Western Ridge Matters of National Environmental Significance Fauna Study

Biologic Environmental Survey

Report to BHP WAIO

July 2021

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TABLE OF CONTENTS

TABLES

FIGURES

APPENDICES

EXECUTIVE SUMMARY

BHP Western Australian Iron Ore (BHP WAIO) are investigating the biological values of the Western Ridge area to provide local and contextual information to inform future environmental approvals. The Western Ridge area is located directly south of BHP WAIO's Whaleback mining operation, approximately eight kilometres (km) south-west of Newman, and covers an area of approximately 33,970 hectares (ha) (hereafter referred to as the Study Area). Previous surveys within and within the vicinity of the Study Area have recorded four species considered to be Matters of National Environmental Significance (MNES) under the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act); northern quoll (*Dasyurus hallucatus*), Pilbara leaf-nosed bat (*Rhinonicteris aurantia* 'Pilbara form'); ghost bat (*Macroderma gigas*), and Pilbara olive python (*Liasis olivaceus* subsp. *barroni*). To support future environmental approvals, BHP WAIO commissioned Biologic Environmental Survey Pty Ltd to undertake species specific studies of these MNES species within and surrounding the Study Area (this assessment).

The assessment was conducted over four separate field surveys between August and November 2020. Survey methods were focussed on obtaining population information of each of the target species, and comprised a variety of techniques including; targeted searches, camera transects, ultrasonic recordings, ghost bat scat monitoring and analysis, and eDNA water sampling.

Northern Quoll

No evidence of northern quoll occurrence was recorded within the Study Area during the assessment. Although the species has previously been recorded within the Study Area, these records are not representative of present occurrence, owing to their preservation within a stable cave microclimate. Based on the extensive sampling undertaken in the Study Area recently, and the isolated nature of suitable habitat within the Study Area, it is likely these records represent scats from an historical population, potentially transient individuals and/ or a declining population that no longer or rarely occurs within the Study Area. While the species may occur within the Study Area on an infrequent basis or in low abundance, the population is not likely to meet the definition of a "population important for the long-term survival of the northern quoll", as defined by the Department of Environment (DoE (2013)).

Pilbara leaf-nosed Bat

The Pilbara leaf-nosed bat was not recorded within the Study Area during the assessment. The species has not been recorded in the Study Area previously but has been recorded from a single call approximately 50 m to the south of the Study Area. The presence of this record suggests that the species has previously, or is likely to, occur within the Study Area. The lack of records from within the Study Area itself confirm that the species is highly unlikely to reside in the Study Area on a permanent or regular basis (i.e. there is highly unlikely to be any Priority 1 or Priority 2 diurnal roosts present) and may only forage and disperse intermittently in the Study Area. Evidence of a roost within the nightly flight distance of the species was detected during regional sampling for this assessment, indicating that the species may utilise the Study Area occasionally for such purposes. Because the Study Area is highly unlikely to contain any Priority 1 or Priority 2 diurnal roosts for the species, it is unlikely to represent a significant area for the Pilbara leaf-nosed bat.

Ghost Bat

During this assessment, the ghost bat was recorded from a single cave. The species has however previously been recorded from an additional six locations and occupation of the species within the Study Area spans multiple years. Additionally, scat evidence in two caves, CWER-01 and CWER-03, indicates occupation has occurred over a long, and perhaps regular period, and that the area has been used by breeding individuals. The presence of one maternity roost (CWER-01) and one potential maternity roost (CWER-03) within the Study Area, indicates that individuals from the Study Area form part of a 'key source population either for breeding or dispersal', as defined by DoE (2013). Additionally, records from within the Study Area represent the south-eastern extent of the species distribution in the Pilbara region, therefore forming part of a 'population that [is] near the limit of the species range' as defined by DoE (2013). Together these definitions suggest the ghost bats occurring within the Study Area form part of an 'important population' as defined by DoE (2013).

Pilbara olive python

The Pilbara olive python was recorded on 15 occasions during this assessment, comprising nine records of live individuals or secondary evidence (i.e. scats, sloughs) and six occasions from eDNA sampling at selected water features. The species has previously been recorded within the Study Area on 16 occasions, comprising direct observations and records of secondary evidence (including scats, sloughs and eDNA from water features). The Pilbara olive python population occurring within the Study Area is likely to represent a permanently residing and breeding population, and therefore a 'key source population either for breeding or dispersal', as defined by DoE (2013). Additionally, individuals from within the Study Area represent the south-eastern extent of the species distribution, therefore forming part of a 'population that [is] near the limit of the species range', as defined by DoE (2013). Together these definitions suggest the Pilbara olive python's occurrence within the Study Area forms part of an 'important population', as defined by DoE (2013).

Summary

The Study Ares is located at the south-eastern most extent of the Pilbara bioregion and as such, the Study Area represents the south-eastern limit for many species, particularly those that have populations confined to ironstone ranges of the Pilbara bioregion. Two MNES species, known to occur within the Study Area, the ghost bat and Pilbara olive python fall into this category.

1 INTRODUCTION

1.1 Background

BHP Western Australian Iron Ore (BHP WAIO) are investigating the biological values of the Western Ridge area to provide local and contextual information to inform future environmental approvals. The Western Ridge area is located directly south of BHP WAIO's Whaleback mining operation, approximately eight kilometres (km) south-west of Newman, and covers an area of approximately 33,970 hectares (ha) (hereafter referred to as the Study Area; Figure 1.1).

Of particular interest is the potential for the Study Area to support species considered to be Matters of National Environmental Significance (MNES), being species listed as threatened under the Federal *Environmental Protection and Biodiversity Conservation Act 1999* (EPBC Act). Such species are also listed under the Western Australian *Biodiversity and Conservation Act 2016* (BC Act). Previous surveys within, and within the vicinity of, the Study Area (Biologic, 2011, 2020c, 2020f) have recorded four MNES species listed under the federal EPBC Act, the:

- northern quoll (*Dasyurus hallucatus*) – Endangered;
- Pilbara leaf-nosed bat (*Rhinonicteris aurantia* 'Pilbara form') – Vulnerable;
- ghost bat (*Macroderma gigas*) – Vulnerable; and
- Pilbara olive python (*Liasis olivaceus* subsp. *barroni*) – Vulnerable.

BHP WAIO commissioned Biologic Environmental Survey Pty Ltd to undertake species specific studies of these MNES species within and surrounding the Study Area (this assessment).

1.2 Survey Objectives

The overarching objective of this assessment was to undertake further investigation of MNES species previously recorded within the Study Area. Specifically, to further investigate the presence, abundance and distribution of MNES species within the Study Area and the surrounding area. MNES species targeted for the survey were northern quoll, Pilbara leaf-nosed bat, ghost bat and Pilbara olive python.

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Legend

Study Area

Vertebrate Fauna Habitat (Biologic 2020b)

- Breakaway/ Cliff
- Drainage Area/ Floodplain
- Gorge/ Gully
- Hillcrest/ Hillslope
- Minor Drainage Line
- Mulga Woodland
- Stony Plain
- Cleared/ Disturbed

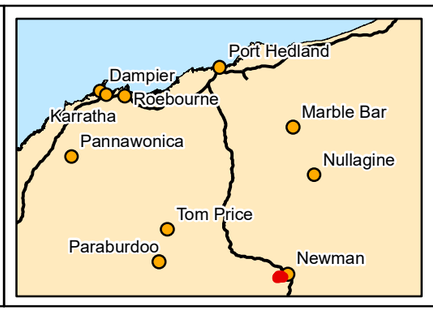
N

biologic Environmental Survey

Scale: 1:30,000

0 0.5 1 1.5 Km

Coordinate System: GDA 1994 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA 1994 Created 30/06/2021



BHP WAIO

Western Ridge MNES Study

Figure 1.1: Study Area and regional location

2 GENERAL FIELD METHODS

2.1 Compliance

This assessment was carried out in a manner consistent with the following documents developed by the Western Australian Environmental Protection Authority (EPA), the Department of Agriculture, Water and the Environment (DAWE - formerly the Department of Environment [DoE], Department of Sustainability, Water, Population, and Communities [DSEWPaC] and Department of Environment, Water, Heritage and Arts [DEWHA]) and BHP WAIO:

- BHP (2017) Guidance for vertebrate fauna surveys in the Pilbara (SPR-IEN-EMS-012);
- DEWHA (2010) Survey guidelines for Australia's threatened bats;
- DoE (2016) EPBC Act referral guideline for the endangered northern quoll (*Dasyurus hallucatus)*;
- DSEWPaC (2011a) Survey guidelines for Australia's threatened mammals;
- DSEWPaC (2011b) Survey guidelines for Australia's threatened reptiles; and
- EPA (2020) Technical guidance: terrestrial vertebrate fauna surveys for environmental impact assessment.

The survey was conducted under Department of Biodiversity, Conservation and Attractions (DBCA) Regulation 27 "Fauna Taking (Biological Assessment)" licences, issued to Ashleigh Jenkins (licence number BA27000215-2). Under Section 40 of the BC Act, threatened species sampling was completed under a DBCA "Authorisation to Take or Disturbed Threatened Species" issued to Ashleigh Jenkins (authorisation number TFA 2020-0014).

2.2 Survey Timing

This assessment was conducted over four separate field surveys between August and November 2020 (Table 2.1). Sampling for the target species was undertaken on trips 1, 3 and 4. trip 2 was specifically completed for the installation of cave monitoring cameras and no targeted species sampling was undertaken. The field survey was undertaken by experienced zoologists, whom collectively have approximately 52 years of experience undertaking fauna surveys within the Pilbara region, including targeted surveys for the MNES that were the focus of this assessment (Table 2.1).

Table 2.1: Survey timing and personnel

Trip	Dates	Survey personnel and position	Years of Pilbara zoology experience
Trip 1	24–31 August 2020	Ryan Ellis – Principal Zoologist	15
		Andrew Hide – Senior Zoologist	15
Trip 2	14–15 September 2020	Thomas Rasmussen – Senior Zoologist	12
Trip 3	9–14 October 2020	Chris Knuckey – Senior Zoologist	10
		Ryan Ellis – Principal Zoologist	15
Trip 4	26–30 November 2020	Chris Knuckey – Senior Zoologist	10
		Ryan Ellis – Principal Zoologist	15

2.3 Climate and Weather

The Pilbara bioregion has a semi-desert to tropical climate, with rainfall occurring sporadically throughout the year, although mostly during summer (Thackway & Cresswell, 1995). Summer rainfall is usually the result of tropical storms in the north or tropical cyclones that impact upon the coast and move inland (Leighton, 2004). The winter rainfall is generally lighter and is the result of cold fronts moving north easterly across the state (Leighton, 2004). The average annual rainfall ranges from 200–350 mm, although there are significant fluctuations between years, with some locations receiving up to 1,200 mm in some years (McKenzie *et al.*, 2009). Long-term climatic data is not available for the Study Area itself; however, long-term data is available from the Bureau of Meteorology (BoM) weather station at Newman Aero (Station 007176), located approximately 14 km east of the Study Area (BoM, 2021). The Newman Aero weather station is expected to provide the most accurate long-term average (LTA) dataset for climatic conditions experienced within the Study Area and was therefore the source of all climatic data presented herein (Figure 2.1).

Rainfall in the months preceding trip 1 were below long-term averages for most months, with the exception of December 2019, which recorded well above the long-term average for the month (Figure 2.1). In total, the rainfall received in the eight months prior to trip 1 (December 2019 to July 2020, 183.40 mm) was well below annual long-term average for the same period, 288.50 mm (BoM, 2021). Observed temperatures during this assessment were slightly above long-term averages on most days (Table 2.2). No rainfall was recorded during trip 1, however, above average monthly rainfall for October was recorded during trip 3 and rainfall consistent with long-term averages for November was recorded during trip 4 (Table 2.2).

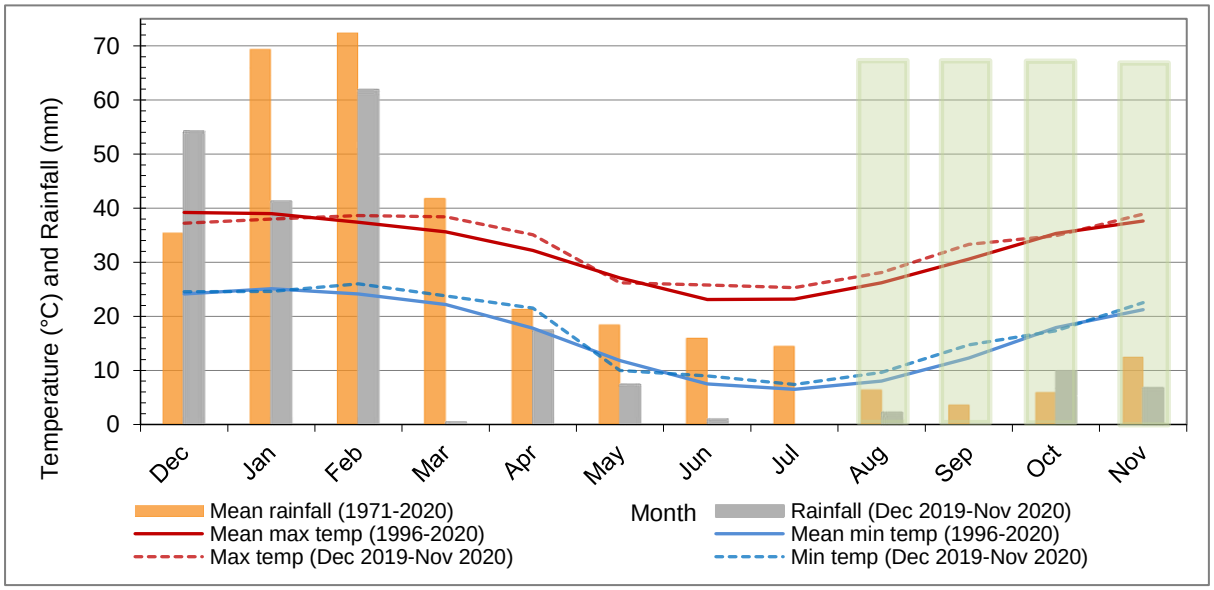


Figure 2.1: Long-term average and contemporary climate data recorded near the Study Area

(BoM, 2021) with approximate survey timing shown in shaded boxes

Table 2.2: Climatic conditions recorded for Newman Airport during the assessment (BoM, 2021)

Date	Min. temp (°C)	Max. temp (°C)	Rainfall (mm)
Trip 1			
24/08/2020	14.7	33.8	0
25/08/2020	12.4	35.6	0
26/08/2020	15.2	34.4	0
27/08/2020	13.7	35.1	0
28/08/2020	16.0	35.0	0
29/08/2020	15.9	33.8	0
30/08/2020	15.7	32.8	0
31/08/2020	12.8	34.4	0
Trip 3			
9/10/2020	7.8	34.7	0
10/10/2020	13.6	36.0	0
11/10/2020	17.6	37.2	6.4
12/10/2020	18.6	38.1	0
13/10/2020	21.9	37.7	0.4
14/10/2020	23.4	40.0	0
Trip 4			
26/11/2020	24.1	37.9	2.4
27/11/2020	24.5	39.6	0
28/11/2020	25.6	40.0	4.2
29/11/2020	29.7	42.1	0
30/11/2020	27.4	37.9	0

2.4 Habitat Assessments

Habitat assessments were undertaken in the field to characterise the suitability to species of conservation significance. Habitat assessments were undertaken at all sampling locations (*n* = 110) both within and at regional sites outside of the Study Area (Appendix A). Habitat assessments were conducted and attributes assessed using attribute terminology prescribed by BHP, which have been modified from the *Australian Soil and Land Survey Field Handbook* (National Committee on Soil and Terrain, 2009). The characteristics recorded during the habitat assessments were:

- Site information: photo and location;
- Landform: slope, relative inclination of slope, morphological type and landform type;
- Vegetation: leaf litter %, wood litter, hollow bearing trees, broad floristic formation, vegetation structure (tall, mid and low), and dominant species;
- Land surface: abundance and size of coarse fragments, rock outcropping, water bodies;
- Soil: texture, colour;
- Substrate: bare ground, rock size, rock type, rock outcropping; and
- Disturbance: time since last fire, evidence of weeds, grazing, or human disturbances.

2.5 Significance of Habitat

For the purposes of this assessment, definition on 'critical habitat' followed that of DoE (2013), being areas necessary "for activities such as foraging, breeding, roosting, or dispersal". For each species, suitable habitat was categorised as providing primary foraging, breeding, roosting, or dispersal habitat (i.e. critical habitat as per the definition above). Due to differing habitat preferences of conservation significant species (including habitat features and/or microhabitats), habitat significance was assessed on a species by species basis. Unsuitable habitat was defined by habitat which is unlikely to support the species and impact upon its presence – note individuals may be recorded in these habitats intermittently, though are not expected to be reliant on them.

It should be noted that assessment of habitat significance applies only to habitat occurring within the Study Area, and therefore may not be representative of significance applied to the same habitat in other areas outside the Study Area. For example, a habitat within the Study Area may be deemed unsuitable due to the absence of certain habitat features which are required for the species persistence, despite the same habitat occurring outside the Study Area being considered of greater significance. The significance of a habitats within the Study Area may also be influenced by other habitats occurring within the Study Area and more broadly, including areas adjacent to the Study Area, particularly if representative of primary habitat.

3 TARGET SPECIES

3.1 Northern Quoll (*Dasyurus hallucatus*)

3.1.1 Species Profile

The northern quoll is listed as Endangered under the EPBC Act and BC Act. The species was once widely distributed across northern Australia; however, it is now restricted to three isolated populations; the Pilbara, the Kimberley and Northern Territory, and Queensland (DoE, 2016). Northern quolls are opportunistic omnivores, consuming a wide range of invertebrates and small vertebrates also in addition to fruit, nectar, carrion and human refuse (Dunlop *et al.*, 2017).

As a result of facultative die-off, the abundance of the species is cyclical, and the annual reproduction is highly synchronised (Oakwood *et al.*, 2001). In the Pilbara, abundance is lowest toward the end of winter into early spring after the mating season, as a significant proportion of adult males die off and young have not yet begun to forage independently (Braithwaite & Griffiths, 1994; Oakwood, 2000). Conversely, the population density is thought to be highest in the summer months, prior to the mating season and when juveniles have begun foraging independently (Oakwood, 2000). Schmitt *et al.* (1989) reported relatively small home ranges in rugged habitat in the Kimberley (i.e. 2.3 ha for females and 1.8 ha for males), whereas in the western Pilbara, minimum activity areas are 75–443 ha for females and 5–1,109 ha for males (King, 1989).

The northern quoll is both arboreal and terrestrial, inhabiting ironstone and sandstone ridges, scree slopes, granite boulders and outcrops, drainage lines, riverine habitats (Braithwaite & Griffiths, 1994; Oakwood, 2002), dissected rocky escarpments, open forest of lowland savannah and woodland (Oakwood, 2002, 2008). Rocky habitats tend to support higher densities, as they offer protection from predators and are generally more productive in terms of availability of resources (Braithwaite & Griffiths, 1994; Oakwood, 2000). Other microhabitat features important to the species include: rock cover; proximity to permanent water and time-since last fire (Woinarski *et al.*, 2008). Dens occur in a wide range of situations including rock overhangs, tree hollows, hollow logs, termite mounds, goanna burrows and human dwellings/infrastructure, where individuals usually den alone (Oakwood, 2002; Woinarski *et al.*, 2008). At present, northern quolls are relatively common in the northern Pilbara region (generally within 150 km of the coast) but are much less common in southern and south-eastern parts of the region (Cramer *et al.*, 2016b).

The species has experienced a precipitous decline in much of its former range in northern Queensland and the Northern Territory in direct association with the spread of the cane toad (*Bufo marinus*) (Braithwaite & Griffiths, 1994; Fitzsimons *et al.*, 2010). Other threats include predation from feral predators such as foxes and cats, inappropriate fire regimes, disease, habitat degradation through grazing and weed invasion, and habitat destruction through mining and agriculture (Woinarski *et al.*, 2011). The potential invasion of the Pilbara by the cane toad is regarded as the most significant future threat to the northern quoll in the Pilbara; however, there is little knowledge of the relative impact of the other key threats, and their interactive effects, currently and in the future (Cramer *et al.*, 2016b).

3.1.2 Previous Records

The Study Area falls within the current distribution of the northern quoll, whereby the species or species habitat may occur (Department of the Environment and Energy; DoEE, 2019). A recent desktop assessment completed by Biologic (2020f) identified the nearest northern quoll record as being that of a roadkill juvenile individual from 2007. The record was made from the main access bridge into Whaleback, located approximately 5.5 km north east of the Study Area (Onshore & Biologic, 2009) (Figure 3.1). This record represents the south-eastern limit of the species occurrence in the Pilbara region (DBCA, 2021). The next nearest record of the species is located approximately 52 km west of the Study Area, dated from 2014 (DBCA, 2021).

Subsequent to the desktop assessment, Biologic (2020f) recorded the species on two occasions within the Study Area, both from secondary evidence (scats) in two caves; CWER-10 (located near the eastern edge of the Study Area) and CWER-16 (located along the southern edge of Western Ridge) (Table 3.1; Figure 3.1). Analysis of the scats by Georgianna Story of ScatsAbout, confirmed one scat (collected from CWER-10) was northern quoll, with scat age determined to be greater than 12 months old (Table 3.1). The remaining scats (collected from CWER-10 and CWER-16) were determined to be probable northern quoll; however, due to their very old age (>36 months) and degraded condition, identification could not be confirmed (Table 3.1). The occurrence of a certain northern quoll scat within the same cave as one of the probable scats provides greater confidence in the probability that the very old scat was also northern quoll. It should be noted that microclimatic conditions present within caves may also provide a degree of preservation of scats and determined age may not accurately reflect the true age of the scats.

Table 3.1: Previous records of northern quoll within the Study Area

Site	Record type	Habitat	Latitude	Longitude	Age
VWER-26 (Cave CWER-10)	Scat (certain)	Gorge/ Gully	-23.4030	119.6603	Old (>12 months)
VWER-44 (Cave CWER-16)	Scat (probable)	Gorge/ Gully	-23.4051	119.6055	Very old (>36 months)
VWER-26 (Cave CWER-10)	Scat (probable)	Gorge/ Gully	-23.4031	119.6604	Very old (3–10 years)

Despite extensive targeted sampling for northern quoll in the Study Area, including targeted searches undertaken at 32 sites (totalling approximately 61 person hours of searching) and five camera trap transects (totalling 200 camera trap sampling nights), no evidence of current occupation was recorded within the Study Area by Biologic (2020f). With consideration of the limited evidence of northern quoll occurrence within and in the vicinity of the Study Area, results of the sampling undertaken (including the age and condition of scats collected during the survey) suggested records were representative of historic occupation, transient individuals or a declining population that no longer or rarely occurs within the Study Area (Biologic, 2020f).

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7412000

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Legend

- Study Area

Vertebrate Fauna Habitat (Biologic 2020b)

- Breakaway/ Cliff
- Drainage Area/ Floodplain
- Gorge/ Gully
- Hillcrest/ Hillslope
- Minor Drainage Line
- Mulga Woodland
- Stony Plain
- Cleared/ Disturbed

Northern quoll Record (Biologic 2020b)

- Scat (Old)
- Scat (Very Old)

Northern quoll Record (Onshore 2009)

- Individual (Dead)

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biologic Environmental Survey

Scale: 1:30,000

0 0.5 1 1.5 Km

Coordinate System: GDA 1994 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA 1994 Created 01/07/2021

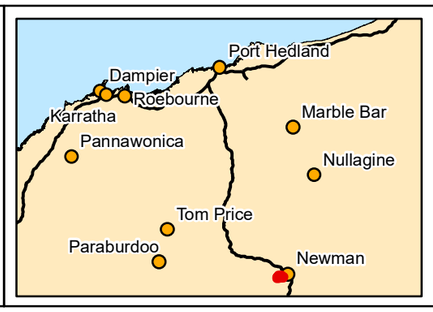

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Figure 3.1: Previous northern quoll records in the Study Area and region

3.1.3 Survey Methods

Targeted Searching

Targeted diurnal searches for northern quoll were conducted during trip 1 and trip 3. Targeted searches focused on areas providing suitable denning/ shelter and/or foraging habitat, particularly within Gorge/ Gully, Breakaway/ Cliff and Hillcrest/ Hillslope habitats (Table 3.2; Figure 3.2). Searches were also undertaken at the location of previous northern quoll records (Biologic, 2020f), sites VWER-26 and VWER-44 (Table 3.1; Figure 3.1). Searches focused primarily on detecting the species from secondary evidence, including scats, tracks, foraging remains or remains of deceased individuals. A total of 13 targeted searches were undertaken at 11 sites over the two trips, equating to a total of 24.1 person hours of targeted searches (Table 3.2).

Table 3.2: Targeted searches completed for northern quoll within the Study Area

Field survey	Site name	Date	Sampling type	Habitat	Latitude	Longitude	Person hours
Trip 1	VWER-53	25/08/2020	Targeted search	Breakaway/ Cliff	-23.3876	119.6170	4
	VWER-55	25/08/2020	Targeted search	Breakaway/ Cliff	-23.4118	119.5868	2.2
	VWER-26	26/08/2020	Targeted search	Gorge/ Gully	-23.4037	119.6605	2.5
	VWER-44	26/08/2020	Targeted search	Gorge/ Gully	-23.4047	119.6052	3.5
	VWER-17	27/08/2020	Targeted search	Gorge/ Gully	-23.3939	119.6176	2
	VWER-59	29/08/2020	Targeted search	Breakaway/ Cliff	-23.4218	119.5728	2
	VWER-60	30/08/2020	Targeted search	Breakaway/ Cliff	-23.3952	119.5914	1.8
	VWER-61	30/08/2020	Targeted search	Hillcrest/ Hillslope	-23.3902	119.6379	1.1
Trip 3	VWER-60	9/10/2020	Targeted search	Breakaway/ Cliff	-23.3952	119.5914	1
	VWER-62	9/10/2020	Targeted search	Breakaway/ Cliff	-23.3880	119.6083	1
	VWER-63	9/10/2020	Targeted search	Hillcrest/ Hillslope	-23.3936	119.5978	1
	VWER-68	9/10/2020	Targeted search	Hillcrest/ Hillslope	-23.3978	119.5796	1
	VWER-61	10/10/2020	Targeted search	Hillcrest/ Hillslope	-23.3902	119.6379	1
						Total	**24.1**

Camera Trap Transects

Camera trap transects were established at eight sites within the Study Area during and between trip 1 and trip 3 of the assessment (Table 3.2; Figure 3.2). In line with methods recommended by DoE (2016), each transect comprised ten camera traps placed approximately 50 to 100 m apart and were left *in-situ* for between four and 42 nights, for a total of 1,800 camera trap sampling nights (Table 3.3). Four camera transects were deployed and retrieved during trip 1, with a further four transects deployed during trip 1 and retrieved during trip 3 (Table 3.3).

Where possible, cameras were oriented to facilitate differentiation of individuals via spot patterning (following Hohnen *et al.*, 2012) and baited with universal bait mixture (comprising oats, peanut butter and sardines or sardines only) contained within a non-reward receptacle (perforated and capped PVC pipe). Transects were established within habitats providing denning/ shelter and/or foraging/ dispersal habitats where the species was most likely to be recorded, including Gorge/ Gully, Breakaway/ Cliff and Hillcrest/ Hillslope habitats (Table 3.3; Figure 3.2). Additionally, camera transects were also deployed in areas where northern quoll scats were recorded during previous surveys (Biologic, 2020f), sites VWER-26 and VWER-44.

Table 3.3: Camera transects sampled for northern quoll within the Study Area

Site	Habitat	Latitude	Longitude	Deployment	Retrieval	Sampling nights	Total trap nights
VWER-17	Gorge/ Gully	-23.3939	119.6176	29/08/2020	9/10/2020	41	410
VWER-26	Gorge/ Gully	-23.4037	119.6605	26/08/2020	30/08/2020	4	40
VWER-44	Gorge/ Gully	-23.4047	119.6052	26/08/2020	30/08/2020	4	40
VWER-55	Breakaway/ Cliff	-23.4118	119.5868	25/08/2020	29/08/2020	4	40
VWER-53	Breakaway/ Cliff	-23.3876	119.6170	25/08/2020	29/08/2020	4	40
VWER-59	Breakaway/ Cliff	-23.4218	119.5728	29/08/2020	10/10/2020	42	420
VWER-60	Breakaway/ Cliff	-23.3952	119.5914	30/08/2020	9/10/2020	40	400
VWER-61	Hillcrest/ Hillslope	-23.3902	119.6379	30/08/2020	10/10/2020	41	410
						Total	**1,800**

3.1.4 Survey Results

Targeted Searching

No evidence of northern quoll was recorded within the Study Area during the 24.1 person hours of targeted searches, including at locations of previous records, or opportunistically during other targeted sampling during this assessment.

Camera Transects

Camera transects deployed at eight sites within the Study Area for a total of 1,800 sampling nights (including sites of previous Biologic (2020f) records), did not record any northern quoll.

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VWER-53
VWER-61
VWER-60
VWER-17
VWER-44
VWER-26
VWER-55
VWER-59

Legend

- Study Area
- **Vertebrate Fauna Habitat (Biologic 2020b)**
  - Breakaway/ Cliff
  - Drainage Area/ Floodplain
  - Gorge/ Gully
  - Hillcrest/ Hillslope
  - Minor Drainage Line
  - Mulga Woodland
  - Stony Plain
  - Cleared/ Disturbed
- Camera Trap Transect
- **Targeted Search**
  - Diurnal Search
  - Nocturnal Search

N

biologic Environmental Survey

Scale: 1:30,000

0 0.5 1 1.5 Km

Coordinate System: GDA 1994 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA 1994 Created 01/07/2021

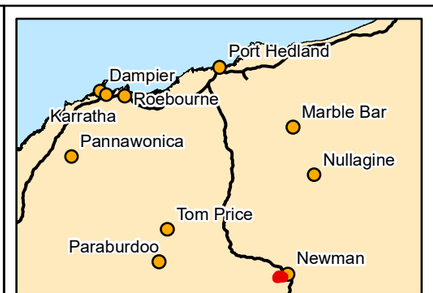

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Figure 3.2: Northern quoll sampling in the Study Area

3.1.5 Discussion

Although the Study Area falls within the current distribution of the northern quoll, whereby the species or species habitat may occur (DoEE, 2019), the lack of contemporary records during the current or recent (Biologic, 2020f) surveys suggest the species' occurrence within the Study Area is limited to historic occurrence or, if present, only intermittently. No evidence of the species was recorded during this assessment and previous records from Biologic (2020f) were representative of old (>12 months) or very old (>36 months) records. Although the extent of preservation cannot be determined, considering the microclimatic conditions present within caves, the scats may in fact be much older than suspected. Furthermore, scats potentially belonging to a regionally extinct species, the Lesser Stick Nest Rat (*Leporillus apicalis*), were also recorded from one cave (CWER-10) by Biologic (2020f), highlighting the potential for scats to be preserved in better condition and retained in these cave environments.

Despite the lack of contemporary records of northern quoll within the Study Area, critical habitat (i.e. denning/ shelter habitat as defined by DoE (2016)) for the species occurs in Gorge/ Gully, and Breakaway/ Cliff habitats (as mapped by Biologic (2020f)). Rocky habitats typically (i.e. Gorge/ Gully and Breakaway/ Cliff) offer protection from predators and are generally more productive in terms of availability of resources (Braithwaite & Griffiths, 1994; Oakwood, 2000) and in the Pilbara provide a higher abundance of possible denning features. The Gorge/ Gully (3.5%, 165.7 ha) and Breakaway/ Cliff (1.1%, 53.1 ha) habitats mapped within the Study Area (Figure 3.3) are consistent with this definition and offer microhabitat features comparable in extent and quality to those which support northern quoll elsewhere in the region (Hernandez-Santin *et al.*, 2016) (refer to Plate 3.1). This, in conjunction with the fact that northern quoll has been recorded in the Study Area (albeit limited to historic occurrence or, if present, only intermittently), Gorge/ Gully habitat has the potential to support breeding activity. Moreover, the recent desktop assessment completed by Biologic (2020f) identified the nearest northern quoll record (5.5 km north east of the Study Area) as being that of a roadkill juvenile individual from 2007. This suggests that breeding may have historically occurred in the area. As such, the Gorge/ Gully and Breakaway/ Cliff habitat is considered primary breeding and foraging habitat for the northern quoll within the Study Area. However, there is a no evidence to suggest the existence of a breeding population in the Study Area at the time of this study. Additionally, the limited occurrence of critical habitat in areas adjacent to or in the broader vicinity of the Study Area, and scarce availability of dispersal corridors between other areas and the Study Area, indicate movement into and from the Study Area by the species is likely to be limited.

Plate 3.1: Gorge/ Gully habitat within the Study Area (Site VWER-40)

Minor Drainage Line (2.1%, 101.7 ha) and Hillcrest/ Hillslope (40.6 %, 1,936.6 ha) habitats mapped within the Study Area (Figure 3.3) represent primary foraging and/or dispersal habitat for the species. However, the presence of northern quoll within these habitats is very likely tied to their occurrence relative to Gorge/ Gully and Breakaway/ Cliff habitat, and they are unlikely to support the species if they are not adjacent to or in close proximity to these habitats. The Minor Drainage Line in the Study Area is represented as an extension of the Gorge/ Gully habitat and thus provides similar habitat features to this habitat type, although to a much lesser extent. Foraging or dispersal habitat is recognised to be any land comprising predominantly native vegetation in the immediate area (i.e. within 1 km) of denning/ shelter habitat (DoE, 2016). While individuals may be recorded within the remaining habitats, this is likely to be on a temporary basis only and confined to areas in close proximity to Gorge/ Gully and Breakaway/ Cliff habitat.

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Legend

- Study Area

Vertebrate Fauna Habitat (Biologic 2020b)

- Breakaway/ Cliff
- Drainage Area/ Floodplain
- Gorge/ Gully
- Hillcrest/ Hillslope
- Minor Drainage Line
- Mulga Woodland
- Stony Plain
- Cleared/ Disturbed

Northern quoll Record (this Survey)

- Camera Trap Null Record

Northern quoll Record (Biologic 2020b)

- Camera Trap Null Record
- Scat (Old)
- Scat (Very Old)

N

biologic Environmental Survey

Scale: 1:30,000

0 0.5 1 1.5 Km

Coordinate System: GDA 1994 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA 1994 Created 01/07/2021

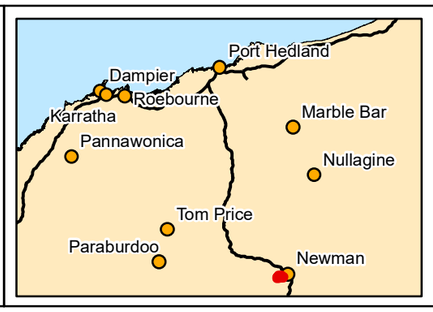

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Figure 3.3: Northern quoll records in the Study Area

3.2 Pilbara Leaf-nosed Bat (*Rhinonicteris aurantia*)

3.2.1 Species Profile

The Pilbara leaf-nosed bat is listed as Vulnerable under the EPBC Act and the BC Act. Within the Pilbara, the species is recognised as a geographically isolated population (or form) of the orange leaf-nosed bat, distributed across northern Australia and separated from the Pilbara population by approximately 400 km of the Great Sandy Desert (Armstrong, 2001). The Pilbara population is regarded as representing a single interbreeding population comprising multiple colonies (TSSC, 2016b). The most updated conservation advice (TSSC, 2016b) stated that there were at least 10 confirmed day roosts (including maternity roosts) and a further 23 unconfirmed roosts throughout the Pilbara region, although this is likely to be an underestimate based on unpublished data.

Pilbara leaf-nosed bats typically roost in undisturbed caves, deep fissures or abandoned mine shafts (Armstrong, 2000, 2001). The species' limited ability to conserve heat and water (Baudinette *et al.*, 2000) means they require warm (28–32ºC) and very humid (85–100%) roost sites to persist in arid and semi-arid climates (Armstrong, 2001; Churchill, 1991). Roost sites with such attributes are relatively uncommon in the Pilbara and the limiting factor of the species' distribution (Armstrong, 2001). During the dry season (June to November), individuals are believed to aggregate in roosts that provide a suitably warm, humid microclimate (Armstrong, 2000, 2001; Bullen & McKenzie, 2011). While in the wet season (December to May), when conditions are generally wetter and more humid, individuals typically disperse roosting in seasonally suitable features (Armstrong, 2000, 2001; Bullen & McKenzie, 2011). TSSC (2016b) categorised underground refuges used by the species into four categories:

- **Permanent Diurnal Roosts** (Priority 1 – critical habitat for daily survival): are occupied year-round and are likely to be the focus for some part of the 9-month breeding cycle.
- **Non-Permanent Breeding Roosts** (Priority 2 - critical habitat for daily and long-term survival): are used during some part of the 9-month breeding cycle but not year-round.
- **Transitory Diurnal Roosts** (Priority 3 – critical habitat for daily and long-term survival): are occupied outside the breeding season and could facilitate long distance dispersal.
- **Nocturnal Refuge** (Priority 4 – not considered critical but important for persistence in a local area): are occupied or entered at night for resting, feeding or other purposes (excluding overhangs).

The species forages within and in the vicinity of roost caves and more broadly along waterbodies with suitable fringing vegetation supporting prey species (TSSC, 2016b). Foraging sites surrounding known or suspected roosts can be critical to the survival of the species. TSSC (2016b) categorised foraging habitat into five categories: gorges with pools (Priority 1), gullies (Priority 2), rocky outcrops (Priority 3), major watercourses (Priority 4) and open grassland and woodland (Priority 5) (TSSC, 2016b). The species is predicted to travel up to 20 km from roost caves during nightly foraging (Cramer *et al.*, 2016a); however, seasonal variation is known to occur, with foraging occurring up to 20 km in the dry season and up to 50 km during the wet season (Bullen, 2013).

3.2.2 Previous Records

The Study Area is located at the southern extent of the species current distribution, whereby the species or species habitat may occur (DoEE, 2019). A total of 156 records of the Pilbara leaf-nosed bat are known to occur within 26 km of the Study Area (Biologic, 2020f). The nearest record of the species is located approximately 50 m south of the Study Area from November 2019 (Biologic, 2020c), followed by four records from Cathedral Gorge, approximately 11-18 km north of the Study Area, recorded between 2013 and 2015 (Biologic, 2014, 2016a) (Figure 3.4). The remaining records occur between 20–26 km north of the Study Area, primarily along Kalgan Creek, from 2013–2017 (DBCA, 2020) (Figure 3.4). Despite a lack of sampling within the intervening area, the scarcity of records in the broader vicinity of the Study Area suggests the species is relatively uncommon locally and in the vicinity of Newman.

Despite numerous surveys, the species has not been confirmed within the Study Area itself. The lack of records within the Study Area relative to the amount of sampling effort expended, almost certainly rules out the presence of a diurnal roost occurring within the Study Area. A total of 19 caves have however previously been recorded within the Study Area, each of which has been assessed visually for presence of the species and six of the most prospective have been sampled with ultrasonic recorders. Additionally, a total of 14 water features have been recorded within the Study Area and six of the most prospective have been sampled with ultrasonic recorders.

The nearest and most recent record of the species, from 50 m south of the Study Area (Biologic, 2020c), was of a single ultrasonic call recording at 21:03 within Mulga Woodland habitat on 30 November 2019. The timing and nature of the record is consistent with that of a foraging and/ or dispersing individual; however, based on the late timing of the call, an indicative roosting location/ distance cannot be determined. Prior to this assessment, the nearest known roost of the species was located approximately 36 km north of the Study Area, near Kalgan Creek (R. Bullen *pers. comms*.). The species is capable of moving such distances, though further survey effort in the intervening area was recommended to discount the possibility of a roost existing closer to the Study Area (Biologic, 2020f).

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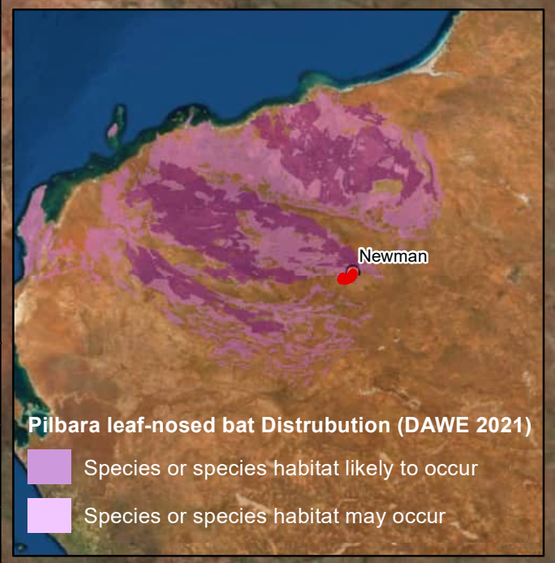

Legend

- Study Area

Vertebrate Fauna Habitat (Biologic 2020b)

- Breakaway/ Cliff
- Drainage Area/ Floodplain
- Gorge/ Gully
- Hillcrest/ Hillslope
- Minor Drainage Line
- Mulga Woodland
- Stony Plain
- Cleared/ Disturbed

Pilbara leaf-nosed bat Record (Biologic 2016)

- Ultrasonic Call (Single)

Pilbara leaf-nosed bat Record (Biologic 2020a)

- Ultrasonic Call (Single)

Pilbara leaf-nosed bat Record (BHP WAIO 2021)

- Ultrasonic Call

N

biologic Environmental Survey

Scale: 1:30,000

0 0.5 1 1.5 Km

Coordinate System: GDA 1994 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA 1994 Created 30/06/2021

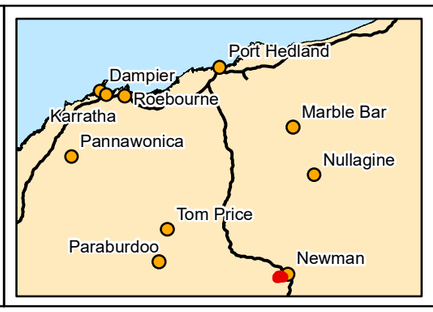

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Figure 3.4: Previous Pilbara leaf-nosed bat records in the Study Area and region

3.2.3 Survey Methods

Ultrasonic Recorders

Sampling effort during this assessment was completed to verify the results and conclusion of Biologic (2020f). Specifically, to complete additional sampling within the Study Area to gain further insight into the regularity of occurrence of the species within the Study Area, and to complete regional sampling to identify the likely location of the nearest roost and thus provide context for the record recorded 50 m south of the Study Area. Overnight recordings of bat echolocation calls were undertaken with SongMeter (SM; Wildlife Acoustics Inc.) ultrasonic bat recorders. Units were deployed at 54 locations during trip 1, trip 3 and trip 4 of this assessment, comprising 21 locations within the Study Area and 33 at regional locations outside of the Study Area (Table 3.4; Figure 3.5). Sampling was focussed on locations where bat occurrence or usage was likely to be highest, such as at the entrance of caves, at water features and within primary foraging habitats (such as along watercourses). At each location, ultrasonic recordings were performed for between one and four nights, resulting in a total of 90 recording nights, comprising 49 within the Study Area and 41 regional locations outside of the Study Area (Table 3.4; Figure 3.5). The audio settings used for the SM units followed the manufacturer's recommendations (Wildlife Acoustics, 2011, 2017) and were set to account for all species known to occur within the region (McKenzie & Bullen, 2009). All recordings were analysed by Robert Bullen of Bat Call WA for the presence of Pilbara leaf-nosed bat and ghost bat calls only.

Table 3.4: Ultrasonic sampling locations within the Study Area

Field survey	Site	Habitat feature- ID	Habitat	Sampling nights
Study Area Sampling				
Trip 1	VWER-29		Gorge/ Gully	3
Trip 1	VWER-30	Water Feature - WWER-12	Stony Plain	1
Trip 1	VWER-51		Stony Plain	3
Trip 1	VWER-52		Breakaway/ Cliff	3
Trip 1	VWER-53		Breakaway/ Cliff	3
Trip 1	VWER-54		Breakaway/ Cliff	3
Trip 1	VWER-55		Breakaway/ Cliff	4
Trip 1	VWER-57		Gorge/ Gully	3
Trip 1	VWER-58		Breakaway/ Cliff	3
Trip 3	VWER-62		Breakaway/ Cliff	2
Trip 3	VWER-63		Hillcrest/ Hillslope	1
Trip 3	VWER-64		Mulga Woodland	1
Trip 3	VWER-65		Mulga Woodland	1
Trip 3	VWER-66		Mulga Woodland	2
Trip 3	VWER-67		Gorge/ Gully	1
Trip 3	VWER-68	-	Hillcrest/ Hillslope	1
Trip 4	VWER-01	Cave - CWER-01	Breakaway/ Cliff	4
Trip 4	VWER-10	Water Feature - WWER-01	Gorge/ Gully	1
Trip 4	VWER-101		Drainage Area/ Floodplain	3
Trip 4	VWER-102		Drainage Area/ Floodplain	3
Trip 4	VWER-103		Drainage Area/ Floodplain	3
			Total	**49**

Field survey	Site	Habitat feature- ID	Habitat	Sampling nights
Regional Sampling				
Trip 1	VWER-56		Mulga Woodland	3
Trip 3	VCAT-01	Cave - CCAT-09	Gorge/ Gully	1
Trip 3	VCAT-08	Cave - CCAT-08	Breakaway/ Cliff	1
Trip 3	VWER-100	Water Feature - WWER-27	Medium Drainage Line	1
Trip 3	VWER-104		Minor Drainage Line	1
Trip 3	VWER-47		Mulga Woodland	2
Trip 3	VWER-69		Medium Drainage Line	1
Trip 3	VWER-70		Medium Drainage Line	1
Trip 3	VWER-71		Breakaway/ Cliff	1
Trip 3	VWER-72		Medium Drainage Line	1
Trip 3	VWER-73		Breakaway/ Cliff	2
Trip 3	VWER-74		Breakaway/ Cliff	2
Trip 3	VWER-75		Gorge/ Gully	2
Trip 3	VWER-76		Major Drainage Line	1
Trip 3	VWER-77		Gorge/ Gully	1
Trip 3	VWER-78	Water Feature - WWER-19	Gorge/ Gully	1
Trip 3	VWER-79	Water Feature - WWER-20	Gorge/ Gully	1
Trip 3	VWER-81		Gorge/ Gully	1
Trip 3	VWER-82	Water Feature - WWER-26	Major Drainage Line	1
Trip 3	VWER-83	Water Feature - WWER-23	Gorge/ Gully	2
Trip 3	VWER-84	Water Feature - WWER-24	Major Drainage Line	1
Trip 3	VWER-86	Water Feature - WWER-17	Gorge/ Gully	2
Trip 3	VWER-87		Medium Drainage Line	1
Trip 3	VWER-90		Major Drainage Line	1
Trip 3	VWER-91		Gorge/ Gully	1
Trip 3	VWER-92	Water Feature - WWER-22	Gorge/ Gully	1
Trip 3	VWER-93		Major Drainage Line	1
Trip 3	VWER-94	Water Feature - WWER-24	Major Drainage Line	1
Trip 3	VWER-95		Major Drainage Line	1
Trip 3	VWER-96		Medium Drainage Line	1
Trip 3	VWER-97		Medium Drainage Line	1
Trip 3	VWER-98	Water Feature - WWER-25	Major Drainage Line	1
Trip 3	VWER-99		Medium Drainage Line	1
			Total	**41**

Targeted Searches

Targeted searches were undertaken both within the Study Area and outside of the Study Area to identify prospective ultrasonic recorder sampling location (e.g. water features and caves). Within the Study Area, targeted searches occurred in areas previously unsurveyed. Regional sampling was targeted at features of interest (i.e. caves and water features) identified from previous surveys in the region (e.g. Biologic, 2014, 2016a) and was facilitated by a helicopter. Where prospective habitat was identified, on-foot targeted searches were undertaken. Additionally, due to the overlap and close association of critical habitats for the target species, opportunistic sampling was also undertaken while completing other sampling methods, such as cave assessments and deployment of camera transects.

Cave Assessments

During the survey, cave assessments were completed at five regional caves that were previously known to occur, but had not previously been described and assessed, caves CCAT-03, CCAT-06, CCAT-08, CCAT-16 and CCAT-21 (Appendix C). The cave assessments were designed to characterise the physical features of each cave and to identify the potential importance to the ghost bat and the Pilbara leaf-nosed bat. Each cave assessment recorded the following characteristics:

- entrance location;
- entrance photograph;
- entrance type, position, aspect, exposure, width, and height;
- floor slope;
- cave depth;
- chambers: main chamber height, and number of total chambers;
- water presence;
- indicative roost type; and
- presence of target species: no. individuals, and/or secondary evidence.

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VWER-47
VWER-102
VWER-103
VWER-101
VWER-10
VWER-62
VWER-53
VWER-57
VWER-52
VWER-63
VWER-58
VWER-64
VWER-68
VWER-29
VWER-30
VWER-54
VWER-65
VWER-55
VWER-01
VWER-66
VWER-56
VWER-67
VWER-51

Legend

- Study Area
- **Vertebrate Fauna Habitat (Biologic 2020b)**
  - Breakaway/ Cliff
  - Drainage Area/ Floodplain
  - Gorge/ Gully
  - Hillcrest/ Hillslope
  - Minor Drainage Line
  - Mulga Woodland
  - Stony Plain
  - Cleared/ Disturbed
- Ultrasonic Recorder
- **Targeted Search**
  - Diurnal Search
  - Nocturnal Search
  - Helicopter Flight Path

N

biologic Environmental Survey

Scale: 1:30,000

0 0.5 1 1.5 Km

Coordinate System: GDA 1994 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA 1994 Created 30/06/2021

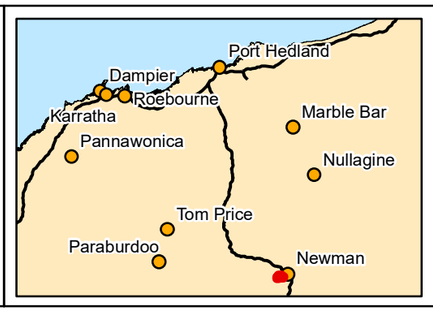

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Figure 3.5: Pilbara leaf-nosed bat sampling in the Study Area and region

3.2.4 Survey Results

No evidence of Pilbara leaf-nosed bat occurrence was recorded within the Study Area during this assessment. The species was however recorded from four of the 33 regional ultrasonic recorder sampling locations (VWER-82, VWER-84, VWER-86 and VWER-91) (Table 3.5, Figure 3.6). All four locations are located along Ophthalmia Range, which runs in an east-west direction, north of the Study Area, including where it is intersects the Fortescue River at Ophthalmia Dam. The distance of the records to the Study Area, ranged from approximately 11 km at site VWER-86 in the north-west and approximately 22 km at site VWER-84 in the north-east (Table 3.5, Figure 3.6). The number of calls per night at these sites ranged from a single call, recorded at sites VWER-82 and VWER-86, to six, recorded at site VWER-91. The earliest calls were recorded at site VWER-86, at 19:23, approximately 36 minutes after dusk civil twilight - dusk civil twilight approximating the time when individuals start to leave a roost (Bullen & Reiffer, 2020). Given the species mode flight speed of approximately 22 kilometres per hour (kph), it is somewhat unlikely that the individual which made the calls was roosting at the known Kalgan Creek roost, located approximately 32 km north-east of VWER-86, but rather originated from somewhere within 10 km of this location. The remaining records were recorded after an hour of dusk civil twilight and therefore could have originated from the Kalgan Creek roost.

All but one of the recordings were taken from, or adjacent to, water features. A single recording site was a cave (CCAT-06), which now exists as the only confirmed nocturnal refuge for the species in the area. All remaining caves assessed as part of this survey, or as part of previous assessments at Western Ridge remain as potential nocturnal refuges only.

Table 3.5: Summary of Pilbara leaf-nosed bat records from this assessment

Site	Distance from Study Area	Location details	Record details
VWER-82	11 km	Ophthalmia Range – Water Feature WWER-26	- 2 calls (from one sampling night) - Earliest call received +9 hours after dusk civil twilight
VWER-84	22 km	Ophthalmia Dam – Water Feature WWER-24	- 4 calls (on each of two sampling nights) - Earliest call received 36 minutes after dusk civil twilight
VWER-86	11 km	Ophthalmia Range (Mt Newman) – Water Feature WWER-17	- 1 call (from one sampling night) - Earliest call received ~2 hours after dusk civil twilight
VWER-91	12 km	Ophthalmia Range – Cave CCAT-06	- 6 calls (from one sampling night) - Earliest call received +1 hours after dusk civil twilight

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Pilbara leaf-nosed bat Record (this Survey)

- Ultrasonic Call (1-6)
- Ultrasonic Recorder Null Record

Pilbara leaf-nosed bat Record (Biologic 2020a)

- Ultrasonic Call (Single)
- Ultrasonic Recorder Null Record

Pilbara leaf-nosed bat Record (Biologic 2020b)

- Ultrasonic Recorder Null Record

Legend

- Study Area

Vertebrate Fauna Habitat (Biologic 2020b)

- Breakaway/ Cliff
- Drainage Area/ Floodplain
- Gorge/ Gully
- Hillcrest/ Hillslope
- Minor Drainage Line
- Mulga Woodland
- Stony Plain
- Cleared/ Disturbed

N

biologic Environmental Survey

Scale: 1:30,000

0 0.5 1 1.5 Km

Coordinate System: GDA 1994 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA 1994 Created 30/06/2021

Port Hedland
Dampier
Roebourne
Karratha
Pannawonica
Marble Bar
Nullagine
Tom Price
Paraburdoo
Newman

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Figure 3.6: Pilbara leaf-nosed bat records in the Study Area and region

3.2.5 Discussion

Despite the species not being recorded within the Study Area during this assessment, nor any prior survey, the presence of a record approximately 50 m from the southern border, ensues that the species has previously, or is highly likely to occur within the Study Area. The extent of ultrasonic sampling within the Study Area, particularly that focussed on types of features regularly used by the species (i.e. caves and water features), has almost eliminated any likelihood of a diurnal roost occurring in the Study Area itself. Furthermore, the sampling to date supports previous conclusions by Biologic (2020f) that the species' occurrence within the Study Area is likely to be limited to occasional and temporary visitation by foraging and dispersing individuals only.

The results of the regional sampling undertaken as part of this assessment, support the desktop findings by Biologic (2020f), which demonstrated that the Pilbara leaf-nosed bat is relatively scarce within the general Newman area (e.g. within 10 km of the Study Area). This is likely due to a lack of potential roosting habitat (i.e. banded iron stone ranges) outside of the Study Area, in the west, south and east, and in the north until the southern reaches of the Ophthalmia Range (which lies on average, 12 km to the north of the Study Area). With that being said, the results of the regional sampling indicate the potential existence of a diurnal roost within the vicinity of Mt Newman along Ophthalmia Range. Prior to this assessment, the nearest Pilbara leaf-nosed bat roost to the Study Area was thought to be the Kalgan Creek roost, located approximately 32 km north of the northern margin of the Study Area. While, the species is capable of traveling such distances (as demonstrated by Biologic, 2020e), a flight between the Study Area and the Kalgan Creek roost would be unusual given the low quality of foraging habitat in the intervening area (particularly compared to that occurring in closer proximity to the Kalgan Creek roost), and the dry conditions experienced during and preceding the record. Should a roost near Mt Newman exist, the Study Area would then fall within the dry season nightly foraging range of an individual, approximately 20 km (Bullen, 2013), and constitute potential foraging grounds for the species.

The Study Area does comprise foraging habitat for the species, both in the form of preferred habitat types, as defined by TSSC (2016b) and foraging features, such as caves and water features. The species is known to frequent cave-like structures during the night, termed nocturnal refuges, presumably for feeding or for resting (TSSC, 2016b). Such caves are not however considered critical for the persistence in a local area. A total of 19 caves have been recorded within the Study Area. Although the species has not been recorded at any of these caves, the likely occurrence of the species within the Study Area, although likely to be infrequent and temporary, suggests that any caves could be intermittently utilised by foraging and dispersing individuals. Additionally, there are 14 water features known to occur within the Study Area, which, similarly to the caves, may all provide an intermittent drinking and /or foraging source for visiting individuals. This is particularly the case for the two seep-fed rock pools (WWER-01 and WWER-04), which likely hold water throughout the year of most years and constitute Priority 1 foraging habitat for the species, 'Gorges with Pools' (TSSC, 2016b).

With regard to fauna habitats, approximately 3.5% (165.7 ha) of the Study Area is represented by the Gorge/ Gully habitat type, which represents Priority 1 (gorges with water pools) and Priority 2 (gullies) foraging habitats as defined by TSSC (2016b). Approximately 1.1% (53.1 ha) is represented by Breakaway/ Cliff habitat, which is regarded as Priority 3 foraging habitat by the TSSC (2016b), as are the limited instances where outcropping occurs within the Hillcrest/ Hillslope (40.6 %, 1,936.6 ha). The Minor Drainage Line habitat (2.1%, 101.7 ha; particularly those with water features) is regarded as Priority 4 foraging habitat by the TSSC (2016b) and also categorised as primary foraging habitat for the species (Biologic, 2020f). Finally, open grasslands and woodlands contained within the remaining habitats; Stony Plain (30.3%, 1,444.8 ha), Mulga Woodland (11.7%, 555.5 ha) and Drainage Area/ Floodplain (9.8%, 468.8 ha); are considered Priority 5, marginal foraging habitat for the species (TSSC, 2016b - refer to Section 2.3).

3.3 Ghost Bat (*Macroderma gigas*)

3.3.1 Species Profile

The ghost bat is listed as Vulnerable under the EPBC Act and the BC Act. The species occurs in disjunct colonies across northern Australia (TSSC, 2016a). In the Pilbara region, the species occurs in all four subregions. The Pilbara population is estimated to comprise between 1,300 and 2,000 individuals (TSSC, 2016a). The largest population occurs within the Chichester subregion (estimated at approximately 1,500 individuals), where known populations are largely restricted to disused mines (TSSC, 2016a).

The distribution of ghost bats in the Pilbara is determined by the presence of suitable roosting sites. Natural roosts generally comprise deep, complex caves beneath bluffs or low rounded hills (Armstrong & Anstee, 2000). Centralised breeding sites in the Pilbara are largely restricted to abandoned mines in the Chichester Ranges; however, there are also a number of smaller maternity roosts in the Chichester and Hamersley Ranges (Armstrong & Anstee, 2000). Based on available data, breeding has been documented in natural caves at Mining Area C, Mt Brockman and West Angeles in the Hamersley sub-region, and at Callawa and Tambrey Station in the Chichester subregion (Armstrong & Anstee, 2000). Ghost bats are known to move between a number of caves seasonally, or as dictated by weather conditions, and require a range of cave sites (Hutson *et al.*, 2001). Outside the breeding season, male bats are known to disperse widely, most likely during the wet season when conditions would allow bats to use caves that would otherwise not be suitable (Worthington-Wilmer *et al.*, 1994). Genetic studies indicate that females are likely to stay close to the maternity roosts (Worthington-Wilmer *et al.*, 1994).

Caves used by the species can be classified into five categories (Biologic, 2016b):

- **Potential Night Roosts:** caves that are only utilised during the night, mostly to feed on prey items or to rest, and are typically shallow caves and shelters/overhangs that can be well lit during the day.
- **Potential Day Roosts:** in addition to being utilised to feed during the night, these caves are more complex in structure and provide suitable temperature and humidity conditions for roosting during the day, but no ghost bats have been recorded using them during the day.
- **Day Roosts:** caves that are used for shelter during the day.
- **Potential Maternity Roosts:** in addition to being used for shelter during the day, these caves have attributes suitable to support breeding, and ghost bats have been recorded in these caves during the breeding season.
- **Maternity Roosts:** in addition to being used for shelter during the day, these caves have attributes suitable to support breeding, and ghost bats have been recorded in these caves during the breeding season, including pregnant females or females with pups.

Ghost bats appear to have a short-range foraging strategy of up to 3 km (average 1.9 km), with vantage points changing approximately every 15 minutes, and average foraging areas of 61 ha having been recorded in the Northern Territory (Tidemann *et al.*, 1985). It also appears that bats generally return to the same area each night (Tidemann *et al.*, 1985), although it has been suggested that ghost bats in the arid zone are semi-transient through most areas and will readily travel large distances (>4 km) (Biologic, 2020d). Ghost bats have a ‘sit and inspect’ foraging strategy; whereby they hang on a perch

and visually inspect their surroundings for movement. Once their prey is detected it may be captured in the air, gleaned (taken from the surface of a substrate by a flying bat) from the ground or vegetation, or dropped on from a perch (Boles, 1999). Recent studies of ghost bat home range and foraging behaviour in the Pilbara region have indicated that the species prefers plains and low-lying habitat for foraging, particularly Drainage Area/ Floodplain (ghost bats have also been observed exiting caves and moving immediately towards broad drainage plains), Mulga Woodland and Major Drainage Line (Biologic, 2020d). Such areas are highly productive and comprise an abundance of foraging structures (Biologic, 2020d).

3.3.2 Previous Records

The Study Area is located at the southern extent of the species current distribution, whereby the species or species habitat may occur (DoEE, 2019). In Western Australia, the species has experienced a significant north-westward contraction, presumably associated with increasing aridity (TSSC, 2016a), thus the Study Area represents a population at the forefront of this decline. A desktop assessment completed by Biologic (2020f) returned 37 records of ghost bat within 24 km of the Study Area (Figure 3.7). There are few publicly available records between 26 km and 50 km of the Study Area, as indicated by DBCA (2020). The nearest records to the Study Area are located between 50 and 100 m from the north-eastern boundary of the Study Area, in an area that has since been developed as part of the current Whaleback mining operation (Biologic, 2020f). The next nearest record is located approximately 4.5 km east (2012) (DBCA, 2020). The remaining records are from the Cathedral Gorge area approximately 12-19 km north of the Study Area (13 records from 7 locations, BHP WAIO, 2021), within the ranges that surround Kalgan Creek approximately 25 km north of the Study Area, and at Jimblebar approximately 40 km norther-east of the Study Area (Figure 3.7). There are no contemporary records (<20 years) of the species south of the Study Area (DBCA, 2020), indicating the species occurrence within and in the vicinity of the Study Area likely represents the south-eastern extent of the species occurrence. The desktop assessment had returned eight previous records of the species within the Study Area, occurring from four main locations (Biologic, 2020f). Four records were documented in 2010 (Biologic, 2011) at three cave locations via direct observation and secondary evidence (Figure 3.7). Additionally, one record was documented in 2011 (ENV, 2012) and three records were documented in 2016 (DBCA, 2020) (Figure 3.7).

During the most recent previous survey of the Study Area, the species was recorded on nine occasions at seven locations, all which were caves. Thus the species has previously been recorded, via direct and/or secondary evidence, from seven on the 19 caves known to occur within the Study Area (table 3.6). During the survey by Biologic (2020f) a direct observation of a single individual was recorded from cave CWER-02 in December 2019. A succession of four calls was recorded at this cave during the survey in March 2020, at 3:45 AM, indicative of a foraging bat (Biologic, 2020f). The remaining records were of scats, which ranged from 8 to 500 individual scats per cave (Biologic, 2020f).

table 3.6: Ghost bat records taken by Biologic (2020f)

Cave ID	Habitat	Coordinates		Ghost bat	
		Latitude	Longitude	Records	Significance
CWER-01	Breakaway/ Cliff	-23.4127	119.5835	**December 2019**: 14 recent scats	Maternity
CWER-02	Breakaway/ Cliff	-23.3923	119.6663	**December 2019**: 2 recent scats	Night Roost
CWER-03	Gorge/ Gully	-23.3970	119.6607	**December 2019**: 1 Individual ghost bat observed, 500 fresh scats **March 2020:** four calls in succession at 0345AM (March 2020)	Confirmed Diurnal Roost, Potential Maternity
CWER-06	Gorge/ Gully	-23.3827	119.6137	**March 2020:** 8 old scats	Night Roost (Biologic, 2014b)
CWER-10	Gorge/ Gully	-23.4030	119.6603	**March 2020:** 10 old scats	Potential Diurnal Roost
CWER-14	Gorge/ Gully	-23.4115	119.6474	**March 2020:** 9 old scats	Night Roost
CWER-16	Gorge/ Gully	-23.4048	119.6056	**March 2020:** 50 recent scats	Potential Diurnal Roost

Additionally the species has been recorded, via direct and/or secondary evidence, on multiple occasions at CWER-01 and CWER-03, between 2016 and 2019 as part of a regional ghost bat monitoring program (Biologic, 2017a, 2017b, 2018, 2020a, 2020b) (table 3.7). A significantly high number of scats deposited between January and May 2018 were indicate of a large group using cave CWER-03 during this period.

Genetic analysis suggests that at least 11 unique individuals have utilised CWER-01 between 2016 and 2019 and at least seven individuals utilised CWER-03 in 2016. One individual (genotype #135) was identified in both caves in 2016. Moreover, several individuals have been recorded across multiple sampling periods; most notably individual #492 and #508 have been recorded on seven and five sampling events respectively.

Elevated levels of progesterone (>970 ng/g) indicating presence of pregnant females, was also recorded from scats collected from CWER-01 in 2016 through to 2019, indicating its use as maternity roost (Biologic, 2020f; McKenzie *et al.*, 2009) (table 3.7). This continued use as a diurnal roost highlights the local and regional significance of the cave and the presence of pregnant females using the area. The irregular occurrence of large scat numbers and high portions of scats with elevated progesterone, does however suggest that these caves are irregularly used for breeding activity.

table 3.7: Previous monitoring conducted at CWER-01 and CWER03

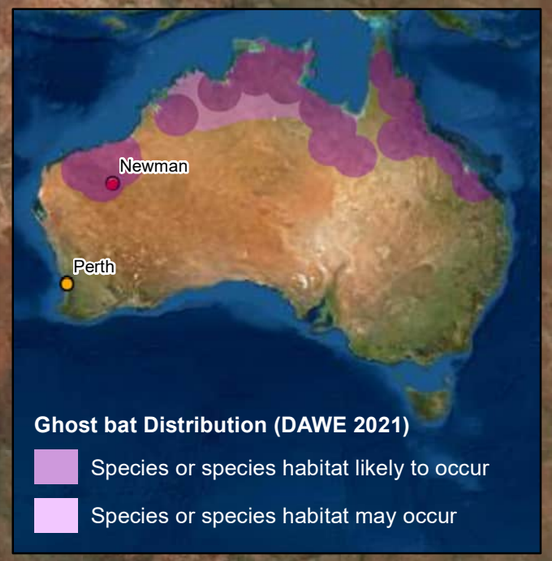
Cave ID	Monitoring year	Date	Total scats on sheets	Deposition rate	No. of ghost bats present	No. of genotyped individuals	Scats with elevated progesterone (pregnant individuals)
CWER-01 (previously OB35-02)	2016-2017 (Biologic, 2018)	14/10/16	0 (35 scats collected off sheet)	0*	2	6 (#135, #140, #141, #142 #143, #144)	5%
		13/12/16	60	1	0		
	2017-2018 (Biologic, 2020a)	20/10/17	65	0.21	0	4 (#135, #492, #494, #499)	80%
		9/01/18	2	0.02	0	1 (#492)	-
		14/05/18	1,000	8	2	4 (#492, #494, #495, #508)	-
		23/07/18	60	0.86	0	3 (#492, #494, #508)	58%
	2018-2019 (Biologic, 2020b)	19/02/19	5	0.02	0	2 (#492, #508)	20%
		27/05/19	50	0.52	0	2 (#492, #508)	18%
		12/08/19	0	0	0	-	-
		8/10/19	0	0	0		-
		6/11/19	0	0	0		-
		2/12/19	14	0.54	0	2 (#492, #508)	50%
	Proceeding current survey (Biologic *unpub. data*)	15/09/20	0	0	0	-	-
		05/05/21	0	0	0	-	-
CWER-03 (previously OB35-01)	2015-2016 (Biologic, 2017a)	13/05/16	19	-	1	7 (#132, #133, #134, #135, #136, #138 and #139)	-
	2016-2017 (Biologic, 2018)	14/10/16	10	0.1	0	-	-
		13/12/16	0	0	0	-	

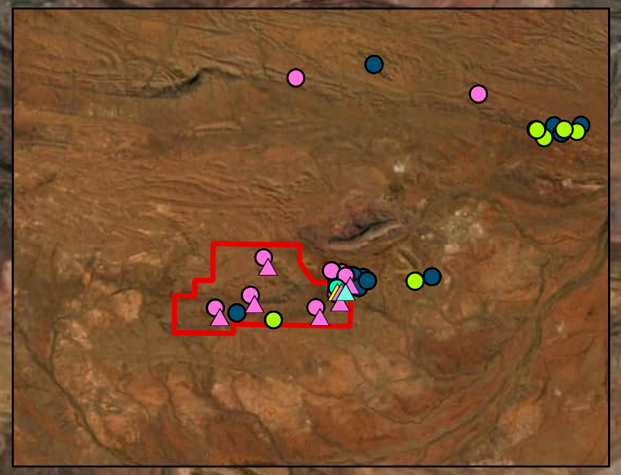
762000 764000 766000 768000 770000 772000

7412000

7410000

7408000





Legend

- Study Area

Vertebrate Fauna Habitat (Biologic 2020b)

- Breakaway/ Cliff
- Drainage Area/ Floodplain
- Gorge/ Gully
- Hillcrest/ Hillslope
- Minor Drainage Line
- Mulga Woodland
- Stony Plain
- Cleared/ Disturbed

Ghost bat Record (Biologic 2020b)

- Individual (Alive)
- Scat
- Other

Ghost bat Record (BHP WAIO 2021)

- Individual (Alive)
- Scat
- Ultrasonic Call
- Unknown

N

biologic Environmental Survey

Scale: 1:30,000

0 0.5 1 1.5 Km

Coordinate System: GDA 1994 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA 1994 Created 30/06/2021

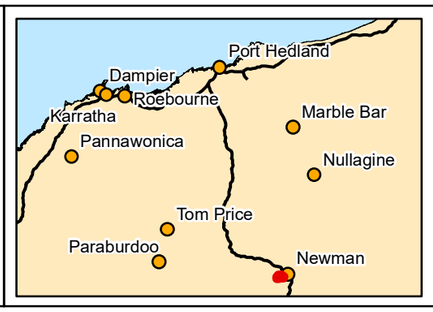


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Figure 3.7: Previous ghost bat records in the Study Area and region

3.3.3 Survey Methods

Ultrasonic Recorders

Overnight recordings of bat echolocation calls were undertaken with SongMeter (SM; Wildlife Acoustics Inc.) ultrasonic bat recorders. Units were deployed at 54 locations during trip 1, trip 3 and trip 4 of this assessment, comprising 21 locations within the Study Area and 33 regional locations outside of the Study Area (Table 3.4; Figure 3.8). Sampling was targeting at areas where bat occurrence and usage was likely to be highest, such as at the entrance of caves, at water features and within primary foraging habitats (such as along watercourses). At each location, ultrasonic recordings were performed for between one and four nights, resulting in a total of 90 recording nights, comprising 49 within the Study Area and 41 at regional locations outside of the Study Area.

The audio settings used for the SM units followed the manufacturer's recommendations (Wildlife Acoustics, 2011, 2017) and were set to account for all species known to occur within the region (McKenzie & Bullen, 2009). All recordings were analysed by Robert Bullen of Bat Call WA for the presence of Pilbara leaf-nosed bat and ghost bat calls only.

Cave Cameras

During trip 2 a motion-triggered Mobotix S16 infrared camera was installed within caves CWER-01 and CWER-03. Each camera was fitted with a single night 180-degree camera lens module. The cameras were powered by a solar-powered battery and accessible remotely via a mobile network. Each camera was placed inside the main roosting chamber of each cave to capture ghost bat activity within the cave itself. These cameras remain deployed at both caves as part of ongoing long-term monitoring of ghost bat occurrence and utilisation within the Pilbara.

Cave Visitation and Scat Monitoring

A selection of the most prospective and easily accessed caves were visited and inspected over the course of the assessment to record the species present. During each visitation, a 'inspector' entered the cave to record any individuals/evidence present, and a 'spotter' waited at the entrance of the cave to record any ghost bats flushed out of the cave and ensure the safety of the inspector.

Scat collection sheets were used to determine ghost bat usage between cave visitations, including visitations prior to this assessment. Scat collection sheets have been deployed within a total of nine caves. Scat collection sheets were previously installed at three caves within the Study Area, CWER-01, CWER-02 and CWER-03 (Biologic, 2020f), and at one regional sampling cave, CCAT-09 (Biologic, *in prep.*). Sheets were also installed at a further five caves during trip 1 of this assessment, CWER-04, CWER-06, CWER-10, CWER-12 and CWER-14. Each sheet measured approximately 18 m^2 and was composed of black plastic. The sheets were installed on the floor of each cave, below sections of ceiling which were considered likely to support roosting by ghost bats or where scats had previously been deposited; thus the number of sheets within each cave varied between one and three.

During this assessment, each of these locations was visited on multiple occasions to assess for the presence of fresh ghost bat scat material. Where ghost bat scats were recorded within the caves, up to 20 scats were collected, for further genetic and hormone analysis. Scats were collected in individual paper envelopes and were stored in a freezer until analysis. All sheets were cleared after every sampling event to ensure new scats were obvious for the next collection period. The number of scats

occurring on-sheet was counted and recorded prior to any collection and were later used to determine a scat deposition rate (the number of scats recorded divided by the number of days since sheets was last cleared).

Table 3.8: Caves visited during this assessment

Cave ID	Visitations			
	Most recent prior to this assessment	Trip 1 August 2020	Trip 3 October 2020	Trip 4 November 2020
Caves Within Study Area				
CWER-01	March 2020 (Biologic, 2020f)	x	x	x
CWER-02	December 2019 (Biologic, 2020f)	Not sampled due to safety concerns (blasting)		
CWER-03	March 2020 (Biologic, 2020f)	x	x	x
CWER-04	March 2020 (Biologic, 2020f)*	x	x	x
CWER-06	March 2020 (Biologic, 2020f)*	x	x	x
CWER-10	March 2020 (Biologic, 2020f)*	x	x	x
CWER-12	March 2020 (Biologic, 2020f)*	x	-	x
CWER-14	March 2020 (Biologic, 2020f)*	x	x	x
Regional Caves				
CCAT-03	October 2015 (Biologic, 2016a)*	-	x#	-
CCAT-06	October 2015 (Biologic, 2016a)*	-	x#	-
CCAT-08	October 2015 (Biologic, 2016a)*	-	x#	-
CCAT-09	December 2019 (Biologic, *in prep.*)	-	x	--
CCAT-16	-	-	x#	-
CCAT-21	-	-	x#	-

* no sheet present before this assessment; # cave not sheeted before or after visitation

Scat Genetic Analysis

Scat samples were analysed by Kym Ottewell and Sun Rujiporn from DBCA. A total of 33 ghost bat scat samples were analysed from the area, comprising nine scats collected from CWER-16 by Biologic (2020f), 21 scats collected from CWER-03 during trip 1 and three scats collected from CWER-03 during trip 2. Samples were genotyped at 44 single nucleotide polymorphism (SNP) loci using a new high-throughput SNP genotyping method. Samples were filtered from the SNP genotyping dataset based on a relatively stringent genotyping success rate of ≥85% (i.e. 37/44 loci amplified) to remove poor quality samples.

SNP genotypes for scat samples were processed using a custom R script written by B. Huntley, R. Thavornkanlapachai & K. Ottewell (unpubl.). The analysis groups scats based on genotype similarity, i.e. by number of per-locus genotype mismatches, to identify the number of unique genotypes present in the sample. DNA samples from 'unique' individuals detected in previous ghost bat studies from microsatellite genotypes (Ottewell *et al.*, 2019; Ottewell *et al.*, 2017; Ottewell *et al.*, 2021) were re-genotyped using the MassARRAY SNP array in order to enable identification of 'recaptured' individuals in this study. Scats from the present study were matched against these 283 reference genotypes obtained from previous studies using the same custom R script as identified above.

Molecular sexing of scat samples from each unique individual was carried out using three custom-designed ghost bat sex-linked markers (DDX3Y, SRY and Zfx) as described in Ottewell *et al.* (2020)

Scat Hormone Analysis

Progesterone hormone analysed was used to detect the presence of pregnant ghost bats occurring through the Study Area. A total of 33 ghost bat scat samples were analysed from the area, comprising nine scats collected from CWER-16 by Biologic (2020f), 21 scats collected from CWER-03 during trip 1 and three scats collected from CWER-03 during trip 2.

Scats were analysed by Dr Tamara Keeley from the University of Queensland for faecal metabolites (hormones) to determine the occurrence of pregnant individuals within caves. The method has been used for numerous other bat species (Keeley *et al.*, 2012a; Keeley *et al.*, 2012b) and has been partially validated for ghost bat in a pilot study using the captive breeding colony from Perth Zoo (Biologic, 2017a). Only whole samples were analysed to avoid the possibility of hormone levels being artificially altered if pooled faecal sample pieces came from different individuals of differing reproductive status.

Faecal samples were analysed for progesterone metabolite concentrations by enzyme-immunoassay (EIA). Prior to analysis for hormone concentrations, each faecal sample was extracted using a basic hormone extraction procedure (Keeley *et al.*, 2012a; Palme *et al.*, 2013). Faecal samples were subsampled to a weight of either 0.1 ± 0.02 or 0.05 ± 0.002 g, to which 5 ml of 80% methanol was added. Samples were rotated gently overnight, centrifuged at 1,000 g for 10 min and then decanted and stored at -20°C until analysis. The supernatant was diluted 1:20 to 1:1000 (dependant on concentration) in assay buffer prior to analysis. Faecal progesterone metabolite concentrations were quantified by double antibody EIA using a goat anti-mouse IgG (Arbor Assays, USA), monoclonal progesterone antiserum (CL425), horseradish peroxidase conjugated label (both provided by C. Munro, University of California-Davis, Davis, USA) and progesterone (Sigma Aldrich Australia Ltd.) standards as previously described with minor modifications (Keeley *et al.*, 2012b).

The antiserum (1:80,000) was incubated on a microtitre plate overnight, horseradish peroxidise conjugate (1:400,000), standards (0.016 - 4 ng/ml) and samples were loaded (50 µl/well) onto the plate and the EIA was performed as described (Keeley *et al.*, 2012b; Pollock *et al.*, 2010). Intra and inter-assay coefficients of variation were both <10%. Cross-reactivities for the EIA antibodies were as described (Graham *et al.*, 2001). Hormone concentrations were expressed as nanograms of hormone metabolites per gram of faeces (ng/g). Progesterone levels were considered to be elevated (i.e. progesterone levels indicating pregnancy or individual) for results of 970 ng/g or greater.

Cave Assessments

During the survey, cave assessments were completed at five regional caves that were previously known to occur, but had not previously been described and assessed, caves CCAT-03, CCAT-06, CCAT-08, CCAT-16 and CCAT-21 (Appendix C). The cave assessments were designed to characterise the physical features of each cave and to identify the potential importance to the ghost bat and the Pilbara leaf-nosed bat. Each cave assessment recorded the following characteristics:

- entrance location;
- entrance photograph;
- entrance type, position, aspect, exposure, width, and height;

- floor slope;
- cave depth;
- chambers: main chamber height, and number of total chambers;
- water presence;
- indicative roost type; and
- presence of target species: no. individuals, and/or secondary evidence.

762000 764000 766000 768000 770000 772000

7412000 7410000 7408000

VWER-47
VWER-102
VWER-103
VWER-101
CWER-06
VWER-10
VWER-53
VWER-62
VWER-57
VWER-52
VWER-63
VWER-68
VWER-58
VWER-64
CWER-03
CWER-04
VWER-29
VWER-30
VWER-54
VWER-65
CWER-10
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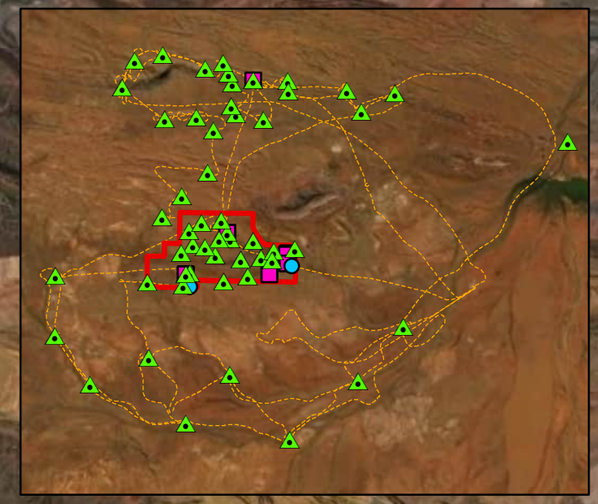

Legend

- Study Area

Vertebrate Fauna Habitat (Biologic 2020b)

- Breakaway/ Cliff
- Drainage Area/ Floodplain
- Gorge/ Gully
- Hillcrest/ Hillslope
- Minor Drainage Line
- Mulga Woodland
- Stony Plain
- Cleared/ Disturbed
- Cave IR Camera
- Scat Collection Sheet
- Ultrasonic Recorder

Targeted Search

- Diurnal Search
- Nocturnal Search
- Helicopter Flight Path

N

biologic Environmental Survey

Scale: 1:30,000

0 0.5 1 1.5 Km

Coordinate System: GDA 1994 MGA Zone 50
Projection: Transverse Mercator
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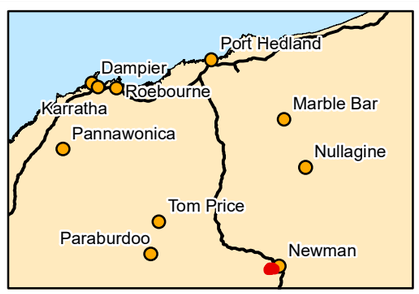

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Figure 3.8: Ghost bat sampling in the Study Area and region

3.3.4 Survey Results

Ultrasonic Recorders

No ghost bats were recorded from the 54 ultrasonic recording sites during this assessment.

Cave Cameras

No ghost bats were detected from the two motion cameras setup in caves CWER-01 and CWER-03. Multiple triggers of other bat species were however detected by these cameras over the sampling period, confirming the absence of ghost bat reflects a lack of ghost bat presence, as opposed to a false negative result.

Cave Visitation and Scat Monitoring

Overall, no ghost bat individuals were recorded from any of the caves visited during the assessment. Ghost bat scats were recorded on-sheet, from one cave and during one visitation, during the duration of the assessment. Approximately 100 scats were recorded from CWER-03 during trip 1 in August 2020. This cave had not been visited between March 2020 and a sheet was present for the full duration, confirming that, these scats were deposited between March and August 2020, a period of 166 days. This equates to a scat deposition rate of approximately 0.60 scats per day between the two survey events, and 0.38 scats per day, over the assessment period (Table 3.9). These totals are considered to be relatively low compared to other caves monitored through the region (Biologic, *in prep.*).

Table 3.9: Ghost bat scat deposition rate recorded during this assessment

Cave ID	Trip 1 August 2020	Trip 3 October 2020	Trip 4 November 2020	Total
Caves Within Study Area				
CWER-01	0	0	0	0
CWER-02	Not sampled due to safety concerns (blasting)			-
CWER-03	0.60	0	0	0.38
CWER-04	0	0	0	0
CWER-06	0	0	0	0
CWER-10	0	0	0	0
CWER-12	0	-	0	0
CWER-14	0	0	0	0
Regional Caves				
CCAT-03	-	0	-	0
CCAT-06	-	0	-	0
CCAT-08	-	0	-	0
CCAT-09	-	0	--	0
CCAT-16	-	0	-	0
CCAT-21	-	0	-	0

Scat Genetic Analysis

A total of seven unique genotypes (unique individuals) were identified from scat samples (Table 3.10). CWER-16 recorded the highest number of individuals (five individuals). In comparison, only two

individuals were recorded from CWER-03. This is likely to be an underestimate of the number of individuals that utilised the cave during the current monitoring survey as only a subsample of scats observed within the cave were collected and analysed. A slightly higher proportion of the individuals identified within the monitoring caves during the current survey were male (57%; Table 3.10). Consequently, CWER-16 contained a higher proportion of male individuals (60%) and CWER-03 contained an equal proportion of females to males.

Most of the individuals recorded during the current survey have previously and only been detected within the Western Ridge Area. Both individuals detected within CWER-03 (#133 and #135) were previously recorded in the cave in 2016 (table 3.7; Table 3.10). Individual #135 was also detected within CWER-01 in 2016 and 2017. Of the five individuals detected within CWER-16, four have previously be detected in CWER-01 (Table 3.10). Specifically, individual #140 was previously detected in 2016, individual #494 was previously detected in 2017 and 2018, individual #495 was previously detected in 2018 and individual #508 was previously detected in 2018 and 2019. However, scats collected at CWER-16 were not collected from a sheet and therefore the age of scats cannot be guaranteed. Consequently, it is unknown when the caves were utilised by the genotyped individuals.

Table 3.10: Detection of unique ghost gat genotypes recorded from this study

Individual ID	Recapture/ new	Sex	CWER-03	CWER-16
133	Recapture (Previously detected in 2016 at CWER-03)	Male	X	
135	Recapture (Previously detected in 2016 and 2017 at CWER-01 and in 2016 at CWER-03)	Female	X	
140	Recapture (Previously detected in 2016 at CWER-01)	Female		X
494	Recapture (Previously detected in 2017 and 2018 at CWER-01)	Female		X
495	Recapture (Previously detected in 2018 at CWER-01)	Male		X
508	Recapture (Previously detected in 2018 and 2019 at CWER-01)	Male		X
715	New	Male		X

Scat Hormone Analysis

Of the 33 scat samples analysed, 12 scats (36%) from both caves sampled contained elevated progesterone (i.e. samples with progesterone levels higher than 970 ng/g) (Table 3.11). This suggests that these caves were likely to be utilised by pregnant individual/s prior to their respective sampling periods.

For cave CWER-16, the presence of scats containing elevated progesterone indicates the cave was likely used by pregnant individual/s. However, all scats observed in the cave at the time of collection (approximately 50) were located near the entrance of the cave. The number and locality of scats observed suggests the cave was being utilised for night roosting by those individuals. Additionally, the number of scats recorded in the cave is low and uncharacteristic of a maternity cave, such as when compared to the thousands of scats recorded at caves CWER-01 and CWER-03. Notably the scats were not collected from a sheet and therefore the age and timing of utilisation is not known.

Four scat samples analysed from CWER-03 contained intermediate levels of progesterone (i.e. samples with progesterone levels between 700-950 ng/g) (Table 3.11). This suggests the hormone was either decaying due to the age of the scat, or the individual may have recently been pregnant or was in early stages of pregnancy. The number of scats containing elevated or intermediately elevated progesterone was relatively low at all caves, consistent with the raw numbers of scats. This indicates that the caves were only being used intermittently by pregnant individuals and were unlikely to be used as maternity roosts over the course of this assessment.

Table 3.11: Summary of hormone analysis

Cave ID	Collection date	Number of scats				Scat-collection sheet
		Analysed	Elevated progesterone	Intermediate levels of progesterone	No elevated progesterone	
CWER-16	16/03/2020 (Biologic, 2020f)	9	6 (66.67%)	0 (0.00%)	3 (33.33%)	No
CWER-03	25/08/2020 (this assessment)	21	5 (23.81%)	3 (14.29%)	13 (61.90%)	Yes
CWER-03	10/10/2020 (this assessment)	3	1 (33.33%)	1 (33.33%)	1 (33.33%)	Yes

Cave Assessments

Of the five regional caves subject to a cave assessment, none contained obvious evidence of the species. However, three of these caves CCAT-03, CCAT-06 and CCAT-21 could not be accessed in their entirety to provide a complete assessment. The rear chamber of CCAT-03 and CCAT-06 could not be accessed due to the size of the passages, and CCAT-21 was not accessed completely due to an active beehive, which was present midway into the cave, restricting access to the most inner chamber. Thus each of these caves may have contained unrecorded ghost bat scats. Of the five caves visited and assessed, three were deemed to represent potential diurnal roosts CCAT-3, CCAT-06, and CCAT-21, and the remaining two, CCAT-08 and CCAT-16, were deemed to represent night roosts.

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7408000

Legend

- Study Area

Vertebrate Fauna Habitat (Biologic 2020b)

- Breakaway/ Cliff
- Drainage Area/ Floodplain
- Gorge/ Gully
- Hillcrest/ Hillslope
- Minor Drainage Line
- Mulga Woodland
- Stony Plain
- Cleared/ Disturbed

Ghost bat Record (this Survey)

- Scat
- Scat Collection Sheet Null Record
- Ultrasonic Recorder Null Record

Ghost bat Record (Biologic 2020a)

- Ultrasonic Recorder Null Record

Ghost bat Record (Biologic 2020b)

- Ultrasonic Recorder Null Record

N

biologic Environmental Survey

Scale: 1:30,000

0 0.5 1 1.5 Km

Coordinate System: GDA 1994 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA 1994 Created 30/06/2021

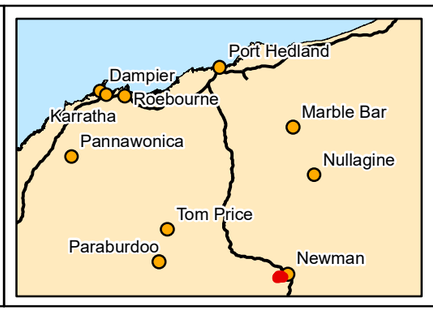

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Figure 3.9: Ghost bat records in the Study Area and region

3.3.5 Discussion

Despite a lack of ghost bat presence recorded during this assessment, the species has been well documented within the Study Area previously, suggesting that the lack of recent records may represent a temporary absence of the species only. Ghost bats are known to move between a number of caves seasonally, or as dictated by weather conditions (Hutson *et al.*, 2001) and it is not uncommon for them to be entirely absent from an area (Biologic, *in prep.*). The reason for the absence within the Study Area is not clear but most likely attributed to resource availability, particularly given the species has been recorded within the Study Area during the same season during previous years (table 3.7) and was known to occur in the Study Area between December 2019 and August 2020. As indicated by the numerous habitat assessments completed by Biologic (2020f) and during this survey, much of the Study Area and surrounding region is in the early stages of regeneration from a large wildfire. Presumably this has impacted prey abundance and productivity of foraging habitats throughout the broader area, potentially explaining the lack of ghost bat activity recorded during this assessment and other recent surveys in the area (Biologic, *in prep.*). However, given the ongoing presence of fire in the Pilbara, this impact should only be considered temporary and part of a natural fluctuation.

Due to the sheer amount of historical ghost bat scats present in caves CWER-01 and CWER-03, it is clear that the species has occupied the Study Area consistently over a long period, and likely on a regular basis. Both of these caves contain chambers, which are dark, deep, high and likely to maintain a steady microclimate. Together, with the evidence of past occupation, these caves are both likely to be used as maternity caves by the species, though only CWER-01 has been confirmed as a maternity roost (Biologic, 2020f). Results from this assessment and previous work at these caves suggests that usage of these caves in such a manner is however irregular and may be separated by multiple years, as is indicated for most natural maternity caves in the Hamersley Ranges (Biologic, *in prep.*). This is supported by the lack of recruitment (new genotypes) identified through scat analysis (only one individual since May 2018). These studies have however demonstrated that the species has used these caves for roosting on a semi-regular basis over the last five years.

Of the remaining 17 caves known from the Study Area, ghost bats have been confirmed from five, CWER-02, CWER-06, CWER-10, CWER-14, CWER-16, either by direct observation or secondary evidence. In summary, of the 19 caves recorded within the Study Area, one is confirmed as a maternity roost, one is considered a potential maternity roost, three are considered potential diurnal roosts, five as night roosts and five as potential night roosts. The remaining four caves showed no evidence of usage by the ghost bat and are unlikely to be suitable for the species. Of the 15 caves that provide or potentially provide habitat for the species, thirteen occur within Gorge/ Gully habitat, two within Breakaway/ Cliff habitat. Within the Study Area, Gorge/ Gully habitat (3.5%, 165.7 ha) and Breakaway/ Cliff (1.1%, 53.1 ha), is prone to forming important habitat features such as overhangs and caves.

The ghost bat is a generalist feeder which feeds on a large array of prey items from invertebrates to small vertebrates, some of which are strongly associated with specific habitats and soil types (Claramunt *et al.*, 2018). Recent studies of ghost bat home range and foraging behaviour in the Pilbara region have identified Drainage Area/ Floodplain (9.8%, 468.8 ha), Gorge/ Gully (3.5%, 165.7 ha), Minor Drainage Line (2.1%, 101.7 ha), Mulga Woodland (11.7%, 555.5 ha) and in instances Stony Plain (30.3%, 1,444.8 ha), as primary foraging habitats for the species (Biologic, 2020d; Knuckey, 2021).

While the suitability of these habitats varies across the Study Area, each of those listed above was recorded as being suitable habitat for the species (Biologic, 2020d), containing areas with a suitable density of understorey vegetation (either at the time of survey or in the process of fire regeneration) and tree/ shrub perches for hunting. While it is possible that all instances of these habitat types will be used for foraging by the species, those instances in closer proximity to roosting caves (night roosting, day roosting and maternity) should be considered of greater significance. Various unpublished studies have confirmed that the nightly foraging ranges of the Pilbara ghost bat are far larger than in tropical populations (~2 km), averaging between 6-15 km per night (Knuckey, 2021), though the species has been recorded travelling >20 km in a night (Biologic, 2020d; Knuckey, 2021).

3.4 Pilbara Olive Python (*Liasis olivaceus barroni*)

3.4.1 Species Profile

The Pilbara olive python is listed as Vulnerable under the EPBC Act and the BC Act. The species is Western Australia's largest snake, averaging 2.5 m, with records up to 4.5 m (Bush & Maryan, 2011; Cogger, 2014). The species has a dull olive-brown upper surface and is pale cream below (Burbidge, 2004; Cogger, 2014). This species is endemic to the Pilbara and northern parts of the Gascoyne bioregions, distributed from Burrup Peninsula, Ord Ranges and Meentheena south to Nanutarra and Newman in the Pilbara, with an isolated population occurring at Mt Augustus in the Gascoyne region (Bush & Maryan, 2011; Storr *et al.*, 2002).

This species is primarily nocturnal and tends to shelter amongst rocky habitats, in small caves or under vegetation during the day. During summer months they will emerge from daytime shelters soon after dark and continue to move until the early hours of the morning (DSEWPaC, 2011b). In the winter months, the species is primarily nocturnal, although adult pythons can sometimes be found basking in the morning sun (DSEWPaC, 2011b; Pearson, 2001). The breeding season of the Pilbara olive python takes place in the cooler months, which extends from June to August and males will travel up three kilometres in search of a mate (DSEWPaC, 2011b). The species is a well-adapted opportunistic ambush predator and common prey items include rock-wallabies, small euros, fruit bats, waterbirds, doves/pigeons and is also likely to include instances of northern quoll and other small mammals (Ellis, 2013; Ellis & Johnstone, 2016; Pearson, 2007; Pearson, 2003; TSSC, 2008).

The species commonly inhabits moist areas such as gorges, rivers, pools and surrounding hills, but can be found in a range of habitats (Burbidge, 2004). In the Hamersley region, the Pilbara olive python is most often encountered in the vicinity of permanent waterholes in rocky ranges or among riverine vegetation (Pearson, 1993). It is a common misconception that the species is reliant and restricted to areas near permanent water; however, the species is likely to be attracted to these areas due to the productivity and abundance of suitably-sized prey (Pearson, 2003).

Threats to the species include major fire events, competition for prey with introduced predators, habitat loss (TSSC, 2008), predation of food sources (quolls and rock-wallabies) by foxes, habitat destruction by gas and mining development, deliberate and accidental road kills, and deliberate killings around tourist and residential areas associated with mistaken identification as a venomous brown snake (Pearson, 2003).

3.4.2 Previous Records

The Study Area is located at the south-eastern extent of the species current distribution, whereby the species or species habitat may occur (DoEE, 2019). Pilbara olive pythons are known to occur across the Pilbara bioregion, particularly within the Hamersley subregion, and are most often encountered in the vicinity of permanent waterholes in rocky ranges or among riverine vegetation (Pearson, 1993). The desktop assessment undertaken by (Biologic, 2020f) returned 14 records of the Pilbara olive python within 33 km of the Study Area (Figure 3.10).

The species has previously been recorded within the Study Area on 16 occasions, comprising direct observations and records of secondary evidence (including scats, sloughs and eDNA from water

features) (Biologic, 2011, 2020f) (Figure 3.10). Biologic (2011) recorded Pilbara olive python on three occasions within the Study Area, comprising a single individual recorded in a small cave, accumulated scats and sloughs at another location in the same gorge and slough recorded approximately 1 km north of these records at Afghan Pool (near water feature WWER-01) (Figure 3.10).

The species was recorded a further 13 occasions during a targeted survey in March 2020 (Biologic, 2020f). These records comprised two records of live individuals (one adult and one juvenile), four records of scats and seven positives (inclusive of equivocal results treated as positive) eDNA results, from water sampled at selected water features (Figure 3.10). A juvenile female (RFID# 690222) was recorded in WWER-07 (rock pool) on 12th March 2020 and an adult female (RFID# 604512) was recorded approximately 500 m from WWER-04 during a nocturnal search on 15th March 2020. Of the four scats recorded during targeted searches undertaken in March 2020 (Biologic, 2020f), two were collected from VWER-17, one from VWER-40 and one was opportunistically collected opportunistically in Gorge/ Gully habitat on the southern edge of Western Ridge (Figure 3.10).

Additionally, Biologic (2020f) recorded Pilbara olive python from eDNA samples collected from seven water features (WWER-01, WWER-04, WWER-07, WWER-11, WWER-13, WWER-14, WWER-16), where positive results (or equivocal results treated as positive therein) were recorded (Table 3.12). It should be noted that where an equivocal result was returned by qPCR, the results of PCR + DNA metabarcoding analysis were used to weight the likelihood of the result representing a positive or negative result (e.g. where qPCR returned an equivocal result for WWER-07 but PCR returned a positive, the result was treated as a positive). The equivocal results were considered positive in two instances, where individuals were recorded at or in the vicinity of water features sampled (WWER-04 and WWER-07), supporting the treatment of results as positive.

Table 3.12: Pilbara olive python eDNA results from Biologic (2020f)

Water feature ID	Date	eDNA sampling and analysis method	
		EnviroDNA (qPCR)	eDNA Frontiers (PCR + metabarcoding)
WWER-01	15/03/2020	Equivocal	Negative
WWER-04	15/03/2020	Positive	Positive
WWER-07	13/03/2020	Equivocal	Positive
	16/03/2020	Equivocal	N/A
WWER-11	13/03/2020	Negative	Positive
WWER-13	15/03/2020	Positive	N/A
WWER-14	15/03/2020	Negative	Positive
WWER-16	15/03/2020	Equivocal	N/A

Outside the Study Area, the nearest record of the species is located at the tailing dam at the Mt Whaleback mine, prior to 1998 (ecologia, 1998), followed by a record located at Pipe Spring, approximately 16 km west of Mt Newman (dated 1967), with few other records within 20 km of the Study Area (DBCA, 2021) (Figure 3.10). The nearest contemporary record of the species is located approximately 9.6 km northwest of the Study Area (dated 2012) (DBCA, 2021). A cluster of records are also known from the Cathedral Gorge area approximately 12 km north (Biologic, 2016a), the Orebody 24 area approximately 15 km north-east (Biologic, 2014), Ophthalmia Dam approximately 22 km north-east (BHP WAIO, 2021) and Jimblebar approximately 35 km north-east of the Study Area (BHP WAIO, 2021). With exception of two 2014 records approximately 50–55 km to the east, that are slightly further south than the Study Area, and the isolated records at Mount Augustus approximately 300 km west-southwest of the Study Area, no further records of the species occur south or southeast of the Study Area (DBCA, 2021). This is likely due to the Study Area occurring within the southern extent of the Hamersley subregion and consequently the southern extent of suitable habitat for the species.

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Newman

Pilbrara olive python Distribution (DAWE 2021)

Species or species habitat likely to occur

Species or species habitat may occur

Pilbara olive python Record (Biologic 2020b)

- Individual (Alive)
- eDNA
- Scat

Pilbara olive python Record (BHP WAIO 2021)

- eDNA
- Individual (Alive)
- Individual (Dead)
- Scat
- Unknown

Legend

- Study Area

Vertebrate Fauna Habitat (Biologic 2020b)

- Breakaway/ Cliff
- Drainage Area/ Floodplain
- Gorge/ Gully
- Hillcrest/ Hillslope
- Minor Drainage Line
- Mulga Woodland
- Stony Plain
- Cleared/ Disturbed

Pilbara olive python Record (DBCA 2020)

- Caught or Trapped
- Day Sighting
- Dead
- Secondary Sign
- Specimen
- Other

N

biologic Environmental Survey

Scale: 1:30,000

0 0.5 1 1.5 Km

Coordinate System: GDA 1994 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA 1994 Created 30/06/2021

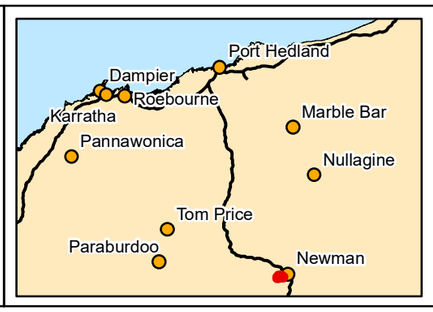

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Figure 3.10: Previous Pilbara olive python records in the Study Area and region

3.4.3 Survey Methods

Targeted Searching

Diurnal and nocturnal searches for the Pilbara olive python were conducted during trip 1, trip 3 and trip 4. A total of 25 targeted searches undertaken at 17 sites over the three trips, equating to a total of 53.30 person hours of targeted searches (Table 3.13). This included a total of 28.05 person hours of diurnal searches and 25.25 person hours of nocturnal searches over the three trips (Table 3.13). Some sites were revisited on multiple occasions, particularly sites where the species had previously been recorded, to increase the likelihood of detecting the species. Furthermore, targeted searches were undertaken at regional sites during trip 3, with the aim to provide contextual data and obtain genetic material for possible use with future genetic studies on the population occurring within the Study Area (Table 3.13).

Captured animals were processed at point of capture, during which standardised measurements were recorded (i.e. snout-vent length, tail length and weight) and overall general condition was assessed (i.e. general body condition, presence of scaring and parasites). A tissue sample (ventral scale clip) was collected from all captured individuals and stored in 100% ethanol for subsequent genetic analysis. Any individuals captured were permanently marked for future identification using RFID microchips, and following procedures developed by (DBCA, 2017). Opportunistic road spotlighting and searches were also undertaken whilst driving between sites.

eDNA Sampling

Environmental DNA (eDNA) is a by-product of the metabolic process, derived from sources such as deceased individuals, faeces, urine, scales mucous secretions and are recoverable from environmental substrates (i.e. water or substrate) (Huerlimann *et al.*, 2020). The extraction of eDNA has emerged as a novel sampling technique in the realm of environmental surveying, monitoring and conservation with potentially greater sensitivity in detecting rare and cryptic species (Bylemans *et al.*, 2019; Harper *et al.*, 2018; Huerlimann *et al.*, 2020).

eDNA sampling was undertaken at a total of seven water features, including five within the Study Area and two regional sites python (Table 3.14). Some water features were samples on multiple trips to maximise the detection of Pilbara olive python and assess species detection temporally between trips (Table 3.14). A selection of water features sampled for eDNA, represented regional sampling sites. Sampling at these sites was undertaken to provide some contextual data outside of the Study Area.

Five 1 litre (L) sampling bottles were filled with water from five different locations at the water feature and stored upright in a cooler of ice. Water samples were then filtered to collect eDNA material present within the sample by passing water samples though a 0.45-micron (µm) filter membrane using a peristaltic Sentino pump. All filtering equipment was sterilised in a 10% bleach solution and rinsed between samples. As a control, a sample of the water used to rinse the filtering equipment (after it had been sterilised in a 10% bleach solution) was also passed through a filter membrane. Once the water had been filtered, the filter membrane was folded, placed into a sample bag and stored at approximately -20^{o}C until it could be delivered to eDNA Frontiers for PCR and metabarcoding analysis (see Appendix D for a detailed description of analysis techniques).

Table 3.13: Targeted searches completed for Pilbara olive python within the Study Area

Trip	Site name	Location (Study Area/ regional)	Water feature ID	Date	Search type	Habitat	Latitude	Longitude	Person hours
Trip 1	VWER-10	Study Area	WWER-01, WWER-02 and WWER-04	26/08/2020	Nocturnal	Gorge/ Gully	-23.3833	119.6146	2
	VWER-10	Study Area	WWER-01, WWER-02 and WWER-04	27/08/2020	Nocturnal	Gorge/ Gully	-23.3833	119.6146	3.5
	VWER-10	Study Area	WWER-01, WWER-02 and WWER-04	30/08/2020	Nocturnal	Gorge/ Gully	-23.3833	119.6146	2.5
	VWER-17	Study Area	WWER-06 and WWER-11	27/08/2020	Diurnal	Gorge/ Gully	-23.3941	119.6171	2
	VWER-26	Study Area	N/A	26/08/2020	Diurnal	Gorge/ Gully	-23.4037	119.6605	2.5
	VWER-27	Study Area	N/A	26/08/2020	Diurnal	Gorge/ Gully	-23.3989	119.6078	2
	VWER-44	Study Area	N/A	26/08/2020	Diurnal	Gorge/ Gully	-23.3855	119.6140	3.5
	VWER-53	Study Area	N/A	25/08/2020	Diurnal	Breakaway/ Cliff	-23.3876	119.6170	4
	VWER-60	Study Area	N/A	30/08/2020	Diurnal	Hillcrest/ Hillslope	-23.3952	119.5915	1.8
	VWER-61	Study Area	N/A	30/08/2020	Diurnal	Hillcrest/ Hillslope	-23.3903	119.6380	1.1
Trip 3	VWER-10	Study Area	WWER-01, WWER-02 and WWER-04	10/10/2020	Nocturnal	Gorge/ Gully	-23.3833	119.6146	2.5
	VWER-60	Study Area	N/A	9/10/2020	Diurnal	Hillcrest/ Hillslope	-23.3952	119.5915	1
	VWER-61	Study Area	N/A	10/10/2020	Diurnal	Hillcrest/ Hillslope	-23.3903	119.6380	1
	VWER-62	Study Area	N/A	9/10/2020	Diurnal	Breakaway/ Cliff	-23.3881	119.6084	1
	VWER-63	Study Area	N/A	9/10/2020	Diurnal	Hillcrest/ Hillslope	-23.3936	119.5978	1
	VWER-68	Study Area	N/A	9/10/2020	Diurnal	Hillcrest/ Hillslope	-23.3979	119.5797	1

Trip	Site name	Location (Study Area/ regional)	Water feature ID	Date	Search type	Habitat	Latitude	Longitude	Person hours
Trip 4	VWER-10	Study Area	WWER-01, WWER-02 and WWER-04	26/11/2020	Nocturnal	Gorge/ Gully	-23.3833	119.6146	4
	VWER-10	Study Area	WWER-01, WWER-02 and WWER-04	29/11/2020	Nocturnal	Gorge/ Gully	-23.3833	119.6146	4
	VWER-17	Study Area	WWER-06	27/11/2020	Nocturnal	Gorge/ Gully	-23.3941	119.6171	4
	VWER-30	Study Area	WWER-12	27/11/2020	Nocturnal	Stony Plain	-23.400585	119.640194	0.25
	VWER-79	Regional	WWER-20	28/11/2020	Diurnal	Gorge/ Gully	-23.2688	119.5953	2.66
	VWER-82	Regional	WWER-26	28/11/2020	Diurnal	Major Drainage Line	-23.2765	119.6581	1
	VWER-83	Regional	WWER-23	28/11/2020	Diurnal	Gorge/ Gully	-23.2835	119.6584	0.16
	VWER-84	Regional	WWER-24	28/11/2020	Nocturnal	Major Drainage Line	-23.3151	119.8706	2.5
	VWER-92	Regional	WWER-22	28/11/2020	Diurnal	Gorge/ Gully	-23.2838	119.7392	4.33
								Total	**53.30**

Table 3.14: Pilbara olive python eDNA sampling locations from this assessment

Water feature	Location (Study Area/ regional)	Description	Habitat	Latitude	Longitude	Trip 1		Trip 2		Trip 3		Total samples
						Sample date	Samples	Sample date	Samples	Sample date	Samples	
WWER-01	Study Area	Seep fed rock pool	Gorge/ Gully	-23.3832	119.6145	26/08/2020	5	13/10/2020	5	29/11/2020	5	**15**
WWER-02	Study Area	Rock pool	Gorge/ Gully	-23.3832	119.6145	Not sampled		13/10/2020	3	29/11/2020	5	**8**
WWER-04	Study Area	Seep fed rock pool	Gorge/ Gully	-23.3825	119.6136	26/08/2020	5	13/10/2020	5	29/11/2020	5	**15**
WWER-11	Study Area	Rock pool	Gorge/ Gully	-23.3943	119.6196	27/08/2020	4	Not sampled (dry)		Not sampled (dry)		**4**
WWER-12	Study Area	Artificial/ Turkeys Nest	Stony Plain	-23.4006	119.6402	28/08/2020	5	Not sampled		Not sampled		**5**
WWER-20	Regional	Seep fed rock pool	Gorge/ Gully	-23.2686	119.5953	Not sampled		13/10/2020	5	28/11/2020	5	**10**
WWER-22	Regional	Rock pool	Gorge/ Gully	-23.2837	119.7392	Not sampled		Not sampled		28/11/2020	5	**5**
Total							**19**		**18**		**25**	**62**

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7412000

7410000

7408000

VWER-10

VWER-17

VWER-30

Legend

Study Area

Vertebrate Fauna Habitat (Biologic 2020b)

- Breakaway/ Cliff
- Drainage Area/ Floodplain
- Gorge/ Gully
- Hillcrest/ Hillslope
- Minor Drainage Line
- Mulga Woodland
- Stony Plain
- Cleared/ Disturbed

eDNA

Targeted Search

- Diurnal Search
- Nocturnal Search
- Helicopter Flight Path

N

biologic Environmental Survey

Scale: 1:30,000

0 0.5 1 1.5 Km

Coordinate System: GDA 1994 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA 1994 Created 01/07/2021

Port Hedland
Dampier
Roebourne
Karratha
Pannawonica
Marble Bar
Nullagine
Tom Price
Paraburdoo
Newman

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Figure 3.11: Pilbara olive python sampling in the Study Area

3.4.4 Survey Results

The Pilbara olive python was recorded on 15 occasions during this assessment, comprising nine records of live individuals or secondary evidence (i.e. scats, sloughs) and six occasions from eDNA sampling at selected water features.

Targeted Searching

The Pilbara olive python was recorded from direct and secondary evidence on eight occasions during this assessment, comprising four records of live individuals (including one recapture), two records of sloughs, one scat and one live individual from camera trap capture (Table 3.16; Figure 3.13; Figure 3.14). Of the eight records, five occurred within the Study Area (including three live individual records, one scat and one live individual from camera trap capture) and three from regional locations (including one live individual and two sloughs) (Table 3.16; Figure 3.14).

All records of the species were from Gorge/ Gully habitat, with four records occurring at VWER-10, two at VWER-79 and one at VWER-17 and an opportunistic site. Of the four individuals captured, one individual was caught on two occasions (both at VWER-10), once during trip 1 and again during trip 3 (RFID# 604460). No individuals previously captured and microchipped by Biologic (2020f) were recaptured during this assessment, despite multiple targeted searches undertaken at locations of previous records (Table 3.13).

With the exception of the male individual caught at the regional site (VWER-79), all other captures were in excellent condition. The condition of the individual caught at VWER-79 was poor, with the individual showing heavy scaring and parasite load, and a general emaciated condition.

Figure 3.12: Pilbara olive python individual (RFID# 604460) recorded from VWER-10

Figure 3.13: Pilbara olive python recorded from camera trap capture at VWER-17

eDNA Sampling

Pilbara olive python was detected from five of the seven water features eDNA sampling was undertaken at during this assessment (Table 3.15; Appendix D). The positive results at four water features (WWER-01, WWER-02, WWER-04 and WWER-20) was supported by the direct observation of Pilbara olive python at or in close proximity to the water features (Table 3.16; Table 3.15; Appendix D).

Of the three water features also sampled by Biologic (2020f) where positive results were recorded (WWER-01, WWER-04 and WWER-11), sampling during this assessment returned a positive result at two (WWER-01 and WWER-04). The third site (WWER-11) was only sampled once during this assessment due to the pool drying out between trip 1 and trip 2; however, the species was not detected at the site from this sampling event.

Where multiple sampling events were undertaken at a water feature, the species was not necessarily detected during every sampling event. This may be attributed to several indeterminate factors, including rainfall (and potential dilution of eDNA at water feature), exposed to other conditions (i.e. drying pool) or elements (sunlight and temperature) or the lack of Pilbara olive python occurrence within a reasonable timeframe of detection. It should be noted that it is unknown how long the species' DNA persists at water features once deposited, therefore it is not known if positive recordings are indicative of present (within hours or days of sampling) or a longer duration.

Table 3.15: Pilbara olive python eDNA results from this assessment

Water feature	Site	Location (Study Area/ regional)	Description	Habitat	Biologic (2020f)	This assessment		
						Trip 1	Trip 2	Trip 3
WWER-01	VWER-10	Study Area	Seep fed rock pool	Gorge/ Gully	**Positive**	Negative	Negative	**Positive**
WWER-02	VWER-10	Study Area	Rock pool	Gorge/ Gully	N/A	N/A	Negative	**Positive**
WWER-04	VWER-10	Study Area	Seep fed rock pool	Gorge/ Gully	**Positive**	Negative	Negative	**Positive**
WWER-11	VWER-17	Study Area	Rock pool	Gorge/ Gully	**Positive**	Negative	N/A	N/A
WWER-12	VWER-30	Study Area	Artificial/ Turkeys Nest	Stony Plain	N/A	Negative	N/A	N/A
WWER-20	VWER-79	Regional	Seep fed rock pool	Gorge/ Gully	N/A	N/A	**Positive**	**Positive**
WWER-22	VWER-92	Regional	Rock pool	Gorge/ Gully	N/A	N/A	N/A	**Positive**

Opportunistic Records

Between trip 2 and trip 3 of this assessment, a Pilbara olive python was opportunistically recorded by BHP personnel within the Study Area. On the night of 19th September 2020, an individual measuring approximately 2 m was observed active at a Turkeys nest (WWER-12) located in the southwest of the Study Area (Dave Butchart *pers. comm.* 28 September 2020) (Figure 3.14). The location of the record is approximately 2.4 km east-southeast of the nearest previous record (including a live individual and scat records) in March 2020 (Biologic, 2020f) (Figure 3.14). However, it cannot be determined if the specimen is a previously recorded individual or an unrecorded individual. Although eDNA sampling was undertaken at this water feature, no evidence of the species was detected. This is likely to be attributed to the high volume of water that is processed through the Turkeys nest on a daily basis, with any potential deposition of genetic material likely to be diluted and/or removed entirely along with the high volume of water cycling through the site.

Table 3.16: Pilbara olive python records from previous surveys and this assessment

Site ID	Location	Record type	Latitude	Longitude	Habitat	Date	Specimen information						
							Recapture	Age	Sex	Total length (mm)	Weight (g)	Condition	RFID microchip #
Biologic (2011)													
VWER-17	Study Area	Individual (alive)	-23.3945	119.6187	Gorge/ Gully	2010	No	-	Undetermined	-	-	-	-
VWER-17	Study Area	Scats	-23.3938	119.6178	Gorge/ Gully	2010	N/A						
VWER-10	Study Area	Slough	-23.3831	119.6144	Gorge/ Gully	2010	N/A						
Biologic (2020f)													
VWER-17	Study Area	Individual (alive)	-23.3944	119.6172	Gorge/ Gully	12/03/2020	No	Juvenile	Female	990	275	Excellent	990000003690222
VWER-17	Study Area	Scat	-23.3939	119.6181	Gorge/ Gully	13/03/2020	N/A						
VWER-10	Study Area	Individual (alive)	-23.3821	119.6134	Gorge/ Gully	15/03/2020	No	Adult	Female	3,200	6,018	Excellent	900193003604512
VWER-17	Study Area	Scat	-23.3944	119.6187	Gorge/ Gully	15/03/2020	N/A						
VWER-40	Study Area	Scat	-23.4047	119.6142	Hillcrest/ Hillslope	15/03/2020	N/A						
Opp	Study Area	Scat	-23.4051	119.6055	Gorge/ Gully	16/03/2020	N/A						
This assessment													
VWER-10	Study Area	Individual (alive)	-23.2887	119.7466	Gorge/ Gully	27/08/2020	No	Adult	Female	2,520	2,090	Excellent	900193003604460
VWER-10	Study Area	Scat	-23.3833	119.6146	Gorge/ Gully	30/08/2020	N/A						
VWER-10	Study Area	Individual (alive)	-23.3828	119.6139	Gorge/ Gully	26/11/2020	Yes	Adult	Female	-	3,355	Excellent	900193003604460

Site ID	Location	Record type	Latitude	Longitude	Habitat	Date	Specimen information						
							Recapture	Age	Sex	Total length (mm)	Weight (g)	Condition	RFID microchip #
VWER-10	Study Area	Individual (alive)	-23.3831	119.6145	Gorge/ Gully	29/11/2020	No	Juvenile/ Immature	Female	1,825	865	Excellent	900193003604459
VWER-17	Study Area	Individual (camera trap) (Figure 3.13)	-23.3826	119.6137	Gorge/ Gully	29/11/2020	N/A						
VWER-79	Regional	Slough	-23.3940	119.6176	Gorge/ Gully	11/09/2020	N/A						
VWER-79	Regional	Individual (alive)	-23.2685	119.5953	Gorge/ Gully	12/10/2020	No	Adult	Male	2,790	1,915	Poor	900193003604505
Opp	Regional	Slough	-23.2690	119.5954	Gorge/ Gully	28/11/2020	N/A						
Other													
Opp (BHP personnel)	Study Area	Individual (alive)	-23.4006	119.6402	Stony Plain (Turkey's nest)	19/09/2020	-	Sub-adult	-	~2,000	-	Excellent	-

762000 764000 766000 768000 770000 772000

7412000

7410000

7408000

Pilbara olive python Record (Biologic 2020b)

- Individual (Alive)
- Scat
- Skin

Pilbara olive python Record (BHP 2020)

- Nest
- Scat
- Skin

Legend

- Study

Vertebrate Fauna Habitat (Biologic 2020b)

- Breakaway/ Cliff
- Drainage Area/
- Gorge/ Gully
- Hillcrest/ Hillslope
- Minor Drainage Line
- Mulga Woodland
- Stony Plain
- Cleared/ Disturbed

Pilbara olive python Record (this Survey)

- Individual (Alive)
- eDNA
- Scat
- Skin

Pilbara olive python Record (Other)

- Individual (Alive)

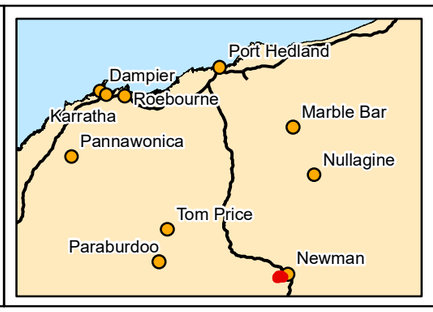
N

biologic Environmental Survey

Scale: 1:30,000

0 0.5 1 1.5 Km

Coordinate System: GDA 1994 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA 1994 Created 01/07/2021



BHP WAIO

Western Ridge MNES Study

Figure 3.14: Pilbara olive python records in the Study Area and region

3.4.5 Discussion

Results from this survey and the work completed by Biologic (2020f) confirms the presence of at least four individuals occurring within the Study Area, within a period of 12 months. This number of individuals within a relatively small area (two Gorge/ Gully system) is considered high indicating a high density for this area. Given the mostly-sedentary lifestyle of the species and the fact that two of these individuals were large mature females of breeding age, it is highly likely that the population existing in the area is permanent and a breeding population. The third female individual known to occur was considered a juvenile further supporting this conclusion.

The Pilbara olive python is regularly encountered in the vicinity of permanent water features in rocky ranges or riverine habitats (Pearson, 1993). Based on previous habitat mapping undertaken for the Study Area (Biologic, 2020f), Gorge/ Gully habitat (3.5%, 165.7 ha) and to a lesser degree Breakaway/ Cliff (1.1%, 53.1 ha) habitats within the Study Area provide primary breeding habitat for the species (Figure 3.14). Additionally, the Gorge/ Gully habitat type is prone to pooling and ponding in areas, therefore providing primary foraging habitat for the species. Primary foraging and dispersal habitat is also provided by Hillcrest/ Hillslope habitat (40.6 %, 1,936.6 ha) and Minor Drainage Line habitat (2.1%, 101.7 ha) (Figure 3.14). The Pilbara olive python is expected to occur throughout the Study Area in these habitats, particularly in areas where it occurs adjacent to and/ or connects Gorge/ Gully and/ or Breakaway/ Cliff habitat.

Water sources are a limiting factor for many ecosystems (James *et al.*, 1995), particularly within arid-zone ecosystems such as the Pilbara (Burbidge *et al.*, 2010; Doughty *et al.*, 2011) and often represent areas of comparatively high ecological productivity (Murray *et al.*, 2003). These features are highlighted because they may provide important sources of food and water for the Pilbara olive python. For Pilbara olive pythons, these water features can often act as primary foraging locations. Therefore, the species is more often than not associated with such features, mainly within rocky habitats, but also (to a lesser degree) within drainage habitats where suitable vegetation cover is present (Pearson, 1993). Overall, a total of 14 water features have been recorded within the Study Area (Biologic, 2020f). Of these 14 water features, two (located in Gorge/ Gully habitat) are fed by a seep (WWER-01 and WWER-04). These water features appear to be potentially permanent or semi-permanent based on a high volume of water observed on all visitations during 2020, including all trips of this assessment. The artificial water feature (Turkey's nest) present within the Study Area (WWER-12) is also likely to contain water on a semi-permanent to permanent basis, while associated activities requiring its operation continue within and in the vicinity of the Study Area. Furthermore, a potentially permanent or semi-permanent non-pooling seep (also located in Gorge/ Gully habitat) was observed at one water feature (WWER-06).

The two seep fed rock pools (WWER-01 and WWER-04) are considered to be of high significance to the Pilbara olive python as they are likely to provide core foraging habitat for the majority of the year. As no ground water dependant vegetation was observed, it is unclear whether these pools are permanent; however, during an opportunistic visit in December 2019 (end of the dry season) water was still observed at these two pools suggesting they may be permanent for most of the year and/or most years. A total of three Pilbara olive pythons (on four occasions) have been recorded within the gorge

containing these water features in 2020 by Biologic (2020f) and during this assessment (trip 1 and trip 3), highlighting the likely significance of the gorge to the species at a local scale. The species has also been detected from eDNA sampling from all three water features present in the gorge (including WWER-01 and WWER-04) during this assessment and previous surveys (Table 3.15). Additionally, the artificial water feature (Turkey's nest) is also likely to provide core foraging habitat for the species, particularly due to the likely permanent to semi-permanent nature of this water feature. Although the Pilbara olive python was not detected at all water features within the Study Area during this assessment, they provide habitat of moderate significance as they are still likely to provide foraging habitat, albeit some only for a temporary period.

The results of this assessment and previous (i.e. Biologic, 2020f) surveys within the Study Area, and the use of RFID microchipping to facilitate individual recognition of captures, suggest at least four unique individuals are known to occur within Study Area. This, however, is likely to remain an underestimation of true species abundance within the Study Area due to the species' cryptic behaviour and difficulty of detection.

3.5 Other Fauna of Conservation Significance

One non-target species of conservation significance was recorded during this assessment, the western pebble-mound mouse (*Pseudomys chapmani*) (Table 3.17). The species was recorded on 11 occasions from secondary evidence (pebble-mounds), including five recently inactive mounds, four inactive mounds and two active mounds (Table 3.17).

Table 3.17: Other species of conservation significance observed during the field assessment

Species	Habitat	Latitude	Longitude	Date	Record type
Pseudomys chapmani	Hillcrest/ Hillslope	-23.3849	119.6143	29/08/2020	Mound (recently inactive)
Pseudomys chapmani	Hillcrest/ Hillslope	-23.4121	119.5824	10/10/2020	Mound (inactive)
Pseudomys chapmani	Hillcrest/ Hillslope	-23.4117	119.5816	10/10/2020	Mound (inactive)
Pseudomys chapmani	Stony Plain	-23.2985	119.6156	13/10/2020	Mound (recently inactive)
Pseudomys chapmani	Gorge/ Gully	-23.2666	119.6080	12/10/2020	Mound (inactive)
Pseudomys chapmani	Stony Plain	-23.3766	119.6047	27/11/2020	Mound (active)
Pseudomys chapmani	Stony Plain	-23.2753	119.5988	28/11/2020	Mound (recently inactive)
Pseudomys chapmani	Stony Plain	-23.2755	119.5996	29/11/2020	Mound (recently inactive)
Pseudomys chapmani	Stony Plan	-23.3865	119.5858	30/11/2020	Mound (recently inactive)
Pseudomys chapmani	Stony Plain	-23.3890	119.5861	30/11/2020	Mound (active)
Pseudomys chapmani	Stony Plain	-23.3788	119.6027	30/11/2020	Mound (inactive)

3.6 Constraints and Limitations

The (EPA, 2020) outlines several potential limitations to vertebrate fauna surveys. These aspects are assessed and discussed in Table 3.18 below. No major limitations or constraints were identified for the survey.

Table 3.18: Survey constraints and limitations

Potential limitation or constraint	Constraint	Applicability to this survey
Sources/availability of data and information (recent or historic) and availability of contextual information	No	A significant amount of survey work has been undertaken in the wider local area and the surrounding region, and most these previous survey results were available for review. These reports were available at the time of reporting.
Competency/ experience of the survey team	No	The field personnel involved in the survey (Chris Knuckey, Ryan Ellis, Andrew Hide and Thomas Rasmussen) are experienced in undertaking fauna surveys of similar nature, including with the species of conservation significance targeted during the survey. Technical personnel with relevant expertise assisted with analysis of ultrasonic recordings (Bob Bullen), genetic analyses (DBCA and eDNA Frontiers) and hormone analysis (UoQ).
Scope (faunal groups sampled and whether any constraints affect this)	No	The scope was a targeted fauna survey and was conducted within that framework (EPA, 2020).
		Northern quoll –The species was sampled following survey guidelines in relation to survey design and effort, site coverage, and detectability (DoE, 2016). Camera trap transects were set during this assessment; 1,800 sampling nights. Targeted searches were undertaken for secondary evidence (e.g. scats).
		Pilbara leaf-nosed bat- The species has been sampled through targeted surveys (ultrasonic recording; 90 sampling nights) and targeted searches (cave searches). Bat detectors were placed at known caves, new caves and at significant habitat areas. Not all caves located have been sampled by SM recorder. Sampling was undertaken during the wet season, when bats are likely to be dispersing, and there is a greater likelihood of detection.
		Ghost bat- The species has been sampled through targeted surveys (ultrasonic recording; 90 sampling nights) and targeted searches (cave searches). Caves have been searched for scats and sheeted for follow up monitoring on subsequent trips. Bat detectors were placed at known caves, new caves, or significant habitat areas. Not all caves located have been sampled by SM recorder.
		Pilbara olive python- Targeted diurnal and nocturnal searches were undertaken in potential habitat for active individuals, scats, and water features likely to support the species. eDNA sampling was also conducted at multiple water features over multiple trips to increase the detectability of the species.
Timing, weather, and season	No	Field surveys occurred over appropriate or optimal periods for sampling the target species. No weather or seasonality constraints or limitations were identified during the surveys.

Potential limitation or constraint	Constraint	Applicability to this survey
Disturbances (e.g. fire or flood)	No	No temporary disturbance impinged on the results of this assessment. Part of a mining pit intersects a small portion of the north-eastern edge of the Study Area; however, is unlikely to have impacted results of this assessment. A recent fire is evident through much of the Study Area and more broadly in the surrounding region, this may have affected ghost bat and Pilbara leaf-nosed bat activity in the Study Area, though conclusions were backed-up by an abundance of historical and contextual information.
Proportion of fauna identified	No	All fauna observed during the field surveys were identified to species level. Species identification of fauna recorded via camera traps and SongMeter ultrasonic recorders were able to be accurately identified with the assistance of technical personnel with relevant expertise.
Adequacy of the survey intensity and proportion of the survey achieved	No	A targeted survey was undertaken across the Study Area to assist with decisions on future environmental approvals. The sampling methods and survey intensity was high and focussed on the species of interest.
Remoteness or access issues	No	With the exception of a portion of the Study Area and some regional sites, the majority of the Study Area was accessible either by vehicle or on foot, thus the sampling techniques used in these areas during this survey were unconstrained by accessibility or remoteness. Due to access constraints in parts of the Study Area and regionally, a helicopter was used to facilitate access to allow sufficient sampling to be undertaken throughout the Study Area and at appropriate regional sites.
Problems with data and analysis, including sampling bias	No	No limitations with data collection and/or analysis were encountered during the field survey or during subsequent analysis.

4 CONCLUSION

The overarching objective of this assessment was to assess the likelihood of occurrence and determine the spatial and quantitative extent of occurrence for four target species considered MNES. The targeted MNES species for the survey comprised the northern quoll, Pilbara leaf-nosed bat, ghost bat and Pilbara olive python.

4.1 Northern Quoll

No evidence of northern quoll occurrence was recorded within the Study Area during this assessment. Although the species has previously been recorded within the Study Area, these records are not representative of present occurrence, owing to their preservation within a stable cave microclimate. Based on the extensive sampling undertaken in the Study Area to date and the isolated nature of suitable habitat within the Study Area, it is likely these records represent scats from an historical population, potentially transient individuals or a declining population that no longer or rarely occurs within the Study Area.

Despite the low likelihood of northern quoll occurrence, suitable habitat with the potential to support the species occurs within the Study Area. Approximately 3.5% (165.7 ha) of the Study Area is represented by Gorge/ Gully habitat and a further 1.1% (53.1 ha) by Breakaway/ Cliff habitat, both of which are considered primary breeding (denning/ shelter) habitat for the species. Minor Drainage Line (2.1%, 101.7 ha) and Hillcrest/ Hillslope (40.6 %, 1,936.6 ha) habitats represent primary foraging and dispersal habitat, particularly in areas that are located adjacent to or in close proximity to Gorge/ Gully and Breakaway/ Cliff habitat.

Based on the paucity of contemporary records within and in the broader vicinity of the Study Area, the species' occurrence within the Study Area is likely to be limited to historic occurrences or, if present, at very low densities. Any records of the species within the Study Area are likely to be representative of individuals on the periphery of the species distribution as it expands and contracts over time and/or dispersing from a permanent population elsewhere. While the species may occur within the Study Area on an infrequent basis or in low abundance, based on current information the population does not meet the definition of a "population important for the long-term survival of the northern quoll", as defined by DoE (2013).

4.2 Pilbara Leaf-nosed Bat

The Pilbara leaf-nosed bat was not recorded within the Study Area during this assessment. The species has not been recorded in the Study Area previously but has been recorded from a single call approximately 50 m to the south of the Study Area. The presence of this records suggests that the species has previously, or is likely to, occur within the Study Area. The lack of records from within the Study Area itself confirms that the species is highly unlikely to reside in the Study Area permanently (i.e. roost) and may only occur to forage and disperse intermittently. Evidence of a roost within the nightly flight distance of the species was detected during regional sampling of this assessment, indicating that the species may utilise the Study Area occasionally for such purposes.

Approximately 3.5% (165.7 ha) of the Study Area is represented by the Gorge/ Gully habitat, which represents primary foraging habitat for the species in the Study Area. Breakaway/ Cliff (1.1%, 53.1 ha), limited instances where outcropping occurs within the Hillcrest/ Hillslope (40.6 %, 1,936.6 ha) and the Minor Drainage Line habitat (2.1%, 101.7 ha) are also categorised as primary foraging habitat for the species. Finally, open grasslands and woodlands contained within the remaining habitats; Stony Plain (30.3%, 1,444.8 ha), Mulga Woodland (11.7%, 555.5 ha) and Drainage Area/ Floodplain (9.8%, 468.8 ha); are considered Priority 5, marginal foraging habitat for the species. Of particular interest are the water features WWER-01 and WWER-04, which constitute Priority 1 foraging habitat and provide a regular, perhaps permanent, water source for visiting individuals.

According to definition prescribed by the TSSC (2016b), the entire Pilbara represents one interbreeding population, meeting the requirements of an 'important population' as defined by DoE (2013) (TSSC, 2016b). Evidence of a roost within the nightly flight distance of the species was detected during regional sampling of this assessment, indicating that the species may visit the Study Area occasionally. However, because the Study Area is highly unlikely to contain any Priority 1 or Priority 2 diurnal roosts for the species, it is unlikely to represent a significant area for the species.

4.3 Ghost Bat

Within the Study Area, Gorge/ Gully habitat (3.5%, 165.7 ha) and Breakaway/ Cliff (1.1%, 53.1 ha) are regarded as primary breeding, roosting and foraging habitat for the species. Stony Plain (30.3%, 1,444.8 ha), Mulga Woodland (11.7%, 555.5 ha), Drainage Area/ Floodplain (9.8%, 468.8 ha) and Minor Drainage Line (2.1%, 101.7 ha) habitat provides primary foraging habitat for the species, particularly when adjacent to roosting caves.

During this assessment, the ghost bat was recorded from a single cave. The species has however previously been recorded from an additional six locations. Occupation of the species within the Study Area spans multiple years and scat evidence in two caves, CWER-01 and CWER-03 indicates that ghost bat occupation has occurred over a long and perhaps regular period. In total, 15 caves suitable for the species are known, comprising one maternity roost, one potential maternity roost (and confirmed diurnal roost), three potential diurnal roosts, five night roosts and five potential night roosts. The maternity roost (CWER-01) and potential maternity roost (CWER-03) are considered to be of high local and regional significance for the species, evident by continued use over the past four years. Evidence of reproducing females has previously been recorded in the Study Area, confirming the Study Area forms part of an area used for breeding.

The presence of one maternity roost (CWER-01) and one potential maternity roost (CWER-03) within the Study Area indicates that individuals from the Study Area form part of a 'key source population either for breeding or dispersal' as defined by DoE (2013). Additionally, records from within the Study Area represent the south-eastern extent of the species distribution in the Pilbara region, therefore forming part of a 'population that [is] near the limit of the species range' as defined by DoE (2013). Together these definitions suggest the ghost bat's occurring within the Study Area form part of a 'important population' as defined by DoE (2013).

4.4 Pilbara Olive Python

The Pilbara olive python was recorded on 15 occasions during this assessment, comprising nine records of live individuals or secondary evidence (i.e. scats, sloughs) and six occasions from eDNA sampling at selected water features. The species was recorded from capture of live individuals on four occasions within the Study Area, including one individual that was captured on two separate occasions. All records of the species within the Study Area were associated with Gorge/ Gully habitat, and in most situations at, or in close proximity, to water features.

Within the Study Area, based on fauna habitat mapping completed by Biologic (2020f), Gorge/ Gully habitat (3.5%, 165.7 ha) and Breakaway/ Cliff habitat (1.1%, 53.1 ha) represent the most significant habitats for the species, as they provide primary breeding and foraging habitat, particularly when occurring in association with permanent or semi-permanent water features. The Minor Drainage Line (2.1%, 101.7 ha) and Hillcrest/ Hillslope (40.6 %, 1,936.6 ha) habitats also represent primary foraging and dispersal habitat, particularly where it facilitates connectivity between areas of primary Gorge/ Gully and/ or Breakaway/ Cliff habitat.

With consideration of the results of targeted sampling for Pilbara olive python within the Study Area during this assessment and previous (i.e. Biologic, 2020f) surveys, and the use of RFID microchipping to facilitate individual recognition of captures, at least four unique individuals are known to occur within the Study Area. Due to the cryptic behaviour of the species, and that, of the four unique individuals marked, only one was recaptured on successive trips, the true abundance of the species within the Study Area is likely to exceed the number of unique individuals captured during these surveys.

The Pilbara olive python population occurring within the Study Area is likely to represent a permanently residing and breeding population, and therefore a ‘key source population either for breeding or dispersal’, as defined by DoE (2013). Additionally, individuals from within the Study Area represent the south-eastern extent of the species distribution, therefore forming part of a ‘population that [is] near the limit of the species range’, as defined by DoE (2013). Together these definitions suggest the Pilbara olive python’s occurrence within the Study Area forms part of an ‘important population’, as defined by DoE (2013).

4.5 Summary

The Study Ares is located at the south-eastern most extent of the Pilbara bioregion and as such, the Study Area represents the south-eastern limit for many species, particularly those that exist as, or have, populations confined to ironstone ranges of the Pilbara bioregion. Two MNES species, known to occur within the Study Area, the ghost bat and Pilbara olive python fall into this category. Any development proposed for this area should seek to minimise impacts to these species to avoid threatening these species’ distributions.

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6 APPENDICES

Appendix A – Habitat Assessments

Site ID	Coord.	Date	Habitat Type	Landform	Aspect	Slope	Soil Type	Soil Avail.	Outcropping Rock Type	Rock Size	Veg. Litter	Dominant Veg. Type	Rocky Cracks/ Crevices	Burrowing Suitability	Hollow' s count	Water present	Disturbance	Last Fire
VCAT-01	-23.2765, 119.6322	10/12/2019	Gorge/ Gully	Gorge	South/ West	Cliff	Clay Loam	Many Small Patches	Extensive Outcropping BIF	Large Rocks (21-60cm)	Few Small Patches	Scattered Eucalypts, Spinifex Hummock Grassland	High	Low	0	None	None Discernible	Moderate (3 to 5 yr.)
VCAT-08	-23.2969, 119.7141	13/10/2020	Breakaway/ Cliff	Breakaway	North	Low	Clay Loam	Few Small Patches	Moderate Outcropping BIF	Large Rocks (21-60cm)	Scarce	Scattered Eucalypts, Spinifex Hummock Grassland	Moderate	Low	0	None	None Discernible	Moderate (3 to 5 yr.)
VWER-01	-23.4127, 119.5835	12/03/2020	Breakaway/ Cliff	Hillcrest/ Upper Hillslope	South/ East	Very Steep	Clay Loam	Scarce	Major Outcropping BIF	Gravel (1-4cm)	Few Small Patches	Scattered Eucalypts, Tussock Grassland	Very High	Nil	0	None	Non-Discernible	Old (6+ yr.)
VWER-03	-23.3970, 119.6607	13/03/2020	Gorge/ Gully	Gully	North	Moderate	Clay Loam	Few Small Patches	Moderate Outcropping BIF	Gravel (1-4cm)	Scarce	Scattered Eucalypts, Spinifex Hummock Grassland	High	Low	0	None	Non-Discernible	Old (6+ yr.)
VWER-10	-23.3833, 119.6146	26/08/2020	Gorge/ Gully	Gully	North/ West	Moderate	Clay Loam	Many Large Patches	Extensive Outcropping BIF	Boulders (>61cm)	Many Small Patches	Fig trees, sedge, Scattered Eucalypts, Tussock Grassland	Moderate	Nil	5	Prone to Pooling	Non-Discernible	Old (6+ yr.)
VWER-16	-23.3981, 119.6593	25/08/2020	Gorge/ Gully	Gully	South/ West	Moderate	Clay Loam	Many Large Patches	Major Outcropping BIF	Boulders (>61cm)	Few Large Patches	Acacia Shrubland, Scattered Eucalypts, Spinifex Hummock Grassland, Tussock Grassland	High	Low	0	None	Non-Discernible	Old (6+ yr.)
VWER-17	-23.3941, 119.6171	27/08/2020	Gorge/ Gully	Gorge	North/ West	Cliff	Loam	Scarce	Extensive Outcropping BIF	Boulders (>61cm)	Many Small Patches	Scattered Eucalypts, Spinifex Hummock Grassland, Tussock Grassland	Very High	Nil	0	Prone to Pooling	Non-Discernible	Old (6+ yr.)
VWER-26	-23.4037, 119.6605	25/08/2020	Gorge/ Gully	Gully	South/ East	Low	Clay Loam	Scarce	Moderate Outcropping BIF	Gravel (1-4cm)	Scarce	Scattered Eucalypts, Spinifex Hummock Grassland	Moderate	Low	2	None	Mining Exploration	Moderate (3 to 5 yr.)
VWER-27	-23.3989, 119.6078	26/08/2020	Gorge/ Gully	Gorge	North	Steep	Clay Loam	Non-Discernible	Extensive Outcropping BIF	Boulders (>61cm)	Scarce	Mulga Woodland, Scattered Eucalypts, Spinifex Hummock Grassland, Tussock Grassland	High	Nil	0	Prone to Pooling	Non-Discernible	Old (6+ yr.)

Site ID	Coord.	Date	Habitat Type	Landform	Aspect	Slope	Soil Type	Soil Avail.	Outcropping Rock Type	Rock Size	Veg. Litter	Dominant Veg. Type	Rocky Cracks/ Crevices	Burrowing Suitability	Hollow' s count	Water present	Disturbance	Last Fire
VWER-28	-23.4117, 119.6467	26/08/2020	Gorge/ Gully	Gully	South	Low	Clay Loam	Scarce	Minor Outcropping BIF	Gravel (1-4cm)	Few Small Patches	Acacia Shrubland, Scattered Eucalypts, Spinifex Hummock Grassland	Moderate	Low	2	None	Mining Exploration	Old (6+ yr.)
VWER-29	-23.3987, 119.6058	27/08/2020	Gorge/ Gully	Gorge	North	Steep	Clay Loam	Non-Discernible	Extensive Outcropping BIF	Large Rocks (21-60cm)	Many Small Patches	Mulga Woodland, Scattered Eucalypts, Tussock Grassland	High	Nil	1	None	Mining Exploration	Moderate (3 to 5 yr.)
VWER-30	-23.4007, 119.6402	28/08/2020	Stony Plain	Stony Plain	Flat	Flat	Clay Loam	Evenly Spread	Negligible	Gravel (1-4cm)	Few Small Patches	Cleared	Nil	Low	0	Permanent	Mining Exploration	Old (6+ yr.)
VWER-44	-23.4047, 119.6052	26/08/2020	Gorge/ Gully	Gully	South	Steep	Clay Loam	Few Small Patches	Extensive Outcropping BIF	Boulders (>61cm)	Few Small Patches	Eucalypt Woodland, Mulga Woodland, Spinifex Hummock Grassland	Very High	Nil	6	None	Mining Exploration	Old (6+ yr.)
VWER-47	-23.3923, 119.6316	11/10/2020	Mulga Woodland	Hardpan Plain	Flat	Flat	Clay Loam	Many Small Patches	Limited Outcropping BIF	Gravel (1-4cm)	Scarce	Scattered Eucalypts, Spinifex Hummock Grassland	Moderate	Low	1	None	Cattle Grazing	Recent (0 to 2 yr.)
VWER-51	-23.4206, 119.5819	24/08/2020	Stony Plain	Gully	South	Moderate	Clay Loam	Scarce	Moderate Outcropping BIF	Pebbles (5-10cm)	Few Small Patches	Scattered Eucalypts, Spinifex Hummock Grassland, Acacia Shrubland	Moderate	Nil	0	None	None Discernible	Moderate (3 to 5 yr.)
VWER-52	-23.3922, 119.5885	24/08/2020	Breakaway/ Cliff	Hillcrest/ Upper Hillslope	South	Moderate	Clay Loam	Scarce	Major Outcropping BIF	Gravel (1-4cm)	Few Small Patches	Acacia Shrubland, Spinifex Hummock Grassland, Scattered Eucalypts	Moderate	Nil	0	None	Mining Exploration	Moderate (3 to 5 yr.)
VWER-53	-23.3876, 119.617	25/08/2020	Breakaway/ Cliff	Hillcrest/ Upper Hillslope	West	Steep	Clay Loam	Scarce	Major Outcropping BIF	Gravel (1-4cm)	Scarce	Spinifex Hummock Grassland, Scattered Eucalypts	Moderate	Nil	0	None	None Discernible	Moderate (3 to 5 yr.)
VWER-54	-23.4017, 119.625	24/08/2020	Breakaway/ Cliff	Gorge	North	Very Steep	Clay Loam	Scarce	Extensive Outcropping BIF	Gravel (1-4cm)	Few Small Patches	Scattered Eucalypts, Spinifex Hummock Grassland	Very High	Nil	0	None	None Discernible	Moderate (3 to 5 yr.)
VWER-55	-23.4119, 119.5868	25/08/2020	Breakaway/ Cliff	Hillslope	South	Low	Clay Loam	None Discernible	Moderate Outcropping BIF	Gravel (1-4cm)	Few Small Patches	Spinifex Hummock Grassland, Scattered Eucalypts	High	Nil	0	None	None Discernible	Recent (0 to 2 yr.)

Site ID	Coord.	Date	Habitat Type	Landform	Aspect	Slope	Soil Type	Soil Avail.	Outcropping Rock Type	Rock Size	Veg. Litter	Dominant Veg. Type	Rocky Cracks/ Crevices	Burrowing Suitability	Hollow' s count	Water present	Disturbance	Last Fire
VWER-56	-23.4172, 119.6123	27/08/2020	Mulga Woodland	Drainage Area/ Floodplain	Flat	Flat	Clay Loam	Many Large Patches	Negligible	Gravel (1-4cm)	Few Small Patches	Mulga Woodland, Acacia Shrubland, Scattered Eucalypts, Spinifex Hummock Grassland	Nil	Moderate	0	Prone to Flooding	Cattle Grazing	Old (6+ yr.)
VWER-57	-23.3885, 119.6343	27/08/2020	Gorge/ Gully	Gully	East	Steep	Clay Loam	None Discernible	Extensive Outcropping BIF	Gravel (1-4cm)	Few Small Patches	Scattered Eucalypts, Spinifex Hummock Grassland, Acacia Shrubland	Very High	Nil	0	None	None Discernible	Moderate (3 to 5 yr.)
VWER-58	-23.3934, 119.6657	27/08/2020	Breakaway/ Cliff	Hillslope	Flat	Flat	Sandy Clay Loam	Few Small Patches	Major Outcropping BIF	Pebbles (5-10cm)	Few Small Patches	Scattered Eucalypts, Spinifex Hummock Grassland	Moderate	Nil	0	None	Mining Exploration	Moderate (3 to 5 yr.)
VWER-59	-23.4219, 119.5728	29/08/2020	Mulga Woodland	Hillslope	South	Moderate	Clay Loam	Few Small Patches	Moderate Outcropping BIF	Gravel (1-4cm)	Few Large Patches	Mulga Woodland, Scattered Eucalypts, Spinifex Hummock Grassland	Moderate	Nil	0	None	Mining Exploration	Moderate (3 to 5 yr.)
VWER-60	-23.3952, 119.5915	30/08/2020	Hillcrest/ Hillslope	Hillcrest/ Upper Hillslope	South	Moderate	Clay Loam	Scarce	Major Outcropping BIF	Gravel (1-4cm)	Few Small Patches	Mulga Woodland, Scattered Eucalypts, Spinifex Hummock Grassland	Moderate	Nil	0	None	Mining Exploration	Old (6+ yr.)
VWER-61	-23.3903, 119.638	10/10/2020	Hillcrest/ Hillslope	Hillslope	East	Moderate	Clay Loam	Few Small Patches	Major Outcropping BIF	Gravel (1-4cm)	Many Small Patches	Spinifex Hummock Grassland, Scattered Eucalypts	Moderate	Nil	0	None	None Discernible	Old (6+ yr.)
VWER-62	-23.3881, 119.6084	9/10/2020	Breakaway/ Cliff	Breakaway	East	Steep	Clay Loam	Scarce	Major Outcropping BIF	Large Rocks (21-60cm)	Many Small Patches	Scattered Eucalypts, Spinifex Hummock Grassland, Tussock Grassland	High	Nil	0	None	None Discernible	Old (6+ yr.)
VWER-63	-23.3936, 119.5978	9/10/2020	Hillcrest/ Hillslope	Breakaway	North	Low	Clay Loam	Scarce	Major Outcropping BIF	Gravel (1-4cm)	Scarce	Acacia Shrubland, Scattered Eucalypts, Spinifex Hummock Grassland	Moderate	Nil	0	None	None Discernible	Old (6+ yr.)
VWER-64	-23.3956, 119.6497	10/10/2020	Mulga Woodland	Drainage Area/ Floodplain	Flat	Flat	Clay Loam	Evenly Spread	Negligible	Gravel (1-4cm)	Many Large Patches	Mulga Woodland, Eucalypt Woodland, Tussock Grassland	Nil	Moderate	4	None	Mining Exploration	Old (6+ yr.)

Site ID	Coord.	Date	Habitat Type	Landform	Aspect	Slope	Soil Type	Soil Avail.	Outcropping Rock Type	Rock Size	Veg. Litter	Dominant Veg. Type	Rocky Cracks/ Crevices	Burrowing Suitability	Hollow' s count	Water present	Disturbance	Last Fire
VWER-65	-23.4018, 119.6487	10/10/2020	Mulga Woodland	Drainage Area/ Floodplain	Flat	Flat	Clay Loam	Evenly Spread	Negligible	Gravel (1-4cm)	Many Large Patches	Mulga Woodland, Scattered Eucalypts, Tussock Grassland	Nil	Moderate	6	None	Road/ Access Track	Moderate (3 to 5 yr.)
VWER-66	-23.4133, 119.6303	10/10/2020	Mulga Woodland	Minor Drainage Line	Flat	Low	Clay Loam	Evenly Spread	Negligible	Gravel (1-4cm)	Few Large Patches	Mulga Woodland, Scattered Eucalypts	Nil	Low	0	None	Mining Exploration	Old (6+ yr.)
VWER-67	-23.4191, 119.5547	10/10/2020	Gorge/ Gully	Gully	South	Steep	Clay Loam	Few Small Patches	Extensive Outcropping BIF	Boulders (>61cm)	Many Large Patches	Mulga Woodland, Eucalypt Woodland, Tussock Grassland	High	Nil	3	None	Mining Exploration	Old (6+ yr.)
VWER-68	-23.3979, 119.5797	10/10/2020	Hillcrest/ Hillslope	Gully	South	Steep	Clay Loam	Scarce	Extensive Outcropping BIF	Boulders (>61cm)	Evenly Spread	Scattered Eucalypts, Spinifex Hummock Grassland, Mulga Woodland	Very High	Nil	2	None	Mining Exploration	Old (6+ yr.)
VWER-69	-23.3578, 119.5794	11/10/2020	Medium Drainage Line	Medium Drainage Line	Flat	Flat	Clay Loam	Evenly Spread	Limited Outcropping BIF	Boulders (>61cm)	Many Small Patches	Scattered Eucalypts, Tussock Grassland	Nil	Moderate	0	Prone to Flooding	Cattle Grazing	Old (6+ yr.)
VWER-70	-23.3413, 119.5987	11/10/2020	Medium Drainage Line	Medium Drainage Line	Flat	Flat	Clay Loam	Evenly Spread	Minor Outcropping BIF	Boulders (>61cm)	Scarce	Tussock Grassland	Low	Low	4	Prone to Flooding	Weed Invasion	Recent (0 to 2 yr.)
VWER-71	-23.312, 119.6025	11/10/2020	Breakaway/ Cliff	Breakaway	North	Low	Clay Loam	Scarce	Moderate Outcropping BIF	Gravel (1-4cm)	None Discernible	Tussock Grassland	Low	Nil	0	None	Frequent Fire	Moderate (3 to 5 yr.)
VWER-72	-23.3049, 119.5653	11/10/2020	Medium Drainage Line	Medium Drainage Line	Flat	Flat	Clay Loam	Evenly Spread	Negligible	Pebbles (5-10cm)	Few Small Patches	Acacia Shrubland, Tussock Grassland	Nil	Low	2	Prone to Flooding	Road/ Access Track	Moderate (3 to 5 yr.)
VWER-73	-23.3029, 119.5893	11/10/2020	Breakaway/ Cliff	Breakaway	South	Steep	Clay Loam	Scarce	Extensive Outcropping BIF	Gravel (1-4cm)	Scarce	Acacia Shrubland, Spinifex Hummock Grassland	High	Low	2	None	Road/ Access Track	Recent (0 to 2 yr.)
VWER-74	-23.3004, 119.6191	11/10/2020	Breakaway/ Cliff	Breakaway	South	Steep	Clay Loam	Scarce	Extensive Outcropping BIF	Boulders (>61cm)	Scarce	Scattered Eucalypts, Spinifex Hummock Grassland	High	Low	7	None	Road/ Access Track	Recent (0 to 2 yr.)
VWER-75	-23.2949, 119.6154	11/10/2020	Gorge/ Gully	Gully	South	Moderate	Clay Loam	Scarce	Major Outcropping BIF	Large Rocks (21-60cm)	Scarce	Acacia Shrubland, Spinifex Hummock Grassland	Very High	Low	2	None	Road/ Access Track	Old (6+ yr.)

Site ID	Coord.	Date	Habitat Type	Landform	Aspect	Slope	Soil Type	Soil Avail.	Outcropping Rock Type	Rock Size	Veg. Litter	Dominant Veg. Type	Rocky Cracks/ Crevices	Burrowing Suitability	Hollow' s count	Water present	Disturbance	Last Fire
VWER-76	-23.2786, 119.6161	11/10/2020	Major Drainage Line	Major Drainage Line	Flat	Flat	Clay Loam	Many Large Patches	Negligible	Pebbles (5-10cm)	Scarce	Eucalypt Woodland, Tussock Grassland	Nil	Low	10	Prone to Flooding	Weed Invasion	Moderate (3 to 5 yr.)
VWER-77	-23.264, 119.5417	12/10/2020	Gorge/ Gully	Gorge	South	Moderate	Clay Loam	Few Small Patches	Extensive Outcropping BIF	Small Rocks (11-20cm)	Many Small Patches	Scattered Eucalypts, Spinifex Hummock Grassland	Very High	Nil	0	None	None Discernible	Old (6+ yr.)
VWER-78	-23.2597, 119.563	12/10/2020	Gorge/ Gully	Gully	North	Very Steep	Clay Loam	Many Small Patches	Extensive Outcropping BIF	Boulders (>61cm)	Few Small Patches	Scattered Eucalypts, Tussock Grassland	Very High	Nil	0	Prone to Pooling	None Discernible	Old (6+ yr.)
VWER-79	-23.2688, 119.5953	12/10/2020	Gorge/ Gully	Gorge	Flat	Flat	Clay Loam	Many Large Patches	Extensive Outcropping BIF	Boulders (>61cm)	Many Large Patches	Eucalypt Woodland, Acacia Shrubland, Tussock Grassland	Very High	Low	6	Prone to Pooling	Cattle Grazing	Old (6+ yr.)
VWER-81	-23.272, 119.613	12/10/2020	Gorge/ Gully	Gorge	South/ West	Steep	Sandy Loam	Few Large Patches	Extensive Outcropping BIF	Pebbles (5-10cm)	Many Small Patches	Scattered Eucalypts, Tussock Grassland	Very High	Nil	0	None	None Discernible	Moderate (3 to 5 yr.)
VWER-82	-23.2765, 119.6581	12/10/2020	Major Drainage Line	Major Drainage Line	Flat	Flat	Clay Loam	Few Large Patches	Limited Outcropping BIF	Pebbles (5-10cm)	Many Small Patches	Scattered Eucalypts, Acacia Shrubland, Tussock Grassland	Nil	Low	2	Prone to Pooling	Cattle Grazing	Old (6+ yr.)
VWER-83	-23.2835, 119.6584	12/10/2020	Gorge/ Gully	Gorge	Flat	Flat	Clay Loam Sandy	Few Large Patches	Extensive Outcropping BIF	Pebbles (5-10cm)	Many Small Patches	Eucalypt Woodland, Tussock Grassland	High	Low	2	Prone to Pooling	Cattle Grazing	Old (6+ yr.)
VWER-84	-23.3151, 119.8706	28/11/2020	Major Drainage Line	Major Drainage Line	Flat	Flat	Sandy Loam	Evenly Spread	Negligible	Negligible	Few Large Patches	Tussock Grassland, Scattered Eucalypts	Nil	Low	0	Prone to Pooling	Road/ Access Track	Old (6+ yr.)
VWER-86	-23.283, 119.5326	12/10/2020	Gorge/ Gully	Gorge	Flat	Flat	Sandy Loam	Many Large Patches	Extensive Outcropping BIF	Boulders (>61cm)	Few Small Patches	Eucalypt Woodland, Tussock Grassland	Very High	Low	10	Prone to Pooling	None Discernible	Moderate (3 to 5 yr.)
VWER-87	-23.3039, 119.64	11/10/2020	Medium Drainage Line	Medium Drainage Line	Flat	Flat	Clay Loam	Few Large Patches	Negligible	Pebbles (5-10cm)	Few Small Patches	Tussock Grassland, Scattered Eucalypts	Nil	Low	0	None	Road/ Access Track	Moderate (3 to 5 yr.)
VWER-90	-23.2647, 119.6087	12/10/2020	Major Drainage Line	Major Drainage Line	Flat	Flat	Sandy Loam	Evenly Spread	Negligible	Negligible	Evenly Spread	Scattered Eucalypts, Tussock Grassland	Nil	Low	0	None	None Discernible	Moderate (3 to 5 yr.)

Site ID	Coord.	Date	Habitat Type	Landform	Aspect	Slope	Soil Type	Soil Avail.	Outcropping Rock Type	Rock Size	Veg. Litter	Dominant Veg. Type	Rocky Cracks/ Crevices	Burrowing Suitability	Hollow' s count	Water present	Disturbance	Last Fire
VWER-91	-23.2821, 119.7024	13/10/2020	Gorge/ Gully	Gorge	Flat	Steep	Clay Loam	Many Small Patches	Extensive Outcropping BIF	Pebbles (5-10cm)	Many Small Patches	Scattered Eucalypts, Tussock Grassland, Acacia Shrubland	Moderate	Low	0	Prone to Pooling	Road/ Access Track	Old (6+ yr.)
VWER-92	-23.2838, 119.7392	13/10/2020	Gorge/ Gully	Gorge	Flat	Very Steep	Clay Loam	Many Small Patches	Extensive Outcropping BIF	Boulders (>61cm)	Few Small Patches	Acacia Shrubland, Tussock Grassland	Very High	Low	3	Prone to Pooling	None Discernible	Old (6+ yr.)
VWER-93	-23.4576, 119.485	13/10/2020	Major Drainage Line	Major Drainage Line	Flat	Flat	Sandy Loam	Evenly Spread	Negligible	Pebbles (5-10cm)	Many Small Patches	Acacia Shrubland, Eucalypt Woodland, Tussock Grassland	Nil	Low	0	None	None Discernible	Old (6+ yr.)
VWER-94	-23.4908, 119.5123	13/10/2020	Major Drainage Line	Major Drainage Line	Flat	Flat	Sandy Loam	Evenly Spread	Negligible	Pebbles (5-10cm)	Many Small Patches	Acacia Shrubland, Eucalypt Woodland, Tussock Grassland	Nil	Low	0	Prone to Pooling	Cattle Grazing	Old (6+ yr.)
VWER-95	-23.517, 119.585	13/10/2020	Major Drainage Line	Major Drainage Line	Flat	Flat	Sandy Loam	Evenly Spread	Negligible	Pebbles (5-10cm)	Many Small Patches	Acacia Shrubland, Eucalypt Woodland, Tussock Grassland	Nil	Low	0	Prone to Flooding	Cattle Grazing	Old (6+ yr.)
VWER-96	-23.4716, 119.5562	13/10/2020	Medium Drainage Line	Medium Drainage Line	Flat	Flat	Sandy Loam	Evenly Spread	Negligible	Pebbles (5-10cm)	Many Small Patches	Acacia Shrubland, Eucalypt Woodland, Tussock Grassland	Nil	Low	0	None	Cattle Grazing	Old (6+ yr.)
VWER-97	-23.4822, 119.6181	13/10/2020	Medium Drainage Line	Medium Drainage Line	Flat	Flat	Sandy Loam	Evenly Spread	Negligible	Pebbles (5-10cm)	Many Small Patches	Acacia Shrubland, Eucalypt Woodland, Tussock Grassland	Nil	Low	0	Prone to Flooding	Cattle Grazing	Old (6+ yr.)
VWER-98	-23.5269, 119.6643	13/10/2020	Major Drainage Line	Major Drainage Line	Flat	Flat	Sandy Loam	Evenly Spread	Limited Outcropping Conglomerate	Pebbles (5-10cm)	Many Small Patches	Acacia Shrubland, Eucalypt Woodland, Tussock Grassland	Nil	Low	0	Prone to Flooding	Cattle Grazing	Old (6+ yr.)
VWER-99	-23.4852, 119.7154	13/10/2020	Medium Drainage Line	Medium Drainage Line	Flat	Flat	Sandy Clay Loam	Evenly Spread	Limited Outcropping Conglomerate	Pebbles (5-10cm)	Many Small Patches	Acacia Shrubland, Eucalypt Woodland, Tussock Grassland, Spinifex Hummock Grassland	Nil	Low	0	Prone to Flooding	Cattle Grazing	Old (6+ yr.)
VWER-100	-23.4465, 119.749	13/10/2020	Medium Drainage Line	Medium Drainage Line	Flat	Flat	Sandy Clay Loam	Evenly Spread	Negligible	Gravel (1-4cm)	Many Small Patches	Acacia Shrubland, Tussock Grassland	Nil	Low	0	Prone to Flooding	Cattle Grazing	Moderate (3 to 5 yr.)
VWER-101	-23.3826, 119.585	27/11/2020	Drainage Area/ Floodplain	Medium Drainage Line	Flat	Flat	Clay Loam	Evenly Spread	Negligible	Gravel (1-4cm)	None Discernible	Scattered Eucalypts, Acacia Shrubland	Nil	Nil	0	None	None Discernible	Recent (0 to 2 yr.)

Site ID	Coord.	Date	Habitat Type	Landform	Aspect	Slope	Soil Type	Soil Avail.	Outcropping Rock Type	Rock Size	Veg. Litter	Dominant Veg. Type	Rocky Cracks/ Crevices	Burrowing Suitability	Hollow' s count	Water present	Disturbance	Last Fire
VWER-102	-23.3744, 119.6092	27/11/2020	Drainage Area/ Floodplain	Medium Drainage Line	Flat	Flat	Clay Loam	Evenly Spread	Limited Outcropping Basalt	Gravel (1-4cm)	None Discernible	Scattered Eucalypts, Acacia Shrubland	Nil	Nil	0	None	None Discernible	Moderate (3 to 5 yr.)
VWER-103	-23.3768, 119.5945	27/11/2020	Drainage Area/ Floodplain	Medium Drainage Line	Flat	Flat	Clay Loam	Evenly Spread	Limited Outcropping Basalt	Gravel (1-4cm)	None Discernible	Scattered Eucalypts, Mulga Woodland	Nil	Nil	0	None	None Discernible	Moderate (3 to 5 yr.)
VWER-104	-23.4155, 119.4849	13/10/2020	Minor Drainage Line	Major Drainage Line	Flat	Flat	Sandy Loam	Evenly Spread	Negligible	Negligible	Many Large Patches	Acacia Shrubland, Tussock Grassland, Scattered Eucalypts	Nil	Low	0	None	None Discernible	Old (6+ yr.)

Appendix B – New Water Features Recorded During the Assessment

Water feature ID	Latitude	Longitude	Date Assessed	Length (m)	Width (m)	Water present above surface	Depth (m)	Water present in intermediate zone	Emergent macrophyte present	Aquatic vegetation	Fauna present	Notes	Photo
WWER-17	-23.283	119.5327	12/10/2020	5	1.2	Yes	30	Yes	No	No	No		
WWER-18	-23.2837	119.5326	12/10/2020	5	5	Yes	0.5	Yes	No	No	No		
WWER-19	-23.2597	119.5629	12/10/2020	2	1.4	Yes	1	No	No	No	No		
WWER-20	-23.2687	119.5953	12/10/2020	5.5	2	Yes	0.5	Yes	Yes	*Typha* (reeds) Aquatic plants (in and on the water)	No	eDNA sampling conducted 12/10/2020	

Water feature ID	Latitude	Longitude	Date Assessed	Length (m)	Width (m)	Water present above surface	Depth (m)	Water present in intermediate zone	Emergent macrophyte present	Aquatic vegetation	Fauna present	Notes	Photo
WWER-21	-23.2816	119.7023	13/10/2020	10	2	Yes	0.25	Yes	No	No	No		
WWER-22	-23.2838	119.7392	13/10/2020	10	10	Yes	2	No	No	No	No	Fish in water	
WWER-23	-23.2835	119.6584	12/10/2020	25	2	Yes	0.4	Yes	No	No	No		
WWER-24	-23.3151	119.8706	12/10/2020	80	8	Yes	0.6	Yes	No	No	No		

Water feature ID	Latitude	Longitude	Date Assessed	Length (m)	Width (m)	Water present above surface	Depth (m)	Water present in intermediate zone	Emergent macrophyte present	Aquatic vegetation	Fauna present	Notes	Photo
WWER-25	-23.5269	119.6643	13/10/2020	3	1	Yes	0.15	No	No	No	No	Post rain pooling	
WWER-26	-23.2765	119.6581	12/10/2020	50	5	Yes	0.5	Yes	No	No	No		
WWER-27	-23.4466	119.749	13/10/2020	40	0.3	Yes	0.5	Yes	No	No	No		
WWER-28	-23.4908	119.5123	13/10/2020	10	2.5	Yes	0.5	Yes	No	No	No		

Appendix C –Caves Assessed During the Current Assessment

Cave ID	Coordinates	Date Assessed	Roost Type	Cave Position	Floor Slope	Aspect	Cave Exposure	Entrance Type	Entrance Shape	Entrance Width (m)	Entrance Height (m)	Cave Depth (m)	No Cham	Cham Height (m)	Water Present	Number of Ghost Bat Scats	Scat Count or Scat Estimate	Scat Age	Bats in cave	Photo
CCAT-03	-23.2720868, 119.6131207	12/10/2020	Day Roost	Lower Slope	Incline	West	Semi Exposed	Cavity	Vertical	0.8	3	2	1	5	None	0	Count	-	*Taphozous georgianus*	
CCAT-06	-23.2821198, 119.7024255	13/10/2020	Potential Day Roost	Mid Slope	Flat	East	Sheltered	Cavity	Horizontal	1	0.5	6	1	3	None	0	Count	-	Nil	
CCAT-08	-23.296965, 119.7141052	13/10/2020	Night Roost	Lower Slope	Flat	East	Semi Exposed	Cavern	Round/Oval	3	2	15	2	2	None	0	Count	-	*Vespadelus finlaysoni*	

Cave ID	Coordinates	Date Assessed	Roost Type	Cave Position	Floor Slope	Aspect	Cave Exposure	Entrance Type	Entrance Shape	Entrance Width (m)	Entrance Height (m)	Cave Depth (m)	No Cham	Cham Height (m)	Water Present	Number of Ghost Bat Scats	Scat Count or Scat Estimate	Scat Age	Bats in cave	Photo
CCAT-16	-23.2949414, 119.6155172	11/10/2020	Night Roost	Mid Slope	Incline	West	Semi Exposed	Cavity	Round/Oval	1.5	1.2	15	1	1.5	None	0	Count	-	Nil	
CCAT-21	-23.2723650, 119.6133530	12/10/2020	Potential Day Roost	Mid Slope	Incline	West	Semi Exposed	Cavity	Vertical	1.5	3	20	2	3	None	0	Count	-	*Taphozous georgianus*	

Appendix D – eDNA Frontiers – Results

eDNA frontiers
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REPORT OF eDNA ANALYSIS

Scope of Work:	EF-093		
Project Title:	eDNA biodiversity audit targeting reptile presence/absence in pools of water in the Pilbara using eDNA metabarcoding		
Client:	Biologic Environmental Survey Pty Ltd (ABN: 55 133 116 131) 24-26 Wickham St, East Perth 6004 Postal: PO Box 179, Floreat 6014		
Contact Details:	Ryan Ellis Senior Zoologist E: ryan@biologicenv.com.au | P: +61 8 6365 5066		
Test Facility	eDNA frontiers Curtin University (ABN: 99 143 842 569) 303.194 Kent Street Bentley WA 6102 Phone: +61 8 9266 4119 Email: ednafrontiers@curtin.edu.au		
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Report Reference:	EF-093_Biologic_Final Report		
Laboratory Start Date:	05/01/2021	**Laboratory End Date:**	08/02/2021
Report Issue Date:	16/03/2021		

APPROVALS

	Name	Signature	Date (DD/MM/YYYY)
Author	Dr Kathryn Dawkins		16/03/2021
Author	Dr Tina Berry		17/03/2021
Reviewer	Melissa Borges Rodriguez		16/03/2021

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1.0 OBJECTIVE

The objective of this study was to assess the presence of *Liasis olivaceus barroni* (Pilbara Olive python) from water samples collected in the Pilbara using eDNA metabarcoding.

1.1 Study Scope

Using environmental DNA (eDNA) testing, eDNA frontiers was tasked with analysing water samples for the presence of *L. olivaceus barroni* at seven different sites across the Pilbara region. The client provided a total of 63 samples (62 samples and 1 control) consisting of water filtrate suspended on filter membranes.

2.0 SAMPLE DETAILS

Table 1. Sample receipt details

Date received:	04/12/2020
Transport:	Frozen
Number of samples:	63
Storage:	All samples were stored at -20ºC prior to analysis.

Table 2. Supplied sample details

eDNA frontiers ID	Client Sample ID	Sample Type	Collection Date
E-093-001	WWER-12 Sample A	Water - filtered to 0.45μm	28/08/2020
E-093-002	WWER-12 Sample B	Water - filtered to 0.45μm	28/08/2020
E-093-003	WWER-12 Sample C	Water - filtered to 0.45μm	28/08/2020
E-093-004	WWER-12 Sample D	Water - filtered to 0.45μm	28/08/2020
E-093-005	WWER-12 Sample E	Water - filtered to 0.45μm	28/08/2020
E-093-006	Control Rinse	Water - filtered to 0.45μm	26/08/2020
E-093-007	WWER-11 Sample A	Water - filtered to 0.45μm	27/08/2020
E-093-008	WWER-11 Sample B	Water - filtered to 0.45μm	27/08/2020
E-093-009	WWER-11 Sample C	Water - filtered to 0.45μm	27/08/2020
E-093-010	WWER-11 Sample E	Water - filtered to 0.45μm	27/08/2020
E-093-011	WWER-01 Sample A	Water - filtered to 0.45μm	26/08/2020
E-093-012	WWER-01 Sample B	Water - filtered to 0.45μm	26/08/2020
E-093-013	WWER-01 Sample C	Water - filtered to 0.45μm	26/08/2020
E-093-014	WWER-01 Sample D	Water - filtered to 0.45μm	26/08/2020
E-093-015	WWER-01 Sample E	Water - filtered to 0.45μm	26/08/2020
E-093-016	WWER-04 Sample A	Water - filtered to 0.45μm	26/08/2020
E-093-017	WWER-04 Sample B	Water - filtered to 0.45μm	26/08/2020
E-093-018	WWER-04 Sample C	Water - filtered to 0.45μm	26/08/2020
E-093-019	WWER-04 Sample D	Water - filtered to 0.45μm	26/08/2020
E-093-020	WWER-04 Sample E	Water - filtered to 0.45μm	26/08/2020
E-093-021	WWER-02 Sample A	Water - filtered to 0.45μm	13/10/2020
E-093-022	WWER-02 Sample B	Water - filtered to 0.45μm	13/10/2020
E-093-023	WWER-02 Sample C	Water - filtered to 0.45μm	13/10/2020

eDNA frontiers ID	Client Sample ID	Sample Type	Collection Date
E-093-024	WWER-01 Sample A	Water - filtered to 0.45μm	13/10/2020
E-093-025	WWER-01 Sample B	Water - filtered to 0.45μm	13/10/2020
E-093-026	WWER-01 Sample C	Water - filtered to 0.45μm	13/10/2020
E-093-027	WWER-01 Sample D	Water - filtered to 0.45μm	13/10/2020
E-093-028	WWER-01 Sample E	Water - filtered to 0.45μm	13/10/2020
E-093-029	WWER-04 Sample A	Water - filtered to 0.45μm	13/10/2020
E-093-030	WWER-04 Sample B	Water - filtered to 0.45μm	13/10/2020
E-093-031	WWER-04 Sample C	Water - filtered to 0.45μm	13/10/2020
E-093-032	WWER-04 Sample D	Water - filtered to 0.45μm	13/10/2020
E-093-033	WWER-04 Sample E	Water - filtered to 0.45μm	13/10/2020
E-093-034	WWER-20 Sample A	Water - filtered to 0.45μm	13/10/2020
E-093-035	WWER-20 Sample B	Water - filtered to 0.45μm	13/10/2020
E-093-036	WWER-20 Sample C	Water - filtered to 0.45μm	13/10/2020
E-093-037	WWER-20 Sample D	Water - filtered to 0.45μm	13/10/2020
E-093-038	WWER-20 Sample E	Water - filtered to 0.45μm	13/10/2020
E-093-039	WWER-02 Sample A	Water - filtered to 0.45μm	29/11/2020
E-093-040	WWER-02 Sample B	Water - filtered to 0.45μm	29/11/2020
E-093-041	WWER-02 Sample C	Water - filtered to 0.45μm	29/11/2020
E-093-042	WWER-02 Sample D	Water - filtered to 0.45μm	29/11/2020
E-093-043	WWER-02 Sample E	Water - filtered to 0.45μm	29/11/2020
E-093-044	WWER-20 Sample A	Water - filtered to 0.45μm	28/11/2020
E-093-045	WWER-20 Sample B	Water - filtered to 0.45μm	28/11/2020
E-093-046	WWER-20 Sample C	Water - filtered to 0.45μm	28/11/2020
E-093-047	WWER-20 Sample D	Water - filtered to 0.45μm	28/11/2020
E-093-048	WWER-20 Sample E	Water - filtered to 0.45μm	28/11/2020
E-093-049	WWER-22 Sample A	Water - filtered to 0.45μm	28/11/2020
E-093-050	WWER-22 Sample B	Water - filtered to 0.45μm	28/11/2020
E-093-051	WWER-22 Sample C	Water - filtered to 0.45μm	28/11/2020
E-093-052	WWER-22 Sample D	Water - filtered to 0.45μm	28/11/2020
E-093-053	WWER-22 Sample E	Water - filtered to 0.45μm	28/11/2020
E-093-054	WWER-04 Sample A	Water - filtered to 0.45μm	29/11/2020
E-093-055	WWER-04 Sample B	Water - filtered to 0.45μm	29/11/2020
E-093-056	WWER-04 Sample C	Water - filtered to 0.45μm	29/11/2020
E-093-057	WWER-04 Sample D	Water - filtered to 0.45μm	29/11/2020
E-093-058	WWER-04 Sample E	Water - filtered to 0.45μm	29/11/2020
E-093-059	WWER-01 Sample A	Water - filtered to 0.45μm	29/11/2020
E-093-060	WWER-01 Sample B	Water - filtered to 0.45μm	29/11/2020
E-093-061	WWER-01 Sample C	Water - filtered to 0.45μm	29/11/2020
E-093-062	WWER-01 Sample D	Water - filtered to 0.45μm	29/11/2020
E-093-063	WWER-01 Sample E	Water - filtered to 0.45μm	29/11/2020

3.0 METHODS

3.1 Sampling Locations

Water samples were collected at seven locations by Biologic staff between 26th August and 29th November 2020, with some sites sampled on multiple occasions. Five replicates were collected at each sampling point, except for the first trip to site WWER-02 (n=3) and the single trip to WWER-11 (n=4).

3.2 Sample Collection

Water samples (1L) were collected and filtered using $0.45\mu m$ mixed cellulose ester (MCE) with a peristaltic Sentino pump to capture eDNA present in the water. All filtering was carried out by Biologic Environmental staff. One control sample of water to clean filtration equipment was included from the first sampling trip (August 26). Filter membranes were transported frozen to eDNA frontiers laboratories where they were stored at -20°C until scheduled for DNA extraction.

3.3 Laboratory Methods

3.3.1 eDNA Extraction and Analysis

DNA was extracted from half of each filter paper using a Qiagen DNeasy blood and tissue kit, following the eDNA frontiers lab's SOPs and detailed in Koziol *et al.,* (2018), Stat *et al.,* (2017), Stat *et al.,* (2018). Each sample was assigned an individual combination of index tags and amplified by PCR using an in-house 16S assay that detects reptiles. A library was generated and sequenced using the Illumina MiSeq. A single field control as well as laboratory extraction and PCR controls were included to test for contamination.

3.3.2 Bioinformatics and Taxonomic assignments

Bioinformatic tools were used to analyse raw sequence data (Mousavi-Derazmahalleh et al, 2021). Results were demultiplexed and trimmed using Obitools and quality filtered with Usearch v11 for sequencing errors (maxee=1) and minimum length. Sequences were then dereplicated and unique sequences were transformed into zero radius operational taxonomic units (ZOTUs) to provide sensitive taxonomic resolution (Usearch v11) (Edgar, 2018). ZOTUs, in contrast to OTUs, are a more exact sequence variant, clustering at 99% to improve taxonomic resolution. Generated ZOTUs were queried against the nucleotide database NCBI (GenBank) and assigned to the species level where possible. Taxonomic assignments were based on an in-house Python script which does further filtering of Blast results (evalue <= 1e-5, %identity >= 95 and qCov >= 100), combines it with ZOTU table results and produces a table containing the taxonomic information available from Blast taxonomy database (accessed February 2021). Additionally, Geneious Prime (version 2021.0.3) was used to align the ZOTU identified as potential *L. olivaceus barroni* against the refence sequence generated by eDNA frontiers in a previous study for the client.

It is important to note that the sequences recovered are converted to the lowest possible taxon based on similarities and differences to a DNA database (NCBI's GenBank). This database, and the taxonomic framework that underpins it, may contain errors. Accordingly, the DNA taxon identifications should be interpreted as the best available assignment based on currently available information and that errors are possible.

4.0 RESULTS

Liasis olivaceus barroni was detected at five of the seven sites in the Pilbara (Tables 3-6); however, where a site was repeatedly sampled, it was not necessarily detected at all sampling time-points or in all replicates. The *L. olivaceus barroni* detected in the samples matched with 100% similarity to the reference sequence generated in a previous study.

In addition to *L. olivaceus barroni*, three other reptile species were detected plus an unidentified member of the Pythonidae family. A range of other taxa were also detected, with the presence of various mammals, birds, frogs, fish, and a freshwater ostracod revealed. Taxa that had ≥95% similarity in the sequence region have been reported (Tables 3-6). The single water control sample taken did not contain any detectible sequence data; however, it should be noted that this control is only applicable to samples collected on 26 August 2020. Laboratory extraction controls were all negative.

Table 3. Taxa detected at Pilbara sites WWER-01 and WWER-02 in 2020. Presence of the species at each site is indicated by the * symbol.

				WWER-01																WWER-02							
				26 August					13 October					29 November						13 October			29 November				
Class	**Order**	**Family**	***Genus/Species***	**A**	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**A**	**B**	**C**	**D**	**E**
Lepidosauria	Squamata	Agamidae	*Ctenophorus* sp.																							
		Pythonidae																								
			Liasis olivaceus barroni												*							*				
		Scincidae	*Lerista* sp.																							
		Varanidae	*Varanus panoptes*																			*				
Mammalia	Artiodactyla	Bovidae	*Bos taurus*																			*				
		Suidae	*Sus scrofa*																							*
	Chiroptera	Vespertilionidae	*Chalinolobus gouldii*																							
	Diprotodontia	Macropodidae	*Macropus robustus*							*	*	*	*										*	*	*	*
			Macropus sp.																							
Amphibia	Anura	Hylidae	*Litoria* sp.	*	*				*	*	*	*	*	*	*	*		*	*	*	*					
		Myobatrachidae	*Uperoleia* sp.																							
Actinopteri	Centrarchiformes	Terapontidae	*Leiopotherapon unicolor*																							
Aves	Accipitriformes	Accipitridae	*Accipiter* sp.		*						*															
	Columbiformes	Columbidae	*Geopelia cuneata*			*	*																*	*	*	*
			Geopelia sp.																							
		Corvidae	*Artamus cinereus*																							
			Corvus coronoides																							
		Estrildidae	*Taeniopygia guttata*																							
		Meliphagidae	*Ptilotula* sp.															*						*		*
	Psittaciformes	Cacatuidae	*Eolophus roseicapillus*																							
Ostracoda	Podocopida	Cypridae	*Cypridopsis vidua*																							

Table 4. Taxa detected at Pilbara site WWER-04 in 2020. Presence of the species at each site is indicated by the * symbol.

				26 August					13 October					29 November				
Class	**Order**	**Family**	***Genus/Species***	**A**	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**
Lepidosauria	Squamata	Agamidae	*Ctenophorus* sp.															
		Pythonidae													*			
			Liasis olivaceus barroni											*				
		Scincidae	*Lerista* sp.															
		Varanidae	*Varanus panoptes*															
Mammalia	Artiodactyla	Bovidae	*Bos taurus*															
		Suidae	*Sus scrofa*					*										
	Chiroptera	Vespertilionidae	*Chalinolobus gouldii*															
	Diprotodontia	Macropodidae	*Macropus robustus*											*	*	*	*	*
			Macropus sp.											*				
Amphibia	Anura	Hylidae	*Litoria* sp.			*		*										
		Myobatrachidae	*Uperoleia* sp.															
Actinopteri	Centrarchiformes	Terapontidae	*Leiopotherapon unicolor*															
Aves	Accipitriformes	Accipitridae	*Accipiter* sp.															
	Columbiformes	Columbidae	*Geopelia cuneata*		*	*		*	*	*		*		*	*		*	
			Geopelia sp.															
		Corvidae	*Artamus cinereus*	*	*	*	*					*						
			Corvus coronoides														*	
		Estrildidae	*Taeniopygia guttata*															
		Meliphagidae	*Ptilotula* sp.					*										*
	Psittaciformes	Cacatuidae	*Eolophus roseicapillus*		*		*											
Ostracoda	Podocopida	Cyprididae	*Cypridopsis vidua*															

Table 5. Taxa detected at Pilbara sites WWER-11 and WWER-12 in 2020. Presence of the species at each site is indicated by the * symbol.

				WWER-11				WWER-12				
				27 August				28 November				
Class	**Order**	**Family**	***Genus/Species***	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**E**
Lepidosauria	Squamata	Agamidae	*Ctenophorus* sp.	*								
		Pythonidae										
			Liasis olivaceus barroni									
		Scincidae	*Lerista* sp.									
		Varanidae	*Varanus panoptes*									
Mammalia	Artiodactyla	Bovidae	*Bos taurus*									
		Suidae	*Sus scrofa*									
	Chiroptera	Vespertilionidae	*Chalinolobus gouldii*							*		
	Diprotodontia	Macropodidae	*Macropus robustus*	*	*			*				
			Macropus sp.									
Amphibia	Anura	Hylidae	*Litoria* sp.		*	*	*					
		Myobatrachidae	*Uperoleia* sp.									
Actinopteri	Centrarchiformes	Terapontidae	*Leiopotherapon unicolor*									
Aves	Accipitriformes	Accipitridae	*Accipiter* sp.	*								
	Columbiformes	Columbidae	*Geopelia cuneata*								*	
			Geopelia sp.									
		Corvidae	*Artamus cinereus*						*	*		
			Corvus coronoides	*								
		Estrildidae	*Taeniopygia guttata*									
		Meliphagidae	*Ptilotula* sp.									
	Psittaciformes	Cacatuidae	*Eolophus roseicapillus*							*		
Ostracoda	Podocopida	Cypridídae	*Cypridopsis vidua*									

Table 6. Taxa detected at Pilbara sites WWER-20 and WWER-22 in 2020. Presence of the species at each site is indicated by the * symbol.

				WWER-20										WWER-22				
				13 October					28 November					28 November				
Class	**Order**	**Family**	***Genus/Species***	**A**	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**
Lepidosauria	Squamata	Agamidae	*Ctenophorus* sp.															
		Pythonidae																
			Liasis olivaceus barroni	*					*	*	*							*
		Scincidae	*Lerista* sp.										*		*	*		
		Varanidae	*Varanus panoptes*															
Mammalia	Artiodactyla	Bovidae	*Bos taurus*															
		Suidae	*Sus scrofa*															
	Chiroptera	Vespertilionidae	*Chalinolobus gouldii*															
	Diprotodontia	Macropodidae	*Macropus robustus*						*			*		*	*			*
			Macropus sp.															
Amphibia	Anura	Hylidae	*Litoria* sp.		*				*	*	*	*						
		Myobatrachidae	*Uperoleia* sp.	*	*													
Actinopteri	Centrarchiformes	Terapontidae	*Leiopotherapon unicolor*											*	*	*	*	*
Aves	Accipitriformes	Accipitridae	*Accipiter* sp.															
	Columbiformes	Columbidae	*Geopelia cuneata*	*	*	*	*	*	*					*				*
			Geopelia sp.		*				*									
		Corvidae	*Artamus cinereus*															
			Corvus coronoides									*	*					
		Estrildidae	*Taeniopygia guttata*							*								
		Meliphagidae	*Ptilotula* sp.		*	*	*	*	*			*						
	Psittaciformes	Cacatuidae	*Eolophus roseicapillus*															
Ostracoda	Podocopida	Cyprididae	*Cypridopsis vidua*	*	*				*		*	*	*					

5.0 SUMMARY

This report documents the successful detection of *Liasis olivaceus barroni* from environmental water samples collected from the Pilbara. The species was detected at five sites and matched with 100% similarity to the reference sequence generated in a previous study. In addition to the target taxon, several other taxonomic groups were identified. This type of survey is useful for detecting change in biological communities if long term monitoring using eDNA is employed (Berry et al, 2019).

The DNA extracts derived from this study will be stored with eDNA frontiers' premises for a period of 12 months. If samples are required to be stored longer, a sample archiving service can be provided.

ARCHIVING OF STUDY DATA

All electronic data relating to the study is stored in an offsite secure server. This includes; all laboratory raw data; personnel records; and the study report. Hard copy documents are archived by study number into a locked area of the test facility located in eDNA frontiers, Curtin University administration area.

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APPENDIX 1

Glossary

Term	Definition
% value in data	Represents the percentage similarity of a DNA sequence recovered from a sample compared to reference sequences in a database (e.g. compared to DNA databases such as GenBank or references generated in-house)
(x) value in data	Represents the frequency the % value was recorded in the dataset
16S rRNA	The 16S rRNA refers to a conserved gene region of mitochondrial DNA, which codes for a subunit of the ribosome. 16S rRNA is found in all eukaryotes making it a good candidate for DNA barcoding
18S rRNA	The 18S rRNA refers to a conserved gene region of nuclear DNA, which codes for a subunit of the ribosome. 18S rRNA is found in all eukaryotes making it a good candidate for DNA barcoding
18S AIS reference database	Reference 18S rRNA sequences of invasive marine species that are available in DNA databases
AIS	Alien Invasive Species
Assay	In the context of eDNA metabarcoding an assay refers to a PCR 'test' that selectively targets a subset of biota from an environmental DNA sample. The use of multiple assay when combined will always detect a wider diversity of taxa than a single assay. eDNA assays should be selected to address the question relevant to the study.
Barcode	Refers to a region of DNA sequenced for many species that is able to (through variation in the DNA sequence) is able to differentiate species. DNA barcodes are the most common targets of eDNA studies that seek to explore taxon assemblages.
COI	The gene region that is being used as the standard barcode for almost all animal groups is a 648 base-pair region of the mitochondrial cytochrome c oxidase 1 gene ("CO1"). COI is proving highly effective in identifying birds, butterflies, fish, flies and many other animal groups. COI is not an effective barcode region in plants because it evolves too slowly, but two gene regions in the chloroplast, matK and rbcL, have been approved as the barcode regions for plants
CO1 AIS reference database	Reference COI sequences of invasive marine species that are available in DNA databases
DNA	Deoxyribonucleic Acid (DNA) is the hereditary material that contains the genetic information of an organism
DNA metabarcoding	Is a genetic technique that simultaneously amplifies and sequences barcode regions (e.g. COI, 18S, 16S) of many different species in parallel

Term	Definition
eDNA	Environmental DNA (eDNA) refers to genetic material that is recovered from an environmental substrate (e.g. water, sediment, air)
Eukaryotes	An organism where cells contain a nucleus surrounded by a membrane and has the DNA bound together by proteins (histones) into chromosomes. The cells of eukaryotes also contain an endoplasmic reticulum and numerous specialised organelles not present in prokaryotes, especially mitochondria, golgi bodies, and lysosomes
Fisheries	Department of Primary Industries and Regional Development, Fisheries Division, Aquatic Biosecurity Section
GenBank	Publicly available repository of genetic information. Contains the barcode information of genes that have previously been sequenced
Genome	A genome is all the genetic material of an organism. It consists of DNA (or RNA in RNA viruses). The genome includes both the genes (the coding regions) and the noncoding DNA. In eukaryotes it refers to the genomes of the nucleus, mitochondria and chloroplasts. In prokaryotes, there is a single genome (as they do not contain mitochondria or chloroplasts)
Illumina MiSeq	Next generation sequencing platform developed by the company Illumina
IMP	Introduced marine pests
Low abundance	Low abundance reads have been defined as those that constitute <0.1% of total reads for a particular sample
Metabarcoding assay	A PCR reaction using a specific set of primers that simultaneously amplifies the same gene target from multiple species. Also see definition of 'assay'.
Mitochondrial DNA (mtDNA)	The mitochondrion (plural mitochondria) is a double membrane-bound organelle found in all eukaryotic organisms. mtDNA markers (e.g. 16S or COI) are common DNA barcodes.
Mitogenomes	Refers to the mitochondrial genome
NGS	Next generation sequencing or second generation sequencing refers to massively parallel sequencing technology, as opposed to first generation sequencing or sanger sequencing where only a single template is sequenced at one time
Nucleotide	A compound consisting of a nucleotide linked to a phosphate group. Nucleotides form the basic structural unit of nucleic acids such as DNA
PCR	Polymerase chain reaction (PCR) is the technique that is used to amplify (akin to photocopying DNA) specific regions of the genome from specific groups of taxa
Primer	A short DNA strand (≈20bp in size) used in PCR to target particular groups of organisms and genes. Two of them are required for PCR (a forward and a reverse)

Term	Definition
Primer binding site	A primer-binding site is the target region of a genome where the primer attaches to start replication. The primer binding site is on one of the two complementary strands of a double-stranded nucleotide polymer, in the strand which is to be copied, or is within a single-stranded nucleotide polymer sequence
Prokaryote	Any of the typically unicellular microorganisms that lack a distinct nucleus and membrane-bound organelles and that are classified as a kingdom (Prokaryotae syn. Monera) or into two domains (Bacteria and Archaea)
RNA	Ribonucleic acid (RNA) is a polymeric molecule implicated in various biological roles in coding, decoding, regulation, and expression of genes
rRNA	ribosomal ribonucleic acid is the RNA component of the ribosome, and is essential for protein synthesis in all living organisms
Sequence	DNA sequencing is the process of determining the precise order of nucleotides within a DNA molecule. It includes any method or technology that is used to determine the order of the four bases—adenine, guanine, cytosine, and thymine—in a strand of DNA
Shotgun sequencing	Refers to randomly sequencing short pieces of DNA (≈150bp in size) after shearing or cutting DNA (e.g. fragmenting a genome)
OTU	Operational Taxonomic Unit is a molecular biology term that describes unique DNA barcode clusters and how they are different from one another. It is usually defined by a % cut-off based on DNA sequence similarity. The value of OTUs is that biodiversity can be compared without the need to assign each sequence into a taxonomic framework and is most appropriate when there are large deficiencies in the underpinning taxonomic framework. OTU are very similar in function to ZOTUs (see below).
ZOTU	Zero-radius Operational Taxonomic Unit is a molecular biology terms that describes unique DNA barcode clusters and how they are different from one another. It is usually defined by a % cut-off based on DNA sequence similarity. The value of ZOTUs is that biodiversity can be compared without the need to assign each sequence into a taxonomic framework and is most appropriate when there are large deficiencies in the underpinning taxonomic framework. ZOTU are very similar in function to OTUs (see above) but describe more exact sequence variants