

Main Roads Western Australia

Cape Leveque Road Upgrade (SLK 25 – 102.6) Roadkill Monitoring and Adaptive Management Plan

July 2016

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1. Introduction

This plan has been developed in response to requirements under *Environment Protection and Biodiversity Conservation Act* 1999 (EPBC Act) (EPBC 2013/6984) Cape Leveque Upgrade Project (SLK 25 – 102.6). It outlines Main Roads Western Australia's (Main Roads) commitment to monitor and manage roadkill along Cape Leveque from prior to the commencement of construction activities until the expiry of the Approval (31 December 2035). Condition 10 of the Approval requires the development of a Roadkill Monitoring and Adaptive Management Plan to detail baseline data on traffic, fauna abundance and roadkill. The Plan will also outline the methodology for recording roadkill data and the actions to be undertaken should certain thresholds of roadkill be observed.

The Cape Leveque Road is located in the Shire of Broome and runs from the Broome Highway, east of Broome townsite, to the northern Dampier Peninsula for a length of approximately 200 kilometres (km). The road is a main transport link, providing access for Aboriginal communities (including Beagle Bay, Lombadina, Djarindjin Kooglim and One Arm Point), pastoral stations, pearling industries and tourist destinations.

Various sections of the Cape Leveque Road have been upgraded to a sealed standard over the last ten years. Main Roads proposes to upgrade 77.6 km (SLK 25 to 102.6) of the unsealed section of the Cape Leveque Road. The proposed upgrade involves construction of a new sealed road generally parallel to the existing unsealed road. Road user safety and reduced maintenance are two of the key reasons for the proposed upgrade. Depending on the allocation of funding, it is anticipated that construction of the new road will take place during the 2016–2019 dry seasons (May to October).

Upgrading the Cape Leveque Road SLK 25-102.6 will involve the clearing of up to 297 ha of native vegetation. The vegetation to be cleared includes up to 297 ha of suitable foraging, breeding and sheltering habitat for the Greater Bilby (*Macrotis lagotis*), which is listed as vulnerable under the EPBC Act and the *Wildlife Conservation Act 1950* (WC Act). The GHD Targeted Bilby survey (GHD 2013) confirmed the presence of the species in the Project area (photographic, foraging evidence and burrows). While there is a lack of certainty with regards to the distribution and abundance of the Bilby on the Dampier Peninsula (Southgate 2015a), the Bilby on the Dampier Peninsular is considered an important population in accordance with the Department of the Environment (DotE) significant impact guidelines 1.1 (AECOM 2010) and this is supported by Southgate (Southgate 2012). It is one of the most northern populations known in Western Australia.

The Bilby is an omnivorous, usually solitary, nocturnal marsupial that lives in burrows during the day. The species National Recovery Plan is currently under review and an interim plan is being released (Bradley et al. 2015).

The 'National Recovery Plan for the Greater Bilby' directly linked roads to Bilby mortality (Pavey 2006). Bilby mortalities from collisions with vehicles have been recorded on public roads including the Stuart Highway (NT) and Peron Peninsula (Shark Bay, WA), where at least 6 mortalities have been recorded (C. Simms personal communication as cited in Pavey 2006). It is understood there is one known record for Bilby roadkill along the existing Cape Leveque Road (GHD 2013), and records of Bilby roadkill on sealed mine roads in the Pilbara (Stephen van Leeuwen, pers. comm.)

Table 1 describes the specific requirements of the condition (as listed in EPBC 2013/6984) and the means by which this RMAMP meets each requirement.

1.1 Purpose of document

This Roadkill Monitoring and Adaptive Management Plan (RMAMP) has been developed to address the requirements of the DotE EPBC 2013/6984 condition 10:

"To ensure there is no decline in the local Greater Bilby population as a result of roadkill on the sealed Cape Leveque Road, the person taking the action must submit a Roadkill Monitoring and Adaptive Management Plan (RMAMP) for the Minister's approval. The RMAMP must provide sufficient detail (timing, effort and methodology) to detect the level of impact of roadkill on the local Greater Bilby population during the construction phase and operational phase. Commencement of the action must not occur unless the Minister has approved the RMAMP. The approved RMAMP must be implemented".

This document specifies the:

- Methods that Main Roads will employ to monitor the baseline local Greater Bilby population and rate of roadkill of the Greater Bilby during the construction and operational phase of the project (Condition 10b, 10c, 10d)
- Trigger values for assessing the significance of change to the roadkill rate due to the controlled action (sealing the road) (condition 10e)
- Adaptive management approach that will be implemented should the trigger values be exceeded (Condition 10f)
- Reporting schedule for the monitoring and adaptive management programs (Condition 10g)

Condition	Addressed	
10 a) The RMAMP is to be developed and endorsed by a suitably qualified ecologist and a linear infrastructure ecologist (as defined in EPBC 2013/6984)	The primary author Jo Kuiper has over 15 years' experience as an ecologist working with threatened Australian mammals in applied management and consultancy.	
	Craig Grabham who reviewed this plan has over 15 years' experience working as an ecologist with linear infrastructure projects.	
	The RMAMP has been independently reviewed and updated based on comment and advice provided by Department of Parks and Wildlife (DPaW).	
10 b) The RMAMP include survey methodology and	Methodology.	
effort to be implemented that are sufficient to determine the baseline local Greater Bilby population and the location of high density Greater Bilby areas (as defined in EPBC 2013/6984)	Section 2.4 details survey methodology and effort	
10 c) The RMAMP include sufficient monitoring	Methodology.	
methodology and effort to determine baseline Greater Bilby roadkill rates on the unsealed Cape Leveque	Section 2.5 details survey	

Table 1 Specifications of conditon 10 EPBC 2013/6984

Condition	Addressed
Road prior to commencement of the action	methodology and effort
10 d) The RMAMP include sufficient monitoring methodology to determine Greater Bilby roadkill rates on the sealed Cape Leveque Road during the construction phase and operational phase	Methodology. Section 2.6. and 2.7 details survey methodology and effort
10 e) The RMAMP include appropriate Greater Bilby roadkill trigger values (as defined in EPBC 2013/6984) and adaptive management measures to be implemented should Greater Bilby roadkill trigger values be reached during the construction phase and operational phase	Trigger values and management Section 3 details roadkill trigger values (section 3.1) and adaptive management measures (section 3.2)
10 f) The RMAMP include the requirement for ongoing monitoring and adaptive management measures until such time as it can be demonstrated that there is no decline in the local Greater Bilby population as a result of roadkill for three successive years	Trigger values and management Section 3 details roadkill trigger values (section 3.1) and adaptive management measures (section 3.2)
10 g) The RMAMP include the requirement to provide a report on survey findings and effectiveness of adaptive management to the Department annually by 30 June each year following commencement of the action, until such time as it can be demonstrated that there is no decline in the local Greater Bilby population as a result of roadkill for three successive years	Reporting Reporting schedule is provided in section 4.2

1.2 Scope and limitations

This report has been prepared by GHD for Main Roads and may only be used and relied on by Main Roads for the purpose agreed between GHD and the Main Roads as set out in section 1.1 of this report.

GHD otherwise disclaims responsibility to any person other than Main Roads arising in connection with this report. GHD also excludes implied warranties and conditions, to the extent legally permissible. The services undertaken by GHD in connection with preparing this report were limited to those specifically detailed in the report and are subject to the scope limitations set out in the report.

The opinions, conclusions and any recommendations in this report are based on conditions encountered and information reviewed at the date of preparation of the report. GHD has no responsibility or obligation to update this report to account for events or changes occurring subsequent to the date that the report was prepared. The opinions, conclusions and any recommendations in this report are based on assumptions made by GHD described in this report. GHD disclaims liability arising from any of the assumptions being incorrect.

GHD has prepared this report on the basis of information provided by Main Roads and others who provided information to GHD (including Government authorities), which GHD has not independently verified or checked beyond the agreed scope of work. GHD does not accept liability in connection with such unverified information, including errors and omissions in the report which were caused by errors or omissions in that information.

2. Monitoring methods

2.1 Objective of monitoring

The objective of the monitoring is to identify the level of impact of roadkill on the local Greater Bilby population during the construction phase and operational phase of the Project. The monitoring program aims to:

- Determine the baseline local Greater Bilby population and the locations of higher density areas as defined in EPBC 2013/6984
- Determine baseline Greater Bilby roadkill rates on the unsealed Cape Leveque Road prior to commencement of the construction phase of the project
- Determine Greater Bilby roadkill rates on the sealed Cape Leveque Road (the Project area) during the construction phase of the project
- Determine Greater Bilby roadkill rates on the sealed Cape Leveque Road (the Project area) during the operational phase of the project

2.2 Sampling methods consideration

The monitoring methods were chosen in light of the following:

- Estimations of population size and abundance for wild populations of fauna are inherently difficult. There is no currently accepted standard sampling methodology for the Greater Bilby as noted as a key point in the recent (February 2015) National Bilby Summit (M. Dziminski pers. comm.)
- The survey effort will assess all habitats in the Project area due to the temporal variation of the suitability of habitats (mainly due to fire events that occur regularly through the Dampier Peninsula landscape) and spatially to account for the assumed wide ranging and nomadic behaviour of the Bilby.
- The need for repeatable sampling methods after the road is sealed that allow for comparison of roadkill rates before, during and after the project construction phase.
- The paucity of previous studies trying to meet the same (or similar) objective (i.e. there are no published studies evident that have aimed to assess the impacts that a road upgrade has to the roadkill rate to the Greater Bilby). As such, there is a lack of proven and reliable sampling methods for this type of program. The selection of monitoring methods has drawn on studies that have similar aspects such as targeting ground dwelling animals in similar environments and for similar road projects. Selection of monitoring methods have been guided by:
 - Discussions with Rick Southgate and Main Roads (Southgate 2015a)
 - Review of Moseby, Nano and Southgate (2009)
 - Review of Lester and Livesey (2012) (Public Environmental Report, Tarkine Forest Drive Tasmania EBPC ref 2011/6210)
 - Review of outcomes of the February 2015 National Bilby Summit, Gold Cost (<u>https://sites.google.com/site/cbsggreaterbilbies/home/library</u>).
 - Peer review of methods by DPaW
- Given there is a lack of proven and reliable sampling techniques, a wider suite of techniques has been selected to allow for different limitations of each technique

 Previous studies of mitigation measures suffer from a lack of replication and a control group (Lester and Livesey 2012). As such, this study has employed methods that provide before-after-control-impact (BACI) results for more robust comparisons between the roadkill rates before and after project construction and both within and outside the Project area

2.3 Consultation

The Draft Roadkill Monitoring and Adaptive Management Plan was reviewed by DPaW prior to submission to DotE. DPaW provided advice with respect to the monitoring method and effort proposed in this plan.

2.4 Baseline local Greater Bilby population and the location of higher density areas

The local Greater Bilby population (as defined in EPBC 2013/6984) along the length of Cape Leveque Road SLK 25-102.6 will be assessed to determine its distribution and the number of individuals within a 200m wide corridor

This survey will also be used to determine the location of high density Greater Bilby areas. A high density Greater Bilby area is defined in EPBC 2013/6984 as any 6 ha area that includes or is immediately adjacent to the proposed disturbance route that contains 3 or more signs of Greater Bilbies. Signs of Greater Bilby may include sightings (in person or via camera), active burrows, inactive burrows, diggings, or scats.

To determine the local Greater Bilby population and high density Greater Bilby areas the following monitoring methodology will be employed. The survey will be conducted by an experienced Greater Bilby survey team of at least two observers.

Once the monitoring is complete, this RMAMP will be updated with the finalised report included in Appendix A.

2.4.1 Presence / absence survey

- Standard sampling plots will be established and surveyed at 1 km intervals along the proposed road alignment, within the 200 m corridor (100 m either side of the proposed road centreline)
- The plots will be positioned so that they are not impacted directly by the construction of the road
- Each plot would be 2 hectares (ha) in size (approximately 200 m x 100 m) and be searched by two observers for all signs of the Greater Bilby occurrence (i.e. tracks, droppings, burrows, diggings)
- Estimates of recent Greater Bilby occupation of the area will be made, and Greater Bilby scats will be collected for DNA analysis and estimation of numbers of bilbies in that area. The methodology and protocols for DNA analysis will be approved by DPaW
- Observations of other fauna of interest (e.g. olive pythons, foxes, feral cats) will also be recorded
- Where active burrows or diggings are located, motion sensor cameras will be deployed for at least one week to confirm the presence/absence of the Greater Bilby

2.4.2 Distribution and relative abundance survey

Sampling plots will be established along the proposed road alignment to determine the distribution and relative abundance of the local Greater Bilby population. This will involve:

- A minimum of 12 sampling plots will be established (approximately evenly spaced) over the full extent of the Project area at sites where the presence/absence survey (Section 2.4.1) indicated Bilby presence. The 2 ha quadrats may extend beyond the 200m corridor
- Each plot would be 2 ha in size (approximately 200 m x 100 m) and established at locations where the presence / absence survey (Section 2.4.1) indicated Greater Bilby presence
- Plots will be marked with corner dropper posts and the GPS location of these recorded
- Each plot will be systematically searched for Greater Bilby presence / absence and quantitative measures of Greater Bilby presence recorded. These will include number and freshness of bilby tracks, burrows and diggings
- Greater Bilby scats will be collected and DNA analyses undertaken to identify the numbers and identity of individual bilbies occupying each quadrat
- The first baseline monitoring would occur immediately following the establishment of the sampling plots. The second and third baseline monitoring would occur at approximately one monthly intervals thereafter

2.4.3 Data collection for plot sampling

Data from each plot will be recorded using a standardised method and include presence or absence for:

- The type of evidence (i.e. tracks, droppings, burrows, diggings)
- Unique ID for scats collected for DNA analysis
- The age class of each piece of evidence; fresh (within 3 days), recent (within the preceding week) or old (older than a week)
- Data collected from the motion sensor camera will be used to record Bilby visits, however it is recognised that motion cameras will not provide a reliable and accurate method of determining visits per night.

2.4.4 Timing of Survey to determine Baseline local Greater Bilby Population and the locations of high density areas

The survey to identify the local Greater Bilby population and high density Greater Bilby areas will be undertaken prior to the commencement of the action.

The survey will be completed in parallel with the Department of Parks and Wildlife (DPaW) offset program *Bilby Offset Priorities for Dampier Peninsula Populations, West Kimberley, October 2014.* The offset program is intended to extend over 3 years and will identify the abundance of the Greater Bilby regional population, key threats and on-ground threat management actions that could be deployed in an adaptive management program.

2.5 Baseline roadkill data prior to commencement of construction

To better understand processes controlling roadkill rates along the Cape Leveque Road SLK 25-102.6, baseline monitoring of roadkills of vertebrates including threatened, protected, native

and introduced species will be undertaken twice a week for a period of 8 weeks prior to construction. It is not intended that monitoring will occur on the same day each week, rather, monitoring will be undertaken on any day within a given week. Roadkill monitoring will be conducted at 50km/hr.

The following data will be collected:

- Date
- Time
- GPS location coordinates
- Photographic evidence of the roadkill (where the species is not known) and all Bilbies, to assist with animal identification
- A DNA sample of the Bilby (depending on the condition of the roadkill) to determine sex, and where possible, age
- Any noteworthy circumstances including circumstances of injury death where relevant and known
- Lunar cycle
- Weather condition preceding the survey

Additional control sites will be established on sections of the Cape Leveque Road that are outside the Project area and already sealed (SLK 0-12.7 and SLK 102.6-135).

Any roadkill that is identified will be removed from the edge of the road to a distance of approximately 40 meters.

2.5.1 Monitoring of traffic

Vehicle count data will be established at four locations along the Cape Leveque Road and an assessment of the volume of traffic, peak travel times, and the speed of travel will be recorded.

2.6 Roadkill monitoring during construction

Monitoring and recording of roadkill during the construction phase of the project will be undertaken employing the same methodology outlined in section 2.5. The monitoring will be undertaken twice a week during construction.

2.7 Roadkill Monitoring during operation

Once the constructed section of road is operational, monitoring will be undertaken twice a week post construction for 16 weeks then once a week for 3 months (using the same methodology outlined in section 2.5). Roadkill monitoring will be repeated annually following the completion of each of the stages of construction of the Project, until such time as monitoring data can demonstrate there is no decline in the local Greater Bilby population as a result of roadkill for three successive years.

Roadkill data will be compiled into Roadkill Monitoring Reports for each monitoring cycle. This data will be included in the annual compliance report required by the conditions of EPBC 2013/6984.

2.8 Analysis of results

Results from the various monitoring methods will present a range of data including:

- Baseline local Greater Bilby population data will be presented for each sampling plot, for the Project area and for areas abutting (already) sealed sections of Cape Leveque Road outside the Project area.
- Where appropriate an assessment of Bilby population density will occur and be presented. Density will be calculated by dividing the individual animal number estimate by the area surveyed.
- Locations of high density Greater Bilby areas (as defined by EPBC2013/6984) based on areas of higher activity
- Baseline roadkill counts and locations for roadkill incidents of vertebrates, including the Greater Bilby
- An assessment of traffic volume, speed and peak travel times.

There is potential for the Bilby population in the local area to fluctuate in response to factors unrelated to the Project such as the occurrence of fire events in the broader landscape that may cause individuals to move into the Project area. These external factors will be investigated and documented where a detailed investigation is required by the roadkill trigger levels.

3. Adaptive management

3.1 Trigger levels

Consistent with condition 10e of EPBC 2013/6984, this Plan stipulates 'trigger levels' and corresponding actions in relation to construction and operational monitoring results. A conservative approach has been taken with the setting of the trigger level, which is \geq 1 observed Greater Bilby roadkill count per monitoring campaign.

This trigger level will be reviewed and may be revised in consultation and approval from DotE based on the results of the baseline roadkill monitoring data collected prior to the commencement of construction (Section 2.4). The results of the monitoring will also be included in Appendix A.

3.2 Adaptive management approach

Adaptive management will be implemented in a staged approach where the Greater Bilby roadkill trigger level is exceeded within a construction or operational monitoring campaign (Stage One). Stage Two involves a detailed investigation to determine the reason for the roadkill increase. Where the investigation confirms this increase in roadkill prevalence is primarily caused by the road upgrade, Stage Three is initiated. Stage Three will identify, refine and select additional appropriate mitigation and/or management measures to reduce the adverse impacts identified. The staged adaptive management approach is presented in Figure 1.

3.2.1 Stage One – Construction and Operational Monitoring

The roadkill monitoring in Stage One will be consistent with that specified in Sections 2.6 and 2.7.

Trigger level assessment

If no Greater Bilby roadkills are observed during the monitoring campaign, no further action will be implemented other than collate and report these results annually to DotE.

If monitoring over a campaign registers a Greater Bilby roadkill count of \geq 1, then a rapid highlevel response will occur, followed by a more detailed investigation (Stage Two) at the completion of the monitoring campaign.

Rapid response

In the event that a Bilby roadkill incident is recorded during the monitoring campaign this incident will be reviewed via a rapid high-level investigation. The assessment will consider contributing factors that may have clearly contributed to the roadkill incident such as unusual traffic volumes during the monitoring campaign, fire or flooding in the local area and potentially the health of the individual (if detectable). The investigation will include assessment of the location of the incident for any aspects that may have increased the risk of the incident. For example, the area will be checked for:

- Build-up of debris in the culverts at / near the location that may have otherwise facilitated Bilby movement
- Fallen timber or high grass along the verge (that may have reduced visibility / avoidance)

The incident and outcome of the location assessment will be reported to the Main Roads Environment Officer. Remedial maintenance action (i.e. removal of debris from culverts, vegetation clearing along verge) will be implemented as soon as practical if this is determined to be a key contributing factor. Main Roads will complete the monitoring campaign as specified in Section 2 whatever the outcome of the rapid high-level investigation.

3.2.2 Stage Two – Detailed Investigation

The purpose of the detailed investigation will be to identify the possible reasons for higher than expected levels of roadkill observed during the construction or operational monitoring campaign.

Investigative study

A study will investigate if the roadkill rate observed is due to increased animal abundance, traffic patterns or the road environment. As a minimum the study will examine:

- Is the roadkill rate only elevated for the Greater Bilby or several species
- Whether roadkill spatial data are presenting patterns or identifying potential roadkill hotspots
- Are there any notable circumstances relating to the deaths
- If there are any notable changes in animal abundance at a local and regional scale
- Whether there is a change in traffic patterns, and if so, the potential reasons for this
- How environmental and social factors impacting the Greater Bilby may have changed

The outcomes from the rapid response investigations (Section 3.2.1) will be revisited at this stage and supplement the conclusions of the investigative study.

Where the investigative study confirms the increase in the Greater Bilby roadkill rate is primarily occurring as a result of the road upgrade, Stage Three of the adaptive management approach will be triggered. If the increased rate is the result of external factors, extenuating circumstances or a combination of factors, no further action will be implemented as part of this annual monitoring campaign, other than collate and report these results to DotE.



Figure 1 Adaptive management staged approach

3.2.3 Stage Three – Mitigation and Enforcement

The selection of additional mitigation and/or management measures to be incorporated into a response plan needs to consider the practicalities of implementation. Furthermore, it is important to ensure the application of additional measures will be effective and are properly focused (i.e. is a 'regional' solution required, or is there is a localised impact / factor worth targeting).

Adaptive Management

If existing maintenance and mitigation measures are compliant with audit requirements and there is still a clear significant adverse impact on the Greater Bilby primarily caused by the road upgrade, additional appropriate mitigation and/or management measures will be identified. Potential management options will be determined based on the outcome of the detailed investigations (Stage Two) and any other additional information that may be relevant. These targeted measures may include (depending on any identified cause):

- Additional signage informing drivers that the Greater Bilby inhabits the local area
- Installation of additional coloured road pavements
- Increasing vegetation clearances
- Installation of other on-road features
- Other innovative technologies or management actions as they become available

Main Roads will submit the additional targeted mitigation and/or management measures to DotE for approval. Once approved, Main Roads will implement these within the mutually agreed timeframe. Following the implementation of the additional measures, Main Roads will review the need for additional monitoring to assess the effectiveness of the adaptive management approach.

4. Responsibility, reporting and review

4.1 **Responsibility**

Main Roads will be responsible for managing the monitoring program and associated reporting.

Shire of Broome will be responsible for the on-going maintenance of the road/ mowed verge/ drains/ underpasses once the road has been constructed.

In the event that additional mitigation and / or management measures are necessary (as a result of Stage Three of the adaptive management approach) then Main Roads will be responsible for the implementation of these measures.

4.2 Reporting and documentation

In accordance with condition 10g of EPBC 2013/6984 the following information will be provided to the DotE:

- Survey results determining the baseline local Greater Bilby population
- Location of high density Greater Bilby areas
- Annual Roadkill Monitoring Reports, including the effectiveness of adaptive management

4.3 Review of Plan

Although not required as a condition of EPBC 2013/6984, Main Roads may undertake a review of this Plan as required. The review may outline the success of the Plan in meeting its objectives; make recommendations for improving the Plan; and review the appropriateness of the trigger levels and adaptive management approach.

If Main Roads revises the plan following a review, it will be submitted to DPaW for comment and DotE for approval. Following the approval of the revised Plan, it will be made publically available on the Main Roads' website.

5. References

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Southgate R. 2012, Peer review of the Browse Bilby Review, a report detailing the consolidated information relating to the occurrence of the Bilby Macrotis lagotis near the proposed Browse LNG Precinct (close to James Price Point) and more broadly on the Dampier Peninsula. Envisage Environmental Services for Sinclair Knight Merz. Perth.

Appendix A – Monitoring data

Baseline local Greater Bilby population and the location of higher density areas monitoring report (enclosed)

Baseline roadkill monitoring survey (to be included once completed)



Main Roads WA

Cape Leveque Road SLK 25 - 102.6 Targeted Greater Bilby Survey

May 2016

Executive summary

This report outlines the methods and results of the targeted survey between Cape Leveque Road SLK 25 – 102.6 to determine the baseline local Greater Bilby (Bilby) population and identify high density Bilby areas as defined in EPBC 2013/6984. This report is subject to, and must be read in conjunction with, the limitations, assumptions and qualifications contained throughout the Report.

The survey was completed in two Components over approximately three months in 2015. Seventy seven (77) survey plots positioned at 1 km intervals were surveyed during Component 1 as part of a presence/absence survey. From Component 1 twelve plots were selected for longer term monitoring. They were selected based on recent and historic Bilby activity or an increased likelihood of activity based on the habitat present. A quantitative number has been used to interpret the presence or absence of the Bilby within each survey plot. This was supplemented with DNA analysis of Bilby scats collected on site.

Of the 77 plots, three (Plots 62, 65, 100) had active burrows present with multiple signs of evidence present in the form of burrows, digs, tracks and scats. Five plots (Plots 71, 72, 80, 88, 98) had old burrows with and without aprons present (remnants of old burrows) but no other evidence of recent Bilby use was observed at these plots. Five plots had Bilby like digs present but there was no other evidence present to confirm if undertaken by Bilby or another species. The remaining 64 plots had no signs of presence by Bilby recorded.

Component 2 demonstrated five plots in total had active Bilby present over the survey period with between 1 and 3 animals present. These numbers are likely conservative and an underestimate of actual numbers. This is due to the cryptic nature of the species and weather events over the assessment period.

The population density estimate for the survey area was calculated as 2.60 individuals/km2 (0.026 individuals/ha) as a conservative estimate with 5.80 individuals/km2 (0.058 individuals/ha) recorded as an upper limit. These population density estimates should be refined as and when additional Bilby survey data becomes available.

Eleven high density Bilby areas were identified through this targeted survey at Plots 45, 48, 62, 65, 70,71,72,80, 88, 98 and 100, exhibiting three or more signs of bilby activity.

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1. Introduction

1.1 Background

The Cape Leveque Road is located in the Shire of Broome and runs from the Broome Highway, east of Broome townsite, to the northern Dampier Peninsula for a length of approximately 200 kilometres (km). The road is a main transport link, providing access for Aboriginal communities (including Beagle Bay, Lombadina, Djarindjin, Kooljamin and One Arm Point), pastoral stations, pearling industries and tourist destinations.

Various sections of the Cape Leveque Road have been upgraded to a sealed standard over the last ten years. Main Roads Western Australia (Main Roads) proposes to upgrade 77.6 km (Straight Line Kilometre (SLK) 25 to 102.6) of the unsealed section of the Cape Leveque Road. The proposed upgrade involves construction of a new road generally parallel to the existing unsealed road. Road user safety and reduced maintenance are two of the key reasons for the proposed upgrade. Depending on the allocation of funding, it is anticipated that construction of the new road will take place during the 2016 – 2019 dry seasons (May to October).

Upgrade of the Cape Leveque Road SLK 25 - 102.6 will involve up to 297 ha clearing of native vegetation. The vegetation to be cleared includes up to 297 ha of suitable foraging, breeding and sheltering habitat for the Greater Bilby (Bilby), a species listed as vulnerable under the *Environmental Protection Biodiversity Conservation Act 1999* (EPBC Act) and the *Wildlife Conservation Act 1950* (WC Act). GHD completed a targeted Bilby survey (GHD 2013) which confirmed the presence of the Bilby in the Project Area (photographic, denning and foraging evidence).

As a result of residual impact to the Bilby Main Roads referred the Cape Leveque Road upgrade proposal SLK 25 - 102.6 to the Department of the Environment (DotE) August 2013 (EPBC 2013/6984). In July 2015 DotE granted approval of the proposal as a controlled action, with various conditions imposed. In order to satisfy some of the EPBC 2013/6984 conditions a targeted Bilby survey is required to determine the baseline local Bilby population and high density Bilby areas.

To inform future works with respect to Cape Leveque Road SLK 25 – 102.6, Main Roads required the target Bilby survey be completed for SLK 0 – 102.6. A separate survey report will be issued for Cape Leveque Road SLK 0 – 25 (GHD 2016).

1.2 Purpose of this report

Main Roads requires a suitably qualified consultant to complete a targeted Bilby survey to determine the baseline local Bilby population (as defined in EPBC 2013/6984) and high density Bilby areas (as defined in as defined in EPBC 2013/6984) within the Cape Leveque Road Project Area.

This report outlines the methods and results of the targeted survey to determine the baseline local Bilby population and identify high density Bilby areas. This report covers Cape Leveque Road SLK 25 – 102.6.

1.3 Project area

The Project area (survey corridor) is 200 m wide (approximately 100 m width either side of the proposed Cape Leveque Road centreline) between SLK 25 – 102.6 with some small deviations. Some small deviations in the 200 m wide corridor occur at alignment intersections. The survey

encompassed the corridor at the widest points. The location and extent of the Project area is presented in Figure 1, Appendix A.

1.4 Scope of works

The scope of works associated with this report is consistent with the Project brief and GHD proposal to:

- Undertake a desktop assessment to establish pre-determined survey plots at 1 km intervals along the proposed alignment 77 plots in total
- Undertake a targeted survey for the Bilby within the established sampling plots
- Establish and survey 12 permanent quadrats to determine the distribution and relative abundance of the local Bilby population
- Re-survey the 12 permanent quadrats twice at approximately monthly intervals
- Prepare a targeted Bilby survey report, documenting the method and results of the survey to determine the baseline local Bilby population and high density Bilby areas

1.5 Limitations

This report has been prepared by GHD for Main Roads and may only be used and relied on by Main Roads for the purpose agreed between GHD and the Main Roads as set out in section 1.2 of this report.

GHD otherwise disclaims responsibility to any person other than Main Roads arising in connection with this report. GHD also excludes implied warranties and conditions, to the extent legally permissible.

The services undertaken by GHD in connection with preparing this report were limited to those specifically detailed in the report and are subject to the scope limitations set out in the report.

The opinions, conclusions and any recommendations in this report are based on conditions encountered and information reviewed at the date of preparation of the report. GHD has no responsibility or obligation to update this report to account for events or changes occurring subsequent to the date that the report was prepared.

The opinions, conclusions and any recommendations in this report are based on assumptions made by GHD described in this report (refer section 1.6 of this report). GHD disclaims liability arising from any of the assumptions being incorrect.

GHD has prepared this report on the basis of information provided by Main Roads and others who provided information to GHD (including Government authorities), which GHD has not independently verified or checked beyond the agreed scope of work. GHD does not accept liability in connection with such unverified information, including errors and omissions in the report which were caused by errors or omissions in that information.

The opinions, conclusions and any recommendations in this report are based on information obtained from, and testing undertaken at or in connection with, specific sample points. Site conditions at other parts of the site may be different from the site conditions found at the specific sample points.

Investigations undertaken in respect of this report are constrained by the particular site conditions, such as the location of buildings, services and vegetation. As a result, not all relevant site features and conditions may have been identified in this report.

Site conditions (including the presence of hazardous substances and/or site contamination) may change after the date of this Report. GHD does not accept responsibility arising from, or in

connection with, any change to the site conditions. GHD is also not responsible for updating this report if the site conditions change.

1.6 Assumptions

GHD has followed the 'survey methodology' specified in the Consultant Brief. GHD is aware this method is primarily based on the approach recommended by the Department of Parks and Wildlife (DPaW), based on the EPBC approval for the Project. GHD has assumed this method will meet DotE's expectation, but cannot guarantee this.

2. Methodology

A pre-defined method was followed as recommended by DPaW and specified in the consultants brief. The field survey was completed in two components:

- Component 1 Presence / absence
- Component 2 Distribution and relative abundance

2.1 Desktop

GHD undertook a desktop assessment to determine survey plots for the Component 1 aspect of the survey. This includes approximately 77 survey plots positioned at 1 km intervals from SLK 25 – 102.6. This was done prior to the field work to assist in maximising survey effort on the ground. Field maps and shapefile data were uploaded onto a handheld Trimble to assist with reporting and data capture. By having the pre-defined areas also assisted with the establishment of the Component 2 areas.

The pre-determined plots were positioned within the corridor so as not to intersect with the proposed road. To accommodate this, the plots were approximately 70 m in width and 288 m in length (2 ha). Where possible the sites alternated from one side of the road to the other. In some instances this was not possible, for example when material pits were proposed at the 1 km location.

2.2 Survey 1 (October 2015)

Survey 1 included both Component 1 and the set up and first phase of Component 2. The following tasks were undertaken:

2.2.1 Component 1

- Each predetermined plot was searched by two observers for signs of the Bilby occurrence (i.e. tracks, droppings, burrows, diggings)
- Estimates of recent Bilby occupation of the area was made, and Bilby scats collected for DNA analysis and estimation of numbers of Bilbies in that area
- Observations of other fauna of interest (e.g. olive pythons, foxes, feral cats) was also recorded
- Fauna observations were also conducted between the road and the predetermined survey plots to provide additional contextual data and track ability within the area
- A habitat description of each survey plot was collected, including Vegetation community, dominant ground cover and shrubs, percentage cover and estimated time since fire disturbance
- Weather conditions (wind and rain) and time of day were also collected for each survey plot
- Where active burrows or diggings were located, motion sensor cameras were deployed to support the presence/absence determination of the Bilby. Cameras deployed during this survey period were collected during the second survey Campaign (approximately one month later)
- Data were collect using field notes and Trimble and transferred to ArcGIS and excel for interpretation and presentation purposes

Directly after the Component 1 survey was completed, Component 2 was undertaken and included:

2.2.2 Component 2

- Long term study Quadrats were established along the proposed road alignment to determine the distribution and relative abundance of the local Bilby population
- Within each 25 km section of proposed road alignment, four x 2 ha quadrats were established at sites where the presence / absence survey (above) indicated Bilby presence
- Where there were not four survey plots that indicated Bilby presence within the 25 km section, quadrats were selected based on potential likelihood of occurrence in a particular survey plot based on percentage ground cover, grasses present and distance from other plots
- Quadrats were marked with corner dropper posts and the GPS points recorded
- Each quadrat was systematically searched for Bilby presence / absence and quantitative measures of Bilby presence recorded. The following data was collected from each quadrat:
 - The type of evidence (i.e. tracks, droppings, burrows, diggings)
 - The age class of each piece of evidence; fresh (within 3 days), recent (within the preceding week) or old (older than a week)
 - Unique ID for scats collected for DNA analysis Bilby scats were collected for DNA analyses to identify the numbers and identity of individual bilbies occupying each quadrat
 - Data collected from the motion sensor cameras supplemented field observations recording the number of Bilby visits per night, and where possible the number of individuals per night recorded. However, given that the Bilby has no individually distinctive visual markings, the recording of the number of individuals was based on the size and potential sex of individuals photographed. Where individuals could not be clearly demonstrated as different individuals, they were counted as one. (The Cameras deployed as part of Component 1 will align with the 12 plots specified for Component 2 to meet both monitoring requirements.)
- Observations of other fauna of interest (e.g. olive pythons, foxes, feral cats) and recent rain events were also recorded

2.3 Survey 2 (November 2015)

Survey Campaign 2 includes the re-surveying of the 12 survey plots for Component 2. The following tasks were undertaken:

- Revisit the 12 x 2 ha quadrats established along SLK 25 102.6 for Bilby activity
- Each quadrat was systematically searched for Bilby presence / absence and quantitative measures of Bilby presence recorded. The following data was collected from each quadrat:
 - The type of evidence (i.e. tracks, droppings, burrows, diggings)
 - The age class of each piece of evidence; fresh (within 3 days), recent (within the preceding week) or old (older than a week)

- Unique ID for scats collected for DNA analysis Bilby scats will be collected and DNA analyses undertaken to identify the numbers and identity of individual bilbies occupying each quadrat
- Data collected on the motion sensor camera was collected and fresh cameras deployed in areas of high activity that fit the same criteria as in Component 1
- Observations of other fauna of interest (e.g. olive pythons, foxes, feral cats) and recent rain events were also recorded

2.4 Survey 3 (December 2015)

Survey Campaign 3 includes the re-surveying of the 12 survey plots for Component 2. The following tasks were undertaken:

- Revisit the 12 x 2 ha quadrats established along SLK 25 102.6 for Bilby activity
- Each quadrat was systematically searched for Bilby presence / absence and quantitative measures of Bilby presence recorded. The following data was collected from each quadrat:
 - The type of evidence (i.e. tracks, droppings, burrows, diggings)
 - The age class of each piece of evidence; fresh (within 3 days), recent (within the preceding week) or old (older than a week)
 - Unique ID for scats collected for DNA analysis Bilby scats will be collected and DNA analyses undertaken to identify the numbers and identity of individual bilbies occupying each quadrat
 - Data collected on the motion sensor camera was retrieved
- Observations of other fauna of interest (e.g. olive pythons, foxes, feral cats) and recent rain events were also recorded

2.5 Interpretation of the observations

2.5.1 Bilby presence / absence score

A quantitative number has been used to interpret the presence or absence of the Bilby within the survey plots / quadrats. The score of 0 to 3 has been applied using the criteria listed in Table 1.

Table 1	Presence	absence	criteria

Presence / absence score	Criteria
0	No recorded evidence
1	Possible evidence but not confirmed,
2	Evidence present but old and not active,
3	Active area with good evidence

2.5.2 DNA analysis

Scats were collected in the field and stored via 2 methods, 1. Collecting in luncheon paper bags and freezing and 2. Samples collected in vials of silica beads for dry storage. Samples were then given to Fiona Carpenter and Martin Dziminski of DPaW for genetic analysis. The method

used for gene extraction is found in the report "Genotyping of bilby scats collected from Cape Leveque Road, Kimberley" and can be found in Appendix C.

2.5.3 Definitions

For the purpose of the targeted Bilby survey the following definitions were applied as specified in the Commonwealth Approval for the proposed action (EPBC 2013/6984):

- Local Greater Bilby Population: The population size and distribution of Greater Bilby that is determined by a suitably qualified ecologist as likely to be impacted by the proposed action as derived from surveys conducted prior to referral and the baseline population surveys required by this approval.
- High density Greater Bilby area: Any 6 ha area that includes or is immediately adjacent to the proposed disturbance route, containing three or more signs of Greater Bilby. Signs of Greater Bilby may include sightings (in person or via camera), active burrows, inactive burrows, diggings, scratching or scats.
- Suitably qualified ecologist: Refers to an independent person, approved by the Minister, with relevant tertiary qualifications and a minimum of five years' experience in Australian mammal fauna surveys in the region.

2.5.4 Population density Estimate

Bilby population density was calculated by dividing the individual animal number estimate by the area surveyed. For the purpose of this calculation, the area surveyed was based on the assessment of 77 survey plots of 2 ha in size along Cape Leveque Road during Component 1. An estimate of individuals numbers was based on evidence observed for each plot during Component 1 and where available supplemented by genotyping data from Component 2 (Campaign 1). This estimate is considered to be a conservative prediction of the population density within the survey area. A population density upper value was calculated by also considering the estimated number of individuals recorded from Component 2 (Campaign 2 and 3) through evidence observed and genotyping data. It should be noted these estimates are only based on three months of data and should be reviewed and revised as future survey and research is completed in the region.

2.5.5 Survey team

To ensure compliance with the Commonwealth Approval (EPBC 2013/6984) experienced ecologists completed the field surveys and subsequent analysis of the results. The following ecologists were involved with this survey effort:

Glen Gaikhorst

Role – Glen was involved in the desktop assessment and all field survey Campaigns (Survey 1, 2 and 3). Glen also completed analysis of the results.

Experience – Glen has 22 peer-reviewed papers, 3 book chapters and over 20 years' experience with ex and in-situ management of reptiles, amphibians, birds, and mammals, environmental consultancies and related animal industries. Glen Gaikhorst has managed all aspects of vertebrate terrestrial fauna survey. He has been primarily responsible for the survey design, implementation, species identification and analysis of data.

Glen has worked on Bilby projects around Western Australia and the Northern Territory since 2008 including regions of Ti Tree (Northern Territory), Dampier Peninsular (and numerous projects around Broome), numerous projects in the Pilbara and Murchison and Great Sandy Desert (with WA Museum). Glen has also undertaken monitoring via radio tracking of Bilby at

Lorna Glen Conservation Reserve with DPaW. Glen has also undertaken all of the previous Bilby surveys for the Cape Leveque Road upgrades since 2012.

Dr Richard Southgate

Role – Richard was involved in the Survey 1, which included Component 1 (Presence / absence) and the first phase of Component 2 (Distribution and relative abundance).

Experience – Richard has more than 17 peer reviewed papers, one book and over 25 years' experience with fauna research across Australia. Richard spent approximately 18 years at Arid Recovery, an independent, not-for-profit conservation initiative. Whilst with this organisation Richard was involved with Bilby research and the re-introduction of the species at the Arid Recovery reserve.

Richard is recognised by industry and government agencies as a leader in Bilby research and has been engaged to provide expert and peer review advice for projects and proposed actions being assessed by both state and commonwealth environmental regulators.

Craig Grabham

Role – Craig was involved in Survey 2.

Experience – Craig is a Senior Ecologist and Project Manager with GHD's Environmental Assessment and Approvals Group in Perth. He is Team Leader of Terrestrial Ecology with 15 years' experience in conducting ecological survey and impact assessment within Australia. The majority of Craig's work with GHD has involved the assessment and management of ecological impacts resulting from linear infrastructure projects including road, pipeline, powerline and rail and resource extraction projects across Australia.

Craig has been the Project Manager and lead author of multiple biological surveys and Environmental Impact Assessment's (EIA) for Main Roads WA (MRWA) projects. He is also an author and major contributor for other environmental assessments for linear infrastructure projects. Craig has assisted various regions of Main Roads WA including the Kimberley, Pilbara, Goldfields, South- West, Great Southern and Wheatbelt regions with fauna and environmental assessments.

Laura Zimmermann

Role – Craig was involved in Survey 3.

Experience – Laura is a qualified Environmental Scientist / Ecologist with five years' experience. Laura has worked throughout Western Australia, including the Kimberley, Pilbara, Mid-West, Swan Coastal Plain and South-West regions, and has been responsible for a variety of technical and fieldwork tasks and reporting. Laura has experience in fauna and flora field surveys, writing impact assessment reports, environmental approvals documentation, fauna monitoring environmental management and vegetation assessments. She has been involved in a variety of infrastructure (roads, railways and pipelines), mining, land development and government projects whilst working at GHD.

3. Results

3.1 Component 1

Seventy seven plots and the associated road were searched (SLK 25 – 102) for the presence / absence of Bilby.

Of the 77, three plots (Plots 62, 65, 100) had active burrows present with multiple signs of evidence present in the form of burrows, digs, tracks and scats. Five plots (Plots 71, 72, 80, 88, 98) had old burrows with and without aprons present (remnants of old burrows) but no other evidence of recent Bilby use was observed at these plots. Five plots had Bilby like digs present but there was no other evidence present to confirm if undertaken by Bilby or another species. The remaining 64 plots had no signs of presence by Bilby recorded.

In summary three plots had a 'presence/absence criteria' ranking of 3, one had 2, nine had 1 and 64 plots had 0.

Scats were collected from Plots 62, 65 and 100. Scats from Plot 62 were all uniform in size and fresh and old suggesting potentially one animal utilising this site. Samples were scattered over a relatively large area (approximately 6 ha). Plot 65 had scats collect in a relatively small area and all quite small suggesting a young animal. Plot 100 had few samples collected but all of a large similar size suggesting a large male or female is utilising the area.

The compiled results of the presence / absence survey associated with SLK 25 – 102.6 are presented in tabular form in Appendix B. These results are also presented in Figure 2.

3.1.1 Track ability

All plots were assessed for the ability to record evidence of Bilby excluding false negatives and false positives. Track ability was assessed via ability to see evidence of species utilising the area like feral species, native mammals, birds, rodents and goannas. Additionally the conditions (wind and rain events) were recorded to assist in predicting age of signs. Track ability was then given a score out of 4. The score combined with species observed provides a guide as to the ability to record Bilby activity on plot. In this case track ability overall ranged between 1.8 and 3.9 with an average of 2.9. This infers that the conditions were difficult to observe signs of Bilby, primarily due to wind and rain spatter occurring during the survey. However the data suggests that good coverage of fauna groups (from small rodents to Agile Wallaby's) were observed and recorded at each plot.

3.1.2 Feral species recorded

Numerous feral species were recorded at each plot. Of the 77 plots 52 had signs of feral species use or activity. Feral species included dog, cat and cow. No evidence of fox was recorded during this component.

3.1.3 Habitats

A habitat assessment was completed for each plot. Forty Seven (47) plots were Woodlands, 14 plots were Open Woodlands, eight plots were Shrublands, four Open Woodlands/Shrublands two Woodlands/Shrublands and two low open woodland. The ground cover species at all sites was dominated with grasses that ranged between 1 - 70% cover. Dominant shrub species included Acacia sp. Eucalyptus sp. Corymbia sp. and Bauhinia sp. that ranged between 10 - 30% cover. Evidence of fire was observed at most sites with very few sites not burnt within past 12 months.

The habitat present at the three currently active sites consists of Open Woodland and Woodland of Eucalyptus and Corymbia over Acacia and Senna (10-15% cover) over mixed grasses (1-10% cover) with 10-15% cover of ground litter.

The compiled results of the habitat data associated with SLK 25 - 102.6 are presented in tabular form in Appendix B.

3.2 Component 2

From Component 1, 12 plots were selected for longer term monitoring. These plots were 30, 39, 45, 48, 53, 62, 65, 71, 80, 88, 98 and 100. Plots 62, 65 and 100 had active Bilby evidence while Plot 45, 48, 71, 80, 88 and 98 had some evidence of historical use (ie burrows and digs). Plot 30, 39, 53 were selected due to reduced ground cover and grasses present.

The results of the distribution and relative abundance survey associated with SLK 25 – 102.6 are presented in tabular form in Appendix B.

3.2.1 Campaign 1 (October)

Plots 62, 65 and 100 showed definitive signs of Bilby activity such as digs, scats and burrows. Plot 65 and 100 had one active burrow, both freshly dug with prints and digs surrounding. Plot 62 has a large amount of evidence, digs, scats and burrows spread out over a large area. Plots 71, 80, 88 and 98 had multiple old burrows and/or aprons present but no recent activity at the old burrows or surrounds were recorded. Plots 45 and 48 had some possible digs but these were not confirmed by the presence of other activity. No activity was recorded at the remainder of the plots.

Cameras were placed on burrows at 62, 65, 71, 88, 98 and 100.

In summary three plots had a 'presence/absence criteria' ranking of 3, one of 2, five of 1 and three of 0.

3.2.2 Campaign 2 (November)

Consistent with Campaign 1, plots 65 and 100 showed definitive signs of Bilby activity such as digs, scats and burrows. Plot 65 had one active burrow while Plot 100 had no fresh activity at the original burrow but lots of digs were scattered around. A broader search was undertaken to try and locate subsequent burrows but none were found. Plot 62 appears to be abandoned at the time of the assessment. In this plot no fresh digs were present and several burrows were eroded with no signs of recent use. A broader search of the region was undertaken but no evidence of use was found. Plot 80 had 3 older burrows within the base of large termitaria which at the time of component 1 had leaf and debris present suggesting it was long unused. During this assessment two of the entrances had been excavated, however the lack of supportive evidence in the general area suggests an echidna may have dug them out. A series of cameras were placed on this burrow to confirm. Plots 71, 88 and 98 had no recent activity at the old burrows.

No activity was recorded at the remainder of the plots.

Cameras were placed on active and old burrows at 62, 65, 80, 98 and 100.

In Summary two plots have a 'presence/absence criteria' ranking of 3, two plots of 2, three of 1 and five of 0.

Camera results

Two plots (65 and 100) recorded multiple camera hits of Bilby. Plot 62 had 62 hits for the duration of the survey period. This individual is a young animal and has only one burrow. At the

time of the plot assessment observations of recent mound use was present and corresponds to observations made on camera. Unfortunately a cat visits the burrow periodically and sits in front of the apron. The cat appears to wait for approximately 10 minutes before moving on. An image of the Plot 65 individual can be seen below in Plate 1.

Plot 100 had 10 hits of a female Bilby with one set of images showing a juvenile at foot (see Plate 2). No images of Bilby at this camera were recorded beyond a week prior to the camera collection. Suggesting she has moved burrows or has moved out of the area. This correlated to the on ground assessment which showed the burrow (the only burrow located at this site) had not been worked recently.



Plate 1 Young Bilby at burrow at Plot 65



Plate 2 Female Bilby with young at foot at Plot 100

3.2.3 Campaign 3 (December)

A large amount of change in activity had occurred over the period between Campaign 2 and 3. Plot 62 appears to have had some activity (prints at a burrow and along the road) in the week leading to this survey however no fresh signs were present at the time of the survey. A broader search was undertaken of the area but no activity recorded.

The main burrow at Plot 65 was eroded and caved in at the time of the survey however activity at the site was recorded (via camera) till the 25th November (only a week after Campaign 2) with no other evidence found. A broader search of the area was undertaken to see if any burrows were present but none were found. A clear image of a small Bilby at Plot 65 is shown in Plate 3.

Plot 100 had a small amount of fresh digs present and recent activity on the burrow. Digs recorded appeared to be from a small individual with the large conical digs identified previously (and at the base of shrubs) not present. This maybe a seasonal feeding resource switch or the adult female has moved on leaving the offspring. A small individual was confirmed on camera as seen in Plate 4.

Plot 98 and 80 both had previous signs of Bilby present historically with old burrows and digs present. During this survey both sites showed fresh signs of Bilby in the form of fresh burrows, digs and scats. Plot 98 appeared to be utilised by one medium sized individual who dug a fresh burrow approximately 20 m from the previously recorded one. This individual was also recorded on camera assessing one of the old burrows as seen in Plate 5. Scats were collected at this plot for DNA assessment. Plot 80 had evidence of activity (activity at an old burrow) during Campaign 2 however no other evidence was recorded within the plot suggesting an Echidna had dug the burrow. Cameras were placed on all three old burrows and recorded Bilby activity during this survey. In total 3 Bilby were recorded on cameras, a female, large male and one juvenile (see Plate 6, Plate 7 and Plate 8). Interestingly no other evidence was recorded in the plot (apart from some minor plate scratchings) where these Bilby were persisting.

Plot 88 also had old burrows previously recorded but the systems were long unused and mostly comprised of apron or with minor burrow entrances remaining. No active burrows were recorded however an areas of digs was found and one series of scats collected. A broader search was undertaken but no other activity recorded. It is concluded that a transient Bilby utilised the site increasing the known utilisation of the area, however could not be graded as a currently active area.

Plot 45 had no evidence recorded on site however increased in its Bilby presence/absence score due to an old burrow being located at the base of a termite mound during a broader search of the area. This burrow is long unused and partially collapsed.

The remaining plots (Plot 30, Plot 39, Plot 48, Plot 53 and Plot 71) had no change to their previous status with no Bilby activity recorded.

In Summary three plots have a 'presence/absence criteria' ranking of 3, three of 2, two of 1 and four of 0.

Camera results

During Campaign 3 all cameras were collected from plots for assessment. As identified above four plots were identified to have Bilby present. Plots 65, 98 and 100 only have one individual identified (Plate 3, Plate 4 and Plate 5) however Plot 80 recorded 3 individuals, a large male (Plate 7), female (Plate 6) and small individual (Plate 8). Observations on camera correspond to use of activity during the assessment however Plot 80 had very little evidence observed for 3 animals recorded.

Additionally all sites where Bilby were recorded also has activity of predators including dingo and cats. At Plot 80 a cat appears to be utilising a side entrance to the main burrow and was

recorded on most days utilising the burrow. This entrance is connected to the main burrow as the cat is recorded exiting this area after entering the other side. The main burrow appears to have the large male utilising it periodically.



Plate 3 Juvenile Bilby at Plot 65



Plate 4 Juvenile Bilby at Plot 100



Plate 5 Sub Adult Bilby recorded in Plot 98



Plate 6 Adult Female recorded at one burrow in Plot 80



Plate 7 Large Adult male Bilby recorded in Plot 80



Plate 8 Juvenile/sub adult Bilby recorded in Plot 80

3.3 DNA Results

Two rounds of DNA assessment were undertaken for faecal samples collected in Campaign 1, 2 and 3. During Campaign 1 and 2 scats were frozen and provided to DPaW for assessment. See attached DNA reports for methodology. The assessment (of 10 samples) of the scats identified three individuals between Plots 62 and 65 (one individual at Plot 62 and two at Plot 65). The genotyping success rate in this assessment was 50%, which is considered high. Campaign 3 provided an additional 3 samples (from Plots 88 and 98) however no genotyping extraction was achieved and no results provided. The reports of the genotyping is provided in Appendix C and data represented in Table 3.

4. Discussion and conclusions

4.1 Baseline local Greater Bilby population

Targeted Bilby survey data over three months provides an insight into the Bilby population along the Cape Leveque Road on the Dampier Peninsular. Seventy seven plots were initially surveyed (Component 1) with twelve plots identified as either having active Bilby evidence (3), recently active but no fresh evidence (1), old evidence (3) or no evidence but suitable habitat (5). This initial data inferred that three plots had actual Bilby presence and persistence with four being recently active with in the last 3 to 6 months (Component 2- Campaign 1) as seen in Table 2.

Campaign 2 had little change with only one plot reducing in activity. This data inferred that two sites had actual Bilby presence and persistence with five being recently active within the last few months. Campaign 2 coincided with the wet season build up and observations on numerous juvenile Bilbys active on cameras. This included young Bilby at foot or recently disbursed. This was also observed in plot evidence with numerous sites having juvenile scats collected.

Campaign 3 was undertaken in December with several rain events occurring early in the month. These rain events made observational data more difficult for visual assessment however aided in providing a timeline of age of evidence that was observed. Campaign 3 recorded 3 active plots, an increase in Bilby activity at an additional three plots and decrease at another. This data inferred that three plots had actual Bilby presence and persistence with five being recently active with in the last few months. This increased activity at plots appears to be via disbursal of offspring or movements of animals from low lying habitat areas to higher ground due to increased rain activity. Interestingly the increased activity was at previously active plots which had old burrows present (except plot 45 where an old burrow was located outside of the plot). Plot 80 appeared to have 3 individuals move back into the plot between Campaign 2 and 3. A breakdown of the activity scores for each of the components is shown in Table 2. This demonstrates that although a Bilby activity area maybe not active at the time of assessment the habitat is preferred and could be utilised at any time in the future. This activity and high movements of Bilby appears consistent with previous study's (Southgate et al. 2005, Southgate et al. 2007, Cramer et al 2016, In Press) and that the population is dynamic and mobile.

Plot no	Component 1	Component 2			
FIOT NO.		Campaign 1	Campaign 2	Campaign 3	
ch30	0	0	0	0	
ch39	0	0	0	0	
ch45	0	0	0	1	
ch48	0	0	0	0	
ch53	0	0	0	0	
ch62	3	3	2	2	
ch65	3	3	3	2	
ch71	1	1	1	1	
ch80	1	1	1	3	
ch88	1	1	1	2	
ch98	2	2	2	3	
ch100	3	3	3	3	

Table 2 Activity scores for each plot for each of the assessments
The field surveys enabled the assessment of potential numbers of individual Bilby present within each plot. This data was expressed from visual observations supplemented with DNA analysis. Table 3 shows the predicted numbers within each plot. Table 3 demonstrates that five plots had active Bilby present over the survey period with between 1 and 3 animals present. These numbers are likely conservative and an underestimate of actual numbers. This is due to the cryptic nature of the species and weather events over the assessment period.

	Compo	nent 1			Compor	nent 2			Total
	Compo		Campaig	n 1	Campaig	n 2	Campaig	n 3	predicted
Plot no.	Field Survey	DNA	Field Survey	DNA	Field Survey	DNA	Field Survey	DNA	numbers at each plot
ch30	0	N/A	0	0	0		0		0
ch39	0	N/A	0	0	0		0		0
ch45	0	N/A	0	0	0		0		0
ch48	0	N/A	0	0	0		0		0
ch53	0	N/A	0	0	0		0		0
ch62	1	N/A	1	1	1	N/A	0		1
ch65	1	N/A	1	2	1	1	1		2
ch71	0	N/A	0	0	0		0		0
ch80	0	N/A	0	0	0		3		3
ch88	0	N/A	0	0	0		0		0
ch98	0	N/A	0	0	0		1		1
ch100	1	N/A	1	0	2		1		2

Table 3 Shows the predicted individuals at each plot

4.1.1 Population density estimate

The population density estimate for the survey area was calculated as 2.60 individuals/km² (0.026 individuals/ha) as a conservative estimate with 5.80 individuals/km² (0.058 individuals/ha) recorded as an upper limit. These population density estimates should be refined as and when additional Bilby survey data becomes available.

In any case the numbers provided above align with previous studies that determined Bilby population density, albeit towards the upper range. The species has been reported to occur in very low densities of <1 individual /km² or <1 individual /100 ha) (Southgate et al. 1995). Other studies have recorded densities at 0.01 individuals/km² (0.0001 individuals/ha) in central Australia to 16 individuals/ km² (0.16 individuals/ha) in south-west Queensland (Southgate 1990a, Southgate et al. 2007). The Bilby recovery plan states that the species typically occurs at densities of 1-2 per km2 (0.02 to 0.01/ha) (Pavey 2006).

4.2 High density Greater Bilby areas

The high density Bilby areas are shown in Figure 3. For contextual purposes all plots within the SLK 25 – 102.6 has been presented in this figure. In total 11 plots were identified to have sufficient evidence to constitute a high density area for Bilby. There plots were numbered 45, 48, 62, 65, 70,71,72,80, 88, 98 and 100.

5. References

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Appendices

Appendix A - Figures

Figure 1	Project area locality
Figure 2	Bilby field observations for Component 1
Figure 3	High density Greater Bilby areas





Local Road

Paper Size A3 0 50 100 200 300 Kilometres	GHD	WESTERN AUSTRALIA	Main Roads Western Australia Cape Leveque Road Bilby Monitoring Chainage 25-101	Job Number 61-32719 Revision 0 Date 03 May 2016
Horizontal Datum: GDA 1994 Grid: GCS GDA 1994			Locality Map	Figure 1

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- Component 1 and Component 2 Campaign 1 🚳 \bigcirc Camera
- Component 2 Campaign 2 \land
- Component 2 Campaign 3







Component 1 and Component 2 - Campaign 1 🚳 Camera \bigcirc

- Component 2 Campaign 2 \land
- Component 2 Campaign 3







- Component 1 and Component 2 Campaign 1 🚳 \bigcirc Camera
- Component 2 Campaign 2 \land
- Component 2 Campaign 3







Component 1 and Component 2 - Campaign 1 🚳 \bigcirc Camera

- Component 2 Campaign 2 \land
- Component 2 Campaign 3







- Component 1 and Component 2 Campaign 1 🚳 \bigcirc Camera
- Component 2 Campaign 2 \land
- Component 2 Campaign 3







Component 1 and Component 2 - Campaign 1 🚳 Impact Area \bigcirc Camera

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- Component 2 Campaign 2 \land
- Component 2 Campaign 3





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- Component 2 Campaign 2 \land
- Component 2 Campaign 3



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- Component 1 and Component 2 Campaign 1 🚳 \bigcirc Camera
- Component 2 Campaign 2 \land
- Component 2 Campaign 3







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- Component 2 Campaign 3







- Component 1 and Component 2 Campaign 1 🚳 \bigcirc Camera
- Component 2 Campaign 2 \land
- Component 2 Campaign 3







ACTIVE SITE (Represented by 3 or more observations of Bilby evidence)

NON-ACTIVE SITE (No activity recorded)



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G:(61/32719/GIS/Maps/MXD/SLK_25-100/6132719_003_Rev0_Fig3BilbyActivity.mxd 999 Hay Street, Perth, WA, 6000 Australia T 61 8 6222 8252 F 61 8 6222 8555 E permail@ghd.com.au © 2016. Whilst every care has been taken to prepare this map, GHD, MRWA, Landgate and Geoscience Australia make no representations or warranties about its accuracy, reliability, completeness or suitability for any particular purpose and cannot accept liability of any kind (whether in contract, tort or otherwise) for any expenses, losses, damages and/or costs (including indirect or consequential damage) which are or may be incurred by any party as a result of the map being inaccurate, incomplete or unsuitable in any way and for any reason. Data source: Geoscience Australia: Topo 250K Series 3; GHD: Site Status - 20151111, Chainage - 20151016; Landgate: Roads. Created by:mmikkonen

Appendix B – Tabulated survey data

Component 1 Component 2 (Campaign 1) Component 2 (Campaign 2) Component 2 (Campaign 3)

Component	1	Data

	Compon	nent 1 Data																												
	Bilby				dog	fox	cat	t c	COW	agile	echidna	coucal	bustard		Habitat					Est.	Small	D	escriptor	of tracking wind	conditions			Notes		
				Presence/	<mark>(</mark>															time	animals	plot	plot	strongest	rain 2	wind at	rain		ci	start obs
plot no.	trax o	digs burrow	scats	score	rd plo	t rd p	olot rd	plot	rd plot	rd plot	rd/plot	rd/plot	rd/plot	Broad habitat	Ground_dom. Sp.	g%cov	lit%c	Shrub_dom. Sp.	s%cov	fire	rd&plot	trackability	ODS	previous	previous	sample	at sample	notes1	date ti	ime name
ch25	0	1 0	0	1	1 0	0	0 1	0	1 0	1 0	0	0	0	shbl	mixed grass /vaaus/sorg	35	15	acc/euc	20	2 vr	0	2.7	7.7	2	0	1	0	possible digs, no other evidence to confirm	23oct15 1	620 ga.rs
ch26	0	0 0	0	0	1 0	0	0 1	0	1 0	1 0	0	0	r	shbl	mixed grass /yaaus/sorg/spin	40	20	acc/euc	25	2 yr	1	2.9	8.9	2	0	1	0		23oct15 1	655 gg,rs
ch27	0	0 0	0	0	0 0	0	0 1	0	0 0	1 0	0	0	r	wdl	mixed grass/sorg	40	25	euc/acc	25	5 yr	1	3.2	9.2	2	0	1	0		23oct15 1	720 gg,rs
ch28 ch29	0	0 0	0	0	0 0	0	0 1	0	0 0		0	0	0	wdl	mixed grass/sorg/xpin mixed grass/sorg/xpin	45 50	20	euc/acc euc/acc	25 30	5 yr 5 vr	1	3.3	9.3 9.3	1	0	0	0		2400115 c	705 gg,rs
ch30	0	0 0	0	0	0 0	0	0 0	1	0 0	0 1	0	0	0	wdl	mixed grass/sorg/xpin	30	20	euc/acc	20	5 yr	1	2.4	8.4	1	0	0	0		24oct15 7	735 gg,rs
ch31	0	0 0	0	0	0 0	0	0 1	0	0 0	0 0	0	0	0	wdl	spin/mixed grass	40	20	euc/acc	20	5 yr	1	3.1	10.1	1	0	0	0		24oct15 8	305 gg,rs
ch33	0	0 0	0	0	0 0	0	0 0	1	0 0	0 1	0	0	0	wdl	spin/mixed grass	45 50	15	euc/acc	20	3 yı 4 yr	1	3.4	10.1	1	0	1	0		240ct15 8	
ch34	0	0 0	0	0	0 0	0	0 0	0	0 0	0 0	0	0	0	wdl	spin/mixed grass	50	20	euc/acc	20	5 yr	1	3.4	10.4	1	0	1	0		24oct15 9	∂30 gg,rs
ch35 ch36	0	0 0	0	0		0	0 0	0	0 0		0	0	0	wdl	spin/mixed grass	50 60	20 25	euc/acc euc/acc/senna	20 10	5 yr 5 yr	1	3.4	10.4 10.5	1	0	1	0		24oct15 1	000 gg,rs
ch37	0	0 0	0	0	0 0	0	0 1	0	0 0	0 0	0	0	0	wdl	mixed grass	60	25	euc/acc/senna	15	5 yr	1	3.5	10.5	1	0	1	0		24oct15 1	055 gg,rs
ch38	0	0 0	0	0	0 0	0	0 1	0	0 0	0 0	0	0	0	wdl	mixed grass	50	10	euc/acc/senna	20	5 yr	1	3.5	10.5	1	0	2	0		24oct15 1	330 gg,rs
ch39 ch40	0	0 0	0	0	0 0	0	0 1	0	0 0	1 0	0	0	0	wdi	mixed grass mixed grass	60 65	20	euc/acc/senna euc/cor/acc/senna	20 15	5 yr 5 vr	0	3.6 3.5	11.6	1	0	2	0		240ct15 1	355 gg,rs 415 aa.rs
ch41	0	0 0	0	0	0 0	0	0 0	0	0 0	1 0	0	0	0	wdl	mixed grass	65	20	euc/cor/acc/senna	15	5 yr	0	3.5	11.5	1	0	2	0		24oct15 1-	435 gg,rs
ch42	0	0 0	0	0	0 0	0	0 1	0	0 0	1 0	0	0	0	wdl	mixed grass	70	25	euc/cor/acc/senna	20	5 yr	0	3.8	11.8	1	0	2	0		24oct15 1	500 gg,rs
ch43	0	0 0	0	0	0 0	0	0 1	0	0 0	1 0	0	0	0	wdl	mixed grass	75	25	euc/cor/acc/senna	20	5 yr	0	3.8	12.8	1	0	2	0		240ct15 1	525 gg,rs
																												possible digs, no other		
ch45 ch46	0	0 0	0	0	0 0	0	0 1	0	0 0	1 0	0	0	0	wdl	mixed grass /spin mixed grass	75 70	25 25	euc/cor/acc/senna euc/cor/acc/senna	20	3-5 yr 5 vr	0	3.6 3.7	12.6	1	0	2	0	evidence to confirm	240ct15 1 240ct15 1	610 gg,rs
	-		-			-		-					-							-).	-				-	_	-	one possible dig, no other		33
ch47	0	1 0	0	1	0 0	0	0 1	0	0 0	1 0	0	0	0	wdl	mixed grass	70	25	euc/cor/acc/senna	20	5 yr	0	3.7	12.7	1	0	2	0	evidence to confirm	24oct15 1	700 gg,rs
																												second visit. Possibledigs but		
ch48	0	1 0	0	1	0 0	0	0 1	0	0 0	1 0	0	0	0	wdl	mixed grass	70	25	euc/cor/acc/senna	20	5 yr	0	3.7	12.7	2	1	2	0	no other evidence to confirm	25oct15 7	/00 gg,rs
ch49	0	0 0	0	0	1 0	0	0 0	0	0 0	1 0	0	0	0	wdl open	mixed grass sorg	3	10	euc/cor/acc/senna	10	4mth	0	3.2	11.2	2	1	2	0	second visit	25oct15 7	720 aa.rs
						-		-													-							rain afternoon before on		35/
ch50	0	0 0	0	0	0 0	0	0 0	0	0 0	0 0	0	0	0	wdl open	mixed grass sorg	3	15	euc/cor/acc/senna	10	4mth	0	3.2	11.2	2	1	2	0	second visit	25oct15 8	340 gg,rs
ch51	0	0 0	0	0	0 0	0	0 0	0	1 0	1 0	0	0	0	wdl open	mixed grass sorg	10	10	euc/cor/acc/senna	10	4mth	0	3.2	11.2	2	1	2	0	second visit	25oct15 9	∂10 gg,rs
ah50	0	0	0	0	0 0				1 0				0	uudi on on	mined areas says	10	10		10	Amath	0	2.0	0.0	2	1	2	0	rain afternoon before on		020
CN52	0	0 0	0	0	0 0	0	0 0	0	1 0	0 0	0	0	0	wai open	mixed grass sorg	10	10	euc/cor/acc/senna	10	4mth	U	3.0	9.0	2	I	2	0	rain afternoon before on	250C[15 9	730 gg,rs
ch53	0	0 0	0	0	0 0	0	0 0	0	1 0	0 0	0	0	0	wdl open	mixed grass sorg	15	10	euc/cor/acc/senna	10	4mth	0	3.0	9.0	2	1	2	0	second visit	25oct15 1	015 gg,rs
ch54	0	0 0	0	0	0 0	0	0 0	0	1 0	0 0	0	0	0	low open wdl	mixed grass sorg	15	10	euc/cor/acc/senna	10	4mth	0	3.0	9.0	2	1	2	0	rain afternoon before on second visit	25oct15 1	045 aa rs
chor	0	0 0	0	Ŭ	0 0		Ũ	Ŭ	1 0		Ū	Ŭ	0	ion open nur	Thinked grass sorg	10	10	cut/con/uco/sching	10		0	0.0	7.0	2	·	2	0	rain afternoon before on	2000110	99,13
ch55	0	0 0	0	0	1 0	0	0 0	0	0 0	1 0	0	0	0	shbl/open wdl	mixed grass sorg	15	10	euc/cor/acc/senna	10	4mth	0	3.0	9.0	2	1	2	0	second visit	25oct15 1	045 gg,rs
ch56	0	0 0	0	0	1 0	0	0 0	0	0 0	1 0	0	0	0	open wdl	mixed grass sorg	10	10	euc/cor/acc/senna	20	4mth	0	3.0	9.0	2	1	2	0	second visit	25oct15 1	115 gg,rs
	_																											rain afternoon before on		
ch57	0	0 0	0	0	1 0	0	0 0	0	0 0	1 0	0	0	0	open wdl	mixed grass sorg	10	10	euc/cor/acc/senna	10	4mth	0	2.7	8.7	2	1	2	0	second visit rain afternoon before on	250ct15 1	550 gg,rs
ch58	0	0 0	0	0	1 0	0	0 0	0	0 0	1 0	0	0	0	low open wdl	mixed grass sorg	10	10	euc/cor/acc/senna	15	4mth	0	2.7	8.7	2	1	2	0	second visit	25oct15 1	610 gg,rs
chEQ	0	0 0	0	0	1 0	0		0	0 0	1 0	0	0	0	wdl	mixed grace corg	10	10	ouclearlaceleanna	10	4mth	1	27	07	r	1	2	0	rain afternoon before on	25oct15 1	620 aa re
0115 9	0	0 0	0	U	1 0	0		0	0 0	1 0	0	0	0	war	Thined grass sorg	10	10	euc/cor/acc/serina	10	411111		2.1	0.7	2	1	2	0	rain afternoon before on	2300113 1	99,13
ch60	0	0 0	0	0	0 0	0	0 0	0	0 0	0 0	0	0	0	wdl	mixed grass sorg	10	10	euc/cor/acc/senna	10	4mth	0	2.7	8.7	2	1	2	0	second visit	25oct15 1	700 gg,rs
ch61 ch62	0 1r	0 0	0	0		0		0	0 0	0 1	0	0	0	wdl	mixed grass sorg	5	10	euc/cor/acc/senna euc/cor/acc/senna	10	4mth 4mth	1	2.0	7.0	2	0	0	0		260ct15 / 260ct15 7	720 gg,rs
ch63	0	0 0	0	0	0 0	0	0 0	0	0 0	1 0	0	0	0	wdl	mixed grass sorg	0	15	euc/cor/acc/senna	10	2mth	0	1.9	8.9	2	0	0	spatter		260ct15 8	340 gg,rs
ch64	0	0 0	0	0	0 0	0	0 0	0	0 0	0 0	0	0	0	wdl	mixed grass sorg	1	10	euc/cor/acc/senna	15	2mth	1	1.9	7.9	2	0	0	spatter		260ct15 9)10 gg,rs
ch66	0	0 0	0	3 0	0 0	0	0 0	0	0 0	0 0	0	0	0	wdl	mixed grass sorg	1	8	euc/cor/acc/senna	10	2mth	0	1.9	8.9 7.9	2	0	0	spatter		260ct15 9	,015 aa.rs
ch67	0	0 0	0	0	0 0	0	0 0	0	0 0	0 0	0	0	0	wdl	mixed grass sorg	2	15	euc/cor/acc/senna	10	2mth	0	1.9	8.9	2	0	0	0		26oct15 1	045 gg,rs
ch68	0	0 0	0	0	0 0	0	0 0	0	0 0	1 1	0	0	0	wdl	mixed grass sorg	3	10	euc/cor/acc/senna	10	2mth	0	1.9	8.9	2	0	0	0	conttored chowers am	26oct15 1	115 gg,rs
0109	0	0 0	0	U	0 0	0		0	0 0	1 0	0	0	0	wai	mixed grass sorg	10	10	euc/cor/acc/serina	12	411101	0	3.2	10.2	Z	I	0	1	scattered showers am, Some	2700115 7	uu yy,is
. = 0																												possible digs but no other		
ch70 ch71	U O	I O 1 old open	U O	1	1 0	0	0 1	0	U 0 0 0	0 1	0	0	r O	wdl shbl	mixed grass sorg	15 5	10 15	euc/cor/acc/senna acc/mel/euc	15 20	4mth 4mth	0	3.2 3.1	10.2 9.1	2	1 1	0	1 1	evidence to confirm scattered showers am	27oct15 7 27oct15 8	30 gg,rs 810 ag rs
ch72	0	1 old apron	0	1	0 0	0	0 0	õ	0 0	0 0	0	Ő	0	open wdl	mixed grass sorg	2	10	acc/euc	20	2mth	Ő	2.9	10.9	2	1	0	1	spatter	27oct15 9	700 gg,rs
ch73	0	0 0	0	0	0 0	0	0 1	0	0 0	0 0	0	0	0	open wdl	sorg/ spin/ mixed grass	1	10	acc/euc	10	2mth	0	2.7	10.7	2	1	0	1	spatter	27oct15 9)40 gg,rs
ch74 ch75	0	U 0 0 0	0	0		0		0	υ 0 0 1		0	0	0	open wdl shbl	sorg/ spin/ mixed grass sorg/ spin/ mixed grass	2	15 15	euc/cor/acc acc/euc/baubin	17 15	2mth 2mth	0	2.7	10.7 9 7	2	1 1	0	1 1	spatter	27oct15 1	015 gg,rs
ch76	0	0 0	0	0	0 0	0	0 1	õ	0 0	0 0	0	Ő	0	shbl	sorg/ spin/ mixed grass	1	15	acc/euc/bauhin	15	2mth	Ő	2.4	9.4	2	1	0	1	spatter	27oct15 1	110 gg,rs
ch77	0	0 0	0	0	0 0	0	0 1	0	0 0	0 0	0	0	0	shbl	sorg/ spin/ mixed grass	1	15	acc/euc/bauhin	15	2mth	0	2.4	9.4	2	1	0	1	spatter	27oct15 1	145 gg,rs
cn/8 ch79	0	0 0	0	0	0 0	0	0 0	0	0 0	1 1	0	0	0	shbl	sorg/ spin/ mixed grass sorg/ spin/ mixed grass	ı 1	15 20	acc/euc	15 20	2mth 2mth	0	2.4	9.4 9.4	2	1	0	ı 1	spatter	270ct15 1	210 gg,rs 245 aa.rs
ch80	0	0 old open + aprons	0	1	0 0	0	0 0	0	0 1	1 1	0	0	0	shbl	sorg/ spin/ mixed grass	1	15	acc/euc/bauhin	15	2mth	0	2.5	9.5	2	1	0	1	spatter	27oct15 1	310 gg,rs
ch81	0	0 0	0	0	1 0	0	0 0	0	1 1	1 0	0	0	0	wdl	sorg/ spin/ mixed grass	70	20	acc/euc/bauhin	20	5 yr	0	3.7	10.7	2	1	0	1	spatter	27oct15 1	346 gg,rs

ch82	0	0 0	0	0	0 0	0	0	0 0	1 1	0	0	1	0	0	0	wdl	sorg/ spin/ mixed grass	70	25	acc/euc/bauhin	15	5 yr	0	3.7	10.7	2	1	0	1	spatter	27oct15 1415 gg,rs
ch83	0	0 0	0	0	1 0	0	0	1 () 1	0	0	0	0	0	0	wdl	sorg/ spin/ mixed grass	70	25	acc/euc/bauhin	15	4 mth	0	3.7	9.7	2	1	0	1	spatter	27oct15 1450 gg,rs
ch84	0	0 0	0	0	1 0	0	0	1 (0 0	0	1	0	0	0	0	shbl/wdl	sorg/ spin/ mixed grass	5	20	acc/euc/bauhin	10	4 mth	0	3.0	8.0	2	1	0	1	spatter	27oct15 1515 gg,rs
ch85	0	0 0	0	0	1 0	0	0	1 (0 0	0	1	0	0	0	0	wdl	sorg/ spin/ mixed grass	60	20	acc/euc/bauhin	20	5yr	1	3.9	9.9	2	1	0	1	prob spatter	27oct15 1545 gg,rs
ch86	0	0 0	0	0	0 0	0	0	0 0	0 0	0	0	0	0	0	0	shbl/wdl	sorg/ spin/ mixed grass	10	20	acc/euc/bauhin	15	4 mth	0	3.1	8.1	2	1	0	1	prob spatter	27oct15 1615 gg,rs
ch87	0	0 0	0	0	0 0	0	0	0 0	0 0	0	1	0	r	r	0	shbl/open wdl	sorg/ spin/ mixed grass	50	20	acc/euc/bauhin	20	5yr	0	3.6	8.6	1	1	0	0	spatter	27oct15 705 gg,rs
ch88	0	0 old open + aprons	0	1	0 0	0	0	1 (0 0	0	0	1	0	0	r	shbl/open wdl	sorg/ spin/ mixed grass	10	15	acc/euc/bauhin	15	4 mth	0	2.7	6.7	1	1	0	0	some spatter	27oct15 740 gg,rs
ch89	0	0 0	0	0	0 0	0	0	1 (0 0	0	1	0	0	r	0	open wdl	sorg/ spin/ mixed grass	40	15	euc/acc	15	4yr	1	2.6	8.6	1	1	0	0	some spatter	27oct15 810 gg,rs
ch90	0	0 0	0	0	0 0	0	0	1 (0 0	0	0	0	r	0	r	wdl	sorg/ spin/ mixed grass	10	20	euc/acc	15	4 mth	1	1.8	4.8	1	1	0	0	some spatter	27oct15 830 gg,rs
ch91	0	0 0	0	0	0 0	0	0	1 1	1 0	0	1	0	0	0	0	wdl	spin/ sorg /mixed grass	40	15	euc/acc	15	6yr	1	2.9	8.9	1	1	0	0		27oct15 910 gg,rs
ch92	0	0 0	0	0	0 0	0	0	1 (0 0	0	1	0	0	0	0	wdl	spin/ sorg /mixed grass	10	15	euc/acc	15	4 mth	1	2.4	8.4	1	1	0	0		27oct15 940 gg,rs
ch93	0	0 0	0	0	0 0	0	0	1 (0 0	0	1	0	0	r	0	wdl	sorg /spin/mixed grass	50	20	euc/acc	20	6yr	1	3.3	9.3	1	1	0	0		27oct15 1015 gg,rs
ch94	0	0 0	0	0	1 0	0	0	1 (0 0	0	1	0	0	r	0	wdl	sorg /spin/mixed grass	40	20	euc/acc	15	6yr	1	3.2	10.2	1	1	0	0		27oct15 1045 gg,rs
ch95	0	0 0	0	0	0 0	0	0	1 (0 0	0	1	0	0	0	0	wdl	spin/ sorg /mixed grass	20	15	euc/acc	15	5 mth	1	2.9	9.9	1	1	0	0		27oct15 1115 gg,rs
ch96	0	0 0	0	0	1 0	0	0	1 (0 0	0	1	0	0	0	0	wdl	sorg /spin/mixed grass	40/20	20	euc/acc	20	6 mth	1	3.0	10.0	1	1	0	0		27oct15 1145 gg,rs
ch97	0	0 0	0	0	0 0	0	0	1 (0 0	0	0	0	0	0	0	wdl	spin/ sorg /mixed grass	10	10	euc/acc	15	5 mth	0	2.8	8.8	1	1	0	0		27oct15 1220 gg,rs
ch98	0	1 1	0	2	0 0	0	0	0 0	0 0	0	1	0	0	0	0	wdl	spin/ sorg /mixed grass	10	15	euc/acc	15	7 mth	0	2.6	8.6	1	1	1	0	some spatter	27oct15 1245 gg,rs
ch99	0	0 0	0	0	0 0	0	0	0 0	0 0	0	0	0	0	0	0	open wdl	spin/ sorg /mixed grass	15	15	euc/acc	10	7 mth	0	2.6	8.6	1	1	1	0	some spatter	27oct15 1320 gg,rs
ch100	0	1 1	1	3	0 0	0	0	0 0	0 0	0	0	1	0	0	0	open wdl	spin/ sorg /mixed grass	10	15	euc/acc	10	7 mth	0	2.6	7.6	1	1	1	0	some spatter	27oct15 1345 gg,rs
ch101	0	0 0	0	0	0 0	0	0	0 0	0 0	0	1	0	0	0	0	open wdl	spin/ sorg /mixed grass	10	15	euc/acc	10	7 mth	0	2.6	7.6	1	1	1	0	spatter	27oct15 1415 gg,rs

Key 0 = None recorded

0 = None recorded 1 = Evidence present r = Evidence on road Small amials recorded = pigeon,quail,invertebrate,small bird, rodent, monitor represented 0 =none, 1 = detected surface trackability: 1=very good ... 4=very poor plot_ODS: 4=very good ... 13=very poor includes light, sun angle, continuity Wind strength: 0=no wind, 1 = light wind, 2 = windy conditions Rain event: 0 = no rain, 1 = light rain (spatter), 2 = rain event

Component 2 Survey 1 (October 2015)

Spp de	e <mark>tails</mark>	bilby				dog		cat			COW			Agile																	
																														Bilby	
																														presence/	
plot		dig dig	g dig	burrows																crow/	qu	ıai				Sm			Last Rain	absence	
no.	1 3	2 plate co	nic shrul	b (No.)	scat	1 2	3 abur	nd 1	2 3	3 abun	d 1	2 3 8	abund	1 2	3 abund	scat	digs	Rodent	bustard	magpie	Coucal I	pigeon	goanna	dragon	invert	bird	echidna	Other	events	score	bilby notes
ch30								1	1 1	r 2		1	1	1				1		r		1	1d		1	r			20/08 spatter	0	No sign of use
ch39								r		1								1		r			r	r					20/08 spatter	0	No sign of use
ch45		1	m				r 1	r	r	2		r	1	r r	1	1f	1f	0											20/08 spatter	1	possible digs present but no other signs
ch48		1	m				r 1	r	r	1				r r	1r 2	1fm	1fm	b					b	r					20/08 spatter	1	possible digs present but no other signs
ch53											r	1	1	1r 1r	1		10												20/08 spatter	0	No sign of use
ch62	r r	r	1	(5) fm	1									1 1	1	1f	1f						b	b		r		snake	14/10 rain	3	Lots of digs over a large area
ch65	r		1	(1) f	1		r 1								r								b	b					26/10 spatter	3	1 small bilby (small scats).
ch71			0	(5) 10		r	1	r	r	1				1r 1	1	10	10			r				b					14/10 rain	1	Numerous old burrows only one open
ch80				(5) o					r	1	1	r	1	r 1	1		10	b											14/10 rain	1	Burrows in road verge, one in termitaria (two entrances)
ch88				(2) 0					r	1				1 1r	2	1mo	1m						b					corella	14/10 rain	1	two old burrows both open
ch98		1	m	(2) m					r	1				r 1r	r 2	1m	1fm						b	b					26/10 spatter	2	few old digs on plot. No scats present
ch100		f	m	(1) f	f									1	r 1	1fm	1fm						b	b					26/10 spatter	3	Large active area, 50% digs into Dodonea hispilura.

Component 2 Survey 2 (November 2015)

Spp de	etail <mark>s</mark>	bilby					dog		ca	t		СС	w		A	Agile																					
																																				Bilby	
																																				presence	/
plot		dig d	lig	dig	burrows																		cro	w/	qu	iai					Sm			Last R	lain	absence	
no.	1 2	plate c	onic	shrub	(no.)	scat	1 2	3 abu	nd 1	2	3 abu	nd	12	3 abu	und	1 2	2 3	abund	scat	digs	Roden	t busta	rd ma	gpie (Coucal I	pigeo	n goanr	a dr	ragon	invert	bird	echidna	Other	event	S	score	bilby notes
ch30										1 1	lr 2		r	1r :	2		r										1d				r			16/11	spatter	0	No sign of use
ch39														r		r	r	1		1m							1r,o	k						16/11	spatter	0	No sign of use
ch45							r	r 1		r	1			r		1 r		1	1f	1f						r	d		b					16/11	spatter	0	No sign of use
ch48																r	1r	1	1m	1m	b				r									16/11	spatter	0	No sign of use
ch53												r	· 1 ·	1r :	2	1r 1r	r	1			b													18/	11 rain	0	No sign of use
																																					no fresh evidence of bilby in the area, rain event 17/11 filled two
ch62			10		(5) m, o	1		r 1								1r	1r	1							1	-	r		r		0			18/	11 rain	2	burrows.
																																					fresh digs and scratching on burrow, only a few minutes of
ch65		1f	1f		(1) f	1			1		1					1	r	1			b													18/	11 rain	3	inspection before large down poor
ch71			0		(10) o			r 1		r	1						1r	1												1				18/	11 rain	1	one open burrow others only aprons
																																					Burrows around termitaria re dug over night possibly echidna as
ch80					(4) o				r		1		-	1r		r	2r	1	10	10												f, digs		16/11	spatter	1	there is no evidence of bilby use in the area
ch88					(2) 0				r		1					1r	1r	1	1m	10							1r, 2r	, b						16/11	spatter	1	Old burrow further eroded
ch98			10		(2) m					r	1						r	1	10	10	b						r, c							16/11	spatter	2	Possible digs near burrow but no fresh evidence or signs of use
ch100		1f	f/m	f/m	(1) m											r	r	1	1m	1m							r, b,	1d						16/11	spatter	3	lots of digs present, burrow not in use

Component 2 Survey 3 (December 2015)

Spp d	etail <mark>s</mark>	bilby	ı –				dog			cat				cow				Agil	е																			
																																					Bilby presence/	
plot		dig	dig (dig	burrows																					crow/		qua	i				Sm			Last Rain	absence	
no.	1 2	plate	e conic s	shrub	(no.)	scat	1 :	2 3	3 abund	1 1	2	3 abı	und	1	2 3	3 abi	und	1	2	3 ab	ound	scat	digs	Rod	ent	bustard magpie	Couca		pigeon	goanna	dragon	invert	bird	echidna	a Other	events	score	bilby notes
ch30							1 1	1			r	•		1	1 1	r	1		1			10	1m			r			r	1bo	b		r			4/12 rain	0	No sign of
ch39														r	r		1	1	1 r		1		1f	b	C	r r				1db	r,1b	r		r		4/12 rain	0	No sign of
ch45					(1) 0			r	1		r		1		1 1				r 1	r	1	1fo	1m					r	r	1b	b					4/12 rain	1	One old bu of the plot
ch48					(.) -						r			1r	r		1	1r	r 1	1	1	1m	1f			r			r	r.1db		1b	r		sm mam	4/12 rain	0	No sign of
ch53								r			r	-		1r -	ı 1r r		2	1r	r r		1			r	r	r				1b			r		frog	4/12 rain	0	No sign of
																																						Four burro
ch62	r		20		(1) m, o			r	· 1		r r		1		r		1	1r	1r r		1		1m	b	C					1bd,r	rb	b				13/12 rain	2	week. Print
																																						Burrow was
ch65			10		(1) o										r r	•	1		1r r	•	1	10								1b	b	b				13/12 rain	2	present bu
ch71					(10) o			r	· 1						r			1r	1	r	1	2fo	2f	b)	1				1bd	b	1				13/12 rain	1	No change
																																						Burrows at
ch80		1f	1f		(4)2f,2o										1			1r	r		1									1b		b				13/12 rain	3	camera doi
ch88		1fm	1fm	1f	(2) 0	1		_	. 1									1r	1r		1	1mo	1f							3hd r		h				13/12 rain	2	Numerous during broa
crioo					(2) 0	'		+														mio								5504,1						13/12 10/11	2	New burrow
ch98		3fm	3fm	1fm	(3) 1f,2o	2												1r	r 1	r	1	10		b	C					1db	b	b				13/12 rain	3	collected. Y
ch100		1f	1fm	1fm	(1) m			r r										2			1			h	h	r				1h	h	b				13/12 rain	3	Some smal

Key

1 = Evidence present (1/2)

2 = Medium amount of evidence (3/5)

3 = Lots of evidence (>5)

r = Evidence on road

d = diggings, b = burrow f = Fresh evidence (within 2 nights)

m = Medium age (3/7 nights)

o = Old evidence (> 1 week)

fs=freshscats,os=oldscats, j=juvenile Track age: 1= 1-2 days, 2= 3-7days, 3=>7 days

abund: 1=few,2=med,3=lots

use

use

urrow found at the base of a termite mound just north t but long unused

use

f use

ows washed away, 1 remaining and used within the last nts also about a week old.

ashed out with only apron remaining. A few old digs It no other evidence, animal no longer present.

on plot burrows and aprons present but not activity t termitaria further dug out and Bilby recorded on oing so.

digs present and scat collected. No burrows found ader search. Possible transient Bilby.

ow dug near to old burrows, lots of digs present, scats Young animal on camera.

Bilby digs but no scats. Small amount of medium burrow. One juvenile animal recorded using on camera.

Bilby presence/absence score: 0= No recorded evidence, 1= possible evidence but not confirmed, 2= Evidence present but old and not active, 3= Active area with good evidence

Appendix C – Genetic Assessment Report

Genotyping of bilby scats collected from Cape Leveque Road, Kimberley



Government of Western Australia Department of Parks and Wildlife Science and Conservation Division Your ref: Our ref: GHDBilb001 Enquiries: Martin Dziminski Phone: 08 9405 5100 Email: <u>martin.dziminski@dpaw.wa.gov.au</u>

18 January 2016

Glen Gaikhorst Principal Zoologist GHD PO Box 3106 Adelaide Terrace PERTH WA 6832

Genotyping of bilby scats collected from Cape Leveque Road, Kimberley Fiona Carpenter and Martin Dziminski

GHD provided 32 greater bilby (*Macrotis lagotis*) scat samples to the Department of Parks and Wildlife for genotyping (Table 1). Samples were collected and kept frozen in labelled brown paper bags within a plastic ziplock bag until DNA extraction was undertaken.

DNA extractions were undertaken over two days from the 22nd to the 23rd December 2015. Genomic DNA was extracted from thawed 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 seven highly polymorphic microsatellite markers (Table 2). 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 (version 5.0.0).

Of the 32 supplied samples, 16 samples yielded DNA (Table 3). PCR using all seven markers and fragment analyses were successful allowing complete genotyping of the 16 samples.

Genotyping using the seven loci identified six distinct individuals present across the plots surveyed (Table 4).

The average genotyping success rate of 50% was higher than the expected rate of 20-25 % from previous trials. The scats provided for genotyping were likely very fresh resulting in this high success rate. GHD provided frozen samples; standard procedure is storage of samples in tubes with dry silica gel beads. The results of this study showed that freezing scat samples also works, and if field and transport facilities and conditions permit, then the technique of freezing scan continue to be used.

Further analyses of these data can reveal the relatedness of individuals within populations (for example if individuals are full- or half-siblings, or other levels of relatedness). When monitoring is completed at other sites across the north of Western Australia and genetic data is available, population genetic analyses can be completed. These analyses can reveal isolation or connectivity between populations using gene flow and transfer.

Sincerely, Dr Martin Dziminski.

References

Moritz, C., A. Heideman, E. Geffen, and P. Mcrae. 1997. Genetic population structure of the Greater Bilby Macrotis lagotis, a marsupial in decline. Mol. Ecol. 6:925–936.

Piggott, M. P., and A. C. Taylor. 2003. Extensive evaluation of faecal preservation and DNA extraction methods in Australian native and introduced species. Aust. J. Zool. 51:341–355.

Smith, S., P. McRae, and J. Hughes. 2009. Faecal DNA analysis enables genetic monitoring of the species recovery program for an arid-dwelling marsupial. Aust. J. Zool. 57:139–148.

	GHD ID (Sample_Plot_I	(ט	
Sample	Plot	ID	DPaW ID
1	24	7	KIM0027
2	24	6	KIM0028
3	24	5	KIM0029
4	24	4	KIM0030
5	24	2	KIM0031
6	24	1	KIM0032
7	24	2/2	KIM0033
8	24	6	KIM0034
9	24	15	KIM0035
10	24	3	KIM0036
11	24	8	KIM0037
1	62	A	KIM0038
2	62	3	KIM0039
1	65	А	KIM0040
2	65	2	KIM0041
3	65	1	KIM0042
4	65	В	KIM0043
5	65	D	KIM0044
6	65	E	KIM0045
7	65	С	KIM0046
8	65	А	KIM0047
1	5	5	KIM0048
2	5	4	KIM0049
3	5	3	KIM0050
4	5	2	KIM0051
5	5	1	KIM0052
6	5	5b	KIM0053
7	5	Н	KIM0054
8	5	С	KIM0055
9	5	Е	KIM0056
10	5	2b	KIM0057
1	100	1	KIM0058

Table 1. Bilby scat samples supplied by GHD. GHD ID (Sample Plot ID)

Locus	Primer set	Fluorescent label	Reference
Multiplex 1			
B02	BIL02	6-FAM	Moritz et al. (1997)
B17	Bil17intF	VIC	Moritz et al. (1997) and Smith et al. (2009)
B56	Bil56intF	PET	Moritz et al. (1997) and Smith et al. (2009)
Multiplex 2			
B55	BIL55	6-FAM	Moritz et al. (1997)
B22	BIL22	VIC	Moritz et al. (1997)
B41	BIL41intF	PET	Moritz et al. (1997) and Smith et al.
			(2009)
B63	BIL63	NED	Moritz et al. (1997)

Table 3. Bilby scat samples successfully genotyped.

	7 0 71
KIM0027	
KIM0028	
KIM0029	
KIM0031	
KIM0032	
KIM0034	
KIM0035	
KIM0036	
KIM0037	
KIM0038	
KIM0042	
KIM0043	
KIM0044	
KIM0045	
KIM0047	
KIM0055	
Total: 16	
	KIM0027 KIM0028 KIM0029 KIM0031 KIM0032 KIM0034 KIM0035 KIM0036 KIM0037 KIM0037 KIM0043 KIM0043 KIM0043 KIM0045 KIM0047 KIM0055 Total: 16

Table 4. Individuals identified from scat samples.

Individual	Sample
1	KIM0027
1	KIM0029
1	KIM0031
1	KIM0032
1	KIM0034
1	KIM0035
1	KIM0037
2	KIM0028
2	KIM0036
3	KIM0038
4	KIM0042
4	KIM0043
5	KIM0044
5	KIM0045
5	KIM0047
6	KIM0055


Government of **Western Australia** Department of **Parks and Wildlife Science and Conservation Division**

Your ref: Our ref: GHDBilb001 Enquiries: Martin Dziminski Phone: 08 9405 5100 Email: martin.dziminski@dpaw.wa.gov.au

Glen Gaikhorst Principal Zoologist GHD PO Box 3106 Adelaide Terrace PERTH WA 6832

Genotyping of bilby scats collected from Cape Leveque Road, Kimberley

Fiona Carpenter and Martin Dziminski

GHD provided 20 greater bilby (*Macrotis lagotis*) scat samples to the Department of Parks and Wildlife for genotyping (Table 1). Samples were stored dry, at room temperature, in 30ml tubes, approximately 1/3-filled with silica gel beads, until DNA extraction was undertaken.

DNA extractions were undertaken over two days from the 6th to the 7th April 2016. Genomic DNA was extracted from the 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 seven highly polymorphic microsatellite markers (Table 2). 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 (version 5.0.0). PCR was replicated three times for each sample to ensure maximum genotyping success and confirm genotypes.

Unfortunately, of the 20 supplied samples, only one sample (DPaW ID: KIM0066 – see Table 1) yielded DNA. PCR using seven markers and fragment analyses were successful allowing complete genotyping of this sample. This individual is distinct from the six individuals identified through DNA from scats collected at the Cape Leveque Road survey areas in October and November 2015 and previously reported to GHD on 18th January 2016.

Overall the genotyping success rate of 5% was substantially lower than the rate of 50% from previous scats supplied by GHD in December 2015. This may be due to the age of some of the scats that were collected and provided for DNA extraction and possibly environmental conditions that the scats were exposed to prior to collection.

Sincerely, Dr Martin Dziminski.

References

Moritz, C., A. Heideman, E. Geffen, and P. Mcrae. 1997. Genetic population structure of the Greater Bilby Macrotis lagotis, a marsupial in decline. Mol. Ecol. 6:925–936.

Piggott, M. P., and A. C. Taylor. 2003. Extensive evaluation of faecal preservation and DNA extraction methods in Australian native and introduced species. Aust. J. Zool. 51:341–355.

Smith, S., P. McRae, and J. Hughes. 2009. Faecal DNA analysis enables genetic monitoring of the species recovery program for an arid-dwelling marsupial. Aust. J. Zool. 57:139–148.

Table 1. Bilby	scat samples suppli	ed by GHD.		
GHD ID (Sample_Plot_ID)			DPaW ID	Notes on Samples
Sample	Plot	ID		
1	24	1	KIM0059	Old
2	24	2	KIM0060	Old
3	24	3	KIM0061	Old
4	24	4	KIM0062	Old
5	24	5	KIM0063	Old
1	98	1	KIM0064	Fresh
2	98	2	KIM0065	Fresh
1	5	1	KIM0066	
2	5	2	KIM0067	
3	5	3	KIM0068	
4	5	4	KIM0069	
5	5	5	KIM0070	
6	5	6	KIM0071	
7	5	7	KIM0072	
8	5	8	KIM0073	
9	5	9	KIM0074	
10	5	10	KIM0075	
11	5	11	KIM0076	
12	5	12	KIM0077	
1	88	1	KIM0078	Old

Table 2. Microsatellite markers used in PCR.

Locus	Primer set	Fluorescent label	Reference	
Multiplex 1				
B02	BIL02	6-FAM	Moritz et al. (1997)	
B17	Bil17intF	VIC	Moritz et al. (1997) and Smith et al. (2009)	
B56	Bil56intF	PET	Moritz et al. (1997) and Smith et al. (2009)	
Multiplex 2				
B55	BIL55	6-FAM	Moritz et al. (1997)	
B22	BIL22	VIC	Moritz et al. (1997)	
B41	BIL41intF	PET	Moritz et al. (1997) and Smith et al.	
			(2009)	
B63	BIL63	NED	Moritz et al. (1997)	

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