2017 West Angelas Ghost Bat Monitoring

Rio Tinto Iron Ore

January 2018

DOCUMENT STATUS				
Revision No.	Author	Review / Approved for Issue	Approved for Issue to	
			Name	Date
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EXECUTIVE SUMMARY

Ghost Bats (*Macroderma gigas*) have been known to occur within the West Angelas area since 1978, and have been recorded intermittently since this time. This survey, commissioned by Rio Tinto Iron Ore (RTIO), represent the fifth Ghost Bat monitoring survey at West Angelas since the recommencement of the monitoring program in 2012. The overarching objective of the survey was to undertake a Ghost Bat monitoring survey at five caves which have previously been regarded as significant for the species (A1, A2, L2, L3, AA1). The field survey was undertaken during the dry season, between the 17th and 19th October 2017. Survey methods employed during the survey were consistent with previous surveys: conducting visual inspections for individuals and fresh scat material, and conducting ultrasonic recordings. Additional survey techniques, genetic and hormone analysis on scat samples were also employed to better understand Ghost Bat usage at each monitoring cave.

All five monitoring caves were successfully sampled during the survey. No individuals were recorded during the survey. Fresh scats originating from the species were recorded at each monitoring cave confirming that the species was present within the area since the previous monitoring survey. The number of fresh scats differed substantially between the caves, with totals ranging from 1 to ~2,000. The highest number of fresh scats was recorded at AA1. The genetic analysis revealed a total of 34 unique genotypes (unique individuals), comprising 12 individuals from 2015 and 24 individuals from 2017. The majority of individuals (91%) were recorded at only one cave. None of the genotypes identified have previously been identified in other studies conducted within the region. Of the 168 scat samples analysed 103 (61%) contained elevated progesterone indicating the presence of pregnant individuals. Elevated progesterone levels were recorded at every cave, with the highest proportion recorded at cave A1.

The results obtained from this survey were relatively consistent with the most recent survey, as well as most surveys preceding this. The number of scats recorded at A2 and L2 indicated these caves were used as night roosts only and are unlikely to be of particular significance to the local population. Cave L3 was also used as a night roost, although it has been regarded as a diurnal roost in the past and therefore should be considered of moderate significance. Eleven individuals were recorded at A1 over a three-year sampling period. The cave has been confirmed as a diurnal roost in the past and contained a high proportion of scats with elevated progesterone. These results suggest that the cave may potentially be a maternity roost for the species and should be considered of high significance. The largest amount of Ghost Bat activity was recorded at AA1, with over 2,000 fresh scats recorded. A total of 19 individuals were recorded at the cave, although there was convincing evidence to suggest more individuals would have been recorded with more analysis. Together these traits indicate the cave is of very high conservation significance and all efforts should be made to minimise disturbance at the cave as this may impact upon the species both locally and regionally.

No additional disturbance was recorded at any of the monitoring caves since the last survey and there was no indication that mining activity had a significant impact on the species activity between this survey and the previous. With this is mind, current management procedures to minimise impacts to the species should be maintained.

1 INTRODUCTION

1.1 Background

The West Angelas Iron Ore Mine is located approximately 130 kilometres (km) west north-west of Newman, in the Pilbara region of Western Australia (Figure 1.1). Ghost Bats (*Macroderma gigas*) have been known to occur within the West Angelas area since 1978 (Integrated Environmental Services, 1980), and have been recorded intermittently since this time.

In 1997, as part of the West Angelas Iron Ore proposal, a Ghost Bat Management Plan was developed prescribing a series of monitoring surveys between 1997 and 2003. During each survey five caves were monitored, comprising four in close proximity to Deposit B (A1, L2, L3 and I1) and one near Deposit F (AA1). In 2012, monitoring was resumed at the site following amendments to the mine layout. One cave (I1) from the original monitoring program could not be located during the survey and was subsequently removed from the monitoring program. The cave was replaced by an additional cave, A2 (located in close proximity to cave A1), which contained evidence of the species during the 2012 monitoring survey (Biologic, 2013). This survey, commissioned by Rio Tinto Iron Ore (RTIO), represents the fifth monitoring survey at West Angelas since the recommencement of the Ghost Bat monitoring program in 2012.

1.2 Objectives

The overarching objective of the survey was to undertake a Ghost Bat monitoring survey at five caves which have previously been regarded as significant for the species (A1, A2, L2, L3, AA1), hereafter referred to as the 'monitoring caves'. Specifically, the objectives of the assessment were to:

- Survey the monitoring caves at West Angelas to determine presence of Ghost Bat and/or recent usage;
- Undertake scat analysis to assess the relative importance and conservation significance of caves at West Angelas;
- Review previous surveys conducted at West Angelas and compare results obtained from this survey to discuss temporal trends;
- Discuss potential impacts on the Ghost Bat population at West Angelas from current and proposed mining activities; and
- Review the frequency of Ghost Bat monitoring at West Angelas and provide recommendations on how RTIO can monitor against the management actions and triggers detailed in the Fauna Management Plan section of the EMP.

677500 680000 682500 685000 687500 690000 692500 695000

7442500 7440000 7437500 7435000 7432500

A1 A2 L2 L3 AA1

Port Hedland
Roebourne
1
95
Millstream-Chichester National Park
Wittenoom
Tom Price
Karijini National Park
Newman

Sources: Esri, HERE, DeLorme, USGS, Intermap, INCREMENT P, NRCan, Esri Japan, METI, Esri China (Hong Kong), Esri Korea, Esri (Thailand), MapmyIndia, NGCC, © OpenStreetMap contributors, and the GIS User Community

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Legend

- Monitoring Caves

biologic Environmental Survey

N

1:52,000

0 0.75 1.5 3 km

Rio Tinto Iron Ore - West Angelas

2017 Ghost Bat Monitoring

Fig. 1.1: West Angelas mine site and location of monitoring caves

Coordinate System: GDA 1994 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA 1994

Size A3. Created 14/12/2017

1.3 Ghost Bat (*Macroderma gigas*)

The Ghost Bat comprises the monotypic genus *Macroderma* (TSSC, 2016). Individuals can weigh up to 150 g, with an average weight of 130 g, and have an average wing span of 686 mm (McKenzie & Bullen, 2009). Individuals have a pale grey or light brown fur with a lighter belly and pale cream to brown wing membranes (Churchill, 2008). They have large ears, measuring on average over 50 mm, which join above the head, large eyes and a long simple-shaped nose leaf extending along the muzzle (Churchill, 2008).

The range of the Ghost Bat is now restricted to the Pilbara, Kimberley, the northern part of the Northern Territory, coastal and near coastal Queensland from Cape York to near Rockhampton (Churchill, 2008), and Western Queensland (TSSC, 2016, Figure 1.2). In the Pilbara region, the species occurs in all four subregions (Figure 1.2), and was recorded in 21 of the 24 areas surveyed by the Department of Environment and Conservation during the Pilbara Biological Survey (2002-2007, McKenzie & Bullen, 2009). The Pilbara population is estimated to be between 1300 and 2000 individuals with the greater majority of individuals occurring in disused mines of the Chichester subregion (TSSC, 2016). The species is listed as Vulnerable under the *Environment Protection and Biodiversity Conservation Act 1999* and Schedule 3 (Vulnerable) under the *Wildlife Conservation Act 1950*.

The distribution of the species in the Pilbara is determined by the presence of suitable roosting sites, either natural caves or man-made mines and adits (Armstrong & Anstee, 2000). Natural roosts within the Hamersley Ranges generally comprise deep, complex caves beneath bluffs or low rounded hills predominantly within Marra Mamba iron ore formations (Armstrong & Anstee, 2000). Roosting sites are suspected to contain high relative humidity as indicated at two known maternity roosts in the Hamersley Ranges (82-84 %). Caves used by the species can be classified into five types: night roost, night/possible day roost, diurnal roost, diurnal roost/possible maternity roost and maternity roost.

There are few known maternity roosts in natural caves in the Pilbara. Centralised breeding sites in the Pilbara are largely restricted to abandoned mines in the Chichester Ranges; however, there are a number of smaller maternity roosts in the Chichester and Hamersley Ranges (Armstrong & Anstee, 2000). Based on available data, breeding has been documented in natural caves at Mining Area C, Mt Brockman and West Angeles in the Hamersley sub-region, and at Callawa and Tambrey Station in the Chichester subregion (Armstrong & Anstee, 2000).

It is believed that Ghost Bats move between a number of caves seasonally, or as dictated by weather conditions, and require a range of cave sites (Hutson *et al.*, 2001). Outside the breeding season, males are known to disperse widely, most likely during the wet season when conditions would allow bats to use caves that would otherwise not be suitable (Armstrong & Anstee, 2000). Genetic studies indicate that females are likely to stay close to the maternity roosts (Worthington-Wilmer *et al.*, 1994). Populations are potentially distinct at local and regional scales (Worthington-Wilmer *et al.*, 1994).

There are currently no studies on the home range of Ghost Bats in the Pilbara. A study in the Northern Territory (Tidemann *et al.*, 1985) provides some information; however, there are likely to be differences in the ecology and foraging behaviour of individuals from the Pilbara region. Tidemann *et al.* (1985) recorded an average foraging area of 61 ha, with foraging areas centred around 1.9 km from the day

roost and found that individuals generally returned to the same foraging areas each night. Ghost Bats in the Pilbara are believed to mate in July and August, with the females giving birth approximately three months after in October (Richards *et al.*, 2008). Young are weaned on prey captured by the mothers, and hunt with the mothers until they become completely independent.

Ghost Bats have a 'sit and inspect' foraging strategy; they hang on a perch where they visually inspect their surroundings for movement (Boles, 1999). Once their prey is detected it may be captured in the air, gleaned from the ground or vegetation (taken from the surface of a substrate by a flying bat), or dropped on from a perch (Boles, 1999).

200000 300000 400000 500000 600000 700000 800000 900000

7800000 7700000 7600000 7500000 7400000

Port Hedland
Karratha
Marble Bar
Onslow
Pannawonica
Tom Price
Paraburdoo
Newman

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Legend

DBCA Ghost Bat Records

Species Distribution (DoEE, 2017)

Species of Species Habitat May Occur

Species of Species Habitat Likely to Occur

biologic Environmental Survey

N

1:2,000,000

0 30 60 120 km

Rio Tinto Iron Ore - West Angelas

2017 Ghost Bat Monitoring

Fig. 1.2: Ghost Bat Distribution and Regional Records

Coordinate System: GDA 1994 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA 1994

Size A3. Created 04/01/2018

1.4 Historical Ghost Bat Occupation

An overview of previous Ghost Bat monitoring at West Angelas is summarised in Table 1.1.

Table 1.1: Summary of Ghost Bat monitoring at West Angelas from 1979 – 2015

Survey	Summary
An ecological appreciation of the West Angelas environment, Western Australia 1979 Integrated Environmental Services (1980)	Ghost Bats were reported from a cave near Deposit E (Cave 1) and presence at two further caves, including one near Deposit B (Cave 3). Caves 1 and 3 were considered to be maternity roosts on the basis of their large middens and the presence of a possibly pregnant female captured at Cave 1. Based on coordinates, Cave 1 appears to be AA1, and Cave 3, appears to be in the vicinity of A1, as documented by of ecologia (1998) and later reports.
West Angelas Iron Ore Project Vertebrate Fauna Assessment Survey ecologia (1998a)	A vertebrate fauna assessment undertaken between June and October 1997. Four Ghost Bats were recorded in rocky gully habitat (sites WA4, WA12 and WA13) in the mine area including a cave near Deposit B. Eight Ghost Bats were observed roosting in a cave overlooking the Mulga plains in the Coondewanna West section of the rail corridor.
West Angelas Project Ghost Bat (*Macroderma gigas*) Assessment Survey ecologia (1998b)	A targeted Ghost Bat assessment of gullies adjacent to Deposits A, B, E and F, undertaken between August and September 1998. The survey sought to clarify the distribution and abundance of Ghost Bats at West Angelas. A total of 60 caves in 27 gullies were searched for Ghost Bats, scat material and animal remains. Many cave-like structures were found; although few were regarded as suitable roosts for bats. Only one individual was recorded, none were found in overhangs or other geomorphological features. One mature female Ghost Bat, presumed pregnant due to swelling in abdomen, was captured in a very large cave (AA1) near Deposit F. The abundance of scats and feeding remains in the AA1 suggested long term utilisation. The cave was thought to be a Ghost Bat maternity cave and was considered to be of considerable conservation significance. A total of six caves and an adit contained evidence of Ghost Bat. The condition of scat material in the other five caves and the adit suggested relatively recently usage, at least within the last year. It appeared that these caves were subject to only temporary, intermittent or seasonal use only, and regarded as feeding sites only (A1, L2, I1, AB1 and the adit).
West Angelas Minesite Ghost Bat Assessment Survey, September 2000 ecologia (2000)	A targeted survey was undertaken in August 2000 for evidence of Ghost Bats in five caves previously identified a significant for the species (AA1, A1, L2, L3, I1). Of the five caves surveyed, recent evidence of Ghost Bats was recorded in three caves (I1, A1 and AA1) and a Ghost Bat was sighted in cave A1. Caves L2 and L3 showed signs that Ghost Bat habitation in the past, but it was difficult to evaluate how long ago this occurred.

Survey	Summary
West Angelas Minesite Ghost Bat Monitoring Survey, September 2001 ecologia (2001)	A survey of caves identified as supporting Ghost Bats during the September 2000 survey was undertaken during September 2001. Bat occupation was based on the presence of scats and condition of scat material. Of the five caves known to contain evidence of Ghost Bats, recent activity was recorded at only three caves. Evidence collected in two of the caves (AA1 and A1) comprised bone fragments and scats. In the third cave (AB1) only scats were collected. No Ghost Bats were found roosting in any cave searched during the 2001 survey.
Ghost Bats at West Angelas: 2002 Survey, Data Review and Future Directions Biota (2002)	Seven Ghost Bat roosts were examined for current or recent signs of occupancy (A1, I1, L2, L3, AB1, AA1, Adit) in November 2002. No Ghost Bats were observed in any feature. Recent signs of occupancy were recorded in three caves (AA1, AB1 and L3) and the Adit. The remainder of the caves (A1, I1 and L2) showed no signs of recent activity.
Monitoring of Ghost Bat Roosts at West Angelas 2003 Biota (2004)	Seven Ghost Bat roosts were examined for current or recent signs of occupancy (A1, I1, L2, L3, AB1, AA1, Adit) in December 2003. One Ghost Bat was observed in A1, as well as a small amount of recent scat. Recent signs of occupancy were also present in two other caves (AA1 and AB1). The West Angelas adit and the remainder of the caves (I1, L2 and L3) showed no signs of recent activity.
West Angelas – Deposit B Ghost Bat Assessment Biologic (2013)	Four caves were surveyed for Ghost Bat presence in October 2012 (A1, A2, L2, L3), I1 could not be located. Caves A1 and L3 were also surveyed using passive ultrasonic recorders. Ghost Bat presence was confirmed at A1 and L3 by the presence of a significant quantity of recent scats, and by Ghost Bat calls recorded on two nights outside cave L3. These two caves were categorised as feeding/ night roosts and occasional day roosts. The size and complexity of these caves, together with the quantities of scats, suggests occasional use by maternal females.
West Angelas – Deposit B Ghost Bat Assessment Biologic (2014)	Five caves were surveyed for Ghost Bat presence in November 2013 (A1, A2, L2, L3, AA1), by scat count and ultrasonic recordings. Evidence of Ghost Bat usage was observed at four of the five monitoring caves (AA1, A1, A2 and L3): One Ghost Bat and a large fresh scat pile was recorded at cave AA1; cave A1 contained fresh scat piles; cave A2 contained fresh scats and Ghost Bat calls recorded; cave L3 had Ghost Bat calls recorded but no scats observed.
West Angelas – Deposit B and F Ghost Bat Assessment: December 2014 Biologic (2015)	Five caves were surveyed for Ghost Bat presence in December 2014 (A1, A2, L2, L3, AA1), by scat count and ultrasonic recordings. Evidence of Ghost Bat usage was observed at two (AA1 and A1) of the five monitoring caves. No evidence of the Ghost Bat was recorded by ultrasonic recordings.
West Angelas – Deposit B and F Ghost Bat Monitoring 2015 Biologic (2016)	Five caves were surveyed for Ghost Bat presence in October 2015 (A1, A2, L2, L3, AA1), by scat count and ultrasonic recordings. Fresh and/or recent scats were recorded in caves A1, A2, L3, and AA1. A potential Ghost Bat was call recorded at cave L2 although no recent scats were recorded. A single Ghost Bat was also recorded at AA1.

2 METHODOLOGY

2.1 Project Team

The following personnel were involved in the project:

- Chris Knuckey (Senior Zoologist) – project manager, field team leader and primary author;
- Thomas Rasmussen (Senior Zoologist) – field member;
- Morgan O'Connell (Principal Ecologist) – technical review and quality assurance; and
- Robert Bullen (Specialist Bat Consultant of Bat Call WA) - analysis of bat calls.

Assistance in the field was provided by RTIO Health, Safety and Environmental personnel. Both Biologic field survey personnel (Chris Knuckey and Thomas Rasmussen) were trained and competent in confined space entry (RIIWHS202D) and atmospheric gas testing (MSMAPMOHS217A) as per RTIO requirements and relevant Australian Standards. The survey was completed under a Regulation 17 – Licence to take fauna for scientific purposes, administered by the Department of Biodiversity, Conservation and Attractions (DBCA).

2.2 Field Survey

2.2.1 Timing and Weather

The field survey was undertaken during the dry season, between the 17th and 19th October 2017. Local weather conditions experienced during the Survey, as recorded at the Newman Aero weather station, were typical for the time of year (Table 2.1, Figure 2.1). Maximum daytime temperatures during the survey ranged from 32.5°C to 39.5°C with an average of 36.9°C. Minimum overnight temperatures ranged from 19.0°C to 20.2°C (Table 2.1). Relative humidity averaged 28% and 14% for readings at 0900 and 1500, respectively. No rainfall fell during the survey period (Table 2.1). Rainfall recorded in the twelve months prior to the survey (544.9 mm) was well above the average annual rainfall of 332.6 mm (Figure 2.1), thus removing any influence of below average rainfall on activity of the species.

The survey was timed to occur between the last quarter and a new moon, which is considered the optimal period for recording bat activity for most northern tropical species (Milne *et al.*, 2005).

Table 2.1: Daily weather and moon observations recorded at Marble Bar during the Survey

Date	Temperature (°C)		Rainfall (mm)	Humidity (%)		Moon cycle		
	Min	Max		900	1500	Rise	Set	Phase (%)
17/10/2017	19.0	34.9	0.0	40	23	0339	1507	15
18/10/2017	16.1	33.3	0.0	25	9	0427	1607	8
19/10/2017	20.2	35.6	0.0	18	10	0512	1706	3
Average	**18.4**	**34.6**	**0 (total)**	**28**	**14**	**-**	**-**	**8.7**

Source: BoM (2017) and USNO (2017)

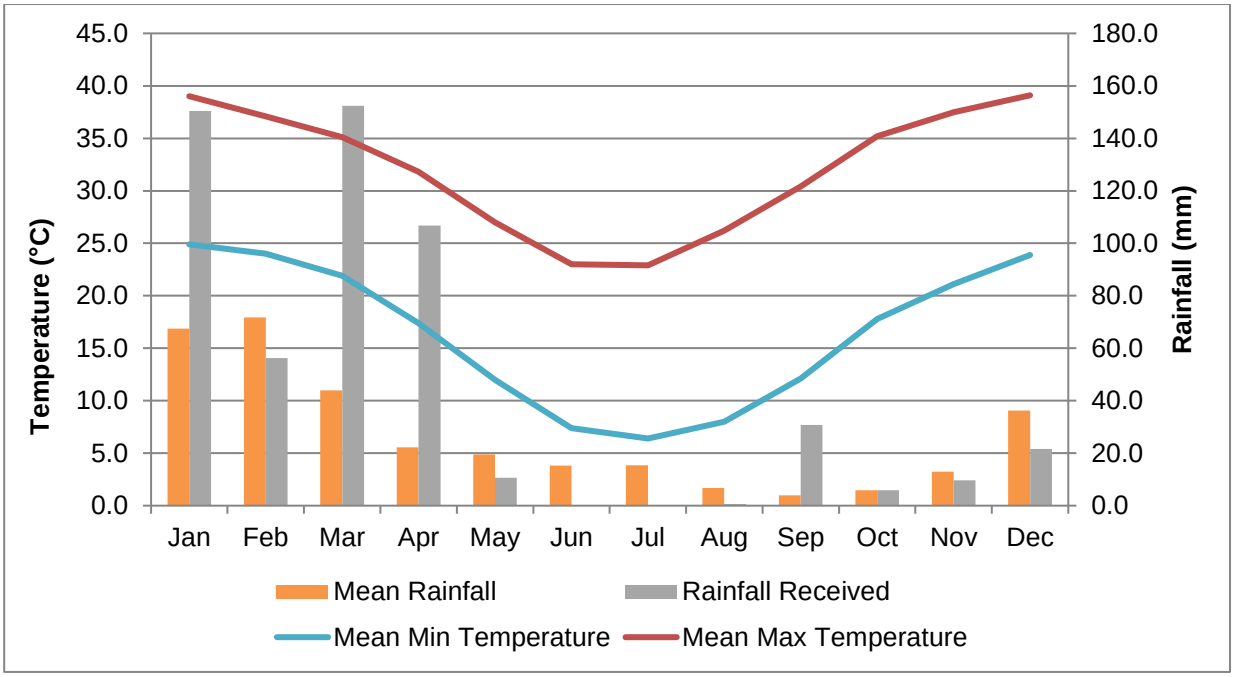

Figure 2.1: Long-term average and recent climatic data for the Study Area

2.2.2 Visual Assessment

Each of the five monitoring caves was visited and inspected by Biologic personal for the presence of Ghost Bat individuals. Two zoologists were involved with each visitation, one to enter the cave (inspector), and the other to wait at the entrance of the cave (spotter). The spotter was positioned, as a safety precaution and to record any Ghost Bats if flushed out of the cave. The Ghost Bat is distinctive from all other cave-dwelling bats within the Pilbara region, being both larger in size and lighter in colouration.

The inspector also searched the floor of each cave for scat material. Scats and middens are also distinctive for this species, with scats being almost twice the size of other cave-dwelling species. Black sheets (measuring approximately 1.5 m^2) were deployed on top of middens or large scat piles within each cave (except caves L3 and A2) during the previous survey (Biologic, 2016), thus any material on the sheet was known to have been deposited since this time. The number of sheets within each cave varied according to the number and spatial spread of scat piles within each cave, and was not dictated by usage at each cave. A representative number of fresh scats were collected from each cave, from numerous sheets where possible, to be used in subsequent analysis (see Sections 2.2.4 and 2.2.5). Searches were also conducted for scats off sheet to identify new scat piles. The number of scats occurring both on-sheet and off-sheet were recorded. Scat recorded on-sheet was used to determine a scat deposition rate, using the number of scats recorded divided by the number of days the sheets were present.

At the mouth of each monitoring cave an assessment on cave stability was undertaken to determine if caves were safe prior to entering. Gas concentrations in the cave atmosphere (oxygen, carbon dioxide, hydrogen sulphide and the lower explosive limit) were monitored using a calibrated hand-held gas meter during each visitation to ensure all conditions were safe for human entry.

2.2.3 Ultrasonic Recordings

Song Meter (SM) ultrasonic bat detectors (both SM2BAT+ and SM4BAT FS, Wildlife Acoustics, USA) were deployed at each of the entrance of each of the caves. Units were fitted with an ultrasonic (U1) omnidirectional microphone. Each unit was configured to activate and record between astronomical sunset and astronomical sunrise the following morning, and were deployed for one to two nights for a total of seven recording nights. Units were configured to record both ultrasonic and social calls of the Ghost Bat.

The recordings, once reformatted, were reviewed using COOL EDIT 2000 (Now available as AUDITION from Adobe Systems Inc.). All recording were analysed by Mr Bullen and confirmed using a database of reference calls.

Table 2.2: Song Meter deployment details

Monitoring Site	SM Deployed	SM Retrieved	Total
A1 and A2	17/10/2017	19/10/2017	2
L2	17/10/2017	19/10/2017	2
L3	17/10/2017	19/10/2017	2
AA1	18/10/2017	19/10/2017	1
		Total	7

2.2.4 Scat Genetic Analysis

A total of 123 scats were collected from the five monitoring caves during this survey (Table 2.3). A further 45 scats collected from four of the monitoring caves (A1, A2, L3, AA1) in 2015 were also included in the analysis Biologic (2016). These scats were stored in a freezer since the day of collection. Each of these scats was collected from sheets that were deployed approximately a year earlier during the previous monitoring survey (Biologic, 2015).

Table 2.3: Allocation of scats collected and analysed from the two sampling periods

Monitoring Site	2015	2017
A1	7	59
A2	4	9
L2	0	1
L3	10	5
AA1	24	49

DNA was obtained from the Ghost Bat faecal samples by scraping the outer surface of frozen scats with a blade (Appendix A). Genetic analysis was undertaken to determine the number of unique individuals which had visited the caves between the last two monitoring periods, the movement of individuals between caves, and to recommend sampling effort for future surveys. See Appendix A for a complete description of analysis techniques.

2.2.5 Scat Hormone Analysis

Scat samples collected for genetic analysis were also used for the hormone analysis. Hormone analysis of the collected scats was completed by Dr Tamara Keeley from the University of Queensland (Keeley, 2018). The use of faecal progesterone levels to indicate pregnancy within a population is a technique which has been used for multiple species (Keeley *et al.*, 2012a; Keeley *et al.*, 2012b) and validated for Ghost Bat in a pilot study using a captive bred breeding population from the Perth Zoo (Keeley, 2018). Analysis methods were conducted and detailed by T. Keeley, and are as follows:

Faecal samples were analysed for progesterone metabolite concentrations by enzyme-immunoassay (EIA). Prior to analysis for hormone concentrations, each faecal sample was extracted using a basic hormone extraction procedure (Keeley *et al.*, 2012a; Palme *et al.*, 2013). Faecal samples were subsampled to a weight of either 0.1 ± 0.02 or 0.05 ± 0.002 g to which 5 ml of 80% methanol was added. Samples were rotated gently overnight, centrifuged at 1000 g for 10 min and then decanted and stored at -20°C until analysis. The supernatant was diluted 1:20 to 1:1000 (dependant on concentration) in assay buffer prior to analysis. Faecal progesterone metabolite concentrations were quantified by double antibody EIA using a goat anti-mouse IgG (Arbor Assays, USA), monoclonal progesterone antiserum (CL425), horseradish peroxidase conjugated label (both provided by C. Munro, University of California-Davis, Davis, USA) and progesterone (Sigma Aldrich Australia Ltd.) standards as previously described with minor modifications (Keeley *et al.*, 2012b).

The antiserum (1:80,000) was incubated on a microtitre plate overnight, horseradish peroxidise conjugate (1: 400,000), standards (0.016 - 4 ng/ml) and samples were loaded (50 µl/well) onto the plate and the EIA was performed as described elsewhere (Keeley *et al.*, 2012b; Pollock *et al.*, 2010). Intra and inter-assay coefficients of variation were both <10%. Cross-reactivities for the EIA antibodies were as previously described (Graham *et al.*, 2001). Hormone concentrations were expressed as nanograms of hormone metabolites per gram of faeces (ng/g).

3 RESULTS

3.1 Monitoring Sites and Disturbance

All five monitoring sites were successfully sampled during the Survey. Visual structural assessments were conducted by both Biologic field personnel and prior to the survey by structural engineers of RTIO. All assessments confirmed that the caves were safe to enter and therefore unlikely to have had been structurally compromised since the previous survey. Additionally, no obvious signs of erosion or collapse were detected.

Gas monitoring conducted at each cave during visitation was completed with no triggers suggesting that all chemical levels and oxygen levels tested adhere to standards; however, dust was prominent at all caves visited, as well as generally across the mining area. Since the last survey, mining activity has encroached towards cave AA1 by approximately 70 m due to the development of Deposit F (Table 3.1, Figure 3.1). Distance to mining activity remained the same as was recorded during the 2015 survey for all other caves.

Table 3.1: Distance between monitoring caves and mining activity

Cave	Distance to Mining Activity	
	2015	2016/2017
A1	500	500
A2	535	535
L2	70	70
L3	90	90
AA1	210	140

677500 680000 682500 685000 687500 690000 692500 695000

7442500 7440000 7437500 7435000 7432500

A1 A2 L2 L3

A2 A1 L3 L2

AA1

AA1

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Legend

- Monitoring Caves
- Disturbance Footprint

biologic Environmental Survey

N

1:52,000

0 0.75 1.5 3 km

Rio Tinto Iron Ore - West Angelas

2017 Ghost Bat Monitoring

Fig. 3.1: West Angelas mine site and location of monitoring caves

Coordinate System: GDA 1994 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA 1994

Size A3. Created 14/12/2017

3.2 Ghost Bat Activity

No individuals were recorded within any of the monitoring caves during this survey and no calls of the species were recorded via the SM units. Fresh scats belonging to the Ghost Bat were recorded at all five of the monitoring caves. The number of fresh scats recorded on-sheet differed substantially between the caves, with totals ranging from 1 to ~2,000. Cave AA1 recorded the highest number of fresh scats on sheet (~2,000). An additional ~1,000 fresh scats were also recorded off-sheet within this cave. The second highest number of fresh scats was recoded at cave A1 (59), all of which were on-sheet. No scats were recorded on-sheet at cave L3, although five fresh scats were recorded off-sheet. Scats collected off-sheet at L3 were taken from a dome near the entrance of the cave, rather than where the sheets are located at the back of the cave which lay over old very large scat piles. No sheets were present at caves L2 and A2, although one and five scats were recorded and collected respectively – no old scats were present in these caves. Scat deposition rates for AA1 and A1 were estimated at 2.77 and 0.14 scats per day, respectively.

3.3 Scat Genetic Analysis

The genotyping success rate was high for samples collected in 2017, with 113 of 123 samples tested producing useable genotypes (92%). Samples collected in 2015 had comparatively low success, with only 18 of 45 samples producing useable genotypes (40%). In total, of 168 scat samples genotyped, 131 were successful (Appendix A).

3.3.1 Number of Individuals

A total of 34 unique genotypes (unique individuals) were identified from the 131 successfully genotyped scat samples. This comprised 12 individuals from 2015 and 24 individuals from 2017 (Appendix A, Table 3.2). Two individuals were detected in both sampling periods (#378 and #379), both at cave AA1 (Appendix A, Table 3.3). AA1 also recorded the highest number of individuals (19) as well as the highest number of individuals per scats sample 0.260 (Appendix A, Table 3.3). Cave A1, which recorded the second highest number of individuals, recorded 11 individuals from 66 samples, giving a number of individuals per scat average of 0.167.

3.3.2 Cave Use

The majority of individuals (91%) were recorded at only one cave, although three individuals (#366, #369 and #370) were recorded at multiple caves (Appendix A, Table 3.3). All records of multiple cave use were recorded within the same sampling period. Individual #366 was detected at all caves in the north of the Study Area (A1, A2, L2 and L3), although the majority of detections of this individual were at A1 (Appendix B). Records of individual #370 were also confined to the northern caves (A1, A2), with similar numbers of scats identified in each cave (Appendix B). Individual #369 was primarily detected in cave A1 but a single scat was also detected at cave AA1, ~10km to the southeast (Appendix B).

Genotypes identified during this study were not synonymous with any genotypes identified from other studies conducted within the region (Ottewell *et al.*, 2017). However, the closest locations in the corresponding studies were 10-15 km from the West Angelas site, which is the scale at which dispersal

frequency appears to decline in the species (Ottewell *et al.*, 2017), and the portion of scats analysed during this study was limited, specifically from cave AA1.

Spatial autocorrelation analysis was conducted to determine the relatedness of sites based on relative location (Appendix A). Although the small sample size limited the results and analysis, the results were consistent with previous studies (Ottewell *et al.*, 2017; Spencer & Tedeschi, 2016) showing higher relatedness of individuals (r) at small spatial scales (~50m, in this case amongst neighbouring caves) that declines with increasing distance, and with low relatedness of individuals in the most distantly located caves (Appendix A).

Table 3.2: Individuals recorded between years

	Individual #																																	
Year	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394
2015	x	x										x	x		x	x	x	x	x	x													x	x
2017			x	x	x	x	x	x	x	x	x			x				x	x		x	x	x	x	x	x	x	x	x	x	x	x		

Table 3.3: Detection of unique Ghost Bat genotypes by monitoring cave

	Individual #																																	
Site	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394
A1	x	x	x	x	x	x	x	x	x	x	x																							
A2						x				x		x	x	x																				
AA1									x						x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
L2						x																												
L3						x																											x	x

3.4 Scat Hormone Analysis

Of the 168 scat samples analysed, 103 (61%) contained elevated progesterone (i.e. samples with progesterone levels higher than 970 ng/g) indicating pregnant individuals (Appendix B). Every monitoring cave contained scats with elevated progesterone levels in both sampling periods, with the exception of L2, which had no scats analysed from the 2015 sampling period (Table 3.4).

Aside from cave L2, which contained only one scat, Cave A1 recorded the highest proportion of scats containing elevated progesterone levels, 95% (63 scat samples, Table 3.4). The neighbouring cave, A2, recorded the second highest proportion of scats containing elevated progesterone, although the sample size used to determine this (11 scat samples) was relatively low. Thirty-four percent of scats analysed from AA1 contained elevated progesterone levels, comprising 46% (11 samples) from the 2015 sampling and 29% (14 samples) from 2017 (Table 3.4). The overall proportion of scat samples containing elevated progesterone at AA1 was 34% (25 samples). Cave L3 contained the lowest proportion of scats containing elevated progesterone levels with a total 27% over the two sample periods (Table 3.4) – although this was based on a limited number of scat samples.

Table 3.4: Number and portion of scats with elevated progesterone levels

Year	Portion of scat contain elevated progesterone (raw no. samples containing)				
	A1	A2	L2	L3	AA1
2015	71% (5)	100% (4)	-	20% (2)	46% (11)
2017	97% (57)	78% (7)	100% (1)	40% (2)	29% (14)
Average (Total)	95% (63)	85% (11)	100% (1)	27% (4)	34% (25)

4 DISCUSSION

4.1 Temporal Trends

4.1.1 West Angelas Population

The results obtained from this survey are relatively consistent with the most recent survey as well as most surveys preceding this. The absence of Ghost Bat individuals has also occurred during several previous surveys (Armstrong & Anstee, 2000; Biologic, 2015; Biota, 2002; ecologia, 2001) and is common for Ghost Bat roosts within the Hamersley Ranges (Armstrong & Anstee, 2000). It is believed that individuals move between caves on a regular basis, most likely in response to disturbance, microclimate, social factors (Armstrong & Anstee, 2000) and possibly seasonal conditions. This scenario is particularly evident at West Angelas given that Ghost Bats are consistently confirmed visiting the caves, as indicated by fresh scat material, but rarely encountered – although this is also partly due to the limited annual sampling effort.

The inclusion of the genetic analysis in this assessment enabled broad comparisons between the 2015-2017 monitoring period and the 2014-2015 monitoring period. Twelve unique individuals were detected from 18 scats sampled in 2015, and 24 individuals from 113 scats sampled in 2017, and only two individuals (#378 and #379) were recorded in both periods. Detailed comparisons between the two periods was not possible due to the unequal sampling effort (one year vs. two years), number of scats analysed and the differences in genotyping success rates between the two periods; however, using supplementary data, such as the number of unanalysed scats recorded at AA1 in 2017 and the rarefaction analysis which indicated a high likelihood of more genotypes (Appendix A), it can be assumed that the average number of individuals present was higher during the most recent monitoring period. These results suggest that the population in the area did not decrease between the two periods and potentially increased.

The exact number of scats recorded from each cave was rarely documented in previous surveys, reducing the ability to compare results from this survey; however, based on comparisons of known data, it appears that the number of scats at most caves (A1, A2, L2) was consistent with the last monitoring period. The exception being a reduction in fresh scats at L3 and an increase in fresh scats at AA1. Detailed accounts of each cave are described in section 4.1.2, but in summary are most likely to reflect sporadic and intermittent occupation by the Ghost Bat, occurring in cycles of long intervals.

4.1.2 Cave Usage and Conservation Significance

Based on the number of scats recorded, it appears that most caves were used in an equivalent manner to previous surveys (Table 4.1). For caves A2 and L2, this was as a night roost. Cave A2 was incorporated into the monitoring program five years ago (Table 4.1) and has showed signs of Ghost Bat in four out of five years, including the current survey. Cave L2 has been surveyed on nine occasions and has only shown reliable evidence of recent Ghost Bat activity on one other occasion (Integrated Environmental Services, 1980, Table 4.1). Additionally, both caves are small, relatively exposed and uncharacteristic of Ghost Bat roosting sites (Armstrong & Anstee, 2000). A large, unknown quantity of these types of caves

are potentially scattered throughout the Pilbara region. Given the lack in contemporary and historical activity recorded at these sites, it is unlikely that these sites represent features of particular significance. Furthermore, the presence of better suited roosting caves near, which consistently have more scats, suggests these caves are unlikely to be of particular significance to the local population.

Cave L3 has now been surveyed on 10 occasions. Complete absence of the species has only been recorded on one occasion (Biologic, 2015) and only one survey has encountered an individual (Table 4.1). Historical data from L3 indicates that the site was historically used as a diurnal roost or even maternity roost (Integrated Environmental Services, 1980). During this survey, fresh scats were only recorded off-sheet, scattered near the entrance of the cave, suggesting night visitation only. No scats were recorded at the back of the cave where the sheets were laid over large old scat piles of several thousand scats. The cave had experienced a similar 'low' in activity prior to surveys in 2012 and 2015, although a 'substantial amount' of scat material was recorded on sheets during these surveys. The low activity levels recorded during this survey may therefore be due to natural fluctuations as documented from previous surveys, the presence of nearby mining activity or the recently erected sound barrier at the cave entrance. Genetic results indicate that the site was visited by multiple individuals (three were confirmed) and the hormone analysis indicates that pregnant females did visit the cave. On this basis, the cave should be considered a potential diurnal roost, potentially being used as a maternity roost, and should be considered of moderate significance as only a small number of such caves are known to occur within the Hamersley Ranges.

The number of fresh scats at A1 were slightly higher than recorded during the previous survey. Eleven individuals were recorded at the cave over the three-year sampling period, making this cave the second richest in terms of the number of individuals. The genetic analysis demonstrated that three individuals visited multiple sites, and all of these were recorded at A1. Though the data on dispersing individuals is limited, results potentially indicate a different role for cave A1 in comparison to other caves at this site (Appendix A). The hormone analysis indicated that 95% of all scats analysed from A1 showed levels of elevated progesterone, suggesting high visitation by pregnant females. In 1980, A1 was suggested as a maternity cave (Integrated Environmental Services), although no evidence has confirmed this. Diurnal roosting has been confirmed at the cave previously (Table 4.1) and the amount of activity recorded at the cave supports this. As such, the cave should be considered of high significance, representing a diurnal roost and potential maternity roost for the species, for which there is few known to occur in the Hamersley subregion.

AA1 has been surveyed on ten occasions, all of which have confirmed recent presence of the species, including during the current survey (Table 4.1). AA1 recorded the highest amount of fresh scat of any cave during this survey and 19 individuals were confirmed over the two monitoring periods. Additionally, the cave was noted as having the highest rate of detection of new individuals for the number of scats analysed, potentially representing a larger or more transient population (Appendix A). AA1 was the only cave to have recorded a significant increase in the number of scats between this survey and the previous, suggesting an increase in Ghost Bat activity at the cave. Hormone analysis identified 34% of scats with elevated progesterone (25 total), confirming the cave was visited by pregnant females and probably

represents at maternity cave for the species, as acknowledged by Integrated Environmental Services (1980) and Armstrong and Anstee (2000). Together these traits indicate the cave is of very high conservation significance and all efforts should be made to minimise disturbance to and at the cave, as this may impact upon the species both locally and regionally.

Table 4.1: Summary of Ghost Bat records from current and previous surveys*

Cave	1978 / 79	1997	Sep 1998	Aug 2000	Sep 2001	Nov 2002	Dec 2003	Nov 2012	Nov 2013	Dec 2014	Oct 2015	Oct 2017
Ref	Integrated Environmental	ecologia (1998a)	ecologia (1998b)	ecologia (2000)	ecologia (2001)	Biota (2002)	Biota (2004)	Biologic (2013)	Biologic (2014)	Biologic (2015)	Biologic (2016)	This survey
A1	-	P(4)	R	P(1)	R	O	P(1)	R	R	R	R	R
A2	-	-	-	-	-	-	-	R	R,C	N	R	R
L2	-	-	R	O	-	N	O	O	N	N	C	R
L3	P	-	O	O	-	R	O	R, C	C	N	R	R
AA1	P(1)	-	P(1)	R	R	R	R	-	P(1) R	R	P(1) R	R
AB1	-	P(8)	R	-	O	R	R	-	-	-	-	-
I1	-	-	R	R	-	O	O	-	-	-	-	-
Adit	-	-	O	-	-	O	O	-	-	-	-	-

Legend:

P Ghost bats present (number observed in parentheses)
R Recent signs of occupation from fresh scats
C Calls recorded at night
O Guano accumulation but no fresh scats
N No signs of Ghost Bat occupation
- Not surveyed

4.2 Potential Impacts of Mining

Mining related impacts to Ghost Bats could potentially include loss of roosting and foraging habitat, either directly (e.g. removal of roosts or vegetation during clearing/ constructions works) or indirectly as a result of mining (e.g. noise and/ or vibrations resulting in damage to roosts or abandonment, and degradation of foraging habitat from dust deposition or weed incursion) (TSSC, 2016). Impacts to caves and the bat activity levels within them can be measured directly through monitoring, but the indirect impacts on foraging habitat are harder to quantify as Ghost Bats hunt over relatively large areas (as inferred from studies in the Norther Territory; Tidemann *et al.*, 1985) and tend to consume species that are common and widespread in the Pilbara (Biologic, *unpub. data*). Without specific data on preferred feeding habitats in the Pilbara region, it can only be assumed that if there is a variety of intact, native vegetation communities surrounding day roosts and maternity roosts (within a radius of 2-3 km), then there should be opportunities for Ghost Bats to hunt and therefore continue to occupy roosts; however, widespread clearing or mining around Ghost Bat roosts would be expected to impact on their occupation or use, as would fire, at least in the short-term prior to the recovery of the vegetation (Bullen & McKenzie, 2011).

There is little available literature on the level or types of disturbance that would lead Ghost Bats to abandon a roost. Repeated disturbance by human visitation, for example, is believed to cause roost abandonment (TSSC, 2016), as is the rapid take-off by adults in fright (believed to potentially cause dislodgement and abandonment by juveniles) (K. Armstrong *pers. comm.*, cited in Woinarski *et al.*, 2014). Meanwhile, a study by Bullen and Creese (2014) suggested that drilling up to 50 m from a Ghost Bat roost was unlikely to cause abandonment. Bullen and Creese (2014) suggested that Ghost Bats could tolerate some audio disturbance from machinery; however, the levels of noise from a drill rig are conceivably much less disruptive than those from blasting. At BHP Billiton Iron Ore's Goldsworthy operations a long-term (10 year) study of Ghost Bats and Pilbara Leaf-nosed Bats (*Rhinonicteris aurantia*) was undertaken at a cave located approximately 400 m from an active pit (Gleeson & Gleeson, 2012), and this study showed no change in bat activity for either species over the duration of the monitoring.

The cave monitoring results do not appear to show any obvious impact of mining at the current time. Caves A1 and A2 are located in a gully approximately 450 m north of the Deposit B pit boundary (Figure 3.1), and the foraging habitat in the immediate vicinity of these caves (to the north) is relatively undisturbed by mining. Given the distance of these caves from the mining operation and their location on the opposite side of the range to the mine pit at Deposit B, it is considered unlikely that these caves would be directly affected by mining.

Caves L2 and L3 (which are located 70 m and 90 m from the Deposit B pit wall respectively) are in closest proximity to active mining areas (Figure 3.1) and would therefore be considered most likely to be impacted by noise and vibration or other forms of disturbance. Any potential Ghost Bat hunting grounds to the immediate south of these caves has been cleared for mining; however, significant areas of unaffected vegetation remain within 2 km to the east, north east, north and west. Therefore, the indirect impacts of mining may not have a pronounced effect on bat occupancy of these caves despite their proximity to the mine. Further monitoring of Ghost Bat usage of these caves over the next few years would be required to assess this.

During the previous and current surveys, it was observed that cave L3 had a sound barrier erected in front of the mouth of the cave to dampen the impact of sounds from blasting at nearby Deposit B. The sound barrier (comprising dense foam matting suspended from a metal fence) covers the lower part of the cave entrance from the floor to within approximately 1 m of the roof of the cave, allowing for uninterrupted access to and from the cave. This is important, as it is unlikely that Ghost Bats would tolerate a significant reduction in the size or change in shape/ aspect of the cave entry. At the time of the survey it was observed that the noise of mining machinery at Deposit B was still apparent within the cave, questioning the effectiveness of this barrier as a tool.

The high conservation value maternity cave AA1 is currently located approximately 140 m to the north of an active pit boundary at Deposit F. The long-term monitoring results do not show any adverse impact of mining within the nearby area to date. The results from his survey show a probable increase in activity despite new development at Deposit F. The entrance of the cave AA1 faces away from the nearby mining disturbance at Deposit F, and the high mounds of rocks at its entrance potentially mask any noise from blasting and earthworks. The mining at Deposit E and F has already, and will further affect, a considerable proportion of the native vegetation in the valley to the south of cave AA1, although significant areas to the east, north and west (mainly on the flanks and hill crests) appear to remain unaffected. Further monitoring of Ghost Bat usage at cave AA1 would be required to assess whether these changes will have an impact on Ghost Bat usage, although the most recent results at the Deposit B caves, particularly AA1, L3 and L2, which are much closer to active mining areas, currently do not suggest any adverse impacts.

4.3 Survey Limitations

The survey team was adequately experienced and resourced to achieve the project scope. Thomas Rasmussen has undertaken four previous Ghost Bat monitoring surveys at West Angelas and both Chris and Thomas have undertaken an extensive number of Ghost Bat surveys in the Pilbara.

The sampling methods employed, and the season within which they occurred, were appropriate for the scope. New methodology was employed in this survey to better address objectives of the scope and monitoring program i.e. scat genetic and hormone analysis; however, all survey techniques employed during the previous survey were also undertaken in this survey to ensure that this survey could be interpreted alongside the results of previous work. The exception to this was the time between monitoring events. The time between this survey and the previous was twice that of the last three surveys and limited the conclusions that could be drawn.

For the genetic analysis, the number of samples that were successfully genotyped was high for samples collected in 2017, but low for the samples collected in 2015, despite best practice storage. This reduced the number of individuals that could be identified from 2015. The lack of survey in 2016 may have also reduced the success rate of genotyping in the 2017 samples, although to a much lesser extent as indicated by the overall high rate of genotyping success for these samples (92%).

All samples sent for hormone analysis were successfully analysed; however, because the degradation rate of progesterone in scats is unknown, it is possible that dataset contains false negative samples of elevated progesterone. Additionally, the ability for progesterone to be transferred from one scat to

another, either by direct contact or by urine is unknown, therefore potentially leading to the occurrence of false positive detections of elevated progesterone in the dataset. Due to this, results were interpreted with caution and discussion focussed on the presence of pregnant females at sites rather than discussing individuals which recorded elevated levels.

5 CONCLUSIONS

Although no Ghost Bats were recorded during the survey, fresh scat belong to the species were recorded at each monitoring cave confirming that the species was present within the area since the previous monitoring survey. For most caves the levels of usage at each cave, inferred from the number of fresh scats, was similar to the previous surveys, the exceptions being caves L3 and AA1. For L3 this was a drop in the number fresh scats and a lack of diurnal roosting evidence. The number of scats recorded in AA1 was much higher than has previously been documented in the cave. This, together with the genetic analysis, confirms that AA1 was used by the highest number of individuals relative to any of the other monitoring caves. High numbers of scats containing elevated progesterone levels, as detected by the hormone analysis, were also recorded at AA1 and A1, indicating their potential use as maternity roosts. Scats containing elevated progesterone levels were also recorded within all other caves during the monitoring period. The number of scats recorded at these caves (A2, L2, L3) indicated that visitation by pregnant individuals was temporary or confined to night time only.

Based on these results, and the fact that no additional disturbance was recorded at the monitoring caves since the last survey, there is no indication that mining activity has had a significant impact on the species between this survey and the previous. With this is mind, current management procedures to minimise impacts to the species should be maintained. Although no significant impact was detected, fluctuations at the site are still not understood, such as the change in usage patterns at L3 (from diurnal roost to night roost).

Inclusion of the genetic analysis into the program provided insight into the number of individuals that were visiting each of the caves over the monitoring periods. The lack of successfully amplified genotypes from 2015 and the unequal length of monitoring periods reduced the ability to accurately compare population dynamics at the site and across each of the monitoring caves. Inclusion of the hormone analysis also provided additional detail on the use of the caves by individuals, successfully demonstrating that each cave was utilised by pregnant females. The number of pregnant individuals was not reported due to potential contamination of scats derived from non-pregnant individuals by pregnant individuals. In either case, the results confirm that the area was visited by pregnant females and strongly suggests caves A1 and AA1 to be maternity caves.

The genetic work conducted at West Angelas indicates that the individuals which visited the caves each year varied substantially, i.e. few repeat visitations. This, together with the lack of Ghost Bat sightings, indicates that individuals using these caves are also roosting elsewhere. Additionally, the lack of matching genotypes with other work conducted within the region indicates that alternate roosting sites are most likely located in areas not monitored by RTIO or third parties. Such sites may be within areas directly surrounding West Angelas, as well as areas further afield to the south and west.

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7 APPENDICES

Appendix A – Scat Genetic Report (Ottewell et al., 2018)

Cave use by the Ghost Bat (*Macroderma gigas*) at the West Angelas mine site

Final Report

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January 2018

GOVERNMENT OF WESTERN AUSTRALIA

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Executive Summary

- 168 faecal samples were analysed from five cave sites at West Angelas sampled in 2015 and 2017.
- Useable genotypes were obtained from 131 samples, from which we identified 34 unique individuals.
- Genotyping success rates were lower for older (2015) samples than recent (2017) samples, potentially highlighting DNA degradation issues with increased storage time.
- Of the 34 individuals identified, only two were detected in both sampling years. Both were located in AA1.
- The great majority of individuals were detected in a single cave. However, three individuals were detected in multiple caves.
- These individuals were primarily detected using closely-located caves to the north of the site, though one individual used both A1 and AA1 (~10 km distant). Based on scat abundance, all three individuals were most frequently detected in cave A1.
- Though based on limited numbers of dispersing individuals, these patterns are consistent with that reported elsewhere in the Pilbara (Ottewell et al., 2017). Spatial autocorrelation analysis also indicated higher relatedness among closely-located caves that declines with distance.
- Genetic diversity was consistently high across caves and was similar to that reported in other Pilbara populations (Ottewell et al., 2017) indicating that the population is in similar genetic health.
- Analyses indicated the genetic effective population size was 12 individuals (95% CI 10-16) that can be tentatively extrapolated to a census population size of 100-160 individuals using the site over two years.
- Individuals in this study have not been previously detected at other sampling locations (Ottewell et al., 2017).

Introduction

The Ghost Bat (*Macrodema gigas*) is a monotypic bat species native to the Pilbara and Kimberley regions of Western Australia (WA), the Northern Territory (NT) and eastern Australia. Throughout northern Australia (QLD, NT, northern WA), they are coastal and occur up to 400 km inland, generally north of the Tropic of Capricorn. They appear to occupy a wide range of habitats from rainforest, monsoon and vine scrub in the tropics to open woodlands and arid areas, such as the Pilbara, which is geographically isolated from extant northern Australian populations (and the historical central Australian populations) by extensive sandy deserts. The Ghost Bat is an obligate troglodyte, and survival is critically dependent on finding natural roosts in caves, crevices, deep overhangs and artificial roosts such as abandoned mines (Hall et al., 1997). Populations of this species appear to have regionally centralised maternity roosts that are genetically isolated from each other (Worthington-Wilmer et al., 1994). The species is characterised by high maternal philopatry and male-biased dispersal (Worthington-Wilmer et al. 1994). Populations are known to disperse in the non-breeding (dry) season (Toop, 1979, 1985).

Recent genetic work has demonstrated the utility of faecal (scat) DNA sampling as a method of 'molecular tagging' to identify individuals in mark-recapture analyses. This approach has been used to detect the spatial and temporal movement patterns of individual ghost bats amongst roost sites in the Pilbara, as well as assessing genetic connectivity amongst populations at a larger, landscape scale (Spencer & Tedeschi, 2016; Ottewell et al., 2017). Using this approach, the current study identifies individual ghost bats using caves at the Rio Tinto West Angelas mine site in the eastern Hammersley Ranges region of the Pilbara to examine patterns of movement amongst roost sites.

Study aims

The study aimed to:

1. *Extract DNA from* M. gigas *faecal samples (168 samples) and provide genetic profiles using existing microsatellite markers following methods in Ottewell et al. (2017)*
2. *Identify the number of unique individuals represented in scat samples*
3. *Temporal analyses:* Using genotype matching, identify individuals present at multiple time points
4. *Spatial analyses*:
    a. Using genotype matching, identify individuals using multiple caves and estimate observed dispersal distances amongst caves
    b. Using spatial autocorrelation analysis, estimate the genetic neighbourhood-size and infer the spatial scale of dispersal
5. *Population genetic analyses*:
    a. estimate the genetic effective population size (N_e), and, if sufficient sampling, an estimate of the census population size
    b. provide an assessment of the 'genetic health' of the population, i.e. genetic diversity, inbreeding.

Materials and Methods

<u>Sampling locations and material</u>

Ghost bat faecal and tissue samples were collected from five roost sites (**Error! Reference source not found.**) at two time points, 26 October 2015 and 18 October 2017 (details in Appendix 1). At

each sampling site *Macroderma gigas* scats were collected into envelopes and kept frozen until DNA extraction.

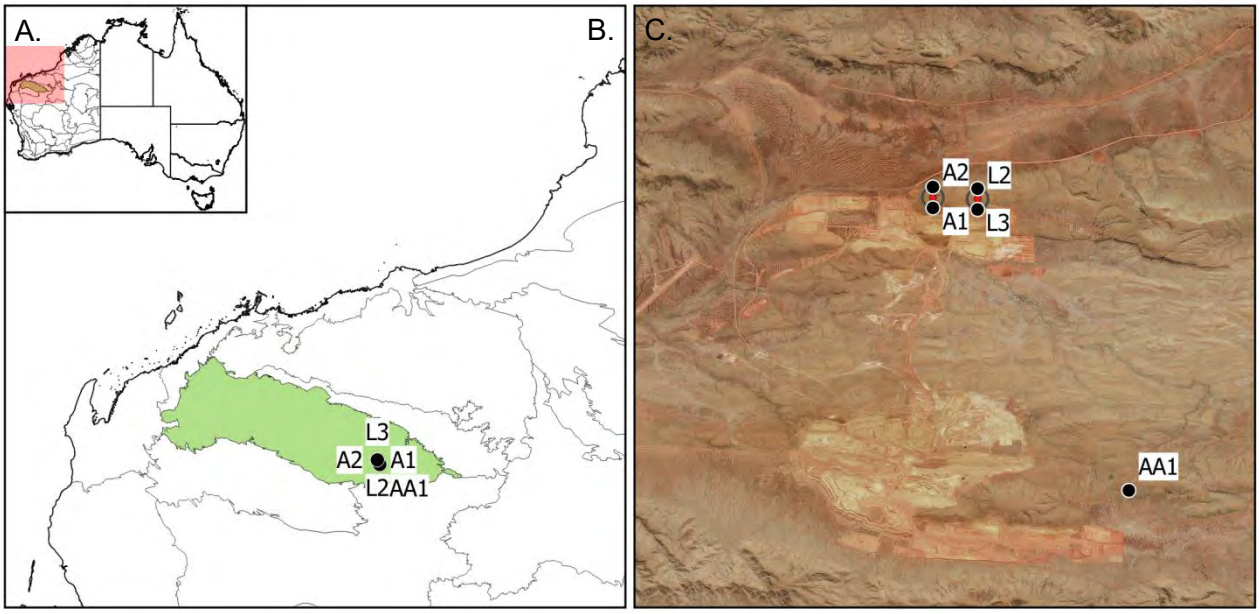

Figure 1: Map of the sampling location (A, B) and sampled *Macroderma gigas* roost sites (C) included in this study. Note that caves A1/A2 and L2/L3 are closely located and point displacement has been used in panel C.

DNA extraction

DNA was obtained from ghost bat faecal samples by scraping the outer surface of frozen scats with a blade using the same methodology as Spencer & Tedeschi (2016). The scraped material was processed using the QIAamp Fast DNA Stool mini kit (Qiagen Cat No: 51604). DNA was resuspended and stored in TE buffer prior to PCR amplification.

Microsatellite genotyping

Ghost bat DNA samples were analysed at 11 hyper-variable microsatellite loci, including four (gigas01, gigas10) sourced from Worthington Wilmer et al. (1999), three unpublished microsatellites developed by J. Hughes (GB18, GB33, GB42; cited in Spencer & Tedeschi, 2016) and six new microsatellites developed by DBCA for Biologic (MG32, MG20, MG05, MG26, MG28, MG21; Ottewell et al., 2017). Microsatellites were arranged in three PCR multiplexes and amplified using the Qiagen Mulitplex PCR kit (Cat No: 206143) as per the manufacturer's instructions. Microsatellite allele sizes were determined by co-running microsatellite PCR products with the Genescan500 size standard (Applied Biosystems, Melbourne). Fragment analysis was carried out on a 3730xl DNA Analyser (ABI systems, Melbourne) using a commercial service (State Agricultural Biotechnology Centre, SABC) and scored using the Genemapper v5 software (Applied Biosystems, Melbourne). Details of microsatellite loci, allele size ranges and multiplexes are provided in Appendix 2.

Previous analyses showed that the combined probability of identity (P_{ID}) of six markers was sufficient to discriminate between related individuals (Ottewell et al., 2017); consequently, we included samples with partial genotypes where six or more loci were successfully genotyped.

Genotyping errors are frequently observed in studies using scat DNA due to the low quality and quantity of DNA sourced from these samples (Knapp et al., 2009, Taberlet et al., 1999). Genotyping error rates were assessed for ghost bat microsatellite loci by re-analysing a subset of samples and comparing the resultant genotypes. Two specific types of errors were assessed: (1) allele dropout

and (2) false alleles. Type 1 errors occur more frequently than Type 2 errors with scat DNA (Sethi et al., 2016). Per locus genotyping error rates are in Appendix 2.

Genotype matching - estimation of the number of unique individuals

To determine the number of unique individuals present in each sample locality we used the software COLONY (Jones & Wang, 2010) to cluster identical scat genotypes. COLONY uses an error-tolerant likelihood-based sample matching protocol, combining the probability of obtaining a pair of true genotypes given population allele frequencies and hypotheses about the relationship between the two samples (e.g. samples from full siblings or unrelated individuals). This is coupled with the probability of observing the sample genotypes given a genotyping error model and genotyping error rates. Both known allele frequencies and locus-specific error rates were input into the sibship models in COLONY. Genotype clusters produced by the software were checked by eye and some minor adjustments were made. In a few cases COLONY identified similar genotypes as being sibs where the observed pattern could also be explained by allelic dropout (Type 1 genotyping errors). Without appropriate reference samples to assess the true sib-ship structure of ghost bat colonies, we thought it more conservative to consider these types of genotypes as duplicates rather than sibs. This may have the effect of underestimating the total number of individuals present in a cave, but should only mean that highly related individuals have not been properly detected.

Genotypes were also matched against a database of previously detected ghost bats from Ottewell et al. (2017) to potentially identify roost use away from the study site.

Assessment of sampling effort

The rate of accumulation of new individuals with increasing sample size was assessed using rarefaction analysis. A single, sample-based rarefaction curve was calculated in the software EstimateS v9.1.0 (Colwell, 2013). We used non-parametric extrapolation to explore the trajectory of the rarefaction curve if sampling effort was roughly doubled (n=350 scats analysed in total). The census population size can be estimated from the rarefaction curve at the point where the curve reaches an asymptote (Eggert et al., 2003).

Population genetic analyses

Summary population genetic diversity statistics, such as observed (H_o) and expected heterozygosity (H_e), number of alleles (N_a) and the inbreeding coefficient (F_{is}) were calculated in GENALEX v6.5 (Peakall & Smouse, 2012). Sample sizes (number of individuals) per cave were low so we pooled genotypes for caves that were closely-located (A1/A2); however, insufficient samples were present in L2/L3 to accurately estimate diversity statistics. In population genetics, sample sizes of 25-30 individuals are typically required for accurate estimation of diversity statistics (Sinclair & Hobbs, 2009, Hale et al., 2012).

Spatial autocorrelation of pairwise genetic relatedness amongst individuals was used to visualise the spatial genetic neighbourhood for ghost bats at the study site. The spatial autocorrelation correlogram plots the autocorrelation coefficient (*r*) as a function of distance, as well as the 95% confidence interval about the null hypothesis of no spatial association of genotypes (random) as determined by permutation. In this analysis, when significant positive genetic structure is present, the estimated value of *r* will decrease with increasing distance. The distance class size at which the estimate of *r* is no longer significant provides an approximation of the extent of detectable positive spatial genetic structure (Peakall et al., 2003). Spatial autocorrelation analyses were performed in GENALEX V6.5. Due to the limited spatial sampling in this study, this analysis provides information on local-scale dispersal only (<10 km).

The program LDNE is used to estimate the contemporary effective population size (N_e) based on genotypic linkage disequilibrium data (Waples & Do, 2008). The program calculates separate

estimates using different criteria for excluding rare alleles (suitable for microsatellite data). Simulations presented in Waples & Do (2010) suggest using allele frequencies >0.02 represents the best precision-bias trade-off for the LD method. The program also implements a jack-knife technique to calculate the 95% confidence intervals of the N_e estimate.

Results

Genotyping success rate

Overall, the genotyping success rate was high for samples collected in 2017, with 113 of 123 samples tested producing useable genotypes (i.e. >6 loci) (92%, Appendix 1). Samples collected in 2015 unfortunately had low success, with only 18 of 45 samples producing useable genotypes (40%). In total, of 168 scat samples genotyped 131 were successful.

Number of unique individuals

Based on genotype matching across scat samples, we detected 34 unique genotypes (i.e. unique individuals) from across the study site. Twelve unique individuals were detected from 18 scats sampled in 2015, and 24 individuals from 113 scats sampled in 2017. There was little overlap in the individuals identified in the 2015 sample with the 2017 sample, except for two individuals (#378 and #379) that were detected in both sampling years (Table 1). Both were present in cave AA1 at each sampling time.

Across the roost sites sampled, AA1 had the largest number of individuals present, and the largest number of individuals detected for the number of samples analysed (Figure 2). While a similar number of scats were analysed from A1, fewer individuals were detected in total (n = 11 c.f. 19). Of the scats collected at A1, a high proportion were attributed to a single individual: 31 of 56 scats tested were from individual #366 (Appendix 3).

As a higher number of unique individuals were detected for the number of scats analysed in AA1, this may indicate a larger total population size, despite similar levels of scat 'activity' as A1.

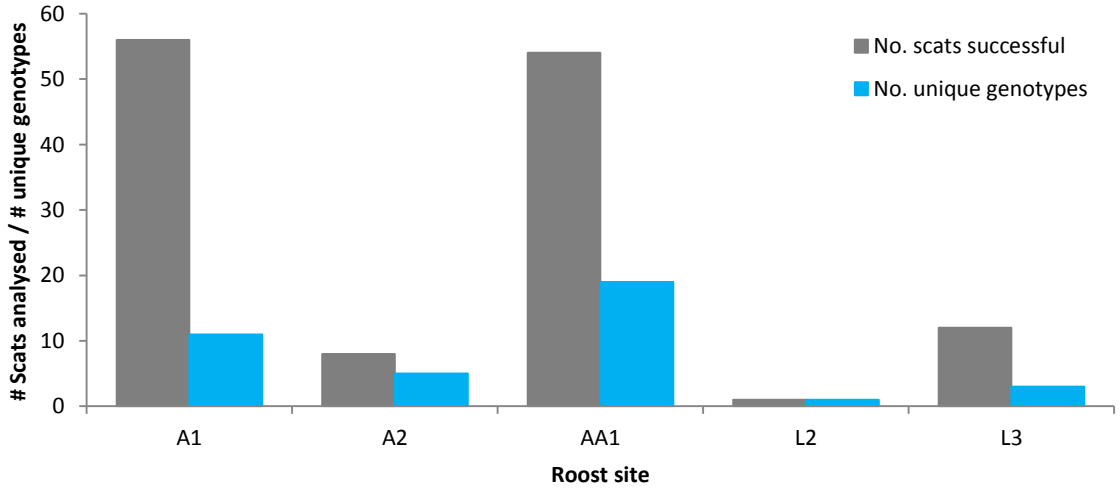

Figure 2: Number of scats successfully genotyped and number of unique genotypes (individuals) identified from each of five roost sites included in the study.

Cave use

Overall, the majority of individuals identified across the study site were detected only using a single cave (31 of 34 individuals; Table 2). Three individuals (#366, #369 and #370) were detected using

different caves, all in the same time period (highlighted in red, Table 2). Individual #366 was detected concurrently using all of the closely located caves to the north of the site (A1, A2, L2 and L3), though was most frequently detected in cave A1 (Appendix 3). Individual #370 also was detected in the northern caves (A1, A2) with similar numbers of scats identified in each cave. Individual #369 was primarily detected in cave A1 but a single scat was detected in cave AA1, ~10km to the southeast. A list of inferred dispersal distances for these individuals is provided in Table 3.

Assessment of sampling effort

The raw sample accumulation curve (blue line, Figure 3) showed a slow rate of increase in the number of unique individuals identified with increasing sample size, particularly due to the large number of scats identified from a single individual (Individual #366, cave A1). Rarefaction analysis showed that the number of individuals detected across the study site was continuing to increase with increasing sample size and did not reach an asymptote (grey line, Figure 3). Further scat sampling would be required to gain a robust estimate of census population size at the site using this method.

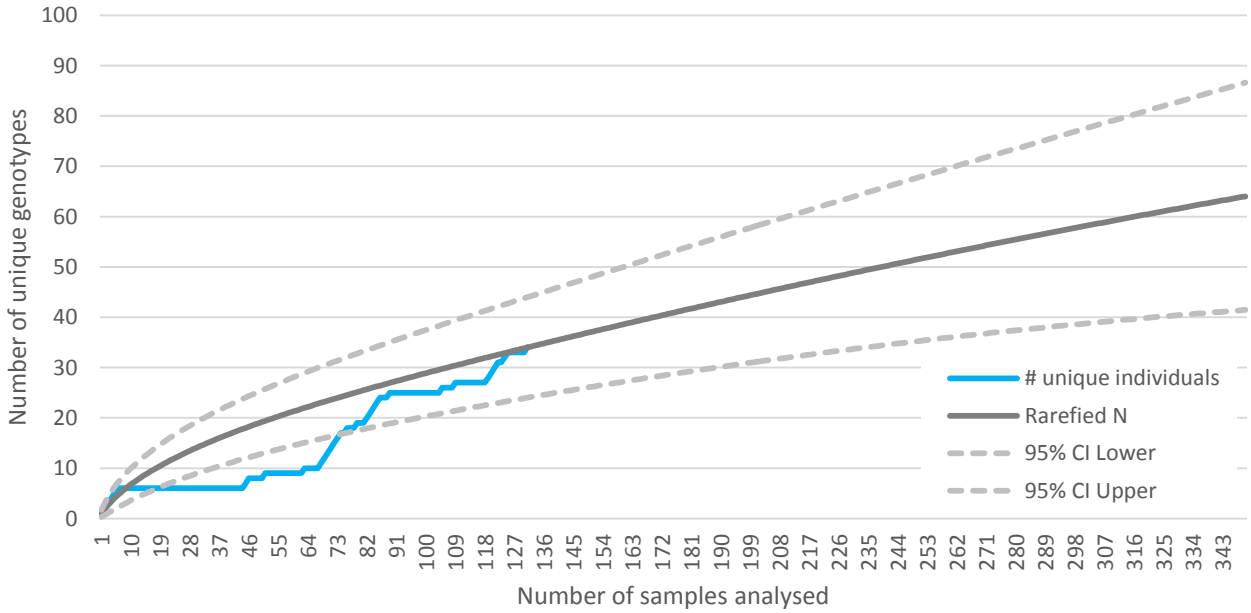

Figure 3: The number of unique individuals identified with increasing numbers of scats analysed and the estimated rarefaction curve.

Table 1: Detection of unique ghost bat genotypes by sampling year. Only two individuals (#378 and #379) were detected in both years (highlighted in red). Both were from the same cave (AA1).

	Individual #																																		
Year	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394
2015	●	●										●	●		●	●	●	●	●	●													●	●
2017			●	●	●	●	●	●	●	●	●			●				●	●		●	●	●	●	●	●	●	●	●	●	●	●		

Table 2: Detection of unique ghost bat genotypes by roost site. Only three individuals (#366, #369 and #370) were detected in multiple caves (highlighted in red). The remaining were only detected in a single cave.

	Individual #																																		
Roost	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394
A1	●	●	●	●	●	●	●	●	●	●	●																							
A2						●				●		●	●	●																					
AA1									●						●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		
L2						●																													
L3						●																											●	●

Table 3: List of inferred dispersal distances for ghost bats detected using multiple caves

Individual #	Cave	Distance
366	A1 → A2	33 m
	A1/A2 → L2	1.1 km
	L2 → L3 (presumed)	32 m
369	A1 → AA1	9.0 km
370	A1 → A2	33 m

Genetic Effective Population Size

The genetic effective population size (N_e) is estimated to be 12 individuals (95% CI's 10 – 16) (Table4). Given that the effective population size is typically around 10% of the census population size (Frankham, 1995), we can tentatively extrapolate that the population of ghost bats using the study site is between 100 – 160 individuals.

Table 4: Estimates of effective population size (N_e) of ghost bats sampled in this study estimated from linkage disequilibrium amongst genotypes, excluding alleles with decreasing frequencies. [a] Using alleles with frequency >0.02 is expected to give the most robust estimate of effective population size (Waples & Do, 2010).

	Lowest allele frequency		
	0.05	**0.02** [a]	**0.01**
# comparisons	1256	1901	2015
Estimated Ne	10.9	12.4	14.0
95% CI (jackknife)	8.4-14.4	9.9-15.6	11.2-17.9

Genetic diversity

Genetic diversity statistics were calculated by combining individuals found in closely-located caves (i.e. A1/A2; sample sizes are low for L2/L3 so statistics were not calculated). Genetic diversity statistics were largely similar amongst the two larger caves (A1/A2 and AA1). Observed and expected heterozygosity were not significantly different amongst the two caves (standard errors overlap) though inbreeding was marginally higher in the A1/A2 caves than in AA1 (Table 5).

Table 5: Genetic diversity statistics (mean and standard error) for ghost bat roost sites. Caves A1 and A2 have been pooled to increase sample sizes. Insufficient samples were available to estimate parameters for cave L2/L3 but individuals from these caves have been included in the Total. N_a = Number of alleles, N_e = Effective number of alleles, H_o = Observed heterozygosity, uH_e = unbiased expected heterozygosity, F = inbreeding coefficient.

Pop	Pop size	N_a	N_e	H_o	uH_e	F
A1/A2	16	6.4 ± 0.5	3.9 ± 0.4	0.68 ± 0.05	0.75 ± 0.02	0.07 ± 0.04
AA1	19	5.5 ± 0.4	3.7 ± 0.3	0.72 ± 0.04	0.72 ± 0.03	-0.03 ± 0.05
Total	34	7.5 ± 0.7	4.5 ± 0.5	0.68 ± 0.03	0.76 ± 0.02	0.10 ± 0.03

Spatial autocorrelation analysis

The small sample size and limited spatial distribution of roost sites somewhat limits the spatial autocorrelation analysis in this report to local-scale patterns (Figure 4). However, the results are broadly consistent with previous studies (Spencer & Tedeschi, 2016; Ottewell et al., 2017) showing higher relatedness of individuals (r) at small spatial scales (~50m, in this case amongst neighbouring caves) that declines with increasing distance, and with low relatedness of individuals in the most distantly located caves.

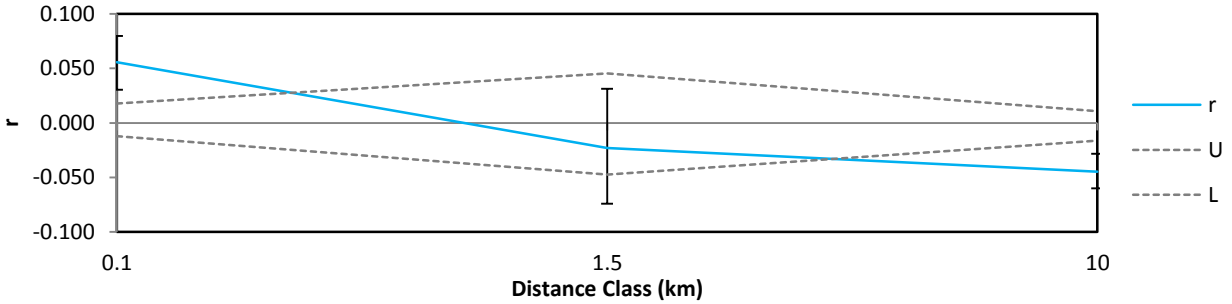

Figure 4: Spatial autocorrelation analysis of ghost bats amongst roost sites in this study. r = autocorrelation coefficient, U / L = upper and lower 95% confidence intervals around null hypothesis that r = 0.

Discussion

Faecal DNA analysis has provided an opportunity to use a non-invasive technique to gain insight into the patterns of movement, spatial and temporal cave use by Ghost Bats at the West Angelas mine site located in the eastern Hamersley Ranges of the Pilbara bioregion.

Of the samples analysed, we found a high genotyping success rate for recent scat collections with 92% of samples collected in 2017 giving a useable genotype (i.e. >6 loci genotyped). A much lower success rate was observed (40%) for scats collected two years previously, in 2015, suggesting that there may be degradation of DNA with increased storage time. This is an important consideration for future studies.

Overall, we detected 34 unique genotypes (individuals) from 131 scat samples taken from the five caves at the mine site. There was some difficulty in identifying unique genotypes from the microsatellite data due to the prevalence of allelic dropout in scat samples (a common problem in scat DNA studies; Knapp et al., 2009, Taberlet et al., 1999). We conservatively grouped like genotypes, but in doing so may have mis-classed a small proportion of scat samples as being from the same individual when they were from different, but highly related, individuals. As a result, the number of unique individuals detected in this study should be considered a minimum estimate.

Of the identified individuals, only two were detected in both sampling periods (2015 & 2017), and both were detected in cave AA1. The great majority of individuals were only detected in one cave, though three individuals were detected in multiple caves. These individuals were primarily detected using closely-located caves to the north of the site (A1, A2, L2, L3), though one individual was detected in both A1 and AA1, located ~10km apart. This pattern and scale of movement is similar to that reported at other Pilbara sites; i.e. predominant dispersal amongst neighbouring caves with less frequent dispersal to greater distances (Ottewell et al., 2017).

Overall, caves A1 and AA1 had the largest numbers of individual ghost bats identified (n = 11 and 19, respectively). AA1, in particular, had a high rate of detection of new individuals for the number of scats analysed, which may indicate a larger population, or a more transient population, at this cave site. Further sampling at this cave is likely to identify more individuals (Appendix 4).

Rarefaction analyses can be used to infer the census population size (N_c) from non-invasive genetic sampling (Kohn et al., 1999; Eggert et al., 2003; Luikart et al., 2010), similar to mark-recapture approaches. The ‘true’ population size can be inferred when the cumulative number of individuals detected with increasing sample size reaches an asymptote. Sample sizes in this study were too low to reach an asymptote so we were unable to estimate the census population size of ghost bats at West Angelas using this method. Genetic estimates of the *effective* population size (N_e), however, suggest the genetic sample represented between 10-16 individuals (mean = 12). It is a

common premise that N_e is 10% of N_c (Frankham, 1995), suggesting our estimates tentatively extrapolate to a census population size of 100-160 individuals using the West Angelas site over the two year time period. However, as the true relationship of N_e:N_c is not known for ghost bats this estimate is speculative.

Genetic diversity estimates indicated that the two main populations (A1/A2 and AA1) have very similar levels of allelic diversity (number of alleles) and heterozygosity. A1/A2 had slightly higher inbreeding (F) than AA1, possibly indicating a smaller population size or that more related individuals are found in A1/A2 than AA1. Overall, genetic diversity across the West Angelas study site is consistent with values reported in other populations in the eastern Hamersley Range (Ottewell et al., 2017).

The individual ghost bats identified in this study have not been previously identified (Ottewell et al., 2017). However, the closest locations in the Ottewell et al. study were 10-15 km distant to the West Angelas site which is the scale at which dispersal frequency appears to decline in ghost bats. Further systematic sampling could increase the probability of detecting movement patterns at these spatial scales to better estimate connectivity amongst ghost bat roosts in the Pilbara.

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Appendices

Appendix 1

Sample localities, year of sampling, numbers of *Macroderma gigas* scat samples analysed, number successfully genotyped and the number of unique individuals detected at each location. Genotyping of a sample was considered successful if genotypes were obtained at six or more microsatellite loci (see text).

Roost site	Year sampled	No. scats analysed	No. scats successful	No. individuals	No. individuals (years pooled)
A1	2015	7	2	2	11
	2017	59	54	9	
A2	2015	4	2	2	5
	2017	9	6	3	
AA1	2015	24	7	6	19
	2017	49	47	15	
L2	2017	1	1	1	1
L3	2015	10	7	2	3
	2017	5	5	1	
Total		168	131		

Appendix 2

Details of *Macroderma gigas* microsatellite loci, including locus name, PCR multiplex number, fluorescent label used, allele size range in base pairs, number of alleles (N_a), information index (I), observed heterozygosity (H_o), unbiased expected heterozygosity (uH_e) and inbreeding coefficient (F), and Type 1 and 2 genotyping error rates (see text) for the current data set.

Locus	Multiplex	Label	Size Range (bp)	N_a	I	H_o	uH_e	F	Type 1 error	Type 2 error
GB18 [a]	1	FAM	84-104	5	1.28	0.84	0.67	-0.25	0.00	0.00
gigas10 [b]	1	VIC	116-124	5	1.29	0.80	0.69	-0.17	0.12	0.04
GB33 [a]	1	NED	174-192	10	1.29	0.70	0.62	-0.14	0.00	0.00
gigas01 [b]	1	PET	137-157	10	1.91	0.66	0.82	0.19	0.08	0.04
MG32 [c]	4	FAM	198-228	11	1.45	0.79	0.70	-0.14	0.08	0.01
MG20 [c]	4	VIC	134-146	7	1.69	0.77	0.79	0.03	0.04	0.08
MG05 [c]	4	PET	93-114	6	1.39	0.62	0.72	0.12	0.04	0.04
MG26 [c]	4	PET	158-178	5	1.57	0.59	0.79	0.25	0.07	0.04
MG28 [c]	5	VIC	157-191	11	2.09	0.86	0.86	-0.01	0.15	0.00
MG21 [c]	5	NED	139-157	6	1.27	0.77	0.66	-0.18	0.11	0.04
GB42 [a]	5	PET	182-204	9	1.60	0.79	0.75	-0.06	0.11	0.00

Source of microsatellite loci:
[a] JH = Jane Hughes, unpublished
[b] WW = Worthington Wilmer et al., 1999
[c] DBCA = Department of Biodiversity, Conservation and Attractions, unpublished

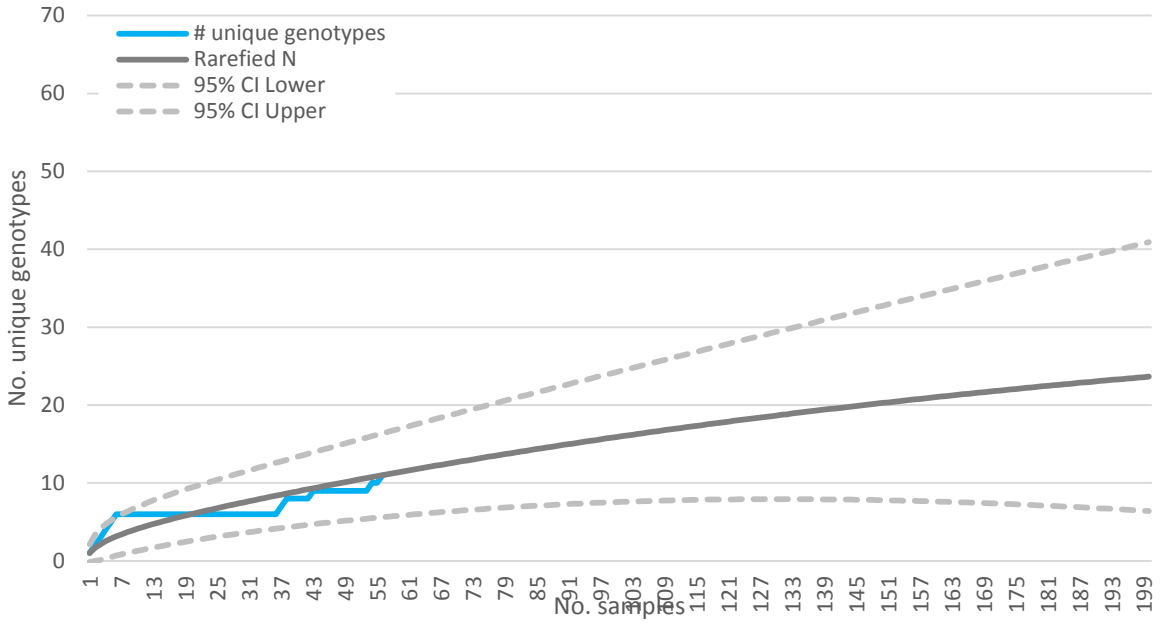
Appendix 3
Details of the number of scats detected for each individual by roost site and by year.

Site	Year	Individual #																																					
		361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394
A1	2015	1	1																																
	2017			1	1	1	31	1	5	11	2	1																							
A2	2015												1	1																					
	2017						2				3				1																				
AA1	2015															1	1	2	1	1	1														
	2017									1									2	2		1	1	1	3	16	4	10	1	1	1	2	1		
L2	2017						1																												
L3	2015																																	6	1
	2017						5																												

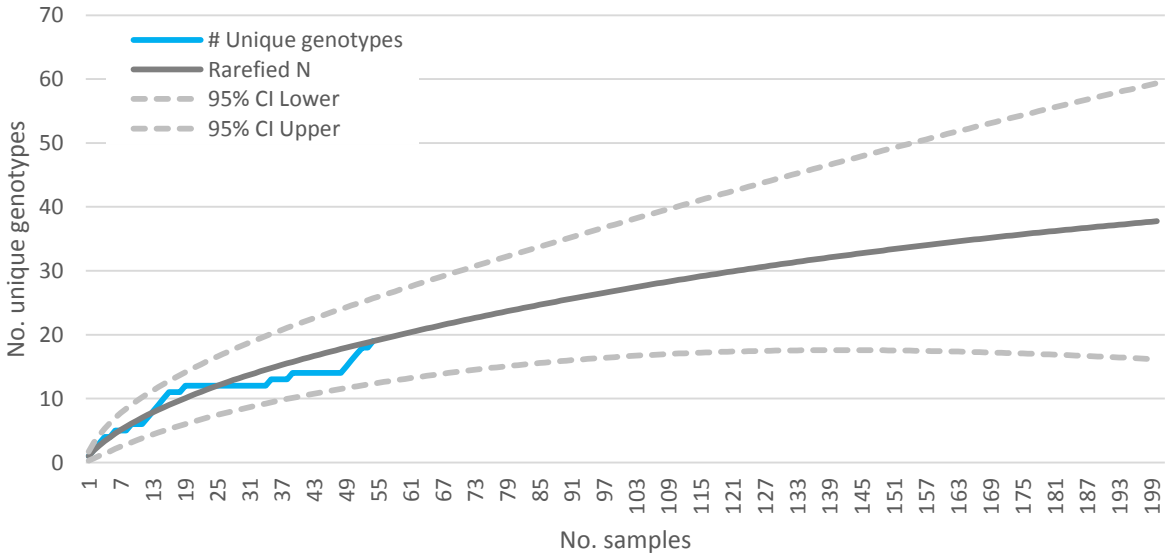
Appendix 4

Rarefaction curve for (A) cave A1 and (B) cave AA1, extrapolated to a sample size of N=200 to assess the rate of detection of new individuals with increased sampling effort.

A. Cave A1



B. Cave AA1



Appendix B – Scat Hormone Analysis (Keeley, 2018)

Sample ID	Biologic ID	Collection Date	Site	Genotype ID#	Lat	Long	Progesterone ng/g
17-1181	A1 171018-01	18/10/2017	A1	368	-23.1134	118.7764	2273.68
17-1183	A1 171018-03	18/10/2017	A1	364	-23.1134	118.7764	5868.88
17-1184	A1 171018-04	18/10/2017	A1	368	-23.1134	118.7764	3762.60
17-1185	A1 171018-05	18/10/2017	A1	368	-23.1134	118.7764	5680.00
17-1186	A1 171018-06	18/10/2017	A1	368	-23.1134	118.7764	3759.04
17-1188	A1 171018-08	18/10/2017	A1	369	-23.1134	118.7764	2999.17
17-1190	A1 171018-10	18/10/2017	A1	369	-23.1134	118.7764	1968.72
17-1191	A1 171018-11	18/10/2017	A1	369	-23.1134	118.7764	2211.02
17-1192	A1 171018-12	18/10/2017	A1	366	-23.1134	118.7764	2178.66
17-1193	A1 171018-13	18/10/2017	A1	369	-23.1134	118.7764	2610.28
17-1194	A1 171018-14	18/10/2017	A1	366	-23.1134	118.7764	3401.67
17-1195	A1 171018-15	18/10/2017	A1	366	-23.1134	118.7764	2757.89
17-1196	A1 171018-16	18/10/2017	A1	366	-23.1134	118.7764	3406.90
17-1198	A1 171018-18	18/10/2017	A1	369	-23.1134	118.7764	4046.69
17-1199	A1 171018-19	18/10/2017	A1	365	-23.1134	118.7764	1905.98
17-1200	A1 171018-20	18/10/2017	A1	366	-23.1134	118.7764	1970.97
17-1201	A1 171018-21	18/10/2017	A1	366	-23.1134	118.7764	2538.10
17-1202	A1 171018-22	18/10/2017	A1	366	-23.1134	118.7764	2507.61
17-1203	A1 171018-23	18/10/2017	A1	366	-23.1134	118.7764	5555.02
17-1204	A1 171018-24	18/10/2017	A1	366	-23.1134	118.7764	2050.41
17-1205	A1 171018-25	18/10/2017	A1	366	-23.1134	118.7764	158.82
17-1206	A1 171018-26	18/10/2017	A1	366	-23.1134	118.7764	1691.57
17-1207	A1 171018-27	18/10/2017	A1	366	-23.1134	118.7764	4200.78
17-1208	A1 171018-28	18/10/2017	A1	366	-23.1134	118.7764	1976.00
17-1209	A1 171018-29	18/10/2017	A1	366	-23.1134	118.7764	2060.92
17-1210	A1 171018-30	18/10/2017	A1	366	-23.1134	118.7764	2184.00
17-1211	A1 171018-31	18/10/2017	A1	366	-23.1134	118.7764	2204.12
17-1212	A1 171018-32	18/10/2017	A1	366	-23.1134	118.7764	1690.00
17-1213	A1 171018-33	18/10/2017	A1	366	-23.1134	118.7764	2750.41
17-1214	A1 171018-34	18/10/2017	A1	366	-23.1134	118.7764	2715.79
17-1215	A1 171018-35	18/10/2017	A1	366	-23.1134	118.7764	2219.76
17-1216	A1 171018-36	18/10/2017	A1	366	-23.1134	118.7764	2400.00
17-1217	A1 171018-37	18/10/2017	A1	366	-23.1134	118.7764	2991.51
17-1218	A1 171018-38	18/10/2017	A1	366	-23.1134	118.7764	2457.81
17-1219	A1 171018-39	18/10/2017	A1	366	-23.1134	118.7764	2547.37
17-1220	A1 171018-40	18/10/2017	A1	366	-23.1134	118.7764	2546.06
17-1221	A1 171018-41	18/10/2017	A1	366	-23.1134	118.7764	1832.95
17-1222	A1 171018-42	18/10/2017	A1	366	-23.1134	118.7764	1545.38
17-1223	A1 171018-43	18/10/2017	A1	366	-23.1134	118.7764	3852.63
17-1225	A1 171018-45	18/10/2017	A1	363	-23.1134	118.7764	3913.40
17-1226	A1 171018-46	18/10/2017	A1	370	-23.1134	118.7764	1349.19
17-1227	A1 171018-47	18/10/2017	A1	367	-23.1134	118.7764	3328.00
17-1228	A1 171018-48	18/10/2017	A1	369	-23.1134	118.7764	2325.82

Sample ID	Biologic ID	Collection Date	Site	Genotype ID#	Lat	Long	Progesterone ng/g
17-1229	A1 171018-49	18/10/2017	A1	369	-23.1134	118.7764	1659.14
17-1230	A1 171018-50	18/10/2017	A1	369	-23.1134	118.7764	2261.79
17-1231	A1 171018-51	18/10/2017	A1	371	-23.1134	118.7764	3065.04
17-1232	A1 171018-52	18/10/2017	A1	366	-23.1134	118.7764	4669.39
17-1233	A1 171018-53	18/10/2017	A1	366	-23.1134	118.7764	2440.00
17-1234	A1 171018-54	18/10/2017	A1	369	-23.1134	118.7764	2477.17
17-1235	A1 171018-55	18/10/2017	A1	366	-23.1134	118.7764	2019.42
17-1236	A1 171018-56	18/10/2017	A1	370	-23.1134	118.7764	1698.35
17-1237	A1 171018-57	18/10/2017	A1	369	-23.1134	118.7764	1495.00
17-1238	A1 171018-58	18/10/2017	A1	369	-23.1134	118.7764	1754.47
17-1239	A1 171018-59	18/10/2017	A1	368	-23.1134	118.7764	554.10
17-1113	A1 20151026-04	26/10/2015	A1	361	-23.1134	118.7764	3878.33
17-1114	A1 20151026-05	26/10/2015	A1	362	-23.1134	118.7764	266.67
17-1072	A2 20151026-01	26/10/2015	A2	372	-23.1131	118.7764	1726.40
17-1075	A2 20151026-04	26/10/2015	A2	373	-23.1131	118.7764	1400.40
17-1118	A2 20171018-01	18/10/2017	A2	370	-23.1131	118.7764	1118.49
17-1119	A2 20171018-02	18/10/2017	A2	370	-23.1131	118.7764	1049.72
17-1120	A2 20171018-03	18/10/2017	A2	366	-23.1131	118.7764	4753.13
17-1121	A2 20171018-04	18/10/2017	A2	370	-23.1131	118.7764	5709.39
17-1123	A2 20171018-06	18/10/2017	A2	366	-23.1131	118.7764	2884.38
17-1124	A2 20171018-07	18/10/2017	A2	374	-23.1131	118.7764	1458.54
17-1132	AA1 171018-01	18/10/2017	AA1	384	-23.1883	118.8266	282.81
17-1133	AA1 171018-02	18/10/2017	AA1	385	-23.1883	118.8266	244.53
17-1134	AA1 171018-03	18/10/2017	AA1	383	-23.1883	118.8266	3160.78
17-1135	AA1 171018-04	18/10/2017	AA1	386	-23.1883	118.8266	395.14
17-1136	AA1 171018-05	18/10/2017	AA1	382	-23.1883	118.8266	493.75
17-1137	AA1 171018-06	18/10/2017	AA1	385	-23.1883	118.8266	268.70
17-1138	AA1 171018-07	18/10/2017	AA1	385	-23.1883	118.8266	244.18
17-1139	AA1 171018-08	18/10/2017	AA1	385	-23.1883	118.8266	225.10
17-1140	AA1 171018-09	18/10/2017	AA1	387	-23.1883	118.8266	2842.11
17-1141	AA1 171018-10	18/10/2017	AA1	387	-23.1883	118.8266	2858.96
17-1142	AA1 171018-11	18/10/2017	AA1	387	-23.1883	118.8266	2547.37
17-1143	AA1 171018-12	18/10/2017	AA1	379	-23.1883	118.8266	216.26
17-1144	AA1 171018-13	18/10/2017	AA1	384	-23.1883	118.8266	2629.43
17-1145	AA1 171018-14	18/10/2017	AA1	387	-23.1883	118.8266	2649.81
17-1146	AA1 171018-15	18/10/2017	AA1	387	-23.1883	118.8266	3617.39
17-1147	AA1 171018-16	18/10/2017	AA1	387	-23.1883	118.8266	2100.39
17-1148	AA1 171018-17	18/10/2017	AA1	384	-23.1883	118.8266	1976.86
17-1149	AA1 171018-18	18/10/2017	AA1	388	-23.1883	118.8266	375.81
17-1150	AA1 171018-19	18/10/2017	AA1	379	-23.1883	118.8266	353.53
17-1151	AA1 171018-20	18/10/2017	AA1	389	-23.1883	118.8266	273.68
17-1152	AA1 171018-21	18/10/2017	AA1	378	-23.1883	118.8266	279.69
17-1153	AA1 171018-22	18/10/2017	AA1	378	-23.1883	118.8266	412.70

Sample ID	Biologic ID	Collection Date	Site	Genotype ID#	Lat	Long	Progesterone ng/g
17-1155	AA1 171018-24	18/10/2017	AA1	386	-23.1883	118.8266	151.56
17-1156	AA1 171018-25	18/10/2017	AA1	387	-23.1883	118.8266	1848.44
17-1157	AA1 171018-26	18/10/2017	AA1	390	-23.1883	118.8266	460.41
17-1158	AA1 171018-27	18/10/2017	AA1	385	-23.1883	118.8266	481.93
17-1159	AA1 171018-28	18/10/2017	AA1	385	-23.1883	118.8266	277.43
17-1160	AA1 171018-29	18/10/2017	AA1	385	-23.1883	118.8266	379.61
17-1161	AA1 171018-30	18/10/2017	AA1	387	-23.1883	118.8266	204.58
17-1162	AA1 171018-31	18/10/2017	AA1	385	-23.1883	118.8266	346.34
17-1163	AA1 171018-32	18/10/2017	AA1	385	-23.1883	118.8266	285.49
17-1164	AA1 171018-33	18/10/2017	AA1	385	-23.1883	118.8266	196.80
17-1165	AA1 171018-34	18/10/2017	AA1	369	-23.1883	118.8266	1512.35
17-1166	AA1 171018-35	18/10/2017	AA1	385	-23.1883	118.8266	268.22
17-1167	AA1 171018-36	18/10/2017	AA1	385	-23.1883	118.8266	133.88
17-1168	AA1 171018-37	18/10/2017	AA1	385	-23.1883	118.8266	301.98
17-1170	AA1 171018-39	18/10/2017	AA1	386	-23.1883	118.8266	357.14
17-1171	AA1 171018-40	18/10/2017	AA1	385	-23.1883	118.8266	307.09
17-1172	AA1 171018-41	18/10/2017	AA1	386	-23.1883	118.8266	325.81
17-1173	AA1 171018-42	18/10/2017	AA1	391	-23.1883	118.8266	4916.73
17-1174	AA1 171018-43	18/10/2017	AA1	385	-23.1883	118.8266	219.05
17-1175	AA1 171018-44	18/10/2017	AA1	391	-23.1883	118.8266	4948.79
17-1176	AA1 171018-45	18/10/2017	AA1	385	-23.1883	118.8266	206.67
17-1177	AA1 171018-46	18/10/2017	AA1	387	-23.1883	118.8266	1894.74
17-1178	AA1 171018-47	18/10/2017	AA1	381	-23.1883	118.8266	105.26
17-1179	AA1 171018-48	18/10/2017	AA1	392	-23.1883	118.8266	150.61
17-1180	AA1 171018-49	18/10/2017	AA1	387	-23.1883	118.8266	375.21
17-1077	AA1 20151026-02	26/10/2015	AA1	375	-23.1883	118.8266	1309.92
17-1078	AA1 20151026-03	26/10/2015	AA1	377	-23.1883	118.8266	1374.71
17-1080	AA1 20151026-05	26/10/2015	AA1	377	-23.1883	118.8266	1162.60
17-1087	AA1 20151026-12	26/10/2015	AA1	378	-23.1883	118.8266	1853.47
17-1088	AA1 20151026-13	26/10/2015	AA1	379	-23.1883	118.8266	6066.67
17-1091	AA1 20151026-16	26/10/2015	AA1	376	-23.1883	118.8266	395.04
17-1098	AA1 20151026-23	26/10/2015	AA1	380	-23.1883	118.8266	2937.40
17-1117	L2 20171018-01	18/10/2017	L2	366	-23.1138	118.7878	1056.91
17-1101	L3 20151026-02	26/10/2015	L3	393	-23.1138	118.7875	456.47
17-1102	L3 20151026-03	26/10/2015	L3	393	-23.1138	118.7875	1167.72
17-1103	L3 20151026-04	26/10/2015	L3	393	-23.1138	118.7875	515.45
17-1105	L3 20151026-06	26/10/2015	L3	394	-23.1138	118.7875	518.52
17-1106	L3 20151026-07	26/10/2015	L3	393	-23.1138	118.7875	513.39
17-1108	L3 20151026-09	26/10/2015	L3	393	-23.1138	118.7875	517.56
17-1109	L3 20151026-10	26/10/2015	L3	393	-23.1138	118.7875	568.34
17-1127	L3 20171018-01	18/10/2017	L3	366	-23.1138	118.7875	3572.36
17-1128	L3 20171018-02	18/10/2017	L3	366	-23.1138	118.7875	569.23
17-1129	L3 20171018-03	18/10/2017	L3	366	-23.1138	118.7875	1284.71

Sample ID	Biologic ID	Collection Date	Site	Genotype ID#	Lat	Long	Progesterone ng/g
17-1130	L3 20171018-04	18/10/2017	L3	366	-23.1138	118.7875	602.06
17-1131	L3 20171018-05	18/10/2017	L3	366	-23.1138	118.7875	559.06

Appendix C – Monitoring Cave Characteristics

Cave	A1	A2	AA1	L2	L3
Coordinats UTM Zone 50K	681914 E 7442820 S	681918 E 7442857 S	686950 E 7434465 S	683086 E 7442760 S	683054 E 7442766 S
Basic Geology	Marra Mamba Iron formation	Marra Mamba Iron formation	Marra Mamba Iron formation	Marra Mamba Iron formation	Marra Mamba Iron formation
Entrance description	Single horizontal entrance at head of horseshoe-shaped gully	Single horizontal entrance on north side of horseshoe shaped gully	Entrance is open, wide but fairly low and horizontal in aspect.	Single entrance sloping down from boulders from old roof collapse.	Single horizontal entrance at head of horseshoe shaped gully. Currently has sound barrier erected in front of entrance.
Entrance dimensions W x H (m)	5 m X 3.2 m	4.5 m X 4 m	15 m x 4 m	5 m X 2.7 m	12 m x 2.5 m
Cave depth	21 m	14.8 m	70 m	25 m	29 m
Entrance (collapsed, tight or open)	Open	Open	Open	Collapsed	Open
Entrance orientation	NW	WSW	W	W	S
Cave grouping	Loose group of caves and overhangs	Loose group of caves and overhangs	Unknown	Tight group of three caves in gully	Tight group of three caves in gully
Location on slope	Mid slope	Mid slope	Mid slope	Mid slope	Mid slope
Cave interior description	One long chamber with small side chamber	One chamber	Three chambers	One long chamber	One long chamber with smaller side chamber
Rear passages that may have Ghost Bat roosts	Yes	No	Yes	No	Yes
Local Ghost Bat foraging opportunities	Eucalypt woodland and ephemeral pools in gully.	Eucalypt woodland and ephemeral pools in gully.		Eucalypt woodland and ephemeral pools in gully.	Eucalypt woodland and ephemeral pools in gully.
Entrance chamber temperature, relative humidity and light level (Biologic, 2015)	28 °C 25 % 3000 lux	28 °C 25 % 3000 lux	36 °C 19 % 4500 lux	34 °C 24 % 4000 lux	32 °C 26 % 3800 lux

Cave	A1	A2	AA1	L2	L3
Internal temperature, relative humidity and light level (Biologic, 2013)	31.4 °C 23 % 0.19 lux	32.7 °C 20 % 0.15 lux -	29.7 °C 21 % 0.00 lux	33 °C 24 % 1.8 lux	29 °C 31 % 0.15 lux
Type of Ghost Bat roost (most likely)	Confirmed day roost / Possible maternity roost	Feeding cave / night roost	Confirmed maternity roost	Feeding cave / night roost	Feeding cave/ night roost/ Possible day roost
Photo