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Department of **Biodiversity, Conservation and Attractions**

Bilby abundance monitoring at Warralong, Western Australia, 2023

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Bilby abundance monitoring at Warralong, Western Australia, 2023

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Warralong Greater Bilby Offset

June 2024

GOVERNMENT OF WESTERN AUSTRALIA

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Acknowledgments

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1 Background

In 2014, the Department of Biodiversity, Conservation and Attractions (DBCA) Bilby Research Team, together with Warralong community members, identified an active bilby population on the Coongan Pastoral Lease (hereafter referred to as the Coongan colony). In 2018, a collaborative project (hereafter referred to as the Warralong Bilby Project) involving the Warralong Community, DBCA, Roy Hill and Greening Australia was initiated to monitor and manage this population. In 2018, preliminary surveys conducted as part of this project detected an additional bilby population on the Coongan lease, approximately 12 km north-west of the Coongan colony (hereafter referred to as the River colony). Since 2014, bilby abundance has been measured at the Coongan colony in 2016, 2018, 2019, 2022, and at the River colony in 2018, 2019, 2021 and 2022. No abundance survey was conducted in 2020 due to the COVID-19 pandemic which resulted in access not being permitted to the area. Recommencement of the annual abundance surveys in 2021 did not find the Coongan colony, although a 'new' Coongan colony was detected in 2022, 5 km from the original colony's location. Given the extensive search conducted at the previous site in 2022 (Department of Biodiversity, Conservation and Attractions, 2023), and the fact that bilbies are capable of moving large distances (Moseby and O'Donnell, 2003), it is possible that this relocated colony is the same population, and we make this assumption here in this report. Eradicat® feral cat baits have been deployed in both 2022 and 2023 inside the Warralong Bilby Land Management Area during June/July of each year, with abundance surveys conducted post management event. This report documents the most recent abundance measurements recorded at two bilby colonies located on the Coongan Pastoral Lease and De Grey Pastoral Lease in 2023.

2 Methods

2.1 Scat collection

Scat collection at each colony was structured into two stages. Stage one involved delineating the boundary of bilby activity at a site, so as to determine the size of the area bilbies are using (activity area). The second stage involved establishing transects within the bilby activity area, and using those transects to systematically locate scat material.

2.1.1 River colony

Stage one search effort at the River colony comprised ~ 20 km of transects around the known activity areas (Fig. 3). The area of most recent bilby activity measured

approximately ~65 ha in size and was located just north of the Coongan-De Grey station border, similar to where the River colony bilby activity was located in 2021 and 2022 (Fig. 5). Stage two search involved ~6 km of transects located within the identified activity area (Fig. 3).

Scat collection at the River colony occurred on 09/08/23 and was completed by three DBCA personnel (Research Scientist, two Technical Officers).

2.1.2 Coongan colony

Stage one search effort at the Coongan colony comprised ~ 100 km of transects surrounding the 2022 activity area (Fig. 4). The area of most recent bilby activity measured approximately ~45 ha in size, and was located approximately 5 km south of where colony was located in 2022, and 5 km southeast of where the colony was located in 2019 (Fig 5). Stage two search involved ~10 km of transects located within the identified activity area (Fig. 4).

Scat collection at the Coongan colony occurred between 10/08/23 - 12/08/23 and was completed by three DBCA personnel (Research Scientist, two Technical Officers).

2.2 Scat genotyping

Bilby scat samples were initially soaked and gently agitated in ~400 ul of SLP buffer to obtain sloughed cells from the surface of the scat. Supernatant from this mixture was transferred to tubes and genomic DNA extractions were completed using the Omega Bio-tek MagBind® Stool DNA 96 Kit (Omega Bio-tek, Norcross, GA, USA) as per the manufacturer's standard protocol. We eluted DNA in a final volume of 100 ul using a 50% dilution of the final elution buffer to reduce EDTA interference with MassArray typing. Samples were concentrated (60 ul DNA reduced to 30 ul) via vacuum centrifuge prior to analysis to improve genotyping results. DNA samples were genotyped using a custom-designed multiplexed panel of single nucleotide polymorphism (SNP) markers (n = 35 SNP loci; Hogg et al., 2023) on the MassARRAY System (Agena BioScience) at the Australian Genome Research Facility, Brisbane (AGRF). We genotyped 113 samples from 2023 and re-genotyped 97 samples from previous surveys (2021 and 2022) to detect 'recaptures' amongst years.

Molecular sexing of scat samples was carried out using four custom-designed bilby sex-linked primers BRA (Brandies, 2021) included on the MassArray panel. To account for discrepancies in sex identification across scat samples, we followed guidelines established by Sun et al. (2021) for classification. Samples were classified as male if they exhibited successful amplification for at least two Y-linked markers and consistently showed the same sex identification across multiple scats. We defined likely sex as a set of scats with minimal variation between markers and/or scats. Predicted sex referred to a cluster of scats with significant discordance, and the selected sex represented the majority of results. Scats that demonstrated low to no amplification signal from sexing markers or were indistinguishable due to equal probabilities were classified as undetermined.

To improve the stringency of genotype matching, we removed samples and loci with amplification rates below 70% and 70% respectively. MassARRAY SNP results were processed in a custom R package 'ScatMatch' (Huntley, 2021) designed to group scats based on genotype similarity, i.e. by the number of allelic mismatches between samples. A mismatch threshold (h) of h = 4 was used to group scat samples, whereby scats with up to 4 genetic mismatches can be considered the same individual.

2.3 Spatially explicit mark-recapture

To estimate bilby density and each population, we fit spatially explicit mark-recapture (SECR) models using the package 'SECR' in R version 4.2.2 (Efford and Fewster, 2013). SECR models estimate the abundance and density of animal populations by combining capture-recapture data with spatial information using a maximum likelihood approach. SECR models have previously been used to estimate bilby densities in the Pilbara and the Kimberley with high success (Dziminski et al., 2021). All samples at each colony in each year were grouped into a single sampling session and occasion. The models used transect detectors with a hazard exponential (HEX) detection function and Nelder Mead maximisation method following Dziminski et al (2021). The position of each sample was collapsed into the nearest point on the transect line. Activity areas were used as habitat masks in models. Abundance estimates were generated by multiplying density estimates (bilbies per ha) by the size of activity areas.

3 Results

3.1 Scat collection

We collected a total of 55 scats from the River colony, and 109 scats from the Coongan colony. In addition, 3 (two inactive, one active) and 11 (two inactive, nine active) burrows were recorded from the River and Coongan colonies, respectively.

3.2 Scat genotyping

After the data had been filtered, a total of 27 scats from the River colony and 57 scats from the Coongan colony could be identified to the individual level. At the River colony, two males and one female were identified (Fig. 6). At the Coongan colony, nine males and three females were identified (Fig. 7). One male from the Coongan colony was also detected in 2022 at the Coongan colony. The 14 other individuals detected in this survey had not been previously recorded. Comparing scats with data from before 2022 is likely to yield unreliable results given the variation in data quality. There were a number of individuals that were only identified from a single scat (River: 1F, Coongan: 1F, 3M).

3.3 Abundance estimates

The minimum number of bilbies alive (as determined by scat genotyping) was 3 and 12 at the River and Coongan colonies, respectively. This is an increase of 1 individual at the River colony and 7 individuals at the Coongan colony since 2022 (Figure 8).

In the River colony, predicted density was 0.043 (0.014-0.133) bilbies per ha, equating to abundance estimate of 2 (1-6). Predicted bilby density at the Coongan colony was 0.235 (0.133-0.414) bilbies per ha, equating to an abundance estimate of 6 (4-11).

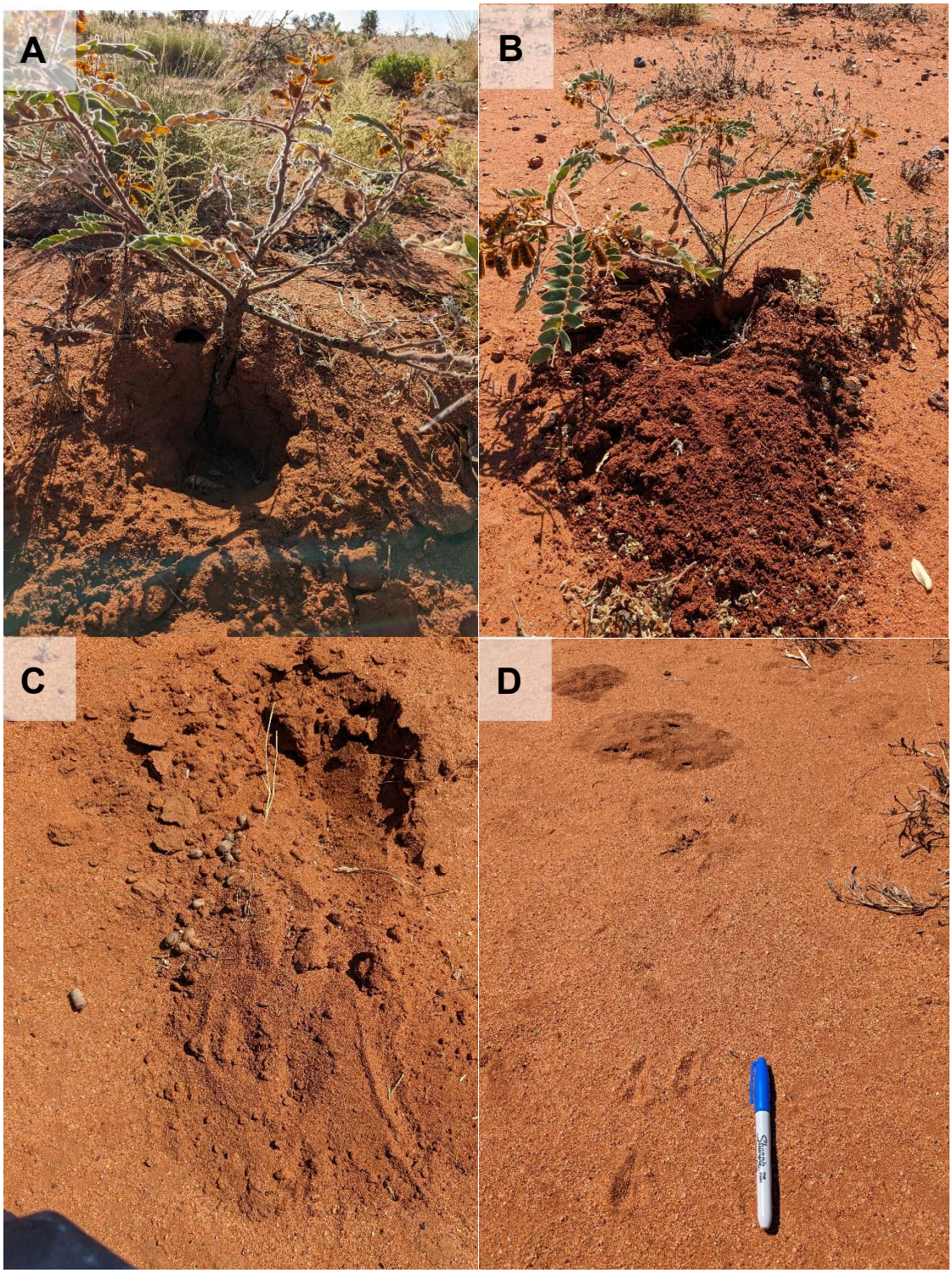

Figure 1. Evidence of bilby activity: (A-B) bilby diggings under *Acacia* trees in the River colony activity area, scats can be seen in the spoil piles; (C) bilby diggings with scats and (D) bilby tracks in the sand made by the Coongan colony.

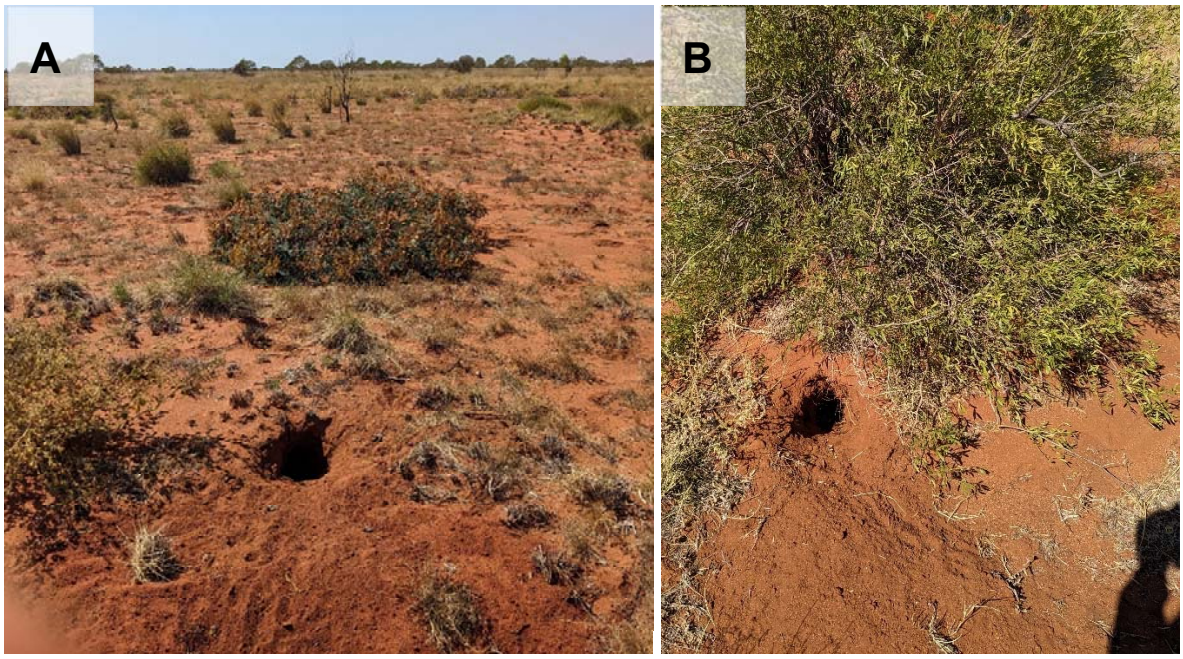

Figure 2. Active bilby burrows in the (A) Coongan and (B) River colonies.

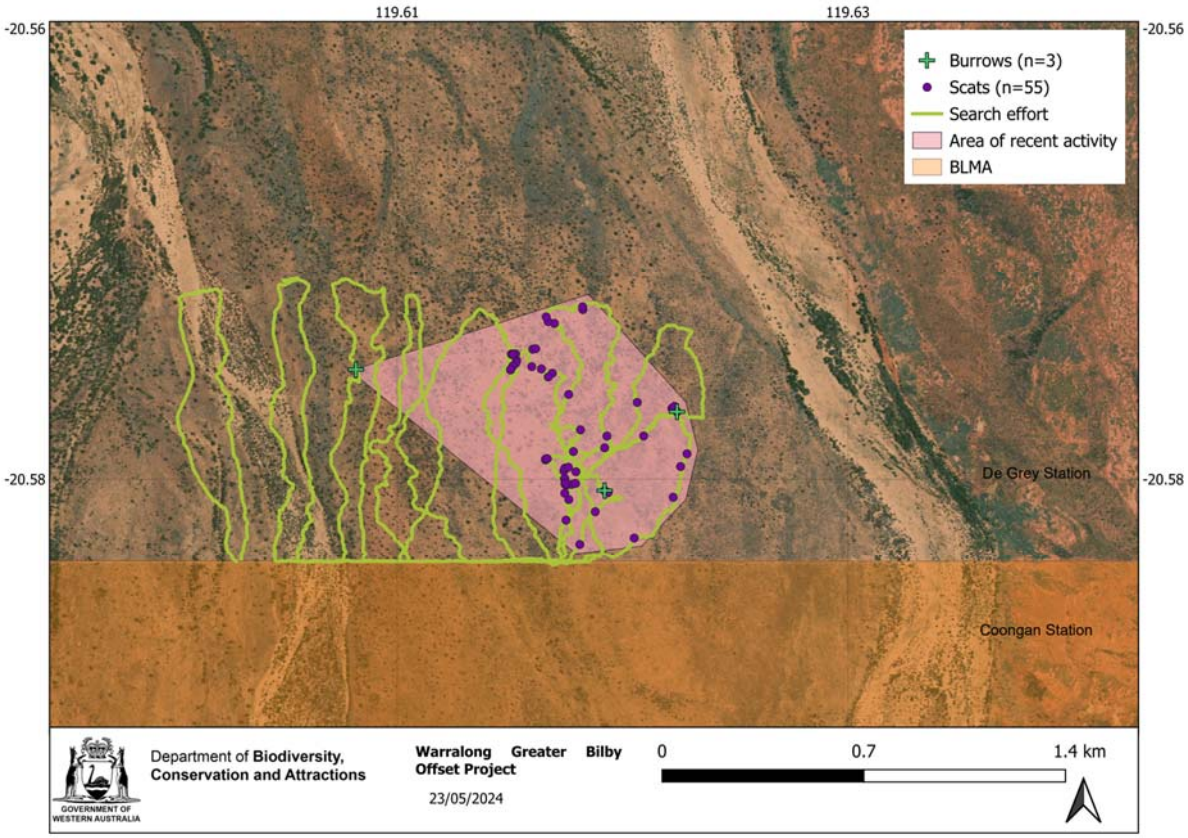

Figure 3. Search effort, activity area, and location of burrows and scats at the River colony on De Grey Station.

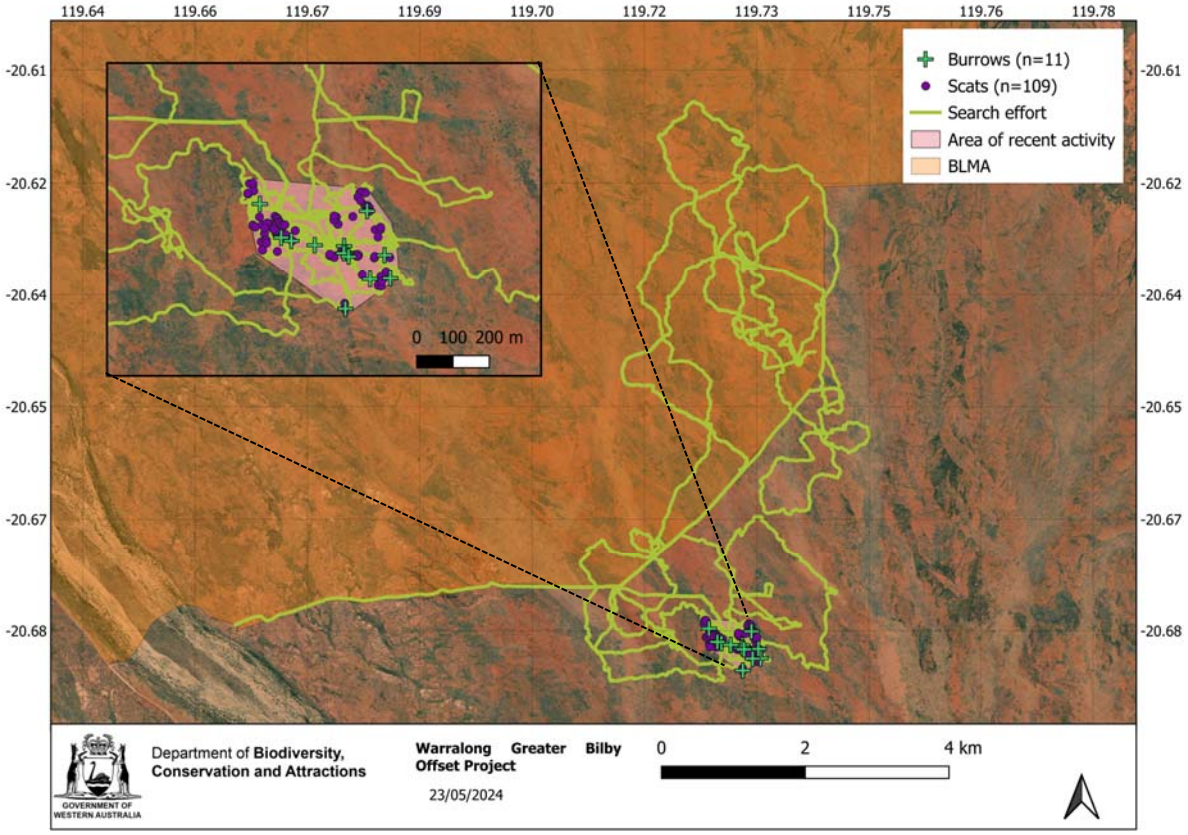

Figure 4. Search effort, activity area, and locations of burrows and scats at the Coongan colony on Coongan Station.

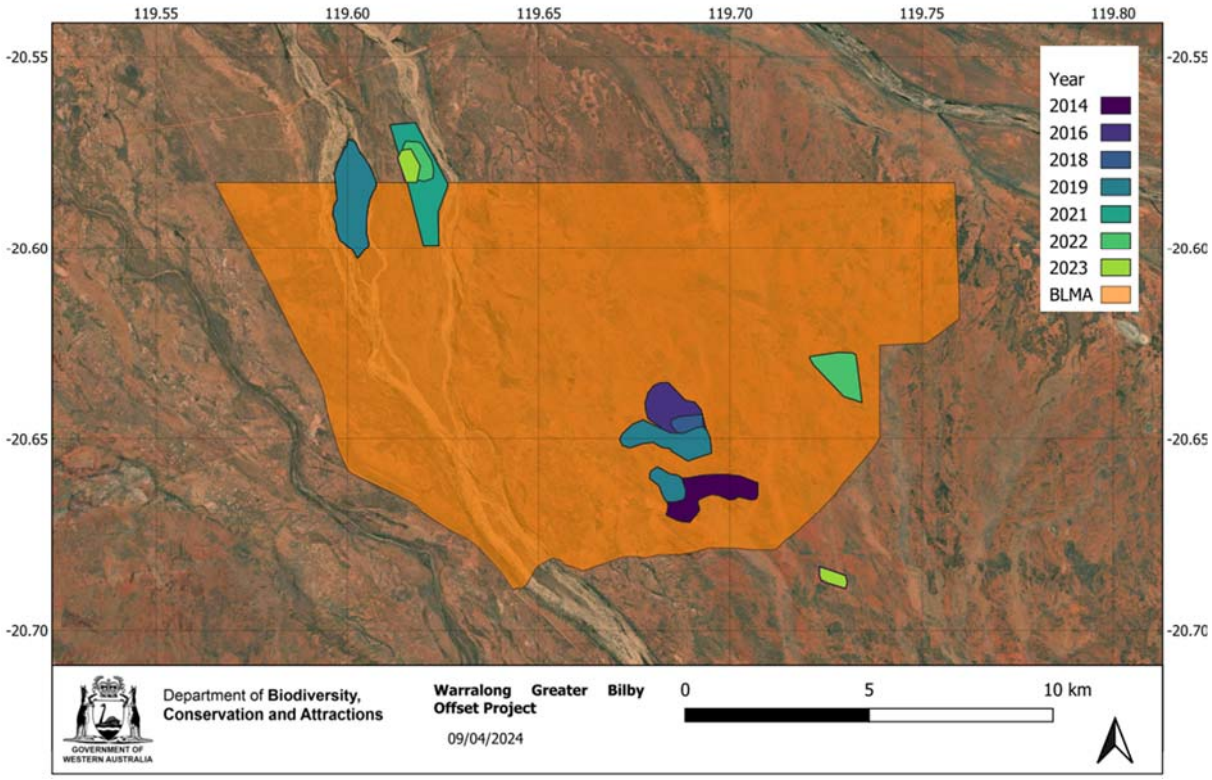

Figure 5. Bilby activity on Coongan Station between 2014 and 2023. In 2023, all activity was found outside of the Bilby Land Management Area (BLMA).

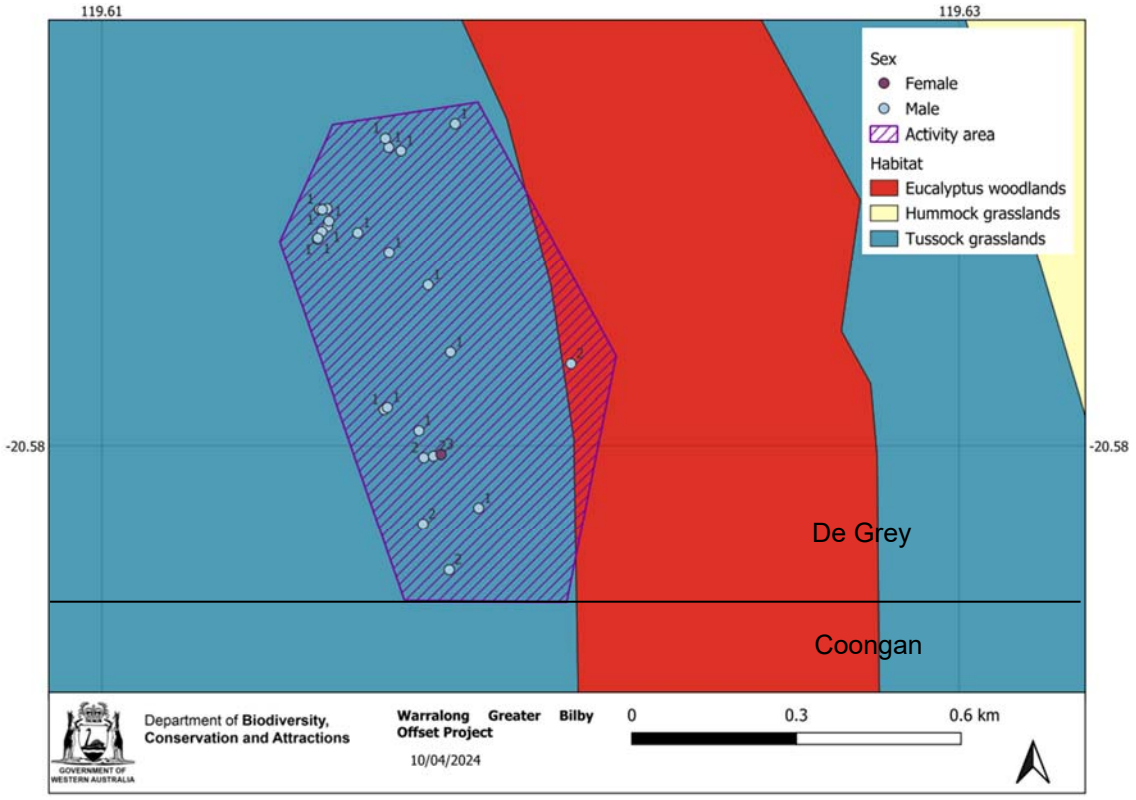

Figure 6. Genotyped scat material from the River bilby colony and habitat type. Numbered labels represent individuals.

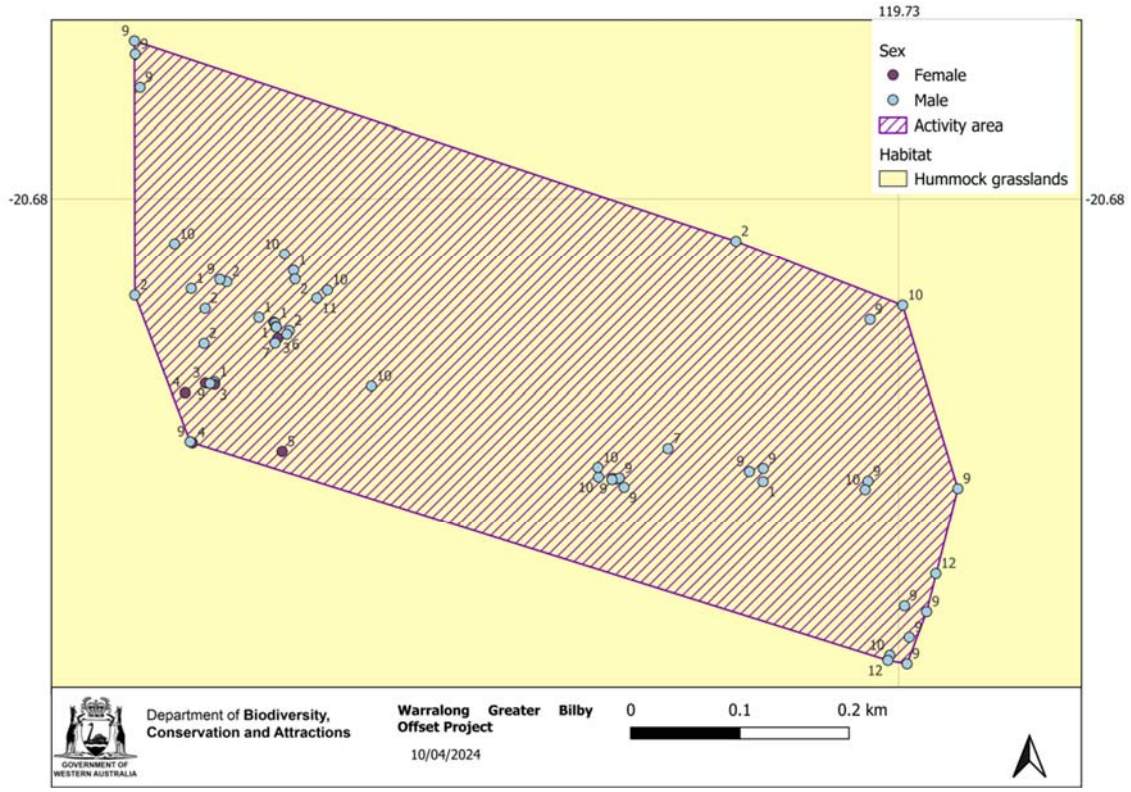

Figure 7. Genotyped scat material from the Coongan bilby colony and habitat type. Numbered labels represent individuals.

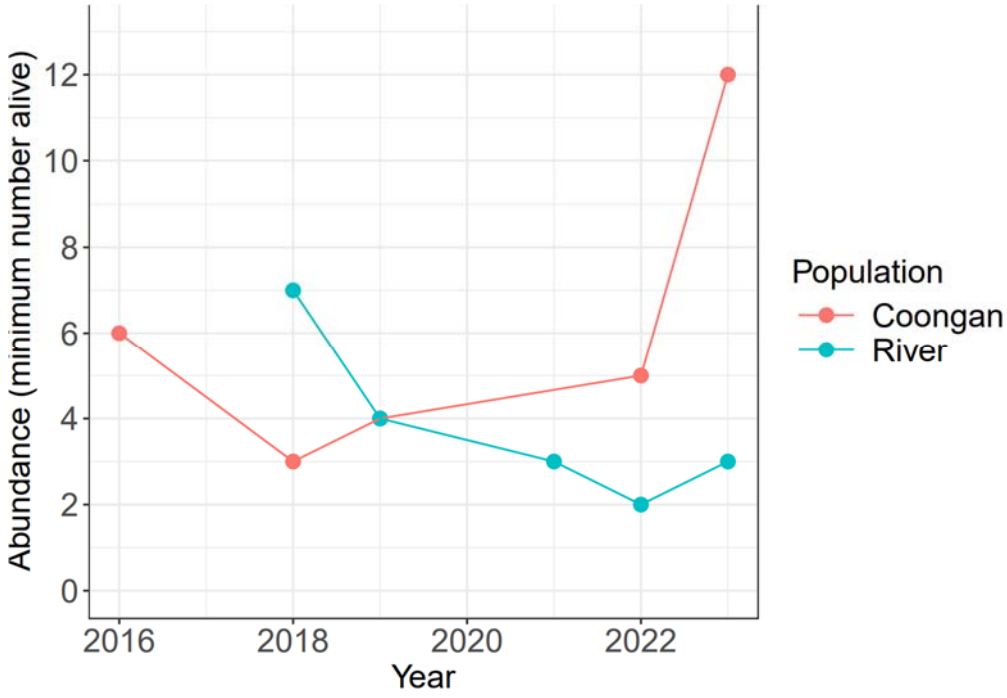

Figure 8. Minimum number of bilbies derived from genotype sequencing of scats from the Warralong Bilby Project site between 2016 and 2023.

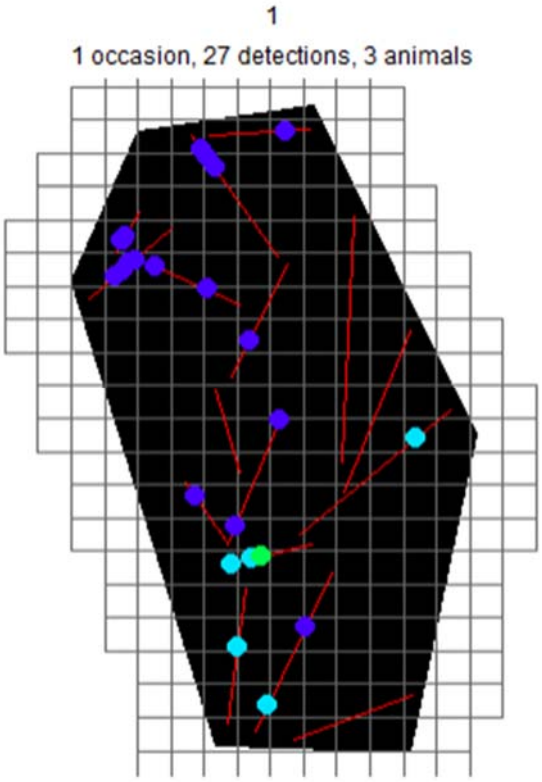

Figure 9. SECR analysis for River bilby colony. Red lines represent transect. Black polygon represents activity area. Grey squares represent the detection mesh, used to define the locations where animals might be present, and the model estimates the density of animals within each grid cell.

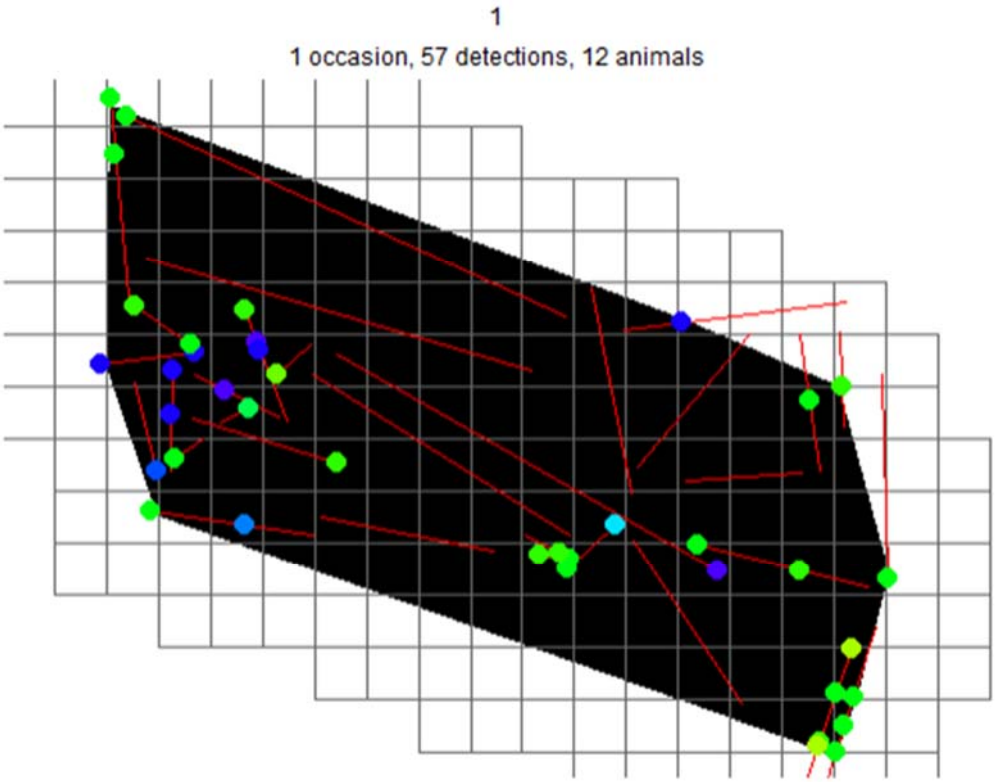

Figure 10. SECR analysis for Coongan bilby colony. Red lines represent transect. Black polygon represents activity area. Grey squares represent the detection mesh, used to define the locations where animals might be present, and the model estimates the density of animals within each grid cell.

4 Future directions

1. There appears to be an increase in population size, particularly at the Coongan colony. Both populations have experienced considerable fluctuations in the past (e.g., Fig. 8). Given the lack of detectable effect of baiting on cat occupancy (Harrison et al., 2024; Moore and Gibson, 2023), this increase in population size is unlikely to be a response to baiting. There were a number of individuals that were only identified from a single scat (River: 1F, Coongan: 1F, 3M), it is possible that these individuals have been misidentified as a result of errors in the amplification of DNA (although quality control is in place to avoid this), or that these individuals are transient, and not permanently part of these colonies.

2. Both the River and Coongan colonies have now relocated outside of the baited BLMA area. Expanding the baited area to include these populations (and a buffer around them to allow for future movement, e.g., 1-3 km) would help to

ensure that the River and Coongan colonies can benefit from any positive effects of baiting.

3. Continued search effort as part of regular 2-ha plot surveys inside and outside the BLMA throughout the monitoring year, will also help to identify changes in bilby occurrence in the area. Maximising the number of colonies or populations monitored will facilitate the detection of changes in abundance in response to management actions implemented as part of this project.

4. To increase the chance of discovering more bilby populations and promote cultural awareness and knowledge sharing, ongoing involvement of local stakeholders continues to be fundamental to the project, particularly the Warralong community and pastoral leaseholders on Coongan and De Grey Stations.

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