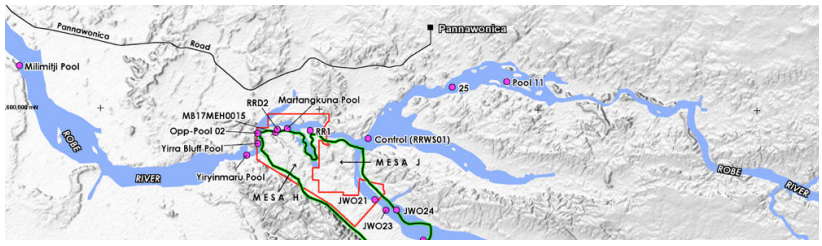
Biota Environmental Sciences

Blind Cave Eel Targeted Survey Interim Report and Assessment



Prepared for Rio Tinto

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Biota Environmental Sciences

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Blind Cave Eel Survey Interim Report and Assessment

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1.0 Introduction

1.1 Project Background

Rio Tinto is evaluating the potential development of a number of iron ore deposits within the Robe River valley in the Pilbara region of Western Australia. This includes the development of the Mesa H deposit, 15 km southwest of Pannawonica. The proposed development of Mesa H is currently being formally assessed under both the State *Environmental Protection Act 1986* (EP Act) and as a controlled action under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* (EBPC Act).

The proposed development of the Mesa H deposit has the potential to impact subterranean fauna, and Rio Tinto commissioned comprehensive surveys and an impact assessment report to inform the State and Commonwealth assessments. One of the key species identified within the area of influence of the proposal was a Threatened stygofauna species, the Blind Cave Eel (*Ophisternon candidum*) (listed as Vulnerable under both the State *Biodiversity Conservation Act 2016* and the Commonwealth EPBC Act).

Given the conservation significance of this species, Rio Tinto commissioned Biota Environmental Sciences (Biota) to carry out further targeted subterranean fauna sampling to improve on the existing data collected on the species during baseline surveys (Biota 2019a).

1.2 Scope and Purpose of this Report

This document summarises the approach, methodology and design of the Blind Cave Eel targeted survey completed to date as of December 2019 (see Section 2.2). It represents an update to the initial interim report completed for Phase 1 of the study (Biota 2019b), and provides the results from the Phase 2 sampling. It will be revised and expanded once the third stage of the targeted study is complete.

Given that new data on the Blind Cave Eel have become available since the completion of the baseline surveys, this document also provides an updated assessment of the species' distribution and habitat use in the Robe River valley, and revisits the impact assessment for the species completed by Biota (2019c). Commentary on EPBC Act significant impact guidelines is also provided.

1.3 Biology of the Species

1.3.1 Overview

The Blind Cave Eel is a depigmented subterranean fish growing up to 40 cm in length, with a long slender body, no eyes, and a thin rayless membrane around the tip of the tail (Moore et al. 2018). The Blind Cave Eel is the world's longest cavefish and one of only three vertebrate animals known from Australia that are restricted to subterranean waters (Humphreys 2001).

1.3.2 Habitat

The Blind Cave Eel inhabits groundwater systems in subterranean caves, transmissive geological formations, fissures and wells (Humphreys 2001). On Cape Range, where it was originally discovered, the Blind Cave Eel utilises cave floor sediments characteristic of crustacean-rich cave habitats. It also occurs in karst aquifers on Barrow Island (G. Humphreys, Biota, pers. obs.; Moore et al. 2018), in addition to alluvial aquifers overlying channel iron deposits (CID) in the western Pilbara; the habitat from which three specimens have been recorded in 2009, 2016 and 2018 at Bungaroo Creek (Biota 2010, 2016, 2018), and the habitat of relevance to the current report.

Limited data have been previously collected on the habitat attributes for confirmed locations where the Blind Cave Eel occurs. The original records from Cape Range come from within fresh and anchialine systems that were studied by the Western Australian Museum (Humphreys and Adams 1991, Humphreys 2001). While much of that work was primarily devoted to the Blind Gudgeon (*Milyeringa veritas*), some conclusions can also be reached for *O. candidum*, as the two species are sympatric.

On Cape Range, *M. veritas* and *O. candidum* both occur in a range of varying salinity waters from fresh to brackish (from 0 to 16 parts per thousand; Humphreys and Adams 1991), which are typically neutral pH (6.8-7.6). Humphreys (2001) reported further that *M. veritas* occurred in a range of oxygen levels from highly oxygenated superficial waters through to hypoxic conditions dominated by sulphur-reducing bacteria. As it is likely that the Blind Cave Eel occupies a similar range of niches, this is indicative of relatively broad environmental tolerance. At Bungaroo Creek, the groundwater is essentially fresh, slightly acidic-neutral and with relatively high dissolved oxygen (Biota 2013).

Past observations from specimens collected alive suggested that the species can survive in suitable conditions outside of subterranean habitats. Past *O. candidum* specimens collected by the Western Australian Museum have been successfully kept alive for several days in aquaria. The first specimen from Bungaroo Creek was similarly collected live, transported to Perth and kept alive in freshwater without special treatment for several days (G. Humphreys, pers. obs.). Subsequent records of specimens from surface water pools in the Robe River support the observations that the species is not wholly obligate to subterranean habitats (see Section 3.1).

1.3.3 Documented Ecology

The Blind Cave Eel has a diet that consists of small aquatic crustaceans, particularly decapods and thermosbaenaceans, though the species is apparently capable of opportunistically taking accidentals from surface sources (Humphreys and Feinberg 1995).

However, little is known of the species' life history, home range, dispersal or reproductive biology. It is thought to generally forage in bottom sediments in cave systems in Cape Range (Humphreys and Adams 1991), and similar foraging behaviour may occur in alluvial systems on the mainland Pilbara. Foraging range or reliable estimates of abundance cannot be commented on from the limited data available, though as larger-sized subterranean predators, first principles suggest they are unlikely to occur at high population densities.

1.3.4 Previously Known Distribution

The Blind Cave Eel has only been recorded from three localities: the Cape Range peninsula, Barrow Island (assuming the record from the island is the same taxon; Humphreys et al. 2013), and Bungaroo Creek on the mainland Pilbara (Biota 2010).

Genetic analysis has demonstrated that the species present at Bungaroo Creek is the same as that on Cape Range (Foster and Humphreys 2011). That result implied that it is likely that the species occurs further downstream from Bungaroo, and is probably associated with the regional aquifer of the Robe River and Jimmawurrada Creek (to which Bungaroo Creek is a tributary). This hypothesis has since been supported with additional data (see Section 3.1).

The hypothesis of wider distribution within the Robe River catchment is also supported by distribution patterns in other stygofauna species, with some taxa sympatric with the Blind Cave Eel on Cape Range and Barrow Island also present in the headwaters of the Robe River (Biota 2010). The thermosbaenacean *Halosbaena tulki* is particularly noteworthy in this respect as it is found at Bungaroo Creek, but also at Cape Range and on Barrow Island (Biota 2009a), and represents a key prey item for the Blind Cave Eel (Section 1.3.3).

2.0 Methodology

2.1 Survey Timing and Personnel

Two phases of targeted sampling for the Blind Cave Eel have been undertaken to date, of a planned three-phase study. The first two field exercises were completed between 5th and 10th August 2019 (Phase 1) and 29th October and 4th November 2019 (Phase 2). The third phase of sampling is currently planned to follow a major recharge event in the system, assuming a significant summer rain event occurs in early 2020.

The field work completed to date was undertaken by Jason Alexander, Penny Brooshooft and Scott Werner of Biota on Phase 1, and Jason Alexander, Michael Greenham (of Biota) and Dr Zoë Hamilton (Helix Molecular Solutions (Helix)) on Phase 2. Yvette Hitchen (Helix) carried out the laboratory analysis of environmental DNA (eDNA) samples from both phases. Overall planning and coordination of the study was provided by Garth Humphreys (Biota), in consultation with Rio Tinto.

2.2 Sampling Design and Effort

Sampling design for the study focused on the Robe River catchment, including Jimmawurrada Creek and Bungaroo Creek, both of which have alluvial aquifer systems connected to the Robe River itself. The species has been previously demonstrated to occur in all three systems during baseline studies (Biota 2019a), and focussing effort on the broader Robe River catchment was therefore the logical spatial scope to attempt to widen the known distribution of the Blind Cave Eel.

Given that the previous records of the species had come from both groundwater bores and surface water pools within the drainage systems (Biota 2019a), a selection of 37 sites were sampled incorporating both surface pools (n=14) and groundwater bores (n=23). These spanned the length of the wider Robe River catchment, from the headwaters through to the coastal plain (Figure 2.1). The sites were all associated with the main drainage landforms and geological units that have previously yielded records of the species, and were selected in collaboration with Rio Tinto and in consultation with Dr Glenn Moore, Curator of Fishes at the Western Australian Museum. The majority of sites were sampled on both the Phase 1 and Phase 2 mobilisations, with two pools dry and unable to be sampled on Phase 2 (NWCH Pool and Jimma Pool), and an additional pool added in Phase 2 (Opportunistic Pool 02) (Figure 2.1).

2.3 Sampling Techniques

Sampling was conducted using a combination of conventional sampling (Section 2.3.1) and eDNA methods (Section 2.3.2). eDNA approaches rely on the collection and assaying of environmental samples that contain residual DNA that has been shed into the environment from target species. There is a rapidly growing number of studies that have successfully used eDNA methods to indirectly detect the presence of aquatic species in marine, estuarine and freshwater systems (Lodge et al. 2012, Minamoto et al. 2012, Thomsen et al. 2012). The approach has also been demonstrated to work in groundwater systems (Biota and Helix 2014), and for the Blind Cave Eel in particular (Biota 2019a).

2.3.1 Stygofauna Sampling

Conventional stygofauna sampling was conducted at borehole sites in an attempt to collect eel specimens using modified plankton haul nets, constructed from 70 µm plankton mesh, with 50 mm and 100 mm apertures attached to a stainless steel catch cylinder.

Nets were lowered to the bottom of water bores and drill holes before being hauled slowly through the water column to the surface, where the contents of the cylinder were flushed into a uniquely labelled container. Each site was sampled in this way a minimum of five times. On the final haul, the net was agitated gently to stir the benthos layer and mobilise any fauna present for more effective specimen collection. Specimens were stored in a shaded esky in order to keep the samples cool prior to sorting in an onsite laboratory.

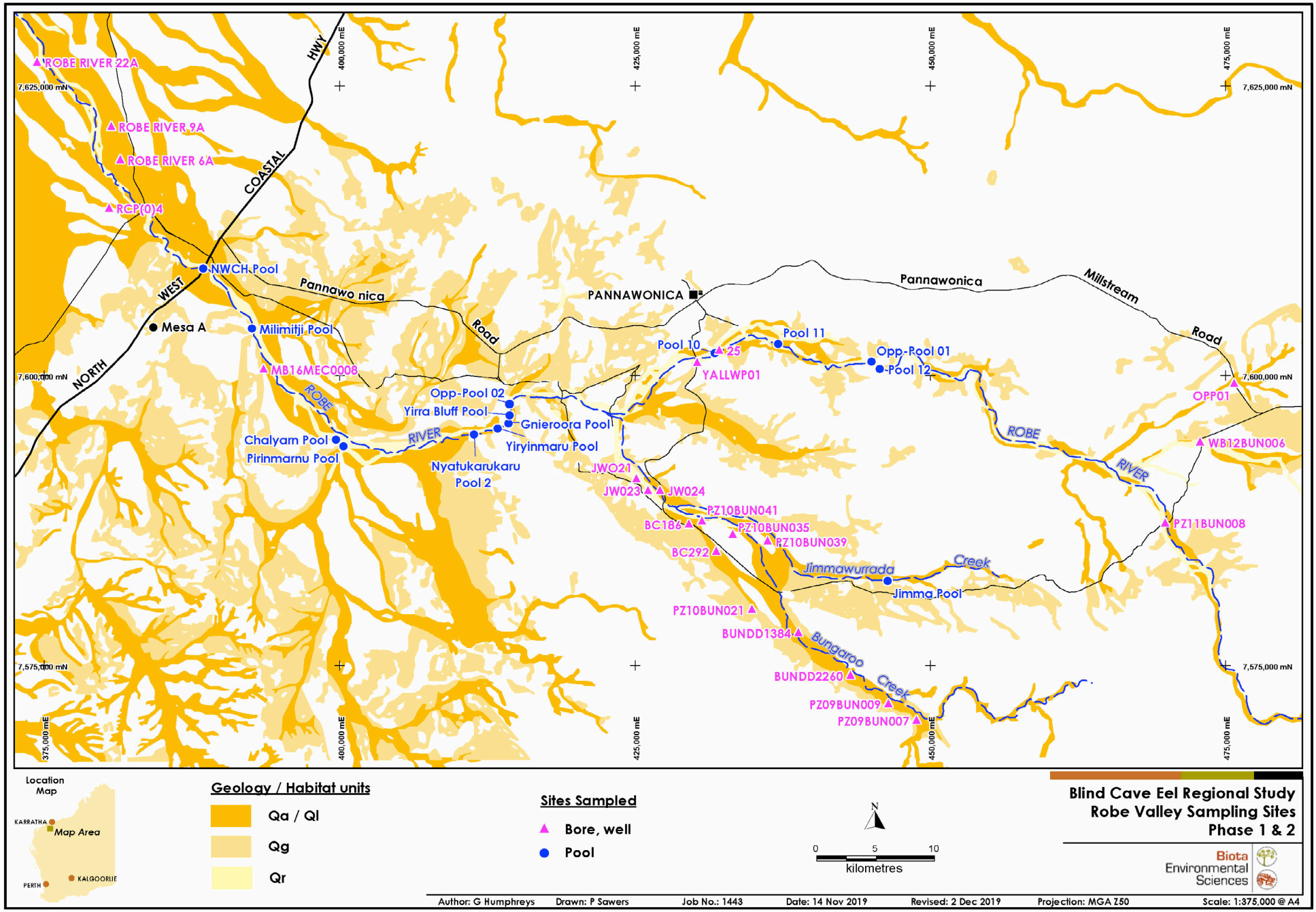

Figure 2.1: Groundwater bore and surface water pool sampling sites for Phase 1 and 2 of the targeted Blind Cave Eel survey.

2.3.2 eDNA Sampling and Sample Processing

eDNA sampling took place on completion of stygofauna haul net sampling at bores and at each of the surface water pool sites. This sampling initially included agitation of the benthos at the base of the borehole, to mobilise DNA molecules and fragments of tissues within the water column. Then, wearing nitrile gloves, a 1 L bailer attached to fishing line, was lowered into the bore until approximately 1 m from the bottom of the hole. The bailer was then removed from the bore and the contents of the bailer emptied into a brand new and uniquely labeled 1 L container. This step was repeated twice more. The line was then discarded after bailing to prevent contamination of eDNA between sites. Samples were collected at surface pool sites by hand dipping brand new sample containers into the pool, while again wearing nitrile gloves. All sample containers were stored in the field in an ice-filled esky until arrival at the on-site laboratory in Pannawonica.

Water samples collected for eDNA analysis were filtered on site through 0.45 µm sterile filter membranes, using both Sentino Microbiology Pumps and manually using specialised Nalgene Filter Flasks and a hand pump. One container of groundwater (1 L) was processed per membrane and on completion of this process, the membrane was folded in half then cut vertically to provide for replication. All laboratory equipment was sterilised between samples using a bleach solution.

2.4 eDNA Analysis

Helix analysed half of the replicate membranes from each sample site using a real-time qPCR method developed previously for the Blind Cave Eel (Biota and Helix 2014). Sequence data from past collections of *O. candidum* from the Jimmawurrada and Bungaroo Creek locality was used to design a species-specific probe using the Integrated DNA Technology (IDT) design tool PrimerQuest and further edited using Oligo Primer analysis software version 6 (Molecular Biology Insights, Cascade, USA). The resulting assay amplified a diagnostic 80 bp fragment of the mitochondrial cytochrome oxidase subunit I gene (COI) (Biota and Helix 2014).

Samples were extracted using the QIAGEN Blood and Tissue Kit (QIAGEN, Hilden, Germany) and, as with previous studies, all starting volumes were doubled to ensure that the filter membrane was covered during incubation. All samples had two elutes of 50 µl each.

The specifically designed qPCR assay was then used to detect the presence of *Ophisternon* DNA. Quantitative real-time PCR assays were performed using the Applied Biosystems StepOne Plus real-time PCR system and software (Biota and Helix 2014). The assay identifies the presence of the target species by the unique fluorescent signal produced during the polymerase chain reaction when both the species-specific primers as well as species-specific probes match DNA present in the sample. The fluorescent response can be visualised in real time as amplification proceeds.

Only samples that showed a positive CT value (the number of cycles required for the fluorescent signal to exceed the background level of fluorescence) and amplification plot were considered to test positive for the target *Ophisternon* DNA.

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3.0 Updated Assessment

3.1 Current Records and Distribution

On completion of the baseline surveys for the Mesa H development, Blind Cave Eel records had been obtained from nine sites, expanding the known range of the species at that time and demonstrating that it occurs within all of Bungaroo Creek, Jimmawurrada Creek and the Robe River (Biota 2019a). The baseline records included both actual specimen collections and eDNA positive water samples, and came from both groundwater sampling and surface pool sampling in Jimmawurrada Creek and the Robe River (Biota 2019a). This is in addition to populations of the same species being present on Cape Range and on Barrow Island.

Phase 1 of the targeted survey work, and other biological sampling conducted in the locality, increased the known number of locations at which the species occurs in the Robe River catchment to 11 sites, with the addition of:

1. an eDNA record from Phase 1 of the targeted survey at Milimitji Pool on the Robe River, 26 km downstream of the previous most western record of the species (Figure 3.1; Plate 3.1); and
2. a specimen collected during aquatic fauna sampling at Martangkuna Pool in September 2019, also on the Robe River (Figure 3.1; Plate 3.2; Wetland Resource Management (WRM) in prep.).

Plate 3.1: Milimitji Pool on the Robe River where Blind Cave Eel DNA was detected during Phase 1 of this study (source: WRM).

Plate 3.2: Blind Cave Eel specimen collected during surface water sampling in the Robe River in September 2019 (source: WRM).

Phase 2 of the targeted survey further increased the known number of locations at which the species occurs in the Robe River catchment, with the addition of five new locations:

1. four new surface water pool sites, comprising from east to west: Yiryinamaru Pool (Plate 3.3; Figure 3.1), Yirra Bluff Pool, Opportunistic Pool 02 and Pool 11 (Plate 3.4); and
2. a groundwater sample collected from Opportunistic Bore 01 (OPP01), in the headwaters or the Robe River, representing the easternmost record for the species to date (see Figure 3.1).

Plate 3.3: Yiryinmaru Pool on the Robe River.

Plate 3.4: Pool 11 on the Robe River.

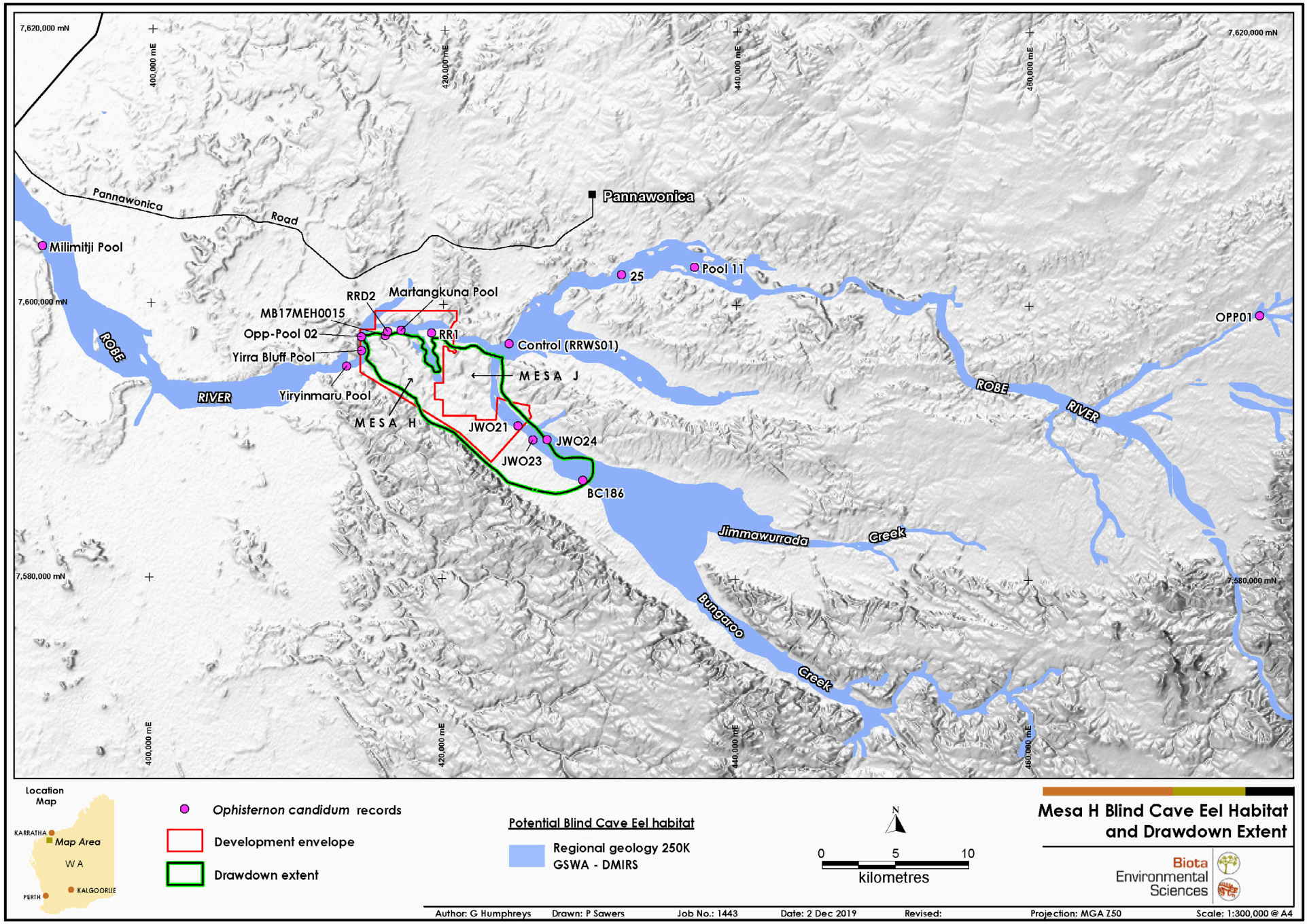

Figure 3.1: All locations in the Robe River catchment where the Blind Cave Eel has been recorded to date and putative habitat.

Both the previous baseline surveys and the current targeted survey completed for the Mesa H proposal have substantially improved the overall knowledge base for the species in the west Pilbara (Moore et al. 2018, Biota 2019a). In 2009, the species was originally only known from a single location in Bungaroo Creek (BC186) (Biota 2009b), and the work completed since that time has now shown the species to be present at 15 additional sites. Specimen records and eDNA evidence also indicate the species occurs within not only the Bungaroo Creek alluvial aquifer but also in the Jimmawurrada Creek and Robe River alluvial aquifers (Biota 2019a).

Further, it is becoming increasingly certain that the species utilises the shallow alluvial, surface habitats of the Robe River, as well as groundwater: one of the baseline eDNA records for the species came from the Control site sampled by Biota (2019a). This site was a surface water pool on the Robe River and consistently yielded eDNA detections for the species from multiple replicate samples (Helix 2018). Additional evidence was provided by the subsequent collection of an additional specimen from the phreatic zone of the Robe River during 2018 aquatic fauna sampling by WRM in a surface pool in the river (Biota 2019a). The two new locations at which the species was recorded during Phase 1 of this study further supported its use of the river surface aquatic habitats, with a specimen collected from another surface pool north of Mesa H (Plate 3.2) and DNA detected at another pool on the Robe River, 26 km downstream of Mesa H during the current study (Figure 3.1). The Phase 2 results were even clearer in this respect, with four additional permanent pools along the Robe yielding eDNA detections for the species (Section 3.1).

These findings demonstrate that the species utilises shallow groundwater habitats in the alluvium of the Robe River, including the phreatic zone, and this ecology may contribute to maintenance of gene flow and population connectivity within the species' overall range; explaining the high level of genetic similarity amongst specimens sequenced to date from the Robe River catchment (Moore et al. 2018, Biota 2019a). This ecological model is also consistent with the Robe River alluvium habitat hypothesis of Moore et al. (2018) and, interestingly: also consistent with gut content analysis of Cape Range specimens that included surface invertebrates such as dragonfly larvae (Humphreys and Adams 1991), again possibly suggesting the use of surface or near-surface aquatic habitats by the species, or at least superficial enough for accidentals from surface sources to be preyed upon.

Lastly, the records show a high spatial correlation to the geological mapping units identified by Biota (2019a) as prospective for the species. Taking account of all confirmed record locations, the distribution of these alluvium units is also consistent with a model of suitable connected habitat for the species occurring along the length of the Robe River and upstream into at least the lower reaches of its tributaries (see Figure 3.1). All records obtained from this Robe River catchment population are summarised in Table 3.1.

Table 3.1: History of all records of the Blind Cave Eel to date from the Robe River catchment.

Site	Site Type	Easting	Northing	Date	Record Type
BC186	Bore	429578	7587212	7/11/09	Specimen
				12/12/17	eDNA positive
JW023	Bore	426138	7590140	22/9/16	Specimen
JW024	Bore	427126	7590154	1/9/17	Specimen
				12/12/17	eDNA positive
				1/6/18	Specimen
				30/9/18	Specimen
JW021	Bore	424138	7589754	10/5/17	eDNA positive
Control (RRWS01)	Surface pool	424478	7597147	12/12/17	eDNA positive
25	Bore	432152	7602229	12/12/17	eDNA positive
MB17MEH0015	Bore	416041	7597690	12/12/17	eDNA positive
RR1	Bore	419176	7597904	12/12/17	eDNA positive
RRD2	Surface pool	416414	7597820	31/5/18	Specimen
Milimitji Pool	Surface pool	392584	7604120	7/8/19	eDNA positive
Martangkuna Pool	Surface pool	417110	7598094	8/9/19	Specimen
Yiryinamaru Pool	Surface pool	413393	7595467	30/10/19	eDNA positive

Site	Site Type	Easting	Northing	Date	Record Type
Yirra Bluff Pool	Surface pool	414417	7596611	30/10/19	eDNA positive
Opportunistic Pool 02	Surface pool	414412	7597603	30/10/19	eDNA positive
Pool 11	Surface pool	437151	7602792	30/10/19	eDNA positive
Opportunistic Bore 01 (OPP01)	Bore	475760	7599356	31/10/19	eDNA positive

3.2 Potential Impacts

3.2.1 Mesa H Context

As discussed in the subterranean fauna impact assessment for Mesa H (Biota 2019c), there are five sites from which the Blind Cave Eel has been recorded that fall within the development's predicted drawdown extent (MB17MEH0015, BC186, JW021, JW023 and JW024; Figure 3.1). While strictly speaking an impacted site, the predicted maximum drawdown at impact site MB17MEH0015 is less than 1 m, which is insignificant compared to the volume and habitat extent of the Robe River which this site is connected to (see Figure 3.1). The impact assessment completed identified that even within the drawdown portion of Jimmawurrada Creek, the alluvial aquifer habitat where the species has been recorded within will retain a substantial saturated thickness along the length of the creek at the peak of groundwater drawdown for the project in 2030 (Biota 2019c).

Figure 3.2 revisits this, showing the four sites where the Blind Cave Eel has been recorded within the drawdown extent on Jimmawurrada Creek, illustrating that a continuous and connected habitat varying between 5 and 17 m thick will remain within the system at the peak of dewatering. This is in addition to the underlying saturated CID, which is in fact structurally more similar to the karst rocks used in other settings by the species, and may also provide potential habitat for the species; particularly during periods when refugia may be needed.

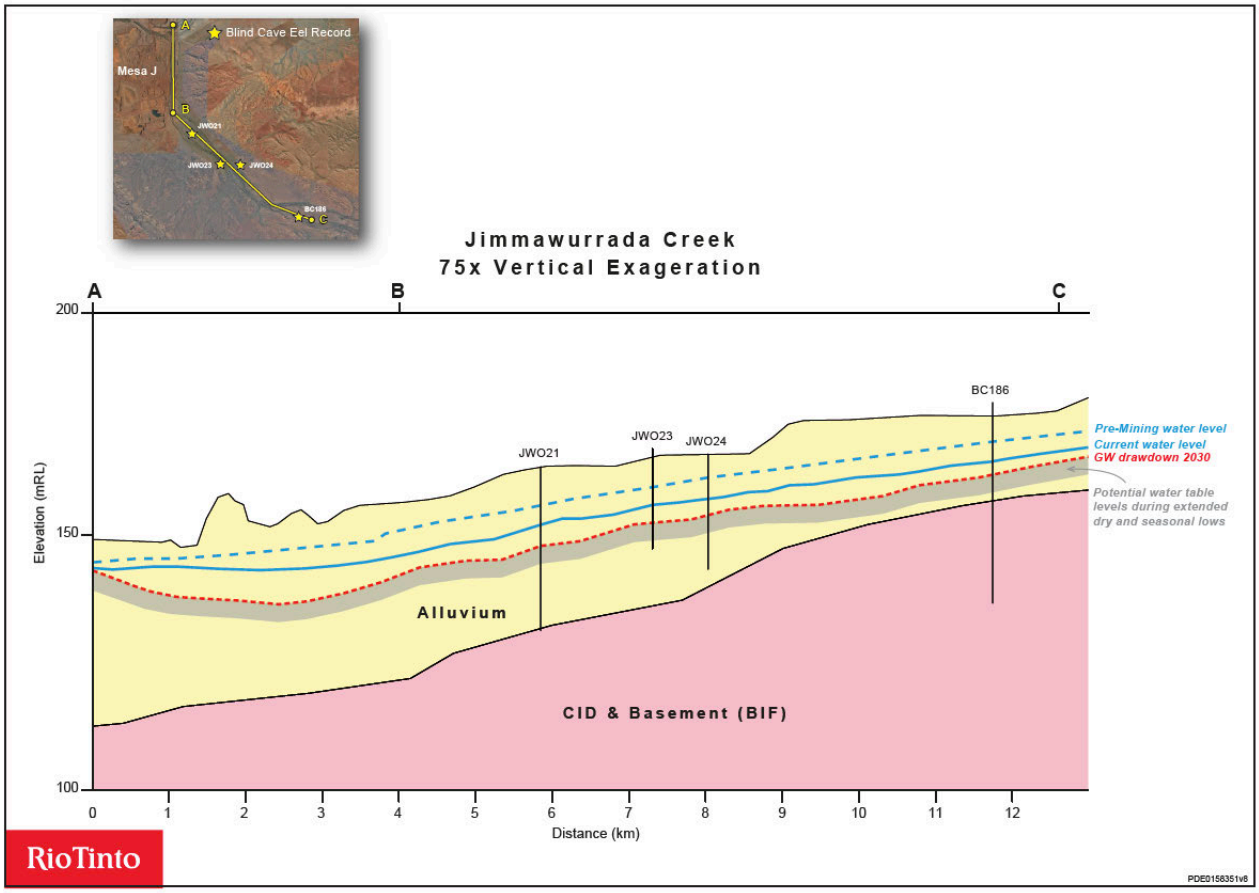

Figure 3.2: Longitudinal cross-section along Jimmawurrada Creek area, showing pre-mining water table (dashed blue line), current water level (blue line), predicted maximum drawdown from the proposal in 2030 (dashed in red) and alluvial and CID stygofauna habitats that will remain saturated (below the red dashed line) (source Rio Tinto).

3.2.2 New Data and Updated Assessment

The new data from Phase 1 and 2 of this study provide improved context to potential impacts on the Blind Cave Eel, particularly the records from Milimitji Pool (Phase 1; (Biota 2019b)) and Opportunistic Bore 01 (Phase 2). If extent of habitat is considered in its most conservative form, as a relatively linear extent following the major drainage systems, then the Phase 1 results doubled the linear area of occupancy for the species in the Robe River from a 25 km stretch of alluvial habitat to a 51 km extent (Biota 2019b). That result in itself strongly suggested the species does actually occur along the entire length of the Robe River as previously hypothesised, from east of site 25 in the upper reaches of the Robe River to the Pilbara coast (the extent of the targeted surveys sampling regime; Figure 2.1). The continuing collections of both specimen and eDNA records from surface water pools within the Robe River support this, and there are no hydrological or physiographic barriers along the course of the river that would suggest any form of aquatic habitat discontinuity.

Given the ongoing incremental knowledge gain from survey effort in the locality, Biota (2019b) predicted that with additional sampling effort, this distribution would be expanded both toward the coast and further inland in the river's headwaters. This was validated to the east by the most recent Phase 2 results, in particular with the new records at Pool 11 and Opportunistic Bore 01 further toward the headwaters of the Robe River (Figure 3.1). The total linear extent of habitat demonstrated for the species has now increased from 25 km during the baseline surveys, through the 51 km extent shown by Phase 1 of this study (Biota 2019b), to 103 km from Milimitji Pool to Opportunistic Bore 01 with the latest Phase 3 findings. The implication from an area of occupancy perspective is that it now appears likely that the linear habitat extent for the species will continue to grow and eventually equate to the majority of the extent of the Robe River.

At the minimum precautionary case, the data now show that the habitat mapped for the species in the Robe River catchment is supported. In the interests of providing a conservative assessment, the area of this habitat was curtailed to the spatial limits at which the species has been proven to occur (Milimitji Pool, BC186 and Opportunistic Bore 01; Figure 3.1). GIS calculation places the extent of this mapped habitat, which includes the Robe River and the lower reaches of Bungaroo and Jimmawurrada Creeks, at 19,175 ha, centred on the major drainage channels. The maximum extent of the drawdown for Mesa H (shown in green on Figure 3.1) represents 1,131 ha or less than 6% by area of the known habitat of the Robe River catchment population of the species. It must also be recognised that, as shown in Figure 3.2 above, even within this impacted area, the habitat availability will only be reduced during dewatering activities, not entirely or permanently lost, as the habitat physical structure will not be physically disturbed in any way and will be become saturated again when groundwater levels recover after closure.

Further, the real consideration is volume of saturated habitat available to the overall Robe River catchment population: while hydrogeological modelling for the entire Robe River catchment does not exist, data from past drilling and localised modelling of the system indicate that the Robe River alluvium stores massive amounts of water even under drought conditions (A. Russo, Rio Tinto, pers. comm. 2019), and that the main channel of the Robe River is likely to be the core and highest quality habitat for this population of the species.

Lastly, in regards to the affected area of habitat, it is also valuable to consider the repeated records of the species from this section of Jimmawurrada Creek over a nine-year period under varying conditions and depths to water table (Table 3.2). Water table levels fluctuate naturally in response to rainfall in the west Pilbara, and the species has clearly persisted at even the individual site level over a range of groundwater conditions.

Table 3.2 shows the water table levels at the time Blind Cave Eel have been recorded for those sites with multiple records of this species. This shows that falls in groundwater levels of 3-6 m have occurred and the species has demonstrably remained present.

Table 3.2: Range of groundwater levels in Jimmawurrada Creek impact bores when Blind Cave Eel have been repeatedly recorded, compared to measured groundwater levels.

Site	Blind Cave Eel record date	Groundwater level at that time (m AHD)	Depth below ground level (m)	Range
BC186	7/11/09	179	2.73	5.66 m
	12/12/17	173	8.39	
JW024	1/9/17	161	8.64	3.02 m
	12/12/17	160	9.11	
	1/6/18	159	10.04	
	30/9/18	158	11.66	

The above data suggests resilience in response to fluctuating conditions in the aquifer and surface systems, and population persistence even during drought conditions. Ecologically, this is probably a function of the animal's high degree of mobility and that it is readily able to undertake local movements, even over relatively dry surfaces (G. Humphreys, pers. obs. and as noted in Moore et al. (2018)). The species has also been shown to occur across a range of physicochemical conditions in aquatic habitats (Humphreys and Adams 1991). These observations are consistent with the long term persistence of the species in the Mesa H locality for at least the last 10,000 years (Moore et al. 2018); presumably during many historical droughts and consequent natural low water table conditions. The current drought conditions have actually been informative to the assessment in this respect, as the species continues to be recorded from both groundwater and surface pool habitats even though 2017-2019 has represented a long-term low rainfall period for the Robe River catchment (A. Russo, Rio Tinto, pers. comm. 2019).

Data from one of the species record sites also provides additional evidence of resilience in the species (site 25; Figure 3.1). This bore is part of the Pannawonica town bore field, which is subject to a low level of groundwater drawdown itself, being pumped for water supply (Rio Tinto 2016). This alluvial aquifer habitat has been abstracted from since 1981 (Rio Tinto 2016), which demonstrates both the significant recharge capacity of the Robe River alluvial aquifer and, by inference, that the Blind Cave Eel is at least tolerant of this level of groundwater impact over several decades, with the bore field having being in operation for 37 years at the time the recent eDNA record was obtained (Biota 2019).

3.3 EPBC Act Significant Impact Guidelines

3.3.1 Policy Framework

This concluding section provides an assessment of the Mesa H development in regards to the significance of the predicted impacts of the action under the terms of the EPBC Act. Specifically, this considers the potential impact of dewatering on the Blind Cave Eel, and we follow a structured assessment based on the framework of the Act's significant impact guidelines (DotE 2013).

In assessing the significance of the impact of the groundwater drawdown on the Blind Cave Eel, the specific circumstances of the proposed action must be considered. The definition of a significant impact under the EPBC Act considers whether the impact is "*…important, notable, or of consequence, having regard to its context or intensity. Whether or not an action is likely to have a significant impact depends upon the sensitivity, value, and quality of the environment which is impacted, and upon the intensity, duration, magnitude and geographic extent of the impacts*".[1]

The EPBC Act significant impact guidelines (DotE 2013) then set out specific criteria to assist proponents with considering this definition of significance for the Threatened Species and Communities controlling provision, and whether actions impacting on identified populations are significant for the purposes of the Act. These criteria are predicated on the population of the Vulnerable species in question being an 'important population' and that the action will impact on 'habitat critical to the survival of the species', as defined in DotE (2013).

[1] https://www.environment.gov.au/epbc/about/glossary#significant

3.3.2 Evaluation

The population of Blind Cave Eel in the Robe River catchment clearly meets the DotE (2013) definition of an important population, as it is "*a population that is necessary for maintaining genetic diversity*" and a "*population near the limit of the species range*" (DotE 2013).

The second defined component in considering the significant impact criteria relates to whether the action would affect habitat critical to the survival of the species. Again, the alluvial aquifer of Jimmawurrada Creek within the predicted drawdown extent meets at least two of the requirements, as this habitat is necessary "*…for activities such as foraging, breeding, roosting, or dispersal*" and to "*maintain genetic diversity and long term evolutionary development*" (DotE 2013). The affected portion of the creek system and its associated alluvial aquifer would therefore be considered habitat critical to the survival of the species for the purposes of the Act.

The significant impact criteria for a Vulnerable species can then be considered in the framework that an important population is present and that an area of habitat critical to the survival of the species may be affected. Each of the nine criteria defined by DotE (2013) are evaluated on the basis of the available data below.

An action is likely to have a significant impact on a Vulnerable species if there is a real chance or possibility that it will:

1. **Lead to a long-term decrease in the size of an important population of a species**

   Dewatering for Mesa H will initially commence for approximately a year's duration in 2025, and then re-commence in 2030 for a further seven years (Rio Tinto 2019). While dewatering at Mesa J will cease by 2029 and abstraction from the Southern Cutback bore field will continue until 2037 with variable rates and frequencies to meet operational water demand, with reduced levels of drawdown, the peak of dewatering will be reached in 2037, with lower levels of drawdown affecting a portion of Jimmawurrada Creek until that time. Considering that over 90% of the Robe River catchment population's habitat will be entirely unaffected by this, and that the area that will be affected will retain saturated habitat even at the peak of dewatering, there is little objective evidence to suggest that sufficient individuals would be lost from the population such that it would result in a long term decrease in population size.

   The duration of the dewatering will be a relatively small number of years, not dissimilar to the duration of drought periods that have occurred in the region (the current drought conditions are now already 2-3 years), which the species has clearly experienced multiple times over the long term.

2. **Reduce the area of occupancy of an important population**

   The partial dewatering of less than 6% of the Robe River catchment population's minimum habitat extent will not affect the overall area of occupancy of the population as currently understood. As discussed in Section 3.2.2, the species occurs along a 100 km plus length of the Robe River and has an overall area of occupancy estimated at 19,175 ha. A relatively small proportion (less than 6%) in the southeast of this area of occupancy will be temporarily and partially dewatered, but the overall extent of the Robe River catchment population's habitat and area of occupancy will not be reduced.

3. **Fragment an existing important population into two or more populations**

   The only potential mechanism by which the Mesa H development could fragment the Robe River catchment population is dewatering. Alluvial habitat and major drainages will not be directly impacted or interrupted by the project, and existing patters of surface hydrology in the river and creek channels will be maintained. This will not only provide recharge to underlying aquifers, but will provide the same periodic connectivity of surface water pools and saturation of surface alluvium that currently occurs in the catchment's drainage lines. It is highly plausible that it is these events that contribute to, or entirely enable, the dispersal of individuals along the catchment the population occupies, particularly for juvenile individuals given the high flow rates. Genetic data support this model, with the individual specimens sequenced to date being genetically very similar (Biota 2019a), as does the

recent evidence that the species routinely uses surface pools (Section 3.2.2). If such events do provide the primary means of dispersal and maintenance of gene flow within the population, then this will continue unchanged.

Even in the event that surface and near surface recharge and flood events do not function in this way, and that gene flow within the population is mediated by individuals moving at depth in the saturated alluvium, the dewatering still will not result in any barrier to gene flow. At the peak of dewatering, the habitat to the southeast in Bungaroo Creek will remain connected through to the Robe River via saturated alluvium 5-17 m thick (Section 3.2.2). In the longer term, the population will again be fully connected through this area, as the physical structure of Jimmawurrada Creek will not be affected and will become fully saturated habitat again after significant rainfall events and post-closure (Section 3.2.2).

4. **Adversely affect habitat critical to the survival of a species**

   The proposed Mesa H development will adversely affect a portion of known habitat in Jimmawurrada Creek. While this meets one of the significant impact criteria, the potential impact needs to be considered in context as required by the overall definition of a significant impact in the EPBC Act (Section 3.3.3).

5. **Disrupt the breeding cycle of an important population**

   The breeding cycles of the Blind Cave Eel are poorly understood. However, as discussed in response to Criterion 3 above, breeding cycles will clearly be linked to the maintenance of connected aquatic habitat along the catchment, both in surface and alluvial aquifer settings. As discussed above; as this will occur, and over 90% of the population's habitat will not be affected at all, there is no reason to expect the breeding cycle of the population to be disrupted. There is also evidence of current breeding occurring even though relatively low water table levels (two juvenile eels collected in 2018 from Jimmawurrada Creek; Table 3.1), again indicating the resilience of the species to environmental change.

6. **Modify, destroy, remove, isolate or decrease the availability or quality of habitat to the extent that the species is likely to decline**

   Genetic work has demonstrated that the listed Vulnerable species, *Ophisternon candidum*, occurs at Cape Range, Barrow Island and in the Robe River catchment (including the Robe River itself, and its tributaries of Bungaroo Creek and Jimmawurrada Creek) (Moore et al. 2018, Biota 2019a). It is the same species across this entire area of occupancy. The proposal will have no effect whatsoever on the populations of the species at Cape Range and on Barrow Island, nor will it have any impact on the majority of the Robe River population's habitat, with the entirety of the Robe River habitat, and the majority of Jimmawurrada and Bungaroo Creeks habitat, unaffected. It is therefore very unlikely that the impact on the habitat within a section of Jimmawurrada Creek would cause the entire species to decline. This is particularly so when one considers that even within the affected area, habitat availability will only be reduced for a finite period, and that in this extent of the creek 5-17 m of alluvium overlaying potentially suitable CID habitat will remain below water table at the peak of dewatering (Section 3.2.2).

7. **Result in invasive species that are harmful to a vulnerable species becoming established in the vulnerable species' habitat**

   There is no pathway by which the dewatering of the Mesa H deposit could lead to an invasive species that might be harmful to the Blind Cave Eel becoming established in the section of Jimmawurrada Creek within the dewatering influence.

8. **Introduce disease that may cause the species to decline**

   There is no pathway by which the dewatering of the Mesa H deposit could lead to the introduction of a disease that would cause the Blind Cave Eel to decline.

9. **Interfere with the recovery of the species**

   There is no recovery plan for the Blind Cave Eel, as it has been deemed that one is not required for the species[2], and the proposed action would therefore not interfere with any recovery actions.

The above evaluation of the significant impact criteria for the Blind Cave Eel in respect of the proposed drawdown for the Mesa H proposal are summarised below in Table 3.3.

Table 3.3: Review of DotE (2013) significant impact criteria for Vulnerable species as they relate to the Mesa H proposal and the Blind Cave Eel.

Significant Impact Criteria	Met?
1. Lead to a long-term decrease in the size of an important population of a species?	No
2. Reduce the area of occupancy of an important population?	No
3. Fragment an existing important population into two or more populations?	No
4. Adversely affect habitat critical to the survival of a species?	Yes
5. Disrupt the breeding cycle of an important population?	No
6. Modify, destroy, remove, isolate or decrease the availability or quality of habitat to the extent that the species is likely to decline?	No
7. Result in invasive species that are harmful to a vulnerable species becoming established in the vulnerable species' habitat?	No
8. Introduce disease that may cause the species to decline?	No
9. Interfere with the recovery of the species?	No

3.3.3 Conclusion

This review finds that eight of the nine significant impact criteria for Vulnerable species are not met (Table 3.3), and the Mesa H development would not have a significant impact on the Blind Cave Eel in respect of those considerations.

The only significant impact criterion that appears relevant relates to adverse effects on habitat critical to the survival of the species (Table 3.3). Although a portion of habitat will be cumulatively affected by the Mesa H development, the scale, nature and context for this must be taken into account. Less than 6% of what is conservatively estimated as the population's area of occupancy will be adversely affected, and even this will still remain viable habitat for the period of the dewatering. At the broader scale, it appears very likely that the species' local distribution is still underestimated and that the context for the adverse impact should be revised again once further information is to hand. As discussed in Section 3.3.2 above, the context for this impact is that:

- the habitat affected is spatially limited to at most 6% of the local extent for the population;
- even within this habitat, the impact will only temporarily desaturate a portion of the habitat from a vertical or volumetric perspective;
- major rainfall events will likely offset this predicted worse-case level of desaturation; and
- the broader context for the Robe River catchment population is that the major and core habitat for the species in the locality is the saturated alluvium along the main channels of the Robe River itself – the true habitat critical to the survival of the species – which data now indicates covers a 100 km plus length of the system, and will remain completely unaffected by the groundwater drawdown arising the Mesa H project.

We therefore conclude that as almost all significant impact criteria would not be met, and the adverse affects on habitat critical to the survival of the species are localised and partial in scale, the potential impacts from the proposal do not appear to be significant, as defined within the policy framework of the EPBC Act.

[2] https://www.environment.gov.au/cgi-bin/sprat/public/publicspecies.pl?taxon_id=66678

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Appendix 1

Helix Molecular Solutions Interim Report

Helix Molecular Solutions

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28 November 2019

Garth Humphreys
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Via email

Re. Helix Job 576 - Preliminary report for the analysis of environmental water samples for the presence of *Ophisternon candidium* DNA from phase 2 sampling.

Executive Summary

- Fifty-eight samples from 36 sites were extracted and analysed for the presence of *Ophisternon candidium* DNA;
- Amplification was observed in five sites (Opportunistic pool 02, Pool 11, Yirra Bluff, Yiryinmaru Pool and Opportunistic bore 01). None of the other sites resulted in a positive amplification for the presence of *O. candidium* DNA;
- To assess whether DNA was successfully amplified from each sample, all samples were run with the *16S* mammal probe as an extraction positive control. Of the total 58 samples, 33 samples amplified for the presence of mammal DNA;

Methods

Filter papers were stored in a sterile vial at -20°C for transport and upon arrival at the lab were then stored at -80°C.

Samples were extracted using the QIAGEN Blood and Tissue Kit (QIAGEN, Hilden, Germany) as described in Helix (2017a and b). In keeping with previous methodology, all starting volumes were doubled to ensure that the filter was covered during incubation. All samples had two elutes of 50µl each.

The extracted DNA was run with the species diagnostic assay for *O. candidium* and the mammal control assay (*16S*) as per previous studies.

Results

Thirty-six sites were sampled with one to three replicate filters per site. Of the 58 filters tested, five sites amplified for the presence of *O. candidium* DNA (Figure 1).

Amongst the total 58 filters analysed, 33 amplified with the control probe. The 25 filters that failed to amplify may have been due to the absence of mammal DNA in the bore or alternatively the presence of only degraded DNA.

Three replicate analyses were conducted for each sample for the *O. candidium* probe and one analysis was conducted per sample for the control probe. Amplification of the *O.*

candidium at Opportunists pool 02, Pool 11, Yirra Bluff and Yiryinmaru Pool was observed in all three replicates from at least one of the filters collected from that site. Opportunistic bore 01 only amplified in one of the three replicates. The absence of amplification in the remaining two replicates is most likely the result of degradation of DNA.

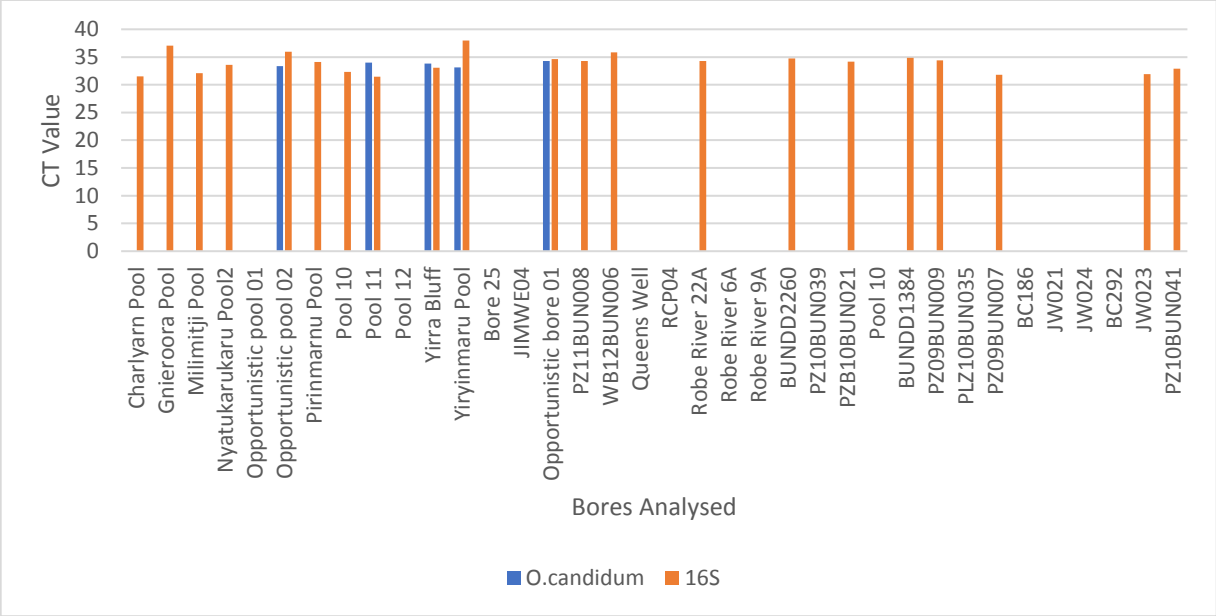

Figure 1: Sites sampled during the collection and the results for *O. candidium* specific qPCR assay. No bar indicates no amplification.

Conclusion

Blind eel DNA, *Ophisternon candidium*, was detected in five bores (Opportunistic pool 02, Pool 11, Yirra Bluff, Yiryinmaru Pool and Opportunistic bore 01). The amplification was observed in one to three replicates. Failure to detect *O. candidium* in the some replicates may be due to the degradation that can occur between replicates as noted in Helix (2017a).

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Thank you once again for collaborating on this project with Helix. We hope we can continue to provide you with useful information, and feel free to contact us if you have any questions or would like to discuss the results in detail.

Sincerely,

Yvette Hitchen, Dr Zoë Hamilton and Dr Terrie Finston

Helix Molecular Solutions Pty Ltd