Biota Environmental Sciences

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5 February 2020

Sharon Green
Senior Environmental Advisor
Rio Tinto
Via email

Dear Sharon,

Winu Project – Bilby Abundance Study

Further to completion of the fauna assessment of the Winu Project Area (WPA) (Biota 2019), we provide a memorandum addressing results of a population abundance study undertaken on the Bilby (*Macrotis lagotis*).

This letter comprises:

- rationale and background of the study;
- methodology employed;
- results; and
- discussion of results in a regional context.

This letter is intended to support the findings of the main fauna assessment of the WPA, the results of which are presented in a separate report (Biota 2019).

Please contact us should you have any queries in relation to this study.

Yours sincerely,

Biota Environmental Sciences Pty Ltd

Penny Brooshooft and Roy Teale
Senior Zoologist and Principal Zoologist/Director

1. Introduction

1.1 Rationale for Study

The Bilby is a Threatened species listed as Vulnerable under the Federal *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) and the State *Biodiversity Conservation Act 2016* (BC Act). As the Bilby is a nationally Threatened species under the EPBC Act, it is a Matter of National Environmental Significance (MNES) for the purposes of the Act (DoE 2013). The fauna assessment of the WPA recorded sign evidence positively attributable to the Bilby, comprising diggings, scats, tracks and active burrows, in one primary locale (Biota 2019). Given that the Bilby is an MNES species, and that it was recorded from the WPA, advice was sought from the Department of Biodiversity, Conservation and Attractions (DBCA) on the level of survey effort required to address likely expectations of regulators on survey adequacy for the Bilby. The DBCA recommended conducting an abundance study to determine the number of individuals within the WPA. The aim was to assess whether the individuals within the WPA represent part of a significant source population, compared to other documented source populations regionally.

It is important to note here that survey effort to detect Bilby presence within the WPA during the fauna assessment was undertaken in accordance with current guidelines (DBCA 2017a). The recommendation provided by the DBCA to undertake an abundance analysis is not a requirement under current guidance, but is considered best practice.

1.2 Background to the Study

Population abundance monitoring of the Bilby has been implemented by the DBCA in the Pilbara and southwest Kimberley over a number of years (DBCA 2016, 2017b, 2018a, 2018b). Abundance monitoring was employed to provide information on actual numbers of bilbies within populations, so that demographic fluctuations over time could be ascertained (DBCA 2016, 2017b). To do this, scats were collected, and molecular analysis of epithelial DNA was undertaken to genotype individuals. This method was preferable over others, including capture-mark-release-recapture and counts of tracks and burrows, as it was non-invasive and enabled accurate calculation of Bilby numbers (DBCA 2017b). The technique also has specific application to field situations, as Bilby scats are relatively easy to detect and collect, and DNA can reliably be obtained from scats deposited up to two weeks prior to collection (DBCA 2016). Genotyping individuals from molecular analysis of scats is therefore the recommended technique for population abundance studies (DBCA 2016, 2017b), and was undertaken here.

2. Methodology

The methodology employed aligned with that recommended by the DBCA (2016, 2017b). Broadly, the steps undertaken were:

1. On-ground mapping of the extent of Bilby evidence within the WPA;
2. Traversing transects through that extent to collect scats;
3. Extracting DNA from scats to genotype individuals; and
4. Statistical analysis of spatial and genetic data using spatially-explicit capture-recapture (SECR) to estimate abundance.

The specific methodology for each of the above steps is discussed further in Sections 2.1 to 2.4 below.

2.1 Mapping

Unbounded foot traverses through the known area of Bilby activity were undertaken by zoologists during the fauna assessment of the WPA (Biota 2019). The aim was to record sign (scats/tracks/diggings/burrows) until evidence of occupancy ceased, which was interpreted as the extremity of local Bilby activity.

2.2 Collection of Scats

The mapping was utilised to overlay strip transects to be traversed. Strip transects are a common field-based approach used to record evidence of a species (in this case scats), from which the resulting data may be used to estimate population abundance. Each strip transect was 20 m wide and separated by 100 m (Attachment 1). Transects were searched by three zoologists (Dr. Stewart Ford, Mr Joshua Keen and Mr Nathan Beerkens) on the 23 September 2019. All scats that were judged to be less than two weeks old were collected. Bilby scats are difficult to age based solely on appearance, however the age of scats can be assessed by examining decomposition of diggings which are typically associated with scat deposition (DBCA 2018b). Therefore, only scats associated with fresh diggings (as determined by recently turned over substrate) were collected.

A drawback of utilising scats for molecular analysis is that they contain low quantity and quality DNA (Piggott and Taylor 2003). To reduce DNA degradation and optimise the likelihood of obtaining a useable molecular sequence, the storage protocol recommended by the DBCA (2017b) was followed. Scats were stored in 30 ml vials filled with 10 ml of silica gel beads and placed in between two balls of cotton wool that acted as buffers to movement. Singular scats were stored in unique vials, unless it could be determined with certainty that scats came from one animal (i.e. scats were deposited in a pile and stuck together).

2.3 DNA Extraction

Twenty scat specimens were extracted using the QIAGEN QIAamp Fast DNA Stool mini kit (Qiagen, Hilden, Germany). Samples were washed in buffer as per Carpenter and Dziminski (2017). Six tissue reference samples were extracted using the QIAGEN DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) as per protocol.

Seven microsatellite loci for *Macrotis lagotis* (Table 2.1) were amplified using the QIAGEN Multiplex PCR kit in triplicate. PCR products were analysed on an ABI3730XL Sequencer using Genescan-500 LIZ internal standard and scored using GeneMarker V1.91

Table 2.1: Microsatellite markers used in the DNA analysis.

	Locus	Primers (5' –> 3')	Dye	Reference
Multiplex 1	B22	GGT ATG AGG AAT TAG AAT TAC AGG CGG TAT TAA ATG GGC TAT GGA GT	VIC	(Moritz et al. 1997)
	B63	CTT AGG CAA ATA GGG TGA AGT GG CAG AAC CAT TAG GAA GGA GTT TC	NED	(Moritz et al. 1997)
	B41	TGA CTT TCT TTT GCT ACA ACA ACC GGA AAA GTT TTT AGC CTA ATA GTG G	PET	(Moritz et al. 1997, Smith et al. 2009)
	B55	GCA CCA ACC TAT CCT CTT CAT TC CTA CAA GTC TGA TAA TTC CAG GC	FAM	(Moritz et al. 1997)
Multiplex 2	B02	GCA TGT ACT TAA CCC CCT TTG CC CCC GAC AAT CCA GCC TGT TAT TC	FAM	(Moritz et al. 1997)
	B17	AGC CTG TGT GTC TTA AAA TGC CTC CAA TTC ACT TTT CCT GAG AC	VIC	(Moritz et al. 1997, Smith et al. 2009)
	B56	CACACTTATACATACACGTACACG CAC TAA CAA ATA TGC TTG GGA AAG G	PET	(Moritz et al. 1997, Smith et al. 2009)

2.4 SECR Analysis

SECR (Efford 2004, Efford and Fewster 2012) is a recognised and robust approach to estimating abundance of bilbies (DBCA 2018a). SECR analysis was investigated here, however limitations with the available data prevented its effective use (see Section 3.2 below).

3 Results

3.1 DNA Extraction

Scats were collected from 20 locations and sequenced. Of the 20 collections, six failed to amplify DNA and a further five failed at the requisite number of loci, leaving nine scats (45%) that were assigned to one of three different individuals (Table 3.1).

Table 3.1: Scats used in the DNA Analysis.

Helix ID	Scat ID	Transect ID	Alleles	Individual Match
PM12	S20190923.WINWBT07-01	WINWBT07	0	-
PM13	S20190923.WINWBT07-02	WINWBT07	7	Bilby01
PM14	S20190923.WINWBT07-03	WINWBT07	5	Bilby03
PM15	S20190923.WINWBT07-04	WINWBT07	4	-
PM16	S20190923.WINWBT07-05	WINWBT07	4	-
PM17	S20190923.WINWBT07-06	WINWBT07	4	-
PM18	S20190923.WINWBT08-01	WINWBT08	7	Bilby02
PM19	S20190923.WINWBT08-02	WINWBT08	7	Bilby01
PM20	S20190923.WINWBT01-01	WINWBT01	0	-
PM21	S20190923.WINWBT01-02	WINWBT01	0	-
PM22	S20190923.WINWBT01-03	WINWBT01	7	Bilby02
PM23	S20190923.WINWBT02-01	WINWBT02	3	-
PM24	S20190923.WINWBT02-02	WINWBT02	5	-
PM25	S20190923.WINWBT02-03	WINWBT02	7	Bilby01
PM26	S20190923.WBT06-01	WINWBT06	7	Bilby01
PM27	S20190923.WBT06-02	WINWBT06	0	-
PM28	S20190923.WBT06-03	WINWBT06	7	Bilby01
PM29	S20190923.WBT05-01	WINWBT05	7	Bilby01
PM30	S20190923.WBT04-01	WINWBT04	0	-
PM31	S20190923.WBT04-02	WINWBT04	0	-

3.2 SECR Analysis

A minimum of 10 recaptures are generally required for successful estimation of density using SECR with at least 20 cited as a more realistic minimum[1]. This survey recorded six recapture events of which five were from a single individual (Attachment 1). While SECR estimation was successful in this instance, SECR has limited utility with these very small data sets and therefore has not been reported here. In such instances, and where a robust sampling approach is followed, the sequence data can be considered representative of a census. In this case, the sequencing identified three different individuals from within the WPA.

[1] (http://www.phidot.org/forum/).

4 Regional Context

Bilby population abundance monitoring has been undertaken within five populations in the Pilbara (Turner River, Hillside, Nullagine, Yarrie and Warralong), one in the central rangelands (Matuwa), one in the Gibson Desert (Kiwirrkurra), and three in the La Grange region (Anna Plains, Nita Downs and Thangoo Stations, located along the coastline between Broome and Sandfire Roadhouse) (DBCA 2017b, 2018b). The population numbers estimated for each of these locations, for each monitoring year, are presented in Table 4.1.

The largest population recorded to date was at Anna Plains in 2017, with 44 (±7) individuals (Table 4.1). The population at Matuwa was also large, however this population was in an area that has benefited from predator control, stock exclusion and fire management for more than 12 years (DBCA 2017b). By contrast, the Pilbara, Gibson Desert and La Grange populations are unmanaged (DBCA 2017b).

Table 4.1: Regional population abundance estimates for the Bilby.

	Pilbara					Central Rangelands	Gibson Desert	La Grange		
Monitoring Year	**Turner River**	**Hillside**	**Nullagine**	**Yarrie**	**Warralong**	**Matuwa**	**Kiwirrkurra**	**Anna Plains**	**Nita Downs**	**Thangoo**
2014	2	4	2	-	-	-	-	-	-	-
2015	1	at least 1	5	10	-	23	-	-	-	-
2016	2	at least 1	8	2	6	25	4	-	-	-
2017	-	-	-	-	-	-	-	44 (±7)	10 (±3)	2 (±2)

Comparison of Bilby abundance within the WPA with regional data allows quantification of the significance of the area compared to other known Bilby populations. Significance in this context relates to the number of individuals compared to regional estimates: numbers akin to those of Anna Plains would suggest a regionally significant source population, while low numbers comparative to those of Turner River, Hillside, Nullagine, Yarrie, Warralong, Kiwikurra, Nita Downs and Thangoo populations would suggest an average source population size (Table 4.1). The findings of this abundance study (three individuals) suggest that the source population here is of low abundance and does not represent a large regionally significant source population. It should be noted however, that as 45% of the collected scats were successfully sequenced, it is possible that more individuals are present. Even if this was the case, it is unlikely that it would significantly change the overall assessment of the source population being of low abundance in a regional context.

Given that the Bilby is an MNES species, an additional consideration is the significant impact criteria for Vulnerable species as per Department of the Environment guidelines (2013). While it is beyond the scope of this memorandum to address the significant impact criteria in full, we can provide comment on the 'important population' aspect of the criteria, as outlined in the guidelines (DotE 2013). Important populations, as defined by the guidelines include those that are key source populations for breeding or dispersal; are necessary for maintaining genetic diversity; and/or are near the limit of the species range (DotE 2013). While the Anna Plains and Matuwa populations represent important source populations, the remaining populations that contain a low number of individuals collectively form part of an important population from a regional perspective. The individuals occurring within the WPA are therefore considered part of an important population of the Bilby within the Great Sandy Desert in this context.

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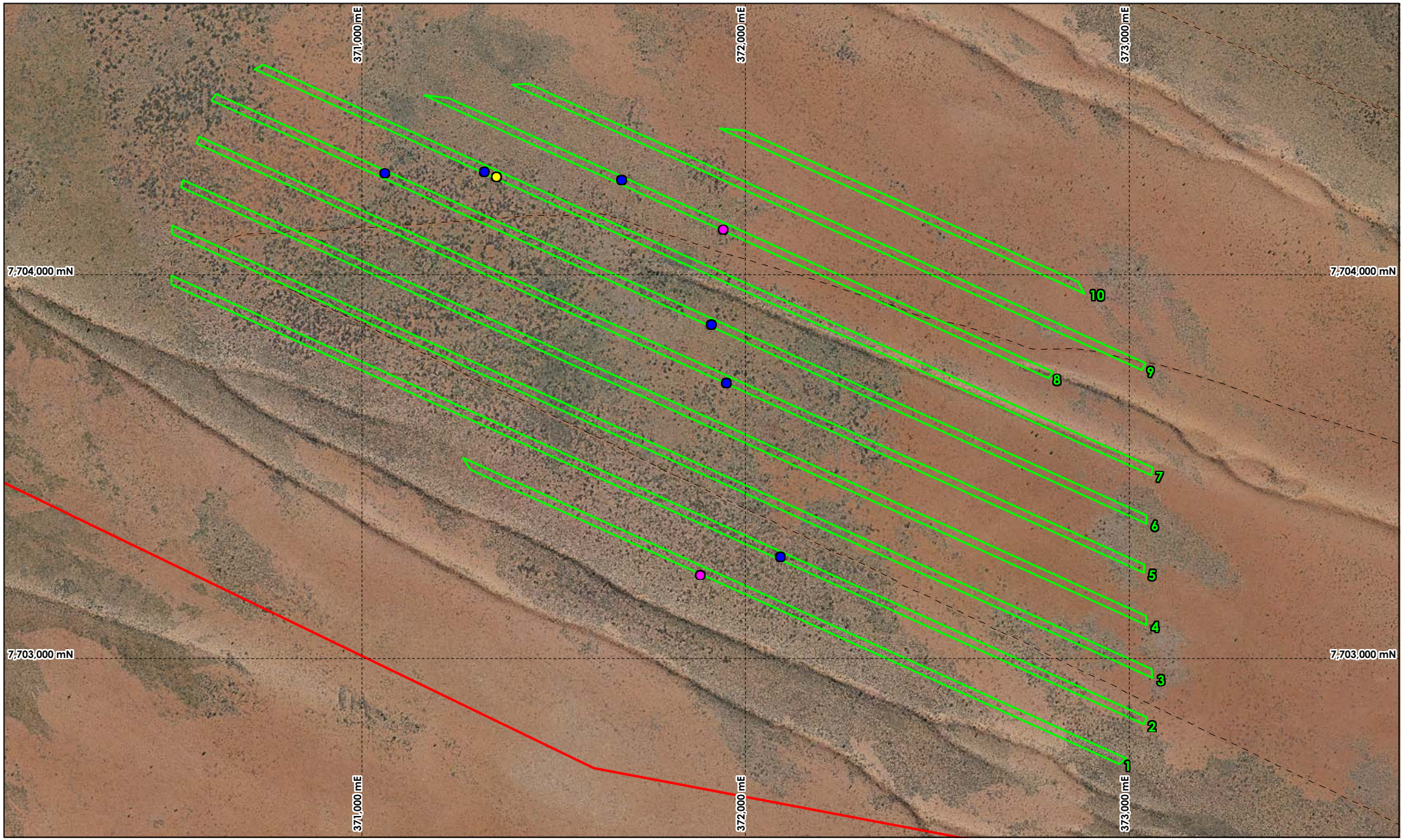

Location Map

Bilby scats:

- Bilby 1
- Bilby 2
- Bilby 3

- WPA
- Strip transect
- Track

N

0 250 500

meters

Winu Project Fauna
Bilby Searches

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Author: P Brooshooft | Drawn: B King | Job No.: 1442 | Date: 29 Jan 2020 | Revised: | Projection: MGA Z51 (GDA94) | Scale: 1:12,500 @ A4