Department of **Biodiversity, Conservation and Attractions**

GOVERNMENT OF WESTERN AUSTRALIA

Biodiversity and Conservation Science

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Abundance monitoring in 2019 of bilbies at Warralong, Western Australia, using DNA extracted from scats.

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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. Since 2014, further observations of bilby presence have been recorded in 2015, 2016, 2018 (Dziminski *et al.* 2019) and 2019. The Coongan Colony shifted approximately 2 km to the north between 2014 and 2016 and the River Colony was identified in 2018 (Figure 1). A collaborative project involving the Warralong Community, DBCA, Roy Hill and Greening Australia was initiated in 2018 to monitor and manage the bilby population at Warralong.

Field sampling

Abundance monitoring was undertaken in 2019 at the Coongan and River Colonies. The activity area (extent) of each colony was initially mapped (Figure 1), so that sampling could be focused on occupied rather than unoccupied surrounding habitat. This was done using vehicles, all-terrain vehicles (quad bikes or ATVs), and on foot, depending on vegetation and terrain. The GPS coordinates of the extent of bilby activity were plotted on electronic devices, and where no more sign of activity (tracks, scats, diggings, burrows) existed, the population boundary was delineated. This process was usually completed in 1-2 days at each colony, then transects to be traversed were overlaid to ensure the population was evenly sampled. Population extent was also used as the habitat mask in spatially explicit capture-recapture (SECR) analyses (see below). Transects across activity areas were traversed by foot and ATV. In 2019 DBCA staff collected 151 scat samples. Samples were stored dry, at room temperature, in 30 ml tubes, approximately 1/3-filled with silica gel beads and cotton wool, until DNA extraction was undertaken.

Laboratory analyses

DNA extractions were undertaken following the protocol in Carpenter and Dziminski (2017). Genomic DNA was extracted from scats using the Qiagen QIAamp Fast DNA Stool Mini Kit with some modifications from Piggott and Taylor (2003) to the recommended procedures included in the kit. DNA was screened using eight highly polymorphic microsatellite markers (Table 1). These were multiplexed into two polymerase chain reactions (PCR) using the Qiagen Multiplex PCR Plus Kit. PCR amplification was performed using cycling conditions modified from the Qiagen Multiplex PCR Plus Kit. The PCR product was then analyzed on an ABI3730XL Sequencer, sized using Genescan-500 LIZ internal size standard, and genotyped using Genemapper Software 5.

Genotyping analyses

Allele matching was completed using the R package '*AlleleMatch*' (Galpern *et al.* 2012). Unclassified samples and samples that matched multiple unique genotypes were examined manually and excluded if they could not be matched or classified as new unique genotypes. Any

remaining mismatched alleles were flagged and examined to determine if they were genotyping errors.

Genotypes identified along transects only provide information on the number of individuals detected specifically on transects, and further analysis is required to calculate the number of individuals within the extent of the population. Genotyping identified four distinct individuals present along surveyed transects at the Coongan Colony, and four at the River Colony.

Abundance analyses

Spatially explicit capture-recapture analyses (SECR: Efford 2004) were used to calculate densities and numbers of animals within the Activity Areas. Maximum likelihood SECR analyses were undertaken using the R package '*secr*'. Spatial analyses were completed using ArcGIS (Esri®) and QGIS software.

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 halfnormal (HHN) detection function and Nelder-Mead maximisation method. The position of each sample was collapsed onto the nearest point on the transect line. The habitat mask for each colony in each year ("Activity Areas" in Figure 1) was constructed by generating the integration mesh using a buffer of 4×σ and clipping with the "Activity Area" polygon (Figure 2 and Figure 3).

Numbers of individuals and densities from maximum likelihood SECR analyses are shown in Figure 4 and Table 2. No individuals were detected at both colonies in 2019, or between or within any previous years, indicating no recent movement between colonies. At the Coongan Colony, one individual was detected present in 2016, 2018 and 2019, and another individual that was detected in 2016 was detected again in 2019. At the River Colony, two individuals that were detected in 2018 were detected again in 2019. Further analyses of the genetic data can reveal the relatedness of individuals within this population (for example if individuals are full- or half-siblings, or other levels of relatedness) as well as geneflow between other populations in the Pilbara.

Recommendations

Management of feral predators and fire (Burrows 2019; Dziminski *et al.* 2019) was to commence in 2020, however, due to the COVID19 Pandemic, this has been delayed until 2021. It is therefore recommended to repeat abundance monitoring prior to the commencement of management in 2021 to ensure a continuous pre-management dataset to compare to the post-management dataset. Abundance monitoring after the commencement of management is recommended to be undertaken in 2022, which ensures enough breeding time for bilbies between the implementation of management and subsequent monitoring, which should then be undertaken annually in subsequent years, concurrently with management for at least three occasions.

At the River Colony, it is recommended to survey similar habitat that exists to the south, east and north ("islands" between channels along the river course - Figure 1) in the days prior to the next abundance monitoring event to detect any shift in the activity area, similar to what is occurring at the Coongan Colony. This habitat has not been surveyed extensively, and may require coordination with the neighbouring pastoral lease to the north, De Grey Station, for access. Habitat types are shown in Figure 5.

Sincerely,

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Table 1. Microsatellite markers used in PCR.

Locus	Primer set	Fluorescent label	Reference
Multiplex 1			
B02	BIL02	6-FAM	Moritz et al. (1997)
B17	BIL17intF	VIC	Moritz et al. (1997) and Smith et al. (2009)
B56	BIL56intF	PET	Moritz et al. (1997) and Smith et al. (2009)
B66	BIL66	NED	Moritz et al. (1997)
Multiplex 2			
B55	BIL55	6-FAM	Moritz et al. (1997)
B22	BIL22	VIC	Moritz et al. (1997)
B41	BIL41intF	PET	Moritz et al. (1997) and Smith et al. (2009)
B63	BIL63	NED	Moritz et al. (1997)

Table 2. Abundance and densities of bilbies at Warralong derived from maximum likelihood SECR analyses.

Year	Colony	Activity area (ha)	Number of individuals (± SE)	5-95% CI	Density (individuals ha^{-1} ±SE)	5-95% CI
2016	Coongan	143	7 (± 3)	3 - 16	0.049 (± 0.021)	0.022 - 0.110
2018	Coongan	26	3 (± 2)	1 - 9	0.114 (± 0.072)	0.037 - 0.355
2019	Coongan	213	4 (± 2)	2 - 11	0.019 (± 0.010)	0.007 - 0.050
2018	River	259	9 (± 4)	4 - 20	0.036 (± 0.015)	0.017 - 0.078
2019	River	258	7 (± 4)	2 - 21	0.026 (± 0.016)	0.008 - 0.080

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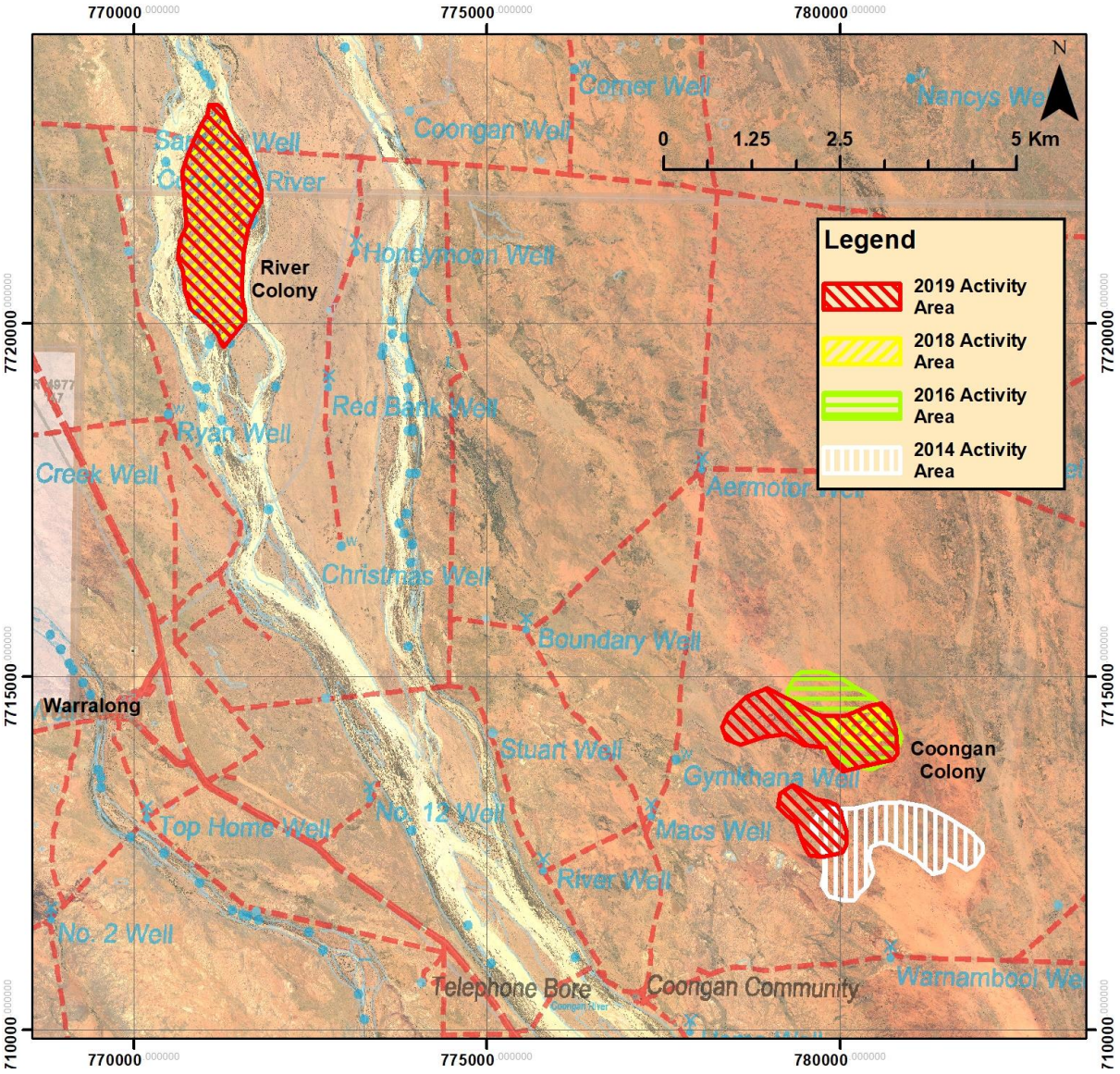

Figure 1. Bilby activity areas 2014 to 2019.

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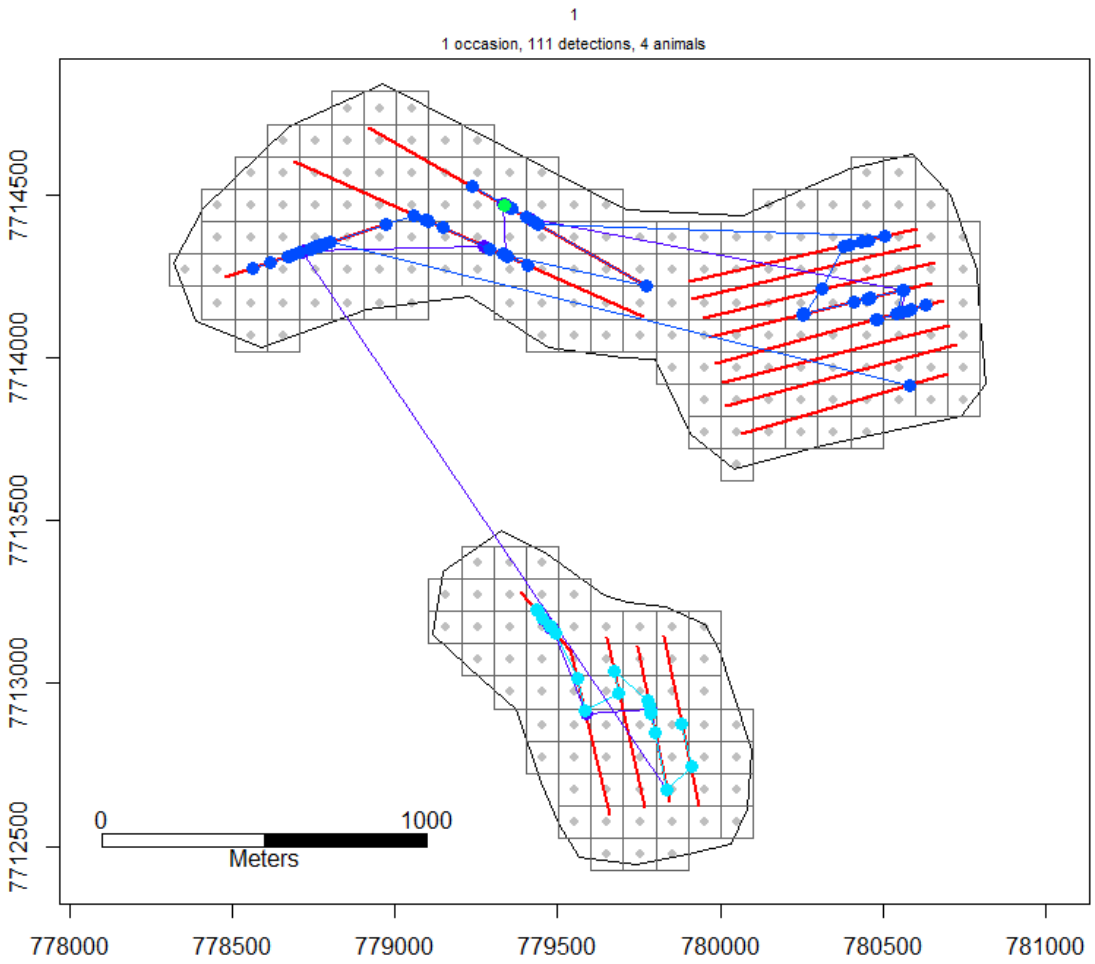

Figure 2. Coongan Colony 2019: Transect detectors (red), habitat mask and integration mesh (grey) and detections of individuals (coloured) with “recaptures” adjoined by lines.

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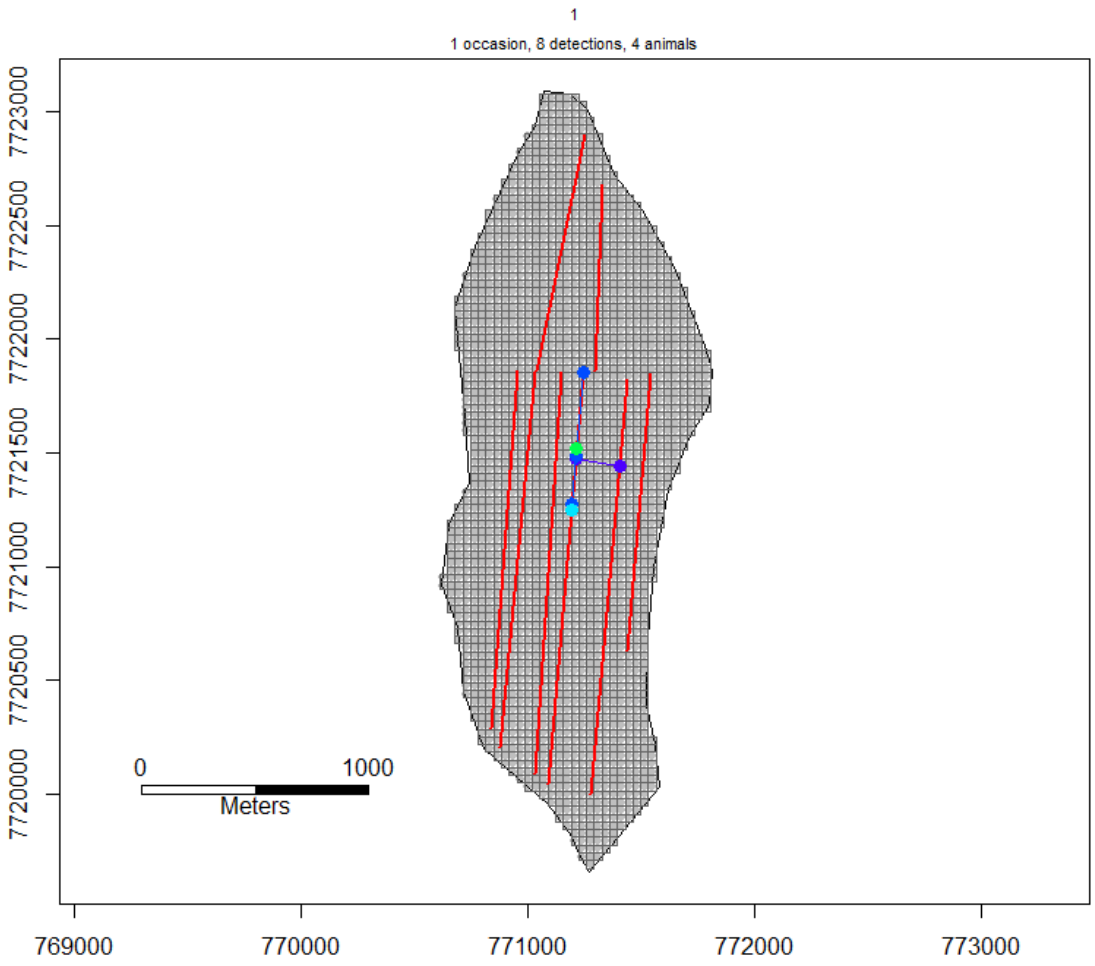

Figure 3. River Colony 2019: Transect detectors (red), habitat mask and integration mesh (grey) and detections of individuals (coloured) with “recaptures” adjoined by lines.

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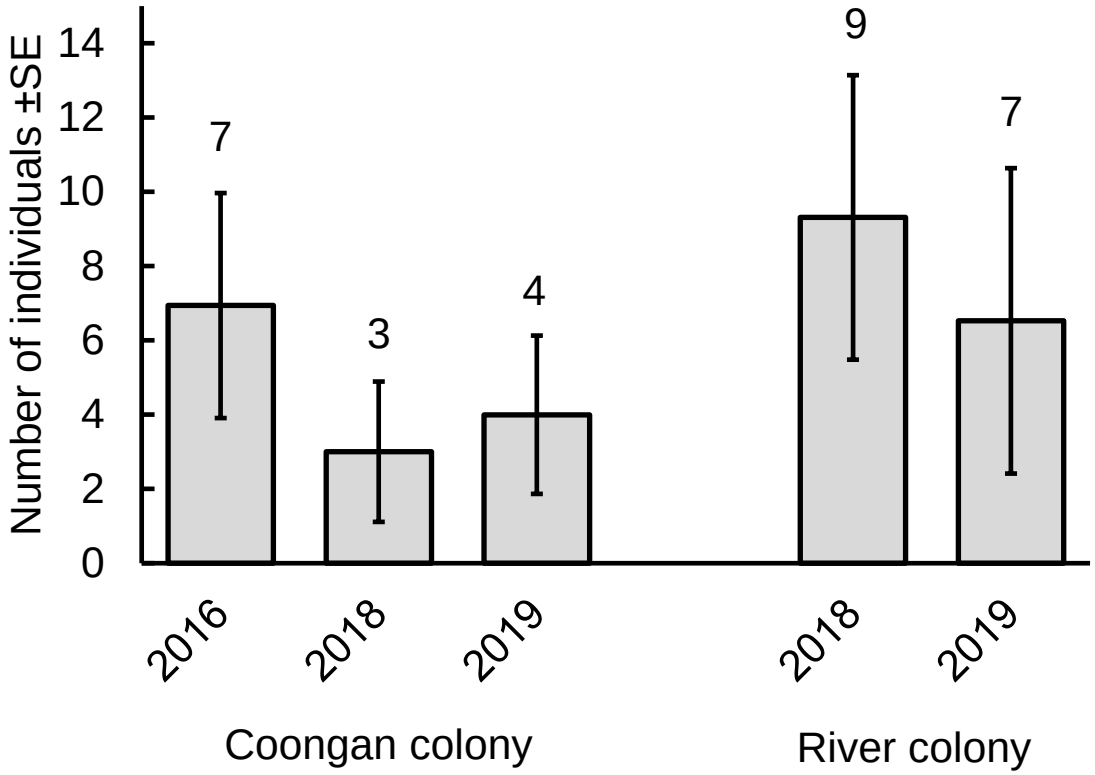

Figure 4. Abundance of bilbies from Spatially explicit capture-recapture (SECR) analyses for each monitoring year at each colony at Warralong. Numbers above error bars indicate numbers of individuals.

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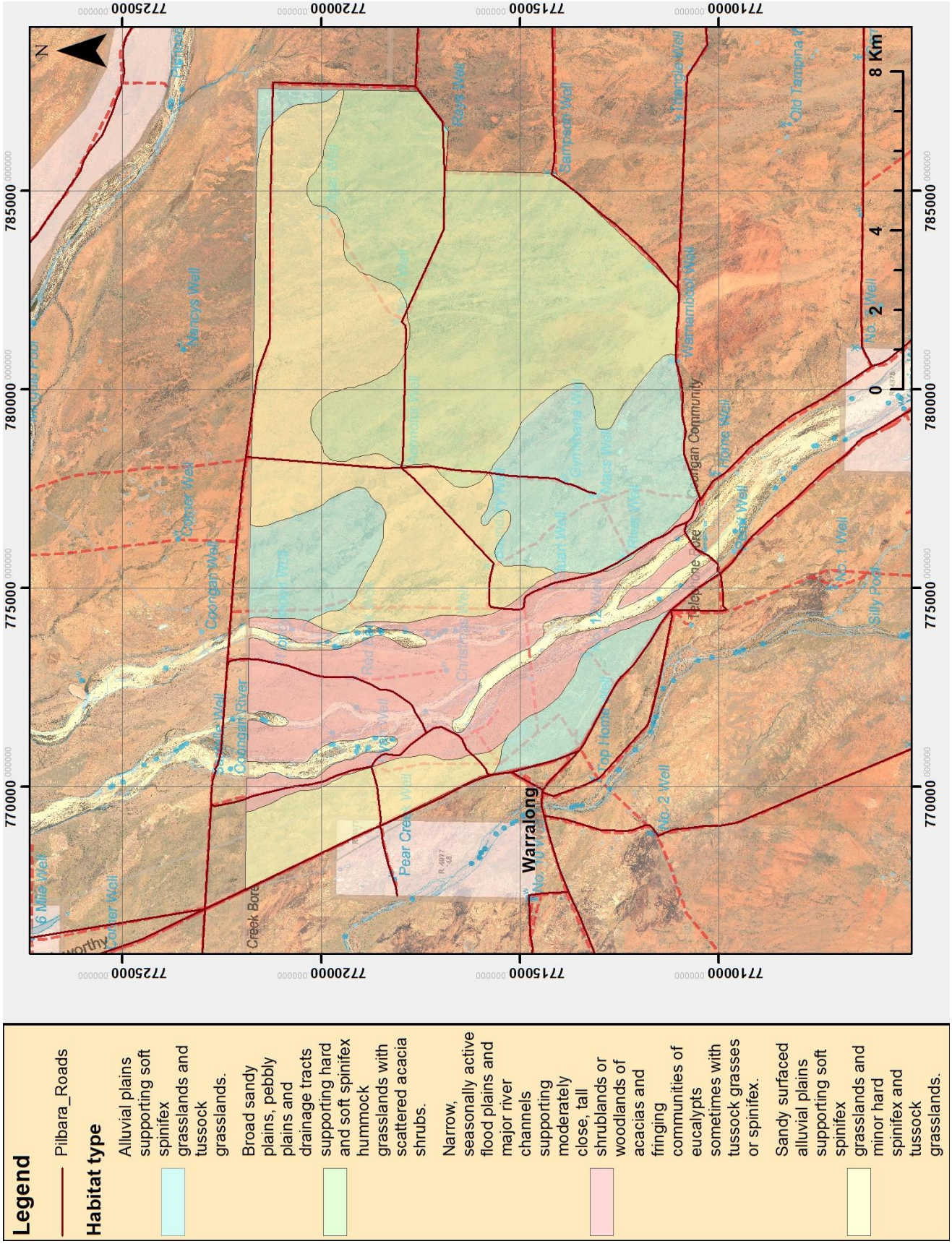

Figure 5. Major habitat types within the Warralong bilby management area.

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