *et al.* 2009). Additionally, use of alternating frequencies in successive pulses may allow for detection of near targets in a cluttered forest (Jones & Corben 1993). The other positive relationship between prey abundance and bat activity in the forest habitat was observed for clutter-tolerant taxa. *Aedes vigilax* abundance was correlated with the activity of *Vespadelus* spp.. Since this bat taxa is suited to foraging close to the edges of cluttered vegetation, as well as detecting small prey such as mosquitoes, it is not surprising that a strong positive relationship was observed between the activity of this taxon and *Ae. vigilax* abundance. However, small lepidopterans (<5 mm) also were abundant in forest habitat, yet did not significantly account for variability in the activity of this bat taxon. One possible explanation for this may be the lower profitability of tympanate moths, able to detect and avoid echolocating bats, making their capture more difficult than other non-tympanate taxa by bats. However, since no attempts in this study were made to identify presence of tympanal organs on lepidopteran samples, further investigation is required.

2.3.4 Conclusion

While prey (*Ae. vigilax* and all non-mosquito prey) were generally most abundant in saltmarsh and forest habitats, positive associations between bat activity and prey abundance as well as greater feeding activity occurred in less cluttered saltmarsh habitat, indicating a potential interaction of effects of clutter and prey abundance on bat activity. *Aedes vigilax* abundance was positively correlated with activity of bats of the *Vespadelus* genus supporting suggestions that *Ae. vigilax* may be an important prey resource for these bats.

Table 2.4 Minimum detection sizes of prey and energetic requirements of each bat taxa recorded in this study.

Species	Mass (g)	Echolocation frequency (kHz)	Min detectable size[a] (mm)	Detectability based on mosquito:		FMR[b] (kjd^{-1})	No. mosquitoes required[c]	No. moths required[c]	Required foraging time[d] (hrs)	
				length (5.2mm)	wingspan (glint situation) (10.7mm)				Mosquito	Moth
Chalinolobus dwyeri	7.7	26.0	10.5		✓	28.91	1 373	355	5.4	1.4
C. gouldii	13.8	34.0	8.1		✓	44.31	2 104	545	8.4	2.2
C. morio	8.9	53.0	5.2	✓	✓	32.14	1 526	395	6.1	1.6
Falsistrellus tasmaniensis	20.5	39.0	7.0		✓	59.20	2 811	728	11.2	2.9
Kerivoula papuensis	6.9	160.0	1.7	✓	✓	26.68	1 267	328	5.0	1.3
Miniopterus australis	6.7	65.0	4.2	✓	✓	26.11	1 240	321	4.9	1.3
Mi. schreibersii oceanensis	14.1	48.0	5.7		✓	45.02	2 138	553	8.5	2.2
Mormopterus sp2	9.0	31.0	8.8		✓	32.41	1 539	398	6.1	1.6
Nyctophilus spp.	12.3	80.0	3.4	✓	✓	40.73	1 934	501	7.7	2.0
Rhinolophus megaphylus	11.5	70.0	3.9	✓	✓	38.78	1 841	477	7.3	1.9
Scoteanax rueppellii	25.4	37.0	7.4		✓	69.26	3 289	851	13.1	3.4
Scotorepens orion	9.3	37.0	7.4		✓	33.19	1 576	408	6.3	1.6
Tadarida australis	37.6	15.0	18.3			92.29	4 383	1 134	17.4	4.5
Vespadelus darlingtoni	7.2	44.0	6.2		✓	27.52	1 307	338	5.2	1.3
Vespadelus spp.	4.4	53.0	5.2	✓	✓	19.19	911	236	3.6	0.9

[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; calorific value of mosquito (n=500) = 10.5 KJg^{-1}, moth (n=250) = 25.5 KJg^{-1}

[d] Foraging time required to obtain enough mosquitoes or moths (<5 mm) to meet FMR requirements assuming 50 % prey attack success based on attack rate reported by Encarnação and Dietz (2006).

3. Importance of vegetation interfaces to foraging bats in Coastal Saltmarsh

3.1 Methods

3.1.1 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 1 km from the Cockle Bay site, and a further 0.75 km 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 (*C. glauca* Sieb.) >10 m in height on the landward side, a seaward side bordered by stands of Grey Mangrove (*Avicennia marina* (Forssk.) Vierh) <5 m in height and an interior primarily composed of characteristic saltmarsh species (e.g., *Sarcocornia quinqueflora* (Bunge ex Ung.-Sternb.) A.J.Scott, *Sporobolus virginicus* (L.) Kunth). Throughout the saltmarsh interior there was a small number of juvenile mangroves transgressing into saltmarsh (as is evident in many saltmarshes in eastern Australia: Saintilan & Williams 1999).

3.1.2 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. Ambient conditions were favourable for bats, with temperature minima experienced during the sampling period ranging from 14.7-15.5° C and temperature maxima 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 1 m height from the marsh surface and 45° 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 navigational and feeding

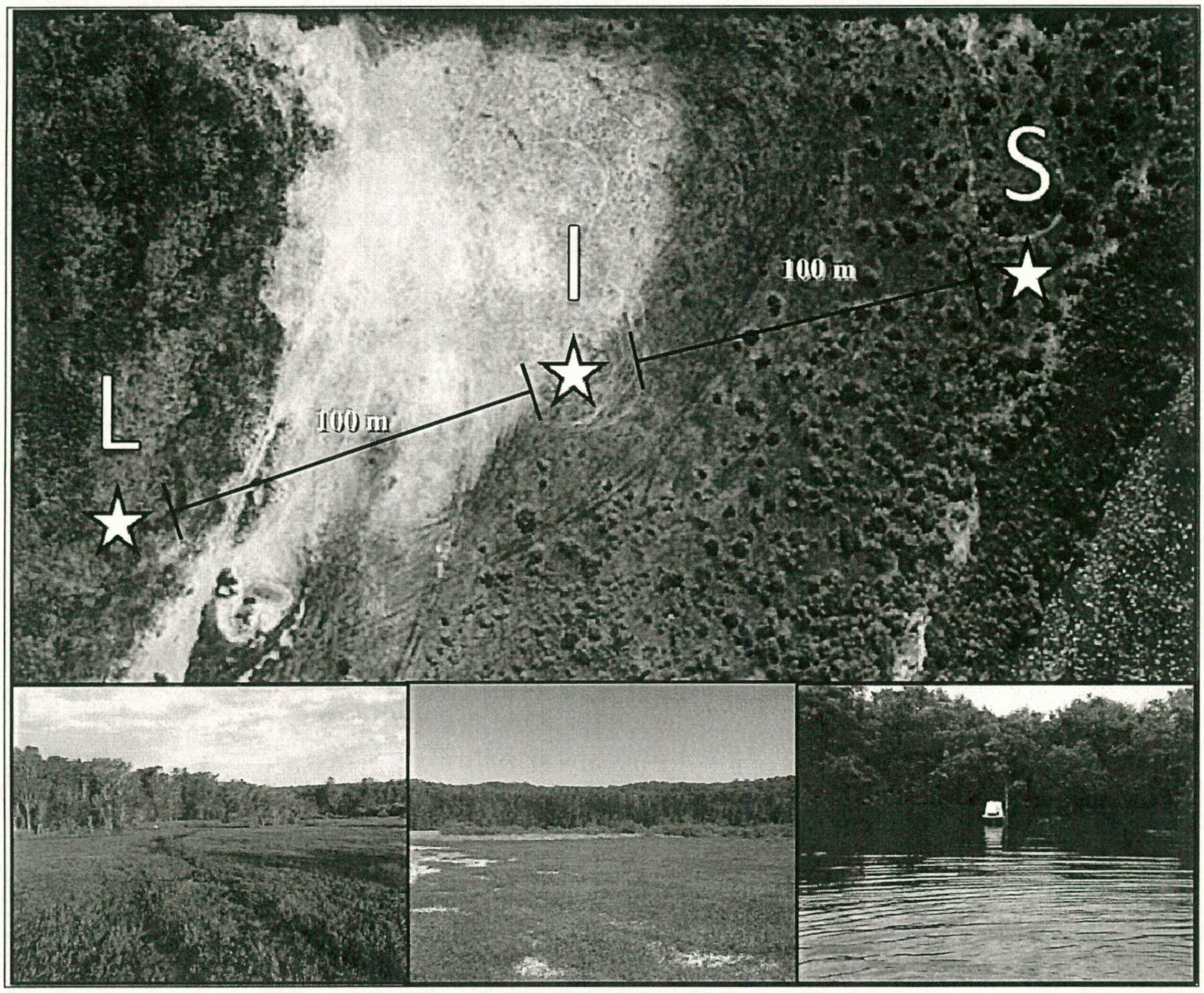

Figure 3.1 Detector location in landward (L), saltmarsh interior (I) and seaward zones (S). Star represents detector position.

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.

3.1.3 Call identification

Recorded bat calls were identified to species level where possible using the automated call identification software, Anascheme (Gibson & 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 only were 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 *N. geoffroyi* (Leach) = *Nyctophilus* spp.; *Vespadelus pumilus* (Gray), *V. troughtoni* (Kitchener, Jones & Caputi), *V. 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 1958; Pennay *et al.* 2004). For each detector and each night, number of bat passes and number of feeding buzzes for each species was tabulated.

3.1.4 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 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 assumptions of sphericity, a Greenhouse-Geisser correction was adopted. Wilcoxon-pairwise comparison was used to identify which zones or sites differed from each other.

3.2 Results

A total of 1 162 bat calls was recorded, representing 12 species and one species group (*Vespadelus* spp.). 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.

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).

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), which both supported greater species richness than the Palmers Lane site (4.8±0.5), but only significantly greater for the Cockle Bay site (Z=-2.221; P=0.026) (Fig. 3.3).

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 Act 1995; * represents taxa detected feeding in saltmarsh (number of buzzes recorded).

Species	Landward	Interior	Seaward
Chalinolobus dwyeri	0.7 ± 0.3	-	-
_Chalinolobus 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
Miniopterus schreibersii oceanensis	0.6 ± 0.3	0.4 ± 0.2	-
Mormopterus norfolkensis	-	-	0.5 ± 0.2
Mormopterus species 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**

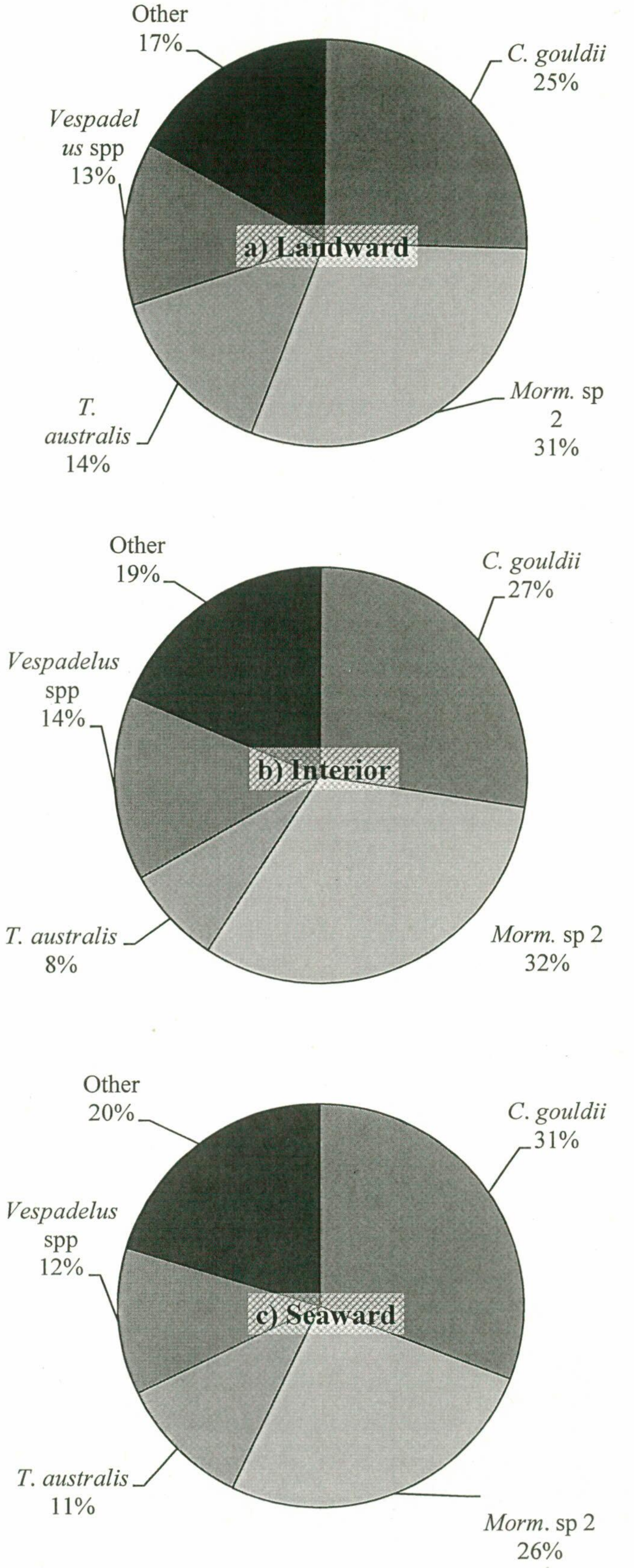

Figure 3.2 Percentage of total bat activity made up by each species in a) Landward edge, b) Interior, and c) Seaward edge. "Other" represents activity from all other species recorded and any calls that could not be assigned to a single taxa.

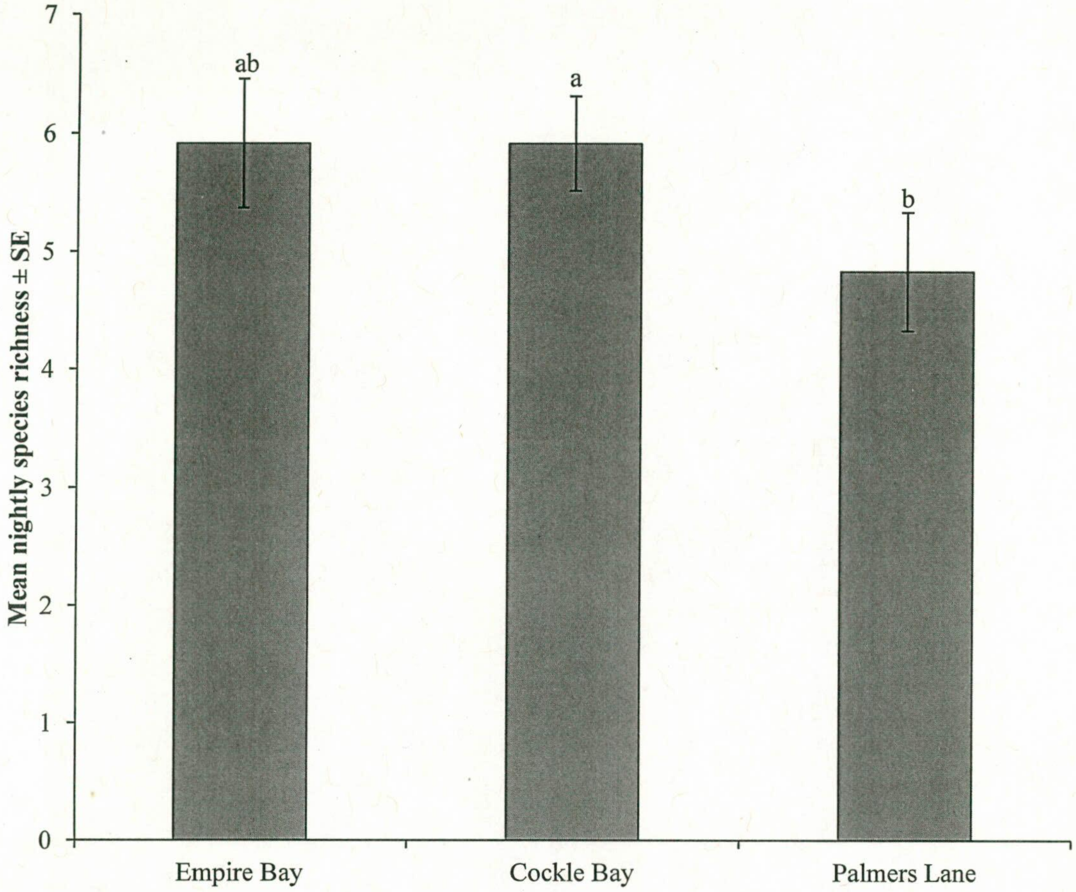

Figure 3.3 Mean nightly species richness of insectivorous bats detected at three coastal saltmarsh sites.

There was a significant difference in mean nightly bat activity (number of bat passes/night) 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).

There was also a significant difference in mean nightly bat activity 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).

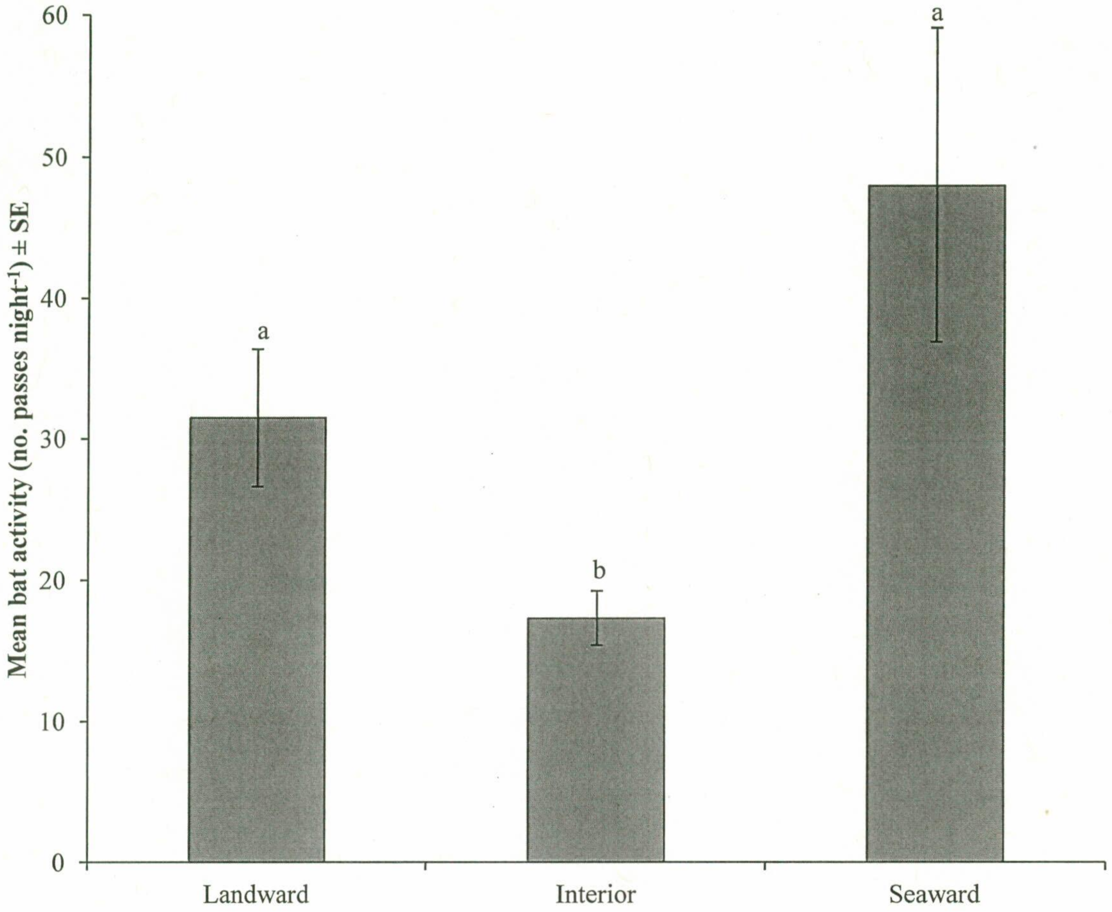

Figure 3.4 Mean nightly bat activity detected in each saltmarsh zone.

Activity (number of bat passes night^{-1}) of individual species was found to differ between saltmarsh zones and also between sites. Activity of *C. dwyeri* (Ryan), *C. gouldii*, *Scotorepens orion* (Troughton), *T. australis* and *V. darlingtoni* (Allen) differed between zones (Table 3.2, Fig. 3.6a,b,d-f), while the difference in activity between zones for *Mormopterus* sp2 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 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 (Figs. 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).

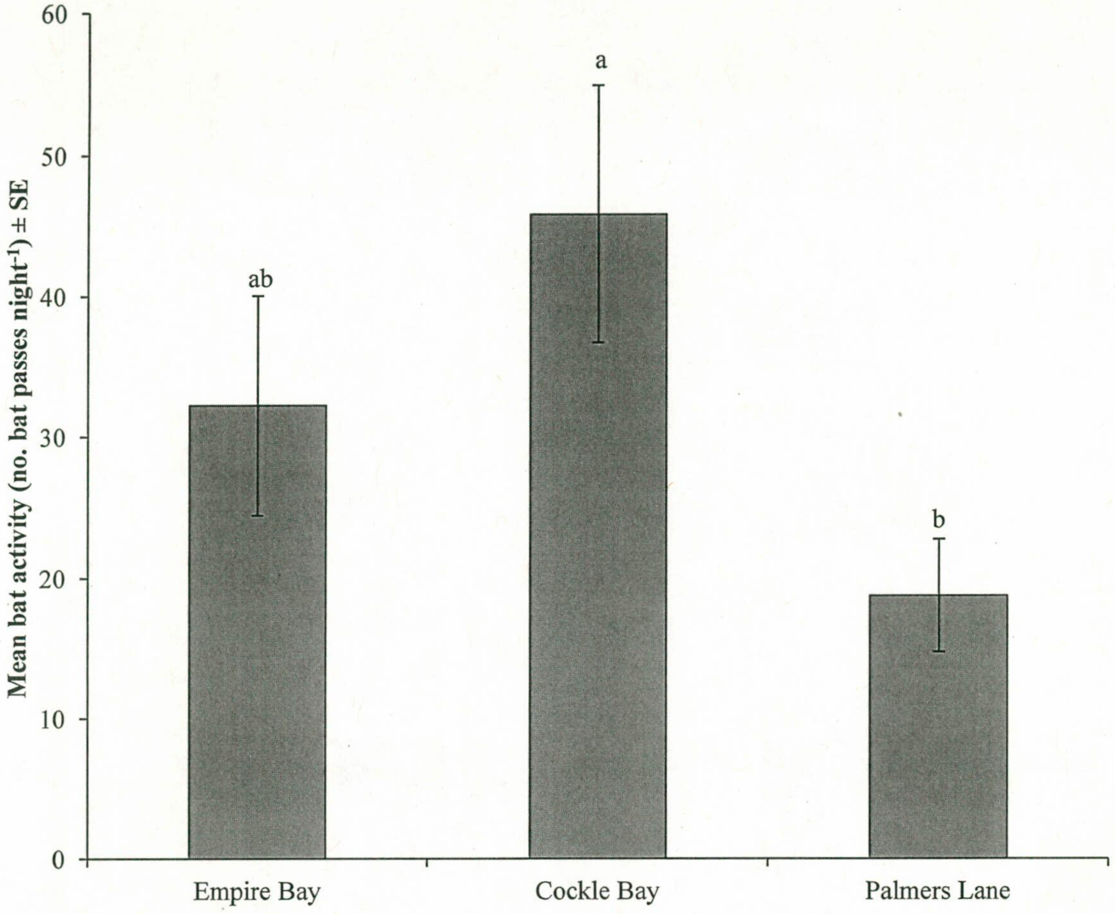

Figure 3.5 Mean nightly bat activity detected in each site. Means denoted by different letters are significantly different from one another

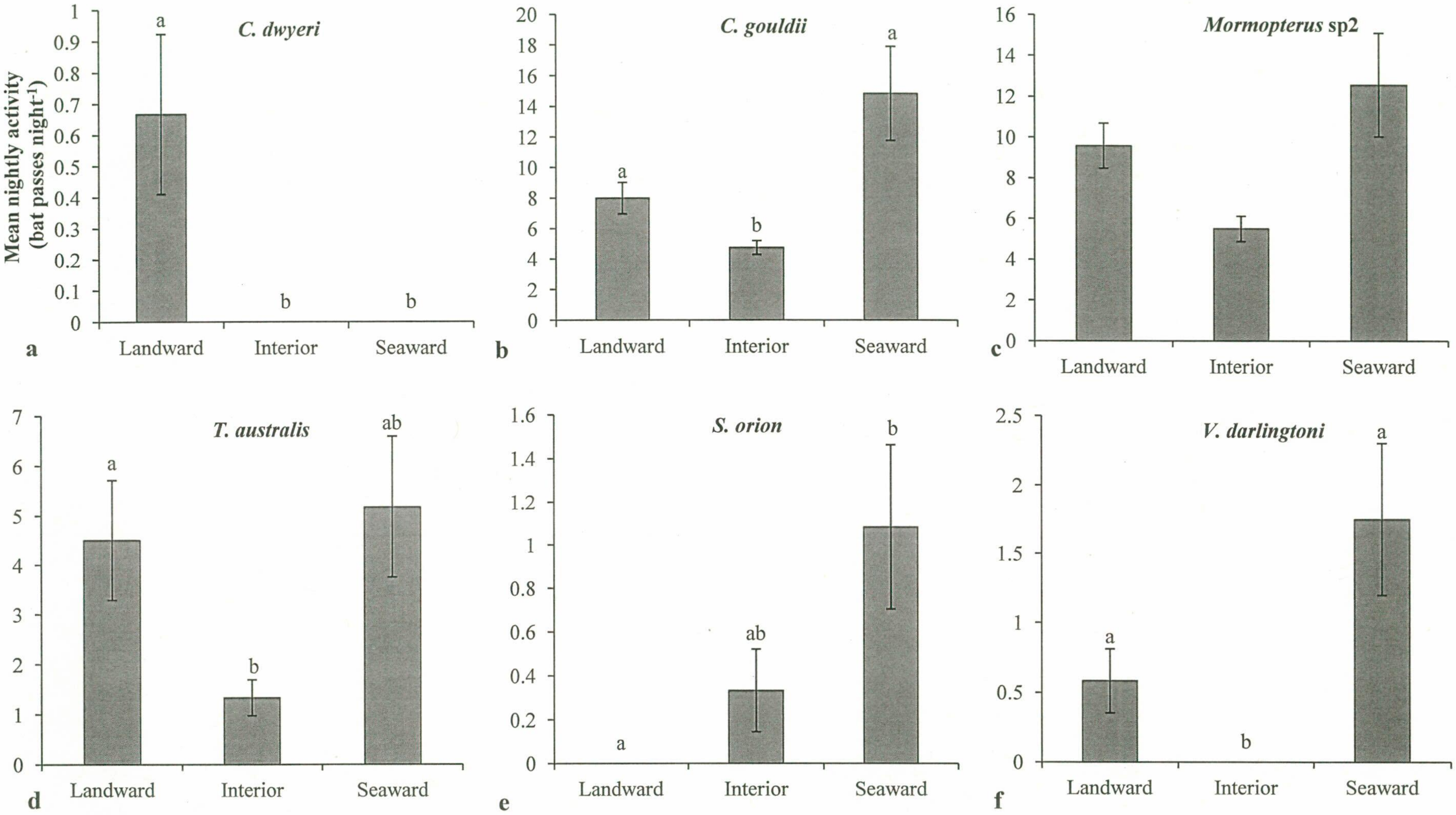

Figure 3.6. Mean nightly activity in each saltmarsh zone: a) *Chalinolobus dwyeri;* b) *Chalinolobus gouldii;* c) *Mormopterus* sp2*;* d) *Tadarida australis;* e) *Scotorepens orion;* f) *Vespadelus darlingtoni.* NB. Means denoted by different letters are significantly different from one another.

Activity of *C. gouldii*, *Mormopterus* sp2 and *T. australis* differed significantly between sites (Table 3.2, Figs. 3.7a-c). The activity of these species was significantly higher in Cockle Bay when compared to Palmers Lane (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	**$24.000_{1,3\ df}$= 0.016**	$1.114_{1.923,\ 5.769\ df}$= 0.387
C. gouldii	**$6.750_{2,6\ df}$= 0.029**	**$7.982_{2,6\ df}$= 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	$5.091_{4.000,\ 12.000\ df}$= **0.012**
M. 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
Mormopterus sp2	**$7.194_{2,6\ df}$= 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
Scotorepens orion	$1.780_{1.003,\ 3.010\ df}$ = 0.247	**$14.778_{2,6\ df}$= 0.005**	$2.714_{2.038,\ 6.113\ df}$= 0.143
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
Tadarida australis	**$12.019_{2,6\ df}$= 0.008**	**$11.420_{2,6\ df}$= 0.009**	$2.464_{2.355,\ 7.064\ df}$= 0.151
Vespadelus darlingtoni	$0.866_{2,6\ df}$= 0.467	**$9.106_{2,6\ df}$= 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

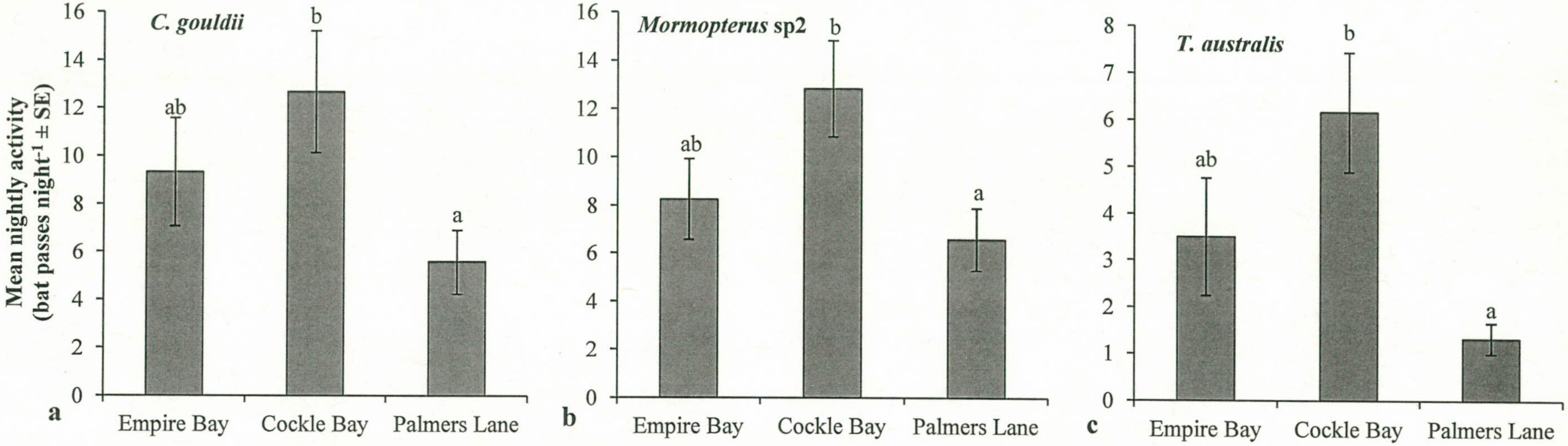

Figure 3.7 Mean nightly activity at each site: a) *Chalinolobus gouldii*; b) *Mormopterus* sp2; c) *Tadarida australis*. Means denoted by different letters are significantly different from one another.

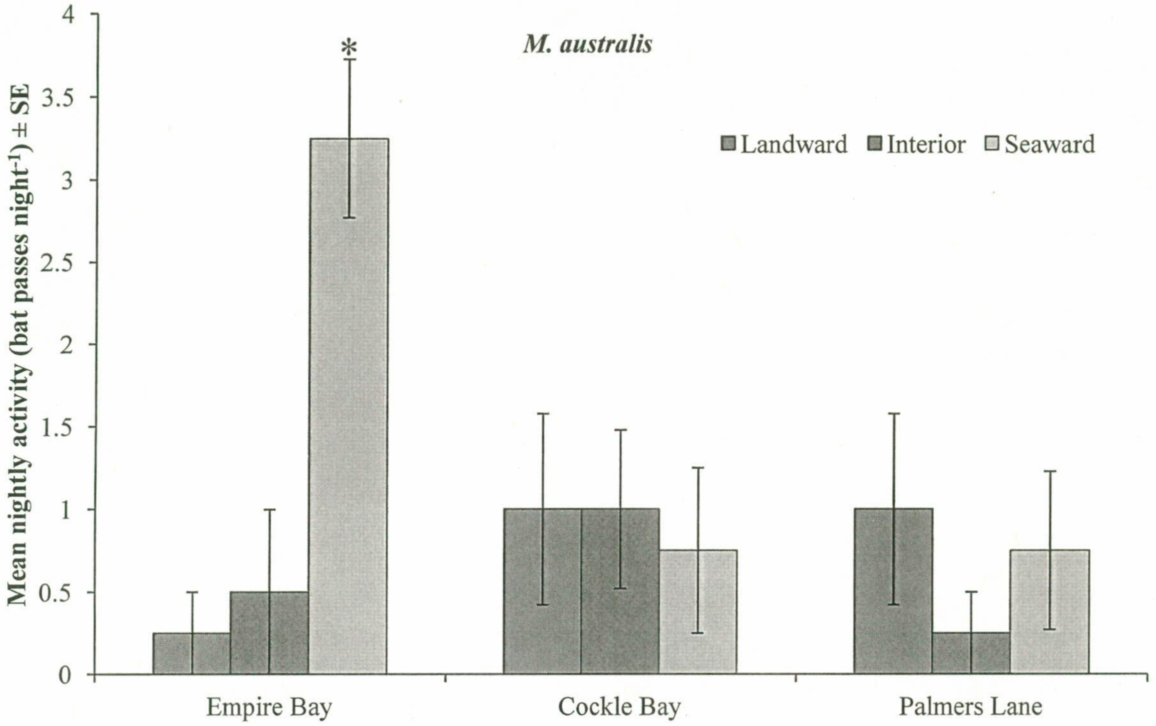

Figure 3.8 Column graph illustrating mean nightly activity of *Miniopterus australis* in each saltmarsh zone at each site. NB. Asterisk indicates a significant interaction effect of zone and site on activity.

3.3 Discussion

Total bat activity and 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 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 predicted elevations in sea level alter the intertidal dynamic (Nicholls *et al.* 1999).

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.

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 navigational 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. Lamb (2009) recorded an average of 12±3 passes night^{-1} in the saltmarsh interior during surveys in 2008-2009, compared to an average of 17±2 passes night^{-1} recorded during our study. The level of bat activity recorded in both studies was low relative to the level of activity reported for forest habitat (119±15 passes night^{-1}) in the study area (Lamb 2009). 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, Lamb (2009) recorded up to nine species and two species groups of insectivorous bat fauna in the saltmarsh interior (Lamb 2009), while only seven species and one species group were recorded during our 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, rarely were recorded by Lamb (2009) (0.014 % of total bat activity), but were detected in the saltmarsh edge microhabitats during our study. Furthermore, three additional bat species (*Mo. 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, Mormopterus* sp2, *T. australis* and *Vespadelus* spp. (species complex including *V. pumilus*, *V. troughtoni* and *V. vulturnus*)). Three of these taxa (*C. gouldii*, *Mormopterus* sp2 and *T. australis*) are clutter-sensitive (Fullard *et al.* 1991; Law & 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 *Mormopterus* sp2 represent the edge-space foraging guild. 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 & Taylor 1986; Rhodes 2002). Consisting 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 (Unpublished data - L. Gonsalves).

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 our 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 & de Jong 1996; Verboom & Spoelstra 1999; Kusch *et al.* 2004). For example, within forests of British Columbia, Canada, activity of foraging bats was reported to be greatest along the edges of cutblocks (Grindal & Brigham 1999). During the same study, insect availability followed the same trend and was greatest along edges. In a study on the NSW central-western slopes, 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 has been documented, there is some uncertainty as to whether the structural differences have a direct or indirect influence on bat activity. Wind speed affects 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 greater bat activity recorded along both saltmarsh edges reflected higher concentrations of insects along these edges. While this may be a reasonable hypothesis, most 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 & 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 & Huitema 1997), while larger insects, not as dependent on the wind-sheltering effect may be available in more open habitats (Taylor 1974; Verboom & 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, availability of these larger insects will be restricted to larger bat species that are morphologically capable of consuming these prey items. Since activity recorded in the saltmarsh interior was not typically restricted to larger bats, it is unlikely that lower levels of bat activity in the saltmarsh interior reflect more profitable foraging conditions.

Although edges are important, we recorded moderately high activity within the interior of saltmarshes. Due to landward transgression of mangroves, occurring in saltmarshes of south-eastern Australia (Saintilan & 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 & 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 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.

This 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, retention of strips of mangroves and casuarinas would be beneficial to bats, providing an edge for foraging bats.

4. Importance of *Aedes vigilax* in insectivorous bat diets

4.1 Methods

4.1.1 Study site and 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).

Within the study area, large areas of estuarine habitats (primarily coastal saltmarsh and mangrove swamps) support highly abundant populations of *Ae. vigilax* throughout the austral summer. Population abundances of *Ae. vigilax* are 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 variation in *Ae. vigilax* population abundances, sampling was undertaken during spring and neap tides during the austral summer of 2009/10. Faecal samples for dietary analysis were obtained from five insectivorous bat species that range in size (mean mass, 4-14 g). These species were the Gould's wattle bat (*Chalinolobus gouldii*) (Gray), little bent wing bat (*Miniopterus australis*) (Tomes), Gould's long-eared bat (*Nyctophilus gouldi*) (Tomes), eastern forest bat (*Vespadelus pumilus*) (Gray) and little forest bat (*V. vulturnus*) (Kunz & Whitaker Jr). While the five species range in size (*C. gouldii*, 14 g, 43.7 mm; *M. australis*, 6.7 g, 39.3 mm; *N. gouldi*, 12 g, 44 mm; *V. pumilus*, 4.4 g, 30.9 mm; *V. vulturnus*, 4.0 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. *Nyctophilus 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).

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 (Fig. 4.1). 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 *M. australis* were collected during a one-off trapping event in a sea cave within the national park in February 2011, during which no prey abundance data were collected (Fig. 4.1). Mitochondrial DNA (mtDNA) belonging to prey items within faecal samples was then used to identify the prey of the five insectivorous bat species.

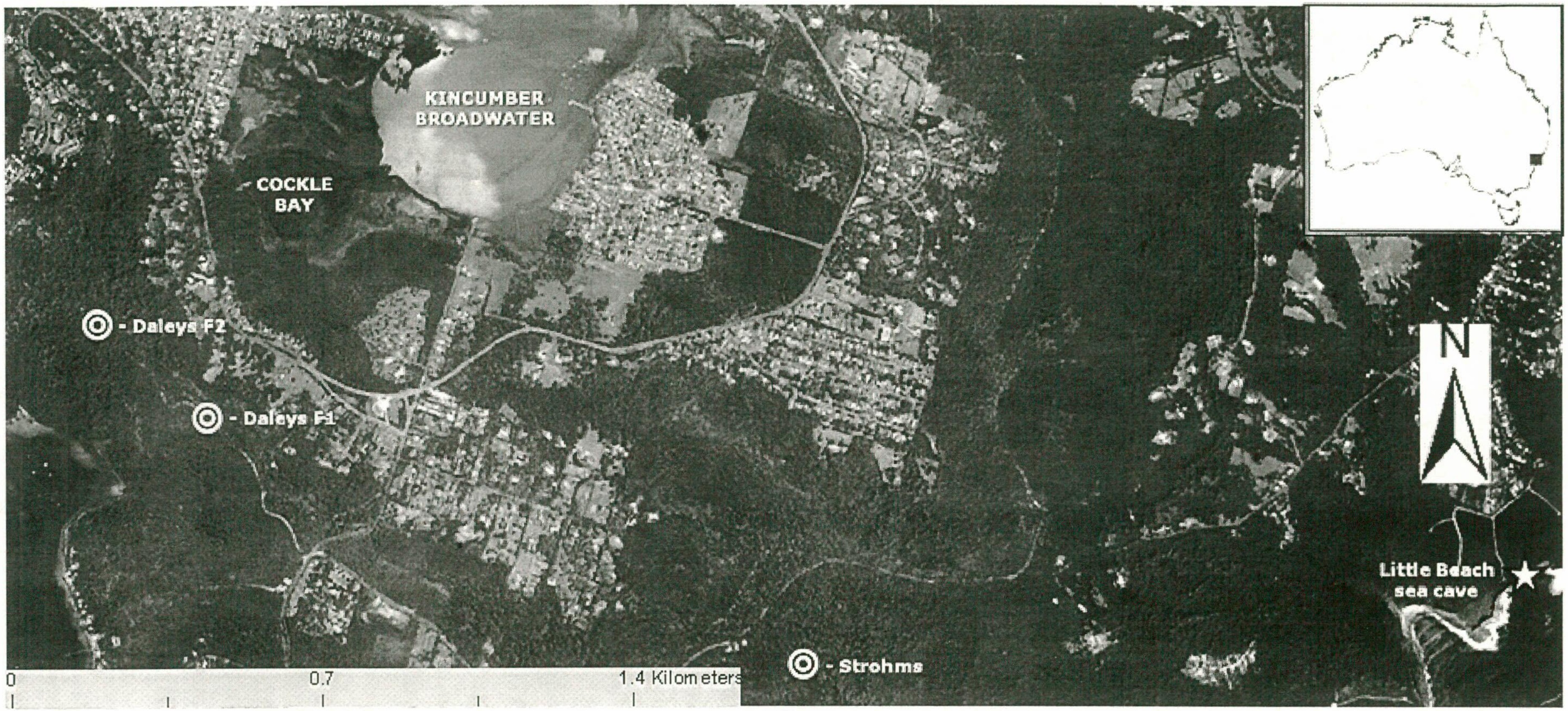

Figure 4.1 Satellite image of study area (adapted from Google Earth). Doughnuts represent harp trapping locations along Daleys Point and Strohms fire trails in Bouddi National Park. Star represents location of sea cave in which *Miniopterus australis* individuals were trapped in 2011.

4.1.2 Collection of guano

General patterns such as peaks in population abundances of saltmarsh mosquitoes can be predicted (Kokkinn *et al.* 2009), with more abundant populations tending to be present two weeks after inundation of saltmarshes by spring tides and/or heavy rainfall. For this reason, where possible, each sampling occasion coincided with either a spring or neap tide, predicted to sustain relatively large and relatively smaller mosquito abundances. To sample predicted peaks and troughs of mosquito abundance, bat trapping was conducted for two consecutive nights in each of eight fortnights during the austral summer of 2009-2010 using harp-traps (Tidemann & Woodside 1978) set along flyways on two fire trails within Bouddi National Park. Traps were not set in saltmarsh habitats where *Ae. vigilax* emerges because of difficulty in trapping bats in open habitats where trees are 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 (little bent wing bat, *M. australis*, eastern bent wing bat, *M. schreibersii oceanensis* (Maeda) and the eastern horseshoe bat, *Rhinolophus megaphyllus* (Gray)). Captured bats (*M. 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. Guano produced by bats in calico bags was transferred to 1.5 mL microcentrifuge tubes and was frozen immediately to reduce the chance of further degradation of faecal DNA. Guano was collected from 52 individuals (five *C. gouldii*, seven *M. australis*, ten *N. gouldi*, ten *V. pumilus*, 20 *V. vulturnus*) (Fig. 4.2).

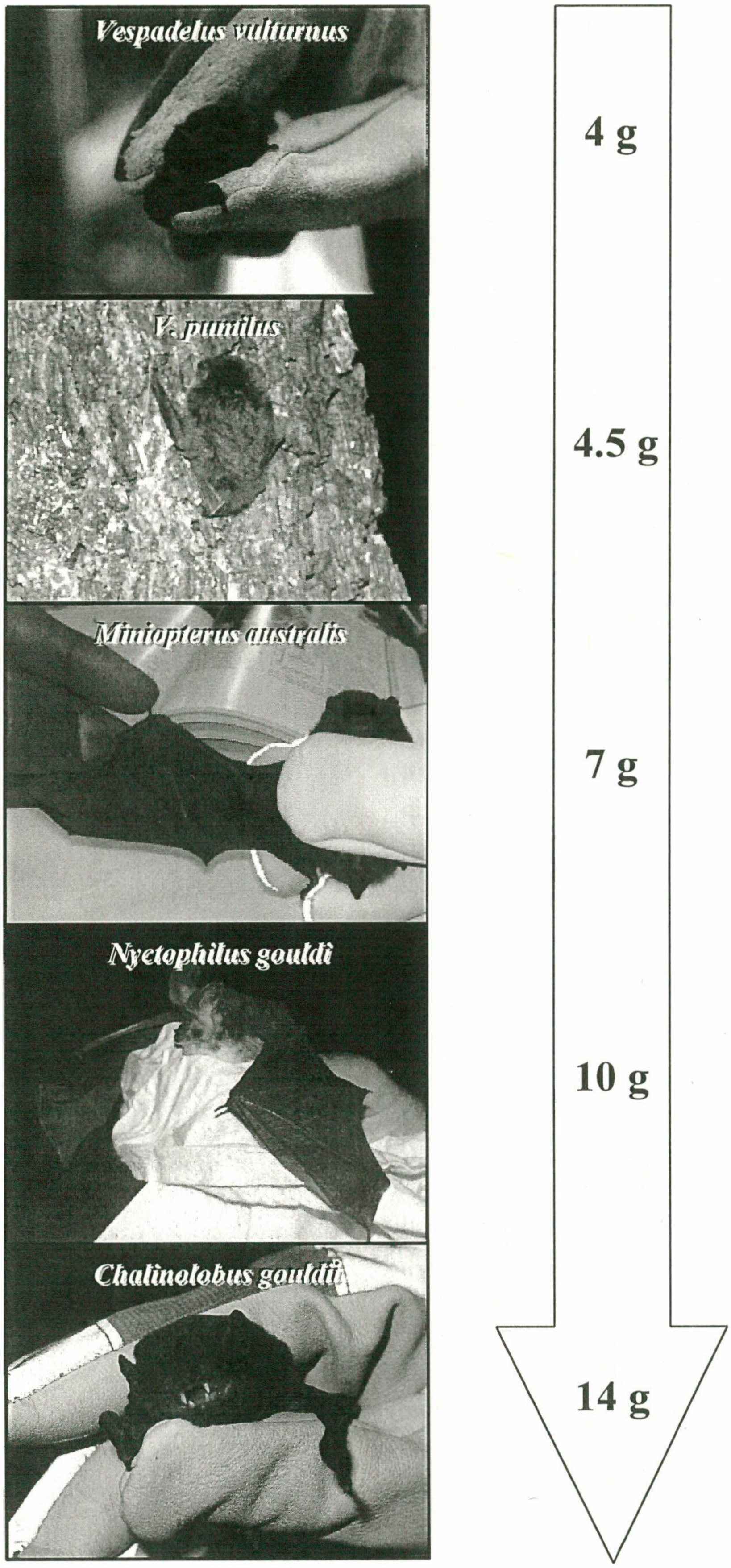

Figure 4.2: Bat species investigated in dietary study. Average mass of bat (taken from Churchill (2009)) is listed beside its picture.

4.1.3 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 & 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. Nightly abundance of each insect order was recorded.

4.1.4 Analysis of guano samples

Genomic DNA was extracted from a pooled sample of five guano pellets for each trapped individual using the Ultraclean Fecal DNA kit (Mo Bio Laboratories) and QIAamp DNA Stool Mini Kit (Qiagen). The Ultraclean Fecal DNA kit was used to extract DNA from the guano of *V. vulturnus*, while the QIAamp DNA Stool Mini Kit was used for all other extractions. Using the Ultraclean Fecal DNA kit, extractions were carried out following manufacturer's instructions, with an additional step in which fecal pellets were broken apart using sterile toothpicks. The QIAamp stool mini kit was used to extract DNA as described by Zeale *et al.* (2011). Taxon-specific primers, ZBJ-ArtF1c and ZBJ-ArtR2c (Zeale *et al.* 2011) were used to amplify a 157bp section of the DNA barcoding region, cytochrome oxidase I, prior to cloning and sequencing. For each individual bat, a sub-sample of 16 clones was purified and sequenced at the Australian Genome Research Facility (Westmead Millennium Institute, Sydney). DNA sequences were entered into the identification engine on the barcoding of life database (BOLD) and the nearest sequence match and percent similarity of each sequence was recorded. Taxonomic assignment to order, family, genus or species was made 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.

4.1.5 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 guano was manufactured with mealworms and varying concentrations of mosquitoes (by volume). One millilitre of each mosquito solution - 0 %, 5 %, 10 %, 15 %, 20 % and 100 % of the mixture was used in DNA extraction and subsequent procedures as described above for bat guano, except that sequencing occurred without the cloning step. Each mosquito concentration was tested in triplicate. If a sequence appeared to be a mixed sequence, it was inferred that both the mosquito and mealworm DNA had been amplified and therefore detected. To confirm this inference, one

PCR product from each mosquito with ten clones sequenced from each clone library.

4.1.6 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 paired samples-t-test was used to investigate whether consumption of each prey taxa reflected prey availability.

4.2 Results

4.2.1 Prey abundance

Fourteen mosquito species were sampled across all sites during the study (Table 4.1). Of these, 12 were recorded during spring and neap tides, respectively. The most abundant species irrespective of tidal cycle was the saltmarsh mosquito (*Aedes vigilax*), representing 77.81 % of all mosquito species recorded during spring tides, and 83.9 % of all mosquito species sampled during neap tides. The other commonly collected species were *Ae. multiplex* (Theobald), *Ae. notoscriptus* (Skuse) and *Culex sitiens* (Wiedemann). As expected nightly *Ae. vigilax* abundances were greater during neap tides, however they were not significantly concentration was cloned and sequenced, different during spring tides (F(1) = 2.125. p = 0.152; Figure 4.3).

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) = 1.982, p = 0.166; Fig. 4.4).

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 (Table 4.2). Coleopterans were the second most abundant taxa, representing 23.8 % of all insects trapped during both spring and neap tides (Table 4.2), while dipterans were the least abundant taxa, 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 (Table 4.2). Abundance of Lepidopterans, Coleopterans, Dipterans and all other taxa did not differ between spring and neap tides (F(1)=3.632, p=0.063; F(1)=0.491, p=0.487; F(1)=0.462, p=0.500; F(1)=3.463, p=0.070; Fig. 4.4).

Table 4.1 Nightly abundances (averaged across sites) ± standard error of mosquito species trapped during spring and neap tides. NB. 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		0.10 ± 0.07 (0.02)
Cx. molestus	4.10 ± 0.77 (1.39)	2.13 ± 0.11 (0.49)
Cx. quinquefasciatus	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

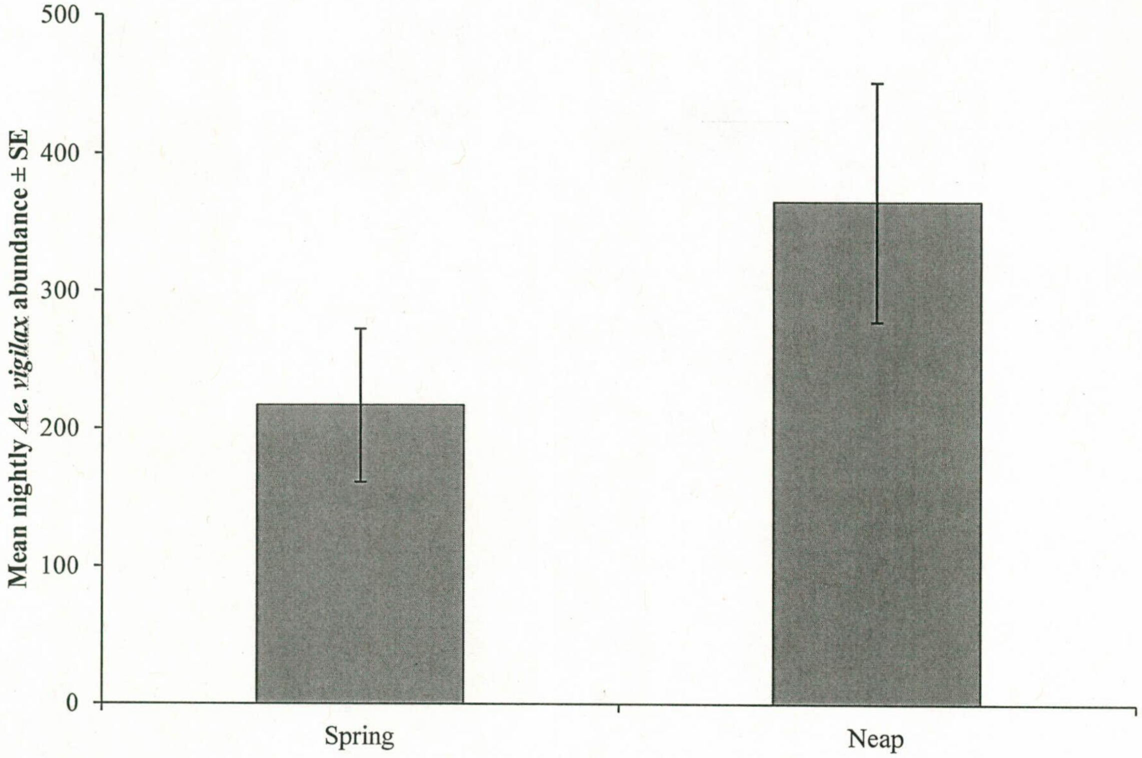

Figure 4.3 Nightly *Aedes vigilax* abundance during spring and neap tides.

Table 4.2 Mean nightly abundance of each insect order ± SE. Values in brackets represent percentage of total nightly insect abundance.

Taxa/class	Spring	Neap
Lepidoptera (moths)	121.71 ± 6.31 (45.53)	138.95 ± 6.45 (48.58)
Coleoptera (beetles)	63.58 ± 3.93 (23.79)	68.19 ± 5.41 (23.84)
Diptera (flies)	34.79 ± 2.04 (13.02)	36.67 ± 1.81 (12.82)
Other	47.21 ± 1.56 (17.66)	42.19 ± 2.27 (14.75)
Total	267.29 ± 9.69	286.00 ± 8.90

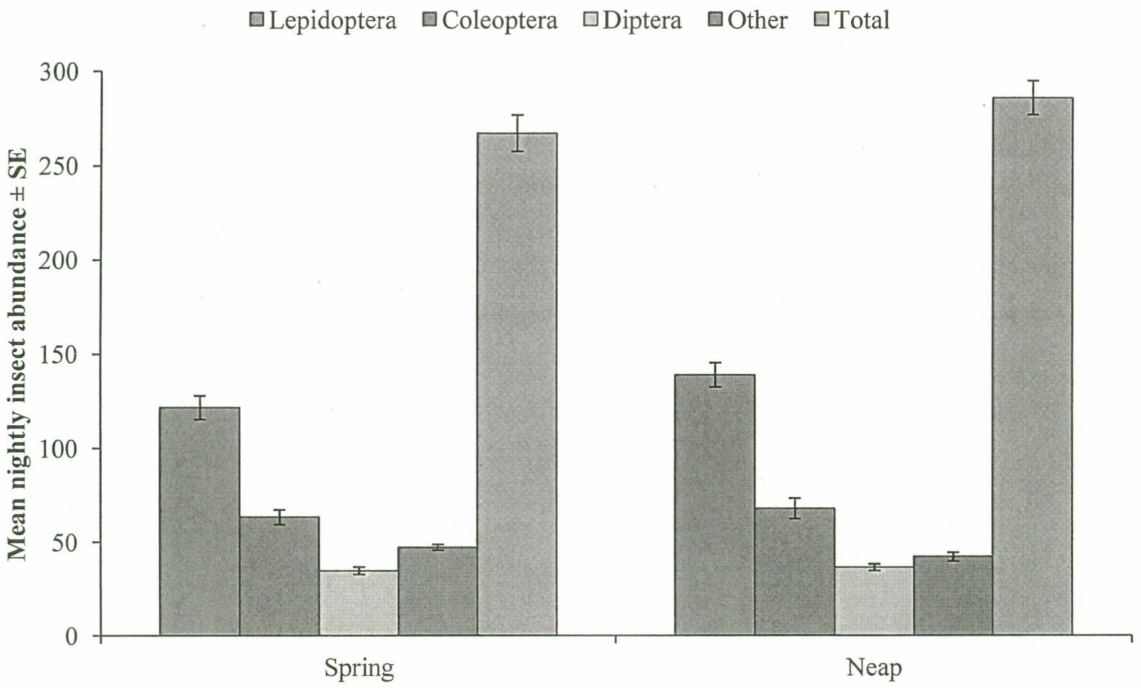

Figure 4.4 Nightly insect abundance during spring and neap tides.

4.2.2 Bat diet

4.2.2.1 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 % *Aedes aegyptii* produced readable sequences that provided species-level matches to either *Tenebrio molitor* (0 % mosquito) or *Ae. aegyptii* (100 % mosquito) (Table 4.3). For each of the other mosquito-mealworm mix (i.e. 5 %, 10 %, 15 %, 20 % and 25 % mosquito), direct sequencing (without cloning) provided mixed DNA sequences that were not readable (Table 4.3). Sequences from one clone library of each different mosquito-mealworm mixture revealed the presence of both *Ae. aegyptii* and *T. molitor*, though the ratio of sequences belonging to *Ae. aegyptii* and *T. molitor* did not appear to be related to the ratios of mosquito-mealworm in the various mixtures (Table 4.3).

Table 4.3: Detectability of mosquito DNA artificial guano with increasing concentrations of *Aedes aegyptii* (by volume; 0-100 %). ✓ represents successful PCR amplification with a corresponding readable DNA sequence matching either *Ae. aegyptii* or *T. molitor* on Barcoding of Life Database (BOLD); × represents successful PCR amplification with a corresponding mixed DNA sequence that could not be attributed to either *Ae. aegyptii* or *T. molitor* without cloning; ° represents a non-readable sequence due to excessive loss of PCR products during purification prior to sequencing.

% *Aedes aegyptii* (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

4.2.2.2 Diets of wild trapped bats

A total of 41 prey taxa was identified from the faeces of 52 insectivorous bats representing five species. All bat species consumed lepidopterans, while three species consumed coleopterans and four species consumed representatives of dipterans and 'other' taxa (Table 4.4). Two bat species consumed the saltmarsh mosquito, *Ae. vigilax* (Table 4.4).

The diet of *Chalinolobus gouldii* consisted of representatives of lepidoptera, coleoptera and blattodea. Lepidopterans were detected in the faeces of 80 % of *C. gouldii* individuals (n=5), while coleopterans and representatives of Blattodea were identified in 40 % of *C. gouldii* individuals, respectively (Table 4.4, Fig. 4.5).

Lepidopterans, dipterans and blattodeans were detected in the faeces of *Miniopterus australis* individuals. Both lepidopterans and dipterans were detected in 71 % of individuals, respectively (n=7), while representatives of blattodea were identified in 29 % of individuals. (Table 4.4, Fig. 4.5).

The diet of *Nyctophilus gouldi* individuals consisted of representatives of lepidoptera, diptera, hemiptera and blattodea. Lepidopterans were detected in the faeces of all individuals (n=10), while dipterans and other taxa (blattodea and hemiptera) were present in 30 % of individuals, respectively (Table 4.4, Fig. 4.5).

Lepidopterans, coleopterans and dipterans were detected in the faecal samples of *Vespadelus pumilus* individuals (n=10). Lepidopterans were detected in the faeces of all individuals, while coleopterans and dipterans were present in the faeces of 10 % and 30 % of individuals, respectively (Table 4.4, Fig. 4.5).

One *V. pumilus* individual also consumed a representative of the non-insect order, Aranae (Table 4.4, Fig. 4.5). *Aedes vigilax*, along with an unknown mosquito species (*Aedes* sp.) was detected in the faecal samples of two (20 %) individuals of *V. pumilus*.

The diet of *V. vulturnus* consisted of representatives of lepidoptera, coleoptera and mosquito-dipterans, which were present in 100 %, 5 % and 55 % of individuals (n=20), respectively (Table 4.4, Fig. 4.5). *Aedes vigilax*, along with an unknown mosquito species (*Aedes* sp.) was found in the faecal samples of 11 individuals of this species.

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.

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
M. 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	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

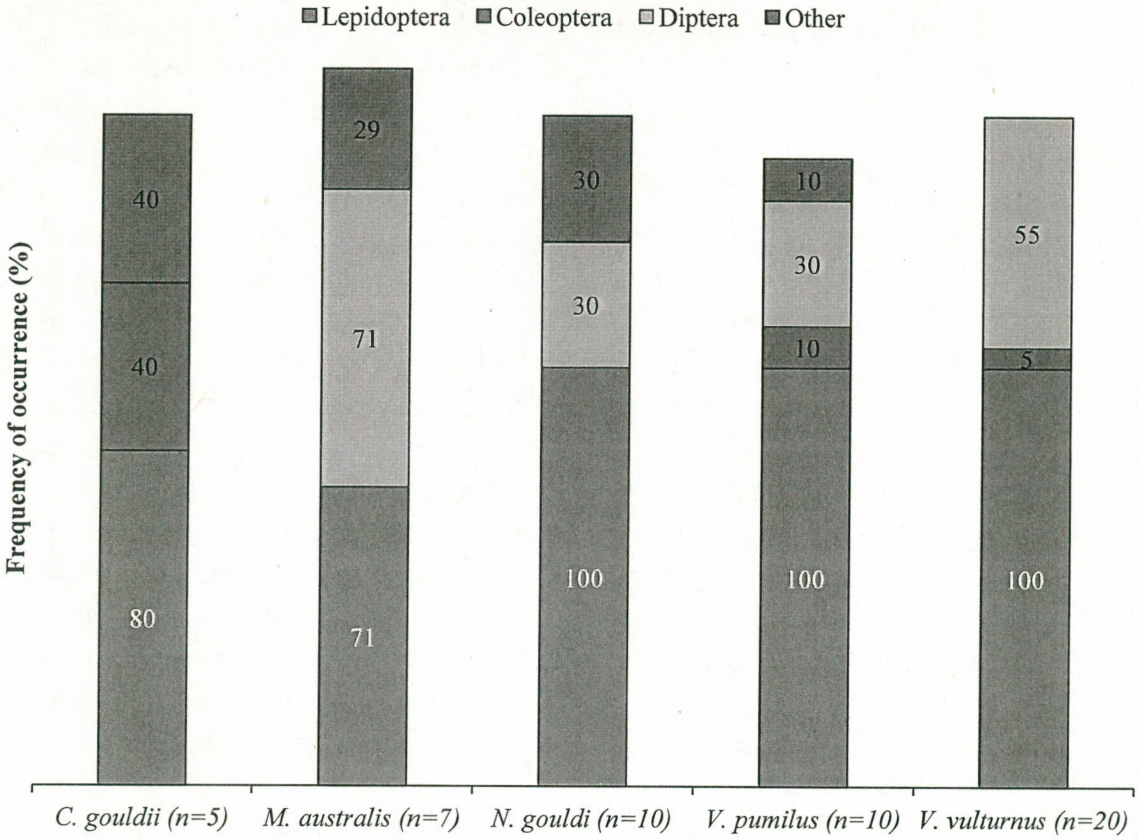

Figure 4.5 Frequency of occurrence of each insect taxa in the diets of the five insectivorous bats (i.e. percentage of individuals of a species that consumed each insect taxa).

For all bat species (excluding *M. australis* for which no prey abundance data were collected), the consumption of prey reflected the abundance of arthropods in light trap collections (*C. gouldii*, t=1.256, p=0.298; *N. gouldi*, t=1.665, p=0.195; *V. pumilus*, t=0.204, p=0.851; *V. vulturnus*, t=0.463, p=0.675).

4.3 Discussion

This study successfully utilised prey DNA within bat guano 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 & Hall 1977; O'Neill & Taylor 1989). While the consumption of prey reflected its abundance in light trap collections, the consumption of mosquitoes was restricted to two smaller-sized bats, *Vespadelus pumilus* and *V. vulturnus*, with the genus *Vespadelus* having previously been observed either hawking (Law *et al.* 2005) or opportunistically feeding on mosquitoes (Hoye 2002). These findings suggest that smaller-sized bats are likely to be more sensitive to broadscale mosquito control in environments with highly abundant vector mosquito populations that pose a threat to public health.

4.3.1 Limitations of dietary study

The findings of this study indicate that while molecular identification of prey within guano provides greater taxonomic resolution than can generally be achieved using traditional techniques and is able to identify small, soft-bodied prey, it still remains difficult to quantify consumed prey. 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 guano 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.

Although the molecular technique used in this study provided great resolution about consumed prey, 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 has been avoided in a previous study (Clare *et al.* 2009) by separating out insect fragments within guano 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. 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 more 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.

4.3.2 Prey abundance

While 14 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). 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. Additionally, the abundance of *Ae. vigilax* in the study area does not differ between saltmarsh and forest habitats (see Section 2.2.3).

The population abundance of *Ae. vigilax* is driven heavily by tidal and rainfall inundation of larval habitats (i.e. coastal saltmarsh and mangrove communities). While general patterns such as peaks in population abundances can be predicted (Kokkinn *et al.* 2009), population abundances can be highly variable, both spatially and temporally. Generally, larger populations tend to be present two weeks after inundation of saltmarshes by spring tides and/or heavy rainfall). The abundance of *Ae. vigilax* populations during this study reflected the variability associated with *Ae. vigilax* populations. In line with predictions based on forecasted extensive tidal inundation of *Ae. vigilax* larval habitats, population abundances of *Ae. vigilax* were generally higher during neap tides. However, great variability within tidal treatments, masked any differences between treatments. Although general patterns of *Ae. vigilax* population abundances can be predicted (Kokkinn *et* *al.* 2009), population abundances are likely to be influenced by a wide range of factors such as rainfall, evaporation, the frequency of saltmarsh inundation and the abundance of adults in the preceding month (De Little *et al.* 2009).

The most abundant taxa present in light trap collections were the lepidopterans (Table 2). 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 also were 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, where lepidopterans represented the greatest amount of biomass in light traps followed by coleopterans and dipterans (Unpublished data – L. Gonsalves). The varying abundances of each insect order in light trap collections provided an opportunity to assess the foraging selectivity (opportunistic, random or selective) across the bat species.

Since two different trapping techniques were used to survey mosquito populations and aerial insect fauna, it is not possible to compare the abundance of mosquitoes directly 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 design (Barclay & Brigham 1991), if the investigated bats were opportunistic in nature, they would be expected to consume both *Ae. vigilax* and lepidopterans.

4.3.3 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 & Hall 1977; O'Neill & Taylor 1989; Churchill 2009). In this study, lepidopterans were most frequently detected in the faeces of all bat species, ranging from 71-100 % frequency of occurrence. Lepidopterans were also the most abundant insect taxa in light trap collections at each site. We assume 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), with three of the five species producing feeding buzzes. it appears as though all species (except *M. australis* for which no prey abundance data were collected) were opportunistic in nature. If this was strictly true, the relatively high abundance of *Ae. vigilax* recorded in EVS traps would suggest that mosquitoes would be consumed by all bat species. However, only two of the five bat species studied (*Vespadelus pumilus* and *V. vulturnus*) were found to consume mosquitoes. Given that mosquito DNA was still detectable when mosquitoes were present as 5 % of insect material in artificial guano (approximately equivalent to the mass of 5 mosquitoes), it is unlikely that larger (> 6 g) bats actively seek mosquitoes as prey in forest. However, the level of sensitivity of the molecular technique to the detection of mosquito DNA in artificial guano could be an over-estimate of the detectability of mosquito DNA in the guano of wild caught bats. In this study, no attempts were made to replicate the effects of enzymatic degradation of prey known to occur in the guts of bat species. Additionally, the complexity and diversity of prey in the faeces of wild bats is greater than what was used in the artificial guano in this study.

It has been suggested (Kunz 1988) and demonstrated (Boonman *et al.* 1998; Rainho *et al.* 2010) that prey abundance does not necessarily equate to 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 small and large prey (Rosenzweig 1968). However, this generalisation may not be appropriate for echolocating aerial foraging bats that may be restricted to prey of a certain size due to detectability constraints imposed by echolocation design (Barclay & Brigham 1991). It is thought that bats that employ high-frequency echolocation (with corresponding long wavelengths) are more suited to detecting small prey (Barclay & 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 ability of larger bats to detect small prey may be reflected by the absence of small prey in the diets of these bats. In this study, moths consumed by all bat species are all larger than *Ae. vigilax*, and may have been more easily detected than *Ae. vigilax*.

Vespadelus pumilus and *V. vulturnus*, are very similar morphologically 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 (4.0 and 4.5 g) and employ high frequency echolocation (51-55 kHz). Unlike other studies in Europe which found diets of many medium-large sized bats were primarily composed of small dipterans (Rydell 1989; Waters *et al.* 1995), the larger bats in this study (*Nyctophilus gouldi* and *Chalinolobus gouldii*) were not found to consume small prey, such as *Ae. vigilax*. The failure to detect *Ae. vigilax* in the faeces of larger bat species in the study area may be indicative of the foraging habitats used by these bats. While the echolocation frequency employed by all species (except *C. gouldii*) is thought to be suitable for detection of small prey, the reduced manoeuvrability of larger bats is likely to inhibit their ability to capture these prey successfully while also negotiating and avoiding collisions in more cluttered habitats.

4.3.4 Quantity of mosquitoes required by Vespadelus *spp. to satisfy their energy requirements*

Using the equation of Speakman & Thomas (2003), the minimum energy required to maintain day-to-day activity (field metabolic rate) for the two bats species found to consume mosquitoes is 17.89 kjd^{-1} (*V. vulturnus*) and 19.51 kjd^{-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 ~850 and ~930 mosquitoes, respectively, each night just to maintain day-to-day activity (assuming a mosquito weighs 0.002 g and provides 10.5 KJg^{-1} of energy). Conversely, if the two bats consumed only lepidopterans (5-10 mm in size), *V. vulturnus* and *V. pumilus* would need to consume ~220 and ~250 moths, respectively (assuming a moth weighs 0.004 g and provides 25.5 $KJg^{-1}$of energy). Given the much larger quantity of mosquitoes that are required to maintain field metabolic activity relative to moths, and assuming similar energy expenditure for the detection, pursuit, capture and digestion of both prey types, optimal foraging theory would suggest that bats would consume moths in preference to mosquitoes.

4.3.5 Conclusion

This study revealed that while all investigated bat species consumed moths, reflecting the abundance of this insect taxa in the environment, the consumption of mosquitoes, particularly *Ae. vigilax*, was restricted to small-sized bats (*V. pumilus* and *V. vulturnus*) that utilise similar high frequency echolocation call designs. Larger bats that also use high frequency calls were not found to prey on mosquitoes. While the impacts of broadscale mosquito control on bat diet and health are yet to be established, the findings of this study suggest that small-sized bats are likely to be most sensitive to any impacts of broadscale mosquito control.

5. Habitat use of the Little Forest Bat (*Vespadelus vulturnus*)

5.1Methods

5.1.1 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.

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 Gould's wattle bat, *Chalinolobus gouldii* (Gray), Eastern freetail bat, *Mormopterus* sp2 (Peters), and the little forest bat, *Vespadelus vulturnus*. The latter was selected for this study as it is a small bat (~4.0 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 (see Section 4.2.2).

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 & 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. & 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 to the 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 to the west of the nature reserve.

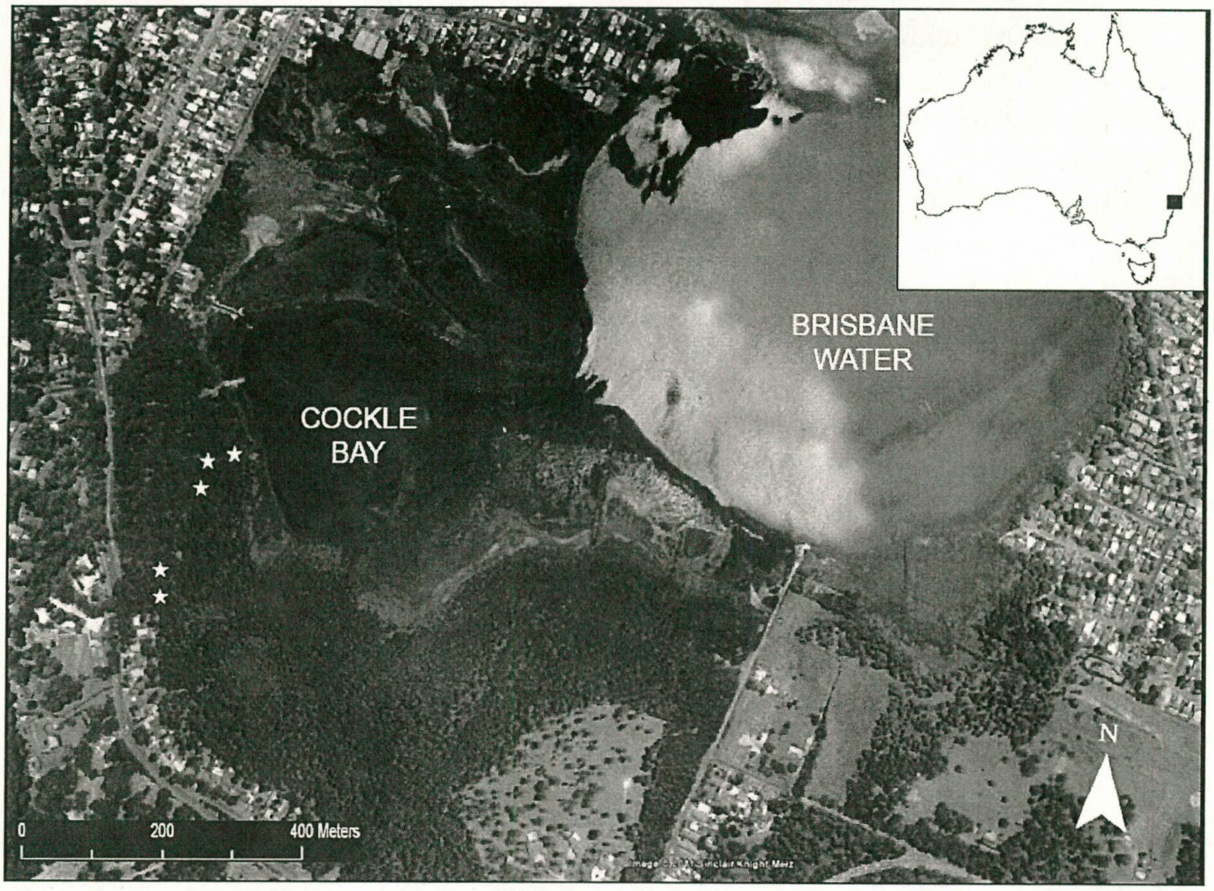

Figure 5.1: Satellite image of study area (adapted from Google Earth). Stars represent harp trapping locations within coastal swamp forest habitat. Saltmarsh areas are visible around Cockle Bay between the strip of mangroves and coastal swamp forest

5.1.2 Harp trapping, attachment of radio-transmitters and tracking methods[2]

Habitat use by *V. vulturnus* was investigated by radio-tracking in two periods during the austral late summer (February) and early autumn (March) of 2010. Bats were trapped in harp-traps (Tidemann & 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 months. The sex ratio of bats tracked during February was 3:7 (M:F), while in March 2010 there were equal numbers of male and female bats. 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 had previously been tracked (based on bare patch where 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.

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[2] All trapping and radio-tracking of free-living bats was done after approval of the NSW Director-General (Agriculture) Animal Care and Ethics Committee.

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* mass (mean), with an aerial length of 10 cm. While the radio-transmitters exceeded the guideline of 5 % of body mass suggested by Aldridge & 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 & 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. 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 also were tracked during the second half of the night (the hours preceding dawn) for at least two nights in each tracking period. Location data were only obtained over a maximum of 10 consecutive nights in each tracking period as it was predicted that mosquito population abundances after the tenth 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.

5.1.3 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. 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 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 & 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 <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. The use of foraging data collected from individuals of multiple social groups has the potential to confound foraging preferences at the 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).

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-squared 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. 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 population scale, since habitats available to members of one social group may not necessarily be available to members of availability. A Wilcoxon-pairwise comparison was used to ascertain the significance of these ranks.

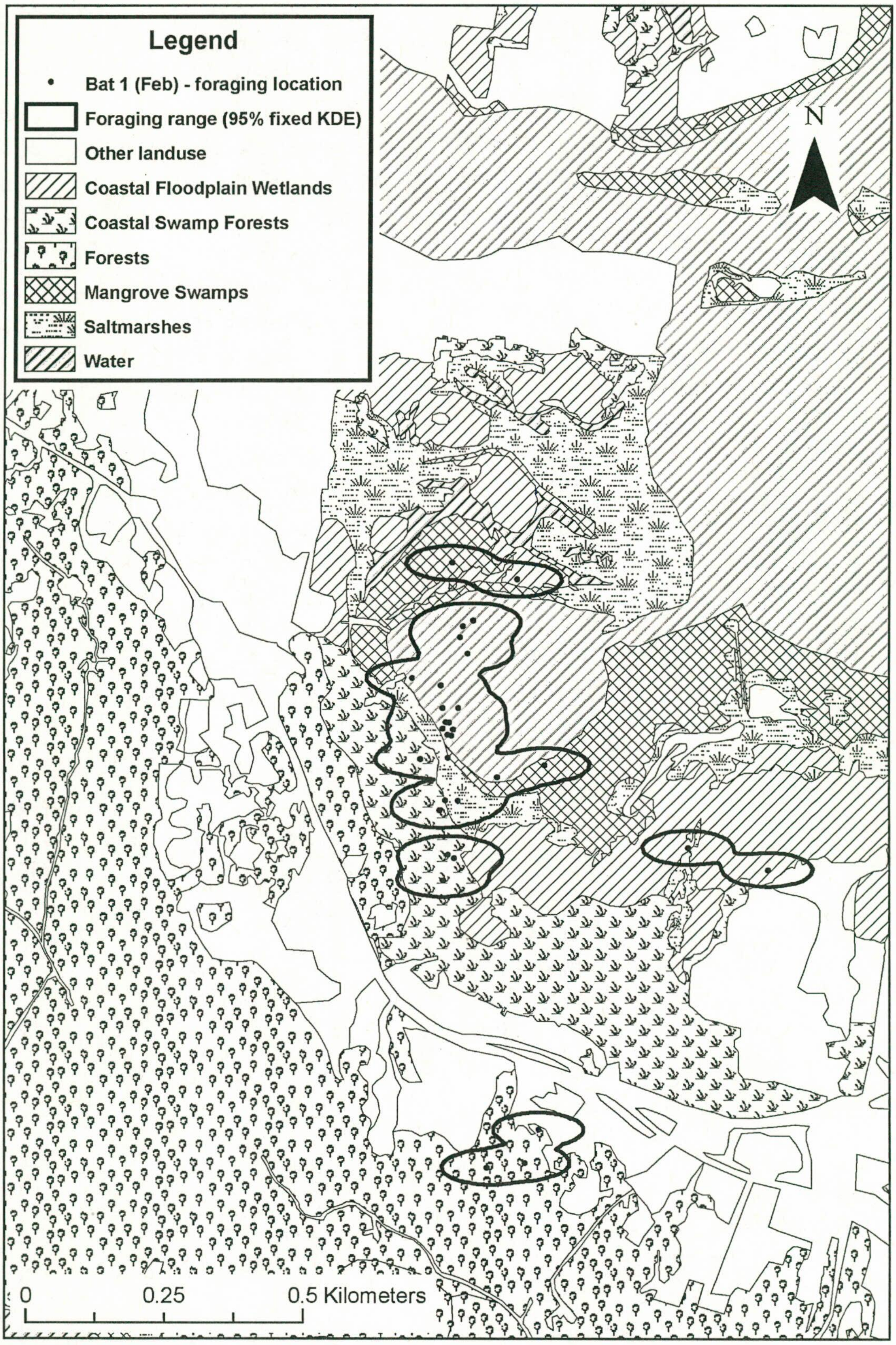

Legend
Bat 1 (Feb) - foraging location
Foraging range (95% fixed KDE)
Other landuse
Coastal Floodplain Wetlands
Coastal Swamp Forests
Forests
Mangrove Swamps
Saltmarshes
Water
N
0
0.25
0.5 Kilometers

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').

5.1.4 Surveillance of available prey

In each habitat, mosquito abundance was surveyed nightly using two CO_2–baited encephalitis virus surveillance (EVS) traps (Rohe & 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 then were 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 x 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).

5.2 Results

5.2.1 Prey abundance

A total of 13 243 mosquitoes representing 13 species was collected over both tracking periods (Table 5.1). *Aedes vigilax* was the most abundant species in each habitat irrespective of tracking period, representing $\approx$74 % of specimens trapped in both habitats during the February tracking period, and $\approx$56 % and $\approx$85 % of specimens trapped in saltmarsh and coastal swamp forest habitats, respectively during March 2010. The other commonly collected species were *Ae. alternans* (Westwood) and *Culex sitiens* (Wiedemann), two species closely associated with estuarine habitats, and *Cx. 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 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 habitats during both tracking sessions. NB. 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. quinquefasciatus	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

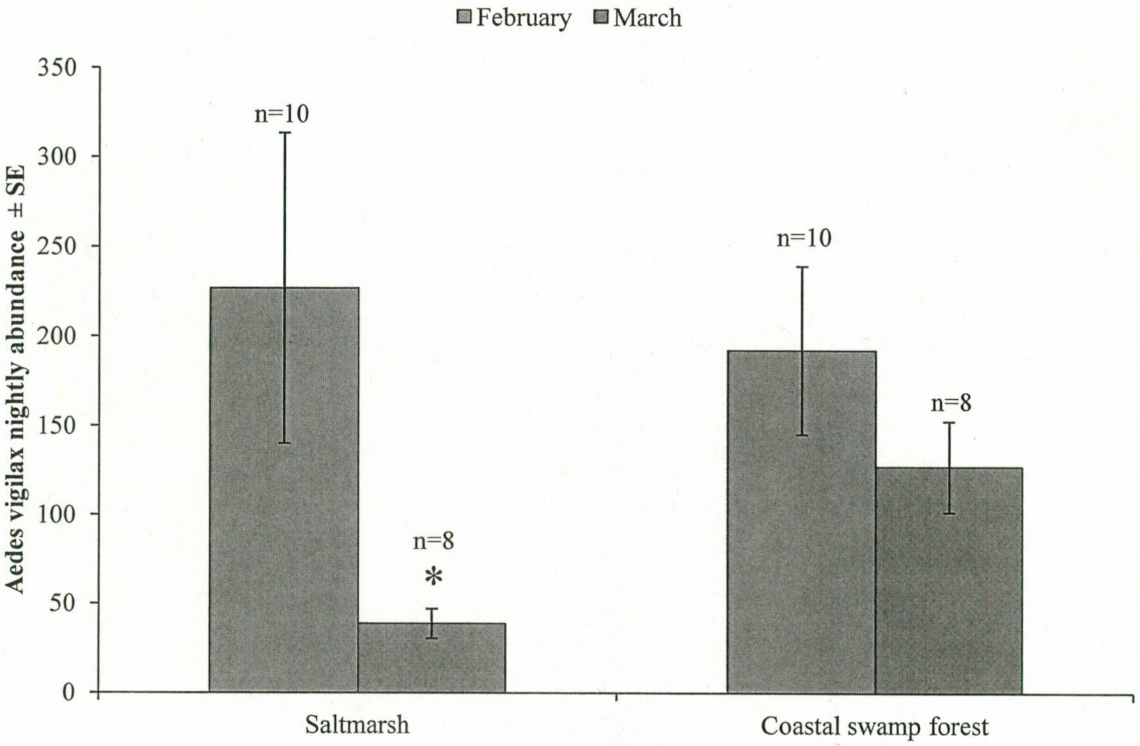
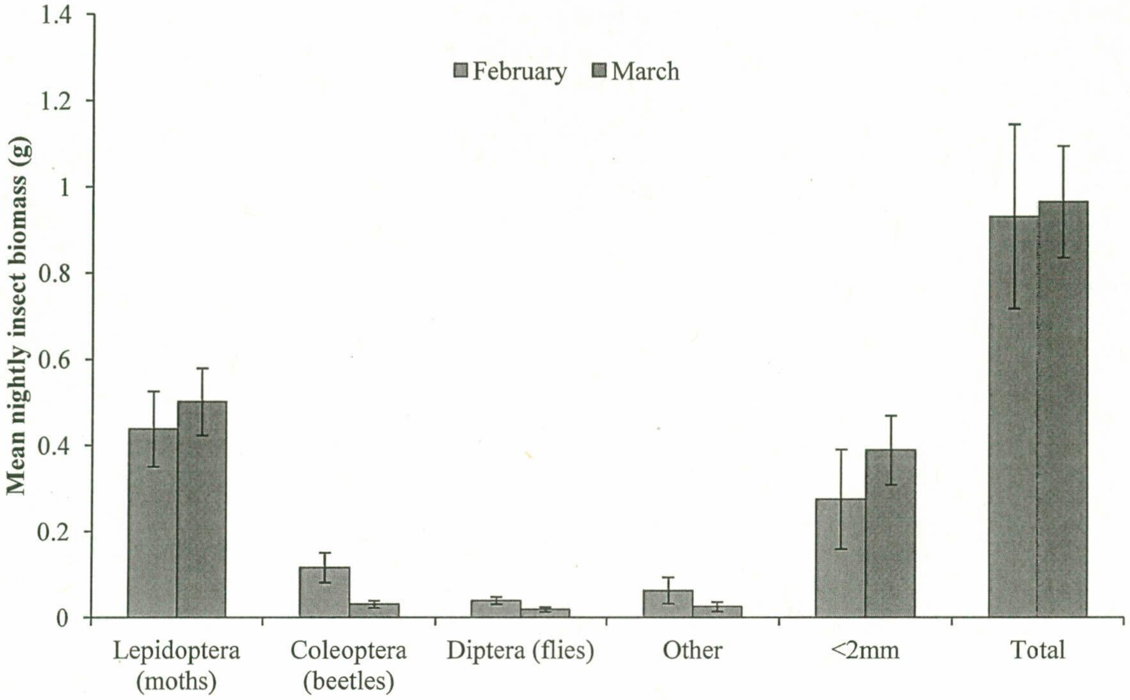


Figure 5.3 Mean nightly abundance of the saltmarsh mosquito, *Aedes vigilax*, in Saltmarsh and Coastal swamp forest habitats during both tracking periods. Error bars represent ±1 standard error from the mean. * Indicates interaction effect.

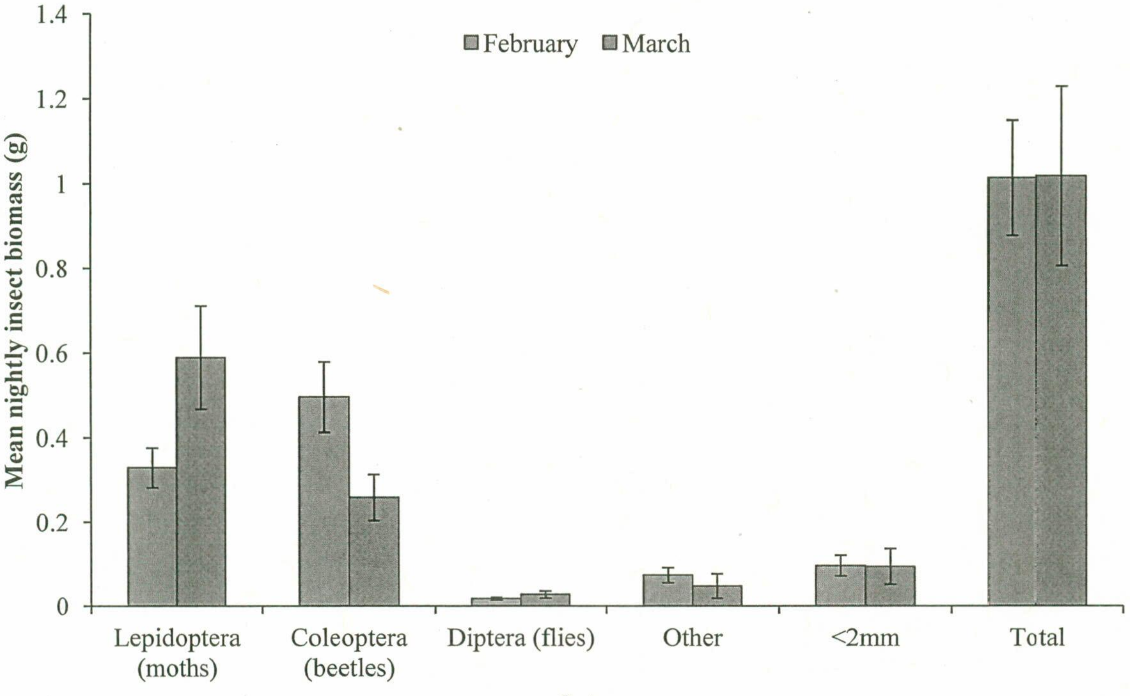
A total of 37.27 g of insect biomass was collected in light traps over both tracking periods (Table 5.2). During February 2010, 10.24 g and 11.15 g of insect biomass was collected in light traps in saltmarsh and coastal swamp forest habitats, respectively. In March 2010, 7.72 g and 8.14 g of insect biomass 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. 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 <2mm in size did not differ significantly between tracking periods or habitats (L.R. χ^2 (1) = 0.019, p = 0.991; L.R. χ^2 (1) = 0.097, p = 0.953; L.R. χ^2 (1) = 0.463, p = 0.793; L.R. χ^2 = 2.479, p = 0.290; Figs. 5.4a & 5.4b). Coleopteran biomass, however, was significantly greater in coastal swamp forest habitat (L.Rχ^2 (1) = 6.597, p = 0.037; Figs. 5.4a & 5.4b).

Table 5.2 Total insect biomass (g) collected in light traps in Saltmarsh and Coastal swamp forest habitats during both sessions of tracking. NB. Values in brackets represent percent of total insect biomass in each habitat.

Taxa/class	FEBRUARY 2010 (n=10)		MARCH 2010 (n=8)	
	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)
<2mm	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



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.

5.2.2 Radio-tracking and habitat selection of 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 (±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 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 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 - $\chi^2 = 28.802$, df = 5, $P < 0.001$; March 2010 - $\chi^2 = 56.480$, df = 5, $P < 0.001$). In February 2010, use of saltmarshes (relative to use of other habitats) was significantly greater than the availability of the habitat (relative to availability of other habitats) ($\chi^2 = 3.846$, df = 1, $P = 0.05$), 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 swamps (Table 5.3). Use of saltmarshes was significantly greater than use of coastal floodplain wetlands ($Z=-2.380$; $P=0.017$), mangrove swamps ($Z=-1.960$; $P=0.050$) and forests ($Z=-2.380$; $P=0.017$).

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 swamps, 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).

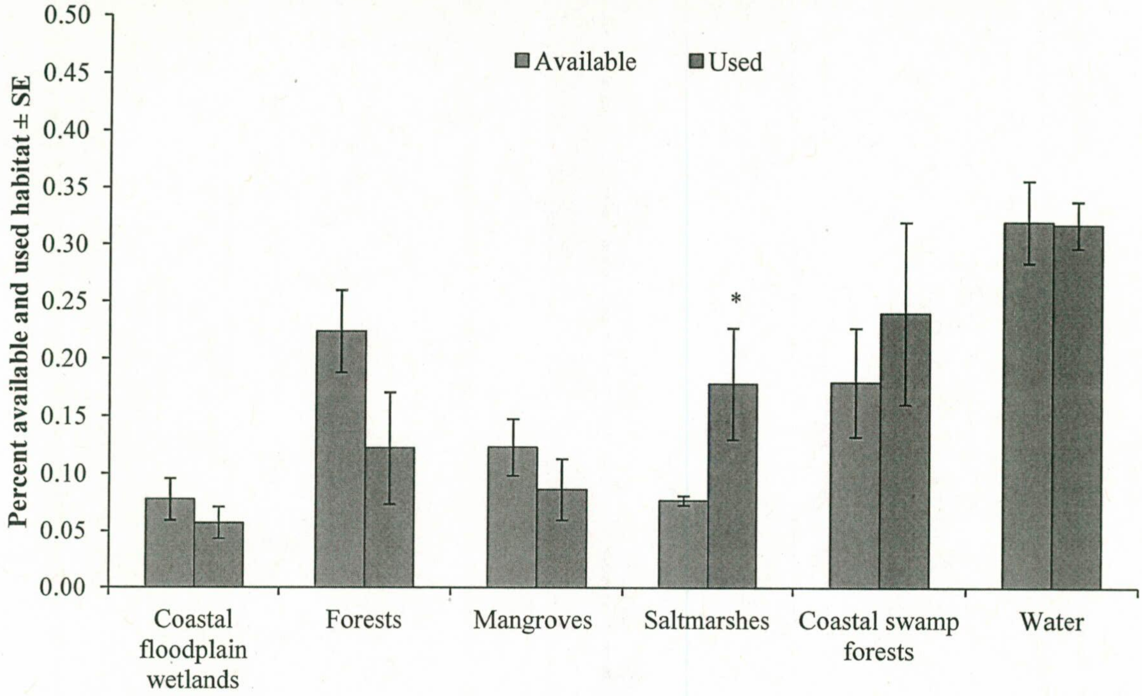


Figure 5.5 Percentage of available and used habitat in February 2010.

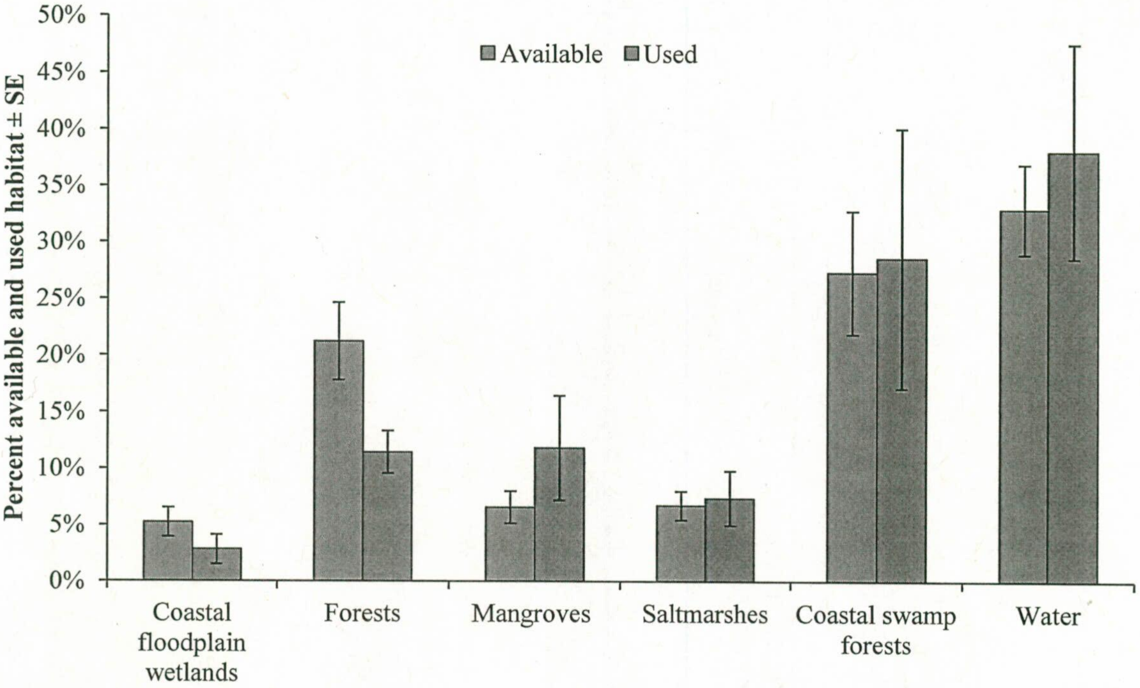


Figure 5.6 Percentage of available and used habitat in March 2010.

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 swamps	Saltmarshes	Water	Rank
February							
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 swamps	-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							
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 swamps	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**

5.3 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 habitat use of *V. vulturnus* changed in association 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 broad-scale 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).

5.3.1 Prey biomass

Aedes vigilax was the most abundant mosquito species in both saltmarsh and coastal swamp forest habitats during both tracking periods. 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). 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 period 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 mosquito 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 (1.18 g) and coastal swamp forest (0.99 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 (0.20 g) and coastal swamp forest (0.66 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 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 & 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 clearcuts (Klein 1989), forest clearings (Howden & Nealis 1975;Driscoll & 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 our results directly to previous investigations given the unknown influence of species-specific ecological or behavioural factors.

5.3.2 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. 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 (see Section 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 this, 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, with disproportionately greater use of saltmarsh than would be expected based on the habitat's availability. While *V. vulturnus* has been recorded echolocating and feeding in saltmarsh previously (Lamb 2009), this is the first study to identify the preferential use of saltmarsh for foraging by insectivorous bats. Gould's wattled bat (*Chalinolobus gouldii*) was the species most commonly recorded by ultrasonic detectors in saltmarsh (Lamb 2009), 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, *V. vulturnus* preferred to forage over water and in coastal swamp forest, with reduced use of saltmarsh for foraging. However, the maintained use of both threatened vegetation communities reaffirms that they are important foraging patches for *V. vulturnus*.

5.3.3 Relationships between prey biomass and habitat selection

If prey abundance is influencing the habitat use of foraging *V. vulturnus* individuals, one would expect that a change in prey abundance in one habitat from February 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, the habitat use of 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 may suggest that *V. vulturnus* chooses to forage in a habitat that while 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 6.1 kcal g^{-1} (DeFoliart 1992) and 5.1 kcal g^{-1} (Chena *et al.* 2004) of energy, respectively. Mosquitoes, however, provide lower levels of energy to predators, representing 1.51-3.54 kcal g^{-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 and thus increased energy expenditure (De Gueldre & 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 1989).

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 of *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 this period to mitigate the risk of predation associated with foraging in open habitats (Speakman 1991; Baxter *et al.* 2006). However, variables including cloud cover may act to mitigate any influence of lunar illumination on the habitat use of bats. At least two studies have found no influence of moonlight on the activity of insectivorous bats (Negraeff & 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 greater than 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 estimated for the similar-sized *V. pumilus* (Law & Anderson 2000).

5.3.4 Conclusion

In conclusion, our 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 broad-scale 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 broad-scale mosquito is required. However, in the interim, control programs should avoid early season treatments that coincide with the lactation period of bats, when energetic demands are greatest.

6. Summary of major findings and management implications

6.1 Major Findings

Four studies conducted independently of one another provided data that could be used to address one or more of the aims of the overall project. The findings of each study indicated saltmarsh is a habitat actively used by insectivorous bats, many of which are threatened species. Population abundances of *Aedes vigilax* in saltmarsh and forest habitats were significantly greater than population abundances in the urban habitat. Relationships between bat activity and the availability of mosquito prey were identified for one bat taxa (*Vespadelus* spp.), suggesting that *Ae. vigilax* is likely to be an important prey resource.

Higher bat activity along saltmarsh edges suggests that these microhabitats are more optimal for foraging than the interior of the saltmarsh. However, the activity of bats of the *Vespadelus* genus in the saltmarsh interior did not significantly differ from activity recorded along vegetation edges. While the recorded activity in the saltmarsh interior for this bat taxa may have represented commuting activity between vegetation edges, at least some of this activity represented feeding activity as indicated by a feeding buzz recorded in the saltmarsh interior. The overall higher bat activity recorded along the edge vegetation in saltmarsh highlights the importance of vegetation interfaces to foraging bats. Consequently, removal of vegetation neighbouring saltmarsh (e.g., mangroves, or coastal swamp forest) has the potential to reduce the foraging habitat available to bats. The retention of vegetation (strips of mangroves and casuarinas) would be beneficial to bats, providing an edge for foraging bats.

Of the bats considered to be able to detect small prey (high-frequency echolocating bats) and therefore most likely to consume *Ae. vigilax*, only two smaller-sized bats of the *Vespadelus* genus (*Vespadelus pumilus* and *V. vulturnus*) were found to consume mosquitoes. Both bat species have been previously observed either hawking (Law *et al.* 2005) or opportunistically feeding on mosquitoes (Hoye 2002). This finding suggests that smaller sized bats are likely to be most sensitive to broadscale mosquito control in environments with abundant vector mosquito populations that pose a threat to public health.

The habitat use of one bat species found to consume mosquitoes (*V. vulturnus*) shifted in association with fluctuations in the abundance and distribution of *Ae. vigilax* over a tidal cycle. Preferential use of saltmarsh and neighbouring coastal swamp forest communities when *Ae. vigilax* was most available in these habitats suggests that *Ae. vigilax* is an important prey resource for *V. vulturnus*, such that its availability in the environment influences in which habitats this bat species forages.

6.2 Implications of broadscale mosquito control for insectivorous bats

The findings of this project provide a baseline to assess the importance of *Ae. vigilax* to foraging bats. By examining relationships between the activity of bats and the availability of mosquito prey and investigating the diets of bat species most likely to consume *Ae. vigilax*, it was revealed that activity of bats of the *Vespadelus* genus was positively correlated with the abundance of *Ae. vigilax* in multiple habitats within the study area, and that these bats also consumed *Ae. vigilax*. An assessment of the habitat use of one of these bats over a tidal cycle (one spring and one neap) revealed a shift in preferential habitat use that closely corresponded to a shift in the distribution and abundance of *Ae. vigilax* (and no other measured prey taxa), further highlighting the importance of the mosquito to this bat species.

Bats of the *Vespadelus* genus are small, agile bats adapted to fly close to clutter, and employ high frequency echolocation considered to be suited to the detection of small prey. This genus of bats has previously been observed hawking or consuming mosquitoes in estuarine habitats. The failure to detect any positive relationships between the activity of larger (less manoeuvrable bats) and the abundance of *Ae. vigilax*, along with the absence of *Ae. vigilax* in the diets of these bats suggest that *Ae. vigilax* is not likely to be an important prey resource for these 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 (e.g. s-methoprene and *Bacillus thuringiensis isreaelensis*) in Australia, are generally mosquito-specific and target the aquatic immature stages of mosquitoes (Russell & Kay 2008). Appropriate management of *Ae. vigilax* populations requires consideration of potential impacts of broad-scale mosquito control on the diets of insectivorous bats.

The consumption of mosquitoes by *V. pumilus* and *V. vulturnus* individuals trapped at regular intervals throughout the austral summer (incorporating the natural variability of *Ae. vigilax* population abundances associated with the tidal cycle) indicates that mosquitoes form a component of the diet of small-sized bats during periods of relatively high and relatively low mosquito population abundances.

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). 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 & Gerell Lunderg 1993).

Reductions in the abundances of particular prey resources may be associated with reduced incidence of these prey within the diets of bats. It is also possible that invertebrate predators of these prey, 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).

Data obtained after a pilot spraying regime is implemented will allow for conclusions regarding the implications of broadscale mosquito control for insectivorous bats to be made. In the absence of these data, it is only possible to speculate what impacts, if any, broadscale mosquito control will have on foraging insectivorous bats.

To assess the impacts of mosquito control on insectivorous bats more adequately, an adaptive management process is recommended whereby careful monitoring of bats before and after the application of broad-scale mosquito is required. However, it is recommended that in the interim, control programs should avoid early season treatments that coincide with the lactation period of bats (early summer), when energetic demands are greatest. While this period is the time of greatest nuisance biting, the risk of mosquito-borne diseases such as Ross River virus and Barmah Forest virus is greatest outside the lactation period of bats. Mitigation of nuisance biting can be undertaken during early summer by avoiding areas where mosquitoes are most abundant if possible, or by wearing suitable clothing that reduces the exposure of skin to mosquito biting and the use of personal insecticidal agents (Barnard & Xue 2004; Webb & Russell 2009b).

6.3 Evaluation of objectives

1. *To quantify seasonal irruptions of the saltmarsh mosquito (*Aedes vigilax*) in saltmarsh areas of the NSW Central Coast*

A seasonal irruption of *Ae. vigilax* in three major habitats within the study area (saltmarsh, urban and forest) was quantified in the first field season of the study during spring and neap tidal cycles (Dec-Apr 2008-09: See Section 2.2.3). Additionally, in a subsequent season (Dec 2009-Mar 2010), further data were collected to assess/measure irruptions of *Ae. vigilax* over spring and neap tidal cycles that would be related to dietary analyses and radiotracking of focal bat taxa (those considered most likely to consume mosquitoes). No attempt was made to compare *Ae. vigilax* abundance data between separate field seasons.

2. *To collect essential ecological information on the use of saltmarsh for feeding by insectivorous bats*

As reported in the first progress report for this project (May 2009), Objective 2 in the original project proposal (*To quantify the effects of Bti spraying on the use of saltmarsh for feeding by insectivorous bats*) was unable to be met. Preliminary analysis of home range data from *C. gouldii* radiotracked early in 2009 indicated that home range size may be far larger than anticipated (145ha with some individuals moving 5 km in straight line distances). This had implications for the independence of some data from the 12 study sites that were to be used in a BACI (Before-After-Control-Impact) study. In the first progress report we proposed an alternative statistical model. In essence, our data collection would form baseline information prior to any controlled spraying of *Bti*. Consequently, Objective 2 was amended such that ecological information on the use of saltmarsh for feeding by insectivorous bats would be collected. These data then could be used in subsequent monitoring to determine the impact of any future spraying on mosquito abundance and bat activity and diet.

3. *To determine the relative proportion of mosquitoes in the diet of insectivorous bat species using saltmarsh seasonally and by bat taxonomic group*

Using novel molecular techniques, we identified two species of bats that consume *Ae. vigilax* within the study area (*Vespadelus pumilus* and *V. vulturnus*). Due to constraints associated with quantification of prey in bat faeces (using traditional morphological and more recent molecular techniques- discussed in Section 4), it was not possible to provide data about relative proportions of bat diet that mosquitoes formed. Instead, we presented percent frequency of occurrence (FO) data (e.g., for 10 individual bats sampled, all consumed moths, while only 2 consumed mosquitoes; moth FO=100%, mosquito FO=20%). It only was possible to collect samples for dietary analysis during one field season (Dec 2009-Mar 2010) given the other season was used to collect data to meet Objective 1 and to identify which bat taxa were most likely to prey on mosquitoes.

4. *To determine the relative proportion each bat species' geographic home range contributed by saltmarsh*

Although we were unable to provide data about relative proportion of each bat species' home range that was contributed by saltmarsh (since radiotracking of all taxa was not feasible given limitations associated with bat sample sizes as well as financial costs associated with radiotracking), we did provide important data for two bat species (*C. gouldii* (Lamb 2009) and *V. Vulturnus*: Section 5). Data for *C. gouldii* was part of a pilot study and is not presented in this report, but is available in the honours thesis of Lamb (2009) that accompanies this report. Additionally, we used patterns of activity as a proxy for geographical home range using ultrasonic bat detectors to measure patterns of activity of each bat taxa concurrently in three major habitats in the study area (saltmarsh, urban and forest).

7. Outputs

7.1 Conference Presentations

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

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

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

Gonsalves, L., Webb, C., Law, B. & 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. & 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.*

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

7.2 Drafted manuscripts for publication

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

Gonsalves, L., Law, B. Webb. & Monamy, V. (submitted). Are vegetation interfaces important to foraging microchiropteran bats in endangered coastal saltmarshes on the Central Coast of New South Wales? *Journal of Pacific Conservation Biology*.

Gonsalves, L., Lamb, S., Webb, C., Law, B. & Monamy, V. (in prep). The influence of clutter on relationships between bat activity and the availability of mosquito and non-mosquito prey resources on the New South Wales Central Coast. *Ecological Monographs.*

Gonsalves, L., Law, B., Webb, C. & Monamy, V. (in prep). Short-term shift in habitat selection of the little forest bat (*Vespadelus vulturnus*) in response to fluctuating prey resources. *PLoS ONE.*

Gonsalves, L., Bicknell, B., Law, B., Webb, C. & Monamy, V. (in prep). Unravelling the dietary importance of mosquitoes to insectivorous bats: Does bat size matter? *PLoS ONE.*

7.3 Theses

Gonsalves, L. (2012). *The Importance of the Saltmarsh Mosquito,* Aedes vigilax*, to Foraging Insectivorous Bats on the Central Coast, NSW*. Unpublished PhD Thesis. Australian Catholic University, North Sydney.

Lamb, S. (2009) .*The Importance of Saltmarsh and Estuarine Macrohabitat for Insectivorous Bats on the Central Coast, NSW.* Unpublished Honours Thesis, Australian Catholic University, North Sydney.

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Progress and Final reporting template (for grants awarded in 2009 and prior)

Grant reference number:	2007/RD/0071	Total grant amount:	$ 185,552

Project title:	Mosquito control, saltmarsh and insectivorous bats: seeking a balance
Grantee organisation:	Australian Catholic University
Person submitting this report:	Associate Professor Vaughan Monamy
Contact number:	(02) 9739 2848
Are you the primary contact?	Yes
If 'no', what is your role in the project?	
Report type:	Final
What stage of the project are you reporting on:	Stage 3 of 3 stages
Actual period covered in this report:	From September 2008 to January 2012
Note that final reports should cover the life of the project	

Use this template to complete your progress and final reports for grants under Restoration and Rehabilitation; Environmental Education; and Protecting our Places Programs. If necessary, please provide additional information as an attachment to this report.

For 2008 and 2009 grantees you must submit with this report an updated ***Schedule C – Project Measures*** as outlined in the reporting guidelines attached to your Grant Agreement.

Grantees must also complete the Environmental Trust's relevant ***Financial Reporting Spreadsheet***.

Note *you should still refer to your reporting guidelines which are included in Schedule B of your Grant Agreement prior to completing your report.*

You are required to submit **2 hard copies plus an e-copy** of your report (including attachments). For progress reports please include a **tax invoice** for the next instalment of your grant (plus GST if applicable). Late submission of an invoice could delay your next payment.

Post your report to: Grants Administrator
NSW Environmental Trust
PO Box 644
Parramatta NSW 2124

Email to: info@environmentaltrust.nsw.gov.au (limit 10MB per email) and include your grant reference number in the subject line.

If at any stage you require assistance please contact your Grants Administrator on (02) 8837 6093.

Note that the following text boxes will expand to the length required as you write your report.

1. Summary

Provide a summary of progress to date, approximately 100-200 words (cover the life of the project if this is your final report). This may be used for promotional purposes.

Coastal saltmarsh provides suitable breeding habitat for a number of estuarine mosquito species. The saltmarsh mosquito (Aedes vigilax) 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 and Barmah Forest virus and is a known nuisance biting pest. Coast residential areas adjacent to Empire Bay (e.g., Killcare, Pretty Beach & Hardys Bay) on the Central Coast of NSW 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 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; Belbasé 2004; Laegdsgaard et al. 2004) and that control of mosquitoes may impact upon foraging bats (Laegdsgaard et al. 2004), no study to date has specifically investigated the importance of the mosquito in the diet of these bats.

2. Background to and objectives of the project

Complete the table below which shows your original objectives and your progress in achieving them

Objectives as per the application	Progress towards achieving objectives
To quantify seasonal irruptions of the saltmarsh mosquito, Aedes vigilax, in saltmarsh areas of the NSW Central Coast	Objectives achieved. Please see Section 2 of final report.
To quantify the effects of Bti spraying on the use of saltmarsh for feeding by insectivorous bats	This objective was abandoned in Year 1 (Please see First Progress report) once it was determined that mosquitoes could move >5km.
To determine the relative proportion of mosquitoes in the diet of insectivorous bat species using saltmarsh	Objective achieved. Please see Section 4 of final report.
To determine the relative proportion of each bat species' geographic home range contributed by saltmarsh	Objective achieved. Please see accompanying Honours thesis (S. Lamb, 2009) and Section 5 of final report.

3. Project outputs and activities

This should report against the Project Management Plan outputs from your original application. Provide a summary of activities undertaken to date and the progress on each and outline the 3 most important outputs during this reporting period (cover the life of the project if this is your final report).

Conference Presentations

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

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

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

Gonsalves, L., Webb, C., Law, B. & 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. & 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.

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

A final output, a community flyer for residents is in preparation.

4. Project outcomes

Describe any outcomes that have been achieved during this reporting period (with evidence) or alternatively how you are progressing towards achieving those outcomes identified in your Application and your Monitoring and Evaluation Plan. Compare the outcomes with those outcomes projected in Schedule C.

The present study 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 only were 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 swamp).

Of the species able to be identified acoustically, five species were able to be trapped and an assessment was made of their diet. The diets of five bat species trapped in forest habitat adjacent to saltmarsh habitat consisted of a diverse range of prey, dominated by moths. Only two bat species (Vespadelus pumilus and V. vulturnus) consumed Ae. vigilax. Neither species is considered threatened in this area. Aedes vigilax abundance was positively correlated only with the activity of bats of this genus. Habitat use of V. 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.
This study provided a baseline to assess the importance of Ae. vigilax to insectivorous bat diet. Aedes vigilax is an important prey resource for bats of the Vespadelus genus but was absent from the diets of all other bat species sampled. Findings of this study can be used as a benchmark for comparison with future research should a pilot Bti spraying regime be initiated. A precautionary approach is recommended whereby the application of Bti is restricted to later in summer in order to avoid lactation periods of bats (Nov-Jan), when energetic demands are greatest.

5. Reflection on the monitoring and evaluation plan (for grants awarded in 2008 and 2009)

Provide a brief summary on the implementation of your Monitoring and Evaluation Plan. You should include:

- how monitoring has been established
- how data has been obtained

- reflect on what the data is showing
- stakeholder involvement.

N/A

6. Project evaluation

This section gives you an opportunity to evaluate the project as a whole rather than against individual objectives/activities (cover the life of the project if this is your final report).

6.1 Progress

How the project is progressing / has progressed overall.

The project was overwhelmingly successful. A large number (>15) of insectivorous bats were identified in the Central Coast area of which five species were identified as feeding over saltmarsh. However, only two Vespadelus species (V. vulturnus and V. pumulis) were identified as having saltmarsh mosquitoes as a substantial part of their diet. Both species are common. Any future Bti spraying regime should bear in mind breeding and lactational periods when these two species may require an increase in daily dietary requirements.

6.2 Issues/challenges

Any problems you have encountered and how you have dealt with, or intend to deal with them. Please include any issues that the Trust, as the funding body, should know about. Include any significant variations from planned approach as outlined in your application. You should refer to the risk management section of your Monitoring and Evaluation Plan (where applicable) and/or any other issues that have arisen.

Preliminary analysis of home range data from 10 individual bats radio-tracked early in 2009 indicated that home range size was far larger than had been anticipated. (This was reported in the first progress report.) Additionally, it was discovered that the saltmarsh mosquito can move distances greater than 5km. This had implications for the independence of data from the study sites and our data now form baseline information collected prior to any scheduled controlled spraying of Bti. Using a Before-After-Control-Impact (BACI) statistical model, subsequent monitoring can then be used to determine the impact of spraying on mosquito abundance and bat activity.

6.3 Opportunities

Lessons learned, other opportunities and how benefits from the project could be spread more widely.

This project provided a rare opportunity to study a guild of bat species in close detail in conjunction with the principal components of their diet. Recording what bat species were consuming mosquitoes (and what species weren't) has provided ecological information previously unknown. The opportunity to educate a doctoral candidate proved to be extremely successful. Leroy Gonsalves was able to develop a brand new method of identifying dietary items from bat guano using remnant DNA . This method has far reaching implications for the study of dietary ecology in all vertebrate species in Australia and beyond. Mr Gonsalves is currently preparing this information for publication in scientific journals.

7. Issues raised from previous progress report(s)

Did you receive specific points to address in this report from the technical review of your previous progress report? If so, please complete the table below.

Points to address	Response/action
Not applicable	

8. Other

Is there anything else you would like to raise in this report?
