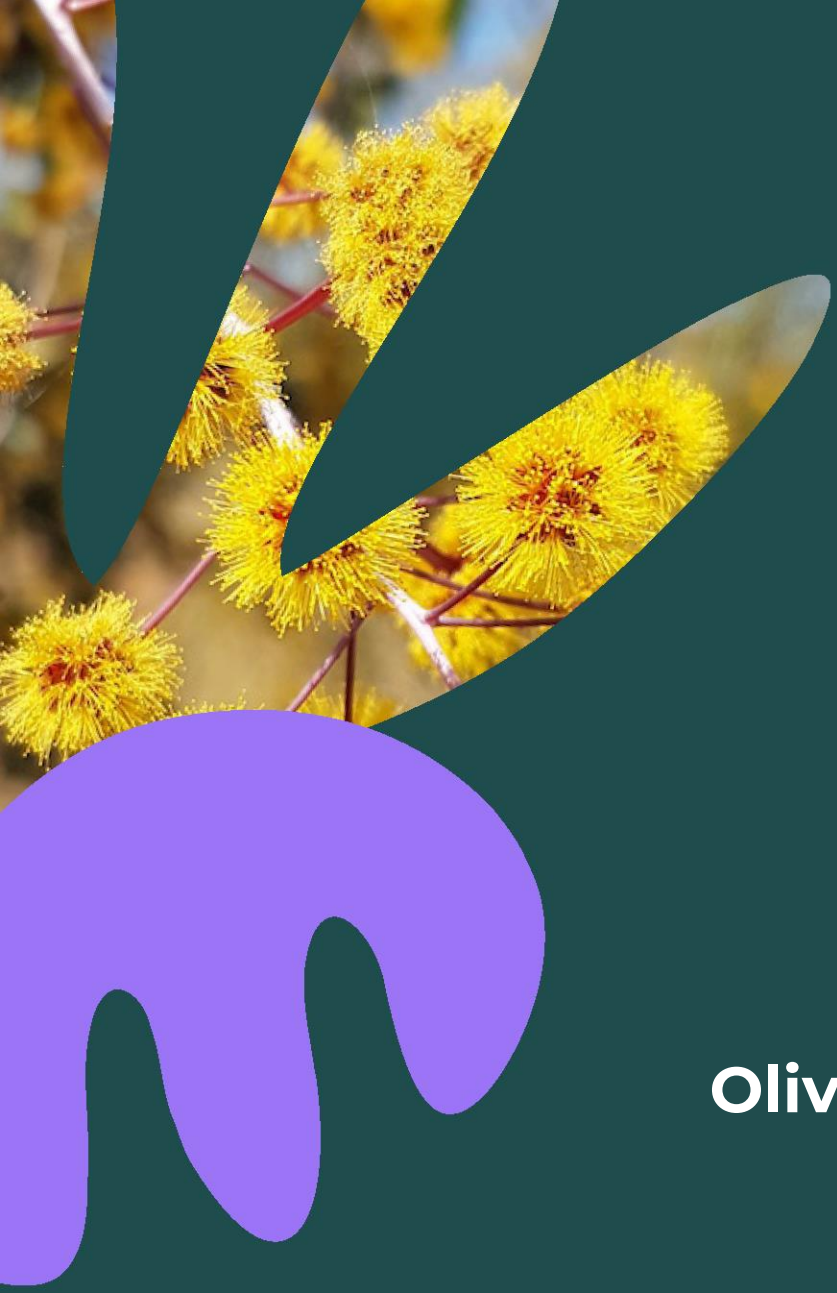

Biologic
ENVIRONMENTAL
SURVEY

Targeted Pilbara Olive Python Survey: South Flank and Mining Area C

Report to BHP Western Australia Iron Ore

28 August 2023

Document Status				
Revision No.	Author	Review / Approved for Issue	Approved for Issue to Name	Date
1	Verity Steptoe	Andrew Hide, Ryan Ellis	Daman Sharma	24/07/2023
2	Verity Steptoe	Ryan Ellis	Daman Sharma	28/08/2023
3				

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Executive Summary

BHP Western Australia Iron Ore commissioned Biologic Environmental Survey Pty Ltd (Biologic) to undertake a single season targeted Pilbara olive python (*Liasis olivaceus barroni*) survey within and surrounding the Mining Area C (MAC) and South Flank (SF) development envelope (hereafter the Study Area). Additional survey effort was also undertaken in an area surrounding the Study Area (hereafter the Regional Study Area). The Study Area is located approximately 80 km north-west of Newman.

The objective of the survey was to determine whether the presence of Pilbara olive python is consistent with baseline data, in accordance with the MAC/ SF Validation Notice. Sampling effort during the current survey aimed to replicate sampling undertaken during the baseline survey and included diurnal and nocturnal targeted searches for Pilbara olive python in suitable habitat (including Gorge/ Gully, Major Drainage Line, Medium Drainage Line and Drainage Area/ Floodplain habitats) and vehicular nocturnal searches along roads. Access constraints during the current survey resulted in a reduced sample effort compared to the 2015 baseline survey, with 44.5 hours of targeted searches completed, compared to 53 hours (40 diurnal and 13 nocturnal) targeted search effort for the species. Additional environmental DNA (eDNA) sampling undertaken during the current survey via water sampling at ten water features within the Study Area and Regional Study Area supplemented traditional sampling and increased the likelihood of detection of the species.

Pilbara olive python were recorded on three occasions within Gorge/ Gully habitat during the current survey. These included one visual observation and one eDNA detection of the same individual at a water feature (WCPH-303) in the Regional Study Area, and the third record from eDNA detection of the species at a water feature (WPSF-20) in the Study Area. The species has previously been recorded within the Study Area (n = 1), Regional Study Area (n = 2), and within 50 km of the Study Area (n = 29). One additional significant species, northern quoll (*Dasyurus hallucatus*) was opportunistically detected from eDNA sampling at a water feature within the Study Area.

The results of the current survey, in addition to records from previous surveys within and in the vicinity of the Study Area, demonstrates that Pilbara olive python is likely to occur as a resident within the Study Area and broader Regional Study Area. Records of the species in these areas show that the critical Gorge/ Gully and Major Drainage Line habitats, along with supporting Medium Drainage Line habitat present within the Study Area are likely to be important to the survival of the species within the Study Area and surrounding region.

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1 Introduction

1.1 Background

BHP Western Australian Iron Ore (BHP WAIO) commissioned Biologic Environmental Survey Pty Ltd (Biologic) to undertake a single season targeted Pilbara olive python (*Liasis olivaceus barroni*) survey within and surrounding the Mining Area C (MAC) and South Flank (SF) development envelope (hereafter referred to as the Study Area). A broader area surrounding the Study Area was also surveyed (hereafter referred to as the Regional Study Area). The Study Area and Regional Study Area are located approximately 80 km north-west of Newman, within the Pilbara region of Western Australia, (Figure 1.1).

The objective of this survey was to determine whether the presence of Pilbara olive python is consistent with baseline data from a previous survey completed in 2015, in accordance with the MAC Validation Notice (BHP, 2018).

1.2 Compliance

This assessment was carried out in acknowledgement of the following guidelines and recommendations developed by the relevant state and federal regulatory bodies, relevant survey-specific license conditions and, where relevant, BHP WAIO procedures:

- BHP WAIO (2022b) Guidance for terrestrial vertebrate fauna surveys in WA (Document Number: SPR-IEN-EMS-012) Version: 9.0;
- BHP WAIO (2020) Biodiversity Survey Spatial Data Requirements Procedure (Document number: SPR-IEN-EMS-015) Version: 11.0;
- EPA (2020) Technical guidance – terrestrial vertebrate fauna surveys for environmental impact assessment;
- DoE (2013) Significant impact guidelines 1.1: matters of national environmental significance;
- DSEWPaC (2011) Survey guidelines for Australia's threatened reptiles; and
- TSSC (2008) Approved Conservation Advice for *Liasis olivaceus barroni* (olive python - Pilbara subspecies).

The survey was conducted under the *Animal Welfare Act 2002* Licence to Use Animals for Scientific Purposes (License No. U244/2022-2024), administered through the Department of Primary Industries and Regional Development. This is enabled through Biologic's chosen Animal Ethics Committee, Murdoch University, under permit RW3354/21. Department of Biodiversity, Conservation and Attractions (DBCA) Regulation 27 "Fauna Taking (Biological Assessment) Licence", was issued to Chris Knuckey (Licence No. BA27000784). Under Section 40 of the *Biodiversity Conservation Act 2016* (BC Act), threatened species sampling was completed under a DBCA "Authorisation to Take or Disturbed Threatened Species" issued to Chris Knuckey (Authorisation No. TFA 2223-0171).

1.3 Species Profile

The Pilbara olive python is listed as Vulnerable under the Federal *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) and State BC Act. The species is Western Australia's largest snake, averaging 2.5 metres (m), with records up to 4.5 m (Bush & Maryan, 2011; Cogger, 2014). The species has a olive-brown upper surface and is pale cream below (Burbidge, 2004; Cogger, 2014). The 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 and is therefore often difficult to detect. During summer months they will emerge from daytime shelters soon after dark and continue to move until the early hours of the morning (DSEWPaC, 2011). In the winter months, the species is primarily nocturnal, although adult pythons can sometimes be found basking in the morning sun (DSEWPaC, 2011; 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, 2011). 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; DSEWPaC, 2011). 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 (DSEWPaC, 2011; 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 by foxes, habitat destruction from development, deliberate and accidental road kills, and deliberate killings around tourist and residential areas associated with mistaken identification (Pearson, 2003).

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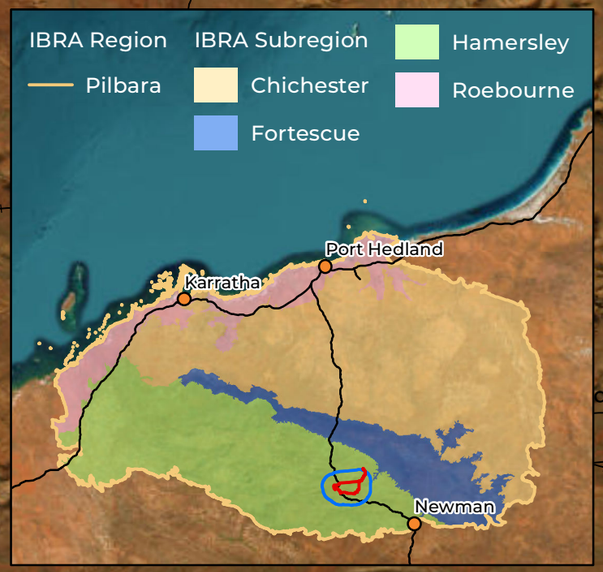


West Angelas Rd

Great Northern Hwy

LEGEND

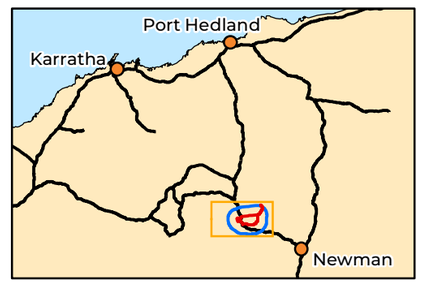
- Study Area
- Regional Study Area
- Local Road
- State Road
- Rail

Biologic

Scale 1:250,000

0 4 8 Km

Coordinate System: GDA2020 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA2020 Created 09/08/2023



BHP WAIO
South Flank and Mining Area C
Targeted Vertebrate Fauna
Survey: Pilbara Olive Python

Figure 1.1: Study Area and regional context

2 Methods

2.1 Desktop Assessment

A desktop assessment, comprising database searches and a literature review has previously been undertaken in the vicinity of the Study Area, as part of a targeted survey of the Central Pilbara Hub (CPH) (Biologic, 2023). The results of the CPH desktop assessment apply to the current survey, with relevant Pilbara olive python records utilised herein for this assessment.

2.1.1 Database Searches

Four fauna databases reviewed as part of the previous desktop assessment were searched for Pilbara olive python records in the vicinity of the Study Area (Table 2.1).

Table 2.1: Details of database searches conducted

Database	Data Access/ Receival Date	Search Area
DBCA (2022a) NatureMap	14/03/2022	Centre point of -22.9600 S, 118.7700 E) with a 50 km buffer. Note: Desktop search area previously undertaken over a broader area covering multiple study Areas; however, encompasses the Study Area in its entirety.
DBCA (2022b) Threatened and Priority Fauna Database	14/03/2022	
DCCEEW (2022) Protected Matters Search Tool	03/03/2022	
BHP WAIO (2022a) Fauna Records Database	24/05/2022	

2.1.2 Literature Review

A total of 79 survey reports were reviewed for records of Pilbara olive python, comprising 53 detailed surveys, six targeted surveys, 24 basic surveys and one desktop assessment within approximately 50 km of the Study Area (Biologic, 2023).

2.2 Field Survey

2.2.1 Survey Timing and Personnel

The field survey was undertaken from the 7–14 March 2023 by experienced zoologists whom collectively have over 19 years' experience undertaking fauna surveys within the Pilbara region and more broadly throughout Western Australia (Table 2.2).

Access to two of the previously sampled and proposed sample sites at water features WJIN-01 and WBEN-01 was restricted during the current survey due to heritage approval delays and therefore unable to be sampled at the time of the current survey. A separate field survey (hereafter referred to as the 'second survey') targeting Matters of National Environmental Significance (MNES) species, was conducted in the broader Regional Study Area from the 21-31 March 2023 by experienced zoologists (Table 2.2) (Biologic, 2023 in prep.). This second survey undertook sampling (targeted searches and/or eDNA) at the two proposed sites once access had been approved, with relevant survey effort and results identified and included in the current survey report.

Furthermore, one area previously surveyed on vehicular nocturnal searches during the baseline survey were inaccessible due to current mining operations. Additional nocturnal searches were undertaken at selected sample sites during the current survey to offset previous survey effort.

Table 2.2: Field survey personnel

Personnel	Position and Role	Qualifications	Experience
7-14 March survey			
Jari Cornelis	Senior Zoologist Field survey	BSc Zoology and Ecology	5 years' Field survey 4 years' EIA (consulting) 5 years' Vertebrate fauna zoology/ ecology
Verity Steptoe	Senior Zoologist Field survey Data management Reporting	BSc Zoology and Marine Biology	14 years' Field survey 13 years' EIA (consulting) 13 years' Vertebrate fauna zoology/ ecology
21-31 March survey			
Claire Brooks	Senior Zoologist Field survey Reporting	BSc (Hons) Conservation Biology and Zoology Postgraduate Certificate in Environmental Management and Assessment	13 years' Field survey 10 years' EIA (consulting 13 years' Vertebrate fauna zoology/ ecology
Aleesha Turner	Zoologist Field survey Data management	BSc (Hon) Applied Science (Wildlife Biology)	5 years' Field survey 3 years' EIA (consulting) 5 years' Vertebrate fauna zoology/ ecology
Emma de Mamiel	Zoologist Field survey	BSc (Hons) Zoology and Marine Science Certificate III Captive Animal Management	4 years' Field survey 2 years' EIA (consulting) 9 years' Vertebrate fauna zoology/ ecology
Samantha	Senior Zoologist	BSc (Hons) Marine Biology	9 years' Field survey

Personnel	Position and Role	Qualifications	Experience
Lostrom	Field survey	and Zoology	7 years' EIA (consulting) 9 years' Vertebrate fauna zoology/ ecology

2.2.2 Climate and Weather

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 74 km south-east of the Study Area (BoM, 2023). 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).

For four of the eleven months prior to the March survey (April 2022 to February 2023), mean minimum and maximum temperatures at Newman Aero Station were comparable to the long-term averages, with temperatures for the other months ranging from 3.6°C below to 2.8°C above the long-term averages (Figure 2.1). Rainfall in the eleven months preceding the survey was below the long-term averages for most months, except for May, June and September 2022, which recorded above the long-term averages (Figure 2.1).

Observed minimum and maximum temperatures during the survey were up to 6.4°C and 4.6°C higher than the long-term average, respectively (Table 2.3). Minimum and maximum temperatures during the current survey varied between 20–28.6°C and between 38.2–40.2°C, respectively (Table 2.3). No rainfall was recorded during the survey (Table 2.3).

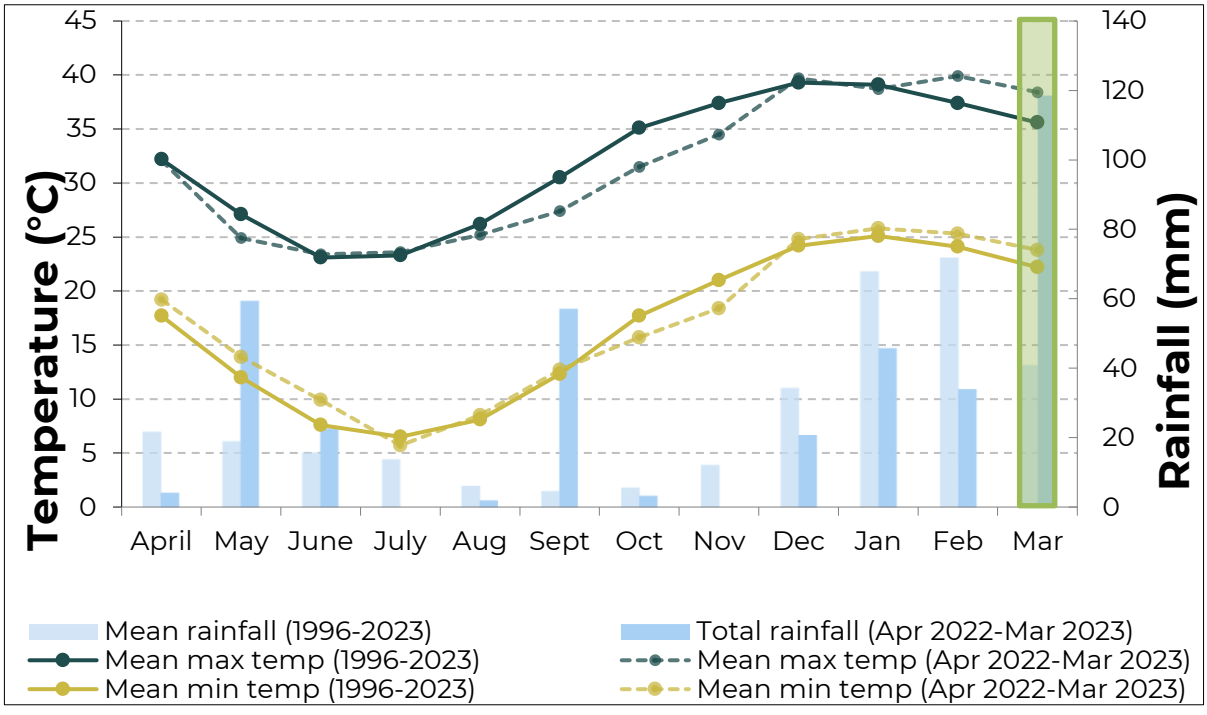


Figure 2.1: Long-term average and contemporary climate data recorded near the Study Area (BoM, 2023)

Note: approximate survey timing shown in shaded box

Table 2.3: Climatic conditions recorded during the survey (Newman Aero weather station) (BoM, 2023)

Date	Minimum temperature (°C)	Maximum temperature (°C)	Rainfall (mm)
7/03/2023	28.6	39.2	0
8/03/2023	27.0	38.2	0
9/03/2023	21.3	39.2	0
10/03/2023	21.6	40.2	0
11/03/2023	22.0	39.3	0
12/03/2023	23.1	39.7	0
13/03/2023	21.1	39.5	0
14/03/2023	20.0	39.9	0
Average/ Total	23.1	39.4	0

2.2.3 Habitat Assessments

Habitat assessments were undertaken in the field to define broad fauna habitats present and their suitability to significant species. Habitat assessments (*n* = 19) were undertaken at all sampling locations (Figure 2.3; Appendix A). Habitat assessments were conducted and attributes assessed using terminology prescribed by BHP WAIO (2020), 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 and dominant species;
- Land surface: abundance and size of coarse fragments, rock outcropping, water bodies;
- Substrate: soil texture, soil colour, bare ground, rock size, rock type, rock outcropping; and
- Disturbance: time since last fire, evidence of weeds, grazing, or human disturbances.

2.2.4 Targeted Searches

Although critical habitats are common within the Study Area and Regional Study Area, particularly Gorge/ Gully habitat and associated permanent and semi-permanent waterbodies, Pilbara olive python are rarely sighted, as the species are known to be cryptic and difficult to survey (DSEWPaC, 2011).

Sampling during the current survey included diurnal and nocturnal targeted transects, and nocturnal vehicle transects, with effort focusing on similar locations as previous sampled (Figure 2.2; Figure 2.3). Where possible, survey effort replicated sampling previously undertaken during the 2015 baseline survey (Biologic, 2016). Sampling was undertaken during the optimal time of year for the detection of the species (summer wet season), allowing for increased likelihood of recording the species within the Study Area. A total of 44.5 person hours sampling effort (including 16 hours diurnal targeted searches and 28.5 hours nocturnal targeted and vehicular searches) was undertaken during the current survey (including effort from the second survey), compared to 53 hours sampling effort (including 40 hours diurnal targeted searches and 13 hours nocturnal targeted and vehicular searches) undertaken during the 2015 baseline survey (Biologic, 2016).

Additional survey effort during the current and second survey, including alternate locations, multiple nights sampling at the same location, and eDNA sampling of water features increased the likelihood of detection for the species within the Study Area and Regional Study Area.

2.2.4.1 Diurnal Targeted Searches

In total, six diurnal targeted searches for the Pilbara olive python were conducted within suitable habitat during the current survey (*n* = 5) and second survey (*n* =1, VJIN-21) (Figure 2.3; Table 2.4). These included three searches within the Study Area (Gorge/ Gully *n* = 2 and Medium Drainage Line *n* = 1) and three searches within the Regional Study Area (Gorge/ Gully *n* = 1 and Major Drainage Line *n* = 2). Searches focused on observing active individuals and/or secondary evidence such as scats, sloughs or remains. A total of 16 person hours of diurnal targeted searches were completed over the two surveys, including 12 hours within the Study Area and four hours within the Regional Study Area.

2.2.4.2 Nocturnal Targeted Searches

A total of 12 nocturnal targeted searches were undertaken during the current survey, 11 within the Study Area and one in the Regional Study Area. Of these, four nocturnal targeted searches were conducted on foot in the Study Area (Gorge/ Gully *n* = 3 and Medium Drainage Line *n* = 1 habitats) and one on foot in the Regional Study Area (Gorge/ Gully

habitat), for a total of 17 person hours (Table 2.4; Figure 2.3). Seven nocturnal targeted searches were undertaken by vehicle along roads and access tracks in the Study Area over four nights (total of 11.5 person hours) (Table 2.4; Figure 2.3).

649980 679980 709980 739980

7483697 7458697 7433697

Packsaddle Rd

West Angelas Rd

Great Northern Hwy

LEGEND

Study Area
Regional Study Area

Local Road
State Road
Rail

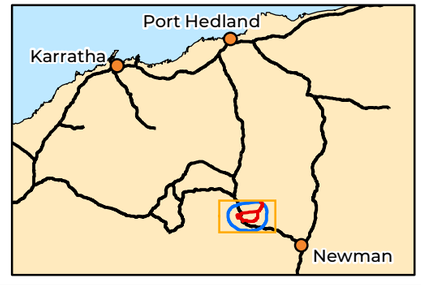
Sampling Type
Diurnal targeted search/ traverse
Nocturnal targeted search/ traverse

Biologic

Scale 1:230,000

0 4 8 Km

Coordinate System: GDA2020 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA2020 Created 09/08/2023



BHP WAIO
South Flank and Mining Area C
Targeted Vertebrate Fauna
Survey: Pilbara Olive Python

Figure 2.2: Baseline survey fauna sample sites and traverses (Biologic, 2016)

649980 679980 709980 739980

7483697 7458697 7433697

VPSF-27
VCSF-302
VPSF-02
VJIN-01
VPSF-17
VPSF-16
VPSF-29
VBEN-01
VPSF-20
VPSF-23
VPSF-24
VPSF-01
VPSF-26
VPSF-21
VPSF-22
VPSF-25
VPSF-30
VPSF-28
VPSF-15

Packsaddle Rd
West Angelas Rd
Great Northern Hwy

LEGEND

- Study Area
- Regional Study Area
- Local Road
- State Road
- Rail

Sampling Type

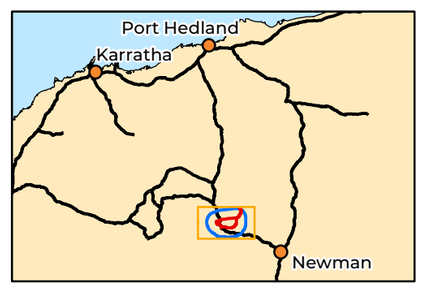
- eDNA
- Habitat Assessment
- Diurnal targeted search/ traverse
- Nocturnal targeted search/ traverse

Biologic

Scale 1:230,000

0 4 8 Km

Coordinate System: GDA2020 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA2020 Created 09/08/2023



BHP WAIO
South Flank and Mining Area C
Targeted Vertebrate Fauna
Survey: Pilbara Olive Python

Figure 2.3: Current survey fauna sample sites and traverses

Table 2.4: Survey effort during the current survey

Targeted Searches	Site ID	Transect ID	Date	Latitude	Longitude	Habitat Type	Sample Effort (person hours)
Diurnal (on foot)	VPSF-01	TPSF-01	8/03/2023	-22.9509	118.9851	Medium Drainage Line	2
	VPSF-15	TPSF-15	9/03/2023	-22.9939	118.9712	Gorge/ Gully	1
	VPSF-16	TPSF-16	10/03/2023	-23.0486	118.6374	Gorge/ Gully	4
	VPSF-17	TPSF-17	10/03/2023	-22.9047	118.7240	Major Drainage Line	1
	VPSF-25	TPSF-25	12/03/2023	-22.9621	118.9438	Gorge/ Gully	4
	VJIN-21	TJIN-21	24/03/2023	-22.9164	119.2017	Major Drainage Line	4
		Total diurnal					**16**
Nocturnal (on foot)	VPSF-21	TPSF-21	10/03/2023	-22.9628	118.9144	Gorge/ Gully	2
	VPSF-22	TPSF-22	11/03/2023	-22.9592	118.9252	Gorge/ Gully	5.5
	VPSF-26	TPSF-26	12/03/2023	-22.9562	118.9498	Gorge/ Gully	3.5
	VPSF-29	TPSF-29	12/03/2023	-23.0781	118.6135	Gorge/ Gully	2
	VPSF-01	TPSF-01	13/03/2023	-22.9509	118.9851	Medium Drainage Line	4
		Subtotal nocturnal (on foot)					**17**
Nocturnal (vehicle)	VPSF-20	TPSF-20	10/03/2023	-22.9569	118.9018	Road	1
	VPSF-23	TPSF-23	10/03/2023	-22.9540	118.9241	Road	1.5
	VPSF-28	TPSF-28	10/03/2023	-22.9627	118.8384	Road	1.5
	VPSF-30	TPSF-30	10/03/2023	-22.9627	118.8085	Road	1.5
	VPSF-20	TPSF-20	11/03/2023	-22.9569	118.9018	Road	2
	VPSF-20	TPSF-20	12/03/2023	-22.9569	118.9018	Road	2
	VPSF-20	TPSF-20	13/03/2023	-22.9569	118.9018	Road	2
		Subtotal nocturnal (vehicle)					**11.5**

Targeted Searches	Site ID	Transect ID	Date	Latitude	Longitude	Habitat Type	Sample Effort (person hours)
		Total nocturnal					**28.5**
		Total					44.5

2.2.5 Condition Assessment and Individual Identification

Any Pilbara olive pythons captured during the survey were processed, with data collected including sex, general body condition, snout-vent length, tail length and weight. Additionally, photographs of head scalation of capture individuals were collected to facilitate identification of individuals. A tissue sample (ventral scale clip) was collected and stored in 100% ethanol, to provide data on population demographics for the area and facilitate further genetic studies on the individuals and population occurring within the Study Area and Regional Study Area.

2.2.6 Environmental DNA Sampling

Environmental DNA (eDNA) is a by-product of the metabolic process, derived from sources such as 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. A recent study has highlighted the use of eDNA sampling for the detection of Pilbara olive python, with eDNA metabarcoding analysis being used in accurate species detection (readily distinguishing Pilbara olive python from other pythons) and identification to minimise the risk of false positives or false negatives occurring (Mousavi-Derazmahalleh *et al.*, 2023).

Water bodies within the Study Area and Regional Study Area considered suitable for eDNA sampling were identified based on previous survey results and during targeted searches. A total of 13 water features were recorded during the current survey, including ten that were previously identified within the Study Area (n = 3) and Regional Study Area (n = 7), and three additional water bodies identified in the Study Area whilst undertaking targeted searches (Appendix B).

Sampling for eDNA was undertaken at ten water features within the Study Area (n = 3) and Regional Study Area (n = 7), of which eight were collected during the current survey and two during the second survey) (Biologic, 2023 in prep.) (Table 2.5; Figure 2.2; Figure 2.3; Figure 2.4). Eight water features were pre-selected for sampling, while two of the water

features were identified during the current survey and subsequently sampled (Table 2.5). Sampled water features were located in Gorge/ Gully (*n* = 7), Major Drainage Line (*n* = 2) and Drainage Area/ Floodplain (*n* = 1) habitats.

Five 1 litre (L) replicate samples were collected from each water feature, for a total of 50 water samples. Samples were collected and stored in a refrigerator, prior to being filtered through a 0.45-micron (µm) mixed cellulose ester (MCE) filter membrane using a peristaltic Sentino pump. All filtering equipment was sterilised in a 10% bleach solution and rinsed between samples. Once filtering was completed, filter membranes were individually stored in 100% ethanol at room temperature for transport and then stored at -20°C at Curtin University's eDNA Frontiers laboratory prior to analysis. Results of the eDNA analysis are provided in the eDNA Frontiers report in Appendix C.

Table 2.5: eDNA sample locations within the Study Area

Site ID	Water Feature ID	Date	Latitude	Longitude
VPSF-02	WMIN-04	8/03/2023	-22.8485	119.1111
VPSF-15	WPSF-01	9/03/2023	-22.9940	118.9730
VPSF-16	WCPH-303	10/03/2023	-23.0485	118.6374
VPSF-17	WCPH-41	10/03/2023	-22.9047	118.724
VPSF-25	WPSF-20^	12/03/2023	-22.9619	118.9438
VPSF-26	WPSF-23^	12/03/2023	-22.9563	118.9497
VPSF-27	WYAN-10	13/03/2023	-22.7878	119.1526
VPSF-29	WCPH-393	13/03/2023	-23.0781	118.6135
VJIN-21	WJIN-01*	24/03/2023	-22.9164	119.2017
VBEN-01	WBEN-01*	27/03/2023	-23.0535	119.1519

*Sampled during the second survey (Biologic, 2023 in prep.).

^Identified during current survey

2.2.7 Opportunistic Fauna Records

At all times while surveying (including whilst driving between sites as well as during sampling and searches), all records pertaining to species not previously recorded during the survey, species of significance or other fauna of interest were documented. These records include those from primary (i.e. direct observation of species) or secondary (e.g. non-target eDNA detections, burrows, scratching's, diggings, nests and scats) evidence.

649980 679980 709980 739980

7483697 7458697 7433697

Packsaddle Rd

West Angelas Rd

Great Northern Hwy

WCPH-41

WCPH-303

WCPH-393

WPSF-21

WPSF-24

WPSF-23

WPSF-20

WPSF-25

WPSF-01

WMIN-04

WYAN-10

WJIN-01

WBEN-01

LEGEND

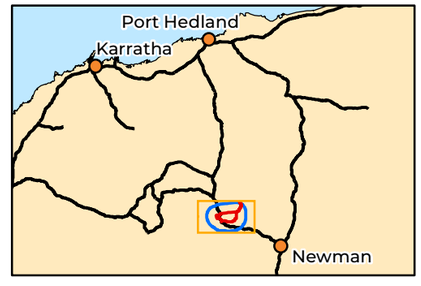
- Study Area
- Regional Study Area
- Local Road
- State Road
- Rail
- Water Feature

Biologic

Scale 1:230,000

0 4 8 Km

Coordinate System: GDA2020 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA2020 Created 09/08/2023



BHP WAIO

South Flank and Mining Area C
Targeted Vertebrate Fauna
Survey: Pilbara Olive Python

Figure 2.4: Water features in the Regional Study Area

3 Results and Discussion

3.1 Fauna Habitats

A total of four broad fauna habitat types were recorded across the Study Area, comprising Gorge/ Gully, Major Drainage Line, Medium Drainage Line and Drainage Area/ Floodplain (Appendix A). Cleared/ Disturbed areas were also observed across the Study Area.

Gorge/ Gully and Major Drainage Line habitats and associated waterbodies (in particular those that are persistent or semi-persistent) present within the Study Area provide critical breeding, foraging and dispersal habitat for Pilbara olive python, with Medium Drainage Line and Drainage Area/ Floodplain habitats providing supporting foraging and dispersal habitat, where proximal to critical habitats. These habitats are contiguous with surrounding areas and are not considered to be uncommon in the broader Hamersley subregion.

3.2 Water Features

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). Pilbara olive python is often encountered in the vicinity of permanent water features in rocky ranges or among riverine vegetation (DSEWPaC, 2011; Pearson, 1993).

Thirteen water features within suitable habitat types, including Gorge/ Gully, Major Drainage Line and Drainage Area/ Floodplain habitats, were searched for evidence of Pilbara olive python (Figure 2.4; Appendix B) (Biologic, 2023 in prep.). It is possible that additional water features occur within the Study Area and Regional Study Area, particularly within the Major Drainage Line, Medium Drainage Line and Gorge/ Gully habitat.

With the exception of water feature WPSF-01, which was surrounded by active mine operations and associated infrastructure (i.e. haul roads), all of the water features visited during the survey were considered to provide foraging opportunities for the Pilbara olive python (Figure 2.4).

3.3 Pilbara olive python

3.3.1 Previous records

The Study Area and Regional Study Area fall within the current distribution of the Pilbara olive python, where the species or species' habitat is likely to occur (DoE, 2022). A total of 32 records of the species were identified as occurring within 50 km of the Study Area during the desktop assessment (DBCA, 2022b) (Figure 3.1). Pilbara olive python have been previously recorded on one occasion within the Study Area, when one dead individual was recorded in 2008 (Outback Ecology, 2008). The species has also been recorded within the Regional Study Area on two prior occasions, where a dead individual was observed on the road near Mulla Mulla camp in 2022 (M. O'Connell, pers. comm) within 70 m of the Study Area and a vouchered specimen collected on an unknown date (DBCA, 2022b) (Figure 3.1). A further 11 records occur within 10 km of the Study Area (dated between 1980 and 2014), 13 records within 10–25 km (2010–2014) and five records within 25–50 km (2012–2014) of the Study Area (DBCA, 2022b) (Figure 3.1).

3.3.2 Current survey

Pilbara olive python were detected on three occasions during the current survey, once from direct observation of an active individual and twice from eDNA sampling (Figure 3.2; Appendix D). An individual was observed lying within water feature WCPH-303 in Gorge/ Gully habitat during a diurnal targeted search in the Regional Study Area (Figure 3.2; Appendix D). One water sample collected from the same water feature (WCPH-303) immediately following the capture and removal of the animal for processing, positively detected the species through eDNA analysis (Appendix C; Appendix D; Appendix E). While it can't be confirmed, it is almost certainly a detection of the same individual. The second detection of the species through eDNA analysis was recorded at water feature WPSF-20 in Gorge/ Gully habitat within the Study Area (Appendix C; Appendix D; Appendix E).

Table 3.1: Pilbara olive python records from the current survey

Water Feature ID	Site ID	Date	Latitude	Longitude	Sample Type	Sex/ Maturity	Length	Weight
WCPH-303	VPSF-16	10/03/2023	-23.0485	118.6375	Individual (alive)	Adult Male	269 cm	3750 g
WCPH-303	VPSF-16	10/03/2023	-23.0485	118.6375	eDNA*	-	-	-
WPSF-20	VPSF-25	12/03/2023	-22.9619	118.9438	eDNA	-	-	-

* Same individual as visually observed

Building upon previous records of the species, results from the current survey confirm their presence within the Study Area and demonstrate their ongoing presence in the Regional Study Area. The presence of critical (i.e. Gorge/ Gully and Major Drainage Line) and supporting (i.e. Medium Drainage Line, Drainage Area/ Floodplain) habitat types and habitat features (i.e. water bodies) indicates the Study Area and broader Regional Study Area are likely to be important for the survival of the species at a local and regional scale.

3.4 Other vertebrate fauna

A total of 25 non-target vertebrate fauna species were recorded during the current survey. These included six mammal species (four native, two introduced), 13 reptiles, four birds and two amphibians (Appendix D). This includes one record of the northern quoll (*Dasyurus hallucatus* – Endangered EPBC Act and BC Act), which was detected via eDNA sampling at water feature WPSF-23 (Appendix C; Appendix D).

533768 633768 733768 833768

7545347 7470347 7395347

Nanutarra Munjina Rd
Karijini Dr
Manuwarra Red Dog HWY
Tom Price
Nanutarra Munjina Rd
Paraburdoo Rd
Tom Price Rd
Paraburdoo
Great Northern Hwy
Marble Bar Rd
Newman

LEGEND

- Study Area
- Regional Study Area
- State Road
- Rail
- DBCA, 2022
- M. O'Connell, pers. comm. 2022
- Outback Ecology, 2008

Biologic

Scale 1:800,000

0 10 20 30 Km

Coordinate System: GDA2020 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA2020 Created 09/08/2023

BHP WAIO
South Flank and Mining Area C
Targeted Vertebrate Fauna Survey: Pilbara Olive Python

Figure 3.1: Pilbara olive python records from the desktop assessment

649980 679980 709980 739980

7483697 7458697 7433697

Packsaddle Rd

West Angelas Rd

Great Northern Hwy

LEGEND

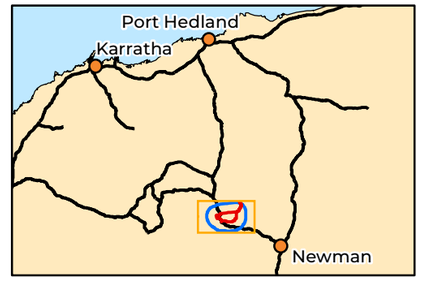
- Study Area
- Regional Study Area
- Local Road
- State Road
- Rail
- Pilbara olive python

Biologic

Scale 1:230,000

0 4 8 Km

Coordinate System: GDA2020 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA2020 Created 09/08/2023



BHP WAIO

South Flank and Mining Area C
Targeted Vertebrate Fauna
Survey: Pilbara Olive Python

Figure 3.2: Pilbara olive python recorded in the Study Area during the current survey

3.5 Constraints and Limitations

The EPA (2020) outlines several potential limitations to vertebrate fauna surveys. These aspects are assessed and discussed in Table 3.2 below. No major limitations or constraints were identified for the survey; however, access was limited in some areas of the Study Area, reducing the ability to directly replicate the survey effort undertaken during the baseline survey in 2015 (Biologic, 2016).

Table 3.2: Survey constraints and limitations

Potential limitation or constraint	Constraint	Applicability to this survey
Scope (faunal groups sampled and whether any constraints affect this)	No	The survey was undertaken using standardised and established techniques considered suitable for the detection of Pilbara olive python.
Survey effort and completeness	Partial	Targeted searches for Pilbara olive python were undertaken (including diurnal and nocturnal searches) on foot in suitable habitat and by vehicle along access roads and tracks. Survey effort focused on locations previously surveyed (Biologic, 2016); however, some areas were deemed inaccessible (due to changes in mining operations since baseline survey or heritage access delays), leading to a reduced total (44.5 hours) sampling effort compared to the 53 hours during the baseline survey in 2015 (Biologic, 2016). However, eDNA sampling during the current survey supplemented targeted search survey effort to increase the likelihood of detection of the species.
Access	Partial	Access to some areas of the Study Area was limited due to heritage or mine operations constraints during the current survey. Two proposed sample sites were able to be sampled on a second vertebrate fauna survey (Biologic, 2023 in prep.) however, survey effort was still reduced due to mining operation constraints when compared to the previous baseline survey.
Competency/ experience of the survey team	No	Field personnel consisted of qualified zoologists with extensive experience in conducting biological surveys in the Pilbara.
Timing of survey, weather, seasonality	No	The timing of this survey (summer wet season) was chosen with consideration of the species' behaviour, allowing for increased likelihood of recording the species within the Study Area. Weather conditions experienced during the survey were considered normal for the time of year and did not impede the ability of the survey to detect the species.
Availability of data and information	No	All contextual resources required to complete the survey were available (previous capture data, environmental information and climate data).

4 Conclusion

The objective of the current survey was to determine the presence of the Pilbara olive python in the Study Area and broader Regional Study Area, in relation to the baseline survey undertaken in 2015. Sampling effort during the current survey replicated the baseline survey as closely as possible, although access restrictions to some areas of the Study Area partially limited survey effort achieved, with a total of 44.5 hours completed, compared to 53 hours during the baseline survey. Current survey effort included diurnal (16 hours) and nocturnal (17 hours) targeted searches for Pilbara olive python in suitable habitat (including Gorge/ Gully, Major Drainage Line and Medium Drainage Line habitats) and vehicular nocturnal searches (11.5 hours) along roads and access tracks. Supplementary survey effort was undertaken via eDNA sampling at ten water features within the Study Area and Regional Study Area to increase the likelihood of detection of the species.

During the current survey, Pilbara olive python were recorded in the Study Area and broader Regional Study Area in Gorge/ Gully habitat on three occasions. This included one positive eDNA detection at a water feature within the Study Area, representing the first detection of the species within the Study Area. An additional two records (one visual observation and one eDNA detection) were collected of the same individual at a water feature within the Regional Study Area.

Current and previous records of Pilbara olive python within and in the vicinity of the Study Area and/or Regional Study Area demonstrates that resident Pilbara olive python occur. These records show that the critical Gorge/ Gully and Major Drainage Line habitats and supporting Medium Drainage Line and Drainage Area/ Floodplain habitats and associated habitat features (i.e. water features) present within the Study Area are likely to be important to the survival of the species at a local scale within the Study Area and more broadly within the surrounding Pilbara region.

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Appendix A: Habitat Assessment

Site ID	Latitude	Longitude	Habitat Type	Landform	Soil Type	Soil Availability	Rocky Outcropping Extent	Rock Size	Vegetation Litter	Woody Debris	Vegetation Type	Availability Rocks, Cracks, Crevices	Suitahility Burrow	Water Presence	Last Fire	Disturburbance	Habitat Condition	Image
VCSF-302	-22.8345	119.1313	Drainage Area/ Floodplain	Drainage Area/ Floodplain	Light Clay	Few Small Patches	Extensive (Ironstone)	Boulders (>61cm)	Few Large Patches	Few Small Patches	Eucalypt Woodland, Tussock Grassland, Spinifex Hummock Grass	Very High	Moderate	Prone to Pooling	Old (6+ yr)	None	Very Good	
VPSF-01	-22.9513	118.9837	Medium Drainage Line	Medium Drainage Line	Clay Loam Sandy	Few Large Patches	Moderate (Conglom.)	Small Rocks (11-20cm)	Few Small Patches	Few Small Patches	Scattered Eucalypts	Low	Moderate	Prone to Pooling	Old (6+ yr)	None	Very Good	
VPSF-02	-22.8484	119.1110	Gorge/ Gully	Gorge	Sandy Clay Loam	Few Small Patches	Major (BIF)	Boulders (>61cm)	Many Small Patches	Many Small Patches	Acacia Shrubland, Eucalypt Woodland, Tussock Grassland	High	Moderate	Permanent	Old (6+ yr)	None	Excellent	
VPSF-15	-22.9939	118.9712	Gorge/ Gully	Gully	Clay Loam	Scarce	Extensive (Ironstone)	Boulders (>61cm)	Few Large Patches	Few Large Patches	Scattered Eucalypts, Tussock Grassland	Very High	Low	Prone to Pooling	Old (6+ yr)	Mining dust	Very Good	
VPSF-16	-23.0485	118.6374	Gorge/ Gully	Gorge	Clay Loam	Few Small Patches	Extensive (BIF)	Boulders (>61cm)	Few Small Patches	Few Large Patches	Scattered. Eucalypts, Spinifex Hummock Grass	Very High	Low	Prone to Pooling	Old (6+ yr)	None	Excellent	
VPSF-17	-22.9045	118.7240	Major Drainage Line	Drainage Area/ Floodplain	Clay Loam	Few Large Patches	Negligible	Gravel (1-4cm)	Few Small Patches	Few Small Patches	Mulga Woodland, Tussock Grassland, Spinifex Hummock Grass, Scattered. Eucalypts	Nil	Moderate	Permanent	Old (6+ yr)	Cattle Grazing	Very Good	
VPSF-20	-22.9569	118.9018	Cleared/ Disturbed		Clay Loam	None Discernible	Negligible	Negligible	None Discernible	None Discernible	None	Nil	Nil	None	Recent (0 to 2 yr)	Road/ Access Track	Very Poor	N/A

Site ID	Latitude	Longitude	Habitat Type	Landform	Soil Type	Soil Availability	Rocky Outcropping Extent	Rock Size	Vegetation Litter	Woody Debris	Vegetation Type	Availability Rocks, Cracks, Crevices	Suitahility Burrow	Water Presence	Last Fire	Disturbance	Habitat Condition	Image
VPSF-21	-22.9629	118.9145	Gorge/ Gully	Gorge	Clay Loam	Few Small Patches	Extensive (BIF)	Boulders (>61cm)	Few Small Patches	Few Small Patches	Scattered. Eucalypts, Tussock Grassland, Casuarina	Very High	Low	Prone to Pooling	Old (6+ yr)	None	Excellent	
VPSF-22	-22.9596	118.9255	Gorge/ Gully	Gorge	Clay Loam	Many Small Patches	Extensive (BIF)	Boulders (>61cm)	Few Large Patches	Few Large Patches	Scattered Eucalypts, Spinifex Hummock Grass	Very High	Low	Prone to Pooling	Old (6+ yr)	None	Excellent	
VPSF-23	-22.9540	118.9241	Cleared/ Disturbed		Clay Loam	None Discernible	Negligible	Negligible	None Discernible	None Discernible	None	Nil	Nil	None	Recent (0 to 2 yr)	Road/ Access Track	Very Poor	N/A
VPSF-24	-22.9535	118.9601	Gorge/ Gully	Gorge	Clay Loam	Few Small Patches	Extensive (BIF)	Boulders (>61cm)	Many Large Patches	Many Large Patches	Scattered Eucalypts, Spinifex Hummock Grass	Very High	Low	Prone to Pooling	Old (6+ yr)	None	Excellent	
VPSF-25	-22.9621	118.9438	Gorge/ Gully	Gorge	Clay Loam	Few Large Patches	Major (BIF)	Large Rocks (21-60cm)	Many Small Patches	Scarce	Scattered Eucalypts, Spinifex Hummock Grass, Tussock Grassland	Moderate	Nil	Prone to Pooling	Old (6+ yr)	None	Excellent	
VPSF-26	-22.9562	118.9499	Gorge/ Gully	Gorge	Clay Loam	Few Large Patches	Extensive (BIF)	Boulders (>61cm)	Many Large Patches	Many Large Patches	Scattered Eucalypts, Spinifex Hummock Grass, Scattered Shrubs	Very High	Moderate	Prone to Pooling	Old (6+ yr)	None	Excellent	
VPSF-27	-22.7866	119.1488	Major Drainage Line	Major Drainage Line	Clay Loam Sandy	Few Small Patches	Negligible	Pebbles (5-10cm)	Many Large Patches	Many Large Patches	Eucalypt Woodland	Nil	High	Permanent	Old (6+ yr)	Cattle Grazing	Very Good	
VPSF-28	-22.9930	118.8384	Cleared/ Disturbed		Clay Loam	None Discernible	Negligible	Negligible	None Discernible	None Discernible	None	Nil	Nil	None	Recent (0 to 2 yr)	Road/ Access Track	Very Poor	N/A
VPSF-29	-23.0781	118.6135	Gorge/ Gully	Gully	Silty Loam	Scarce	Major (BIF)	Boulders (>61cm)	Many Small Patches	Many Small Patches	Shrubland, Scattered.	Very High	Low	Prone to Pooling	Old (6+ yr)	None	Very Good	N/A

Site ID	Latitude	Longitude	Habitat Type	Landform	Soil Type	Soil Availability	Rocky Outcropping Extent	Rock Size	Vegetation Litter	Woody Debris	Vegetation Type	Availability Rocks, Cracks, Crevices	Suitahility Burrow	Water Presence	Last Fire	Disturburbance	Habitat Condition	Image
											Eucalypts, Spinifex Hummock Grass, Tussock Grassland							
VPSF-30	-22.9627	118.8085	Cleared/ Disturbed		Clay Loam	None Discernible	Negligible	Negligible	None Discernible	None Discernible	None	Nil	Nil	None	Recent (0 to 2 yr)	Road/ Access Track	Very Poor	N/A
VBEN-01	-23.0535	119.1519	Drainage Area/ Floodplain	Wetland	Sand	None Discernible	Negligible	Negligible	None Discernible	None Discernible		Nil	Nil	Permanent	Recent (0 to 2 yr)	None Discernible	Excellent	
VJIN-21	-22.9164	119.2017	Major Drainage Line	Major Drainage Line	Sandy Loam	Many Large Patches	Limited	Gravel (1-4 cm)	Many Large Patches	Many Large Patches	Scattered Shrubs, Eucalypt. Woodland	Low	High	Permanent	Old (6+ yr)	Cattle Grazing	Very Good	

Appendix B: Water Features

Water Feature ID	Description	Persistence	Latitude	Longitude	Habitat	eDNA sample collected	Comments	Image
WMIN-04	150 m long 25 m wide 1 m deep	Persistent (will persist all year most years)	-22.8485	119.1111	Gorge/ Gully	Yes		
WPSF-01	1.5 m long 0.8 m wide 0.1 m deep	Temporary (will persist for <3 months)	-22.9940	118.9730	Gorge/ Gully	Yes		
WCPH-303	3 m long 2.5 m wide 0.3 m deep	Temporary (will persist for <3 months)	-23.0485	118.6374	Gorge/ Gully	Yes		
WCPH-41	50 m long 4 m wide 1 m deep	Semi-persistent (will persist 3-9 months of year)	-22.9047	118.7240	Major Drainage Line	Yes		

Water Feature ID	Description	Persistence	Latitude	Longitude	Habitat	eDNA sample collected	Comments	Image
WPSF-20	2.1 m long 0.7 m wide 0.4 m deep	Temporary (will persist for <3 months)	-22.9619	118.9438	Gorge/ Gully	Yes	Recorded as a new water feature during current survey	
WPSF-23	40 m long 8 m wide 1 m deep	Semi-persistent (will persist 3-9 months of year)	-22.9563	118.9497	Gorge/ Gully	Yes	Recorded as a new water feature during current survey	
WYAN-10	1200 m long 15 m wide 4 m deep	Persistent (will persist all year most years)	-22.7878	119.1526	Gorge/ Gully	Yes	Artificial discharge point	
WCPH-393	2 m long 2 m wide 1 m deep	Semi-persistent (will persist 3-9 months of year)	-23.0781	118.6135	Gorge/ Gully	Yes		

Water Feature ID	Description	Persistence	Latitude	Longitude	Habitat	eDNA sample collected	Comments	Image
WPSF-24	11m long 4m wide 0.4m deep	Temporary (will persist for <3 months)	-22.9570	118.9493	Gorge/ Gully	No		
WPSF-25	11.8m long 8m wide 0.7m deep	Semi-persistent (will persist 3 - 9 month of year, most years)	-22.9577	118.9495	Gorge/ Gully	No		
WPSF-21	2 m long 0.8 m wide 0.4 m deep	Temporary (will persist for <3 months)	-22.9597	118.9064	Gorge/ Gully	No	Recorded as a new water feature during current survey	

Water Feature ID	Description	Persistence	Latitude	Longitude	Habitat	eDNA sample collected	Comments	Image
WJIN-01	1000 m long 50 m wide 1 m deep	Semi-persistent (will persist 3-9 months of year)	-22.9163	119.2109	Major Drainage Line	Yes		
WBEN-01	60 m long 16 m wide 1.6 m deep	Persistent (will persist all year most years)	-22.9536	118.8321	Drainage Area/ Floodplain	Yes	Groundwater fed	

Water Feature ID	Description	Persistence	Latitude	Longitude	Habitat	eDNA sample collected	Comments	Image
WJIN-01	1000 m long 50 m wide 1 m deep	Semi-persistent (will persist 3-9 months of year)	-22.9163	119.2109	Major Drainage Line	Yes		
WBEN-01	60 m long 16 m wide 1.6 m deep	Persistent (will persist all year most years)	-22.9536	118.8321	Drainage Area/ Floodplain	Yes	Groundwater fed	

Appendix C: Curtin University eDNA Frontiers Service Report

eDNA FRONTIERS | Curtin University

SERVICE REPORT

eDNA ANALYSIS

ASSAYS


Universal


Fish


Sharks & Rays


Corals


Crustaceans


Bacteria


Plants & Algae


Mammals


Insects


Vertebrates


Molluscs


Reptiles


Birds


Fungi

SAMPLES


Water


Plankton tows


Sediment


Deposition arrays


Biofoul


Bore water


Scats


Tissue


Plants


Fossils


Pollen


Stomach contents

DNA	Deoxyribonucleic acid
eDNA	Environmental DNA
NCBI	National Centre for Biotechnology Information
OTU	Operational taxonomic unit
ZOTU	Zero-radius operational taxonomic unit
AIS	Alien Invasive Species
LULU	A post-clustering algorithm for curation of DNA amplicon data
PCR	Polymerase chain reaction
mtGenome	The full mitochondrial genome
fasta	A formatting type for sequence data
18S	The nuclear gene region, 18S
COI	The mitochondrial gene region, cytochrome c oxidase I
16S	The mitochondrial subunit ribosomal RNA gene region, 16S
12S	The mitochondrial gene region, 12S

Project Details

Scope of Work: EF272

Project Title: eDNA biodiversity audit targeting reptiles (specifically Pilbara Olive Python) from water samples taken in the Central Pilbara Hub Area using eDNA metabarcoding. March 2023

Client Details

Client: Biologic Environmental Survey Pty Ltd (ABN: 55 133 116 131)
24-26 Wickham St, East Perth 6004. Postal: PO Box 174, Floreat 6014

Contact: Andrew Hide | Senior Zoologist
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Report Details

Report reference: EF272_Biologic_RevA

Report issue date: 10/05/2023

Laboratory start date: 21/03/2023 Laboratory end date: 05/05/2023

Test Facility

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Approvals	Dr Kathryn Dawkins (Author)	Dr Tina Berry (Reviewer)

1.0 OBJECTIVE

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

1.1 Study Scope

Using environmental DNA (eDNA) testing, eDNA frontiers was tasked with analysing water samples for the presence of *Liasis olivaceus barroni* (Pilbara Olive python). The client provided a total of 48 samples consisting of water filtrate suspended on filter membranes and preserved in ATL buffer (Tables 1 and 2). In-field control samples were provided for each site visited.

2.0 SAMPLE DETAILS

Table 1. Sample receipt details.

Date received:	15/03/2023
Transport temp:	Ambient – ATL buffer (810µL)
Number of samples:	48
Storage:	All samples were stored at -20°C prior to analysis.

Table 2. Supplied sample details.

eDNA Frontiers ID	Client Sample ID	Sample Type	Volume filtered (mL)	Collection Date
E-272-001	WPSF01-05	Water	50	9/03/2023
E-272-002, E-272-013	WPSF01-04	Water	75 & 70	9/03/2023
E-272-003	WPSF01-01	Water	100	9/03/2023
E-272-004	WPSF01-03	Water	100	9/03/2023
E-272-005	WPSF01-02	Water	20	9/03/2023
E-272-006	WMIN04-04	Water	340	8/03/2023
E-272-007	WMIN04-01	Water	675	8/03/2023
E-272-008	WMIN04-03	Water	625	8/03/2023
E-272-009	WMIN04-02	Water	750	8/03/2023
E-272-010	WMIN04-05	Water	700	8/03/2023
E-272-011	WMIN04-06	Water	1000	8/03/2023
E-272-012	WPSF01-06	Water	1000	9/03/2023
E-272-014	WCPH303-1	Water	60	10/03/2023
E-272-015	WCPH303-2	Water	80	10/03/2023
E-272-016	WCPH303-3	Water	70	10/03/2023
E-272-017	WCPH303-4	Water	80	10/03/2023
E-272-018	WCPH41-2	Water	110	10/03/2023
E-272-019	WCPH41-1	Water	90	10/03/2023
E-272-020	WCPH41-3	Water	75	10/03/2023
E-272-021	WCPH303-6	Water	1000	10/03/2023
E-272-022	WCPH41-4	Water	100	10/03/2023
E-272-023	WCPH41-6	Water	1000	10/03/2023
E-272-024	WCPH303-5	Water	80	10/03/2023
E-272-025	WCPH41-5	Water	50	10/03/2023
E-272-026	WPSF-23-1	Water	500	12/03/2023
E-272-027	WPSF-23-2	Water	530	12/03/2023
E-272-028	WPSF-23-4	Water	480	12/03/2023
E-272-029	WPSF-23-3	Water	580	12/03/2023
E-272-030	WPSF-20-1	Water	120	12/03/2023
E-272-031	WPSF-20-2	Water	170	12/03/2023
E-272-032	WPSF-20-3	Water	170	12/03/2023

eDNA Frontiers ID	Client Sample ID	Sample Type	Volume filtered (mL)	Collection Date
E-272-033	WPSF-20-4	Water	195	12/03/2023
E-272-034	WPSF-23-6	Water	950	12/03/2023
E-272-035	WPSF-20-6	Water	1000	12/03/2023
E-272-036	WPSF-23-5	Water	170	12/03/2023
E-272-037	WPSF-20-5	Water	180	12/03/2023
E-272-038	WYAN-10-1	Water	1000	13/03/2023
E-272-039	WYAN-10-2	Water	1000	13/03/2023
E-272-040	WYAN-10-3	Water	1000	13/03/2023
E-272-041	WYAN-10-4	Water	1000	13/03/2023
E-272-042	WYAN-10-6	Water	1000	13/03/2023
E-272-043	WYAN-10-5	Water	1000	13/03/2023
E-272-044	WCPH-442-1	Water	105	13/03/2023
E-272-045	WCPH-442-2	Water	130	13/03/2023
E-272-046	WCPH-442-3	Water	100	13/03/2023
E-272-047	WCPH-442-4	Water	80	13/03/2023
E-272-048	WCPH-442-5	Water	100	13/03/2023
E-272-050	WCPH-442-6	Water	1000	13/03/2023

3.0 METHODS

3.1 Sample Collection

Water samples were collected at eight locations by Biologic staff between the 9th and 13th March 2023. Five replicates were collected at each sampling point, with water samples filtered using 0.45µM 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 used to clean common filtration equipment was supplied for each site. All samples were transported at ambient temperature while preserved in ATL buffer to eDNA Frontiers' laboratories where they were stored at -20°C until scheduled for DNA extraction.

3.2 eDNA Extraction and Analysis

DNA digestion was performed on each filter paper, with half of the digest taken through for DNA extraction 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), and Stat *et al.,* (2018). Each sample was assigned an individual combination of index tags and amplified by PCR using a 16S assay targeting reptiles. A library was generated and sequenced using the Illumina MiSeq. Laboratory extraction and PCR controls were included to test for contamination.

3.3 Bioinformatics and Taxonomic Assignments

Bioinformatic tools were used to analyse raw sequence data (Mousavi-Derazmahalleh *et al*., 2021) generated from the metabarcoding. The sequencing results were demultiplexed and trimmed using Obitools and quality filtered with Usearch v11 for sequencing errors (maxee=1) with a minimum length of 70 used. 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 further filters the Blast results (evalue ≤1e-5, %identity ≥95, qCov =100, LULU minMatch =97%), combines them with the ZOTU table results and produces a table containing the taxonomic information available from Blast taxonomy database (accessed May 2023). Additionally, Geneious Prime (version 2023.1.1) was used to align any ZOTU identified as potential *L. olivaceus barroni* against the refence sequence generated by eDNA frontiers in a previous study for the client, as well as all previously recovered ZOTUs for this species.

It is important to note that while 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

4.1 Taxonomic Diversity

The species of interest, *Liasis olivaceus barroni*, was detected in one replicate from site WCPH303 (>30,000 sequencing reads) and one from site WPSF-20 (3 sequencing reads). The *L. olivaceus barroni* ZOTU detected in these samples matches the reference sequence generated in a previous study (100%), as well as sequences retrieved from project EF057 (Zotu2, 99.07% similarity; Zotu3, 100% similarity), EF123 (Zotu3, 100% similarity), and EF138 (Zotu5, 100% similarity).

In addition to the target species, several species of fish, frog, bird, and mammal were detected in the samples, as well as other species of reptile (Table 3). Taxa that had ≥95% similarity in the sequence region have been reported, with species-level classification provided for those ≥97% similarity. Although contamination was evident in field control samples, none of these detections were attributable to the target organism. Laboratory extraction controls were negative, and the positive control amplified successfully.

Table 3. Diversity detected from water samples using a 16S assay targeting reptiles. Presence of the species at each site is indicated by the symbol *. Taxonomy was assigned as per NCBI and classifications were standardised according to the Global Biodiversity Information Facility (accessed May 2023). Blank cells indicate where taxa could not be resolved to a lower taxonomic level; species-level taxonomy is only shown for matches ≥97%. Blue text indicates taxa whose distribution is not recorded to extend to the area according to GBIF. Blue highlighting indicates the species of interest. Samples appended with "-6" are control samples.

Phylum	Class	Order	Family	Genus	Species	WCPH303	WCPH303-6	WCPH41	WCPH41-6	WCPH-442	WCPH-442-6	WMIN04	WMIN04-06	WPSF01	WPSF01-06	WPSF-20	WPSF-20-6	WPSF-23	WPSF-23-6	WYAN-10	WYAN-10-6
Bryozoa	Phylactolaemata	Plumatellida	Plumatellidae	*Plumatella*	*Plumatella vorstmani*													*			
Chordata	Actinopterygii	Atheriniformes	Melanotaeniidae	*Melanotaenia*	*Melanotaenia duboulayi*							*								*	
		Perciformes	Terapontidae	*Leiopotherapon*	*Leiopotherapon unicolor*					*		*								*	
	Amphibia	Anura	Limnodynastidae	*Neobatrachus*				*													
			Myobatrachidae	*Uperoleia*								*									
			Pelodryadidae	*Litoria*		*		*	*	*		*	*	*		*		*		*	*
				Ranoidea				*		*		*	*			*		*			
					Ranoidea maini	*	*	*		*		*		*		*	*	*		*	*
	Aves	Anseriformes	Anatidae			*						*						*		*	
		Columbiformes	Columbidae	*Geopelia*	*Geopelia cuneata*	*				*						*					
		Coraciiformes	Meropidae	*Merops*								*									
		Galliformes	Phasianidae	*Gallus*						*											
					Gallus gallus	*				*											
		Passeriformes	Estrildidae	*Taeniopygia*								*									
					Taeniopygia guttata					*					*						
			Meliphagidae	*Ptilotula*														*			
			Ptilonorhynchidae	*Ptilonorhynchus*	*Ptilonorhynchus violaceus*	*				*											
		Psittaciformes	Psittacidae	*Platycercus*		*		*										*			
	Mammalia	Artiodactyla	Bovidae	*Bos*	*Bos taurus*						*					*				*	
		Dasyuromorphia	Dasyuridae	*Dasyurus*	*Dasyurus hallucatus*													*			
	-	Squamata	Pythonidae	*Liasis*	*Liasis olivaceus barroni*	*										*					
	-	Testudines	Chelidae	*Chelodina*				*													
					Chelodina steindachneri			*													
Platyhelminthes	Catenulida	-	Stenostomidae	*Stenostomum*								*									
					Stenostomum cf. simplex AW-2018							*									

5.0 SUMMARY

This analysis successfully detected the target species *Liasis olivaceus barroni* from two environmental water samples collected from sites in the Newman area. The ZOTU detected matched with 100% similarity to the reference sequence generated in a previous study. Several other taxonomic groups were also identified, including fish, frogs, birds, and mammals.

ARCHIVING OF STUDY DATA

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

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.

REFERENCES

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Stat M, John J, DiBattista JD, Newman SJ, Bunce M, Harvey ES (2018). Combined use of eDNA metabarcoding and video surveillance for the assessment of fish biodiversity. *Conservation Biology* 33(1), 196-205.

Appendix D: Vertebrate Fauna Species Recorded During the Current Survey

Site ID	Date	Latitude	Longitude	Species Name	Common Name	Abundance	EPBC Cons. Status	Obs. Method	Record Type
No site	8/03/23	-22.9477	118.9892	*Amytornis striatus*	Striated grasswren	1		Opportunistic	Individual (alive)
No site	8/03/23	-22.9375	118.9526	*Falco berigora*	Brown falcon	1		Opportunistic	Individual (alive)
No site	8/03/23	-22.8497	119.1112	*Gowidon longirostris*	Long-nosed dragon	1		Opportunistic	Individual (alive)
No site	8/03/23	-22.8493	119.1111	*Geopelia cuneata*	Diamond dove	1		Opportunistic	Individual (alive)
No site	8/03/23	-22.7865	119.1489	*Bos taurus*	European cattle	1		Opportunistic	Individual (alive)
No site	9/03/23	-22.9940	118.9728	*Litoria rubella*	Little red tree frog	100		Opportunistic	Individual (alive) (tadpoles)
No site	10/03/23	-22.9040	118.7230	*Haliastur sphenurus*	Whistling kite	1		Opportunistic	Individual (alive)
No site	10/03/23	-22.9606	118.8470	*Furina ornata*	Moon snake	1		Opportunistic	Individual (alive)
No site	10/03/23	-22.9530	118.9097	*Osphranter robustus erubescens*	Euro	1		Opportunistic	Individual (alive)
No site	10/03/23	-22.9603	118.9074	*Gehyra punctata*	-	1		Opportunistic	Individual (alive)
No site	10/03/23	-22.9626	118.9139	*Gehyra variegata*	Variegated gehyra	1		Opportunistic	Individual (alive)
No site	10/03/23	-22.9617	118.9169	*Heteronotia spelea*	Pilbara cave gecko	1		Opportunistic	Individual (alive)
No site	10/03/23	-22.9605	118.9172	*Antaresia perthensis*	Pygmy python	1		Opportunistic	Individual (alive)

Site ID	Date	Latitude	Longitude	Species Name	Common Name	Abundance	EPBC Cons. Status	Obs. Method	Record Type
VPSF-16	10/03/23	-23.0485	118.6375	*Liasis olivaceus barroni*	Pilbara olive python	1	VU	Targeted search	Individual (alive)
No site	10/03/23	-22.9596	118.9064	*Eremiascincus rubiginosus*	Rusty skink	1		Opportunistic	Individual (alive)
No site	11/03/23	-22.9604	118.9255	*Oedura fimbria*	Western marbled velvet gecko	1		Opportunistic	Individual (alive)
No site	11/03/23	-22.9608	118.9253	*Gehyra punctata*	-	1		Opportunistic	Individual (alive)
No site	11/03/23	-22.9642	118.9278	*Oedura fimbria*	Western marbled velvet gecko	1		Opportunistic	Individual (alive)
No site	11/03/23	-22.9540	118.9241	*Brachyurophis approximans*	-	1		Opportunistic	Individual (alive)
No site	12/03/23	-22.9535	118.9601	*Felis catus*	Cat	1		Opportunistic	Individual (alive)
No site	12/03/23	-22.9561	118.9498	*Taeniopygia guttata*	-	3		Opportunistic	Individual (alive)
No site	12/03/23	-22.9565	118.9493	*Pseudechis australis*	Mulga snake	1		Opportunistic	Individual (alive)
No site	12/03/23	-22.9571	118.9494	*Pseudechis australis*	Mulga snake	1		Opportunistic	Individual (alive)
No site	12/03/23	-22.9578	118.9495	*Zyzomys argurus*	Common rock-rat	1		Opportunistic	Individual (alive)
No site	12/03/23	-22.9554	118.9567	*Pteropus scapulatus*	Little red flying-fox	1		Opportunistic	Individual (alive)
No site	12/03/23	-22.9562	118.9497	*Antaresia perthensis*	Pygmy python	1		Opportunistic	Individual (alive)

Site ID	Date	Latitude	Longitude	Species Name	Common Name	Abundance	EPBC Cons. Status	Obs. Method	Record Type
No site	12/03/23	-22.9560	118.9509	*Brachyurophis approximans*	-	1		Opportunistic	Individual (alive)
No site	13/03/23	-22.9453	118.9746	*Oedura fimbria*	Western marbled velvet gecko	1		Opportunistic	Individual (alive)
No site	13/03/2023	-22.9735	118.8369	*Pseudechis australis*	Mulga snake	1		Opportunistic	Individual (alive)
No site	13/03/2023	-22.9847	118.8233	*Pseudechis australis*	Mulga snake	1		Opportunistic	Individual (alive)
VPSF-16	10/03/2023	-23.0485	118.6375	*Liasis olivaceus barroni*	Pilbara olive python	1	VU	Targeted search	eDNA
VPSF-23	12/03/23	-22.9563	118.9497	*Liasis olivaceus barroni*	Pilbara olive python	1	VU	Targeted search	eDNA
VPSF-16	10/03/2023	-23.0485	118.6375	*Cyclorana maini*	Main's frog	1		Opportunistic	eDNA
VPSF-17	10/03/23	-22.9047	118.7240	*Cyclorana maini*	Main's frog	1		Opportunistic	eDNA
VPSF-02	08/03/23	-22.8485	119.1111	*Cyclorana maini*	Main's frog	1		Opportunistic	eDNA
VPSF-15	09/03/23	-22.9940	118.9730	*Cyclorana maini*	Main's frog	1		Opportunistic	eDNA
VPSF-25	12/03/23	-22.9619	118.9438	*Cyclorana maini*	Main's frog	1		Opportunistic	eDNA
VPSF-26	12/03/23	-22.9563	118.9497	*Cyclorana maini*	Main's frog	1		Opportunistic	eDNA
VPSF-27	13/03/23	-22.7878	119.1526	*Cyclorana maini*	Main's frog	1		Opportunistic	eDNA

Site ID	Date	Latitude	Longitude	Species Name	Common Name	Abundance	EPBC Cons. Status	Obs. Method	Record Type
VPSF-16	10/03/23	-23.0485	118.6375	*Geopelia cuneata*	Diamond dove	1		Opportunistic	eDNA
VPSF-29	13/03/23	-23.0781	118.6135	*Geopelia cuneata*	Diamond dove	1		Opportunistic	eDNA
VPSF-25	12/03/23	-22.9619	118.9438	*Geopelia cuneata*	Diamond dove	1		Opportunistic	eDNA
VPSF-29	13/03/23	-23.0781	118.6135	*Taeniopygia guttata*	Zebra finch	1		Opportunistic	eDNA
VPSF-15	09/03/23	-22.9940	118.9730	*Taeniopygia guttata*	Zebra finch	1		Opportunistic	eDNA
VPSF-29	13/03/23	-23.0781	118.6135	*Bos taurus*	European cattle	1		Opportunistic	eDNA
VPSF-25	12/03/23	-22.9619	118.9438	*Bos taurus*	European cattle	1		Opportunistic	eDNA
VPSF-26	12/03/23	-22.9563	118.9497	*Dasyurus hallucatus*	Northern quoll	1	EN	Opportunistic	eDNA
VPSF-17	10/03/23	-22.9047	118.7240	*Chelodina steindachneri*	Flat-shelled turtle	1		Opportunistic	eDNA

Appendix E: eDNA Sampling

Water Feature ID	Site ID	Date	Latitude	Longitude	Sample Type (water/sediment, etc)	Sample No.	Amount of Sample Filtered	Pilbara Olive Python Detected
WPSF-01	VPSF-15	9/03/2023	-22.9940	118.9729	Water - filtered to 0.45uM	1	100	No
						2	20	
						3	100	
						4	145	
						5	50	
WMIN-04	VPSF-02	8/03/2023	-22.8485	119.1111		1	675	No
						2	750	
						3	625	
						4	340	
						5	700	
WCPH-303	VPSF-16	10/03/2023	-23.0484	118.6374		1	60	Yes
						2	80	
						3	70	
						4	80	
						5	80	
WCPH-41	VPSF-17	10/03/2023	-22.9047	118.7240		1	90	No
						2	110	
						3	75	
						4	100	
						5	50	
WPSF-23	VPSF-26	12/03/2023	-22.9563	118.9497		1	500	No
						2	530	
						3	580	
						4	480	
						5	170	
WPSF-20	VPSF-25	12/03/2023	-22.9619	118.9438		1	120	Yes
						2	170	
						3	170	
						4	195	
						5	180	
WYAN-10	VPSF-27	13/03/2023	-22.7878	119.1526		1	1000	No
						2	1000	
						3	1000	
						4	1000	
						5	1000	
WCPH-393	VPSF-29	13/03/2023	-23.0781	118.6135		1	105	No
						2	130	
						3	100	
						4	80	
						5	100	
WBEN-01	VBEN-01	27/03/2023	-22.9536	118.8321		1	1000	No
						2	1000	
						3	1000	
						4	1000	

Water Feature ID	Site ID	Date	Latitude	Longitude	Sample Type (water/sediment, etc)	Sample No.	Amount of Sample Filtered	Pilbara Olive Python Detected
						5	1000	
WJIN-01	VJIN-21	24/03/2023	-22.9200	119.1971		1	1000	No
						2	1000	
						3	1000	
						4	1000	
						5	1000	