



Winu Project Bilby Abundance Study 2025



Prepared for

Rio Tinto Winu Pty Ltd

May 2026

Biota
Environmental
Sciences



© Biota Environmental Sciences Pty Ltd 2026
ABN 49 092 687 119
Level 4, 46 Colin Street
West Perth, Western Australia 6005
Ph: (08) 9328 1900

Project No.: 1716N

Prepared by: H. Newton

Document Review History

Version: Rev 1	Peer review: N. Watson
Version: Rev 1	Director review: G. Humphreys
Version: Rev 1	Format review: G. Humphreys

Approved for issue: G. Humphreys

This document has been prepared to the requirements of the client identified on the cover page and no representation is made to any third party. It may be cited for the purposes of scientific research or other fair use, but it may not be reproduced or distributed to any third party by any physical or electronic means without the express permission of the client for whom it was prepared or Biota Environmental Sciences Pty Ltd.

File path: /Volumes/Cube/Current/1716N (Winu Bilby Abundance 2025)/Documents/Winu Bilby Abundance Study 2025 Rev 1.docx

Bilby Abundance Study 2025

Contents

1.0	Executive Summary	5
2.0	Introduction	6
2.1	Project Background	6
2.2	Scope of Current Work	10
2.3	Study Objectives	10
2.4	Study Area	11
3.0	Methodology	13
3.1	Search Effort	13
3.2	Weather and Climate	15
3.3	Habitat Mapping	17
3.4	Sign Evidence	18
3.5	DNA Extraction and Genotyping	20
3.6	Survey Limitations	21
4.0	Field Survey Results	22
4.1	Habitat Prospectivity	22
4.2	Bilby Signs	25
4.3	Bilby Census	29
4.4	Feral Predators	33
5.0	Discussion	34
5.1	Local Habitat Assessment	34
5.2	Regional Habitat Assessment	37
5.3	Winu Bilby Population	41
6.0	Conclusion	43
7.0	References	44

Appendices

Appendix 1 All Years Survey Effort Mapping

Appendix 2 Genotyping Bilby Scat Samples from Winu – Helix
Molecular Solutions

Tables

Table 2.1:	Bilby survey effort relative to Winu DE.	8
Table 2.2:	Survey area name, extent and years surveyed.	11
Table 3.1:	Summary of targeted search effort from the current survey.	13
Table 3.2:	Criteria used to assess prospective Bilby habitat.	17
Table 3.3:	Criteria used to assess certainty of Bilby tracks.	18
Table 3.4:	Criteria used to assess certainty of Bilby diggings.	19
Table 3.5:	Criteria used to assess certainty of Bilby burrows.	19
Table 4.1:	Summary of Bilby presence in the study area.	26
Table 4.2:	Scat analysis breakdown.	29
Table 5.1:	Summary of the fire history of the Winu DE.	39
Table 5.2:	Regional population abundance estimates for the Bilby.	42

Figures

Figure 2.1:	Consolidated Bilby survey effort in the Winu DE and surrounding locality.	7
Figure 2.2:	Consolidated Bilby records in the Winu DE and surrounding locality.	9
Figure 2.3:	Location of study area and Winu DE.	12
Figure 3.1:	Targeted Bilby search effort from the current study.	14
Figure 3.2:	Climate graph depicting long-term averages and monthly data for the Telfer Aero (#013030) weather station for the years between the first and most recent Bilby survey.	16
Figure 4.1:	Bilby habitat prospectivity in A0, AA1 and AA2.	23
Figure 4.2:	Bilby habitat prospectivity in AA3 and AA4.	24
Figure 4.3:	Bilby signs recorded during the current survey.	27
Figure 4.4:	Compilation of Bilby signs recorded between 2019-2025.	28
Figure 4.5:	Bilby detections across survey years grouped by survey area.	29
Figure 4.6:	Bilby individuals recorded as determined through molecular genotyping - Map 1 of 2.	31
Figure 4.7:	Bilby individuals recorded as determined through molecular genotyping - Map 2 of 2.	32
Figure 5.1:	Rio Tinto Winu clearing footprint (as of October 2025) overlaid onto fauna habitats (Map 1 of 2).	35
Figure 5.2:	Rio Tinto Winu clearing footprint (as of October 2025) overlaid onto fauna habitats (Map 2 of 2).	36
Figure 5.3:	Soil-landscape systems occurring within the Winu DE.	38
Figure 5.4:	Fire history of the Winu DE (data from Northern Australia & Rangelands Fire Information).	40

1.0 Executive Summary

Rio Tinto Winu Pty Limited (RTW) plans to develop a new copper-gold mine, referred to as the Winu Project, approximately 320 km east of Port Hedland in the Pilbara region of Western Australia, within both the Nyangumarta and Martu Peoples' Native Title Determination Areas.

A significant amount of biological survey work has been undertaken by Biota in the Winu DE and the broader locality between 2017 and 2024 for the purposes of environmental impact assessment. Effort summaries and previous Bilby results provide a contextual understanding of Bilby occurrence in the broader locality.

RTW referred the Winu Project to the Department of Climate Change, Energy, the Environment and Water (DCCEEW) and were issued a request for further information (RFI). RTW commissioned Biota Environmental Sciences (Biota) to undertake a targeted Bilby study to address the DCCEEW RFI. The purpose of this study was to conduct an updated census of the number of individual bilbies known to inhabit the areas of suitable habitat in the vicinity of the Winu Development Envelope (DE).

The survey work at Winu was undertaken by two suitably qualified and experienced zoologists from the 9th – 15th October 2025. The targeted Bilby survey comprised strip transect traverses to record sign evidence of the Bilby (tracks, scats, diggings and/or burrows). Habitat assessments were also undertaken at all sites to determine the suitability of the areas in supporting bilbies.

Since 2019, bilbies have occupied (at discrete points in time) four disjunct areas in the vicinity of the Winu DE. No recent Bilby signs were recorded in any of the areas surveyed in the current study. However, additional evidence showing signs of ageing (debris in diggings, crusted spoil piles, and very dry scat) were recorded. Four scat samples were collected and submitted to Helix Molecular Solutions for molecular analysis and genotyping. Two were successfully genotyped; one aligned with an individual identified during the 2024 survey, whilst the other represented a new individual. Despite the latest two records being collected and analysed in 2025, the condition of the scat samples upon collection and the absence of other recent evidence (fresh diggings, active burrows and/or tracks) in the survey area, strongly suggest that the bilbies are not currently utilising the survey area.

Given bilbies' known propensity to move around in response to resource availability, it is likely the population still exists and is currently occupying areas outside of the current scope of this study.

2.0 Introduction

2.1 Project Background

Rio Tinto Winu Pty Limited (RTW) plans to develop a new copper-gold mine, referred to as the Winu Project, approximately 320 km east of Port Hedland in the Pilbara region of Western Australia, within both the Nyangumarta and Martu Peoples' Native Title Determination Areas.

Multiple studies have been undertaken within and in the vicinity of the Winu development envelope (DE) for the Winu Project, with Biota Environmental Sciences (Biota) holding a large proportion of the biological data, having completed all the fauna surveys to date for the project over multiple phases. In addition to this, a portion of the DE was surveyed for a neighbouring project, the Australian Renewable Energy Hub (AREH) (also by Biota), and a data sharing agreement has been reached between Rio Tinto and AREH, enabling the data from those surveys to be utilised here. While the focus of the current scope is on the Bilby population in the vicinity of the Winu DE, sampling effort and Bilby records from outside the Winu DE for the AREH project are presented as they provide regional context and reference data for the work completed within the Winu DE, particularly in an under-surveyed region such as the Great Sandy Desert.

2.1.1 Summary of Consolidated Survey Effort

A significant amount of biological survey work has been undertaken by Biota in the Winu DE and the broader locality between 2017 and 2024 for the purposes of environmental impact assessment for both the Winu and AREH projects (summarised in Biota (2025a)). In summary, the following surveys included effort targeting bilbies:

- A three-phase seasonal detailed fauna survey for the AREH Project (which overlaps with the Winu Road Access Corridor, RAC) (Biota 2018, 2022a);
- A targeted fauna survey for AREH (Biota 2024a);
- A two-phase seasonal detailed fauna survey of the Mine and Infrastructure Area, MIA (Biota 2021);
- Targeted surveys of a Bilby population south of the MIA (2019, 2022 and 2024 censuses) (Biota 2020, 2023a, 2024b) and,
- Six basic and targeted fauna surveys across the MIA, RAC and regional borefield options as the project developed (Biota 2022b, 2023b, 2023c, 2023d).

Survey methodology employed across these surveys includes:

- Targeted searches for secondary evidence in highly suitable habitat;
- General searches for secondary evidence in broadly suitable habitat;
- 2 ha sign plots;
- Nocturnal surveys;
- Deployment of motion cameras; and
- Searches on transect in areas known to support bilbies.

A detailed breakdown of survey effort inside and outside the Winu DE is provided in Table 2.1 with site locations mapped in Figure 2.1.

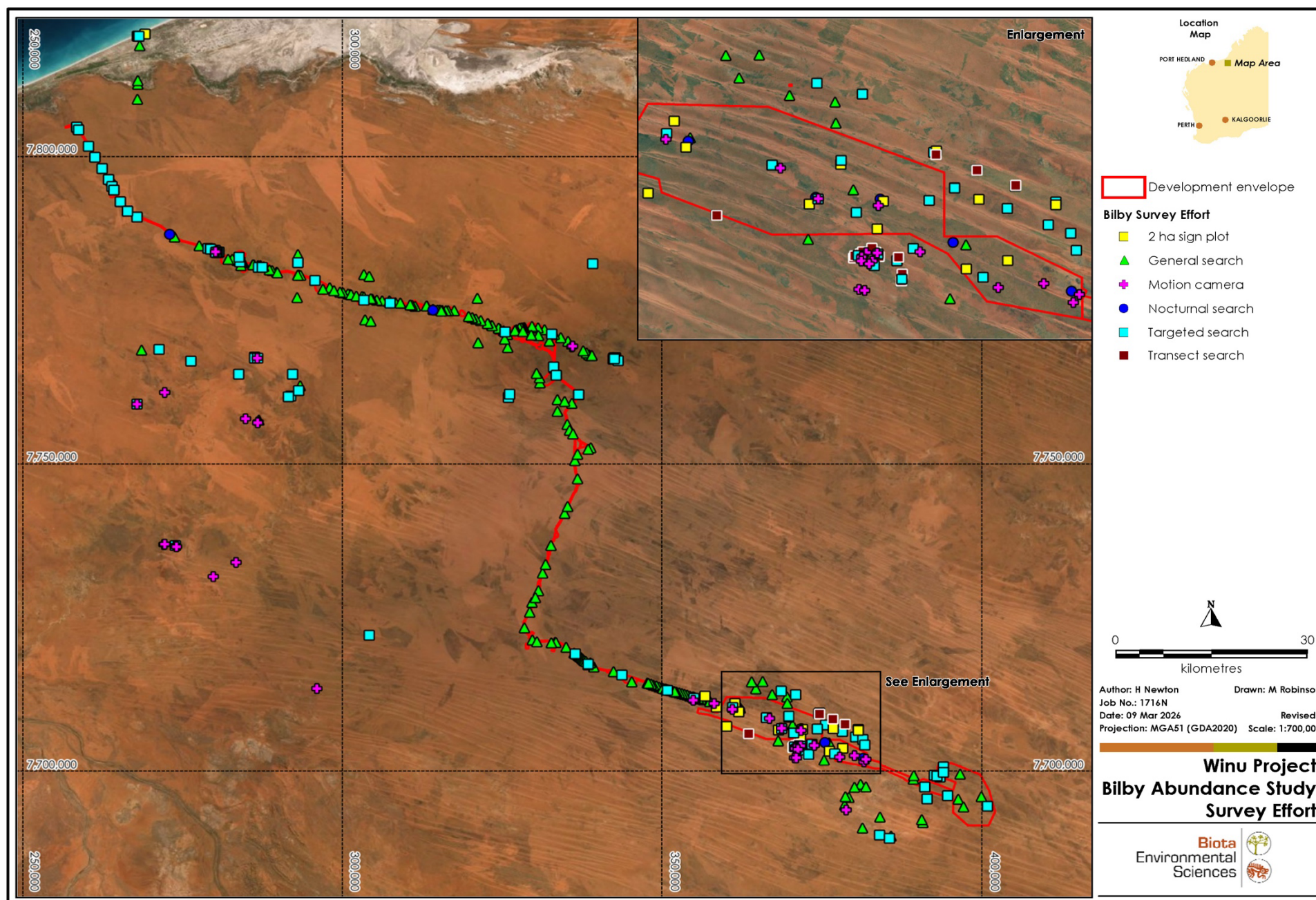


Figure 2.1: Consolidated Bilby survey effort in the Winu DE and surrounding locality.

Table 2.1: Bilby survey effort relative to Winu DE.

Survey Methodology	Survey Effort		
	Inside DE	Outside DE	Total
Targeted searches	67 searches	71 searches	138
General searches	178 searches	59 searches	237
2 ha sign plot	9	6	15
Nocturnal searches	9 searches	1 search	10
Motion camera (short and long-term deployments)	12 sites (44 nights)	31 sites (1,340 nights)	43
Transect searches	-	14	14

2.1.2 Summary of Consolidated Survey Findings

As a result of the extensive survey effort detailed in Section 2.1.1, sign evidence of Bilby indicating past use (inactive burrows, old tracks and scats) was recorded at numerous locations along the RAC and MIA, as well as found in the locality surrounding the DE (Biota 2021, 2022a). Evidence of active use was also recorded for both the Winu (Biota 2020, 2023a, 2024b) and AREH (Biota 2024a) projects. The areas with active, or recently active Bilby sign evidence recorded in proximity to the Winu DE, form the focus of this study.

In early 2019, Biota completed a targeted survey of what was previously referred to as the Winu Project Area (now Mine Infrastructure Area, MIA), resulting in the detection of a population of Bilby (Biota 2021). The area known to support the Bilby population encompassed approximately 344.1 ha of highly prospective Bilby habitat. Following the initial detection of the population, Biota conducted three Bilby abundance surveys within this extent (Phase 1 in 2019, Phase 2 in 2022 and Phase 3 in 2024). Bilbies were recorded here during Phase 1 and 2. In July 2023, the DE boundary was amended, resulting in the area supporting the Bilby population being excised from the DE. Then, after the occurrence of a fire in 2023, RTW advised that much of this area had been burnt and that previously active burrows were now inactive. During Phase 3 (May 2024), the original area was re-surveyed and an additional two areas (Additional Area 1 and 2), located within the vicinity of the area that previously supported bilbies (but outside of the extent of the fire) were also surveyed. The Phase 3 survey recorded recent evidence of Bilby activity within Additional Area 2 (AA2) in the form of tracks, scats, diggings and burrows. No evidence of recent Bilby activity was found in the original survey area or in Additional Area 1 (AA1). Subsequent to these three phases of Bilby abundance surveys, in July 2024, recently active signs of bilbies were recorded during basic fauna surveys of the borefield options at the easternmost end of the DE. Consolidated records of Bilby sign evidence (comprising evidence of both current use and past use) from the Winu and AREH projects from 2017-2024 are presented in Figure 2.2.

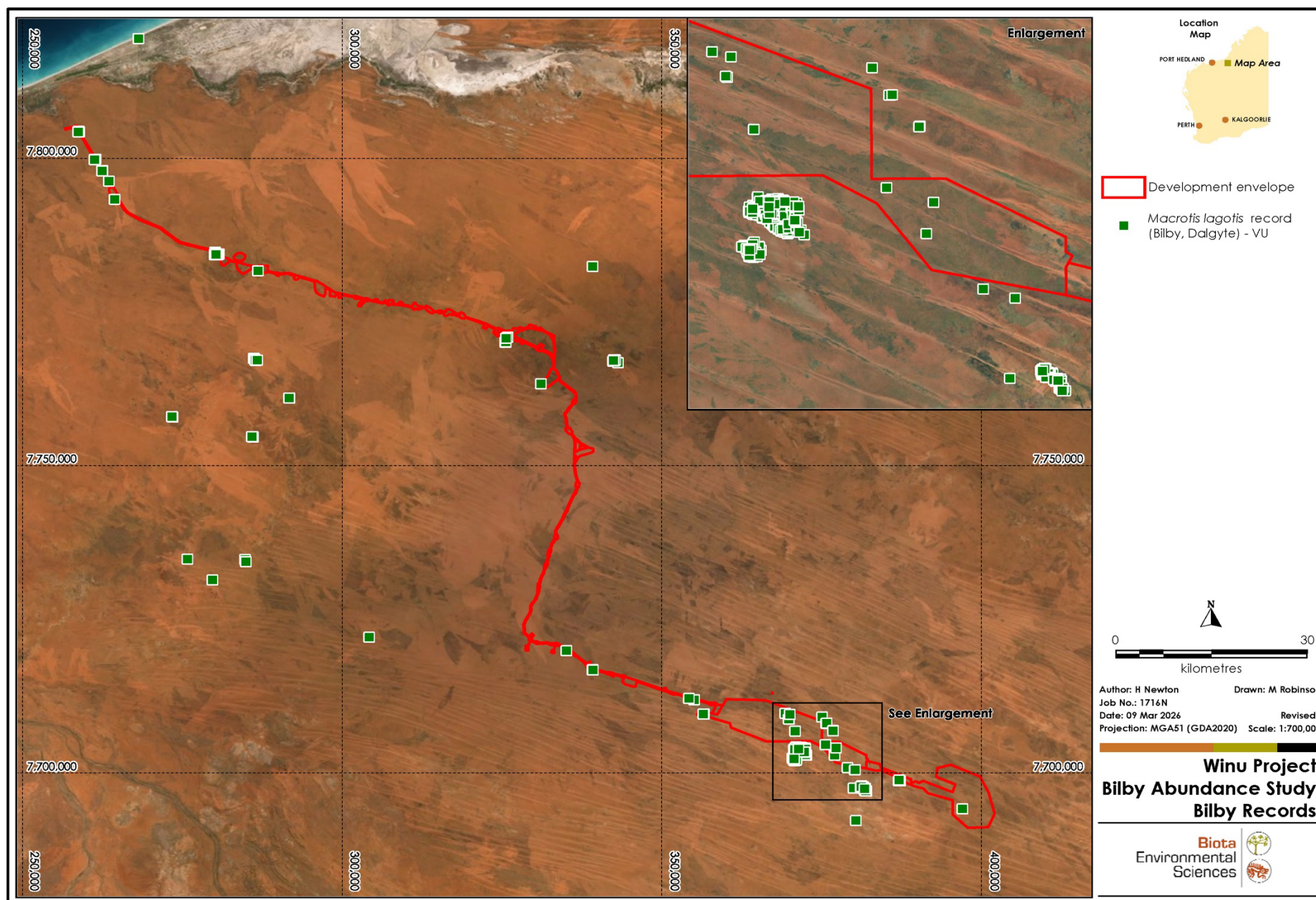


Figure 2.2: Consolidated Bilby records in the Winu DE and surrounding locality.

2.2 Scope of Current Work

RTW referred the Winu Project to the Department of Climate Change, Energy, the Environment and Water (DCCEEW) and were issued a request for further information (RFI), under s95A(2) of the EPBC Act. Section 2 of the RFI requests:

- *“the results of an updated targeted Greater Bilby survey and impact assessment¹ for the development envelope, including the areas not surveyed following the 2023 fire along with the result of the areas surveyed in July 2024 within Rimfire and surrounding areas;*
- *to detail any evidence of use by the Bilby, including burrows, tracks, scats and diggings along with evidence of predator scats;*
- *include evidence and mapping that demonstrates the location of the 4,878 ha of ‘suitable habitat’ for the species that is located within the development envelope; and,*
- *include maps that depict the local and/or regional context for habitat availability for the Greater Bilby”.*

RTW commissioned Biota to undertake a targeted Bilby study to address the DCCEEW RFI requests.

2.3 Study Objectives

The purpose of this study was to conduct an updated census of the number of individual bilbies known to inhabit the areas of suitable habitat in the vicinity of the Winu Development Envelope (DE). The study aims were to:

- conduct targeted Bilby transect searches;
- collect scats (where located) on each transect;
- identify individual bilbies via genotyping of scats;
- consolidate data obtained in 2019, 2022, 2024 and 2025;
- map the current extent of the Bilby population;
- determine robustness of data with the view to conducting spatially-explicit capture-recapture (SECR) analysis (Efford 2004, Efford and Fewster 2012), if valid within the limitations of the data; and,
- address all points relating to Greater Bilby (*Macrotis lagotis*) as set out in Section 2 of the DCCEEW RFI.

¹ Impact assessment addressed within Environmental Review Document.

2.4 Study Area

The study area comprises five disjunct areas in proximity to the Winu DE, including four areas where active Bilby burrows and fresh scats, tracks and diggings have been recorded (Biota 2020, 2023a, 2024b). In addition to these four areas, a fifth area that has not been shown to support bilbies (AA1), constitute the study area of the current scope (Table 2.2, Figure 2.3).

The first area known to support bilbies was identified in early 2019 and at the time, consisted entirely of highly prospective Bilby habitat. A fire in 2023 significantly impacted the area, resulting in a loss of approximately 50% of the suitable habitat. This area, previously referred to as the ‘survey area’ (Biota 2023a), will henceforth be referred to as Area 0 (A0). In May 2024, Biota zoologists censused two additional areas in the vicinity of A0 but outside the extent of the fire; Additional Area 1 and 2 (AA1 and AA2). The two remaining areas known to recently support bilbies were identified during surveys undertaken in July 2024 and are hereafter referred to as Additional Area 3 and 4 (AA3 and AA4). All five areas were surveyed in October 2025 as part of the current study.

Table 2.2: Survey area name, extent and years surveyed.

Area	Extent (hectares)	Years surveyed
Area 0 (A0)	344.1	September 2019, July 2022, May 2024, October 2025
Additional Area 1 (AA1)	131.7	May 2024, October 2025
Additional Area 2 (AA2)	68.9	May 2024, October 2025
Additional Area 3 (AA3)	129.7	July 2024, October 2025
Additional Area 4 (AA4)	39.8	July 2024, October 2025

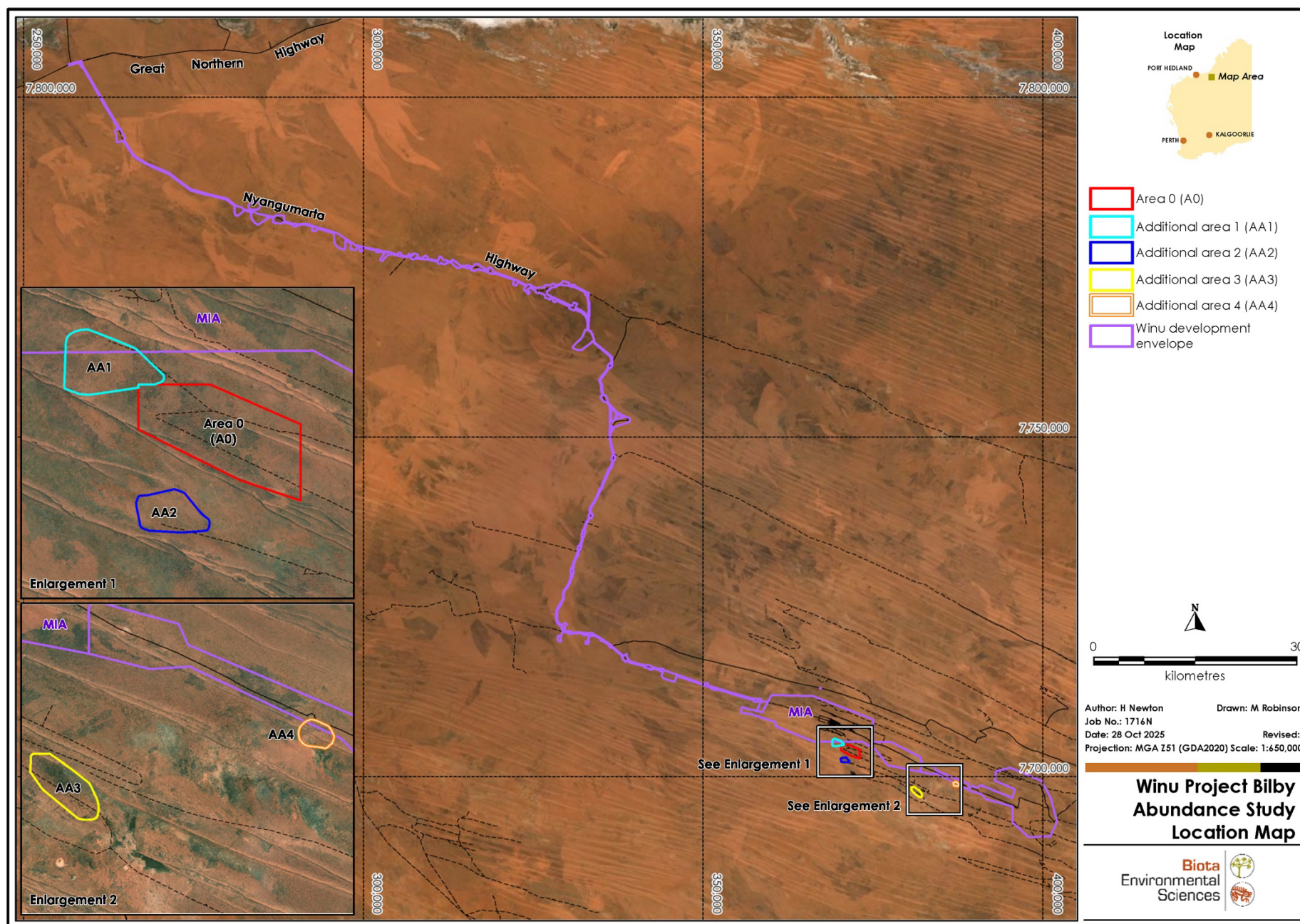


Figure 2.3: Location of study area and Winu DE.

3.0 Methodology

A targeted Bilby survey was undertaken in accordance with relevant Federal and State policy, including:

- Technical Guidance – Terrestrial Vertebrate Fauna Surveys for Environmental Impact Assessment (EPA 2020);
- Guidelines for Surveys to Detect the Presence of Bilbies, and Assess the Importance of Habitat in Western Australia (DBCA 2017a); and,
- Survey Guidelines for Australia’s Threatened Mammals (DSEWPaC 2011).

The targeted Bilby survey comprised strip transect traverses to record sign evidence of the Bilby (tracks, scats, diggings and/or burrows). Habitat mapping was also undertaken (see Section 3.3).

The survey work at Winu was undertaken by Michael Greenham (Senior Zoologist) and Hayley Newton (Zoologist) from the 9th – 15th October 2025. Biota acknowledges the effort and guidance provided by RTW Environmental Advisor, Lily Deveroux and Martu Rangers (JYAC), Louis Janawana and Dion Billycan, during the 2025 survey.

3.1 Search Effort

A summary of the total search effort expended for the current Bilby abundance study is presented in Table 3.1 and Figure 3.1. Appendix 1 maps the combined survey effort of all targeted Bilby abundance surveys completed by Biota for the Winu Project. For details specific to past surveys, please refer to Biota (2020, 2023a, 2024b).

Table 3.1: Summary of targeted search effort from the current survey.

Area	Date	Personnel	Traverse Distance (km) ¹
Area 0 (A0)	12 th Oct 2025	Hayley Newton Michael Greenham Lily Deveroux (RTW) Louis Janawana (JYAC) Dion Billycan (JYAC)	21.5
Additional Area 1 (AA1)	11 th Oct 2025		18.3
Additional Area 2 (AA2)	10 th Oct 2025		9.6
Additional Area 3 (AA3)	10 th -11 th Oct 2025		12.5
Additional Area 4 (AA4)	9 th Oct 2025		5.2

¹Excludes the distances covered by the Martu Rangers.

In addition to the survey effort detailed above, an additional non-systematic targeted search was undertaken in a previously unsurveyed area approximately 1 km south of AA2. This search was undertaken opportunistically as the vegetation was recognised as *Acacia* shrubland with *A. monticola* as a dominant species (a key food source plant for Bilby) (Western Botanical 2025)). Approximately 6 km was traversed during this search (Figure 3.1).

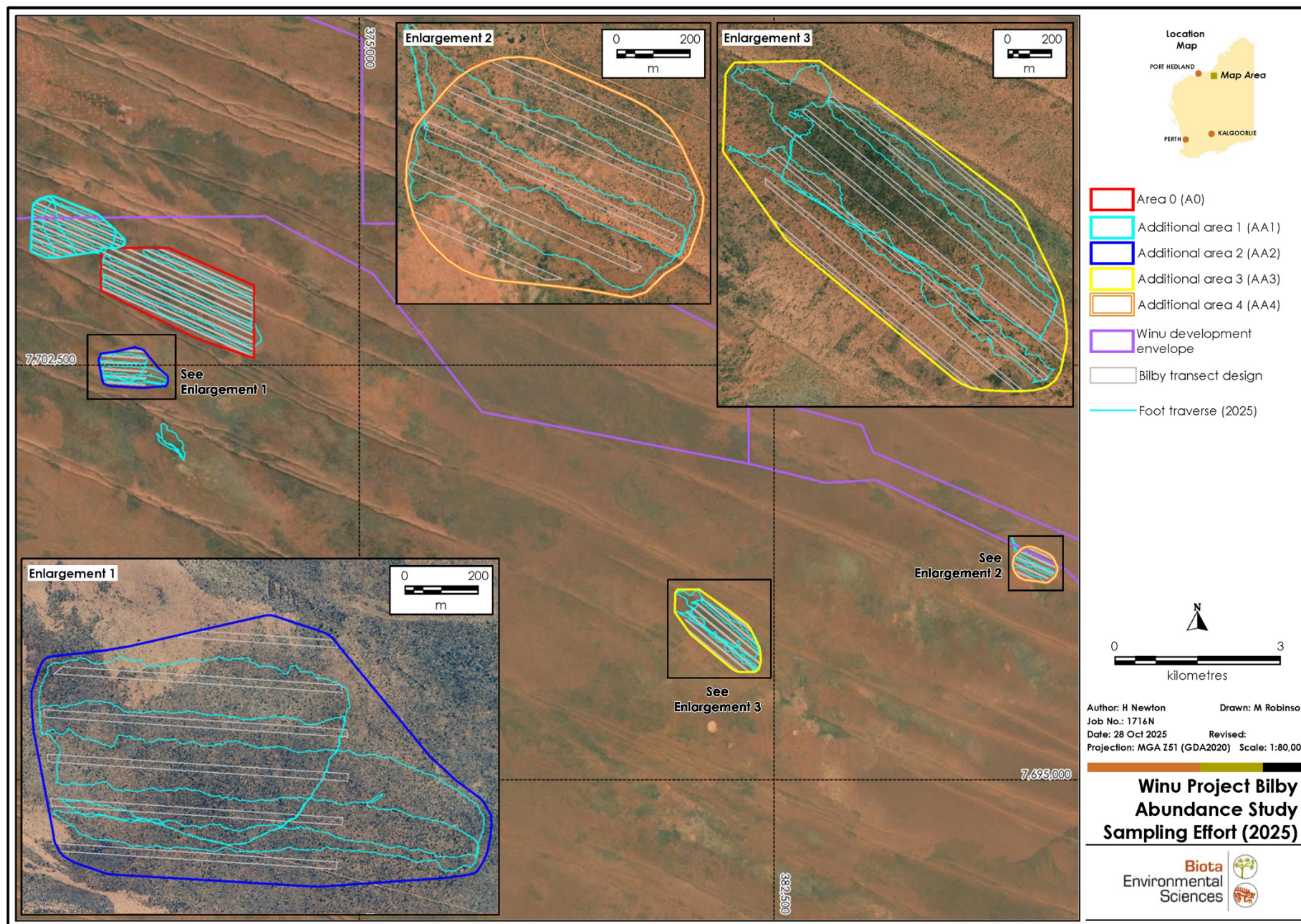


Figure 3.1: Targeted Bilby search effort from the current study.

3.2 Weather and Climate

Weather conditions can influence survey findings, particularly the amount of rainfall preceding a survey. Given the remote location of the Winu project, the closest weather station that can provide a robust dataset of long-term climatic conditions is the Bureau of Meteorology weather station at Telfer Aero (#013030), located approximately 115 km southeast of the survey area.

Figure 3.2 depicts the monthly average rainfall, maximum and minimum temperatures with their respective long-term median values for the years targeted Bilby surveys have taken place. Rainfall in the wet season months of 2019 were much lower than the long-term medians. January and February of 2022 also recorded very little (<3 mm) rain, however March recorded 176 mm, nearly 4-times the monthly long-term median. Rainfall for the remainder of 2022 was generally higher each month compared to the long-term medians. Significant high rainfall events occurred in April 2023, in February and March 2024, and in December 2024 and February 2025.

Generally, average minimum and maximum monthly temperatures hovered close, though slightly higher, than those of the long-term medians (Figure 3.2).

With the exception of the first survey undertaken in September 2019, all other surveys took place in years that recorded significant rainfall in the wet-seasons month, with no significant deviations from long-term maximum and minimum temperatures recorded.

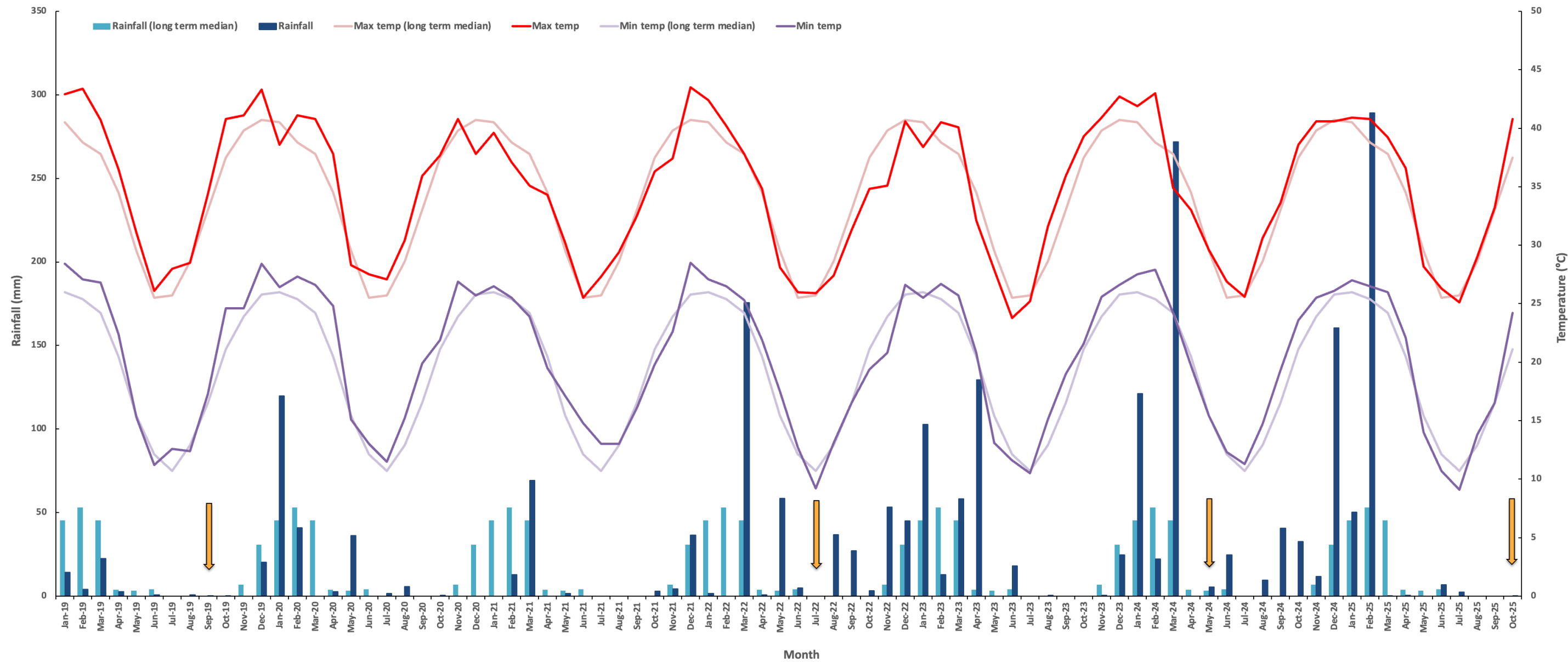


Figure 3.2: Climate graph depicting long-term averages and monthly data for the Telfer Aero (#013030) weather station for the years between the first and most recent Bilby survey.

(data from Bureau of Meteorology; arrows indicate survey timing).

3.3 Habitat Mapping

Although there are many factors which may determine the habitat preferences of Bilby (Cramer et al. 2016), the approach adopted by this study recognises that Bilby presence is strongly linked to the availability of food resources, which are intrinsically dependent on fire history (Southgate 2005).

Bilbies have been recorded in habitats spanning a wide range of fire-ages, including recently burnt (1-2 years), unburnt (3-6 years) and long unburnt (>6 years) areas (Southgate 2005, Southgate and Carthew 2007, DBCA 2017a), with each providing different food resources. For example, recent fire (and subsequent rainfall) promotes regeneration of some short-lived species such as the grass *Yakirra australiensis*, the seeds of which form dominant components of the Bilby diet when available (Southgate 2005, Liddle 2016). Fire also promotes the growth of other short-lived species such as *Senna notabilis*, the roots of which host larvae, another key component in the Bilby diet (Liddle 2016, DBCA 2018). Conversely, fire kills long-lived larvae host species, such as *Acacia hilliana* (Liddle 2016). These long-lived species only develop hosting capacity for larvae after the plant reaches a certain age (Liddle 2016), suggesting that unburnt stands of these species are important from the dietary perspective for the Bilby. The nomadic habit of bilbies allows them to exploit a range of food resources in the landscape.

Considering the above, Biota has developed a set of criteria to aid in determining the prospectivity of Bilby habitat (Table 3.2). Habitats were mapped primarily based on field notes, but mapping was informed by North Australia and Rangelands Fire Information mapping (Charles Darwin University 2022), and vegetation mapping of the area (Western Botanical 2025). The mapping produced is representative of the habitat at the time of writing: many biotic and abiotic factors determine suitability of Bilby habitat, and temporal changes in these factors influence Bilby presence. The habitat map should therefore be viewed as a guide to delineate areas that may currently be of importance to the Bilby, in the absence of significant change in influencing factors (e.g. post-survey fires and rainfall, or increase in prevalence of introduced predators).

Table 3.2: Criteria used to assess prospective Bilby habitat.

Habitat Prospectivity	Criteria
High	1. Sandy habitat supporting long-lived shrubs or forbs known to contain root-dwelling larvae and other important food source plants in unburnt and long unburnt areas (e.g. <i>Acacia monticola</i>, <i>A. eriopoda</i>, <i>A. dictyophleba</i>, <i>A. melleodora</i>, <i>A. stellaticeps</i>, <i>A. hilliana</i>, <i>Senna notabilis</i> and <i>Indigofera georgei</i>), and/or; 2. Sandy habitat supporting short-lived shrubs or forbs known to contain root-dwelling larvae and other important food source plants in recently burnt areas (e.g. <i>Senna notabilis</i> and <i>Yakirra australiensis</i>).
Moderate	1. Sandy habitat supporting plant species that are broadly typical of suitable Bilby habitat in the Great Sandy Desert or Little Sandy Desert (e.g. other <i>Acacia</i> spp., <i>Melaleuca</i> spp. and <i>Triodia</i> grasslands), but with no mapped records of important food source plant species.
Low	1. Habitat does not meet any of the criteria listed above.

3.4 Sign Evidence

3.4.1 Tracks

Bilby foot imprints are distinctive when fresh, and readily distinguished from other species that have an otherwise similar track pattern and gait (Southgate et al. 2019). Hind foot imprints are noticeably longer than front foot imprints and bear few conspicuous indentations, while front foot imprints have three distinct parallel lines produced by the toes and claws (Moseby et al. 2012, Southgate et al. 2019). Other species with similar tracks include the rabbit, bandicoot, dasyurids and rodents, however these can be distinguished from the Bilby based on differences in shape, size and toe and claw prints (Southgate et al. 2019).

The gait of the Bilby is a quadrupedal bounding overstep, which produces a pattern of parallel hind foot imprints and slightly offset or in-line front foot imprints (Moseby et al. 2012, Southgate et al. 2019). This gait pattern is consistent with other similar-sized mammals, including quolls, mulgara, bandicoots, rabbits, rats and occasionally possums (Southgate et al. 2019). As tracks and gait patterns have the potential to be confused with species other than the Bilby, a set of certainty criteria was applied when assessing track evidence in the field (Table 3.3).

Table 3.3: Criteria used to assess certainty of Bilby tracks.

Certainty	Criteria
High	1. Track imprints and gait pattern are clear and consistent with those produced by the Bilby; or 2. Resolution of hind feet and front feet imprints is poor despite a gait pattern consistent with that of the Bilby, however they may be attributed to the Bilby as other species producing similar tracks and gait patterns do not occur, or are unlikely to occur in the survey area.
Low	1. Resolution of hind feet and front feet imprints is poor despite a gait pattern consistent with that of the Bilby, and they cannot reliably be attributed to the Bilby as other species producing similar tracks and gait patterns occur, or are likely to occur in the survey area.

3.4.2 Diggings

Bilbies forage for food at the soil surface, or dig in the subsoil for beetles, termites, root-dwelling larvae, seeds and bulbs (Southgate et al. 2019). Where the Bilby has foraged, diggings are generally conspicuous and distinctive, especially where they occur at the base of shrubs or forbs known to contain root-dwelling larvae, as the Bilby is the only species in mainland Australia that forages for root-dwelling larvae in plant roots (Southgate et al. 2019). Within the Great Sandy Desert bioregion, plant species known to contain root-dwelling larvae include: *Acacia monticola*, *A. dictyophleba*, *A. melleodora*, *A. stellaticeps*, *A. hilliana*, *Senna notabilis* and *Indigofera georgei* (DBCAs 2018, Southgate et al. 2019).

Diggings of the Bilby can be of different size and shape, but are typically:

- shallow, 5-10 cm in depth, with the spoil pile evenly distributed around the dig;
- deep conical or cylindrical digs, usually less than 50 cm deep; or
- large ploughed areas of multiple shallow diggings around termite nests.

Other species such as varanids, echidnas, rabbits, wallabies, bandicoots, mulgara and mice can produce diggings that are similar to the Bilby. Varanid diggings can sometimes superficially appear to belong to the Bilby, but can be distinguished by their notable crescent shape, presence of a distinct mid-ridge at the base of the digging and spoil pile often on one

side (Moseby et al. 2012, Southgate et al. 2019). Given that diggings may be confused with species other than the Bilby, a set of criteria was applied when assessing digging evidence in the field (Table 3.4).

Table 3.4: Criteria used to assess certainty of Bilby diggings.

Certainty	Criteria
High	1. Single or multiple diggings at the base of shrubs or forbs known to contain root-dwelling larvae; and/or, 2. Confirmed Bilby scats detected within spoil piles of diggings; and/or, 3. Other sign, including clear tracks, scats and burrows, recorded in association with diggings.
Moderate	1. Size and shape of diggings are consistent with known characteristics of Bilby diggings, but occur in isolation of other sign; and/or 2. Isolated diggings occur in habitat supporting plant species suitable for foraging, and the Bilby has been recorded historically from the locality.
Low	1. Diggings exhibit some characteristics typical of the Bilby, however they cannot reliably be attributed to the Bilby due to age, location within the landscape (i.e. not at the base of shrubs or forbs), and/or absence of other positive sign.

3.4.3 Burrows

The Bilby constructs burrows that typically have a single round entrance, readily distinguishable from varanids and other reptiles that construct crescent shaped burrows (Southgate et al. 2019). Bilby burrows usually have an apron of excavated sand at the entrance, and are often found under logs, at the base of trees, spinifex tussocks, termite mounds or sand mounds (Triggs 1996, Moseby et al. 2012, Southgate et al. 2019). It is common to observe multiple burrows within an established foraging area, and ‘warrens’ with multiple burrow entrances can sometimes occur (Southgate et al. 2019).

Inactive Bilby burrows can be utilised by a range of other species, including cats, foxes, varanids, echidnas, hopping mice and mulgara, potentially making them appear active (Southgate et al. 2019). Where burrow evidence was detected, a set of criteria was applied to assess the certainty that they were active (or inactive) Bilby burrows (Table 3.5).

Table 3.5: Criteria used to assess certainty of Bilby burrows.

Certainty	Criteria
High (Active)	1. Fresh, confirmed Bilby tracks detected on/surrounding apron in front of burrow entrance; and/or 2. Fresh, confirmed Bilby scats detected on/surrounding apron in front of burrow entrance; and/or, 3. Fresh, confirmed Bilby diggings detected in a foraging area surrounding the burrow entrance; and/or 4. Multiple burrow entrances detected in a foraging area, displaying evidence from 1-3 above.
Low (Inactive)	1. Burrow entrance exhibits characteristics of the Bilby, and some other sign evidence with longevity persists (e.g. scats/diggings), however it cannot reliably be attributed to the Bilby due to age and absence of fresh, positive signs.

3.4.4 Scat Collection

The Bilby produces scats with distinctive characteristics unmatched by any other species on mainland Australia (Southgate et al. 2019). Bilbies produce scats that are oblong in shape, longer than they are wide, firm, usually contain a mixture of sand, plant and invertebrate material, have a smooth coating and rounded ends, and are typically deposited in a group of two to five pellets (Southgate et al. 2019). Scats are most commonly found in association with digging activity, deposited on top of or within spoil piles of diggings, and sometimes at burrow entrances (Southgate et al. 2019).

Molecular analysis of epithelial cell DNA obtained from scats was undertaken to genotype individuals. This method is non-invasive and enables accurate determination of the number of individuals (DBCA 2017b). The technique also has specific application to field situations, as Bilby scats are relatively easy to detect, identify and collect, and DNA can reliably be obtained from scats deposited up to two weeks prior to collection (DBCA 2017b).

To reduce DNA degradation and optimise the likelihood of obtaining a useable molecular sequence, we followed the storage protocol recommended by the DBCA (2017b). Scats were stored in 30 ml vials filled with 10 ml of silica gel beads and placed in between two balls of cotton wool that acted as buffers to movement. Singular scats were stored in unique vials, unless it could be determined with certainty that scats came from one animal (i.e. scats were deposited in a pile and stuck together).

3.4.5 Estimating Age of Signs

Bilbies are often found to occur in low densities, show low site fidelity, and are highly mobile in response to resource availability and habitat modification (DBCA 2021). Therefore, it is not unusual for Bilby signs to be detected in areas they are not currently occupying. Bilby signs can persist in the environment for extended periods of time, with burrows being the most durable. Burrows are generally deemed to be active if there is fresh soil around the entrance and footprints in the soil (Thompson and Thompson 2008). Tracks are the first sign that disappear from the landscape, with other animals or wind altering their appearance after just a few days. The softness and size of a digging spoil pile can be used to estimate age, with recent diggings generally having larger piles of soft sand, and older diggings being flatter and having a crusty layer. Debris in diggings can also be used as an estimator for age, with leaf litter accumulations suggesting diggings have been present for some time (Thompson and Thompson 2008). Bilby scats are difficult to age based solely on appearance, however the age of scats can be assessed by examining decomposition of diggings which are typically associated with scat deposition (DBCA 2018). It is important to consider weather and climate when aging signs as well, with heavy rains and winds altering digging and burrow appearances, and extreme heat likely to dry out scat.

3.5 DNA Extraction and Genotyping

To identify individual bilbies, attempts were made to amplify and sequence seven microsatellite loci for each collected scat. DNA from 59 scat specimens (all Bilby scats collected by Biota since 2019) were extracted using the QIAGEN QIAamp Fast DNA Stool mini kit (Qiagen, Hilden, Germany). Samples were washed in buffer as per Carpenter and Dziminski (2017) and eluted as per manufacturer's protocols. Seven microsatellite loci for *Macrotis lagotis* were amplified using the QIAGEN Multiplex PCR kit in triplicate. PCR products were analysed on an ABI3730XL Sequencer using Genescan-500 LIZ internal standard and scored using GeneMarker V1.91. A genotype accumulative curve was performed, and results revealed that the minimum number of loci to distinguish individuals was five to six (Appendix 2). Examination of genotypes amongst all individual scat specimens was undertaken.

3.6 Survey Limitations

The following limitations should be recognised regarding this study:

- The targeted searches were confined to the five previously identified areas (A0, AA1-AA4) and one additional site. These would not encompass the entire extent of the Bilby population in the locality, but were identified as the areas supporting an active population after extensive survey work throughout the DE.
- Not all collected Bilby scats were successfully sequenced. It is therefore possible that additional bilbies were present in the survey areas during the survey periods.
- The study provides a census snapshot from four occasions (spatially and temporally) and is not an exhaustive study of Bilby population dynamics.

4.0 Field Survey Results

4.1 Habitat Prospectivity

4.1.1 Area 0

Based on the criteria outlined in Section 3.3, 53.5% (184.1 ha) of A0 was assessed as highly prospective Bilby habitat (High prospectivity), mainly due to the presence *Acacia monticola* (a key food source plant). Any areas that were burnt during the fire in 2023 or contained suitable habitat but with few or no food source plants, were assessed as Moderate prospectivity (45.4%, 156.2 ha). *Acacia monticola* has started to grow back in the areas previously burnt, however, are currently too young to harbour the ground-dwelling larvae bilbies favour. The tracks and cleared areas (3.8 ha, 1.1 %) within A0 have been mapped as Low prospectivity habitat (Figure 4.1).

4.1.2 Additional Area 1

The majority (98.3%, 129.5 ha) of AA1 was assessed as Moderate prospectivity as it contained suitable habitat, but with few or no food source plants and no history of recorded Bilby activity (Figure 4.1). All lay-down pads and associated tracks within AA1 were assessed as Low prospectivity (1.7 %, 2.2 ha).

4.1.3 Additional Area 2

Most of AA2 was assessed as High prospectivity (95.3%, 65.6 ha; Figure 4.1); the exceptions being the access track (0.3%, 0.2 ha) and a small area that had been recently burnt (4.4%, 3.0 ha). The area was dominated by *Acacia ancistrocarpa* and *Acacia monticola*, the latter a long-lived forb known to contain root-dwelling larvae favoured by bilbies.

4.1.4 Additional Area 3

Just under half of AA3 is High prospectivity Bilby habitat (41.3 ha, 31.9%, Figure 4.2). The area is dominated by the food source plant *Acacia monticola*, with Bilby foraging activity ceasing in line with the extent of this plant species in the landscape. The remaining area does not contain food source plants though is still broadly suitable for bilbies and is hence assigned a prospectivity rating of Moderate (87.7 ha, 67.6%).

4.1.5 Additional Area 4

A small proportion of AA4 is mapped as High prospectivity habitat (5.9 ha, 14.7%, Figure 4.2), the remaining is Moderate prospectivity habitat (33.9 ha, 85.3%). Given the extent of High prospectivity habitat is quite minimal, we have deduced that AA4 likely represents a temporary area of occupancy for dispersing individuals, rather than core habitat for a small population like A0, AA2 and AA3.

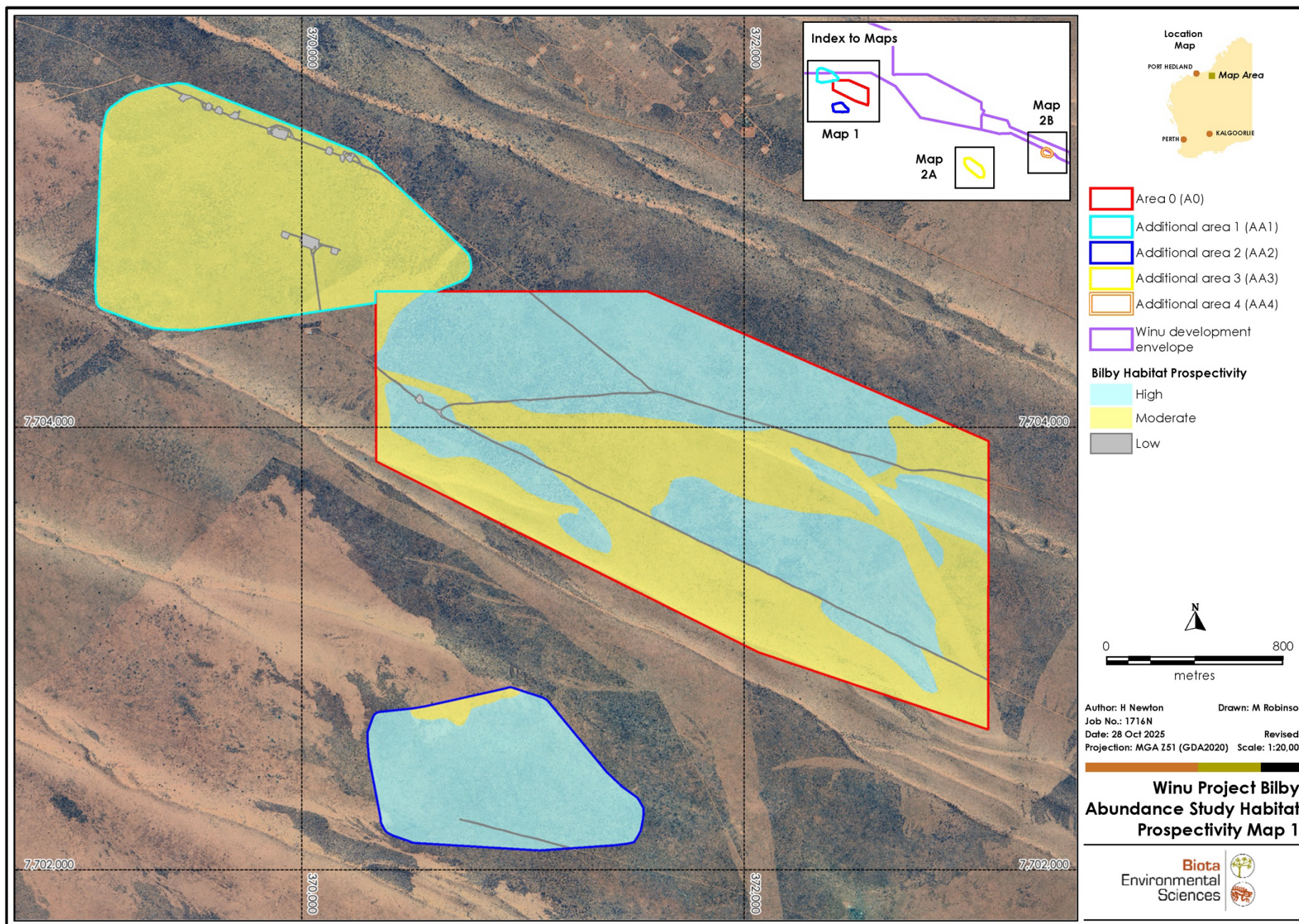


Figure 4.1: Bilby habitat prospectivity in A0, AA1 and AA2.

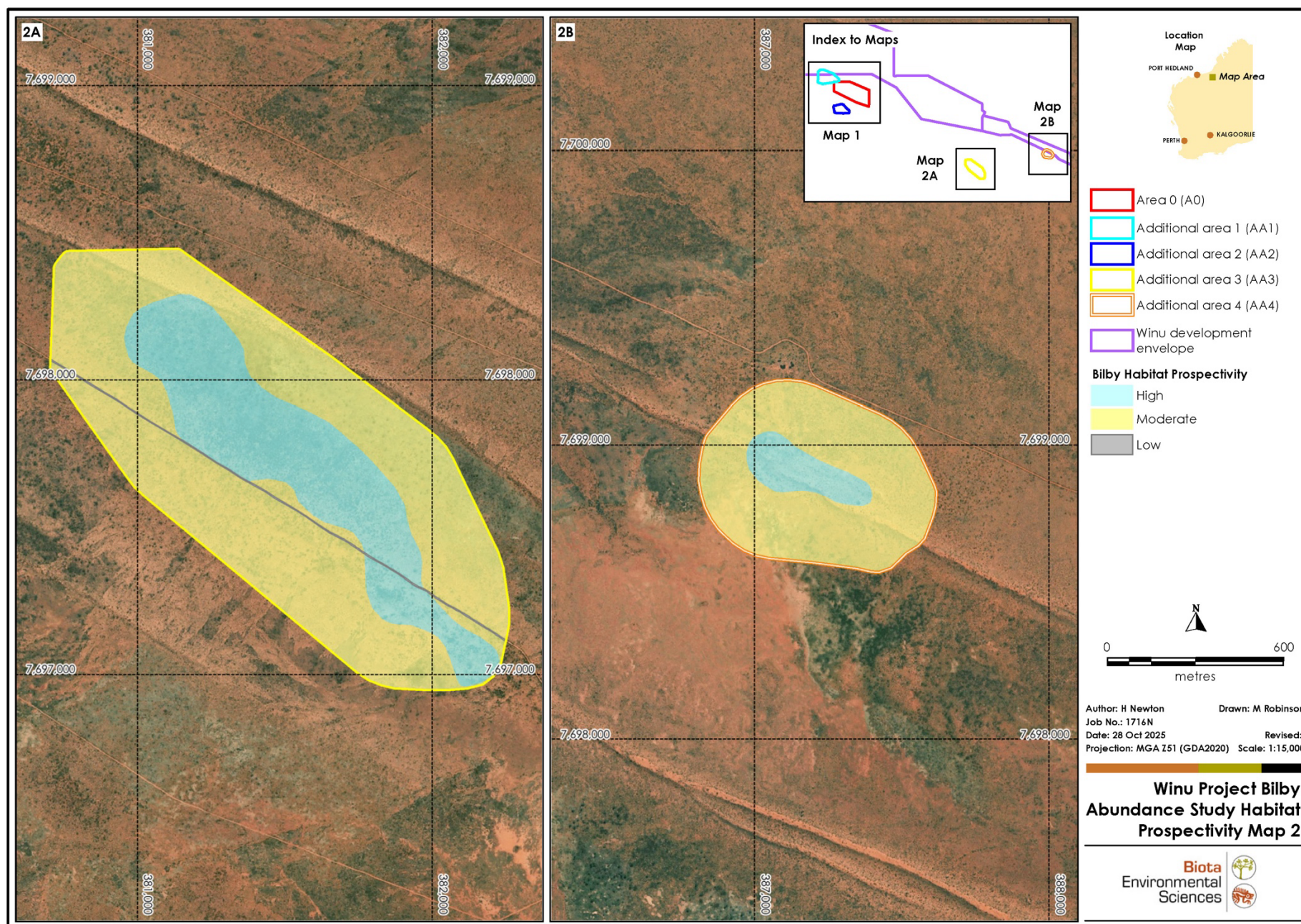


Figure 4.2: Bilby habitat prospectivity in AA3 and AA4.

4.2 Bilby Signs

4.2.1 Area 0

Active Bilby signs were recorded during the 2019 and 2022 searches of A0 (Biota 2020, 2023a) (Figure 4.4). During the third and fourth (current) survey (May 2024, October 2025), no recent Bilby activity was documented in A0.

4.2.2 Additional Area 1

AA1 is situated immediately west of A0; no signs of Bilby activity were recorded at this site in 2024 or 2025.

4.2.3 Additional Area 2

AA2 is located approximately 2 km south of A0 (separated by a longitudinal dune), numerous fresh signs were recorded during the 2024 survey (Biota 2024b). Additional evidence was recorded during the current study, including a single scat sample, though none appeared to be from recent activity (Plate 4.1, Figure 4.3).



Plate 4.1: Example burrow recorded in AA2 during the October 2025 survey; entrance full of debris and lack of tracks or soft soil around entrance indicating it is aged and currently unused.

A targeted search in an area less than 1 km south of AA2 (separated by a longitudinal dune), that consists of highly prospective Bilby habitat, did not return any evidence of the species.

4.2.4 Additional Area 3

AA3 is an area located approximately 9-10 km east of A0 and AA2. Over 50 diggings and 11 burrows were recorded during the 2024 survey (Biota 2024b). Additional signs were recorded during the current survey, including scats diggings and burrows. However, most signs showed evidence of ageing, with diggings filled in with leaves and spoil piles having a layer of crust (Figure 4.3, Plate 4.2). A single burrow on the western edge of AA3 had tracks in the burrow

entrance and a few diggings in the vicinity that appeared to be more recent than all other signs in the area (soft spoil piles, no debris in the digging). However, given the low number of active signs, we have assumed these are the remnants of the last individual that occupied the area.



Plate 4.2: Example scat sample collected from AA3 during the October 2025 survey highlighting the low quality (aged) scat and debris in the associated digging.

4.2.5 Additional Area 4

AA4 is located within the Rimfire pipeline corridor; in 2024 two burrows, one scat sample and ten diggings were recorded here (Figure 4.4). Additional sign evidence was recorded during the 2025 survey, though all were aged, likely attributable to the same period of activity as the records from the 2024 survey (Figure 4.3).

4.2.6 Summary

Since 2019, bilbies have occupied (at discrete points in time) four disjunct areas in the vicinity of the Winu DE. At the time of the current study, it appears as though the Bilby population has moved beyond the bounds of the previously occupied areas into unsurveyed territory (Table 4.1).

Table 4.1: Summary of Bilby presence in the study area.

Area	September 2019	July 2022	May & July 2024	October 2025
A0	Present	Present	Absent	Absent
AA1	-	-	Absent	Absent
AA2	-	-	Present	Absent
AA3	-	-	Present	Absent
AA4	-	-	Present	Absent

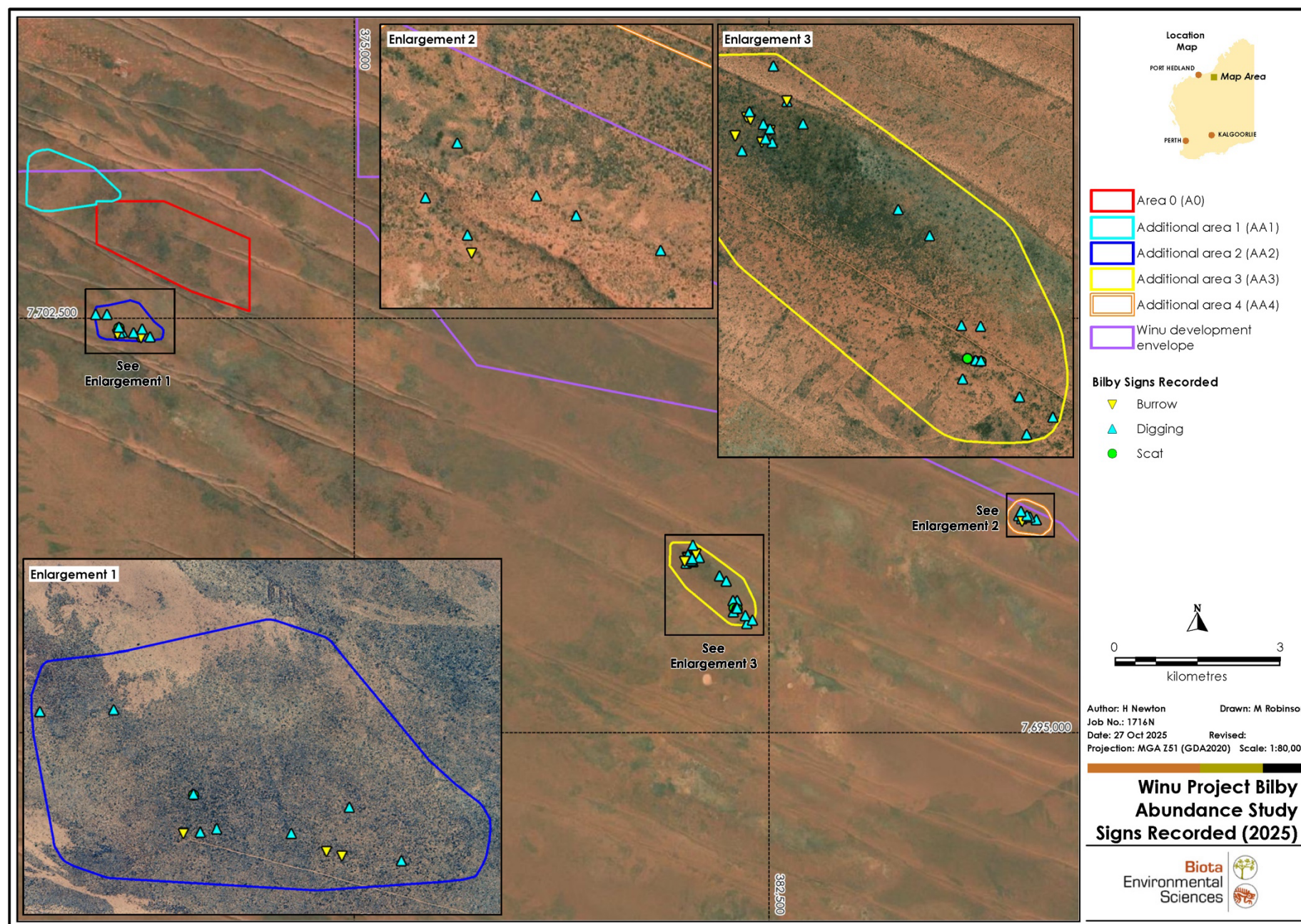


Figure 4.3: Bilby signs recorded during the current survey.

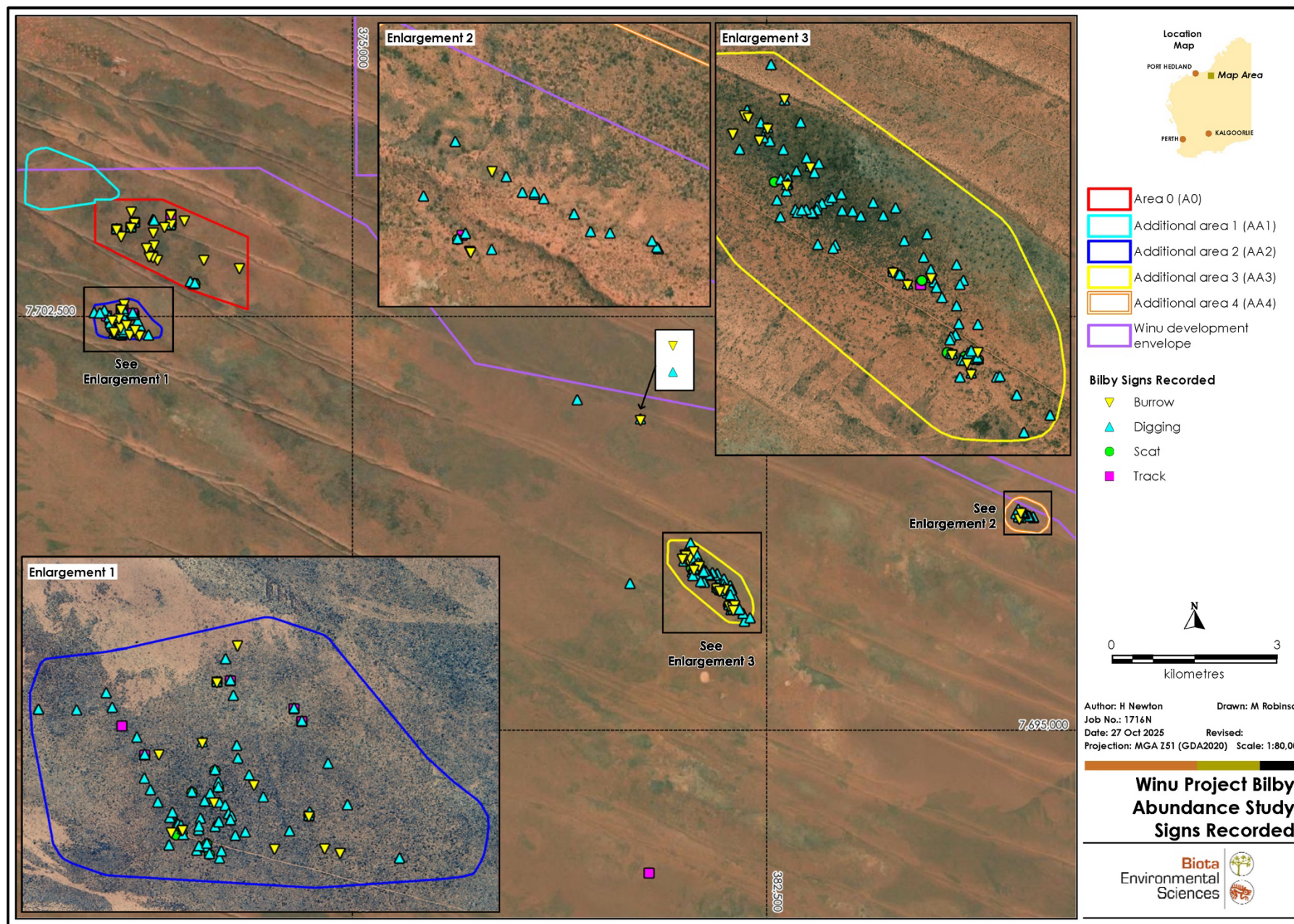


Figure 4.4: Compilation of Bilby signs recorded between 2019-2025.

4.3 Bilby Census

4.3.1 Genotyping of Individuals

A combined total of 59 Bilby scats have been collected within and near to the Winu DE over four years of surveying. Thirty-four of these were successfully genotyped, corresponding to ten individual bilbies (Table 4.2, Figure 4.5; Appendix 2).

Table 4.2: Scat analysis breakdown.

Area	Year	Number of scats collected	Number successfully genotyped
Area 0	2019	20	9
	2022	18	13
	2024	0	-
	2025	0	-
Additional Area 1	2024	0	-
	2025	0	-
Additional Area 2	2024	9	5
	2025	1	1
Additional Area 3	2024	7	4
	2025	3	1
Additional Area 4	2024	1	1
	2025	0	-
Total		59	34

The additional scat samples and improvements in data analysis techniques provides an updated census of the Winu Bilby population. Individual genotype groupings of scat samples are presented in Appendix 2 and displayed below (Figure 4.5). In this study, a ‘detection’ refers to a single scat sample, e.g. 12 scat samples belonging to individual A were recorded in Area 0 across two years. Locations of each individual detection are presented in Figure 4.6 and Figure 4.7.

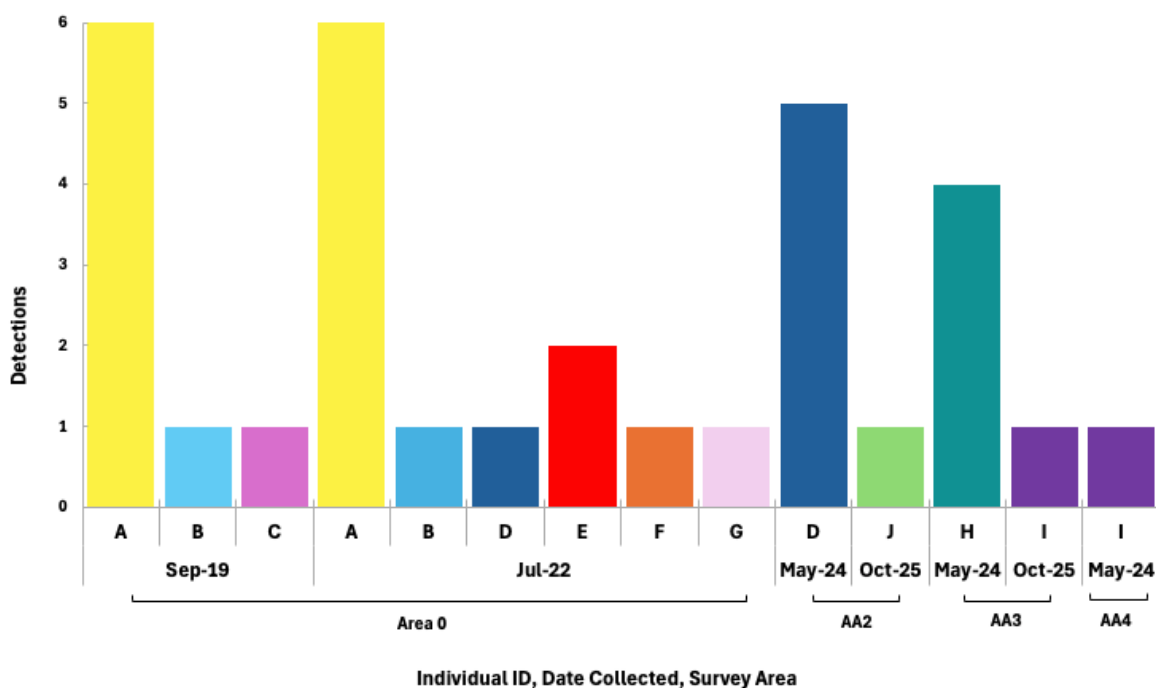


Figure 4.5: Bilby detections across survey years grouped by survey area.

4.3.2 SECR Analysis

A minimum of 10 recaptures are generally required for robust density estimation using SECR, with at least 20 cited as a more realistic minimum². During the combined surveys, only four of the ten individuals were 'recaptured' (detected again from scats collected on a subsequent phase), and only one was recaptured enough times to meet the suggested minimum (Individual A; Figure 4.5). SECR analysis has therefore not been utilised here, as the data obtained in this study is not reliable for the analysis and the findings would be uninformative. In such instances, and where a robust sampling approach is followed, the sequence data can be considered representative of a census only. In this case, the sequencing identified three different individuals from within A0 in 2019, six individuals from within A0 in 2022 (four new, two recaptures), three individuals from three different areas in 2024 (two new and one recapture), and two individuals in 2025 (one new in AA2 and one recapture in AA3) (Figure 4.6, Figure 4.7).

² (<http://www.phidot.org/forum/>).

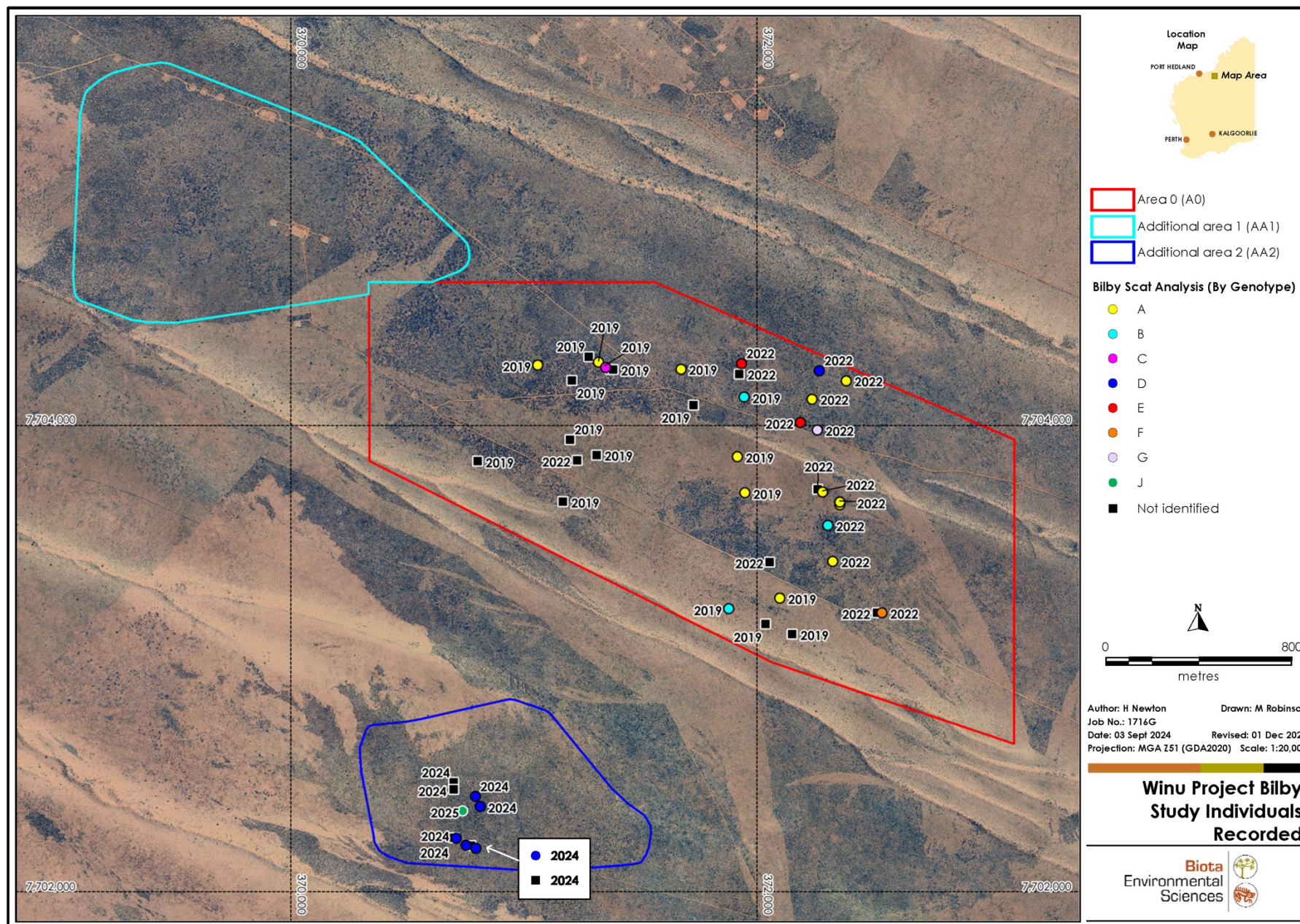


Figure 4.6: Bilby individuals recorded as determined through molecular genotyping - Map 1 of 2.

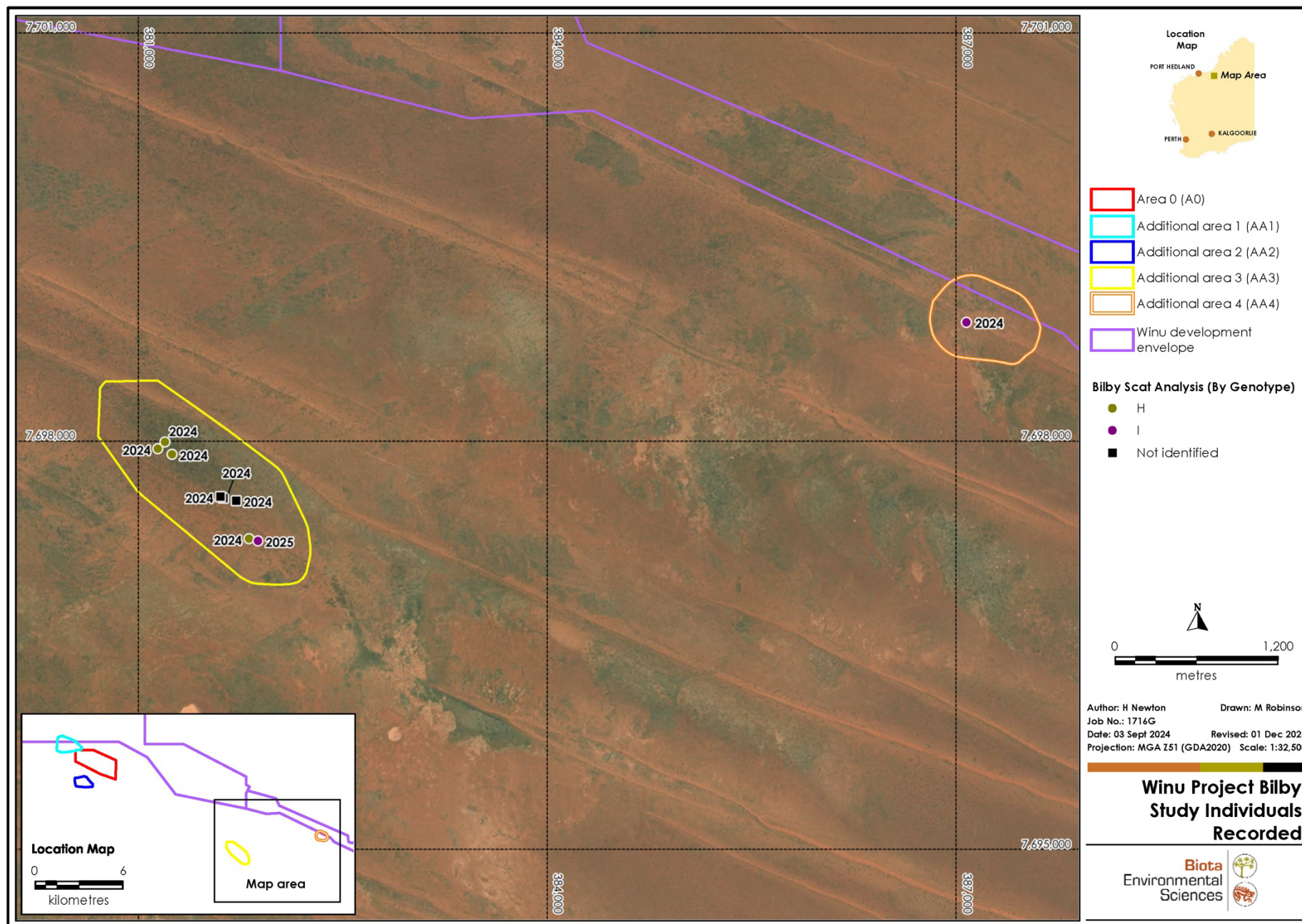


Figure 4.7: Bilby individuals recorded as determined through molecular genotyping - Map 2 of 2.

4.4 Feral Predators

Evidence of feral predators in the Winu DE and the current study area is common and not restricted to specific fauna habitats. Tracks attributable to both feral domestic cats (*Felis catus*) and red foxes (*Vulpes vulpes*) (and the similar tracks of dogs/dingos) are seen in the landscape frequently. No scats of either species or fox dens were recorded in the study area, and there were no obvious signs of the feral predator species occupying abandoned Bilby burrows.

Mining infrastructure such as office and camp establishments can act as predator-prey focal points due to increased availability of shelter and food sources (including human food waste, and small irrigated areas creating attractive natural vegetated patches). There is evidence of this in the Winu DE with cats and foxes being frequently observed on vehicle access tracks, and under transportable offices/accommodation units (pers. comms. on site RTW personnel). Furthermore, a recent trapping initiative undertaken by Animal Pest Management Services captured six foxes in the Winu DE (October 2025, pers. comm. RTW environmental advisor). The Winu Project Felixer trial has begun; one unit has been placed just north of Winu Camp, and the other in A0. At the time of writing, the trial was still in the 'photo only' mode (i.e. no targeted bait firing was occurring).

5.0 Discussion

5.1 Local Habitat Assessment

Five rationalised fauna habitats (plus disturbed/cleared areas) have been mapped as occurring in the Winu DE (Biota 2025a), comprising:

- Shrub and spinifex on sandplain;
- Inland sand dune;
- Gravelly lateritic rises;
- Clayey sandplain with termitaria;
- Ephemeral claypan; and
- Disturbed/cleared;

Two of these include potential areas of core habitat for Bilby; Shrub and spinifex on sandplain and Inland sand dune. The sandy soils provide the appropriate substrate to support burrows and host foraging plant species bilbies are known to favour. Gravelly lateritic rises may provide supporting habitat for the species, particularly if food source plants such as *Acacia hilliana* (Hill's Tabletop Wattle) are present.

The current Winu disturbance footprint constitutes an area of approximately 4,878 ha, 3,682 ha of this is mapped as Shrub and spinifex on sandplain (75.5%), and 815 ha is mapped as Inland sand dune (16.7%) (Figure 5.1, Figure 5.2). Whilst these fauna habitats can provide critical habitat for bilbies, previous surveys of the DE did not reveal any prominent areas of Bilby activity, with evidence of the species being most prevalent south of the DE. The current Winu disturbance footprint also encompasses areas already highly disturbed, with numerous tracks dissecting the area leading to bores, laydowns, and camp infrastructure.

Furthermore, the Winu Bilby population very closely aligns with the presence of *Acacia monticola* in the landscape, showing a clear preference for areas that contain large swathes of this plant species with an understory of *Triodia* spp. The Winu mine area is predominately mapped as Shrub and spinifex on sandplain, but the dominant shrub species here are *Acacia platycarpa*, *A. tumida* var. *kulparn*, *A. sabulosa*, and *Grevillea wickhamii* subsp. *hispidula* (Western Botanical 2025).

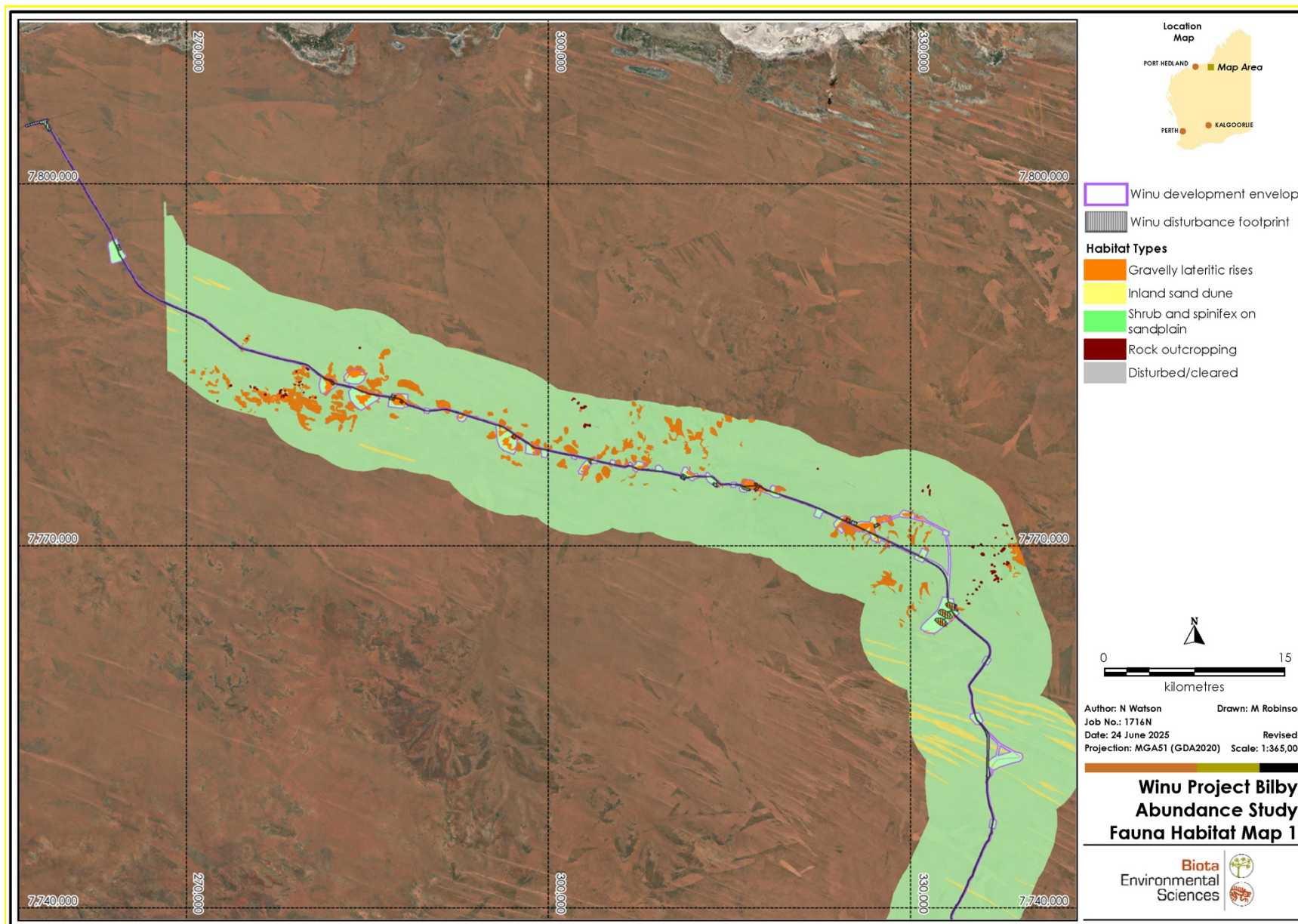


Figure 5.1: Rio Tinto Winu clearing footprint (as of October 2025) overlaid onto fauna habitats (Map 1 of 2).

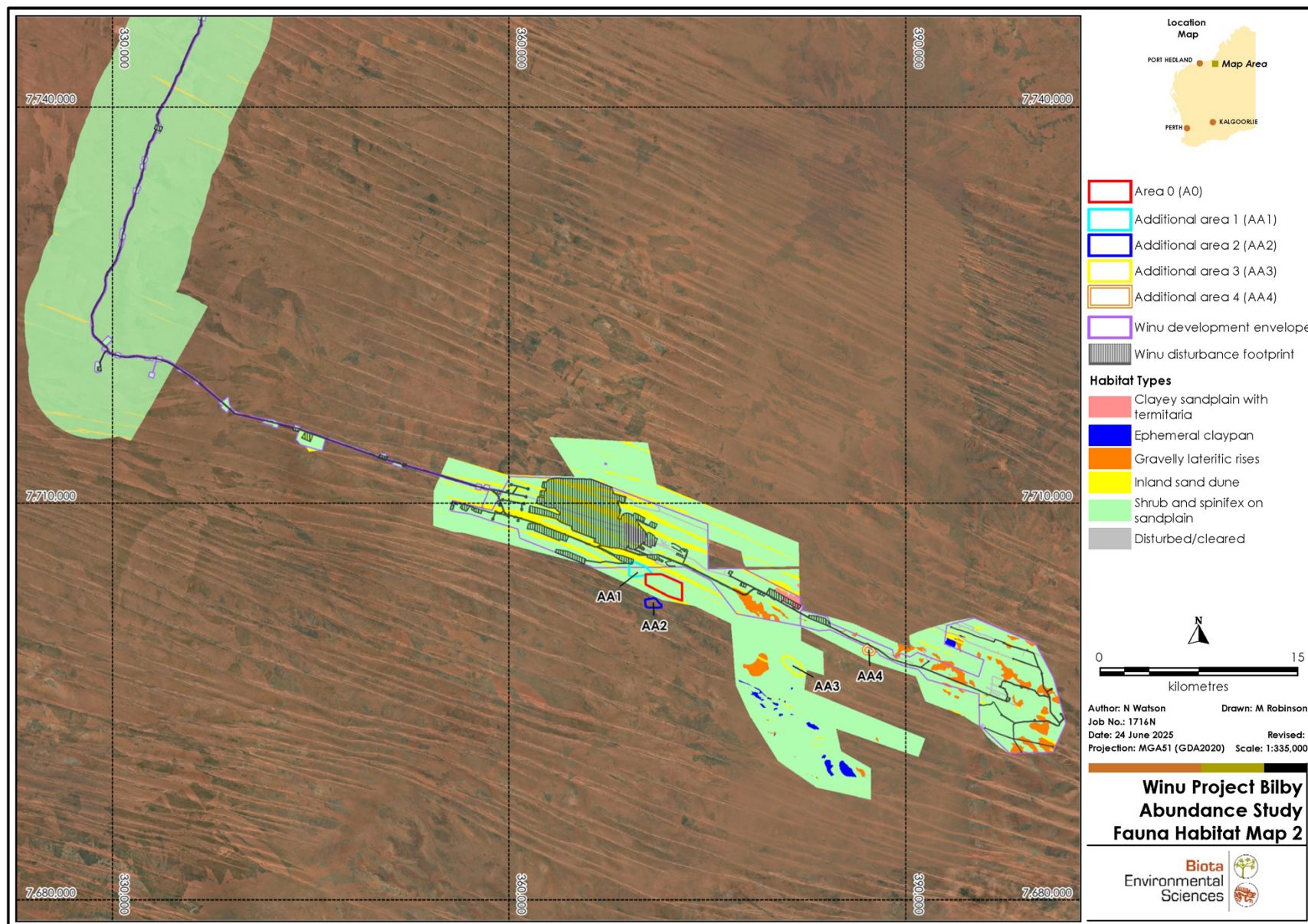


Figure 5.2: Rio Tinto Winu clearing footprint (as of October 2025) overlaid onto fauna habitats (Map 2 of 2).

5.2 Regional Habitat Assessment

5.2.1 Land Systems and Soil Landscapes

As described in Biota (2025), both land systems mapping for the Rangelands regions of WA (van Vreeswyk et al. 2004) and soil-landscape mapping by DPIRD (2018) were examined in order to place the fauna habitats of the Winu DE into a regional context. Four land systems have been mapped as occurring in the DE; Little Sandy, Nita, Callawa and Buckshot, and in the absence of land system mapping to the east of the MIA, two soil landscape units are represented, AB40 and B30 (Figure 5.3). A review of the descriptions of these two soil landscape units determined that they are most comparable to land units of the Little Sandy land system.

Two of the land systems encompass the areas containing suitable habitat for bilbies; Little Sandy and Nita, with each characterised by sandplains and linear and reticulate dunes supporting shrubby (hard and soft) spinifex grasslands, with occasional trees. The extent of these land systems present within the DE ranges from <1% to just over 2.85% of their broader extent across the McLarty, Mackay and Pindanland IBRA Subregions combined (Biota 2025a), indicating that habitat suitable for bilbies is not restricted to the Winu DE, rather is common and widespread in the broader region.

Our consolidated survey findings support this, with evidence of Bilby occurrence recorded at numerous sites in the surrounding locality (see Figure 2.2).

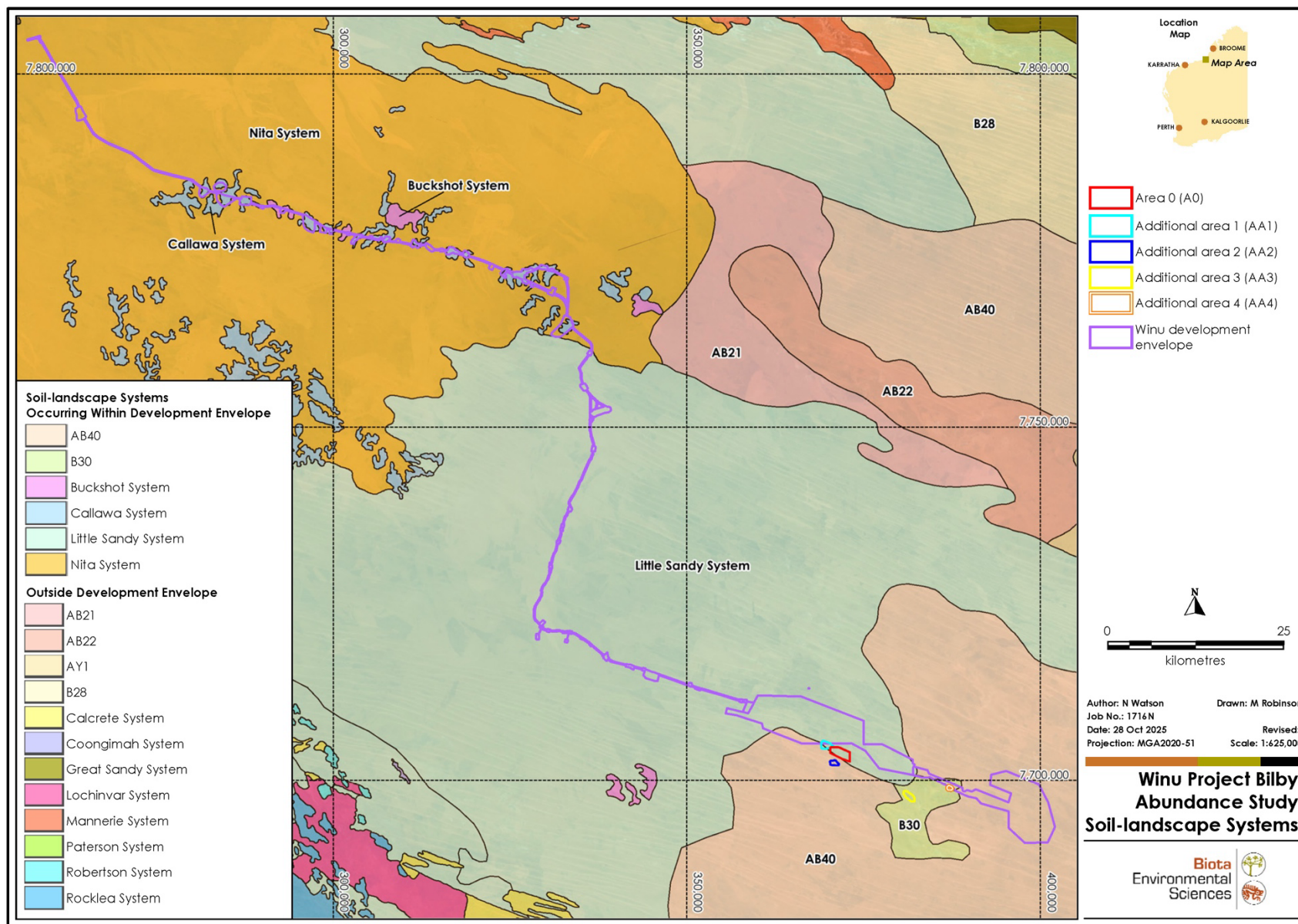


Figure 5.3: Soil-landscape systems occurring within the Winu DE.

5.2.2 Fire History

Fire has played a dominant role in shaping many of Australia’s ecosystems, particularly our desert landscapes. The Winu DE has been directly impacted by fire multiple times prior to, and since biological survey work began. Fire occurrences in the DE are summarised in Table 5.1 and mapped in Figure 5.4.

Table 5.1: Summary of the fire history of the Winu DE.

Area (ha)	Year burnt	Approximate area burnt (ha)
Mine Infrastructure Area	2018	6,663
	2022	1,797
	2023	3,035
Road Access Corridor	2017	836
	2018	2,626
	2019	1,388
	2022	170
	2023	2,923
Rimfire	2018	6,678
	2023	173

Due to their small and isolated nature, Bilby populations in the Pilbara and Great Sandy Desert are highly susceptible to the impacts of fire; large intense wildfires can lead to local extirpations, and lack of fire can lead to reduced habitat quality (Bradley et al. 2015, Northover et al. 2024). The relationship between Bilby occurrence and fire is complex, some studies indicate long-unburnt areas are preferred by the species (DCCEEW 2023, Moore et al. 2024), and others found areas with more homogenous vegetation, such as the Tanami Desert, have a positive relationship between Bilby occurrence and fire frequency (Southgate et al. 2007), likely due to the high availability of fire-promoted food plants (Southgate and Carthew 2007). A recent study found fire frequency to be the most important predictor in contemporary (2000-2022) ecological niche models, indicating that bilbies have contracted to areas with higher fire frequencies (Geyle et al. 2025).

The Winu Bilby population has demonstrated resilience to fire, with evidence of the species persisting in the landscape less than 2 km from where a fire event occurred in 2023 (Biota 2024b).

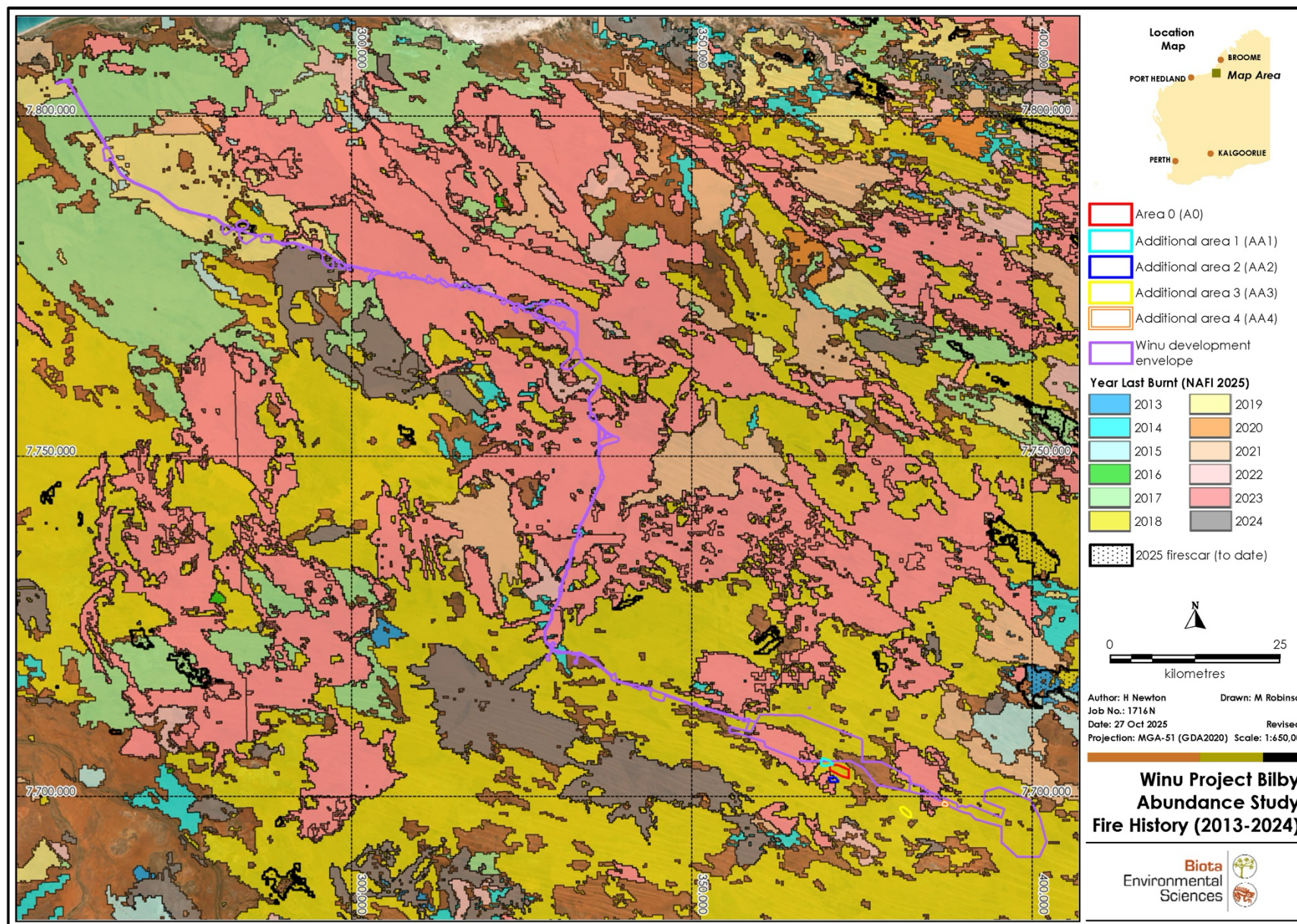


Figure 5.4: Fire history of the Winu DE (data from Northern Australia & Rangelands Fire Information).

5.3 Winu Bilby Population

Targeted surveys to detect signs of bilbies in the vicinity of the Winu DE have been undertaken on four occasions; initially in September 2019, followed by July 2022, May 2024 and lastly, in October 2025. Each survey phase was at different times of the year under varying climatic and environmental conditions (see Figure 3.2). The first targeted abundance survey took place towards the end of the dry season after the locality experienced below than average rainfall. The second survey was undertaken just over a month after a significant rainfall event occurred (May 2022). In 2023, a fire occurred in the locality, impacting around 50% of the suitable habitat in A0. The third survey was undertaken soon after a particularly wet, wet season and the current survey was undertaken at the end of the dry season.

The number of molecularly identified individuals across survey phases is 3, 6, 3 and 2, respectively. During the first three surveys, active burrows and recent diggings were recorded along with finding scat samples, indicating the individuals identified were likely occupying the survey area at the time of the surveys. In contrast, no active signs were recorded during the current survey (October 2025), with the scat collected being dry and aged. We conclude that no bilbies were using the study area at the time of the current survey. However, the recording of additional sign evidence and scat samples suggests bilbies were still utilising the study area for some time after the May 2024 survey.

5.3.1 Population Significance

Under Federal significant impact guidelines (Department of the Environment 2013), nationally threatened species populations are considered important if they:

1. represent key source populations either for breeding or dispersal;
2. are necessary for maintaining genetic diversity; and/or
3. are near the limit of the species' range.

Considered in isolation, the Winu Bilby population would not represent a key source population for breeding or dispersal within the Great Sandy Desert due to its low to moderate abundance, nor is it a population near the limit of the species' range. However, it likely represents a population necessary for maintaining genetic diversity in the bioregion. In this context, the Winu Bilby population is considered important.

5.3.2 Regional Population Comparison

Comparing the Winu Bilby population against regional data allows for a rudimentary comparison of population size. Bilby population abundance monitoring has been undertaken within at least 11 populations in the Pilbara (Dziminski et al. 2020). Monitoring has also been conducted in the Gascoyne, Gibson Desert and Dampierland bioregions (DBCA 2017b, 2018, 2021). Regional population estimates for 21 locations across these regions are summarised in Table 5.2.

The largest population recorded to date was at Anna Plains in 2017, with 44 (± 7) individuals (though this is considered an outlier population) (Table 5.2). The populations at Matuwa (Gascoyne) were also large, however these populations were in an area that has benefited from predator control, stock exclusion and fire management for more than 12 years (DBCA 2017b). By contrast, the remaining Pilbara, Gibson Desert and Dampierland populations for which abundance estimates are available, are largely unmanaged (DBCA 2017b). Bilby populations are generally small and geographically isolated, with most comprising fewer than 10 individuals (Northover et al. 2024). The findings of the Winu abundance study align, with an average of 3.5 individuals being recorded across four years of surveying (Table 5.2).

Table 5.2: Regional population abundance estimates for the Bilby.

	Pilbara													Gascoyne		Gibson Desert	Dampierland					
Monitoring Year	Turner River	Hillside	Nullagine	Yarrie	Warralong	Callawa	MvPhee Creek	Pardoo	Warralong Congan	Warralong River	Warralong O’Grady Well	Rail	Winu	Matuwa (Possum Lake)	Matuwa (New Market)	Kiwirrkurra	Anna Plains	Nita Downs	Thangoo	Yarri Yarri	Kurlku	Bawoorrooga
2013	-	-	-	-	-	-	2	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2014	2	3	2	-	-	-	-	1	-	-	-	2	-	-	-	-	-	-	-	-	-	-
2015	1	-	5	10	-	8	1	0	-	-	-	1	-	26	-	-	-	-	-	-	-	-
2016	2	-	8	2	6	-	0	0	6	-	-	2	-	29	21	4	-	-	-	-	-	-
2017	-	3	-	-	-	-	0	-	-	-	-	1	-	-	-	-	44	10	2	-	-	-
2018	-	-	-	-	-	-	-	-	3	7	-	2	-	-	-	-	-	-	-	-	-	-
2019	-	-	-	-	-	-	-	-	-	4	-	-	3	-	-	-	-	-	-	-	-	-
2020	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	4	13
2021	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-
2022	-	-	-	-	-	-	-	-	5	2	-	-	6	-	-	-	-	-	-	-	-	-
2023	-	-	-	-	-	-	-	-	12	3	-	-	-	-	-	-	-	-	-	-	-	-
2024	-	-	-	-	-	-	-	-	4	0	3	-	3	-	-	-	-	-	-	-	-	-
2025	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-
Average Abundance	1.7	3.0	5.0	6.0	6	8	0.75	1.8	6.0	3.2	3	1.6	3.5	27.5	21	4	44	10	2	6	4	13

Shaded columns indicate managed populations. Feral cat baits were deployed in the Warralong Bilby Land Management Area between 2022-2024 (Harrison et al. 2025).

6.0 Conclusion

The results of the current field survey revealed no recent Bilby activity in the four areas previously known to support the species. A search in an additional area of suitable habitat further south of the Winu DE did not reveal any sign evidence either. Molecular analysis of the four scat samples collected during the current survey had a 50% success rate, with two being successfully genotyped. One record is attributable to an individual identified in the 2024 survey, the other is a previously unidentified individual. The condition of the scat samples upon collection and the absence of other recent evidence in the survey area (fresh diggings, active burrows and/or tracks), strongly suggest that no bilbies were occupying the study area at the time of the survey. Rather, these are representative of individuals occupying the area sometime between the previous survey (May 2024) and the current (October 2025) survey.

Given bilbies' known propensity to move in response to resource availability and environmental variables, it is likely the population still exists and is currently occupying areas outside of the current scope of the study. The alternative hypothesis is that the population has been extirpated; whilst predators are known to occur within the area, the population has persisted since 2019 (and likely for years prior to this), and given no unusually extreme weather events have occurred in the past year, we do not think this to be the case. Future monitoring of the areas of High prospectivity habitat would allow us to verify if the Winu Bilby population returns. Educating on-site personnel on the importance of recording any Bilby sightings is recommended.

7.0 References

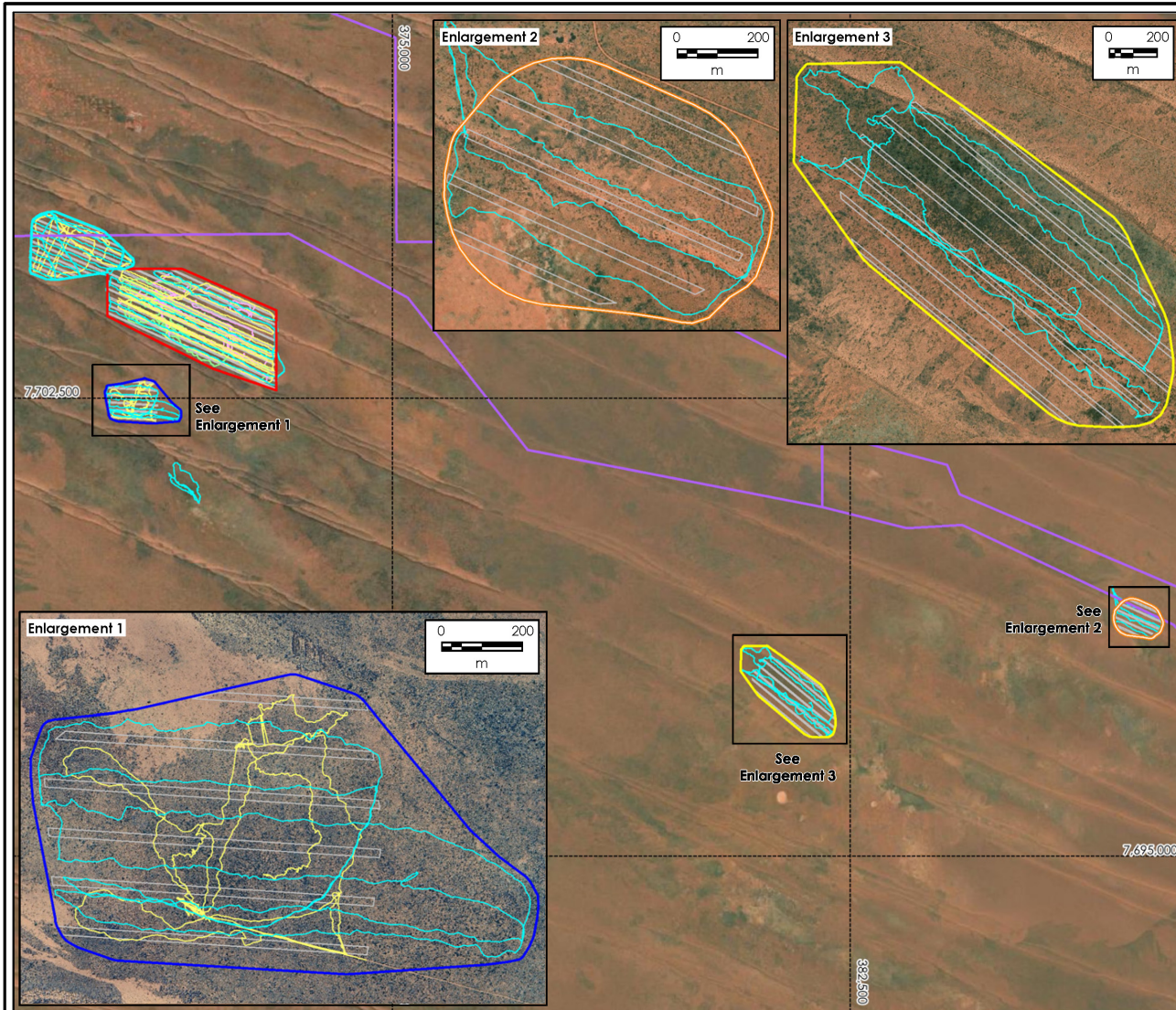
- Biota (2018). Asian Renewable Energy Hub Terrestrial Fauna and SRE Survey. Unpublished report prepared for NW Interconnected Power, Biota Environmental Sciences, Western Australia.
- Biota (2020). Winu Project - Bilby Abundance Study. Unpublished Report Prepared for Rio Tinto Winu Pty Limited, February 2020, Biota Environmental Sciences.
- Biota (2021). Winu Project Fauna Assessment. Unpublished report prepared for Rio Tinto Winu Pty Ltd, Biota Environmental Sciences, Leederville, Western Australia.
- Biota (2022a). Asian Renewable Energy Hub Detailed Fauna Assessment. Unpublished report prepared for NW Interconnected Power, Biota Environmental Sciences, Leederville, Western Australia.
- Biota (2022b). Winu Project Area Extension Targeted Fauna Survey. Unpublished report prepared for Rio Tinto Winu Pty Ltd, December 2022, Biota Environmental Sciences, Leederville, Western Australia.
- Biota (2023a). Winu Project Bilby Population Abundance Study. Unpublished report prepared for Rio Tinto, March 2023, Biota Environmental Sciences, Western Australia.
- Biota (2023b). Winu Project Water Borefield Targeted Fauna Survey. Unpublished report for Rio Tinto Winu Pty Ltd, March 2023, Biota Environmental Sciences, West Perth, Western Australia.
- Biota (2023c). Winu Project Native Vegetation Clearing Permit Fauna Clearance Survey. Unpublished report prepared for Rio Tinto Winu Pty Ltd, August 2023, Biota Environmental Sciences, West Perth, Western Australia.
- Biota (2023d). Winu Project Biological Gap Analysis. Unpublished report prepared for Rio Tinto, March 2023, Biota Environmental Sciences, Western Australia.
- Biota (2024a). Australian Renewable Energy Hub Targeted Fauna Survey. Unpublished report prepared for bp and GHD, Biota Environmental Sciences, West Perth, WA.
- Biota (2024b). Winu Project Bilby Population Abundance Study. Unpublished report prepared for Rio Tinto, August 2024, Biota Environmental Sciences, West Perth, WA.
- Biota (2025a). Winu Project Vertebrate Fauna Consolidation Report. Report prepared for Rio Tinto Winu Pty Limited, June 2025, Biota Environmental Sciences, West Perth, WA.
- Biota (2025b). Winu Project Vertebrate Fauna Consolidation Report. Unpublished Report, Biota Environmental Sciences, West Perth, Western Australia.
- Bradley, K., Lees, C, Lundie-Jenkins, G, Copley, P, R. Paltridge, M. Dziminski, R. Southgate, Nally, S, and Kemp, L (2015). Greater Bilby Recovery Summit 2015 Report and Interim Conservation Plan: an initiative of Save the Bilby Fund. Save the Bilby Fund.
- Carpenter, F., and M. Dziminski (2017). Breaking down scats: degradation of DNA from greater bilby (*Macrotis lagotis*) faecal pellets. *Australian Mammalogy* 39:197–204.

- Charles Darwin University (2022). North Australia and Rangelands Fire Information [WWW Document]. Retrieved from <http://www.firenorth.org.au/nafi3/>.
- Cramer, V. A., M. A. Dziminski, R. Southgate, F. M. Carpenter, R. J. Ellis, and S. van Leeuwen (2016). A conceptual framework for habitat use and research priorities for the greater bilby (*Macrotis lagotis*) in the north of Western Australia. *Australian Mammalogy* 39(2):137–151.
- DBCA (2017a). Guidelines for surveys to detect the presence of bilbies, and assess the importance of habitat in Western Australia. Version 1 - August 2017, Department of Biodiversity, Conservation and Attractions, Western Australia.
- DBCA (2017b). The conservation and management of the bilby (*Macrotis lagotis*) in the Pilbara. Progress Report 2016. Department of Biodiversity, Conservation and Attractions, Western Australia.
- DBCA (2018). Greater Bilby Survey: La Grange Project Area. Department of Biodiversity, Conservation and Attractions, Western Australia.
- DBCA (2021). Monitoring, mapping and safeguarding Kimberley bilbies. Department of Biodiversity, Conservation and Attractions.
- DCCEEW (2023). Recovery Plan for the Greater Bilby (*Macrotis lagotis*). Department of Climate Change, Energy, the Environment and Water, Canberra, Australia.
- Department of the Environment (2013). Matters of National Environmental Significance - Significant Impact Guidelines 1.1 *Environment Protection and Biodiversity Conservation Act 1999*. Department of the Environment, Canberra, ACT. Retrieved from https://www.dcceew.gov.au/sites/default/files/documents/neg-guidelines_1.pdf.
- DPIRD (2018). Soil landscape mapping Western Australia. Department of Primary Industries and Regional Development.
- DSEWPaC (2011). Survey Guidelines for Australia's Threatened Mammals: Guidelines for detecting mammals listed as threatened under the EPBC Act. Department of Sustainability, Environment, Water, Population and Communities, Canberra. Retrieved from <http://www.environment.gov.au/epbc/publications/threatened-mammals.html>.
- Dziminski, M. A., F. M. Carpenter, and F. Morris (2020). Monitoring the Abundance of Wild and Reintroduced Bilby Populations. *Journal of Wildlife Management* 85:240–253.
- Efford, M. (2004). Density estimation in live-trapping studies. *Oikos* 106:598–610.
- Efford, M. G., and R. M. Fewster (2012). Estimating population size by spatially explicit capture-recapture. *Oikos*. Retrieved from <http://onlinelibrary.wiley.com/doi/10.1111/j.1600-0706.2012.20440.x/abstract>.
- EPA (2020). *Technical Guidance: Terrestrial Vertebrate Fauna Surveys for Environmental Impact Assessment*. Environmental Protection Authority, Western Australia.
- Geyle, H. M., A. R. Young, B. P. Murphy, C. R. Dickman, and C. Schlesinger (2025). Declines in greater bilby (*Macrotis lagotis*) geographic range and realised niche are best explained by the invasive red fox (*Vulpes vulpes*). *Biological Conservation* 302. Retrieved from <https://doi.org/10.1016/j.biocon.2024.110872>.

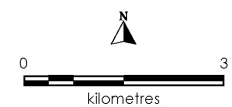
- Harrison, N., K. Ottewell, M. Milar, D. Fandino, and L. Gibson (2025). Bilby abundance monitoring at Warralong, Western Australia, 2024. DBCA.
- Liddle, N. R. (2016). The availability and use of root-dwelling larvae as a food source for the greater bilby, *Macrotis lagotis*, in the fire prone Tanami Desert of the Northern Territory. Charles Darwin University.
- Moore, H. A., Yawuru Country Managers, Bardi Jawi Oorany Rangers, Nyul Nyul Rangers, Nykina Mangala Rangers, L. A. Gibson, M. A. Dziminski, I. Radford, B. Corey, K. Bettink, F. M. Carpenter, R. McPhail, T. Sonneman, and B. R. Greatwich (2024). Where there's smoke, there's cats: long-unburnt habitat is crucial to mitigating the impacts of cats on the Ngarlgumirdi, greater bilby (*Macrotis lagotis*). *Wildlife Research* 51. doi: 10.1071/WR23117.
- Moseby, K., T. Nano, and R. Southgate (2012). *Tales in the sand: a guide to identifying Australian arid zone fauna using spoor and other signs*. Ecological Horizons Pty Ltd.
- Northover, A., M. Dziminski, F. Carpenter, Moore, Harry, K. Ottewell, Palmer, Russell, and L. Gibson (2024). Informing conservation management of the bilby (*Macrotis lagotis*) in the Pilbara: a review of research and future directions. *Australian Mammalogy* 46.
- Southgate, R. (2005). The results from animal track sampling in the Western Desert Tanami Project (Coyote and Larranganni Deposits). Unpublished report prepared for Biota Environmental Sciences, Envisage Environmental Services.
- Southgate, R., and S. Carthew (2007). Post-fire ephemerals and spinifex-fuelled fires: a decision model for bilby habitat management in the Tanami Desert, Australia. *International Journal of Wildland Fire* 16:741–754.
- Southgate, R., M. Dziminski, R. Paltridge, A. Schubert, and G. Gaikhorst (2019). Verifying bilby presence and the systematic sampling of wild populations using sign-based protocol – with notes on aerial and ground survey techniques and asserting absence. *Australian Mammalogy* 41:27–38.
- Southgate, R., R. Paltridge, P. Masters, and S. Carthew (2007). Bilby distribution and fire: A test of alternative models of habitat suitability in the Tanami Desert, Australia. *Ecography* 30:759–776.
- Thompson, G. G., and S. A. Thompson (2008). Greater Bilby (*Macrotis lagotis*) burrows, diggings and scats in the Pilbara. *Journal of the Royal Society of Western Australia* 91:21–25.
- Triggs, B. (1996). *Tracks, Scats and Other Traces - A Field Guide to Australian Mammals*. Oxford University Press.
- van Vreeswyk, A. M. E., A. L. Payne, K. A. Leighton, and P. Hennig (2004). *Technical Bulletin No. 92: An inventory and condition survey of the Pilbara region, Western Australia*. Department of Agriculture, South Perth WA.
- Western Botanical (2025). Consolidated Detailed Flora and Vegetation Assessment of the Winu Project, February 2025. Report prepared for Rio Tinto Winu Pty Limited, Perth WA.



Appendix 1 All Years Survey Effort Mapping



- Area 0 (A0)
- Additional area 1 (AA1)
- Additional area 2 (AA2)
- Additional area 3 (AA3)
- Additional area 4 (AA4)
- Winu development envelope
- Bilby transect design
- Foot traverse (2025)
- Foot traverse (2024)
- Foot traverse (2022)
- Foot traverse (2019)



Author: H Newton Drawn: M Robinson
 Job No.: 1716N
 Date: 28 Oct 2025 Revised:
 Projection: MGA Z51 (GDA2020) Scale: 1:80,000

Winu Project Bilby Abundance Study Sampling Effort



Appendix 2 Genotyping Bilby Scat Samples from Winu – Helix Molecular Solutions

Helix

Molecular
Solutions



Genotyping Bilby (*Macrotis lagotis*) Scat Samples from the Winu Project



Prepared for

Biota Environmental
Sciences

November 2025



© Helix Molecular Solutions Pty Ltd 2025
ABN 32 133 230 243
Building F, 661 Newcastle Street
Leederville, Western Australia 6007

Job No.: 911

Prepared by: Serina McConnell

Document Review History

Version: Rev A Peer review: Dr. Zoë Hamilton

Rev A Director review: G. Humphreys

Rev A Format review: G. Humphreys

Approved for issue: G. Humphreys

This document has been prepared to the requirements of the client identified on the cover page and no representation is made to any third party. It may be cited for the purposes of scientific research or other fair use, but it may not be reproduced or distributed to any third party by any physical or electronic means without the express permission of the client for whom it was prepared or Helix Molecular Solutions Pty Ltd.

Genotyping Winu Bilby (*Macrotis lagotis*) Scat Samples

Contents

1.0	Executive Summary	5
2.0	Introduction	6
2.1	Study Scope	6
3.0	Methods	7
3.1	DNA Extraction, PCR Amplification and Fragment Sizing	10
3.2	Genetic Diversity Analyses	11
4.0	Results	12
5.0	Discussion	16
6.0	References	17

Tables

Table 1.	October 2025 Winu Project area Bilby scat sample identification and collection details. Shaded cells represent samples that did not produce a useable genotype.	7
Table 2 .	Previously genotyped Winu Project area Bilby scat sample identification and collection details from May and July 2024. Shaded cells represent samples that did not produce a useable genotype.	7
Table 3.	Previously genotyped Winu Project area Bilby scat sample identification and collection details from July 2022. Shaded cells represent samples that did not produce a useable genotype.	8
Table 4.	Previously genotyped Winu Project area Bilby scat sample identification and collection details from September 2019. Shaded cells represent samples that did not produce a useable genotype.	9
Table 5.	Summary information for the seven microsatellite loci used on the bilby scat samples in all project phases. T optimal annealing temperature, B_p allele size range.	10
Table 6.	Genetic diversity statistics generated from the seven microsatellite loci for the unique Bilby genotypes ($n = 10$), collected by Biota from the Winu Project area. N_A – number of alleles, N_E – number of effective alleles, I – information index, F_{IS} - inbreeding coefficient, H_o - observed heterozygosity, uH_e - unbiased expected heterozygosity.	13

Table 7	Bilby scat identifiers, number of genotyped loci and genotype identity group assignment for scat samples collected across the Winu Project area during four sampling phases (2019, 2022, 2024, 2025).	13
---------	---	----

Figures

Figure 1	Multilocus genotype curve used to determine required number of loci for discrimination of unknown individuals from scat samples.	12
----------	--	----

1.0 Executive Summary

Helix Molecular Solutions (Helix) was engaged by Biota Environmental Sciences (Biota) to perform DNA extraction, microsatellite amplification and individual genotype identification using faecal (scat) samples suspected to belong to the Greater Bilby (*Macrotis lagotis*).

Suspected bilby scats were collected during a targeted survey in the Winu Project area, where bilby scats have been collected in previous years. The objective of this investigation was to identify unique individuals based on their multilocus genotypes and determine whether these individuals had been detected during previous surveys, or whether they represent 'new' / 'previously undetected' individuals.

DNA was extracted from four separate bilby scat samples, two of which produced DNA of sufficient quality and quantity to yield a clean genotype that was suitable for individual discrimination. Resulting genotypes were assigned to two distinct identity groups, each representing a unique individual.

One individual was new and previously undetected, whilst the other had previously been detected by Helix during the Biota 2024 survey at the Winu Project area (Helix and Biota, 2024). Throughout the four survey phases over the six years (2019, 2022, 2024, 2025), there have been a total of ten individual bilbies (unique genotypes) detected.

2.0 Introduction

2.1 Study Scope

Biota Environmental Sciences (Biota) engaged Helix Molecular Solutions (Helix) to determine the minimum number of individual Bilby (*Macrotis lagotis*) that were represented amongst scat samples collected from the Winu Project area during a single sampling period (10th – 15th October 2025).

This was undertaken by performing DNA extractions on the submitted scat samples, followed by genotyping those that yielded high quality DNA.

3.0 Methods

Scat samples submitted to Helix were stored in a sterile vial in silica-based desiccant to preserve DNA through the provision of a dry environment. These scat samples were medium to large in size and were moderately to very dry in condition upon receipt. Two to three scats (dependent on size and moisture level) were selected from each sample collection for DNA extraction. DNA was extracted and amplified from the four submitted scat samples suspected to belong to the Greater Bilby (*Macrotis lagotis*) (Table 1).

During analysis, reference genetic data were included from previous Biota collections within the Winu Project area to determine if the same individuals were detected across different phases of the survey (see Table 2 - Table 4).

Table 1. **October 2025 Winu Project area Bilby scat sample identification and collection details.**
Shaded cells represent samples that did not produce a useable genotype.

Scat ID	Helix ID	Collection date	Latitude	Longitude
1716N_20251010_AA3_HN-02	WC01	2025-10-10	-20.82083765	121.8651132
1716N_20251010_AA3_HN-03	WC02	2025-10-10	-20.82073872	121.8648234
1716N_20251010_AA3_HN-01	WC03	2025-10-10	-20.82084325	121.8653177
1716N_20251010_AA2_HN-01	WC04	2025-10-10	-20.7741618	121.7581648

Table 2. **Previously genotyped Winu Project area Bilby scat sample identification and collection details from May and July 2024.**
Shaded cells represent samples that did not produce a useable genotype.

Scat ID	Helix ID	Collection date	Latitude	Longitude
1716G.20240514.Scat01MG	TQ01	2024-05-14	-20.773988	121.758867
1716G.20240514.Scat02MG	TQ02	2024-05-14	-20.773596	121.758724
1716G.20240514.Scat03MG	TQ03	2024-05-14	-20.775514	121.758485
1716G.20240514.Scat04MG	TQ04	2024-05-14	-20.775498	121.758322
1716G.20240514.Scat05MG	TQ05	2024-05-14	-20.775205	121.757751
1716G.20240514.Scat06MG	TQ06	2024-05-14	-20.775212	121.757936
1716G.20240515.Scat01OKK	TQ07	2024-05-15	-20.773056	121.7577778
1716G.20240515.Scat02OKK	TQ08	2024-05-15	-20.773333	121.7577778
1716G.20240515.Scat01HN	TQ09	2024-05-15	-20.775612	121.758661
WPC07DS_MG-04	TQ10	2024-07-11	-20.81807396	121.8632568
WPC07DS_MG-03	TQ11	2024-07-11	-20.81790049	121.8624395
WPC07DS_MG-02	TQ12	2024-07-11	-20.81779267	121.8621782
WPC07DS_MG-01	TQ13	2024-07-11	-20.82060455	121.8641743

Scat ID	Helix ID	Collection date	Latitude	Longitude
WPC05DS_MG-01	TQ14	2024-07-10	-20.8145902	121.8578083
WPC02DS_HN-01	TQ15	2024-07-10	-20.80655146	121.9148679
WPC05DS_OKK-01	TQ16	2024-07-10	-20.814964	121.8587919
WPC05DS_OKK-02	TQ17	2024-07-10	-20.8141317	121.8582939

Table 3. Previously genotyped Winu Project area Bilby scat sample identification and collection details from July 2022.

Shaded cells represent samples that did not produce a useable genotype.

Scat ID	Helix ID	Collection date	Latitude	Longitude
S20220719_WAA03BT-01	SA01	2022-07-19	-20.7646475	121.7709263
S20220719_WAA03BT-02	SA02	2022-07-19	-20.766584	121.7753205
S20220719_WAA03BT-03	SA03	2022-07-19	-20.7665963	121.7754804
S20220719_WAA04BT-01	SA04	2022-07-19	-20.7645582	121.7735061
S20220719_WAA06BT-01	SA05	2022-07-19	-20.7618465	121.7729333
S20220719_WAA06BT-02	SA06	2022-07-19	-20.76192697	121.7731446
S20220719_WAA08BT-01	SA07	2022-07-19	-20.7591905	121.772217
S20220719_WAA08BT-02	SA08	2022-07-19	-20.7594693	121.7728629
S20220719_WAA09BT-02	SA09	2022-07-19	-20.7568523	121.7697594
S20220719.WAABT09-01	SA10	2022-07-19	-20.7583271	121.7727307
S20220719_WAA10BT-01	SA11	2022-07-19	-20.757588	121.7740931
S20220719_WAA10BT-02	SA12	2022-07-19	-20.7572303	121.7730023
S20220721.WAABO_rdv01-01	SA13	2022-07-21	-20.7632194	121.7732889
S20220721.WAABOPP_jke-01	SA14	2022-07-21	-20.7624495	121.773845
S20220721.WAABOPP_jke-02	SA15	2022-07-21	-20.7623018	121.7738011
S20220719.WAABTopp02-01	SA16	2022-07-19	-20.7572932	121.7696761
S20220719.WAABTopp03-01	SA17	2022-07-19	-20.75256241	121.8050556
S20220719.WAAopp-01_01.	SA18	2022-07-19	-20.76060883	121.763003

Table 4. Previously genotyped Winu Project area Bilby scat sample identification and collection details from September 2019.
Shaded cells represent samples that did not produce a useable genotype.

Scat ID	Helix ID	Collection date	Latitude	Longitude
S20190923.WINWBT07-01	PM12	2019-09-23	-20.7566412	121.7634752
S20190923.WINWBT07-02	PM13	2019-09-23	-20.7568333	121.7639071
S20190923.WINWBT07-03	PM14	2019-09-23	-20.7569615	121.7642162
S20190923.WINWBT07-04	PM15	2019-09-23	-20.7570847	121.764444
S20190923.WINWBT07-05	PM16	2019-09-23	-20.7570938	121.7645487
S20190923.WINWBT07-06	PM17	2019-09-23	-20.7585271	121.7678107
S20190923.WINWBT08-01	PM18	2019-09-23	-20.7582497	121.769879
S20190923.WINWBT08-02	PM19	2019-09-23	-20.7570561	121.7673443
S20190923.WINWBT01-01	PM20	2019-09-23	-20.7673683	121.7717549
S20190923.WINWBT01-02	PM21	2019-09-23	-20.7669568	121.7707239
S20190923.WINWBT01-03	PM22	2019-09-23	-20.7663859	121.7692259
S20190923.WINWBT02-01	PM23	2019-09-23	-20.7606075	121.75888
S20190923.WINWBT02-02	PM24	2019-09-23	-20.7621728	121.7623916
S20190923.WINWBT02-03	PM25	2019-09-23	-20.7659524	121.7712529
S20190923.WBT06-01	PM26	2019-09-23	-20.7604802	121.7695682
S20190923.WBT06-02	PM27	2019-09-23	-20.75747578	121.7628321
S20190923.WBT06-03	PM28	2019-09-23	-20.75686129	121.761419
S20190923.WBT05-01	PM29	2019-09-23	-20.76185487	121.7699319
S20190923.WBT04-01	PM30	2019-09-23	-20.7603867	121.7637985
S20190923.WBT04-02	PM31	2019-09-23	-20.7598171	121.7627399

3.1 DNA Extraction, PCR Amplification and Fragment Sizing

All scat samples submitted to Helix were extracted using the QIAGEN QIAamp Fast DNA Stool mini kit (Qiagen, Hilden, Germany, cat. no. 51604). Samples were washed in buffer as per Carpenter et al. (2017), followed by an incubation in the buffer solution overnight to allow for the maximum recovery of DNA, and then eluted as per the manufacturer's protocols ("Isolation of DNA from Stool for Pathogen Detection" (Qiagen, Feb 2020)).

Seven microsatellite loci (Moritz et al., 1997 and Smith et al., 2009, see Table 5) were amplified using the QIAGEN Multiplex PCR kit in triplicate. Polymerase Chain Reaction (PCR) products were analysed on an ABI3730XL Sequencer using Genescan-500 LIZ internal standard and scored using the software Geneious Prime® version 2024.0.7. Individual genotypes were based on molecular weights.

Table 5. Summary information for the seven microsatellite loci used on the bilby scat samples in all project phases.
T optimal annealing temperature, *B_p* allele size range.

Loci	Repeat bases	Primer sequences (5' - 3')	<i>T</i>	<i>B_p</i>
B22	(CA) ₁₆	F: GGT ATG AGG AAT TAG AAT TAC AGG	55	183-197
		R: CGG TAT TAA ATG GGC TAT GGA GT		
B63	(CA) ₁₄	F: CTT AGG CAA ATA GGG TGA AGT GG	55	232-268
		R: CAG AAC CAT TAG GAA GGA GTT TC		
B41	(CA) ₁₄	F: TGA CTT TCT TTT GCT ACA ACA ACC	55	171-191
		R: GGA AAA GTT TTT AGC CTA ATA GTG G		
B55	(CA) ₂₄	F: GCA CCA ACC TAT CCT CTT CAT TC	55	184-196
		R: CTA CAA GTC TGA TAA TTC CAG GC		
B02	(CA) ₁₇	F: GCA TGT ACT TAA CCC CCT TTG CC	55	170-183
		R: CCC GAC AAT CCA GCC TGT TAT TC		
B17	(CA) ₁₆	F: AGC CTG TGT GTC TTA AAA TGC	55	212-229
		R: CTC CAA TTC ACT TTT CCT GAG AC		
B56	(CA) ₂₃	F: CACACTTATACATACACGTACACG	55	157-170
		R: CAC TAA CAA ATA TGC TTG GGA AAG G		

3.2 Genetic Diversity Analyses

Genotype discrimination was performed using *R* software (R core team, 2022). The *R* package ‘poppr’ (Kamvar et al. 2014) was used to construct an accumulative genotype curve to assess whether the number of loci was sufficient to differentiate unique ‘multilocus genotypes’ (MLGs) from the genetic data of unknown individuals. Loci were randomly sampled until $n - 1$ loci (n being the total number of loci) and the number of observed genotypes were counted with each iteration. A distribution for each locus was generated by resampling 10,000 times without replacement. A plateau point in the curve indicated that the corresponding loci are sufficient to accurately describe the number of MLGs in the data (Kamvar et al. 2015). The ‘allelematch’ (Galpern et al., 2012) package was then used to discriminate between the identified MLGs and assign them to ‘genotype identity groups’, each of which equates to an individual animal. Assignment was confirmed through manual appraisal of the genetic data.

Verification of data quality and basic population genetic statistics were generated using *R* software (R core team, 2022) and the Excel add-in GenAEx version 6.5 (Peakall and Smousse, 2006, 2012). All unique individuals collected across the four sampling years were included in the following analyses and any duplicate individuals were excluded:

- the *R* package ‘PopGenReport’ (Adamack and Gruber, 2017) was used to assess the data quality in the form of ‘null alleles’, with the frequency of null alleles determined per locus using the method of Brookfield (1996);
- departures from Hardy-Weinberg equilibrium (*HWE*) were assessed for each locus and each sampling year with the *R* package ‘pegas’ (Paradis, 2010), using an exact test with 1,000 Monte Carlo permutations and $\alpha = 0.05$;
- the *R* package ‘poppr’ (Kamvar et al. 2014) was used to evaluate the level of missing data; and
- GenAEx (Peakall and Smousse, 2006; 2012) was used to calculate the number of alleles (N_A), number of effective alleles (N_E), observed and unbiased expected heterozygosities (H_O and uH_E respectively), the Information index (I) and the inbreeding coefficient (F_{IS}).

4.0 Results

Seven loci were successfully amplified in two of the four scat samples. Genotyping fails were associated with those samples of a 'dry and crumbly' consistency - a presentation likely resulting from extended exposure to the DNA degradative effects of the sun's ultraviolet radiation. The two resulting (successful) sample genotypes were combined with those generated during prior collections for analysis.

All seven loci were polymorphic and conformed to Hardy-Weinberg equilibrium. No null alleles were detected. The genotype accumulation curve indicated five loci were sufficient to accurately discriminate the number of unique individuals in this dataset (Figure 1); as such, sample genotypes of less than five loci were excluded from all further analyses (see Table 1 - Table 4).

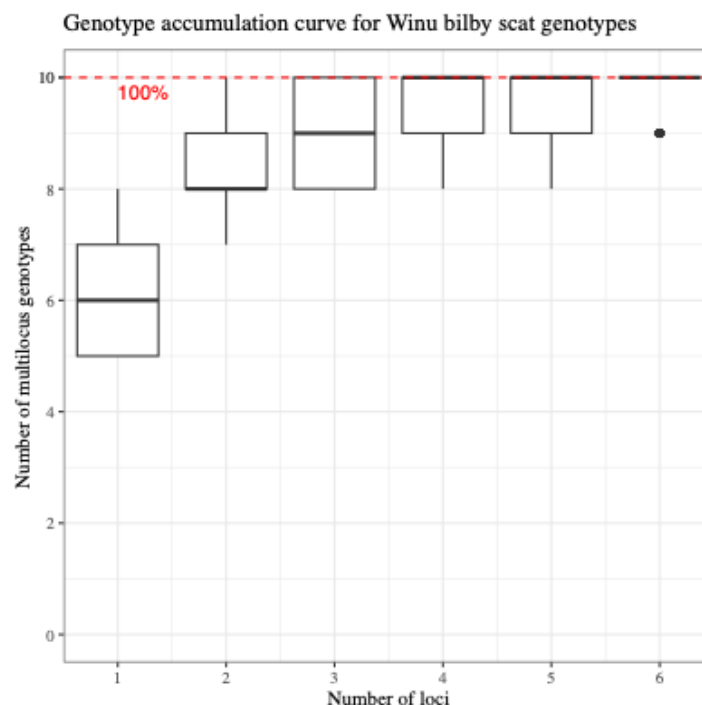


Figure 1. Multilocus genotype curve used to determine the required number of loci for identification of unknown individuals from scat samples.
Dotted line (---) depicts 100% discrimination of genotypes.

Missing data was insignificant and averaged 5% due to the inclusion of two individuals missing data from two loci (PM14, TQ17). The number of alleles (N_A) observed across the seven loci ranged from four to seven and averaged 5.71 (± 0.36) alleles (see Table 6). The number of effective alleles was relatively lower, ranging from 2.71 (locus 'B41') – 4.17 (locus 'B55') with an average of 3.52 (± 0.23) effective alleles (N_E) (see Table 6).

The information index (I) was moderate and averaged 1.42 (± 0.06) across loci (see Table 6). Observed (H_o) and unbiased expected (uH_e) heterozygosity's were similar and high, averaging approximately 75% (mean $H_o = 0.76 \pm 0.05$, mean $uH_e = 0.74 \pm 0.02$). No inbreeding was detected, exemplified by an inbreeding coefficient (F_{IS}) estimate close to zero (mean $F_{IS} = -0.07 \pm 0.06$).

Table 6. Genetic diversity statistics generated from the seven microsatellite loci for the unique Bilby genotypes ($n = 10$), collected by Biota from the Winu Project area.
 N_A – number of alleles, N_E – number of effective alleles, I – information index, F_{IS} – inbreeding coefficient, H_o – observed heterozygosity, uH_e – unbiased expected heterozygosity.

Loci	N_A	N_E	I	F_{IS}	H_o	uH_e
B55	6.00	4.17	1.57	-0.20	0.91	0.80
B22	6.00	2.80	1.32	0.09	0.58	0.67
B63	7.00	3.77	1.56	0.18	0.60	0.77
B41	6.00	2.72	1.24	-0.19	0.75	0.66
B02	4.00	3.17	1.21	-0.22	0.83	0.71
B17	6.00	4.00	1.54	-0.20	0.90	0.79
B56	5.00	4.03	1.49	0.03	0.73	0.79
Mean	5.71 ± 0.36	3.52 ± 0.23	1.42 ± 0.06	-0.07 ± 0.06	0.76 ± 0.02	0.74 ± 0.02

The two genotyped samples from the current October 2025 collection were assigned to two separate genotype identity groups, each representing a unique individual (Table 7). One genotype was newly detected and assigned the genotype identity ‘J’ (Table 7). The other, genotype ‘I’, had previously been detected in the 2024 collection and has now been detected a total of two times (Table 7).

When all four survey phases (2019, 2022, 2024 and 2025) for the project area are considered, a total of ten individual bilbies have been identified from the total 59 scat samples. Three individuals are unique to the 2019 collection (genotype IDs ‘E’, ‘F’ and ‘G’), two are unique to the 2024 collection (genotype IDs ‘C’ and ‘H’) and the newly detected genotype ID ‘J’ is unique to the 2025 collection (see Table 7). Genotypes detected across multiple phases indicate the persistence of individuals and encompass genotype IDs ‘A’ and ‘B’ in 2019 and 2022, genotype ID ‘D’ in 2022 and 2024, and genotype ID ‘I’ in 2024 and 2025 (Table 7).

Table 7 Bilby scat identifiers, number of genotyped loci and genotype identity group assignment for scat samples collected across the Winu Project area during four sampling phases (2019, 2022, 2024, 2025).

Scat Vial ID	Latitude	Longitude	Helix ID	Genotyped loci	Genotype ID
1716N_20251010_AA3_HN-02	-20.8208	121.8651	WC01	0	-
1716N_20251010_AA3_HN-03	-20.8207	121.8648	WC02	7	I
1716N_20251010_AA3_HN-01	-20.8208	121.8653	WC03	0	-
1716N_20251010_AA2_HN-01	-20.7742	121.7582	WC04	7	J
1716G.20240514.Scat01MG	-20.7740	121.7589	TQ01	7	D
1716G.20240514.Scat02MG	-20.7736	121.7587	TQ02	7	D
1716G.20240514.Scat03MG	-20.7755	121.7585	TQ03	3	-
1716G.20240514.Scat04MG	-20.7755	121.7583	TQ04	7	D
1716G.20240514.Scat05MG	-20.7752	121.7578	TQ05	0	-
1716G.20240514.Scat06MG	-20.7752	121.7579	TQ06	7	D
1716G.20240515.Scat01OKK	-20.7731	121.7578	TQ07	0	-
1716G.20240515.Scat02OKK	-20.7733	121.7578	TQ08	0	-
1716G.20240515.Scat01HN	-20.7756	121.7587	TQ09	7	D
WPC07DS_MG-04	-20.8181	121.8633	TQ10	0	-

Scat Vial ID	Latitude	Longitude	Helix ID	Genotyped loci	Genotype ID
WPC07DS_MG-03	-20.8179	121.8624	TQ11	0	-
WPC07DS_MG-02	-20.8178	121.8622	TQ12	3	-
WPC07DS_MG-01	-20.8206	121.8642	TQ13	7	H
WPC05DS_MG-01	-20.8146	121.8578	TQ14	7	H
WPC02DS_HN-01	-20.8066	121.9149	TQ15	7	I
WPC05DS_OKK-01	-20.8150	121.8588	TQ16	7	H
WPC05DS_OKK-02	-20.8141	121.8583	TQ17	5	H
S20220719_WAA03BT-01	-20.7646	121.7709	SA01	0	-
S20220719_WAA03BT-02	-20.7666	121.7753	SA02	6	-
S20220719_WAA03BT-03	-20.7666	121.7755	SA03	7	F
S20220719_WAA04BT-01	-20.7646	121.7735	SA04	7	A
S20220719_WAA06BT-01	-20.7618	121.7729	SA05	1	-
S20220719_WAA06BT-02	-20.7619	121.7731	SA06	7	A
S20220719_WAA08BT-01	-20.7592	121.7722	SA07	7	E
S20220719_WAA08BT-02	-20.7595	121.7729	SA08	7	G
S20220719_WAA09BT_JK-01	-20.7569	121.7698	SA09	7	E
S20220719.WAABT09-01	-20.7583	121.7727	SA10	6	A
S20220719_WAA10BT-01	-20.7576	121.7741	SA11	7	A
S20220719_WAA10BT-02	-20.7572	121.7730	SA12	7	D
S20220721.WAABO_rdv01-01	-20.7632	121.7733	SA13	7	B
S20220721.WAABOPP_jke-01	-20.7624	121.7738	SA14	7	A
S20220721.WAABOPP_jke-02	-20.7623	121.7738	SA15	7	A
S20220719.WAABTopp02-01	-20.7573	121.7697	SA16	0	-
S20220719.WAABTopp03-01	-20.7526	121.8051	SA17	7	D
S20220719.WAAopp-01_01.	-20.7606	121.7630	SA18	0	-
S20190923.WINWBT07-01	-20.7566	121.7635	PM12	0	-
S20190923.WINWBT07-02	-20.7568	121.7639	PM13	7	A
S20190923.WINWBT07-03	-20.7570	121.7642	PM14	5	C
S20190923.WINWBT07-04	-20.7571	121.7644	PM15	4	-
S20190923.WINWBT07-05	-20.7571	121.7645	PM16	4	-
S20190923.WINWBT07-06	-20.7585	121.7678	PM17	4	-
S20190923.WINWBT08-01	-20.7582	121.7699	PM18	7	B
S20190923.WINWBT08-02	-20.7571	121.7673	PM19	7	A
S20190923.WINWBT01-01	-20.7674	121.7718	PM20	0	-
S20190923.WINWBT01-02	-20.7670	121.7707	PM21	0	-
S20190923.WINWBT01-03	-20.7664	121.7692	PM22	7	B
S20190923.WINWBT02-01	-20.7606	121.7589	PM23	3	-
S20190923.WINWBT02-02	-20.7622	121.7624	PM24	5	-
S20190923.WINWBT02-03	-20.7660	121.7713	PM25	7	A

Scat Vial ID	Latitude	Longitude	Helix ID	Genotyped loci	Genotype ID
S20190923.WBT06-01	-20.7605	121.7696	PM26	7	A
S20190923.WBT06-02	-20.7575	121.7628	PM27	0	-
S20190923.WBT06-03	-20.7569	121.7614	PM28	7	A
S20190923.WBT05-01	-20.7619	121.7699	PM29	7	A
S20190923.WBT04-01	-20.7604	121.7638	PM30	0	-
S20190923.WBT04-02	-20.7598	121.7627	PM31	0	-

5.0 Discussion

Scat DNA extraction and genotype assignment has proven successful for determining the minimum number of unique bilby individuals detected from secondary evidence (scat samples) from the Winu Project area.

Genotyping was based on seven variable and informative loci. Analyses determined that at least five loci are required for accurate discrimination between individual genotypes, due to the genetic diversity of the bilby individuals and the variability of the microsatellite loci. Two of the four submitted scat samples produced good quality genomic DNA with a high amplification success rate of all seven loci.

Two unique individuals were detected and assigned the genotype identification codes '*I*' and '*J*'. Individual '*I*' had been previously detected from the 2024 samples from the project area, while Individual '*J*' is new and not previously detected. When all ten unique individuals detected across the four project phases are considered, genetic diversity of the sampled bilby population is high and inbreeding is negligible.

6.0 References

- Adamack A, Gruber B. 2014.** POPGENREPORT: simplifying basic population genetic analyses in R. *Methods in Ecology and Evolution*, **5**: 384-387. doi:10.1111/2041-210X.12158
- Brookfield JF Y. 1996.** A simple new method for estimating null alleles frequency from heterozygote deficiency. *Molecular Ecology*, **5**: 453-455. doi:10.1046/j.1365-294X.1996.00098.x
- Carpenter FM, Dziminski MA. 2017.** Breaking down scats: degradation of DNA from greater bilby (*Macrotis lagotis*) faecal pellets. *Australian Mammalogy*, **39**: 197-204
- Helix and Biota. 2024.** Genotyping Bilby (*Macrotis lagotis*) scat samples from the Winu project. *Report prepared for Biota Environmental Sciences*.
- Kamvar ZN, Brooks JC, Grünwald NJ. 2014.** Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ*, **2**: e281. doi:10.7717/peerj.281
- Kamvar ZN, Tabima JF, Grünwald NJ. 2015.** Novel R tools for analysis of genome wide population genetic data with emphasis on clonality. *Frontiers in Genetics*, **6**: 208. doi: 10.3389/fgene.2015.00208
- Moritz, C., A. Heideman, E. Geffen, and P. Mcrae. 1997.** Genetic population structure of the Greater Bilby, *Macrotis lagotis*, a marsupial in decline. *Molecular Ecology*, **6**:925–936.
- Paradis E. 2010.** pegas: an R package for population genetics with an integrated-modular approach. *Bioinformatics*, **26**: 419-420. doi:10.1093/bioinformatics/btp696
- Peakall R, Smouse PE. 2006.** GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**: 288-295.
- Peakall R. and PE Smouse 2012.** GenALEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*, **28**: 2537-2539.
- R Core Team 2022.** R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Smith S, McRae P, Hughes J. 2009.** Faecal DNA analysis enables genetic monitoring of the species recovery program for an arid-dwelling marsupial. *Australian Journal of Zoology*. **57**: 139–148.