Bilby abundance monitoring at Warralong, Western Australia, 2024

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Warralong Greater Bilby Offset

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GOVERNMENT OF WESTERN AUSTRALIA

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Front image: A bilby at Coongan Station. Credit: Roy Hill.

This document is available in alternative formats on request.

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Acknowledgments

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Figure 1. A bilby entering a burrow on Coongan Station, 16th September 2024. Credit: Roy Hill.

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, 2023 and at the River colony in 2018, 2019, 2021, 2022 and 2023 (see Harrison et al., 2024; Moore, 2022; Moore et al., 2023). 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. In 2023, a southern population was again found 5 km from the original location, and 10 km from where the population was found in 2022. Eradicat® feral cat baits have been deployed annually from 2022-2024 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 estimates recorded at two bilby colonies located on the Coongan Pastoral Lease in 2024.

2 Methods

2.1 Scat collection

Searching initially focused on recent activity points provided by A. Coppin (Outback Beef) observed from a helicopter survey undertaken in August 2024, as well as activity documented on cameras or at sign plots (by Biologic) and areas of bilby activity from previous years. When these were exhausted, we focused our search on areas of suitable habitat (groves of *Acacia sp.* trees). Once activity was located, scat collection was structured into two stages. Stage one involved delineating the boundary of bilby activity at a site (incorporating fresh scats and active burrows), so as to determine the size of the area bilbies were using (activity area). The second stage involved establishing transects within the bilby activity area, and using those transects to systematically locate scat material. Scat collection was conducted by two DBCA personnel (Research Scientist, Technical Officer) and a volunteer between 11/09/24 – 19/09/24. The search effort comprised 303 km of transects (Figure 2).

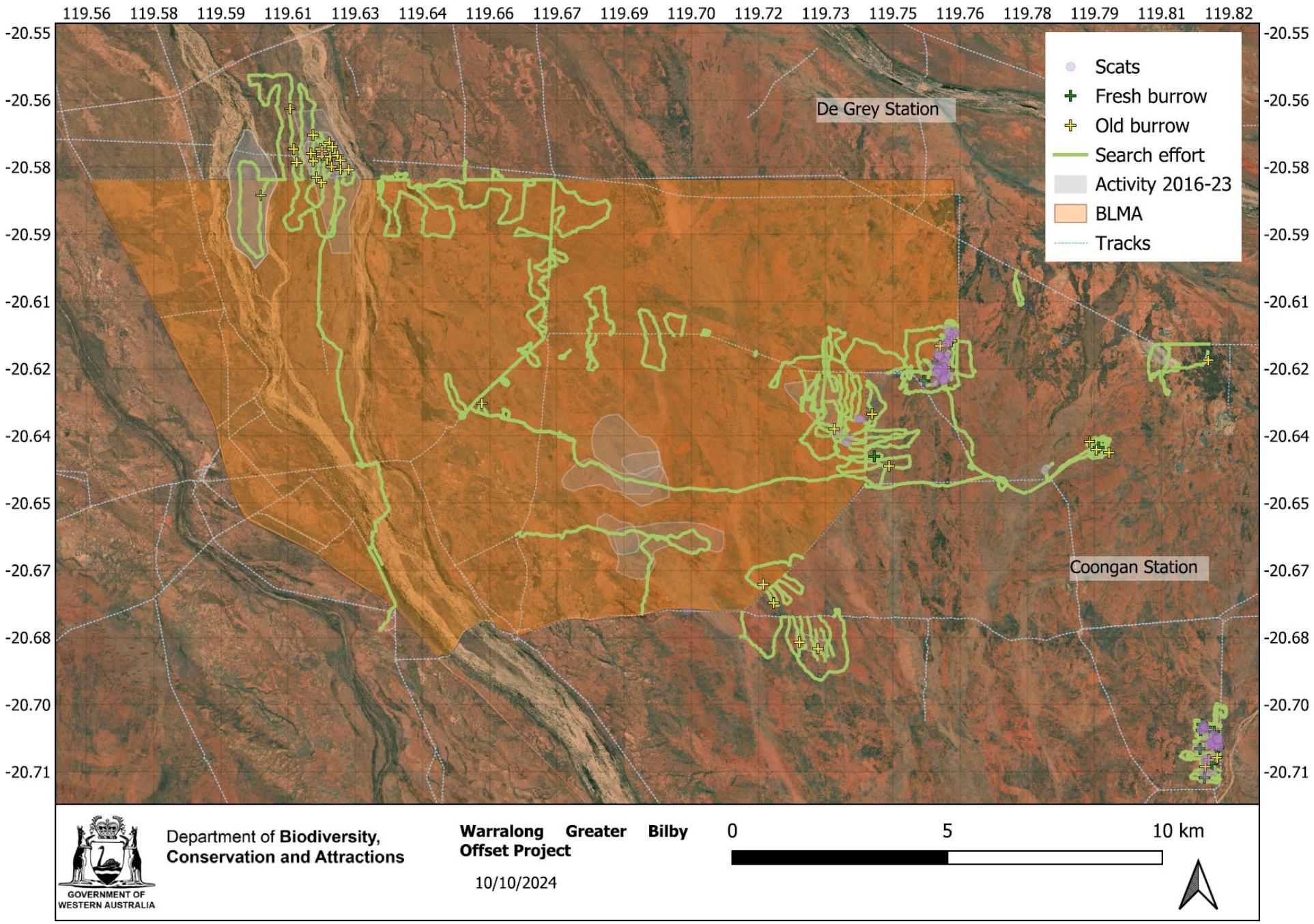

Figure 2. Map of search effort conducted within and around the BLMA in 2024. Two colonies were detected – one on the eastern edge of the BLMA (~2km from the Southern colony's 2022 location) and one located further to the SE, near O'Grady Well. Little recent activity was found within previous years activity areas.

2.1.1 River colony

No bilby activity was located in the area where the northern River colony is usually found (based on activity areas from the past four years). There were a substantial number of disused burrows that appeared to be >6 months old, but no active burrows, diggings, or scats were found. The habitat around this colony's previous location is degraded, with soil compaction and grazing by cattle (Figure 3), as well as invasive flora (*Calotropis procera*). We suggest that this colony may have dispersed or become extirpated.

Figure 3. Spinifex grasslands subject to different stocking rates. The right side of the fence (where the River colony was previously located) shows evidence of overgrazing and soil compaction.

2.1.2 Coongan colony

The area of activity at the Coongan colony was approximately 80 ha. This colony has moved approximately 8 km to the north-east of the 2023 location. We detected 2 active burrows, 5 inactive burrows, and collected 69 scat samples (Figure 4).

2.1.3 O'Grady Well colony

The area of activity at the O'Grady Well colony was approximately 100 ha. This colony was identified by A. Coppin from a helicopter survey and confirmed through the on-ground survey. We detected 6 active burrows, 3 inactive burrows, and collected 50 scat samples (Figure 5).

2.1.4 Dispersing individuals

Ten additional scats were also collected from between areas of activity that may have been deposited by dispersing individuals (Figure 2).

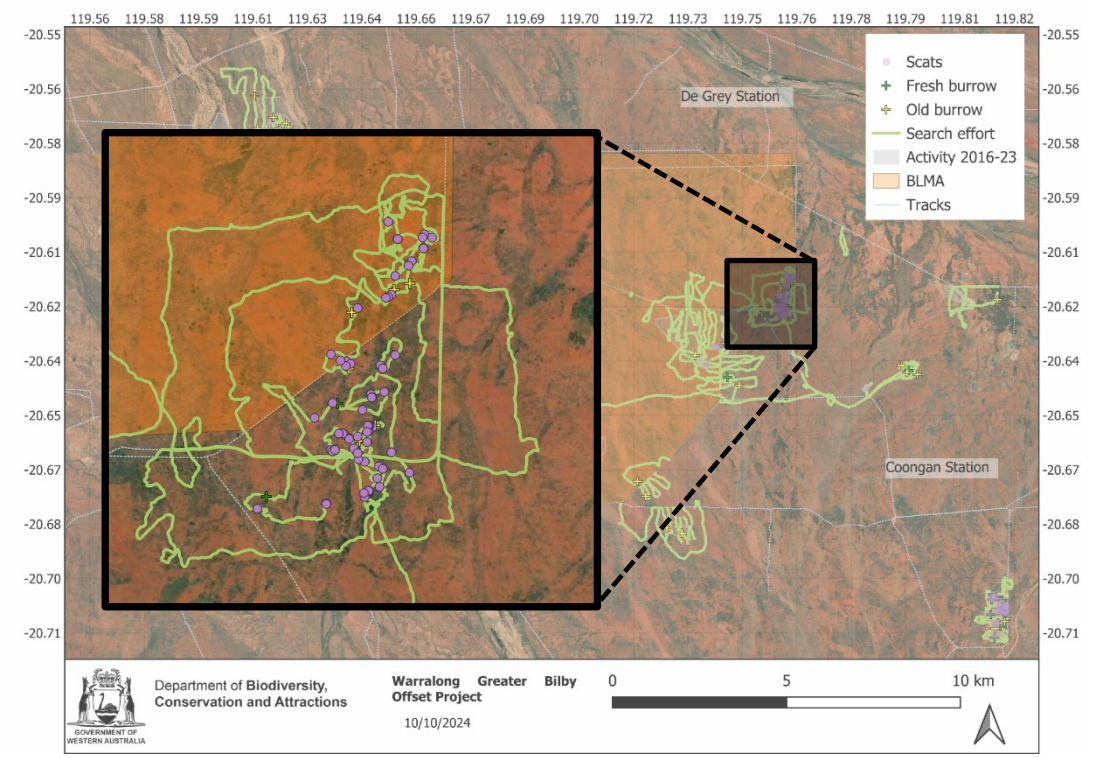

Figure 4. Search effort at the Coongan colony, where scats (purple circles), disused burrows (yellow cross) and active burrows (green cross) are depicted.

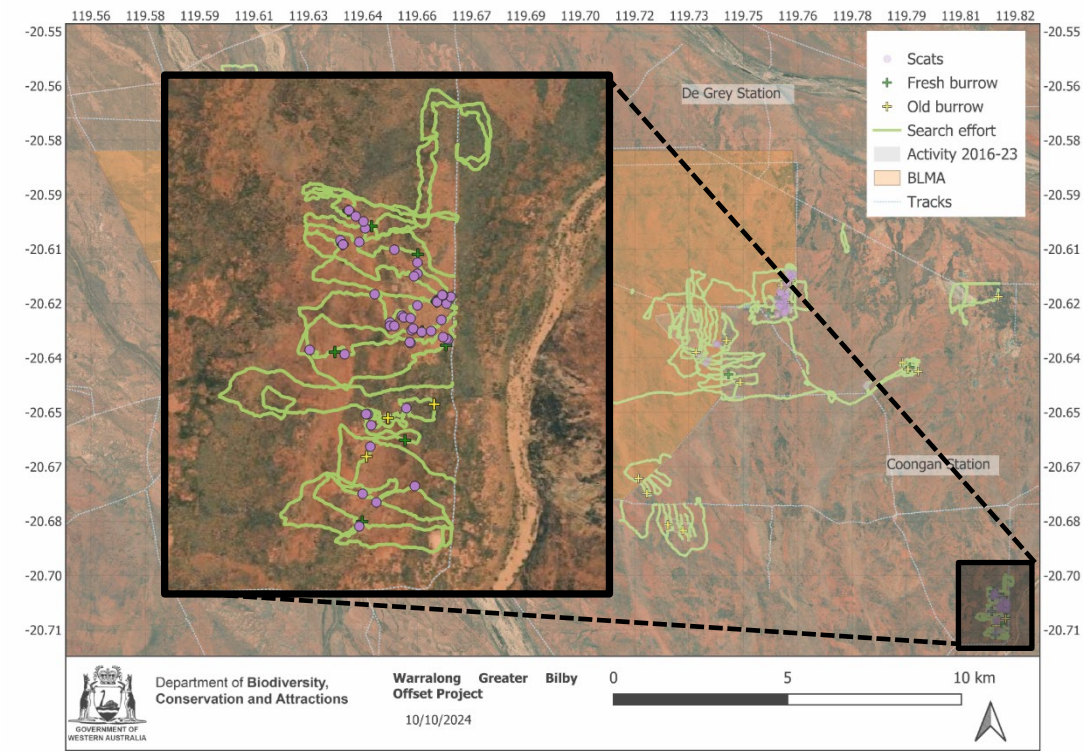

Figure 5. Search effort at the O'Grady Well colony, where scats (purple circles), disused burrows (yellow cross) and active burrows (green cross) are depicted.

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.

Prior to sending samples for SNP genotyping, we screened DNA extracts for the presence of bilby DNA using a quantitative PCR assay. We designed a g-Blocks™ synthetic DNA fragment 125bp in length, based on the primer sequences used to amplify SNP Bilby_404595_38 from the MassArray SNP panel (details in Hogg et al., 2024). Scat DNA was amplified, as were serial dilutions of g-Block DNA to provide bilby target DNA standards ranging in concentration from 1 to 0.00001 ng μl^{-1}, DNA extracted from bilby tissue (5 ng μl^{-1}) as a positive control, and water as a no template or negative control. Amplification reactions contained 5µl of SsoAdvanced™ Universal SYBR® Green Supermix, 1 µl of 10 µM Forward Bilby_404595_38 primer and Reverse Bilby_404595_38 primer, 3 µl of dH2O, and 1 µl of either scat, standard, or control DNA. Amplification was performed on a BioRad, CFX96 Real Time System, C1000 Touch Thermal Cycler with a hotstart of 95°C for 3 min, and 40 cycles of 95°C for 15s, 60°C for 60s and 65°C for 5s, with a final melt curve from 65°C to 95°C increasing 0.5°C every 2s. All standard and control amplifications were performed in triplicate. Averaged standard curves were checked for linearity. The average Relative Fluorescence Units (RFU) of the last 5 cycles was used to determine if bilby DNA is present in scat samples. A tolerance of 10% of the total RFU range and the average of the negative controls was used as a cut off value. Samples with an RFU greater than the cutoff were considered positive for the presence of bilby DNA. Samples that failed to amplify target DNA were omitted from further analysis and passing samples were sent for SNP genotyping analysis (n = 98).

DNA samples were genotyped using a custom-designed multiplexed panel of single nucleotide polymorphism (SNP) markers (n = 35 SNP loci; Hogg et al., 2024) on the MassARRAY System (Agena BioScience) at the Australian Genome Research Facility, Brisbane (AGRF). We genotyped 98 samples from 2024 and compared these to 30 and 149 samples from 2022 and 2023 respectively to detect 'recaptures' amongst years. Data from before 2022 was not of a high enough quality to facilitate robust comparisons.

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 80%. 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 & 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

Of the total 129 scats collected from both colonies and the opportunistic collections, 98 were suitable for sequencing.

3.2 Scat genotyping

After the data had been filtered, a total of 57 scats could be identified to the individual level, yielding 10 unique individuals (7M, 3F; Table 1). At the Coongan colony, three males and one female were identified (Figure 6). At the O'Grady Well colony, three males and two females were identified (Figure 6). In addition, two transient males were identified (males identified from scats collected opportunistically who were not

detected in either colony). Male 1 was identified at both colonies which are approximately 10 km apart from each other. Males 4 and 8 (from the Coongan colony), as well as male 1 (located in both Coongan and O'Grady Well colonies), were all identified in the Coongan colony in 2023. There were a number of individuals that were only identified from a single scat (Coongan: 1M, O'Grady Well: 1F). No individuals from the previous River colony were detected. Both colonies were located outside of the existing BLMA (Figure 7).

Table 1. Identity and sex of bilbies detected between 2022 and 2024 on Coongan Station. Map ID refers to Figure 6 which depicts the location of scats from each individual in 2024. Individuals detected in 2024 are highlighted in grey.

Database ID	Map ID	Sex	Detected
WN1	-	M	2022, 2023 (River)
WN2	-	Unknown	2022 (River)
WN3	-	F	2022 (River)
WN4	-	M	2023 (River)
WN5	-	M	2023 (River)
WN6	-	F	2023 (River)
WS1	-	F	2022 (Coongan)
WS2	-	F	2022 (Coongan)
WS3	-	M	2022 (Coongan)
WS4	8	M	2022, 2023, 2024 (Coongan)
WS5	-	F	2022 (Coongan)
WS6	1	M	2023 (Coongan), 2024 (O'Grady, Coongan)
WS7	4	M	2023 (Coongan), 2024 (Transient)
WS8	-	F	2023 (Coongan)
WS9	-	F	2023 (Coongan)
WS10	-	F	2023 (Coongan)
WS11	-	M	2023 (Coongan)
WS12	-	M	2023 (Coongan)

WS13	-	M	2023 (Coongan)
WS14	-	M	2023 (Coongan)
WS15	-	M	2023 (Coongan)
WS16	-	M	2023 (Coongan)
WS17	2	M	2024 (Coongan)
WS18	5	M	2024 (Coongan)
WS19	6	M	2024 (Transient)
WS20	7	F	2024 (Coongan)
WOG1	3	F	2024 (O'Grady)
WOG2	9	M	2024 (O'Grady)
WOG3	10	F	2024 (O'Grady)

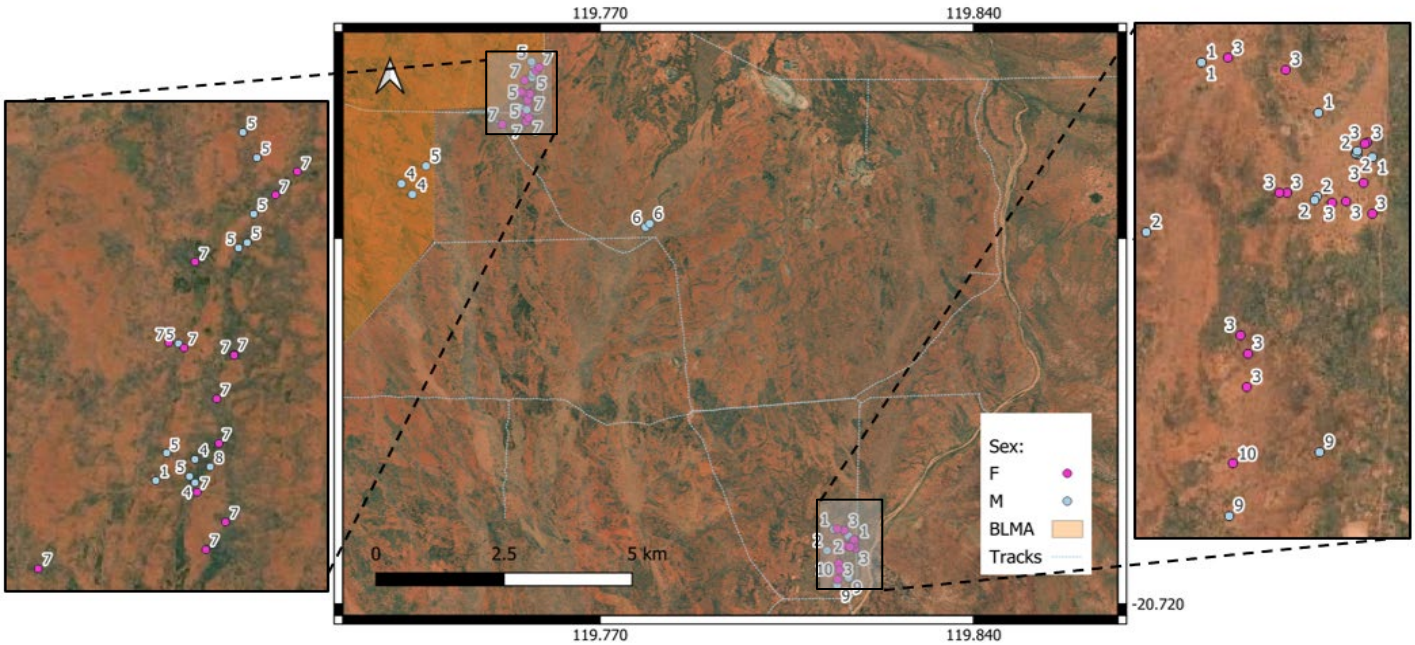

Figure 6. Location of genotyped scats relative to the BLMA. Scats identified as belonging to females are displayed in pink, and males are in blue. Numbers represent unique individuals. Close up views of the Coongan and O'Grady Well colonies can be found to the left and right of the main map respectively.

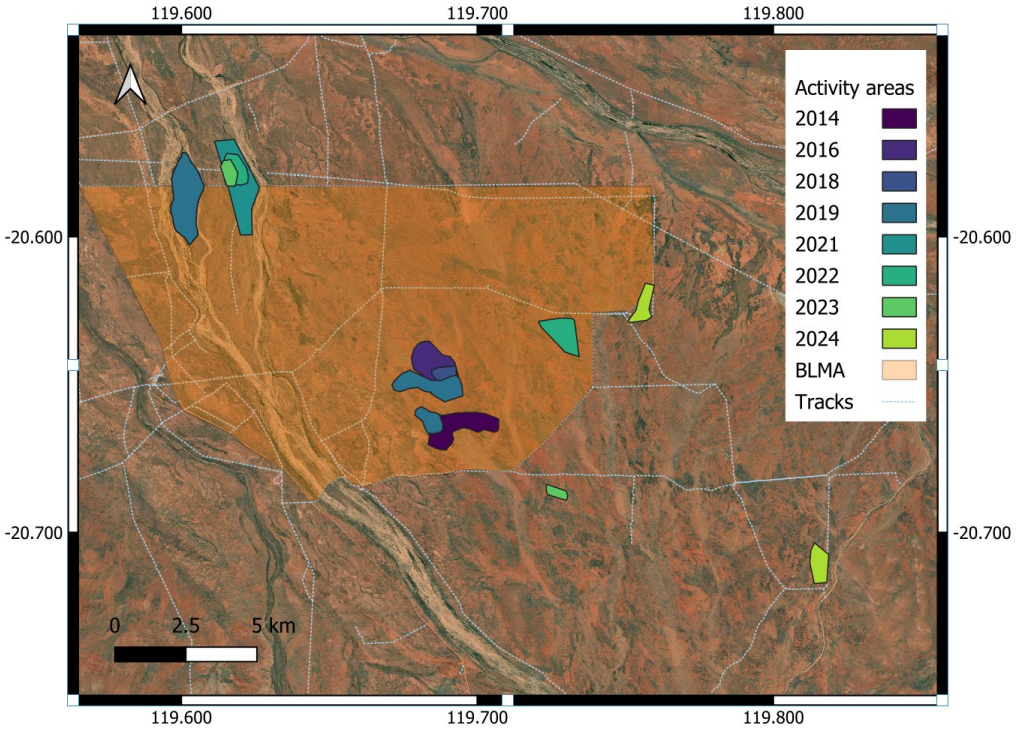

Figure 7. Bilby activity on Coongan Station between 2014 and 2024. In 2024, bilby activity was found outside of the existing Bilby Land Management Area (BLMA).

3.3 Abundance estimates

The minimum number of bilbies alive (as determined by scat genotyping across both colonies and transient individuals) was 10. This is a slight decrease from 2023 (Figure 8).

In the O'Grady Well colony, predicted density was 0.044 (0.018-0.105) bilbies per ha, equating to abundance estimate of 3 (1-8). Predicted bilby density at the Coongan colony was 0.046 (0.019-0.110) bilbies per ha, equating to an abundance estimate of 3 (1-7). These estimates are comparable to estimates from the River colony and smaller than estimates from the Coongan colony in 2023.

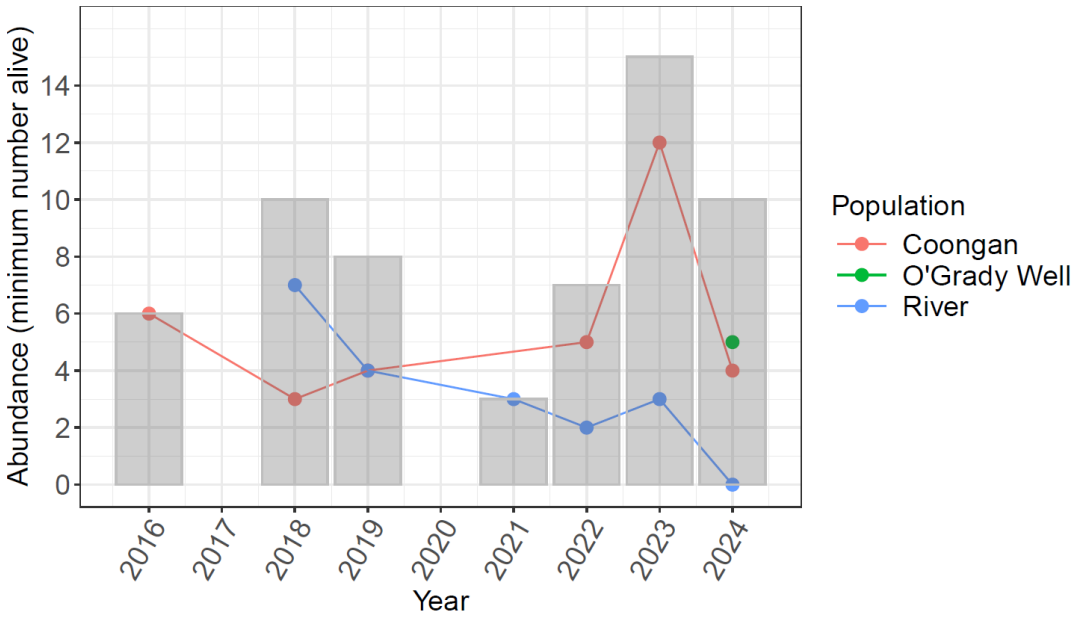

Figure 8. Minimum number of bilbies derived from genotype sequencing of scats from the Warralong Bilby Project site between 2016 and 2024. Grey bars represent the total number of unique individuals identified each year.

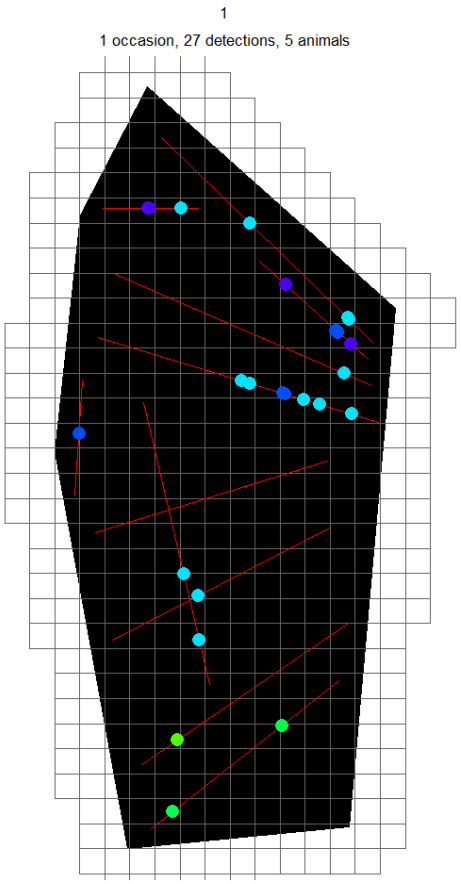

Figure 9. SECR analysis for O'Grady Well bilby colony. Red lines represent transects. Black polygon depicts activity area. Grey squares represent the detection mesh, used to define the locations where animals might be present, and the model estimates the density of animals within each grid cell. Each dot represents a genotyped scat with unique individuals depicted by different colours.

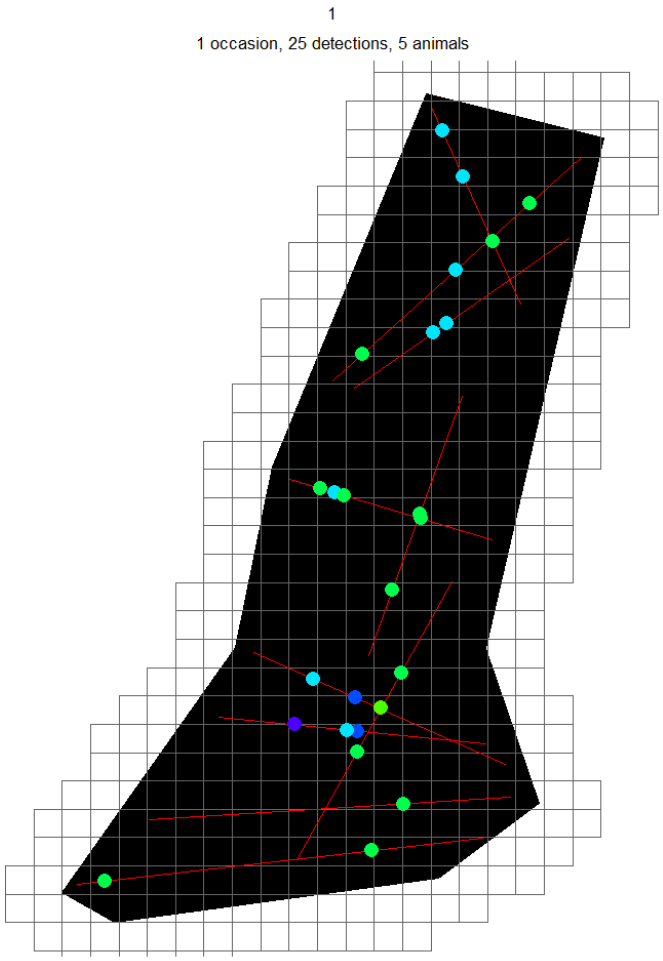

Figure 10. SECR analysis for Coongan bilby colony. Red lines represent transects. Black polygon depict activity area. Grey squares represent the detection mesh, used to define the locations where animals might be present, and the model estimates the density of animals within each grid cell. Each dot represents a genotyped scat with unique individuals depicted by different colours.

4 Future directions

1. The River colony appears to have been extirpated or dispersed, highlighting the vulnerability of small, isolated populations that characterise bilby distribution in the Pilbara (Dziminski et al., 2021). Such populations are more sensitive to interacting threats and stochastic events. Investigation into the factors that are driving bilby movement between years and population dynamics (e.g., resource availability, fire) can improve our ability to design targeted conservation management actions. Continued monitoring of the Coongan and O'Grady colonies is recommended.

2. Both colonies found in 2024 were located outside of the baited BLMA area, with the River colony's 2023 location also outside the BLMA. 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 they 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 the neighbouring stations of De Grey, Eginbah and Muccan Stations.

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