



**Biodiversity and
Conservation Science**

Bilby abundance monitoring at Warralong, Western Australia, 2022



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Warralong Greater Bilby Offset
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Cover image: Bilby on Coongan Station (credit Roy Hill)

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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 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, and at the River colony in 2018, 2019 and 2021. The Coongan colony could not be found in 2021. This report documents the most recent abundance measurements recorded at two bilby colonies located on the Coongan Pastoral Lease in 2022, shortly after deployment of Eradicat® feral cat baits.

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. The second stage involved establishing transects within the bilby activity area, and using those transects to locate scat material.

2.1.1 River colony

Stage one search effort at the River colony population comprised ~112 km of transects around previously known activity areas (Figure 1). The area of most recent bilby activity measured approximately ~210 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 (Figure 3). Stage two search involved ~35 km of transects located within the identified activity area (Figure 2).

Scat collection at the River colony occurred between Tuesday the 12th and Tuesday the 27th of September 2022, and was completed by two DBCA personnel (Research Scientist, Technical Officer).

2.1.2 Coongan colony

Initial search effort for stage one targeted previous known locations of the Coongan colony based on 2019 information (as this colony as not found in 2021). A further search effort was conducted over an area where coordinates of active bilby burrows were provided by the lease holder of Coongan Station, Annabelle Coppin (burrows detected using helicopter) (Figure 1). The search effort revealed an activity area, approximately 5km from the previous 2019 location of the Coongan colony, but it is unknown if this new activity area is the Coongan colony relocated or a new bilby colony. The area of most recent bilby activity measured approximately ~230 ha in size (Figure 3).

Scat collection at the new Coongan colony occurred between Sunday the 30th of October and Tuesday the 1st of November 2022, and was completed by two DBCA personnel (Research Scientist, Technical Officer). Stage two search involved ~29 km of transects located within the identified activity area (Figure 2).

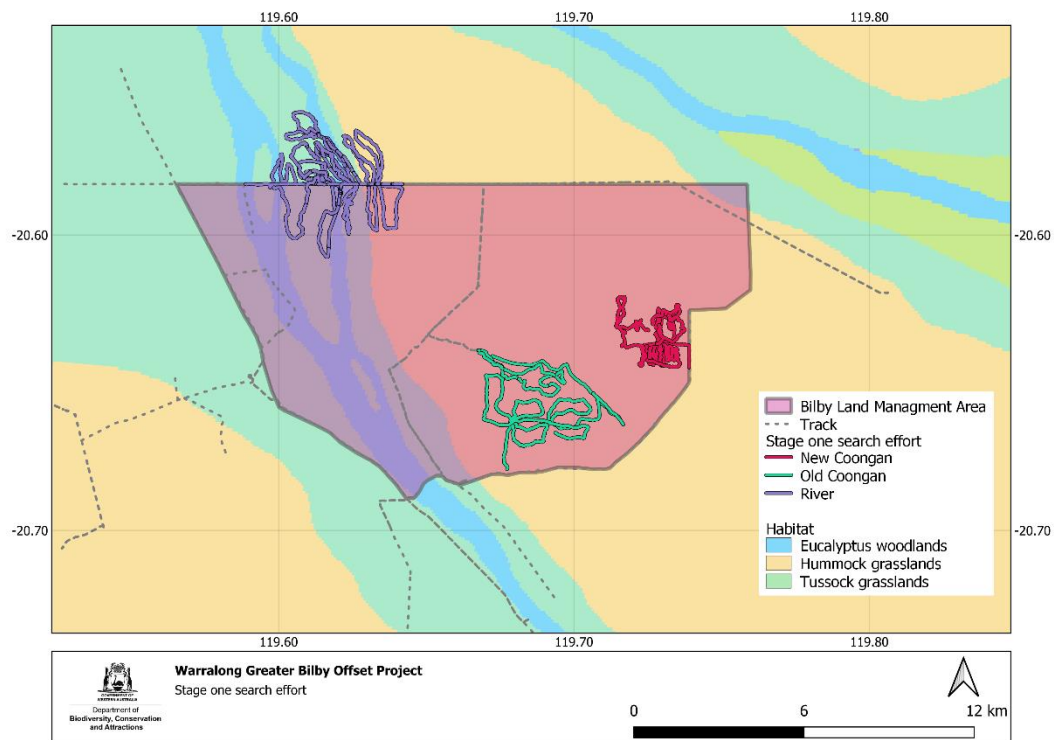


Figure 1 – Stage one search effort at the River and previous and new Coongan bilby colonies, near Warralong community.

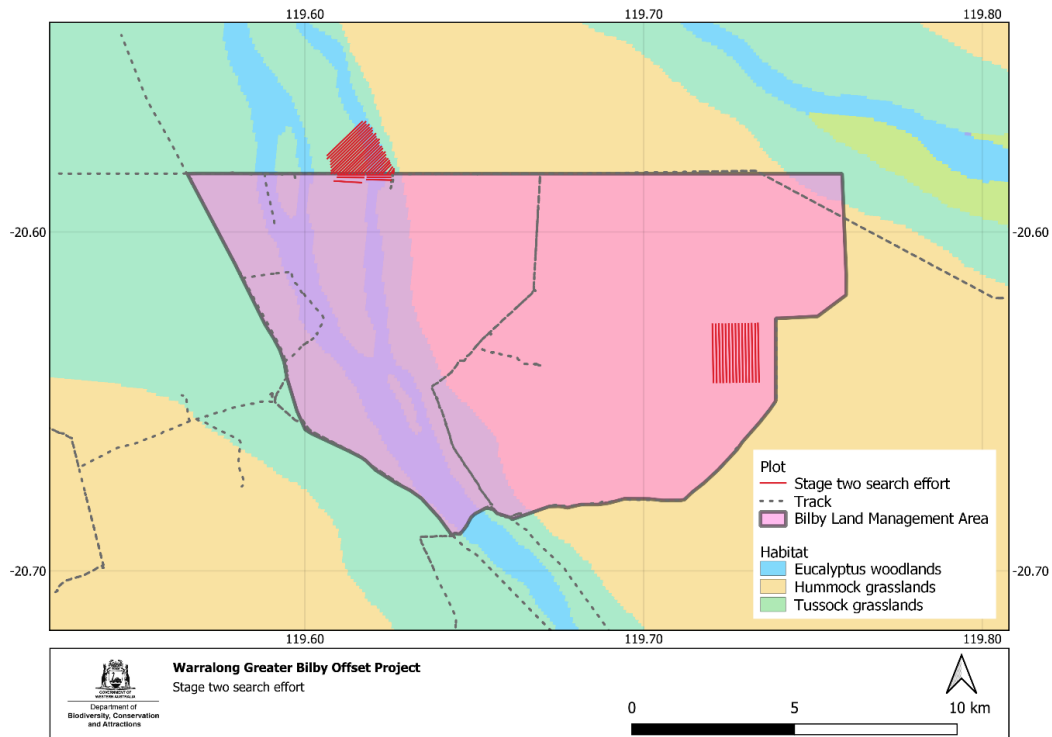


Figure 2 – Stage two search effort at the River and new Coongan bilby colonies, near Warralong community.

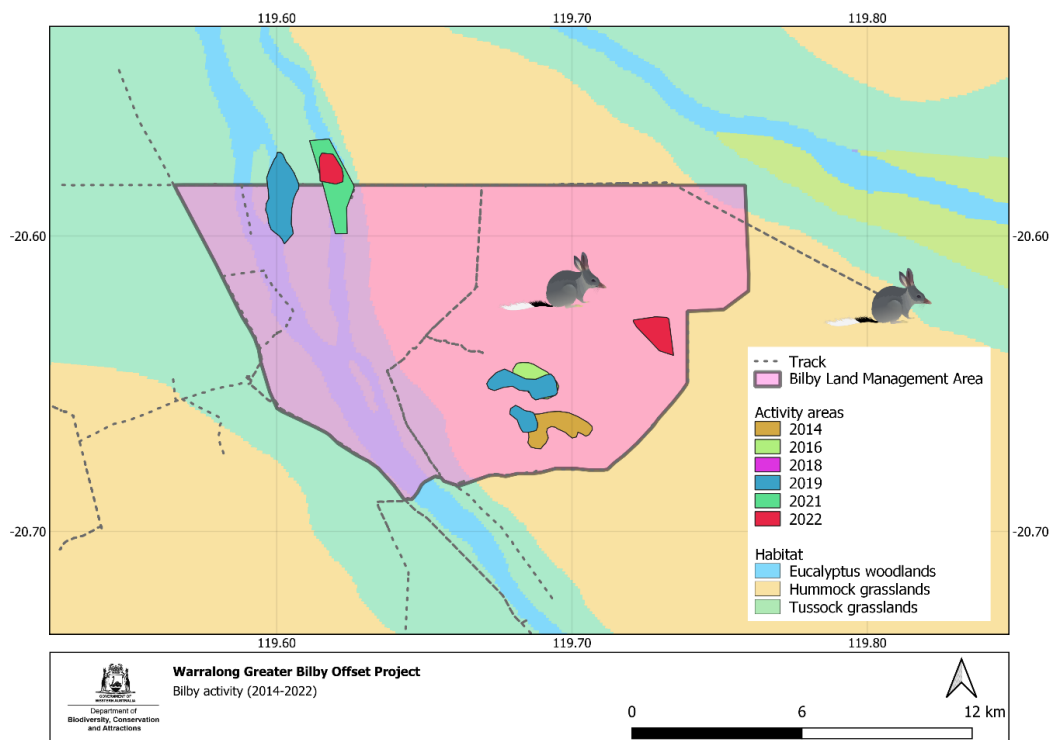


Figure 3 – Bilby activity areas located between 2014-2022 near Warralong community. Bilby symbols represent additional incidental records collected during this time.

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) on the MassARRAY System (Agena BioScience) at the Australian Genome Research Facility, Brisbane (AGRF). We genotyped 84 samples from 2022 and re-genotyped 13 samples from the previous survey (2021) 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 50% and 60% 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.

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 (Dzimirski *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 onto 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 33 scats from River colony, and 84 scats from the new Coongan colony (Figures 4 to 9). In addition, a total of 16 active/semi-active burrows were recorded at the River colony, and 12 at the new Coongan colony (Figure 6).

3.2 Scat genotyping

After data had been filtered, a total of six scats from the River colony, and 24 scats from the new Coongan colony could be identified to the individual level (Figure 9). Two individuals were identified from the River colony (1*M, 1*F), one of which was also identified in the 2021 survey (1*M). Five individuals were identified at the new Coongan colony (2*M, 2*F, 1*Unknown), none of which have previously been recorded (Figure 10). It remains unclear if the new Coongan population identified in 2022 is comprised of the same or related individual to the previous Coongan populations identified in the 2019 abundance surveys.

3.3 Abundance estimate

Due to the low number of successfully genotyped scat samples, reliably estimating bilby density using SECR models was not possible for the River colony. However, the minimum number of animals alive has declined slightly (by 1 individual) since 2021 (Figure 11).

Predicted bilby density at the new Coongan colony was equal to 0.02 (0.001-0.05) bilbies per ha, equating to an abundance estimate of 5 (2-12; Figure 12). Abundance estimates based on minimum number alive indicate the population here (and if presumed to be the previous Coongan colony but has moved 5km since 2019 to the new location) has increased slightly (by 1 individual) since 2019 (Figure 11).

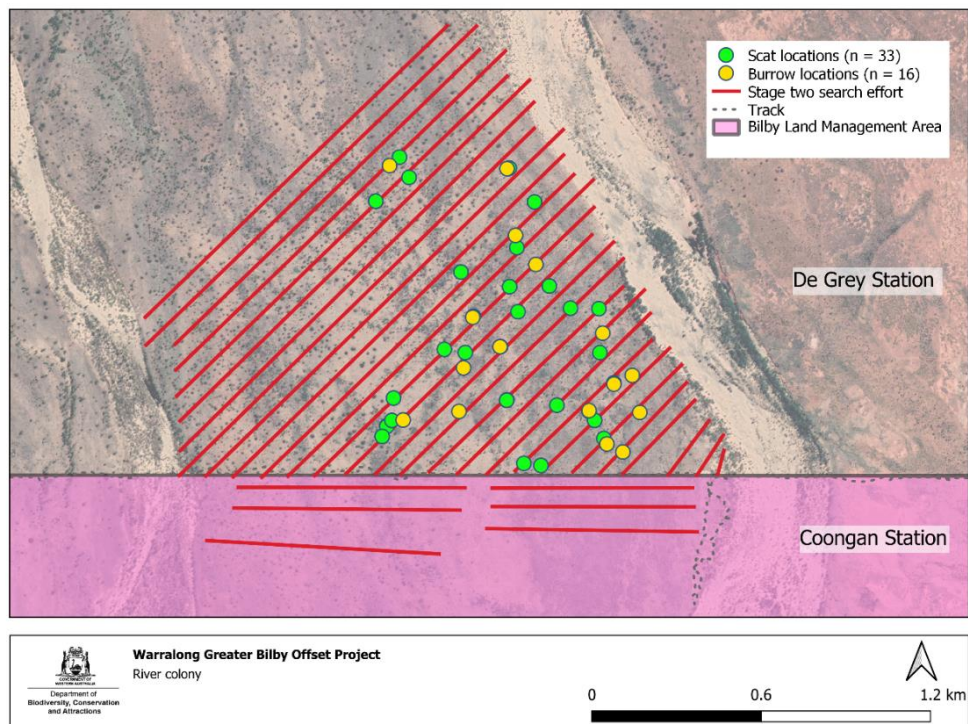


Figure 4 – Stage two search effort at the River bilby colony, near Warralong community.

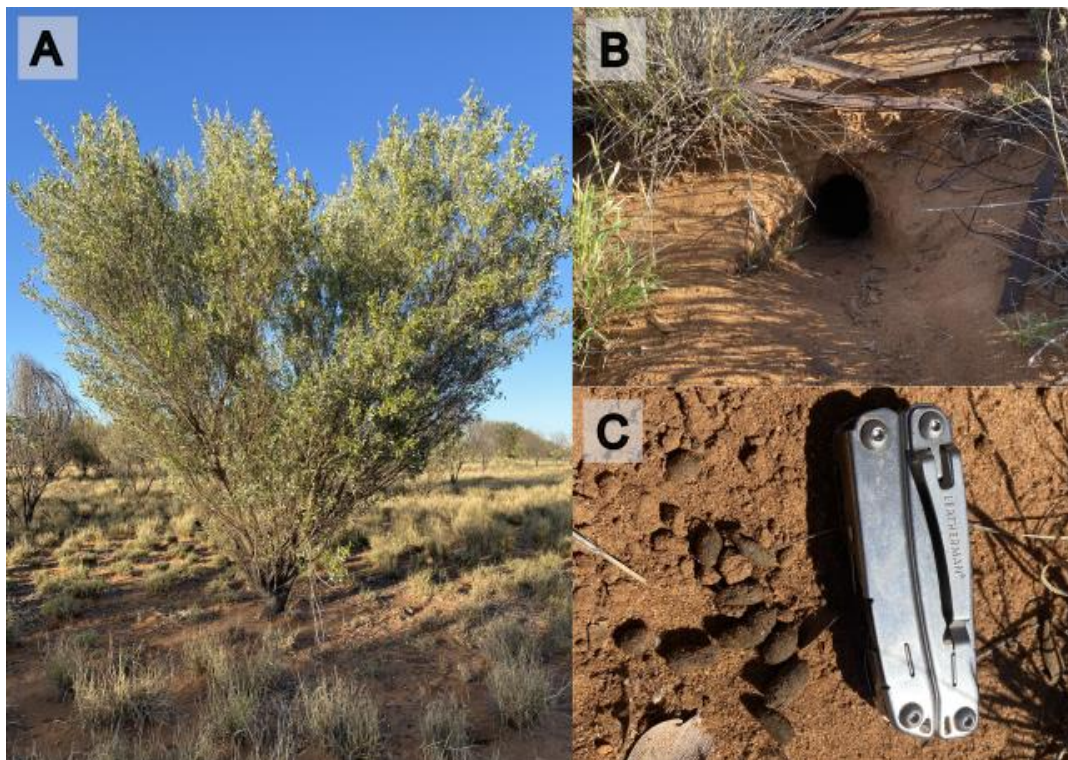


Figure 5 – A) *Acacia spp* under which bilby diggings and scat were most often located at the River colony. B) Bilby burrow with recent evidence of use located at the River colony. C) Fresh bilby scats located at the River colony.



Figure 6 – Students from Warralong Community school investigating an active bilby burrow at the River colony.

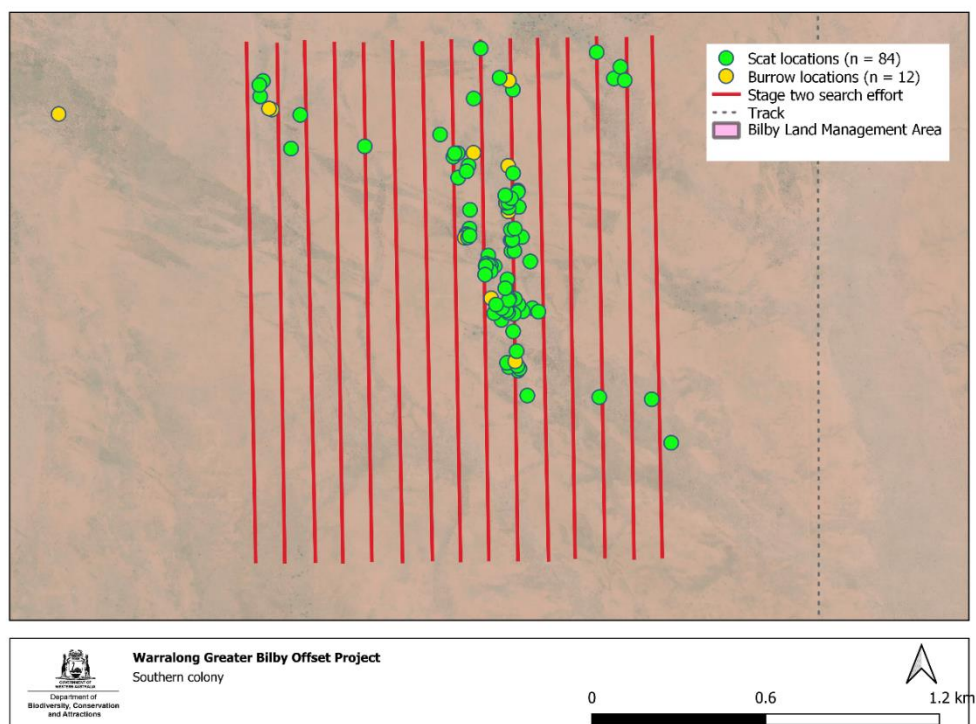


Figure 7 – Stage two search effort at the new Coongan bilby colony, near Warralong community.

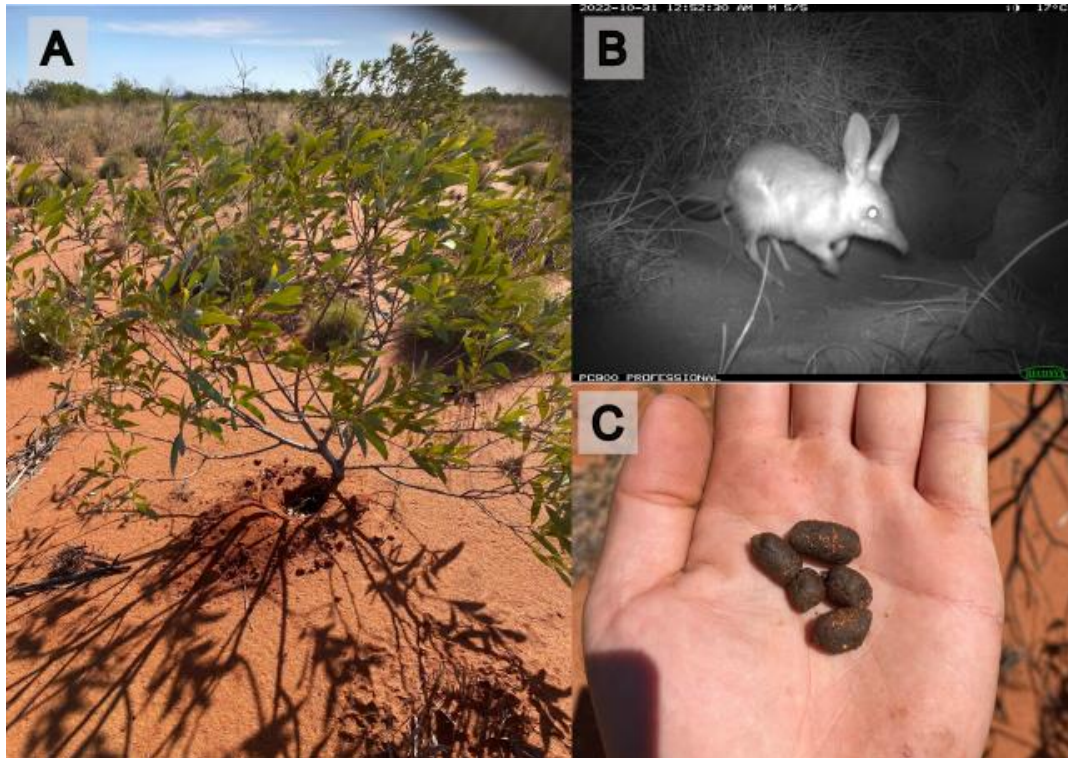


Figure 8 – A) *Acacia colei*, under which bilby diggings and scat were most often located at the new Coongan colony. B) Bilby detected at fresh burrow within the new Coongan colony activity area C) Fresh bilby scat located at the new Coongan colony (Scat material not used in genotyping).

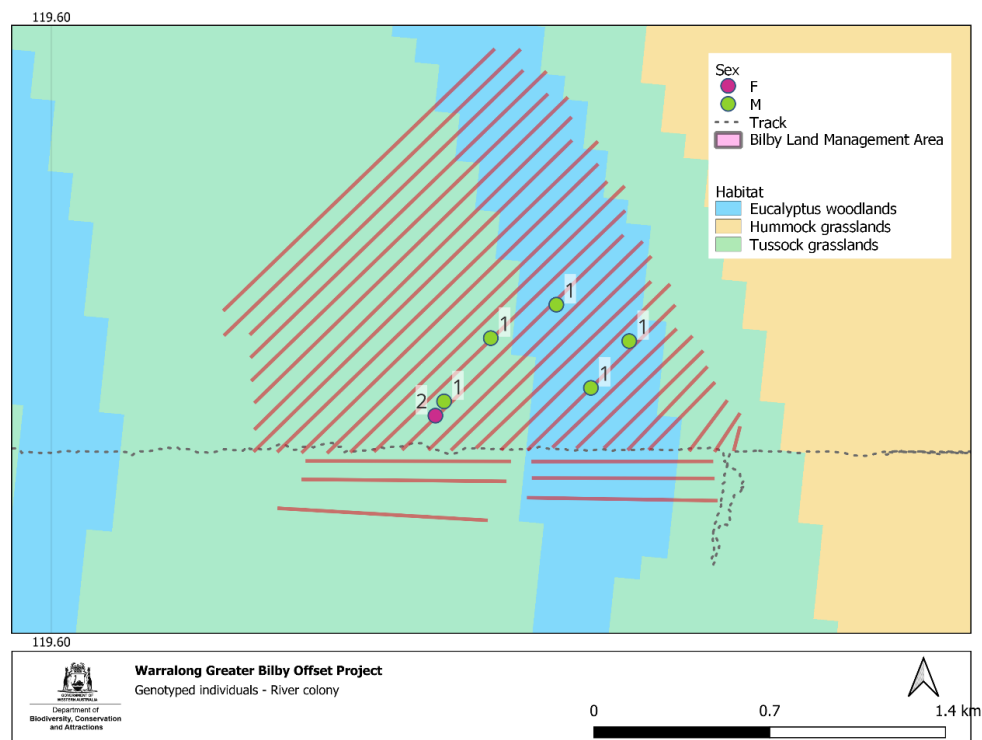


Figure 9 – Genotyped scat material from the River bilby colony near Warralong Community. Numbered labels represent individuals.

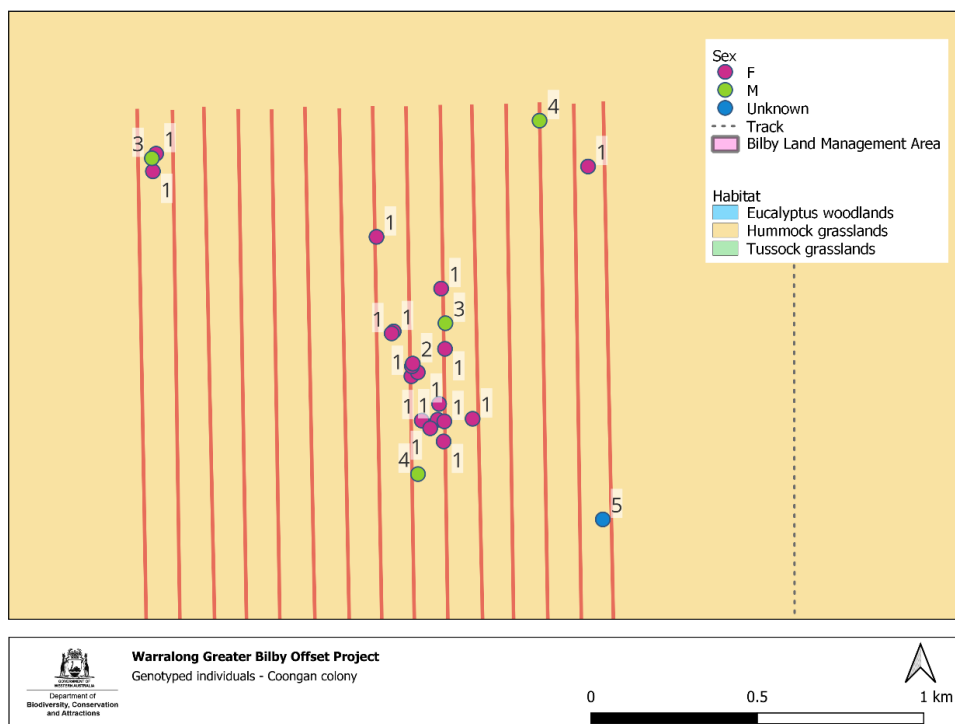


Figure 10 – Genotyped scat material from the new Coongan bilby colony near Warralong Community. Numbered labels represent individuals.

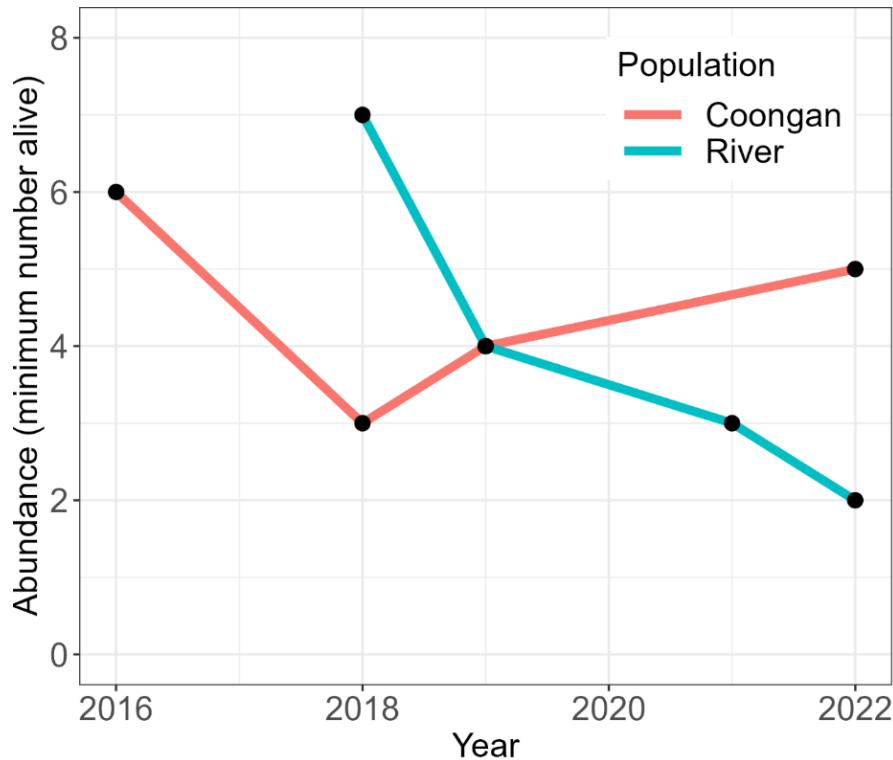


Figure 11 – Minimum number of bilbies alive derived from genotype sequencing of scats on Coongan Station between 2016 and 2022. Assumption: that the new Coongan colony is the relocated previous Coongan colony.



Figure 12 – SECR analysis for Coongan bilby colony, near Warralong community. Red lines represent transect. Black polygon represent 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 Recommendations

1. Feral cat management (via aerial deployment of Eradicat®) was conducted in June 2022. Although population size within both the new Coongan and River colonies appear to have remained relatively stable over the last year, more survey effort is needed to detect any positive impacts of baiting on bilbies. Additional abundance surveys within the BLMA post baiting in 2023 will assist to assess the effectiveness of feral cat management.
2. Continued search effort as part of regular 2 ha plot surveys inside and outside the BLMA 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.
3. To increase the chance of discovering more bilby populations and promote cultural awareness and knowledge sharing, ongoing involvement of local stakeholders is critical, particularly the Warralong community and pastoral leaseholder.

5 References

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