

Distribution and key foraging habitat of the Large-footed Myotis *Myotis macropus* in the highly modified Port Jackson estuary, Sydney, Australia: an overlooked, but vulnerable bat

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ABSTRACT

The Large-footed Myotis *Myotis macropus* is a threatened echolocating bat that uses a specialised 'trawling' foraging strategy to hunt for aquatic prey. While the species is well known in freshwater habitats, in 2014 it was recorded for the first time roosting and foraging in a sheltered bay on Sydney Harbour in the Port Jackson estuary. To investigate how widely distributed *M. macropus* was within the estuary (Parramatta River, Lane Cove River, Middle Harbour, harbour islands, west Harbour and east Harbour), 56 sampling sites were surveyed acoustically. Of these sites, 24 were in harbour bays/coves, 20 were in tributary bays, seven were along tributary channels/creeks, four were on the margins of harbour islands and a single site was located on a freshwater lake. We also investigated relationships between *M. macropus* activity and environmental variables to identify those that should be targeted for management. Radio-tracking of *M. macropus* at one known roost was carried out to assess roost fidelity and identify key foraging areas within the estuary. *Myotis macropus* was widespread in Port Jackson, being present at 92.6 % of sites, but with high activity (>90 passes night⁻¹), including feeding buzzes (≥ 24.5 buzzes night⁻¹) concentrated in a few 'hot spots'. Greatest activity was recorded in east Harbour (~ 70 passes night⁻¹), west Harbour (~ 15.5) and Lane Cove River (~ 14), while lowest activity was on the Parramatta River (2) and Middle Harbour (10). Activity, including feeding, was significantly greater in harbour bays/coves when compared with other habitats. Radio-tracking revealed that bats roosting in west Harbour showed 100 % fidelity to the roost site over a three week period and were only recorded foraging in this zone and the nearby (1.2 km) Lane Cove River. Historical Zinc concentrations in surficial sediments was negatively associated with *M. macropus* activity, though heavy metals were also correlated positively with total suspended solids (TSS). While heavy metal concentrations in sediments were not associated with feeding activity, increased TSS was negatively associated with *M. macropus* activity and feeding. Best subsets regressions found that *M. macropus* activity was associated with TSS (-ve), mangrove cover (+ve), seagrass cover (+ve) and total water extent (-ve). We recommend further research on the negative association between TSS, heavy metals and *M. macropus* activity to identify the sensitivity of this species to past and present pollution events.

Key words: Bio-indicator, Heavy Metals, Estuaries, Sydney Harbour, trawling bat

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Introduction

The Large-footed Myotis *Myotis macropus* is a small echolocating bat weighing less than 10 g, with disproportionately large feet (Churchill 2008). Although the species occurs predominantly in a narrow coastal band in northern and eastern Australia, it has also been recorded along some of the major inland rivers, reflecting its close association with water (McKean and Hall 1965; Lumsden and Menkhorst 1995) and a specialised 'trawling' foraging strategy (Thompson and Fenton 1982). Trawling is a strategy in which individuals fly 5-100 cm above a water surface before dipping to make contact with the surface and raking with their large feet (Dwyer 1970; Robson 1984). Using this strategy, *M. macropus* captures aquatic invertebrates and small fish

(Law and Urquhart 2000; Robson 1984), although fish represent only a small portion of their diet in some forest streams (Law and Urquhart 2000).

Observations of *M. macropus* have mostly been near large, permanent bodies of freshwater at low elevations where the terrain is flat and surrounded by vegetation (Anderson *et al.* 2006). However, the species has also been recorded regularly foraging on brackish water in Lake Victoria (Campbell 2007) and in better quality coastal lagoons in NSW (Clarke-Wood *et al.* 2016), indicating that tidal waterbodies may represent important habitat for *M. macropus*. Given its highly specialised foraging strategy, *M. macropus* could be particularly sensitive to reductions

in water quality affecting food resources. However, no association was detected between the activity of the bat and snap-shots of water quality in forest streams with minimal pollution on the north coast of NSW (Anderson *et al.* 2006). Loss of roosting habitat and clearing of land adjacent to foraging areas also threaten the species. Consequently, *M. macropus* is considered a threatened species in NSW (NSW *Threatened Species Conservation Act 1995*).

Myotis macropus was recently recorded roosting and foraging in a sheltered bay on Sydney Harbour in the Port Jackson estuary (Gonsalves and Law 2014). Given the habitat in which *M. macropus* was observed (sheltered bay), there was potential for the species to be present in other areas of the estuary. However, the estuary encompasses a working harbour and tributaries that are highly urbanised with low levels of remnant bushland and high levels of lighting and contaminants (Irvine and Birch 1998; Birch and Taylor 1999, McCready *et al.* 2000). It remains unclear how widely distributed *M. macropus* is in the Port Jackson estuary and whether the degree of urbanisation, distribution of contaminants and other environmental variables restricts its distribution.

The aim of our study was to describe the distribution and habitat use by *M. macropus* within the Port Jackson estuary. We also investigated relationships between *M. macropus* activity and environmental variables to identify those that could be used to predict areas of suitable foraging habitat and to identify variables that may be prioritised for management. Radio-tracking was used to assess fidelity to a known roost and identify key foraging areas.

Methods

Study area

The Port Jackson estuary is a drowned river valley that is approximately 30 km long, 2 km wide and occupies about 50 km² with a catchment covering 500 km² (Birch 2007). The estuary experiences a maximum 2.1 m tidal range, with flushing times varying across different zones within the estuary from 3-10 days (Birch 2007). Given the relatively small catchment size of the Port Jackson estuary, little fresh water enters the estuary, leaving it mostly saline. Heavy rainfall, however, can lead to stratification of the estuary, resulting in a 1-2 m layer of turbid freshwater that sits above the more dense saltwater (Birch 2007).

Approximately 86 % of the Port Jackson catchment is urbanised and/or industrialised (Birch 2007), with bushland highly fragmented and only occurring in relatively contiguous blocks in Lane Cove, Homebush, Middle Harbour and Sydney Harbour National Park (on the foreshore of east Harbour). The underlying soils of surrounding areas have been derived from Hawkesbury sandstone and are considered to be low in productivity and associated with low insect abundance and bat activity, relative to soils derived from shale (Threlfall *et al.* 2012).

The estuary has had some form of industrial activity since as early as 1800, with metal industries (foundries) established at Darling Harbour, Cockle, Rozelle and Blackwattle Bays (southernmost embayments in west Harbour). Sediment cores suggest major heavy metal contamination commenced in about 1860 in Blackwattle Bay. Between the early 1800s and present there has been considerable change to industrial activities, including early expansion of industry to other parts of the estuary (e.g., Iron Cove, Homebush Bay). This has all contributed to the current condition of the estuary. Today, sediments in parts of the Port Jackson estuary are among the most contaminated in harbours worldwide, with high concentrations of heavy metals, organochlorine pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and dibenzofurans (Birch and Taylor 1999, 2000; McCready *et al.* 2000; Birch *et al.* 2006).

Sampling areas and design

In all, 56 sampling sites, selected to be representative of the Port Jackson estuary, were surveyed for *M. macropus* (Parramatta River, 9; Lane Cove River, 6; Middle Harbour, 13; harbour islands, 4; west Harbour, 7; east Harbour, 17) (Fig. 1). Of these sites, 24 were in harbour bays/coves (Fig. 2a), 20 were in tributary bays (Fig. 2b), while seven were along channels/creeks of the Parramatta River, Lane Cove River and Middle Harbour (Fig. 2c). An additional four sites were located on the margins of four harbour islands (Fig. 2d), while a single site was on a freshwater lake (Lake Parramatta).

Anabat data collection and analysis

A single Anabat detector (Anabat II and external Z-CAIM, Anabat SD1 or Anabat SD2: Titley Scientific, Brendale QLD) was deployed at each site between February and May, 2015. Anabat detectors were attached to ultrasonic microphones that were housed in PVC piping for weather protection and positioned on shore and directed towards the water surface using 2 m extension cables. Each detector recorded bat calls from dusk until dawn for two consecutive nights (Fischer *et al.* 2009). Sampling was not undertaken on cold nights and during heavy rain since bat activity can be significantly reduced under these conditions (Fenton 1970; Bell 1980). Recorded bat calls were identified to species (where possible; see below) using automated call identification software, AnaScheme (Adams *et al.* 2010) in association with an identification key for bats of Sydney (B. Law, unpubl. data). Bat calls with fewer than three valid pulses (i.e., minimum of six data points and model quality of ≥ 0.8) were not analysed by AnaScheme. Because multiple bat species may call simultaneously, calls were assigned to a species only if >50 % of pulses within the sequence were attributed to that species and only passes with a minimum of three pulses classified to the same species were identified. Since linear calls of *M. macropus* and *Nyctophilus* spp. can be difficult to distinguish using automated software, all linear calls were identified as 'linear bats' by AnaScheme and

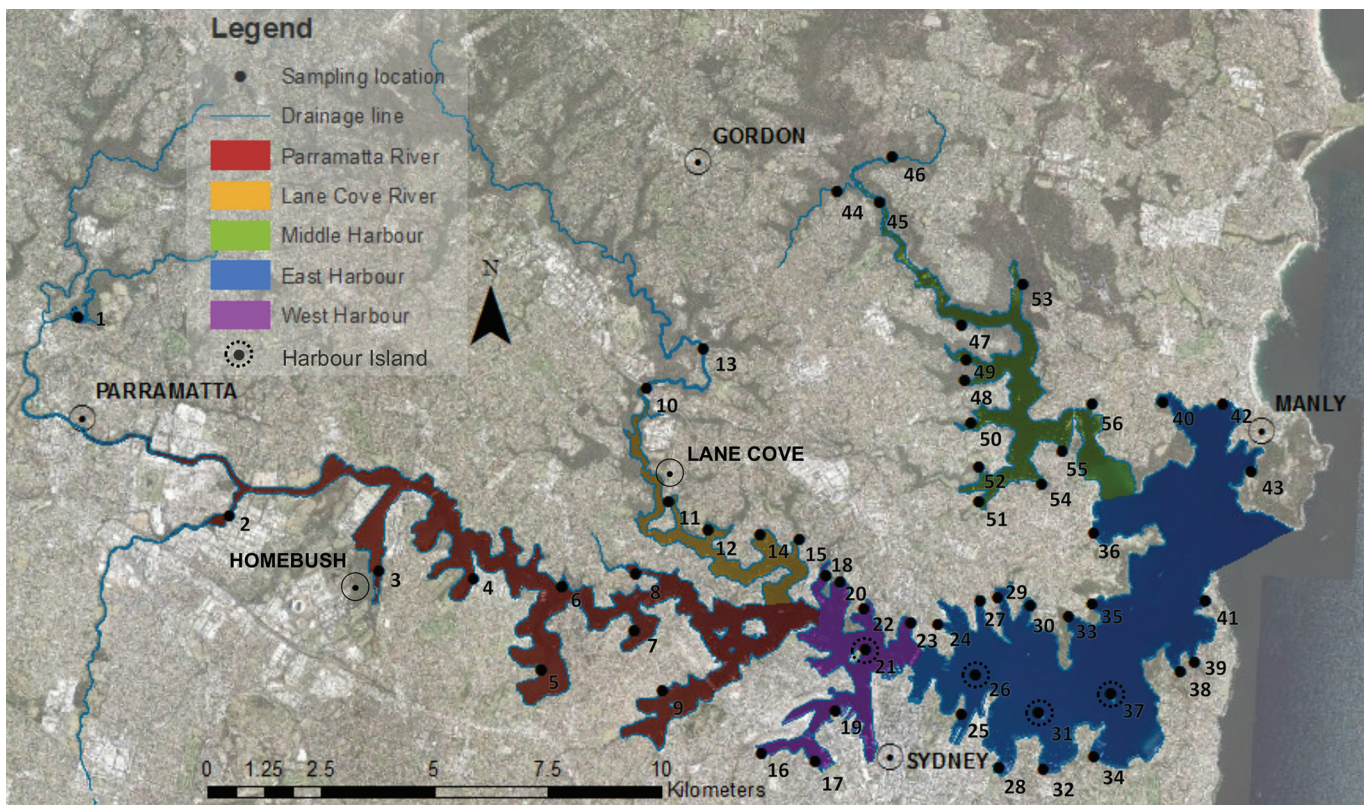


Fig. 1. Map illustrating sampling locations within each zone of the Port Jackson estuary. 1=Lake Parramatta, 2=Duck River, 3=Homebush Bay, 4=Majors Bay, 5=Canada Bay, 6=Looking Glass Bay, 7=Five Dock Bay, 8=Tarban Creek, 9=Iron Cove, 10=Epping Rd Bridge, 11=Burns Bay, 12=Tambourine Bay, 13=Fullers Bridge, 14=Woodford Bay, 15=Gore Creek, 16=Rozelle Bay, 17=Blackwattle Bay, 18=Gore Cove, 19=Pirrama Park, 20=Oyster Cove, 21=Goat Island, 22=Berrys Bay, 23=Lavender Bay, 24=Careening Cove, 25=Woolloomooloo Bay, 26=Fort Denison, 27=Shell Cove, 28=Rushcutters Bay, 29=Mosman Bay, 30=Little Sirius Cove, 31=Clark Island, 32=Double Bay, 33=Taylor's Bay, 34=Rose Bay, 35=Chowder Bay, 36=Hunters Bay, 37=Shark Island, 38=Vaughan Bay, 39=Parsley Bay, 40=Jilling Cove, 41=Camp Cove, 42=Manly Cove, 43=Spring Cove, 44=Twin Creeks, 45=Middle Harbour North, 46=Carroll Creek, 47=Middle Harbour South, 48=Crag Cove, 49=Castle Cove, 50=Sailors Bay, 51=Willoughby Bay, 52=Wreck Bay, 53=Bantry Bay, 54=Quakers Hat Bay, 55=Pearl Bay, 56=Fisher Bay.



Fig. 2. Habitat types sampled across the estuary: a) harbour bay/cove; b) tributary bay; c) tributary channel/creek; d) water surrounding harbour island. (all photos: Leroy Gonsalves)

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were subsequently manually screened to verify whether calls were produced by *Nyctophilus* spp. or *M. macropus* (Reinhold *et al.* 2001). All *M. macropus* calls were then screened manually to identify the presence of a feeding buzz – a rapid increase in pulse repetition rate, slope, frequency and speed (associated with pursuit and capture of prey: Griffin *et al.* 1960, Britton and Jones 1999). This was done by visually examining the calls as time vs. frequency graphs in AnalookW before converting Anabat files to wave files and then playing-back to listen for the sound of at least one ‘buzz’.

For each detector and each night, the number of calls produced by each species and all species combined (hereafter total bat activity) was tabulated. The number of calls (activity) and feeding buzzes (feeding activity) produced by *M. macropus* was averaged across consecutive nights for each site. Following this, each site was allocated to an estuary zone (Parramatta River, Lane Cove River, Middle Harbour, east Harbour, west Harbour, harbour islands), habitat (harbour bays/coves, tributary bays, tributary channel/creeks and open harbour). Sites were also allocated to a heavy metal concentration class (Cu, $\mu\text{g g}^{-1}$: <100, 100-200, 201-300, >300; Pb, $\mu\text{g g}^{-1}$: <200, 200-300, 301-400, >400; Zn, $\mu\text{g g}^{-1}$: <300, 300-500, 501-800, >800) based on concentrations in surficial sediments previously measured in 1995 from 1700 sediment cores taken throughout the estuary, including from our sites (Birch and Taylor 1999). Since there was no relationship between minimum daily temperature and *M. macropus* activity (Observatory Hill weather station: 11.1-22.4°C; Pearson correlation: $df=33$, $r=0.076$, $P=0.668$), sites sampled in late autumn were included in all analyses. Elevated levels of *M. macropus* activity at three sites with known roosts were atypical of the rest of the estuary as bat activity at these sites also encompasses circling behaviour at roost sites. Consequently, these sites were excluded from analyses. Two additional sites were also excluded from analyses since sampling was not undertaken throughout the night due to risk of theft of detectors.

Not all habitats were present within every estuary zone and not all heavy metal classes were present in every habitat or estuary zone (i.e. design was not orthogonal), so it was not possible to test for interactions among estuary zones, habitats and heavy metal classes. Generalised linear models were fit to the data with *M. macropus* activity (normal-identity link) and feeding (inverse gaussian-identity link) used as response variables, while estuary zones, habitats and heavy metal concentration classes were used as fixed effects. Distribution and link functions used to model the data were selected by visually inspecting histograms and using Akaike information criterion (AICc) scores corrected for small sample sizes to assess the fit of each model. Bonferroni post-hoc pairwise comparisons were used to identify which treatments differed from one another. *Myotis macropus* activity and feeding data were transformed ($\log_{10}(x+1)$) prior to analysis.

Best subsets regression was used to test whether environmental variables were associated with *M. macropus* activity and feeding. A number of variables were initially selected for analysis (bushland cover, mangrove cover, saltmarsh cover, seagrass cover, extent of water, salinity, total suspended solids (TSS), total N, total P and chlorophyll-a). These variables were selected for analysis since they are either known to influence bat activity elsewhere or are likely to influence the prey of bats. Bushland cover, mangrove cover, saltmarsh cover, seagrass cover and water extent were calculated as hectares (ha) within a 500 m buffer of sampling locations using ArcGIS (ESRI) and spatial data layers (NSW Department of Primary Industries 2009; Office of Environment and Heritage 2013). Salinity (coefficient of variation=12 %), TSS (55 %), total N (40 %), total P (34 %) and chlorophyll-a (65 %) concentrations measured in each month between January and June 2013 (two years prior to our study) at 25 sites across the estuary (P. Freewater, unpubl. data) were averaged across months and used to interpolate these variables across the harbour. The inverse density weighting (IDW) tool (Spatial Analyst Tools) was used for interpolation with a variable search radius and maximum power (3) in ArcGIS (ESRI). Following this, sampling locations for the *M. macropus* surveys were overlaid before water quality data for each location were extracted using the extraction tool (Spatial Analyst Tools) in ArcGIS (ESRI). Because many variables were highly correlated, a subset of these variables (bushland cover, mangrove cover, seagrass cover, TSS and water extent) was used in the analysis. AICc scores were used to rank models, with lower scores ranking higher.

A principal components analysis (PCA) was undertaken to identify which environmental variables (described above) were associated with each site. Each variable was $\log_{10}(x+1)$ transformed and normalised prior to ordination based on a correlation matrix in PRIMER-6 (PRIMER-E Ltd, Luton UK).

To provide a distribution map (weighted by activity) for *M. macropus* within the Port Jackson estuary, the IDW tool (Spatial Analyst Tools) was used to interpolate activity levels across the estuary using a variable search radius and maximum power (3) in ArcGIS (ESRI).

Estimated population means \pm SE are reported in the results section.

Radio-tracking

Radio-tracking was carried out to provide data on movements (between roost and foraging areas) of *M. macropus* in the Port Jackson estuary. Bats were netted at a known roost (in lift holes underneath a jetty) in west Harbour at low tide on 5th March 2015 (Figs. 3a-d). The site was the only known roost for *M. macropus* in the Port Jackson estuary and was used by approximately 50 pregnant and lactating females as well as males (L. Gonsalves and B. Law, unpubl. data). Since greatest



Fig. 3. a) Jetty roost (Photo: Andrew Scott); b) Bats netted for radio-tracking (Photo: Andrew Scott); c) Captured *M. macropus* individual showing its disproportionately large feet (Photo: Andrew Scott); d) *Myotis macropus* with radio-transmitter attached. (Photo: Leroy Gonsalves)

energetic demands for bats occur during pregnancy and lactation (Speakman and Thomas 2003), we targeted lactating females for radio-tracking. However, we also included males for radio-tracking as foraging areas may differ between sexes, as has been demonstrated for other trawling bat species (Encarnação *et al.* 2005).

Three lactating females and two adult males were selected for radio-tracking. All other bats were released at the point-of-capture. A radio-transmitter (LB-2X, Holohil Ontario) was glued to the bat between the shoulder blades using Uro-bond® IV (BrightSky Australia®, Newington NSW). Each radio-transmitter had an aerial length of 12 cm and weighed 0.31 g, representing 4.4 % of *M. macropus* mean mass, and a 21-day battery life. Netting and radio-tracking was carried out under Scientific Licence (SL100623) and in accordance with conditions of an Animal Research Authority (ARA 13/13).

During each day of the radio-tracking period (23 days), radio-telemetry equipment (Australis 26K scanning receiver and three-element Yagi antennae - Titley Scientific, Brendale QLD) was used to identify whether bats fitted with radio-transmitters (here after 'tagged bats) were roosting within lift holes underneath the jetty roost. If bats were roosting at this site, the location of the lift hole in which the bat was located was recorded, along with a count of numbers of bats within that lift hole (where possible). Following this, for signals of any tagged bats that were not roosting at this site, searches were made on-foot with radio-telemetry equipment at potential roost sites identified in the North Sydney LGA in a previous study (Gonsalves and Law 2014).

Radio-tracking was also undertaken at dusk (when bats emerge from roosts) and during the night (when bats are likely to forage). Data on broad movements of tagged bats were collected using a modification of the 'homing in' approach (White and Garrott 1990). In this approach, tagged bats are followed and encircled over a short period of time (where possible), during which GPS locations are recorded, compass bearings are taken to the direction in which the radio-transmitter signal is strongest and the signal strength is noted. Though this approach does not provide a location for a moving bat, it does provide an estimate of a broad area in which the bat was likely to be using. Since encircling a tagged bat was not possible, we measured time spent in a broad area (e.g., bay/cove) instead.

Radio-tracking was carried out for ~3.5 hr each night, with searches made in several sheltered bays and coves for ~10-15 mins. Given the low flight of *M. macropus* and attenuation of signals by vegetation and other structures, if a signal was detected from a particular bay/cove, it was considered that the bat was within this bay/cove and not neighbouring bays/coves. When tagged bats were located, a GPS location of the observer was

recorded along with the general direction of the bat and signal strength. The signal strength was used to assess whether the bat was likely to be within the bay/cove or flying in a channel of the estuary that drains into the bay/cove. The time the signal was first detected was recorded along with the time at which the signal was lost in order to estimate minimum time spent in each bay/cove. To increase the search area, two tracking teams covered different zones of the estuary (mostly along the Lane Cove River but also east Harbour and sections of the Parramatta River). When bats were not detected in a bay/cove, this was recorded to identify areas that were not being used by tagged bats.

To quantify the minimum time spent by *M. macropus* in the roost bay, an Australis 26K scanning receiver (Titley Scientific, Brendale QLD) fitted with a remote RF data logger (Titley Electronics, Ballina NSW) and an omnidirectional whip antenna (Titley Electronics, Ballina NSW) was set 30 m from the roost and adjacent to the roost bay. The antenna was secured to a tree so that it was parallel to the ground, with the tip of the antenna facing the open bay. The scanning receiver actively scanned through radio-frequencies of tagged bats and the RF data logger logged their presence ("Entry") every four seconds if a pre-determined signal strength was achieved and a minimum of 3 pulses was detected in that period. If a signal was not detected, this was also logged to indicate the bat had left the bay ("Exit") (i.e., either at the roost or in other bays). This was undertaken in the first week of radio-tracking. The time spent by each radio-tracked bat in the area around the RF logger was tallied and averaged across nights for each bat. To quantify the time between foraging bouts in the roost bay, the number of mins between entry and exit times was calculated and averaged across nights for each bat. However, since small differences in time between entry and exit logs were likely to occur as bats shuffled between the bay and the roost during single foraging bouts, average time between foraging bouts would likely be underestimated. To avoid this, only foraging bouts separated by ≥ 15 mins were used to calculate average time between foraging bouts in the roost bay.

In the second week of radio-tracking, to quantify time spent at the roost by each bat, the RF data logger and antenna were moved closer to the roost (within 10 m) with the tip of the antenna facing the roost. Time spent at the roost was calculated as described above for the roost bay and averaged across nights for each bat. Time between extended visits to the roost (≥ 15 mins) was also calculated and averaged across nights for each bat.

Results

In all, 7 362 bat calls were recorded over water across all sites. Of these, 5 110 were identified to species. Those calls that were not identified to species were usually poor quality and of short duration. A total of 3 358 calls were

identified as *M. macropus*, with the species recorded at 92.6 % of sites (54). Other recorded species were Eastern Bentwing Bat *Miniopterus schreibersii oceanensis* (81.5 % of sites), Gould's Wattled Bat *Chalinolobus gouldii* (68.5 %), Eastern Freetail Bat *Mormopterus ridei* (50 %), White-striped Freetail Bat *Austronomus australis* (16.7 %), Little Bentwing Bat *M. australis* (13.0 %) and Little Forest Bat *Vespadelus vulturnus* (3.7 %).

Distribution in Port Jackson

Myotis macropus activity tended to be lowest in tributaries and increased down the estuary, albeit not consistently. 'Hot spots' of activity were identified in several bays in east Harbour and a few bays in west Harbour, while moderately high activity was recorded along small portions of Middle Harbour and Lane Cove River. Lowest activity levels were recorded in larger highly urbanised bays (Canada Bay and Iron Cove where the species was not recorded) and the upper reaches (Duck River) of the Parramatta River zone, in southern embayments (Rozelle Bay and Blackwattle Bay) in west Harbour and in a single bay (Pearl Bay where the species was not recorded) in Middle Harbour (Fig. 4). *Myotis macropus* was detected at two freshwater sites (above tidal Middle Harbour and Lake Parramatta) that were sampled during the study, with low levels of activity recorded at these sites (~5 passes night⁻¹).

Patterns of habitat use

Myotis macropus activity differed significantly among zones within the Port Jackson estuary (GLM: $F_{5,45}=10.243$, $P<0.001$), with activity in east Harbour 7-, 9- and 30-times greater than Middle Harbour, harbour

islands and Parramatta River, respectively (Fig. 5). Activity in Lane Cove River was 6-times greater than Parramatta River, while all other zones did not differ from one another (Fig. 5).

A number of feeding 'hot spots' were identified in east Harbour (Taylors Bay, 97.5 ± 3.5 buzzes night⁻¹; Parsley Bay, 48.5 ± 4.5 ; Camp Cove, 31 ± 9 ; Chowder Bay, 26 ± 12), west Harbour (Balls Head Bay, 45.5 ± 1.5) and Middle Harbour (Crag Cove, 24.5 ± 2.5). *Myotis macropus* feeding activity differed significantly among zones within the Port Jackson estuary (GLM: $F_{5,45}=4.064$, $P=0.004$), with feeding activity in east Harbour 8-, 32- and 53-times greater than Middle Harbour, harbour islands and Parramatta River, respectively (Fig. 6).

In terms of aquatic habitat types, *M. macropus* activity in harbour bays/coves was 6-, 7- and 12-times greater than tributary bays, harbour islands and tributary channels/creeks, respectively (GLM: $F_{3,47}=6.529$ $P=0.001$; Fig. 7). *Myotis macropus* feeding activity showed the same pattern (GLM: $F_{3,47}=4.838$ $P=0.005$; Fig. 8), though the magnitude of differences was greater (harbour bays/coves 7-, 25- and 63-times greater than tributary bays, harbour islands and tributary channels/creeks, respectively).

Relationships with heavy metals

Myotis macropus activity at sites with lowest levels of Zn ($<300 \mu\text{g g}^{-1}$) was 5-6-times greater than all other concentration classes (GLM: $F_{3,47}=3.059$, $P=0.037$; Fig. 9) which did not differ from each other. However,

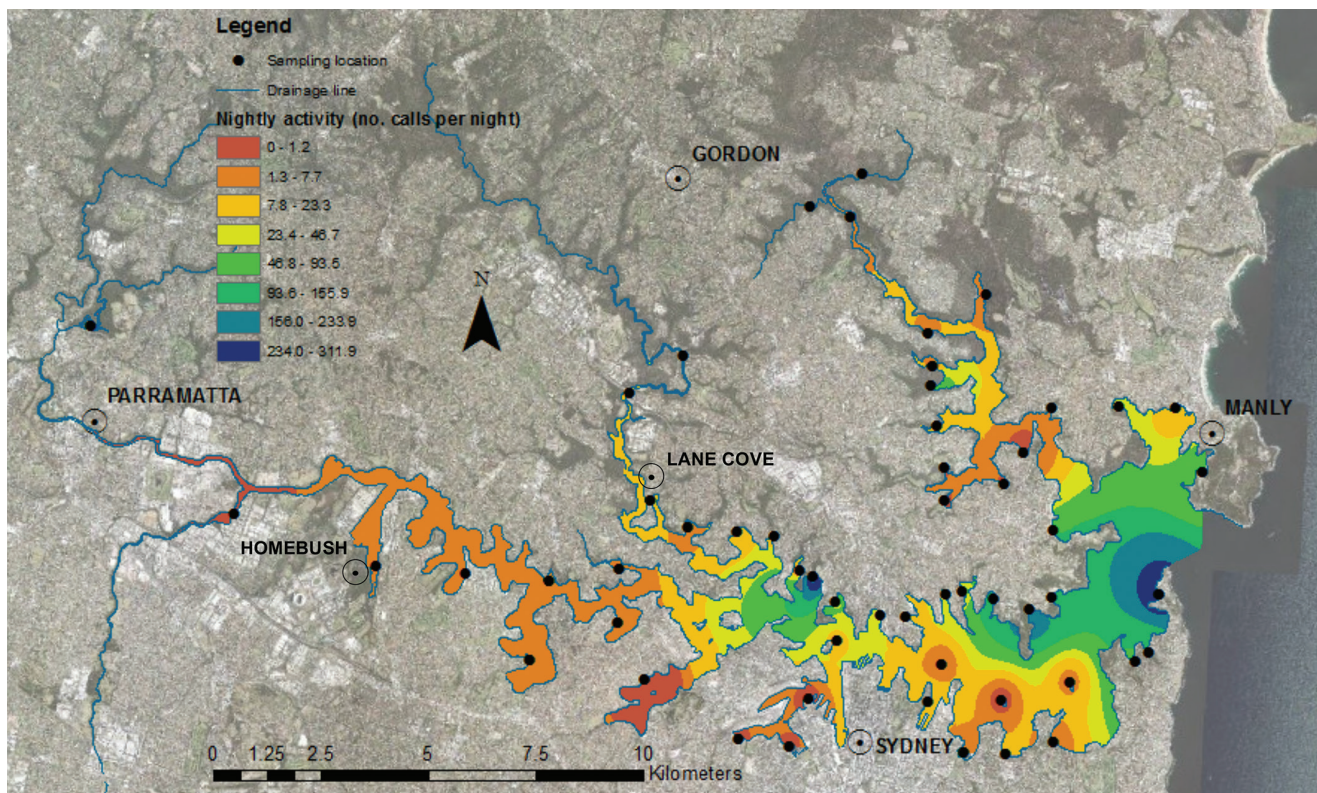


Fig. 4. Map illustrating *M. macropus* distribution weighted by nightly activity recorded at each sampling location.

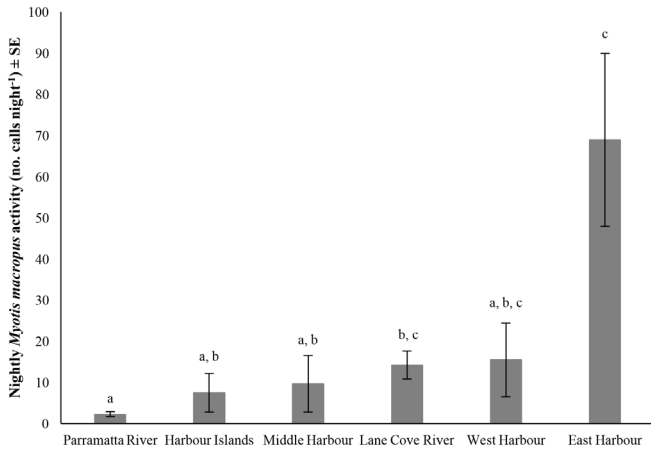


Fig. 5. Nightly *M. macropus* activity in zones within the Port Jackson estuary. Estimated means denoted by different letters are significantly different from one another based on Bonferroni post-hoc pairwise comparisons.

M. macropus feeding activity was not affected by Zn concentration (GLM: $F_{3,47}=0.096$, $P=0.962$).

Sites with the lowest levels of Pb ($<200 \mu\text{g g}^{-1}$) had the greatest level of *M. macropus* activity, but differences between concentration classes were not significant (GLM: $F_{3,47}=2.158$, $P=0.105$). *Myotis macropus* feeding activity was not related to Pb concentrations (GLM: $F_{3,47}=0.235$, $P=0.872$). Similarly, sites with the lowest levels of Cu ($<100 \mu\text{g g}^{-1}$) had the greatest level of *M. macropus* activity, but differences between concentration classes were not significant (GLM: $F_{3,47}=2.270$, $P=0.093$). *Myotis macropus* feeding activity was not related to Cu concentrations (GLM: $F_{3,47}=0.179$, $P=0.910$).

Relationships with environmental variables

A PCA revealed that sites in the Parramatta River zone were associated with high TSS (Duck River, Homebush Bay, Majors Bay, Looking Glass Bay, Tarban Creek) or high water cover and low bushland cover (Five Dock Bay,

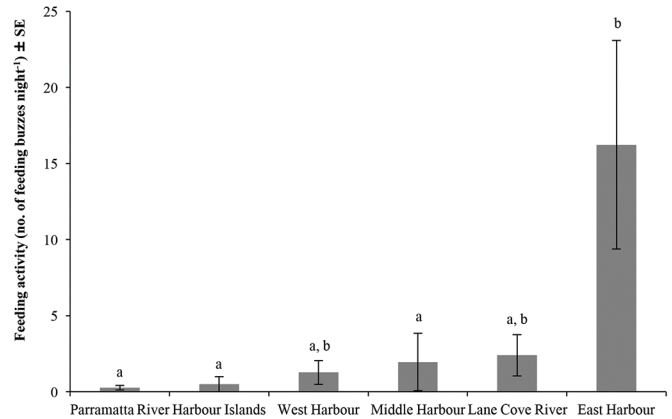


Fig. 6. Nightly *M. macropus* feeding activity in zones within the Port Jackson estuary. Estimated means denoted by different letters are significantly different from one another based on Bonferroni post-hoc pairwise comparisons.

Canada Bay, Iron Cove). A single site (Lake Parramatta) was associated with high bushland cover. In the Lane Cove River zone, sites were highly dispersed in the PCA, with three sites (Epping Bridge, Burns Bay, Tambourine Bay) showing an association with increased mangrove cover and two sites with moderate seagrass (Woodford Bay) or bushland cover (Gore Creek). Sites in the Middle Harbour zone were characterised by moderate to high bushland cover (Carroll Creek, Twin Creeks, Crag Cove, Middle Harbour South, Quakers Hat Bay, Wreck Bay, Willoughby Bay), mangrove cover (Castle Cove) or both (Twin Creeks, Bantry Bay, Middle Harbor North). All other sites (Fishers Bay, Pearl Bay, Sailors Bay) in this zone were more closely associated with seagrass cover. In the west Harbour zone, sites were associated with increased water extent (Blackwattle Bay, Rozelle Bay), seagrass cover (Berrys Bay, Lavender Bay) or bushland cover (Gore Cove). Sites in east Harbour were associated with increased seagrass cover and decreased TSS (Neutral Bay, Shell Cove, Mosman Bay, Taylors Bay, Chowder

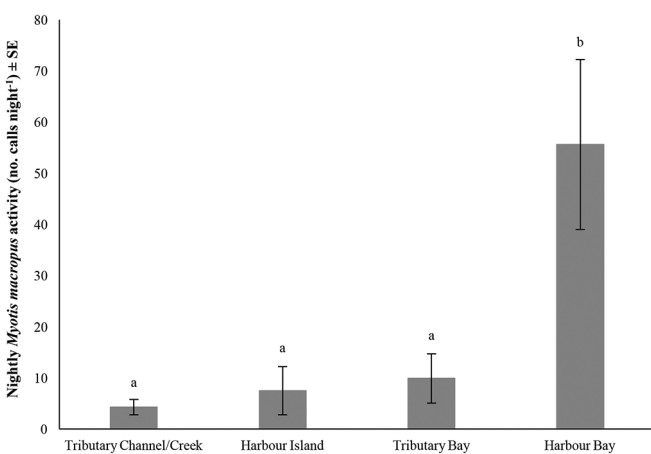


Fig. 7. Nightly *M. macropus* activity in habitats within the Port Jackson estuary. Estimated means denoted by different letters are significantly different from one another based on Bonferroni post-hoc pairwise comparisons.

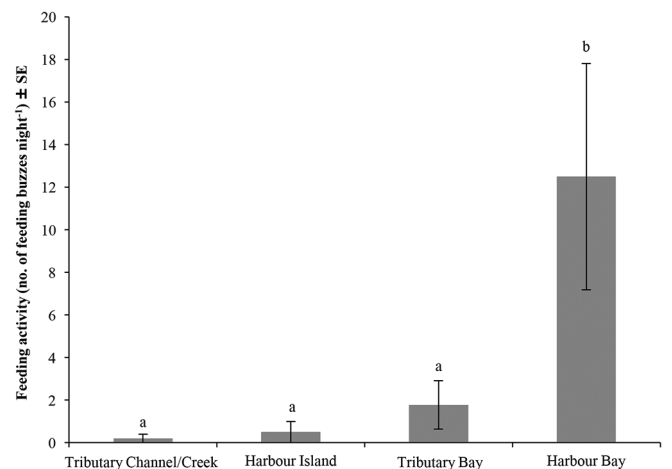


Fig. 8. Nightly *M. macropus* feeding activity in habitats within the Port Jackson estuary. Estimated means denoted by different letters are significantly different from one another based on Bonferroni post-hoc pairwise comparisons.

Bay, Vacluse Bay, Parsley Bay, Jilling Cove, Manly Cove, Spring Cove), or decreased bushland cover and increased water extent (Woolloomooloo Bay, Rushcutters Bay, Double Bay, Rose Bay). All Harbour Islands were associated with increased water extent (Fig. 10).

Best subsets regression in association with AICc scores identified four supported models (within 2 AIC points of the top model) that predicted *M. macropus* activity. Model-1 included TSS (-ve, df=50, R²=0.218, P<0.001), while Model-2, Model-3 and Model-4 also included mangrove cover (+ve, df=49, R²=0.230, P=0.001), water extent (-ve, df=49, R²=0.216, P=0.001) and seagrass cover (+ve, df=49, R²=0.208, P=0.001), respectively (Table 1). Three models that predicted *M. macropus* feeding activity were supported. Model-1 included TSS, which was associated with *M. macropus* feeding activity (-ve, df=50, R²=0.204, P=0.001) (Table 2). Model-2 and Model-3 also included water extent (-ve, df=49, R²=0.216, P=0.001) and mangrove cover (+ve, df=49, R²=0.200, P=0.002), respectively (Table 2). Note that Zinc concentrations in sediments (not included in this analysis) were correlated positively with TSS (df=50, r=0.293, P=0.037).

Radio-tracking of *Myotis macropus*

Radio-tracking of tagged *M. macropus* individuals confirmed that the jetty roost in west Harbour is an important roost in this zone of the Port Jackson estuary, with bats using this site every day during the radio-tracking study (23 days). Only one bat was not detected

after release, all others were radio-tracked for 14±3.5 (range 8-23) days, with the jetty used for roosting each day for the duration of the transmitter battery life or until transmitters were groomed away by bats.

Radio-tracking of *M. macropus* provided a coarse indication of the movements of tagged bats in the Port Jackson estuary. Following emergence from the jetty roost (emergence time; range, ♀:19:48 – 20:01, ♂:19:55 – 20:02; civil twilight range: 19:23 – 19:52) bats would usually spend ~5 mins in the roost bay before moving

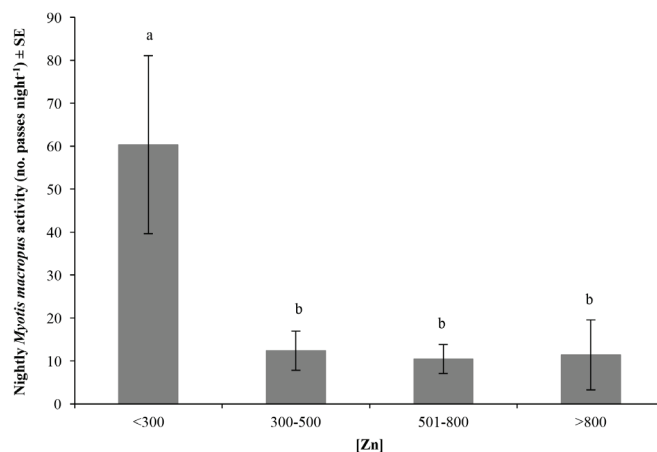


Fig. 9. The association between Zn concentration in surficial sediments and nightly *M. macropus* activity in the Port Jackson estuary. Estimated means denoted by different letters are significantly different from one another based on Bonferroni post-hoc pairwise comparisons.

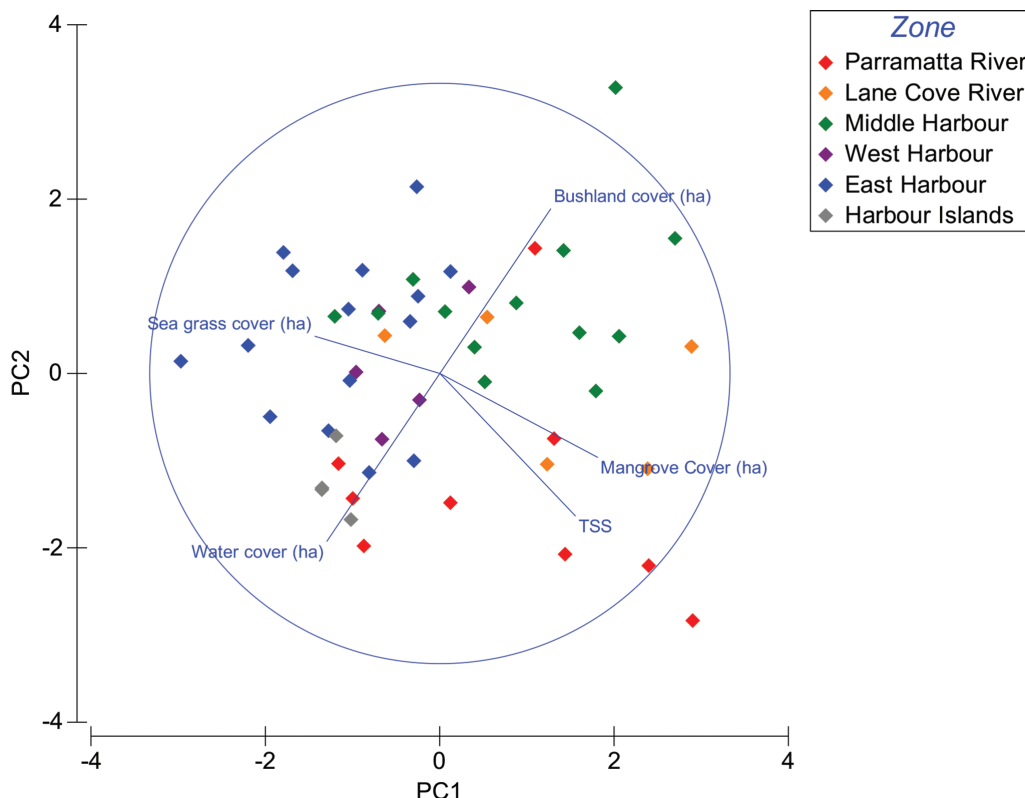


Fig. 10. Principal components analysis indicating which environmental variables were associated with sites in each estuary zone.

elsewhere. Bats generally moved to neighbouring bays in west harbour and Lane Cove River, spending a minimum of 5.5 mins in each bay before moving elsewhere. Variable signal direction in bays indicated bats were likely to be flying. The furthest straight line distance bats were detected from the roost was 4.95 km. Despite searches in other west Harbour bays (Rozelle Bay, Blackwattle Bay, Berrys Bay, Lavender Bay), Parramatta River (Tarban Creek, Iron Cove, Homebush Bay), and east harbour (Careening Cove, Shell Cove, Mosman Bay, Little Sirius Cove), no signals were detected.

On average, tagged bats spent just 54 ± 18 mins in the roost bay each night. The single male bat for which radio-tracking data were collected spent more time in the roost bay (100 ± 40 mins) than female bats (39 ± 14 mins). Time between foraging bouts was shorter for the male bat (46 ± 12 mins) when compared with female bats (221 ± 14 mins).

Table 1. Final regression models for relationships between environmental variables and *M. macropus* activity selected using Akaike information criterion (AICc) score ranking of models.

| Model rank | AICc | Variables | Coefficient | t | P | Model | | | Equation |
|------------|---------|----------------|-------------|--------|--------|----------------|--------|--------|--|
| | | | | | | R ² | F | P | |
| 1 | -54.027 | Constant | 1.595 | 8.377 | <0.001 | 0.218 | 14.933 | <0.001 | <i>Myotis macropus</i> activity (\log_{10}) = 1.595 - 0.190*TSS |
| | | TSS | -0.190 | -3.864 | <0.001 | | | | |
| 2 | -53.586 | Constant | 1.724 | 8.103 | <0.001 | 0.230 | 8.451 | 0.001 | <i>Myotis macropus</i> activity (\log_{10}) = 1.724 - 0.247*TSS - 0.078*Mangrove cover |
| | | TSS | -0.247 | -3.783 | <0.001 | | | | |
| | | Mangrove cover | 0.078 | 1.320 | 0.193 | | | | |
| 3 | -52.703 | Constant | 1.696 | 7.751 | <0.001 | 0.213 | 7.894 | 0.001 | <i>Myotis macropus</i> activity (\log_{10}) = 1.696 - 0.189*TSS - 0.004*Water extent |
| | | TSS | -0.189 | -3.856 | <0.001 | | | | |
| | | Water extent | -0.004 | -0.943 | 0.351 | | | | |
| 4 | -52.178 | Constant | 1.527 | 6.939 | <0.001 | 0.208 | 7.567 | 0.001 | <i>Myotis macropus</i> activity (\log_{10}) = 1.527 - 0.180*TSS + 0.100*Seagrass cover |
| | | TSS | -0.180 | -3.464 | 0.001 | | | | |
| | | Seagrass cover | 0.100 | 0.623 | 0.536 | | | | |

Table 2. Final regression model for relationship between environmental variables and *M. macropus* feeding activity selected using Akaike information criterion (AICc) score ranking of models.

| Model rank | AICc | Variables | Coefficient | t | P | Model | | | Equation |
|------------|---------|----------------|-------------|--------|--------|----------------|--------|-------|--|
| | | | | | | R ² | F | P | |
| 1 | -75.012 | Constant | 0.879 | 5.671 | <0.001 | 0.204 | 13.848 | 0.001 | <i>Myotis macropus</i> feeding activity (\log_{10}) = 0.879 - 0.149*TSS |
| | | TSS | -0.149 | -3.721 | 0.001 | | | | |
| 2 | -74.556 | Constant | 0.993 | 5.622 | <0.001 | 0.216 | 7.892 | 0.001 | <i>Myotis macropus</i> feeding activity (\log_{10}) = 0.993 - 0.148*TSS - 0.005*Water extent |
| | | TSS | -0.148 | -3.743 | <0.001 | | | | |
| | | Water extent | -0.005 | 1.315 | 0.195 | | | | |
| 3 | -73.491 | Constant | 0.946 | 5.406 | <0.001 | 0.200 | 7.233 | 0.002 | <i>Myotis macropus</i> feeding activity (\log_{10}) = 0.946 - 0.179*TSS + 0.040*Mangrove cover |
| | | TSS | -0.179 | -3.325 | 0.002 | | | | |
| | | Mangrove cover | 0.040 | 0.838 | 0.406 | | | | |

Discussion

Myotis macropus was widespread in the Port Jackson estuary, being recorded at 92.6 % of sites, but with high activity (>93 passes night⁻¹) and feeding activity (≥ 24.5 buzzes night⁻¹) concentrated in a few 'hot spots' within the estuary. *Myotis macropus* activity and feeding activity was greatest in east Harbour and lowest on the Parramatta River, with both significantly greater in harbour bays/coves. Radio-tracking revealed that bats roosting in west Harbour showed 100 % fidelity to the roost site and only foraged in bays in this zone and on the nearby Lane Cove River. Historical Zn concentration in surficial sediments was negatively associated with *M. macropus* activity, with a non-significant negative association found for other metals (Cu and Pb). However, this was not the case for feeding activity. TSS was a significant (-ve) variable in all supported models associated with *M. macropus* activity and feeding (heavy metals were only available as categorical data and not included in these analyses).

Distribution in Port Jackson

Although piscivorous bats have been recorded in estuaries previously (Nordlie and Kelso 1975; Kutt 1997; Méndez and Alvarez-Castañeda 2000; Hoye 2002; Bordignon 2006), no studies have specifically investigated the distribution of these species across an entire estuary.

Myotis macropus was first identified on a sheltered saline bay/cove in the Port Jackson estuary in 2014 (Gonsalves and Law 2014), which was unusual in that the species is well known from freshwater streams and creeks, albeit with some records elsewhere on brackish water (Campbell 2011; Clarke-Wood *et al.* 2016). The species was recorded at 92.6 % of sites (n=54), including exposed locations, such as less-sheltered bays/coves and water in the middle of Sydney Harbour (around Harbour Islands – Goat, Fort Denison and Shark). Activity and feeding was variable across the estuary but tended to be low in tributaries and increased down the estuary towards the ocean, with a number of ‘hot spots’ identified in several bays in east Harbour and a few bays in west Harbour. However, radio-tracking revealed that bats roosting in west Harbour would routinely fly up one of the tributaries (Lane Cove River), foraging in bays as they travelled.

Myotis macropus activity and feeding was significantly greater in harbour bays/coves across the estuary than other habitat types. Acoustic data was supported by animal movements revealed during radio-tracking, with bats spending at least 5.5 mins in each bay but briefly recorded along channels during which they were likely to be commuting to other sheltered bays. Harbour bays/coves can provide calmer water surfaces that are more suited to a trawling foraging strategy in which bats detect acoustic glints reflected by prey on water surfaces. Other habitats may experience greater exposure to wind and provide turbulent water which is associated with reduced activity by trawling bats in upland river systems (Warren *et al.* 2000). It is thought that echoes produced by turbulent water may interfere with prey detection of trawling bats (Mackey and Barclay 1989; Rydell *et al.* 1999; Siemers *et al.* 2001).

Relationships between *Myotis macropus*, heavy metal contamination and other environmental variables

Occupying high trophic levels and being sensitive to accumulations of pesticides and water pollution, bats are considered bioindicators (Jones *et al.* 2009). There was a trend for greater *M. macropus* activity in sites with historically lower concentrations of Zn in surficial sediments of the Port Jackson estuary. If this association reflects avoidance of sites on the basis of heavy metal contamination, there is potential for *M. macropus* to be used as an ecological indicator of environmental degradation in the Port Jackson estuary. However, since surficial sediment concentrations of Cu, Zn and Pb have increased in some areas of the estuary and decreased at others since these measurements were undertaken (Birch and Chang 2013),

the negative association between *M. macropus* activity and Zn in surficial sediments should be interpreted with caution. Nevertheless, sediments in the Port Jackson estuary contain some of the highest concentrations of metals reported globally (Birch and Chang 2013) and a high degree of *M. macropus* feeding activity in some parts (e.g., Middle Harbour) that are currently considered to be very severely modified with respect to background levels of Cu, Pb and Zn (Birch and Chang 2013) highlights the vulnerability of *M. macropus* to reductions in water quality (Office of Environment and Heritage 2015). Since historical heavy metal concentrations were strongly correlated with TSS in the Port Jackson estuary, it is unclear whether heavy metal concentrations in sediments or TSS directly affect *M. macropus*. A study investigating the response of bats to coastal lagoon degradation detected heavy metal contamination in *M. macropus* fur, with one individual's fur containing 5 times the lowest-adverse-observable-effects-level of Pb in small mammals (Clarke-Wood *et al.* 2016). In the same study, significantly higher concentrations of heavy metals in sediments of highly degraded lagoons were reflected in the tissues of aquatic invertebrates, indicating a potential pathway for contaminants to move from sediments to *M. macropus* via aquatic prey (Clarke-Wood *et al.* 2016). It remains unclear whether these differences in heavy metal concentrations of sediment and aquatic prey are expressed in the tissues of *M. macropus* since the species was absent from highly degraded lagoons (potentially due to high pollution levels), preventing measurement of heavy metals in tissues at these sites. However, there was a trend for greater metal concentrations in tissues of *M. macropus* at lagoons with moderate levels of degradation when compared with those at lagoons with low levels of degradation (Clarke-Wood *et al.* 2016).

Across the Port Jackson estuary, *M. macropus* activity and feeding was most associated with TSS (-ve), mangrove cover (+ve), water extent (-ve) and seagrass cover (+ve). It should be acknowledged that TSS can be highly variable temporally and is influenced by the amount of runoff resulting from rainfall. While water quality measurements used in our analyses were a 6-month average in 2013, the amount of rainfall recorded in 2015 over the same 6-month period was comparable (Observatory Hill weather station: 995.2 mm (2013); 876.2 mm (2015)). TSS alone explained 21.8 % and 20.4 % of variability in *M. macropus* activity and feeding, respectively. Given TSS (e.g., soil and sediment particles, plankton, algae, etc.) occur in the water column and not on the surface, it is unlikely that TSS directly affects the ability of *M. macropus* to locate prey (e.g., Boonman *et al.* 1998). It is possible that elevated TSS levels may impact prey behaviour and distribution, which for other bat species has been shown to influence where they preferentially forage (Gonsalves *et al.* 2013b). In the Port Jackson estuary, *M. macropus* is known to consume fish (L. Gonsalves and B. Law, unpubl. data). However, levels of TSS in the estuary are markedly lower than those that are known to be

detrimental to some fish species or to result in reduced dissolved oxygen in the water column, thickening of gill epithelium, reduced respiratory function in fish and reduced fish abundance (Horkel and Pearson 1976; Goldes *et al.* 1988; Henley *et al.* 2000). It is also important to note that TSS was correlated with heavy metal concentrations and the latter were not used in analyses of environmental relationships.

TSS only accounted for a small proportion of variation in the activity of the species, indicating that other variables are at play. Several variables examined in this study were found not to be significant predictors of *M. macropus* activity or feeding. Bushland cover was one of these, which is consistent with its poor association with *M. macropus* activity on streams in urban areas (Threlfall *et al.* 2012). Across the urban landscapes sampled in that study, *M. macropus* activity was positively associated with riparian habitats and vegetation gaps.

Mangroves provide hollow resources that are used by roosting insectivorous bats, including *M. macropus* (McConville *et al.* 2013), and along with adjacent habitats, they are used by foraging bats (Gonsalves *et al.* 2012, 2013a, 2013b; McConville *et al.* 2013; McKenzie and Rolfe 1986). Mangrove communities are also generally considered to be important nursery grounds for juvenile fish, with the Transparent Goby *Gobiopsis semivestitus* highly abundant in the Port Jackson estuary (Clynick and Chapman 2002). Though limited mangrove cover

does occur in parts of east Harbour and west Harbour, mangrove cover is more extensive in tributary zones (i.e., Lane Cove River, Parramatta River and Middle Harbour). Yet, mangrove cover alone was not a strong predictor of *M. macropus* activity.

Given other bat species are known to shift foraging ranges in association with key prey items (Gonsalves *et al.* 2013b), it is likely that *M. macropus* activity and feeding activity is influenced by prey abundance and distribution, which may vary seasonally or with prevailing weather. Although it has been confirmed that *M. macropus* in the Port Jackson estuary does consume small fish (L. Gonsalves and B. Law, unpubl. data), it is unclear how common fish are in the diet of the species. In forested streams, fish were found to represent <1 % of *M. macropus* diet, with aquatic invertebrates most commonly consumed (Law and Urquhart 2000). Elsewhere, captive Daubenton's Bat *M. daubentoni* fed insects and fish (~30) showed little evidence of fish consumption, with insect material common in faeces, but only two fish scales and a single bone fragment observed, suggesting that quantification of piscivory may be difficult and probably underestimated for trawling bats (Siemers *et al.* 2001). We recommend characterising *M. macropus* diet in the Port Jackson estuary to confirm the degree of piscivory. If fish are commonly consumed, an understanding of distribution and abundance of small fish may help to explain the variability in *M. macropus* activity, including feeding, across the estuary.

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