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## **Abundance monitoring 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 active an active bilby population on the Coongan Pastoral Lease. Since 2014, further observations of bilby presence have been recorded in 2015, 2016 and 2018 (Figure 1). The Coongan Colony shifted approximately 2 km to the north between 2014 and 2016 and the River Colony was identified in 2018 (Figure 2).

### **Field sampling**

Abundance monitoring was undertaken in 2016 and 2018 at the Coongan Colony, and in 2018 at the River Colony. Transects across activity areas were traversed by foot and ATV (quad bike). In 2016 DBCA staff collected 56 scat samples, and 106 samples were collected in 2018 by DBCA and Greening Australia staff. 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 six distinct individuals present along surveyed transects at the Coongan Colony in 2016, and three in 2018, and seven at the River Colony in 2018.

## 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 2) was constructed by generating the integration mesh using a buffer of  $4 \times \sigma$  and clipping with the "Activity Area" polygon (Figure 3, Figure 4 and Figure 5).

Numbers of individuals and densities from maximum likelihood SECR analyses are shown in Table 2. No individuals were detected at both colonies in 2018, indicating no recent movement between colonies. At the Coongan Colony, two individuals from 2016 were detected still present in 2018. 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.

## Required management area

The area of management surrounding bilby populations needs to be large enough to create habitat heterogeneity and accommodate movement of the population within the managed area (Southgate and Possingham 1995; Southgate *et al.* 2007; Southgate and Carthew 2007). The recommended management area for effective management to achieve a positive impact on the existing bilby population at Warralong, allowing movement of the population and between colonies, and potential to increase the area occupied is shown in (Figure 6). The following management actions should be undertaken:

1. Fire management:
  - a. Establishing and maintaining a suitable firebreak surrounding the managed area to prevent large wildfires destroying vegetation structure and food resources (Wright and Clarke 2007) and allowing easy predator access (McGregor *et al.* 2014; Doherty *et al.* 2015) within managed populations;
  - b. Implementing patch mosaic burning to create fire age heterogeneity, increasing habitat and resource diversity for bilbies (Southgate and Carthew 2006; Southgate and Carthew 2007; Southgate *et al.* 2007).
2. Introduced predator management:
  - a. Baiting the managed area and surrounding buffer zone with Eradicat® (Algar and Burrows 2004; Algar *et al.* 2013; Doherty and Algar 2015) coupled with supplementary trapping (Molsher 2002; Algar *et al.* 2013) and traditional hunting (Taylor 2015) to control feral cats and foxes. Ground baiting can be used within this area.
3. Livestock management:
  - a. Negotiation of reduced stocking densities in the management area;
  - b. Culling feral livestock (camels) within the managed populations;
  - c. Negotiating to close or move artificial water points in the vicinity of managed populations.

Sincerely,

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Table 1. Microsatellite markers used in PCR.

Locus	Primer set	Fluorescent label	Reference
<u>Multiplex 1</u>			
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)
<u>Multiplex 2</u>			
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 derived from maximum likelihood SECR analyses.

Year	Colony	Activity area (ha)	Number of individuals ( $\pm$ SE)	5-95% CI	Density (individuals ha <sup>-1</sup> ) ( $\pm$ SE)	5-95% CI
2016	Coongan	143	7 ( $\pm$ 3)	3 - 16	0.049 ( $\pm$ 0.021)	0.022 - 0.110
2018	Coongan	26	3 ( $\pm$ 2)	1 - 9	0.114 ( $\pm$ 0.072)	0.037 - 0.355
2018	River	259	9 ( $\pm$ 4)	4 - 20	0.036 ( $\pm$ 0.015)	0.017 - 0.078

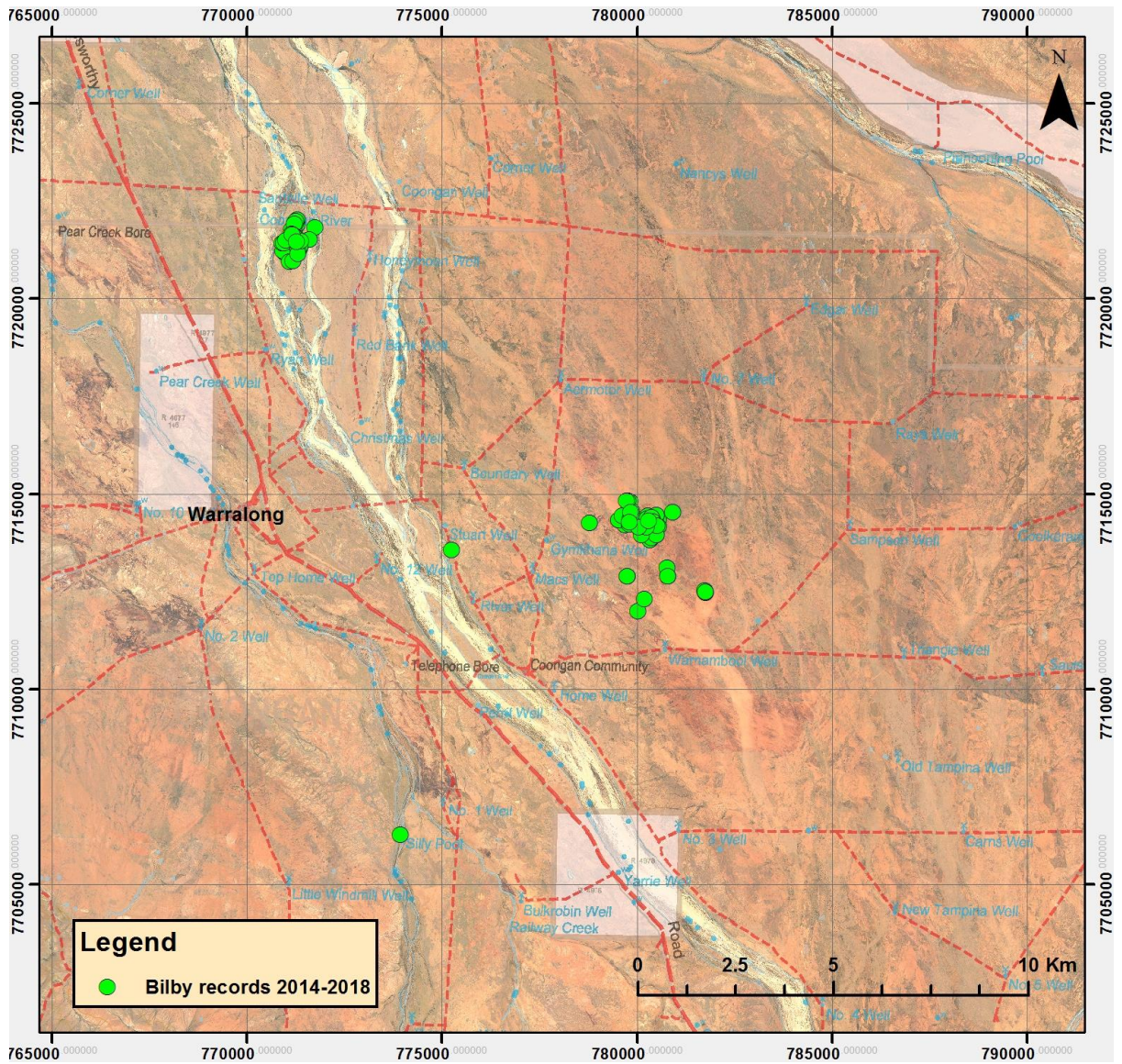


Figure 1. Bilby records in the vicinity of Warralong 2014 to 2018.



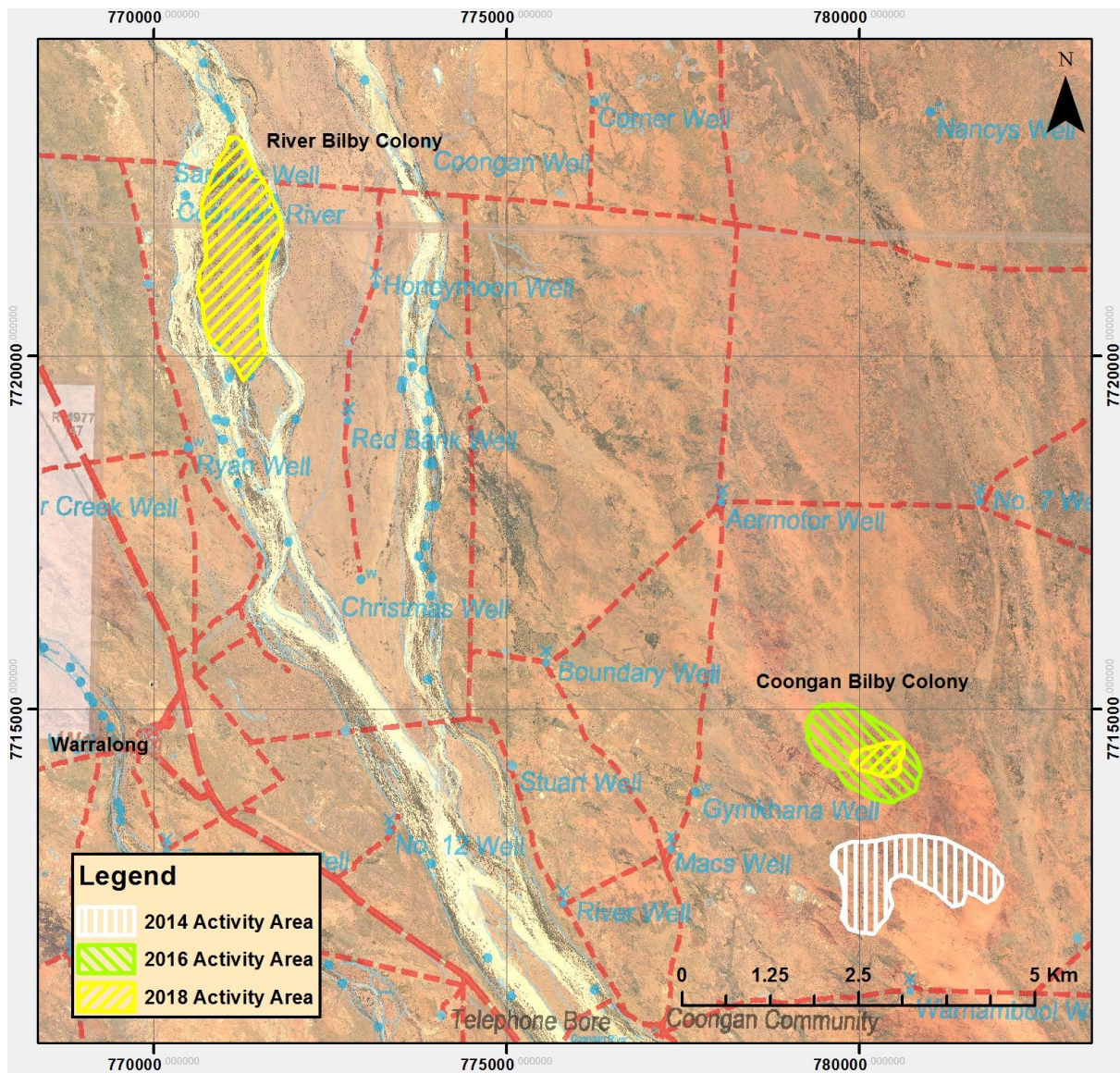


Figure 2. Bilby activity areas 2014 to 2018.

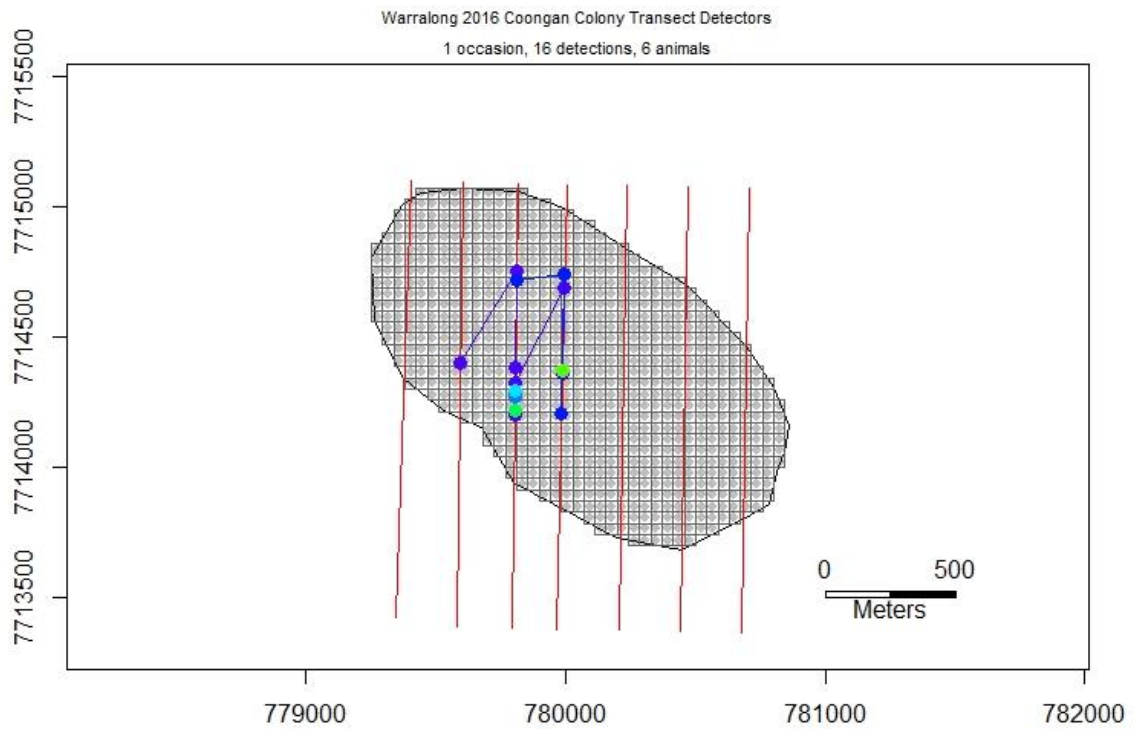


Figure 3. Coongan Colony 2016: Transect detectors (red), habitat mask and integration mesh (grey) and detections of individuals (coloured) with “recaptures” adjoined by lines.

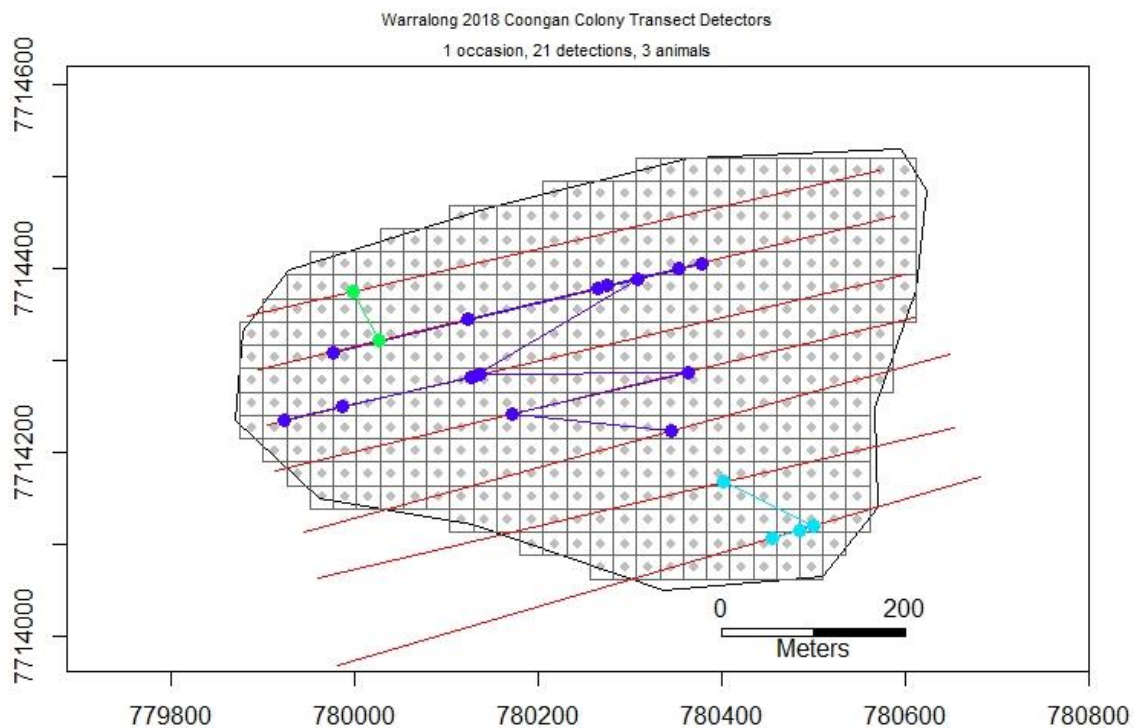


Figure 4. Coongan Colony 2018: Transect detectors (red), habitat mask and integration mesh (grey) and detections of individuals (coloured) with “recaptures” adjoined by lines.

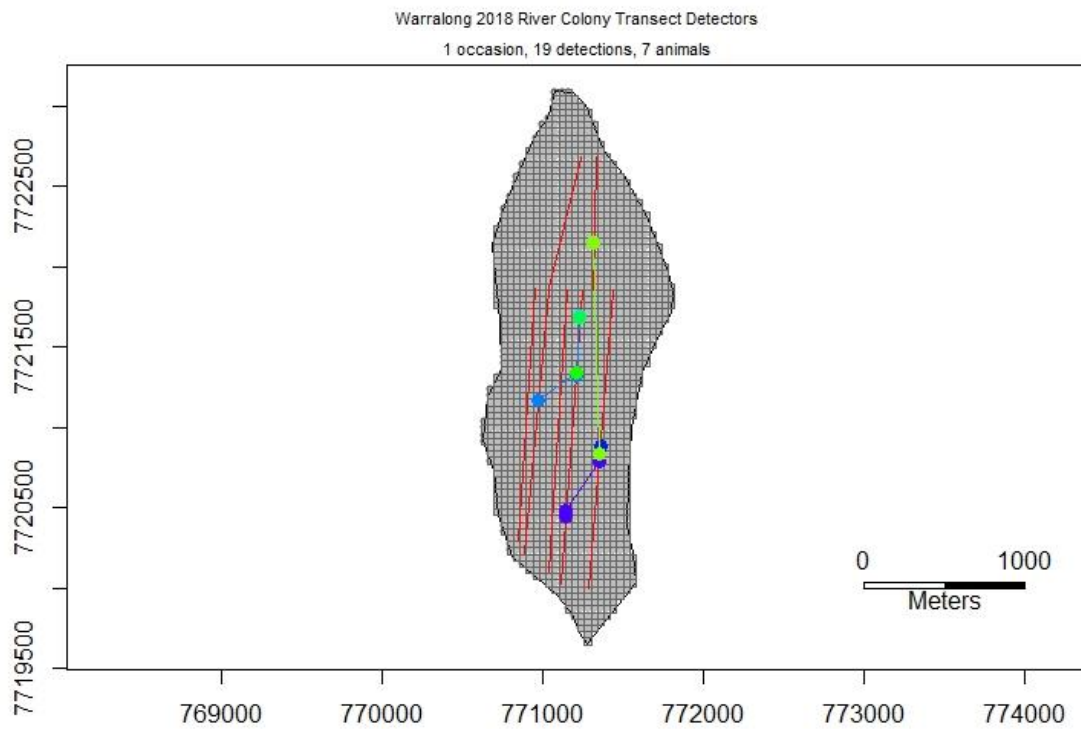


Figure 5. River Colony 2018: Transect detectors (red), habitat mask and integration mesh (grey) and detections of individuals (coloured) with “recaptures” adjoined by lines.



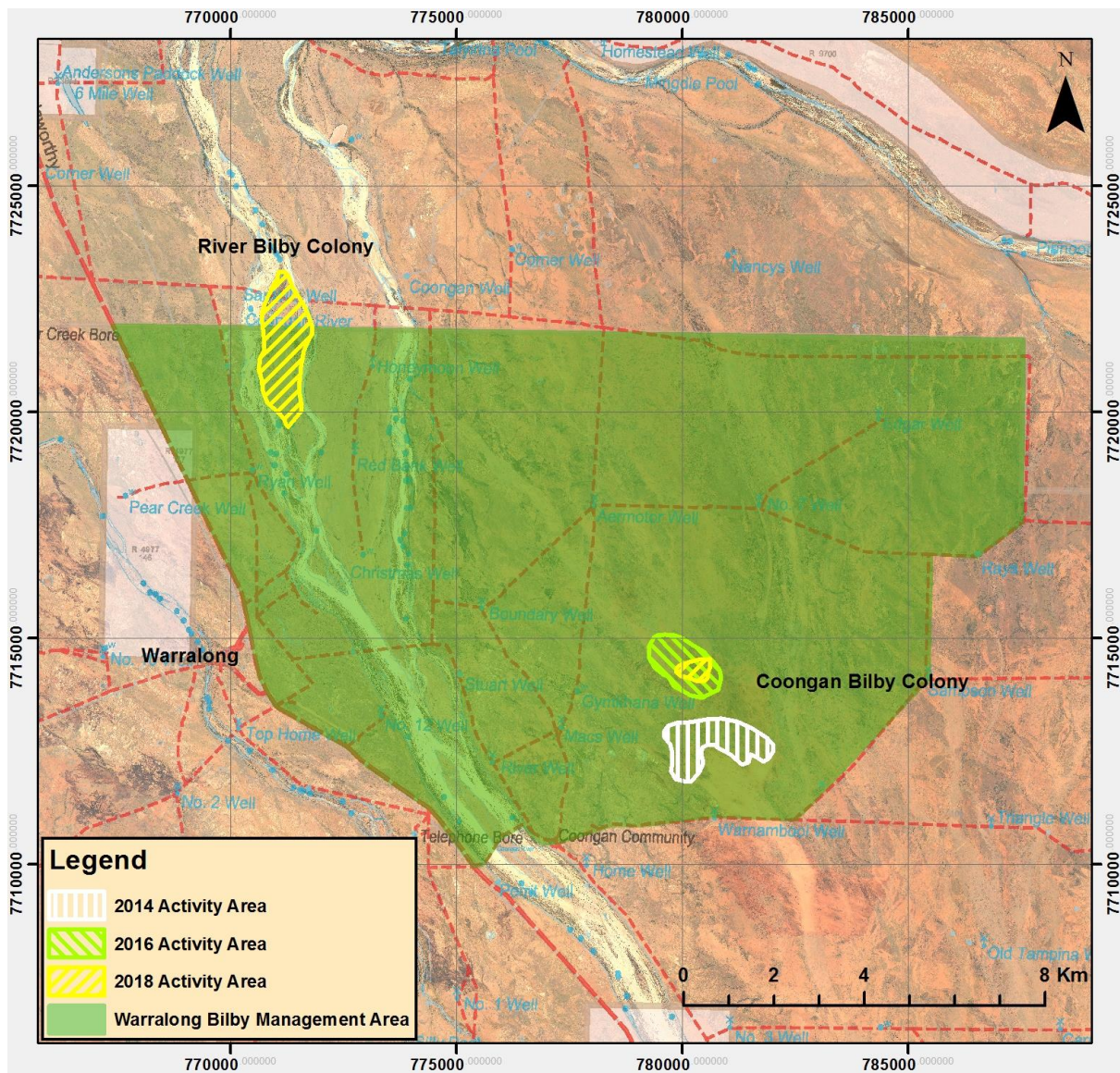


Figure 6. Recommended bilby management area.



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