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Mangroves as maternity roosts for a colony of the rare east-coast free-tailed bat (*Mormopterus norfolkensis*) in south-eastern Australia

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Supplementary Material

A description of the study area (Supplementary Material 1), radio-tracking methods (Supplementary Material 2), candidate variables (Supplementary Material 3), principal components analyses (Supplementary Material 4), bat roost locations (Supplementary Material 5), model response plots (Supplementary Material 6), bat roost exit times (Supplementary Material 7) and temperature measurements (Supplementary Material 8) are provided below.

Supplementary Material S1. Study area

The study was conducted in the Hunter Estuary, NSW, a 3000 ha wave-dominated estuary at the Port of Newcastle and adjacent to the Newcastle central business district (32°55’36”S 151°46’44”E). The area experiences a warm temperate climate (average monthly temperatures 8.4 – 25.6 °C) and average annual rainfall of 1134 mm (Bureau of Meteorology 2012). Harp trapping of bats for radio-tracking was undertaken in the western portion of what is now a 2600 ha landmass, known as Kooragang Island. Approximately 40% is industrial land, 45% mangrove and estuarine and 15% pasture (Hamer, Lane *et al.* 2002). Kooragang Island is bounded by the north and south arms of the Hunter River and was formed by the artificial joining and reclamation of a number of deltaic islands that existed in the estuary. Historical reclamation works involved the disposal of river dredging spoil and industrial waste from nearby steel works (Hamer, Lane *et al.* 2002). The western portion of Kooragang Island was historically utilised for agriculture including cattle grazing, dairy, fruit growing, timber cutting and salt production. Most of the northern part of Kooragang Island, is part of the Hunter Wetlands National Park, a wetland of international importance under *The Convention on Wetlands of International Importance 1971* (Ramsar Convention). The southern portion of Kooragang Island is heavily industrialised with coal export infrastructure being the primary land-use.

The Hunter Estuary contains high quality remnant estuarine vegetation communities, with the second largest area of mangroves (1600 ha) and the third largest area of saltmarsh (600 ha) in NSW (Geoscience Australia 2012). The non-industrial areas of Kooragang Island are dominated by mangroves and saltmarsh, with pasture and smaller areas of freshwater wetland also occurring. The mangrove forest is largely a monoculture of *Avicennia marina* subsp. *australasica* (grey mangrove), with *Aegiceras corniculatum* (river mangrove) occasionally occurring. Mangrove forests in parts of the Hunter Estuary can form relatively tall and closed forests in comparison with the short, sparse mangrove remnants that often occur elsewhere in NSW.

Areas surrounding the Hunter Estuary have been highly modified by a long history of agriculture, coal mining and port-associated industries. The fertile floodplain of the Hunter River has been extensively cleared for agriculture with only small patches of native vegetation remaining. Hexham Swamp, a large 2500 ha wetland dominated by freshwater reeds at the time of the study, occurs to the west. Further west of Hexham Swamp are areas of undulating hills which support young dry sclerophyll forest with few hollow-bearing trees that overlay nutrient-poor soil. The most intact native vegetation occurs to the north of the estuary on the flat, sandy and nutrient poor soils of the Tomago sandbeds, which are an important local groundwater resource. However, even these areas have been subject to disturbances such as sand mining, military facilities and infrastructure.

Supplementary Material S2. Radio-tracking methods

We captured bats using harp traps (Austbat P/L, Victoria, Australia) mostly set in mangrove forests, with some placed on tracks where bush regeneration plantings of young native trees and shrubs occurred. Lactating females were identified by the presence of bare patches around enlarged nipples and / or expression of milk. We attached radio-transmitters to the dorsal surface of bats by parting the fur and gluing transmitters using adhesive glue (VetBond, 3M). Three different types of transmitters were used: LT4-337 (Titley Electronics, Ballina, Australia) with 30cm aerials during 2009; LB-2N (Holohil, Carp, Canada) with 14 cm aerials during early 2010; and a combination of LB-2N and LT6-337 (Titley Electronics) with 15 cm aerials during late 2010. Transmitters weighed between 0.35 g (Holohil) and 0.4 g (Titley Electronics), which represented ≤ 5% of the body weight of individual bats.

We conducted searches for day roosts within a 10 km radius (maximum nightly flight distance; Chapter 6) from the trapping location, with more effort expended in areas closer to the trapping location than those further away. Day roosts were located from a vehicle, boat or on foot using hand-held directional antennae. Signals from roosts were detectable from 20 - 1000 m depending on roost height, topography and vegetation density. The position of the bat within the tree was estimated using triangulation and signal strength and was later confirmed by observations at dusk. Roost trees were located daily for the battery life of the transmitter or until the transmitter fell off.

Roost trees were watched by observers from 30 min before dusk to 1 h after dusk. Mangrove roosts were difficult to observe due to the relatively dense canopy cover and some trees were not accessible at high tide and were not watched. Where bats were observed to exit, the number of bats that emerged and entered were recorded. We subtracted the number of bats that entered the roost during the watching period from the total colony size. Each observer had a radio-telemetry receiver to identify tracked bats and a bat detector (Anabat SD1, Titley Electronics, Balina, Australia) placed on the ground with microphones angled at 45 degrees to record bat echolocation calls. Colony size, time of tracked bat exit, exit direction, hollow aspect and various hollow dimensions were recorded.

Supplementary Material S3. Candidate variables

Table S1. Candidate tree, plot and landscape variables from roost trees, available mangroves and available other trees

Available other trees were selected from sites from each of the major vegetation types present within 10 km radius of the roosts. * maternity roosts only, males were excluded from this analysis. Plot refers to the 10 m radius plot surrounding a focal centre tree which was either a roost or available hollow-bearing tree. Values are mean ± SE (range) or frequency of ordinal values.

Candidate Variables	Scale	Description	Roosts (n = 34)*	Available Mangroves (n = 15)	Other trees (n = 10)
DBH	Tree	Diameter at breast height over bark (DBH) of tree (cm)	24.0 ± 1.2 (14.3 – 41.4)	24.4 ± 1.6 (14.3 – 33.7)	41.3 ± 6.1 (15.9 – 90.1)
TopoPos	Tree	Topographic position: 1- 8 scale 1. hilltop 2. ridge 3. upper slope 4. mid slope 5. lower slope 6. flat 7. gully 8. creek 9. depression	7.9 ± 0.1 (6 – 8)	8 ± 0 (8)	5.4 ± 0.36 (4 – 6)
DecayClass	Tree	Tree decay class (following Gibbons, Lindenmayer *et al.* 2000), 1-8 scale with 1 being healthy with no hollows and 8 being highly decayed	2.6 ± 0.3 (2 – 8)	2 ± 0 (2)	2.9 ± 0.5 (2 – 7)
PFC	Plot	Percent foliage cover (PFC), converted to ordinal categories for analyses (1 is <= 30; 2 is > 30 and <= 50; 3 is > 50 %)	50.9 ± 1.7 (10 – 65)	54 ± 1.4 (50 – 65)	36.5 ± 2.8 (20 – 50)
RoostTreeHt	Tree	Roost Tree Height (m)	9.8 ± 0.6 (3 – 15)	12.5 ± 0.4 (10 – 15)	14.1 ± 1.1 (9 – 20)
DistancetoWB_cat	Tree	Distance to nearest waterbody with open water for drinking. Ordinal category: 1 is ≤ 100 m; 2 is 100 – 1000 m; 3 is ≥ 1000 m.	1 = 28 2 = 6 3 = 0	1 = 9 2 = 6 3 = 0	1 = 0 2 = 3 3 = 7
DistanceForEdge	Tree	Distance to forest edge (m)	47.0 ± 3.6 (0 – 95)	102.8 ± 16.4 (35 – 244)	114.4 ± 37.5 (10 – 330)
Stem Density_ha	Plot	Stem density per hectare derived from the number of stems > 3 cm DBH in each plot	1062.6 ± 78.3 (159.2 – 2387.3)	1468.5 ± 175.3 (668.5 – 2864.8)	1833.5 ± 291.8 (382.0 ± 2896.7)
HBTDensity_ha	Plot	Hollow-bearing tree density per hectare derived from number of hollow-bearing	837.0 ± 47.3	1020.7 ± 84.6	114.6 ± 43.5

Candidate Variables	Scale	Description	Roosts (n = 34)*	Available Mangroves (n = 15)	Other trees (n = 10)
		stems per plot	(95.5 – 1273.2)	(445.6 – 1559.7)	(31.8 – 477.5)
HBTAbundindex	Plot	Number of hollow-bearing trees / number of stems in plot. Converted to ordinal category for PCA (1 is <= 0.5; 2 is > 0.5 and < 0.85; 3 is >= 0.85)	0.78 ± 0.03 (0.18 – 1)	0.74 ± 0.05 (0.22 – 0.97)	0.07 ± 0.04 (0 – 0.42)
AvgOfDecayClass	Plot	Average of stem decay class within plot	2.3 ± 0.1 (1.4 – 3.3)	2.4 ± 0.1 (1.8 – 3.1)	1.3 ± 0.1 (1.1 – 1.7)
AvgofDBH	Plot	Average of stem DBH (cm) within plot	19.8 ± 0.9 (10.1 – 34.2)	17.8 ± 1.5 (8.8 – 27.4)	14.1 ± 3.1 (7.9 – 40.3)
FW_500_pres FW_1000_pres	Landscape	Binary freshwater wetland category (> 5 % freshwater wetland = 1; < 5 % freshwater wetland = 0) within two buffers of tree (500 m; 1 km radii). Wetland boundaries digitised in GIS from aerial photography	500 1 = 0 0 = 34 1000 1 = 21 0 = 13	500 1 = 0 0 = 15 1000 1 = 1 0 = 14	500 1 = 1 0 = 9 1000 1 = 1 0 = 9
VEG_500_pres VEG_1000_pres	Landscape	Binary vegetation category (> 5 % vegetation = 1; < 5 % vegetation = 0) within two buffers of tree (500 m; 1 km radii). Woody vegetation only. Boundaries digitised in GIS from aerial photography	500 1 = 0 0 = 34 1000 1 = 0 0 = 34	500 1 = 1 0 = 14 1000 1 = 2 0 = 13	500 1 = 6 0 = 4 1000 1 = 6 0 = 4
MATMANG_500_pres MATMANG_1000_pres	Landscape	Binary mature mangroves category (> 5 % mature mangroves = 1; < 5 % mature mangroves = 0) within two buffers of tree (500 m; 1 km radii). Mature mangroves only. Boundaries digitised in GIS from aerial photography	500 1 = 33 0 = 1 1000 1 = 29 0 = 5	500 1 = 12 0 = 3 1000 1 = 10 0 = 5	500 1 = 0 0 = 10 1000 1 = 0 0 = 10
URB_500_pres URB_1000_pres	Landscape	Binary urban land-use category (> 5 % urban land-use = 1; < 5 % urban land-use = 0) within two buffers of tree (500 m; 1 km radii). Boundaries digitised in GIS from aerial photography	500 1 = 12 0 = 22 1000 1 = 32 0 = 2	500 1 = 2 0 = 13 1000 1 = 4 0 = 11	500 1 = 2 0 = 8 1000 1 = 4 0 = 6

Supplementary Material S4. Principle Components Analyses

Methods

We conducted a Principle Components Analysis (PCA), using JMP (SAS Institute, version 9.0) to assess the similarity among maternity roosts selected by *M. norfolkensis* compared to available mangroves and available other trees using tree, plot and landscape characteristics (Table S1). Additionally, we conducted a separate PCA to assess the similarity of roost hollows with adjacent available hollows using entrance and internal dimensions. A correlation matrix on normalised data was used in both of the PCA.

Results

The PCA on attributes of roosts selected by lactating females indicated that there was overlap with available mangroves and a high level of separation from available other trees (Figure S1). The first three axes accounted for 63.9 % of the variation in the data, with Component 1 explaining the most variation (36.6 %). Roosts and available mangroves grouped together higher on the Component 1 axis than other trees (Figure S1), with the most important factors describing roosts and available mangroves being the presence of mature mangroves within 500 m, close proximity to water bodies and in patches with greater decay and a high proportion and density of hollow-bearing trees. Additionally, of less importance, shorter trees in plots with greater canopy cover and with freshwater wetland, mature mangroves and urban land-use within 1 km describes roosts and available mangroves on the Component 1 axis.

Roosts were also grouped lower on the Component 3 axis away from available mangroves (Figure S1), which indicates roost trees were closer to the forest edge than available mangroves. Roost trees were also shorter and in plots that had a lower proportion of hollow-bearing trees, less foliage cover, but had freshwater wetland and urban land-use within 1 km than available mangroves on the Component 3 axis.

The PCA on size and depth attributes of maternity mangrove hollows indicated that there was substantial overlap with nearby hollows (Figure S2), suggesting that maternity hollows were similar to adjacent available hollows. The first two axes accounted for 85.3 % of the variation in the data, with Component 1 explaining the most variation (53.7 %).

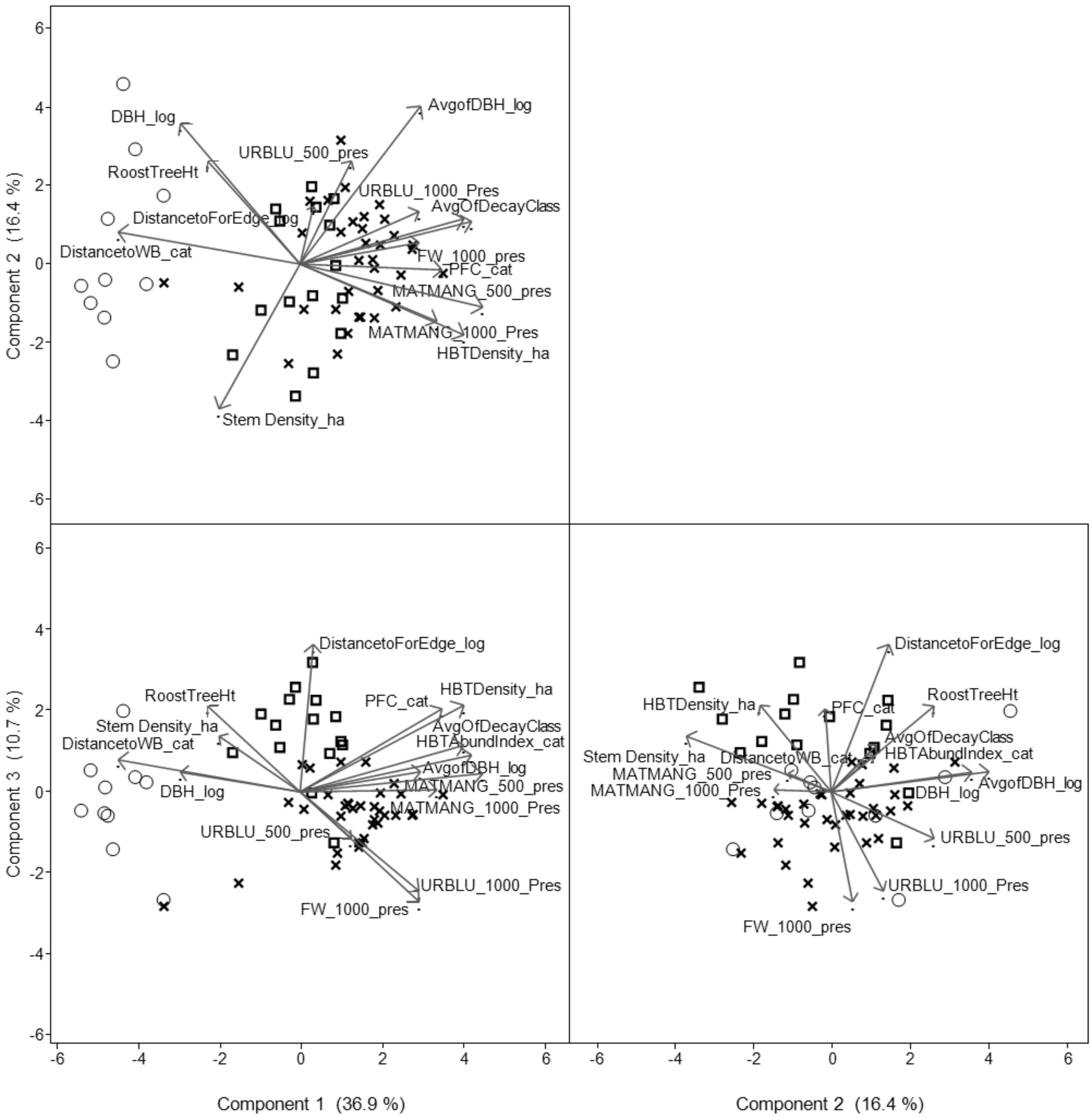


Figure S1. Plots of the first three principal components using tree, patch and landscape variables of known roosts (X), random mangrove (square) and other tree (circle)

The first three axes account for 63.9 % of the variation on the data. See Table S1 for explanation of candidate variables.

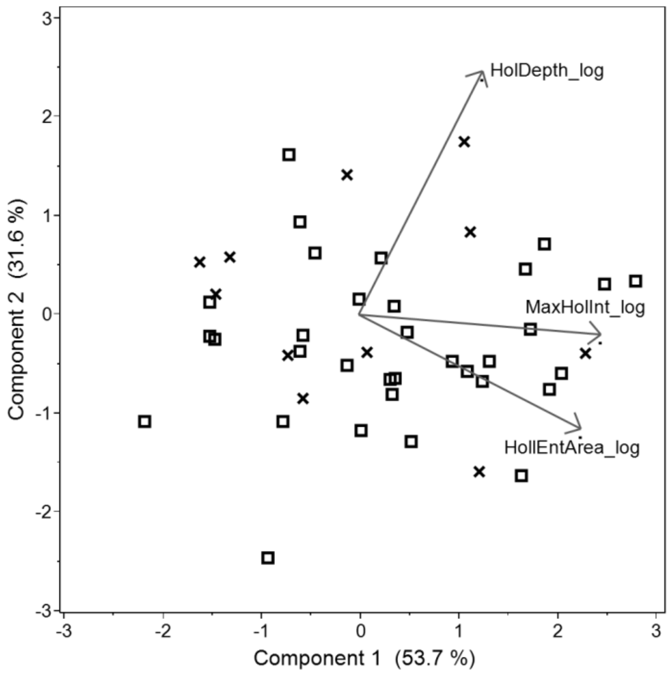


Figure S2. Plot of the first two principal components using hollow depth, hollow internal area and hollow entrance area of known *Mormopterus norfolkensis* maternity roosts (X) and available mangrove hollows (square)

The first two axes account for 85.3 % of the variation on the data.

Supplementary Material S5. Roost location

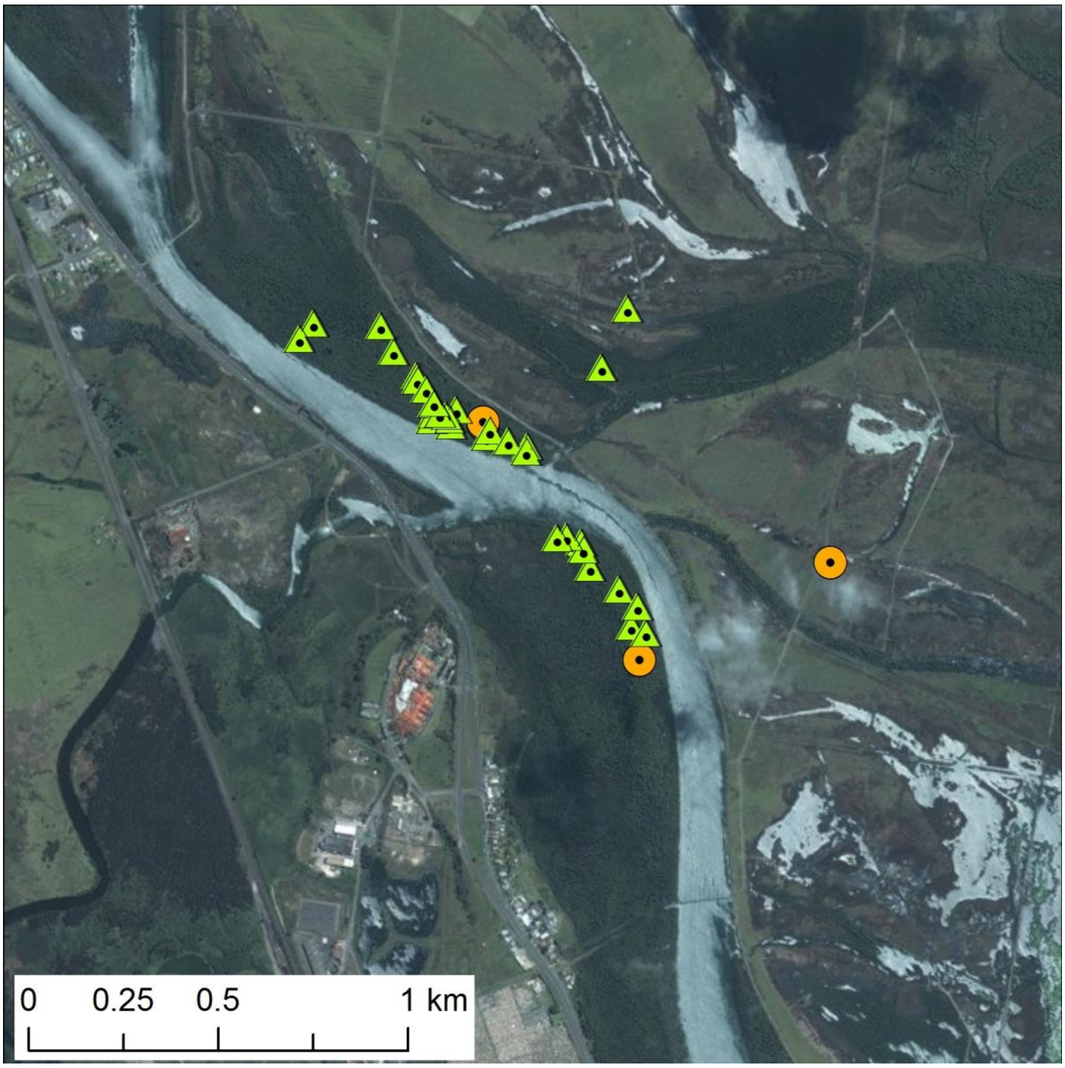
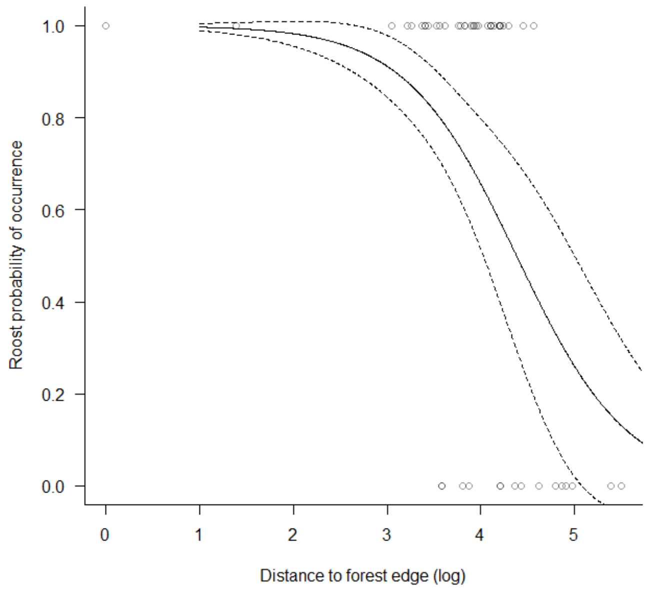
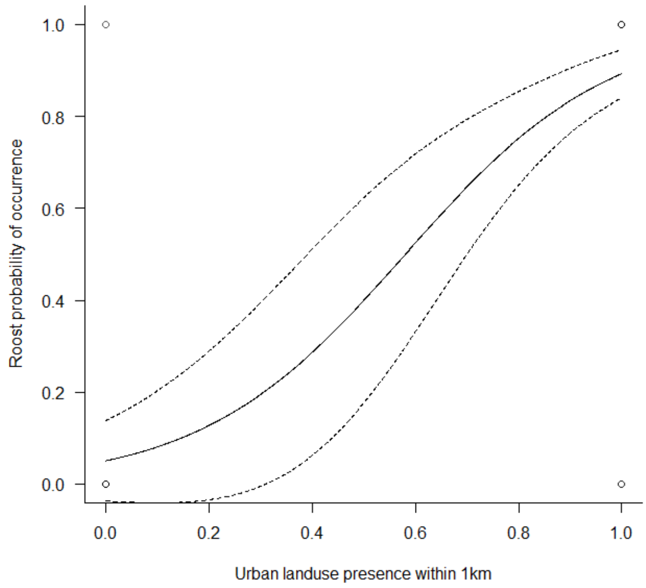


Figure S3. Location of maternity roosts (green triangle) and male roosts (orange circle) on the south arm of the Hunter River, NSW

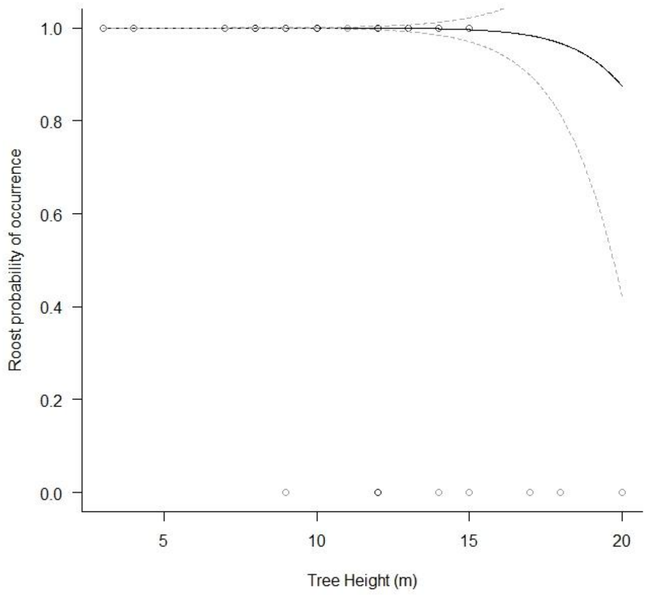
Supplementary Material S6. Model response plots

a)





b)



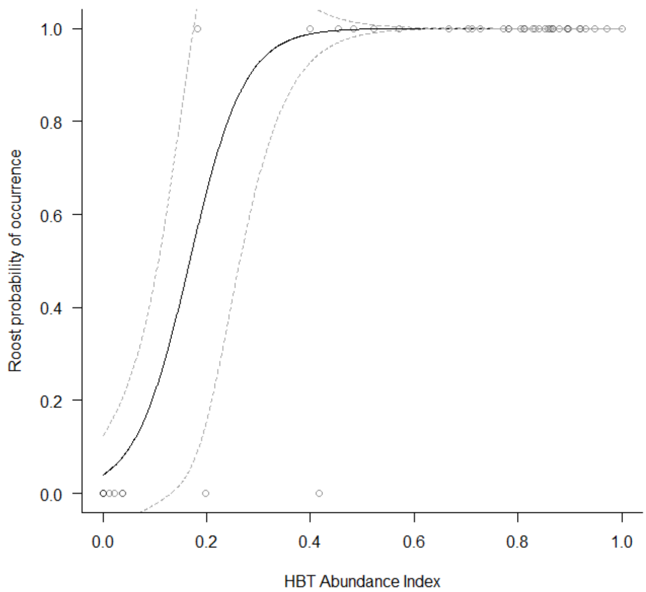


Figure S4. Roost logistic regression model response plots

Partial-plots of the relationship between probability of *Mormopterus norfolkensis* maternity roost occurrence and environmental variables for the best fitting models comparing a) roosts to mature mangroves; and b) roosts to other trees. The dashed lines indicate 95% confidence intervals. The x-axis represents the range of values sampled for each environmental variable. Over-plotting of multiple points is visualized by increasingly darker shades of grey.

Supplementary Material S7. Bat roost exit times

Student's t-tests were used to compare the emergence time of tracked *M. norfolkensis* and the time that the first bat exited the roost to the time of first bat activity in the mangroves obtained from bat echolocation calls recorded using ultrasonic bat detectors (Anabat SD1, Titley Electronics, Balina, Australia).

Tracked bats emerged 34.1 ± 1.9 minutes (n = 26, range 2 – 58 mins) after sunset, which was significantly later than the first bat activity recorded on bat detectors at 13.5 ± 1.5 minutes after sunset ($t_{83} = 9.08$, $p < 0.001$; n = 35, range 0 – 36 mins; Figure S5). Additionally, the first bats out of the roosts exited significantly later (30.5 ± 1.5 minutes after sunset, n = 25) than the first bat activity recorded ($t_{83} = 7.42$, $p < 0.001$). Bats were then usually observed to move quickly out of signal range (average 6.5 ± 1.3 mins; n = 10, range 2 - 15 mins). We occasionally observed solitary bats entering roosts on dusk, which was usually followed by audible noises from bats already located within the roost and then a single bat exiting shortly afterwards.

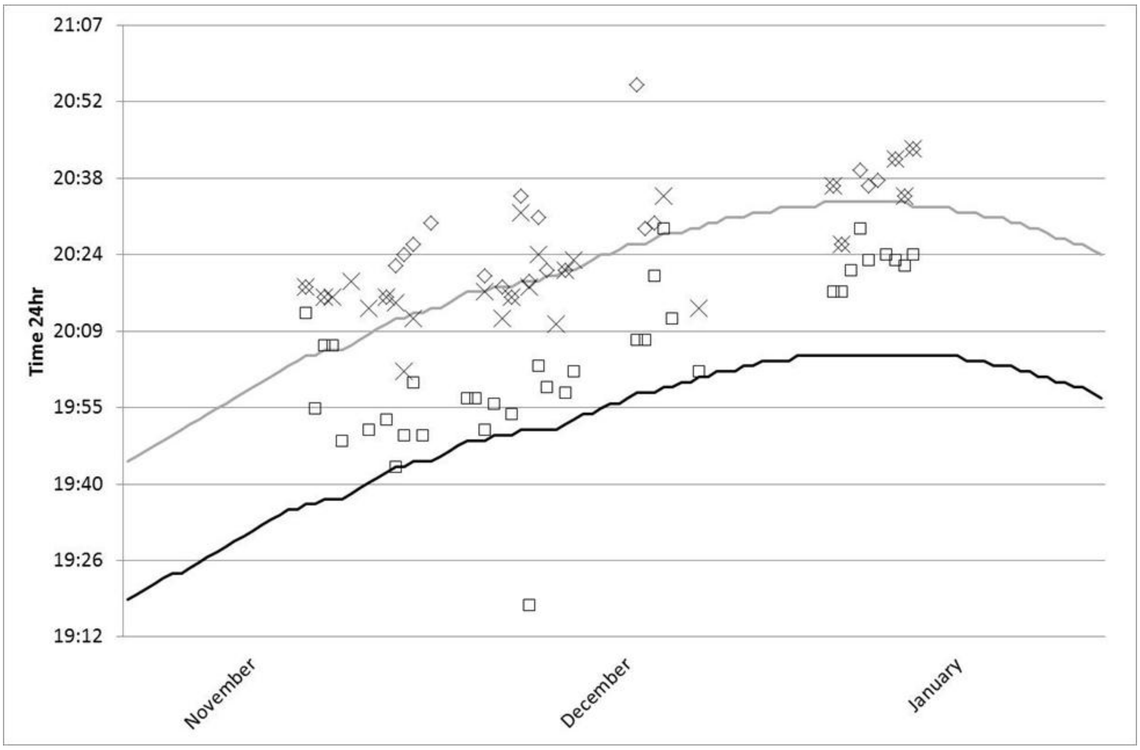


Figure S5. Emergence times for tracked *Mormopterus norfolkensis* (X), first bat activity (square) and start of *M. norfolkensis* roost emergence (diamond)

Emergence data from the Hunter Estuary mangroves during 2009 - 2011 are combined. Dark and light lines represent sunset and civil twilight times respectively.

Supplementary Material S8. Temperature measurements

Table S2. Average temperature measurements

Average temperature (± SE) recorded in mangroves (n = 3) compared with other habitats (n = 3). Temperature was recorded over a 5-day period, with results presented as mean temperature in three time blocks: a) 24hr (1:00 – 24:00 h), b) day (07:00 – 19:00 h) and c) night (20:00 – 06:00 h). Paired t-tests were used to summarise differences (*df* = 2) and significant differences (*) reported at α = 0.05.

Variable	**Mangroves**	**Other Habitat**	**t**	**p**
a) 24 hour				
Average	24.05 ± 1.63	24.68 ± 1.69	5.16	0.0355*
Minimum	20.31 ± 1.42	18.81 ± 0.67	-1.81	0.2126
Maximum	27.60 ± 1.94	31.58 ± 2.96	2.71	0.1131
Range	7.29 ± 1.15	12.77 ± 2.30	2.39	0.1393
Rate of Change	-0.06 ± 0.02	-0.06 ± 0.01	-0.11	0.9218
b) Day				
Average	25.06 ± 1.53	27.26 ± 2.08	2.86	0.1038
Minimum	21.24 ± 1.47	19.79 ± 0.47	-1.31	0.3203
Maximum	27.53 ± 1.94	31.58 ± 2.96	2.65	0.1177
Range	6.28 ± 1.47	11.79 ± 2.5	2.09	0.1721
Rate of Change	-0.21 ± 0.05	0.40 ± 0.02	3.02	0.0946
c) Night				
Average	22.77 ± 1.65	21.50 ± 1.04	-1.90	0.1971
Minimum	21.22 ± 1.72	19.56 ± 1.00	-2.16	0.1637
Maximum	25.23 ± 1.66	25.05 ± 1.23	-0.43	0.7096
Range	4.01 ± 0.13	5.49 ± 0.28	3.75	0.0642
Rate of Change	-0.42 ± 0.03	-0.63 ± 0.01	-4.60	0.0441*

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