



GISERA

Gas Industry Social and
Environmental Research Alliance

Project Order, Variations and Research Progress

Project Title: Characterisation of the stygofauna and microbial assemblages of the Beetaloo sub-Basin, NT

This document contains three sections. Click on the relevant section for more information.

- Section 1: [Research Project Order as approved by the GISERA Research Advisory Committee and GISERA Management Committee before project commencement](#)
- Section 2: [Variations to Project Order](#)
- Section 3: [Progress against project milestones](#)



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GISERA
Gas Industry Social and
Environmental Research Alliance

1 Original Project Order



Project Order

Proforma 2019

1. Short Project Title

Characterisation of the stygofauna and microbial assemblages of the Beetaloo sub-Basin, NT

Long Project Title

A baseline pilot study of the stygofauna and microbial assemblages of the subterranean groundwater dependent ecosystems of the Beetaloo sub-Basin and Roper River system, NT

GISERA Project Number

W18

Proposed Start Date

15/06/2019

Proposed End Date

30/06/2020

Project Leader

Dr Gavin Rees

2. GISERA Region

- | | | |
|--|--|---|
| <input type="checkbox"/> Queensland | <input type="checkbox"/> New South Wales | <input checked="" type="checkbox"/> Northern Territory |
| <input type="checkbox"/> South Australia | <input type="checkbox"/> Western Australia | <input type="checkbox"/> Victoria |

3. GISERA Research Program

- | | | |
|---|---|---|
| <input checked="" type="checkbox"/> Water Research | <input type="checkbox"/> GHG Research | <input type="checkbox"/> Social & Economic Research |
| <input type="checkbox"/> Biodiversity Research | <input type="checkbox"/> Agricultural Land
Management Research | <input type="checkbox"/> Health Research |

4. Project Summary

Objective

This project will provide new knowledge concerning stygofauna and subterranean groundwater dependent ecosystems in the Beetaloo sub-Basin and Roper River system, a critical knowledge gap identified by the Final Report of the Scientific Inquiry into Hydraulic Fracturing in the Northern Territory (2018).

Description

This project will undertake a broad spatial pilot scale survey of bores in the Beetaloo sub-Basin to determine the distribution and abundance of stygofauna and characterise the subterranean groundwater-dependent ecosystems. The project will combine a series of direct sampling and identification of macroinvertebrates with DNA-based approaches to determine animal and microbial assemblages and selected microbial processes, in the Beetaloo sub-Basin and Roper River system. This study will provide the first description of the stygofauna in an understudied region of Australia. It will provide baseline information that is essential for biodiversity conservation and the maintenance of ecological integrity of the groundwater-dependent assets of the region.

As recommended by Gibson et al. (2016), this project will ensure that: 1) survey and sampling protocols are developed and applied in the field to optimise the efficiency of survey and monitoring; 2) accurate, efficient and consistent stygofaunal species identification processes are used to increase taxonomic certainty of organisms; 3) an understanding of habitat requirements is obtained to better define species distributions and provide more in-depth ecological understanding; 4) an understanding of species and community resilience to disturbance is developed to inform mitigation strategies; and 5) ongoing data discoverability and accessibility are supported.

Need & Scope

This project will fulfil the requirement that a strategic regional environmental and baseline assessment (SREBA) that addresses the lack of knowledge of stygofauna and subterranean groundwater dependent ecosystems in the Beetaloo sub-Basin be undertaken (Recommendation 7.20 - Final Report of the Scientific Inquiry into Hydraulic Fracturing in the Northern Territory, 2018).

The project will build upon the existing understanding of stygofauna and subterranean groundwater dependent ecosystems developed in WA, QLD and NSW.

The benefits of undertaking this research have been described by Gibson et al. (2016). They include:

- Increased community trust in the environmental impact assessment process
- Improved knowledge to inform policy and guidelines



- Increased confidence in predictions for decision making
- Promotion of healthy groundwater dependent ecosystems
- Improved understanding of ecosystem services, such as maintenance of good groundwater quality
- Better environmental outcomes
- Stronger social licence to operate
- Reduced delays in a development proposal decision
- Lower survey and monitoring costs through standardised approaches

NT Hydraulic Fracturing Inquiry Recommendations

Given the absence of any information on stygofauna in the proposed regions, this project proposes a pilot-scale, single survey. Results from the pilot study will be used to identify what further work is needed to address each of the recommendations in full. To this end, this project partially addresses the following recommendations:

Recommendation 7.5

That before any further production approvals are granted, a regional water assessment be conducted as part of a SREBA for any prospective shale gas basin, commencing with the Beetaloo sub-Basin. The regional assessment should focus on surface and groundwater quality and quantity recharge and flow, characterisation of surface and groundwater-dependent ecosystems, and the development of a regional groundwater model to assess the effects of proposed water extraction of the onshore shale gas industry on the dynamics and yield of the regional aquifer system.

Recommendation 7.19

That the SREBA undertaken for the Beetaloo sub-Basin must take into account groundwater-dependent ecosystems in the Roper River region, including identification and characterisation of aquatic ecosystems, and provide measures to ensure the protection of these ecosystems.

Recommendation 7.20

That the Beetaloo sub-Basin SREBA must identify and characterise all subterranean aquatic ecosystems, with particular emphasis on the Roper River region.

Recommendation 15.1

That a strategic regional environmental and baseline assessment (SREBA) be undertaken prior to the granting of any further production approvals.

Methodology

This project will undertake a pilot broad-scale survey of bores in the Beetaloo sub-Basin and Roper River region. Laboratory-based studies will be used to characterise the community structure and function of the stygofauna, microbial assemblages and processes, and environmental variables, of the shallow subterranean aquifers of the Beetaloo sub-Basin and Roper River region.

Selection of bores

Before undertaking the field surveys, a desktop study will be undertaken to locate and identify bores that have physical and chemical attributes that are known to be suitable for supporting stygofauna (Hancock & Boulton 2008, Hose et al 2015). Ideally sufficient bores will be identified to give a broad spatial distribution and range of chemical attributes across the Beetaloo sub-Basin and Roper River region. While certain standard ranges of conditions have yielded stygofauna, the sampling will include bores outside the ‘typical range’ of conditions, to determine whether potentially specialised animals may not be existing in conditions not previously thought to harbour animals.

Table 1. Identified attributes of bores suitable for supporting stygofauna (Hancock & Boulton 2008, Hose et al 2015) Western region stygofauna monitoring and assessment plan 2016).

Attribute	Favorable values
Lithology	Alluvial (sand and gravel)
Bore depth	<50 m (max <200 m)
Bore diameter	>50 mm (to permit sampling equipment)
Bore construction	Preferably lined and slotted/screened through the water column Vertically aligned (not angled)
Bore use	Observation/monitoring
pH	6.5-8.51
EC	<2,000 $\mu\text{S}/\text{cm}$ (max <5,000 $\mu\text{S}/\text{cm}$)

Field sampling

Field sampling will be undertaken across a broad selection of landscape features throughout the Beetaloo sub-Basin including lease and potentially off-lease areas. Field sampling locations will be discussed with the Technical Reference Group and relevant experts who are simultaneously carrying out activities in this region, prior to undertaking the field campaign to ascertain that it is fit for purpose for this pilot survey of stygofauna.

Given that this is a pilot scale and that very limited knowledge exists on the approaches that will be required to sample the appropriate bores, we propose an initial sampling event will be carried out during the dry season (July/August) 2019. In the first instance, we propose sampling between 10 and 20 bores, the final number subject to availability of appropriate bore, successful application of a range of sampling methods (see below) and general logistic considerations. This survey will identify the distribution of

stygofauna/microbial communities within the sub-basin and allow assessment of how the stygofauna/microbial communities are distributed in relation to the physical and chemical attributes of the bores. Subject to the outcomes of the first sampling trip, it may be possible to carry out a further sampling round that consists of a smaller team targeting a refined selection of bores. This will allow us to capture a greater range of conditions.

Given the variability in types of bores and their accessibility, we will trial a range of methods that have been used to collect stygofauna. For example, using a peristaltic DC pump as per established methods for sampling stygofauna and water quality in bores (EPA (water) Western Australia policy 2009 Version February 2018). Alternative surface-driven pumping will be trialed since peristaltic pumping can damage organisms. Direct netting is also a recognized method for sampling stygofauna, although published research comparing the two methods has demonstrated that pumping is a more effective means of characterizing stygofauna community (Hancock & Boulton 2009). Further considerations on pumping that will be trialed during the pilot phase will be depth from which water will be pumped and the volume of water retrieved during the sampling. After collection of organisms, all stygofauna samples will be stored in 70% (v/v) ethanol. This is the standard method for preserving macroinvertebrates.

In addition to stygofauna, samples will be collected to assess the microbial community, water quality (pH, salinity, dissolved oxygen), nutrients and dissolved organic carbon. Water samples for these will also be collected using the peristaltic DC pump per established methods for sampling stygofauna and water quality in bores (EPA (water) Western Australia policy 2009 Version February 2018). Portable fridges and freezers will be taken into the field to store samples upon collection. Some development on storage of microbial samples is required and this will be developed in conjunction with GISERA project W17.

An assessment of the above ground vegetation surrounding each bore will be made to determine potential inputs of Dissolved Organic Carbon (DOC).

Laboratory analysis

Analysis of water quality samples and identification and enumeration of stygofauna samples will be undertaken at CDU. Stygofauna will be identified to the lowest possible taxonomic resolution. Since stygofauna taxonomy is limited, it is possible (indeed probable) that we will seek additional taxonomic identification skills from experts in the field.

Water quality samples will be assessed for pH, conductivity, dissolved oxygen, nutrients and DOC.

Molecular analyses, including 16S rRNA (prokaryotes) and 18S rRNA (eukaryotes) metabarcoding, will be undertaken to assist with species identification (stygofauna), the characterization of microbial assemblages, and functional analysis including N cycling genes will be processed by CSIRO at the Albury-Wodonga laboratory. Results from the eukaryote metabarcoding will be used to inform whether DNA barcodes of individuals are required.

Data analysis



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As this is a pilot-scale project, we will use a range of univariate and multivariate techniques to carry out exploratory analysis, rather than developing predictive capacity. Analysis will investigate the spatial distribution of stygofauna and microbial communities within the Beetaloo sub-region and explore how the spatial distribution is influenced by the physical and water quality properties of the bores.

An output of this pilot project will be to identify further work. A key element will be to determine the sampling requirement across time and space, to provide sufficient data for model development (e.g. As per Mokany et al 2018), that can be used to predict distributions of stygofauna across the basins of interest.

5. Project Inputs

Research

The Final Report of the Scientific Inquiry into Hydraulic Fracturing in the Northern Territory (2018) noted that nothing was known regarding the presence of stygofauna in the Beetaloo sub-Basin and Roper River system. This work will contribute to the knowledge of stygofauna in the NT and complement work undertaken in WA, Qld and NSW.

Resources and collaborations

Researcher	Time Commitment (project as a whole)	Principle area of expertise	Years of experience	Organisation
Gavin Rees	25 days	Microbial Ecology, Molecular ecology	>30	CSIRO
Daryl Nielsen	16 days	Invertebrate ecology	>30	CSIRO
Garth Watson	23 days	Molecular ecology	>30	CSIRO

Subcontractors (clause 9.5(a)(i))	Time Commitment (project as a whole)	Principle area of expertise	Years of experience	Organisation
Professor Jenny Davis	44 days (in-kind)	Freshwater ecology	40	Charles Darwin University
Research assistant	220 days	Freshwater ecology	~ 5-10	Charles Darwin University

Budget Summary

Source of Cash Contributions	2018/19	2019/20	2020/21	% of Contribution	Total
GISERA	\$7,467	\$201,057	\$0	63.8%	\$208,524
- Federal Government	\$2,240	\$60,317	0	19 %	\$62,557
- NT Government	\$2,240	\$60,317	0	19%	\$62,557
- Origin Energy	\$996	\$26,808	0	8.6%	\$27,803
- Santos	\$996	\$26,808	0	8.6%	\$27,803
- Pangaea	\$996	\$26,808	0	8.6%	\$27,803
Total Cash Contributions	\$6,349.17	\$201,057	\$0	63.8%	\$208,524
Source of In-Kind Contribution	2018/19	2019/20	2020/21	% of Contribution	Total
CSIRO	\$2,489	\$67,019	\$0	20%	\$69,508
Charles Darwin University	\$0	\$53,858	\$0	16.2%	\$53,858
Total In-Kind Contribution	\$2,489	\$120,877	\$0	36.2%	\$123,366

Cultural Monitoring Program

The cultural monitor program is considered mutually beneficial, increases engagement and participation of the local traditional owners and provides additional safeguards against the research proponent or other fieldworkers inadvertently entering into a sacred site or other culturally sensitive area. These activities will be carried out by the project team in discussion with GISERA and also potential input from CDU staff. Cultural monitors are engaged via the NLC whenever a company or operator goes out in the field.



In GISERA projects where CSIRO researchers are being escorted onto leases by company representatives who have organised permit access, those company procedures will apply.

For all other GISERA projects (particularly environmental and social projects) where CSIRO researchers are not being escorted by industry, CSIRO will work with the NLC to apply this practice.

6. Project Impact Pathway

Activities	Outputs	Short term Outcomes	Long term outcomes	Impact
Literature review	Short literature review describing the published research on stygofauna and microbial communities	<p>Provide information on the biodiversity and distribution on stygofauna and subsurface microbial communities in the Beetaloo sub-Basin. Field sampling will provide information on spatial variability across the project area.</p> <p>Subject to the findings, the project will address key recommendations of the NT Hydraulic Fracturing Inquiry. Where gaps are identified, a clear plan for future works will be provided.</p>	<p>The findings from this work will directly inform communities, from local groups through to Government, on the biodiversity that is present in the subsurface of the Beetaloo basin.</p> <p>These data will provide scientific underpinning of the biodiversity values associated with the Beetaloo sub-Basin, that will lead to improved industry practices</p> <p>Importantly, local communities will be informed of the ecological values associated with groundwater systems.</p>	<p>The main impact will be that industry operates in a financially effective manner, while ensuring biodiversity and values are maintained</p> <p>Policy makers will be informed of the subsurface biodiversity values, leading to better decision making and policy formation</p>
Determine the diversity of stygofauna and microbial communities present in the sub surface environment of the Beetaloo sub-Basin and Roper River system	Milestone reports that provide an update on the field results. A Technical report summarising the entire field programme, outlining the distribution and diversity of stygofauna and microbial communities within the Beetaloo sub-Basin			
Preparation and submit final report.	<p>The final report will form the basis of all the final outputs.</p> <p>A major aspect of the report will be to identify future work and costings with meeting all the recommendations of the NT Hydraulic Fracturing Inquiry.</p>			
Develop fact sheets and share with communities through direct meeting with community members	Fact sheets written in plain English will be available from the GISERA website and a community engagement event.			
Prepare and submit scientific manuscripts for peer-review publication.	Peer reviewed manuscripts that demonstrate the scientific rigour and outcomes to the scientific community			

7. Project Plan

Project Schedule

ID	Activities / Task Title (should match activities in impact pathway section)	Task Leader	Scheduled Start	Scheduled Finish	Predecessor
Task 1	Literature review	Prof Jenny Davis	15 th June 2019	31 th July 2019	Not applicable
Task 2	Identification of suitable bores and sample sites	Research assistant	15 th June 2019	6 th July 2019	Not applicable
Task 3	Stygofauna sampling field trip	Research assistant	1 st July 2019	31 st August 2019	Task 2
Task 4	DNA analysis of microbial community	Dr Gavin Rees	1 st September 2019	30 th January 2020	Tasks 3
Task 5	Taxonomic analysis of stygofauna	Research assistant	1 st September 2019	30 th January 2020	Tasks 3
Task 6	Analysis of data	Project team	1 st February 2020	30 th March 2020	Tasks 4, 5
Task 7	Reporting and future recommendations	Dr Gavin Rees	1 st April 2020	30 th May 2020	Task 6
Task 8	Communication activities	Dr Gavin Rees	1 June 2020	30 June 2020	Task 1 - 8

Task description

Task 1

TASK NAME: Literature review

TASK LEADER: Prof Jenny Davis (CDU)

OVERALL TIMEFRAME: 15 June 2019 – 31 July 2019

BACKGROUND: While there is very limited knowledge on the groundwater dependent systems of the NT, considerable work has been undertaken elsewhere, particularly in Western Australia. Similarly, relevant literature on DNA-based methods continue to emerge. A review that interrogates the available literature will provide the information needed to underpin research activities in the NT.

TASK OBJECTIVES:

- 1) Scan and review literature on stygofauna and ground water dependent organisms
- 2) Examine and compile appropriate field and laboratory-based methods

TASK OUTPUTS AND SPECIFIC DELIVERABLES: A short report that has addressed the objectives.

Task 2

TASK NAME: Identification of suitable bores and sample sites

TASK LEADER: Research assistant (CDU)

OVERALL TIMEFRAME: 15 June 2019 – 6th July 2019

BACKGROUND: Locating and identifying bores which have physical and chemical attributes that are known to be suitable for supporting stygofauna is an important first step in undertaking this project. Ideally sufficient bores should be identified to give a broad spatial distribution across the Beetaloo sub-region and Roper River Region.

TASK OBJECTIVES: Identification of bores within the Beetaloo sub-region and Roper River region

- Identification of suitable bores
- Obtaining permits to access bores
- Planning and co-ordination of field trips to sample bores

TASK OUTPUTS AND SPECIFIC DELIVERABLES: List of bores within the Beetaloo sub-region and the Roper River Region which outlines the physical and chemical attributes of each bore. From this list, bores will be selected for sampling of stygofauna.

Task 3 (Stygofauna sampling trip)

TASK NAME: Field sampling

TASK LEADER: Research assistant (CDU)

OVERALL TIMEFRAME: 1st July 2019 – 30th August 2019

BACKGROUND: Sampling of stygofauna and microbial communities will be undertaken using published methods that were evaluated as part of Task 1 (literature review). Broader ecological understanding is necessary to place any results in a wider context. To this end, ground water chemistry measurements are required, and include Dissolved Oxygen, Dissolved Organic Carbon, Nutrients, Conductivity and pH. Similarly, information is required on the hydrology of the immediate region as well as above ground vegetation.

TASK OBJECTIVES: Collect samples:

- 1) Stygofauna collected directly from water samples
- 2) Water samples filtered for DNA analysis, which will include microbes as well as detecting the presence of any eukaryotes in the water
- 3) Water quality
- 4) Above ground survey for vegetation.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Short summary report following each sampling event detailing changes in bore characteristic.

Task 4

TASK NAME: DNA analysis of microbial community

TASK LEADER: Dr Gavin Rees (CSIRO)

OVERALL TIMEFRAME: 1st September 2019 – 30th January 2020

BACKGROUND: Using samples collected from Task 3, carry out DNA analysis to examine microbial and eukaryote communities detected by the presence of their DNA in water samples.

TASK OBJECTIVES: Identification of microbial communities.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Database listing microbes associated with each bore. Taxa present in samples will be assigned identity subject to availability of appropriate taxonomic information in public databases. Results will be linked to a similar database containing water quality, nutrients and DOC data.

Task 5

TASK NAME: Taxonomic identification of stygofauna

TASK LEADER: Research assistant (CDU)

OVERALL TIMEFRAME: 1st September 2019 – 30th January 2020

BACKGROUND: Using samples collected from Task 3, undertake out taxonomic identification of stygofauna samples from each of the bores.

TASK OBJECTIVES: Stygofauna identification

TASK OUTPUTS AND SPECIFIC DELIVERABLES. Database listing abundance of each stygofauna species identified from each bore. Database will be linked to a similar database containing water quality, nutrients and DOC data.

Task 6

TASK NAME: Analysis of data

TASK LEADER: Project team

OVERALL TIMEFRAME: 1st February 2020 – 30th March 2020

BACKGROUND: Analysis will investigate the spatial distribution of stygofauna and microbial communities within the Beetaloo region and explore how the spatial distribution is influenced by the physical and water quality properties of the bores. From this analysis we will be able to provide predictions on how the stygofauna and microbial communities will respond if changes in the water quality and/or nutrient availability were to occur.

TASK OBJECTIVES: Data analysis

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Outcomes from the analysis will form the base of the final report. From this analysis we will be able to provide predictions on how the stygofauna and microbial communities will respond if changes in the water quality and/or nutrient availability were to occur.

Task 7

TASK NAME: Reporting and recommendations for future activities

TASK LEADER: Dr Gavin Rees

OVERALL TIMEFRAME: 1 April – 30th May 2020

BACKGROUND:

TASK OBJECTIVES: Preparation of the final report

TASK OUTPUTS AND SPECIFIC DELIVERABLES. A Technical report summarising the entire field programme, outlining the distribution and diversity of stygofauna and microbial communities within the Beetaloo sub-Basin. The final report will also include a clear statement of the future work and costings associated with understanding the distribution of stygofauna in the Beetaloo sub-Basin and Roper system.

Task 8

TASK NAME: Communication of outcomes

TASK LEADER: Dr Gavin Rees

OVERALL TIMEFRAME: June 2019 - June 2020

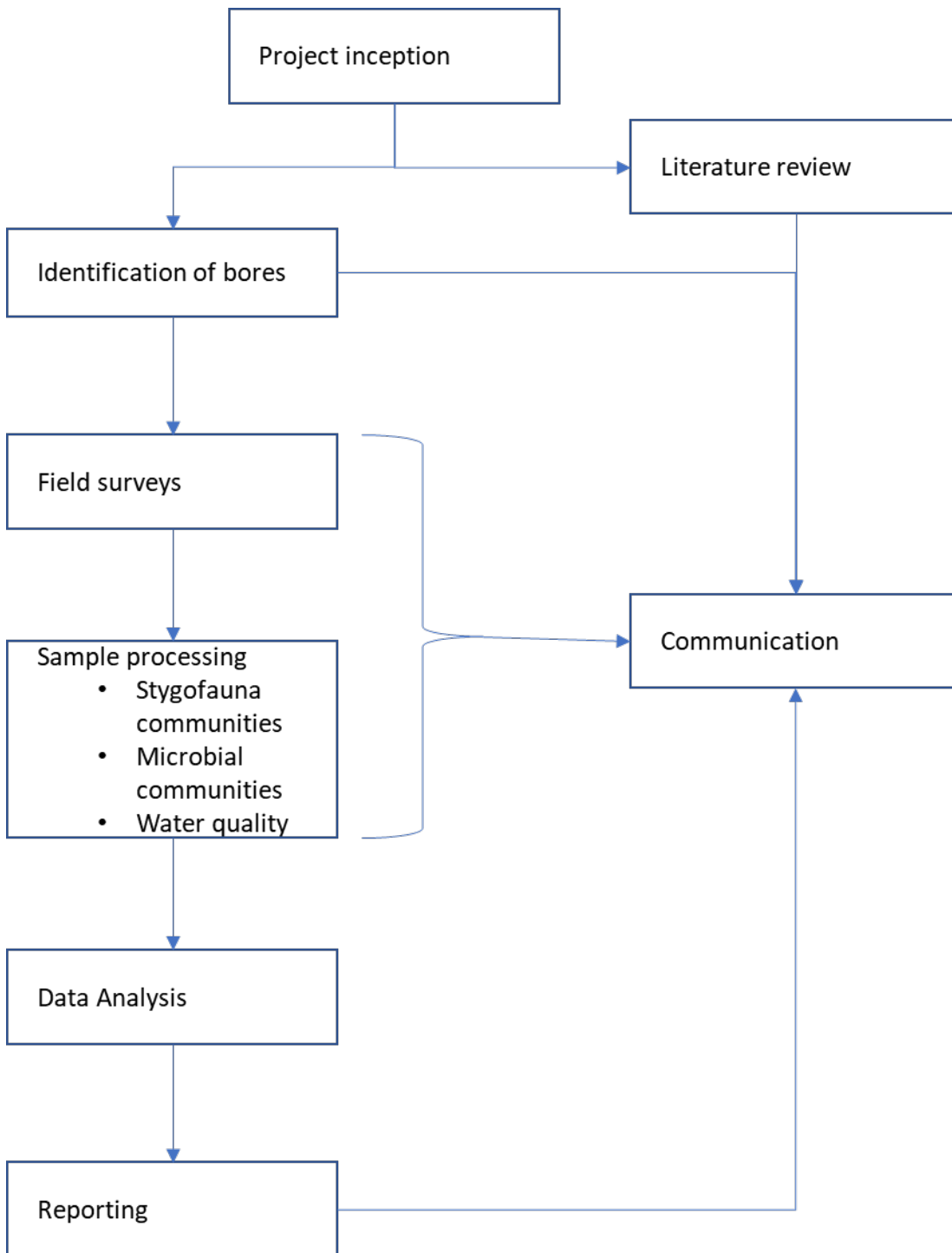
BACKGROUND:

TASK OBJECTIVES: Preparation of factsheets and scientific manuscripts for publication

TASK OUTPUTS AND SPECIFIC DELIVERABLES. Develop factsheets and share with communities through direct meeting with community members and prepare and submit scientific manuscripts for peer-review publication. Factsheets and publications will be available from the GISERA website and made available at a community engagement even.



Flow chart



Project Gantt Chart

Task	Task Description	Task Leader	Jun-19	Jul-19	Aug-19	Sep-19	Oct-19	Nov-19	Dec-19	Jan-20	Feb-20	Mar-20	Apr-20	May-20	Jun-20
1	Literature review	Prof Jenny Davis	█	█											
2	Identification of suitable bores and sample sites	Research assistant	█	█											
3	Stygofauna sampling field trip	Research assistant	█	█	█										
4	DNA analysis of microbial community	Dr Gavin Rees				█	█	█	█	█					
5	Taxonomic analysis of stygofauna	Research assistant				█	█	█	█	█					
6	Analysis of data	Project team									█	█			
7	Reporting and future recommendations	Dr Gavin Rees											█	█	
8	Communication activities	Dr Gavin Rees													█



8. Technical Reference Group

Stakeholders to be invited to participate in the Technical Reference Group include:

- Professor Andrew Boulton, UNE
- Associate Professor Grant Hose, Macquarie University
- Adrian Pinder, Department of Biodiversity, Conservation and Attraction (DBCA) WA
- Representative from the NT Cattlemen’s Association
- Representative from NT Farmers Association
- Representative from NT Government

9. Communications Plan

Stakeholder	Objective	Channel (e.g. meetings/media/factsheets)	Timeframe (Before, during at completion)
Government and Industry	To facilitate a deeper understanding of research findings and implications for policy, programs, planning, and other initiatives	Knowledge transfer sessions and through stakeholder workshops and meetings.	From commencement of project and with updates as they come to hand.
Regional Community/Wider public	To communicate project objectives and key messages from the research	<p>Fact sheets (including development of one at commencement of project which will explain the objective of the project – this will be updated periodically as project progresses).</p> <p>Project progress reported on GISERA website to ensure transparency for all stakeholders including regional communities.</p> <p>Participation in roadshows, community workshops and</p>	<p>From commencement of project and with updates as they come to hand.</p> <p>As required.</p> <p>As required</p>



		meetings and other engagements where appropriate. Maps and visuals - Key findings communicated with the use of maps and visual cues incorporated.	Towards completion
	To report on existing literature on stygofauna and ground water dependent organisms and Examine and compile appropriate field and laboratory-based methods	Literature review	July 2019
Regional Community/ Wider public, Government, Scientific community and Industry	To report on key findings	Technical (final) Report	At completion
Traditional Owner communities	To pursue relations with Traditional Owner communities (via cultural monitors)	Engagement with TO communities – as a wider context as part of CSIRO communications (considered mutually beneficial)	Ongoing
Scientific community	To communicate scientific findings	Journal publication	At completion



10. Budget Summary

Expenditure	2018/19	2019/20	2020/21	Total
Labour	\$1,700	\$94,984	\$0	\$96,684
Operating	\$0	\$36,000	\$0	\$36,000
Subcontractors	\$8,256	\$137,092	\$0	\$145,348
In-kind Charles Darwin University	\$0	\$53,858	\$0	\$53,858
Total Expenditure	\$9,956	\$321,934	\$0	\$331,890

Expenditure per Task	2018/19	2019/20	2020/21	Total
Task 1	\$8,256	\$0	\$0	\$8,256
Task 2	\$1,700	\$1,749	\$0	\$3,449
Task 3	\$0	\$102,604	\$0	\$102,604
Task 3b*	\$0	\$15,000	\$0	\$15,000
Task 4	\$0	\$37,050	\$0	\$37,050
Task 5	\$0	\$54,430	\$0	\$54,430
Task 6	\$0	\$30,499	\$0	\$30,499
Task 7	\$0	\$30,499	\$0	\$30,499
Task 8	\$0	\$11,245	\$0	\$11,245
In-kind Charles Darwin University	\$0	\$53,858	\$0	\$53,858
Total Expenditure	\$9,956	\$321,934	\$0	\$331,890

*Only in the event that CDU can carry out a further sampling round that consists of a smaller team targeting a refined selection of bores. These funds are conditional to this second fieldwork campaign going ahead.

Source of Cash Contributions	2018/19	2019/20	2020/21	Total
Federal Government (19%)	\$2,240	\$60,317	\$0	\$62,557
NT Government (19%)	\$2,240	\$60,317	\$0	\$62,557
Origin Energy (8.6%)	\$996	\$26,808	\$0	\$27,803
Santos (8.6%)	\$996	\$26,808	\$0	\$27,803
Pangaea (8.6%)	\$996	\$26,808	\$0	\$27,803
Total Cash Contributions	\$7,467	\$201,057	\$0	\$208,524



In-Kind Contributions	2018/19	2019/20	2020/21	Total
CSIRO (20%)	\$2,489	\$67,019	\$0	\$69,508
Charles Darwin University (16.2%)	\$0	\$53,858	\$0	\$53,858
Total In-Kind Contributions	\$2,489	\$120,877	\$0	\$123,366

	Total funding over all years	Percentage of Total Budget
Federal Government Investment	\$62,557	19.0%
NT Government Investment	\$62,557	19.0%
Origin Energy	\$27,803	8.6%
Santos	\$27,803	8.6%
Pangaea Resources	\$27,803	8.6%
CSIRO In-kind	\$69,508	20.0%
Charles Darwin University In-kind	\$53,858	16.2%
TOTAL	\$331,890	100%



Task	Milestone Number	Milestone Description	Funded by	Start Date (mm-yy)	Delivery Date (mm-yy)	Fiscal Year Completed	Payment \$ (excluding CSIRO contribution)
Task 1	1.1	Literature Review	GISERA	June 2019	June 2019	2019-20	\$6,192.00
Task 2	2.1	Bore selection	GISERA	June 2019	July 2019	2019-20	\$2,586.00
Task 3	3.1	Field trip	GISERA	July 2019	August 2019	2019-20	\$76,953.00
Task 4	4.1	Microbial analysis of samples	GISERA	September 2019	January 2020	2019-20	\$27,787.50
Task 5	5.1	Stygofauna identification	GISERA	September 2019	January 2020	2019-20	\$40,822.50
Task 6	6.1	Data analysis	GISERA	February 2020	March 2020	2019-20	\$22,874.25
Task 7	7.1	Reporting	GISERA	April 2020	May 2019	2019-20	\$22,874.25
Task 8	8.1	Communication	GISERA	June 2020	June 2020	2019-20	\$8,433.75



11. Intellectual Property and Confidentiality

Background IP (clause 11.1, 11.2)	Party	Description of Background IP	Restrictions on use (if any)	Value
				\$
				\$
Ownership of Non-Derivative IP (clause 12.3)	CSIRO			
Confidentiality of Project Results (clause 15.6)	Project Results are not confidential.			
Additional Commercialisation requirements (clause 13.1)	Not Applicable			
Distribution of Commercialisation Income (clause 13.4)	Not applicable			
Commercialisation Interest (clause 1.1)	Party	Commercialisation Interest		
	CSIRO	N/A		
	Origin Energy	N/A		
	Santos	N/A		
	Pangaea Resources	N/A		



12. References

- EPA (2007, 2016) Sampling methods and survey considerations for subterranean fauna in Western Australia (Guidance Statement 54a) Perth, Environmental Protection Authority.
- Gibson et al. (2016) Shedding new light on the cryptic world of subterranean fauna: a research program for Western Australia. The Western Australian Biodiversity Science Institute.
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2 Variations to Project Order

Changes to research Project Orders are approved by the GISERA Director, acting with authority provided by the GISERA National Research Management Committee, in accordance with the [National GISERA Alliance Agreement](#).

The table below details variations to research Project Order.

Register of changes to Research Project Order

Date	Issue	Action	Authorisation
21 August 2019	There is a need for CDU to undertake an additional sampling round targeting a refined selection of bores. The additional cost for this is \$15,000.	Addition of new task 3B, including addition of \$15,000 (\$11,250 GISERA funding & \$3,750 CSIRO in-kind) to budget.	



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3 Progress against project milestones

Progress against milestones are approved by the GISERA Director, acting with authority provided by the GISERA National Research Management Committee, in accordance with the [National GISERA Alliance Agreement](#).

Progress against project milestones/tasks is indicated by two methods: Traffic Light Reports and descriptive Project Schedule Reports.

1. Traffic light reports in the Project Schedule Table below show progress using a simple colour code:
 - **Green:**
 - Milestone fully met according to schedule.
 - Project is expected to continue to deliver according to plan.
 - Milestone payment is approved.
 - **Amber:**
 - Milestone largely met according to schedule.
 - Project has experienced delays or difficulties that will be overcome by next milestone, enabling project to return to delivery according to plan by next milestone.
 - Milestone payment approved for one amber light.
 - Milestone payment withheld for second of two successive amber lights; project review initiated and undertaken by GISERA Director.
 - **Red:**
 - Milestone not met according to schedule.
 - Problems in meeting milestone are likely to impact subsequent project delivery, such that revisions to project timing, scope or budget must be considered.
 - Milestone payment is withheld.
 - Project review initiated and undertaken by GISERA Research Advisory Committee.
2. Progress Schedule Reports outline task objectives and outputs and describe, in the 'progress report' section, the means and extent to which progress towards tasks has been made.



Project Schedule Table

ID	Activities / Task Title (should match activities in impact pathway section)	Task Leader	Scheduled Start	Scheduled Finish	Predecessor
Task 1	Literature review	Prof Jenny Davis	June 2019	July 2019	Not applicable
Task 2	Identification of suitable bores and sample sites	Research assistant	June 2019	July 2019	Not applicable
Task 3	Stygofauna sampling field trip	Research assistant	July 2019	August 2019	Task 2
Task 3B	Undertake additional sampling targeting a refined selection of bores.	Research assistant	October 2019	October 2019	Task 3
Task 4	DNA analysis of microbial community	Dr Gavin Rees	September 2019	January 2020	Tasks 3
Task 5	Taxonomic analysis of stygofauna	Research assistant	September 2019	January 2020	Tasks 3
Task 6	Analysis of data	Project team	February 2020	March 2020	Tasks 4, 5
Task 7	Reporting and future recommendations	Dr Gavin Rees	April 2020	May 2020	Task 6
Task 8	Communication activities	Dr Gavin Rees	June 2020	June 2020	Task 1 - 8



Project Schedule Report

TASK 1

TASK NAME: Literature review

TASK LEADER: Prof Jenny Davis (CDU)

OVERALL TIMEFRAME: 15 June 2019 – 31 July 2019

BACKGROUND: While there is very limited knowledge on the groundwater dependent systems of the NT, considerable work has been undertaken elsewhere, particularly in Western Australia. Similarly, relevant literature on DNA-based methods continue to emerge. A review that interrogates the available literature will provide the information needed to underpin research activities in the NT.

TASK OBJECTIVES:

- 1) Scan and review literature on stygofauna and ground water dependent organisms
- 2) Examine and compile appropriate field and laboratory-based methods

TASK OUTPUTS AND SPECIFIC DELIVERABLES: A short report that has addressed the objectives.

PROGRESS REPORT:

A draft literature review has been undertaken by the project team. The review has highlighted that the literature on Australian stygofauna is much larger than we expected although there is very little information on stygofauna in the Northern Territory. The review has also indicated that there are more than 750 recorded species of stygofauna within Australian groundwater systems.

TASK 2

TASK NAME: Identification of suitable bores and sample sites

TASK LEADER: Research assistant (CDU)

OVERALL TIMEFRAME: 15 June 2019 – 6th July 2019

BACKGROUND: Locating and identifying bores which have physical and chemical attributes that are known to be suitable for supporting stygofauna is an important first step in undertaking this project. Ideally sufficient bores should be identified to give a broad spatial distribution across the Beetaloo sub-region and Roper River Region.

TASK OBJECTIVES: Identification of bores within the Beetaloo sub-region and Roper River region

- Identification of suitable bores
- Obtaining permits to access bores
- Planning and co-ordination of field trips to sample bores

TASK OUTPUTS AND SPECIFIC DELIVERABLES: List of bores within the Beetaloo sub-region and the Roper River Region which outlines the physical and chemical attributes of each bore. From this list, bores will be selected for sampling of stygofauna.

PROGRESS REPORT:

This project established close links to GISERA project W17 *“Microbial profiling for environmental monitoring and microbial degradation of chemicals in drilling and hydraulic*



fracturing fluid in the Northern Territory". For logistical reasons initial bore selection was based around bores that were to be sampled as part of W17 as these had previously been examined under GISERA projects.

Professor Jenny Davis (CDU) also had a series of discussions with rangers from Elsey National Park and they identified several bores that we would be able to access and sample as part of the first field trip. A further opportunity arose during the field trip to sample three bores at Elliott, which represented sites at the southern end of the region to be examined (detailed below). The latter bores, combined with those at Elsey NP gave us very good coverage for the first field trip. Some modifications to the bore selection were made 'on the fly' as the pumping characteristics had been modified from the initial information. A case in point were some bores that once were accessible now have solar driven fixed pumps that do not allow any access for stygofaunal sampling.

TASK 3

TASK NAME: Field sampling

TASK LEADER: Research assistant (CDU)

OVERALL TIMEFRAME: 1st July 2019 – 30th August 2019

BACKGROUND: Sampling of stygofauna and microbial communities will be undertaken using published methods that were evaluated as part of Task 1 (literature review). Broader ecological understanding is necessary to place any results in a wider context. To this end, ground water chemistry measurements are required, and include Dissolved Oxygen, Dissolved Organic Carbon, Nutrients, Conductivity and pH. Similarly, information is required on the hydrology of the immediate region as well as above ground vegetation.

TASK OBJECTIVES: Collect samples:

- 1) Stygofauna collected directly from water samples
- 2) Water samples filtered for DNA analysis, which will include microbes as well as detecting the presence of any eukaryotes in the water
- 3) Water quality
- 4) Above ground survey for vegetation.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Short summary report following each sampling event detailing changes in bore characteristic.

PROGRESS REPORT:

Sampling of bores took place between the 5th and 15th August. A total of 21 bores were sampled. Ten 10 bores were sampled in the Daly Waters region with the W17 project team. Opportunistically three bores were sampled further south at the township of Elliot and permission was given by national parks to sample bores in and around Elsey National Park. This led to a further six bores being sampled.

Samples were collected by motorised pumps and plankton nets. The choice of pumps and nets was dependant on the type and size of the bore hole. Bores located on private property had fixed pumps in the bore head and samples were collected either directly from pump or via taps. For other bores a pump supplied by Origin was used.

Irrespective of the type of pump, 300 litres of water was sampled. Water was passed through a 50µm mesh net into a catching jar and concentrated before being preserved in 70% ethanol.

In the Mataranka region, the pumping head pressure resulting from the depth to the water was too great for our pump to work successively. Instead a small net 50µm mesh net was lowered to the bottom of the bores and retrieved and sample preserved in 70% ethanol. Of further note, some bores were sufficiently narrow the scale of pump use by Origin would



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also not fit down the bore hole. A range of pumping strategies need to be considered for future stygofaunal sampling.

All samples were returned to the respective labs for analysis. Samples are currently being examined for stygofauna and first indications showed their presence in some samples. This was sufficient evidence to request that our initial tentative activity 3b should occur and we have sought release of this funding for a further field trip. Samples are also currently being processed for eDNA analysis of the groundwater. Results will be presented as part of the subsequent activities.

TASK 3B

TASK NAME: Field sampling

TASK LEADER: Research assistant (CDU)

OVERALL TIMEFRAME: 1st October 2019 – 31st October 2019

BACKGROUND: Additional round of sampling of stygofauna and microbial communities will be undertaken using published methods that were evaluated as part of Task 1 (literature review). Broader ecological understanding is necessary to place any results in a wider context. To this end, ground water chemistry measurements are required, and include Dissolved Oxygen, Dissolved Organic Carbon, Nutrients, Conductivity and pH. Similarly, information is required on the hydrology of the immediate region as well as above ground vegetation.

TASK OBJECTIVES: To undertake an additional sampling round targeting a refined selection of bores.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Short summary report following each sampling event detailing changes in bore characteristic.

PROGRESS REPORT:

The objective of milestone 3B was to undertake an additional sampling round targeting a refined selection of bores. The field trip took place from 12th – 20th October and sampled 17 bores. In some instances, the team used the opportunity to re-sample some of the bores that yielded stygofauna in our previous field trip. We also took the opportunity to refine our sampling methods. It was clear from the first field trip that hand netting, using a small-scale hand held net, would yield fauna from some bores. We refined the net design to cope with some of the narrow bore diameters present throughout the sampling region.

Blind shrimp were again collected from bore RN34032, with field observations suggesting shrimp were also present in some of the Larrimah bores.

Samples have been returned to the lab and are now being examined thoroughly for the presence of stygofauna.

TASK 4

TASK NAME: DNA analysis of microbial community

TASK LEADER: Dr Gavin Rees (CSIRO)

OVERALL TIMEFRAME: 1st September 2019 – 30th January 2020

BACKGROUND: Using samples collected from Task 3, carry out DNA analysis to examine microbial and eukaryote communities detected by the presence of their DNA in water samples.



TASK OBJECTIVES: Identification of microbial communities.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Database listing microbes associated with each bore. Taxa present in samples will be assigned identity subject to availability of appropriate taxonomic information in public databases. Results will be linked to a similar database containing water quality, nutrients and DOC data.

PROGRESS REPORT:

We have received all our molecular DNA data from the bores and data analysis is complete. Our project worked directly with project W17 “ Environmental monitoring and microbial degradation of onshore shale gas activity chemicals and fluids”, with our team sampling from a sub set of those bores that were sampled for eDNA microbial analysis by the project W17 team. To avoid duplication of analysis on the same samples with W17 we shifted our eDNA focus to measuring all animals – those from the microscopic amoeba through to macroinvertebrates. We also carried out an assay that would resolve the fungal communities. Given this was a baseline survey, we could attempt to establish a complete baseline of biota in bore water, then attempt to unravel the data to a point where we could attribute those that are true stygofauna, those that are likely subsurface and those that are terrestrial contaminants. These data have been related to bore water chemistry. As a final addition to the molecular analysis, we carried out some first bar-coding of stygofauna blind shrimp that were retrieved from bores. This an important first step in determining whether there is endemism within these animals. Once project W17 have completed their microbial analysis, we will compare our findings with theirs, to present a wider discussion on biological communities in the NT bores.

TASK 5

TASK NAME: Taxonomic identification of stygofauna

TASK LEADER: Research assistant (CDU)

OVERALL TIMEFRAME: 1st September 2019 – 30th January 2020

BACKGROUND: Using samples collected from Task 3, undertake out taxonomic identification of stygofauna samples from each of the bores.

TASK OBJECTIVES: Stygofauna identification

TASK OUTPUTS AND SPECIFIC DELIVERABLES. Database listing abundance of each stygofauna species identified from each bore. Database will be linked to a similar database containing water quality, nutrients and DOC data.

PROGRESS REPORT:

All specimens collected from bores have been identified to their best resolution. We have had additional identifications carried out by specialists in particular fields. For example, while we identified the blind shrimp to genus, given their clear importance in the some of the bore samples, we chose to have our identifications confirmed by experts who have previously worked on the stygofaunal shrimp species. Full taxonomic lists have been generated for all bores.

TASK 6

TASK NAME: Analysis of data

TASK LEADER: Project team

OVERALL TIMEFRAME: 1st February 2020 – 30th March 2020



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BACKGROUND: Analysis will investigate the spatial distribution of stygofauna and microbial communities within the Beetaloo region and explore how the spatial distribution is influenced by the physical and water quality properties of the bores. From this analysis we will be able to provide predictions on how the stygofauna and microbial communities will respond if changes in the water quality and/or nutrient availability were to occur.

TASK OBJECTIVES: Data analysis

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Outcomes from the analysis will form the base of the final report. From this analysis we will be able to provide predictions on how the stygofauna and microbial communities will respond if changes in the water quality and/or nutrient availability were to occur.

PROGRESS REPORT:

First drafts of data analysis have been completed. Data analysis and reporting are occurring together and we will refine some analyses as we prepare our report. For example, we have prepared first draft of maps where stygofauna have been found and we have carried out analyses to relate the presence of biota to water quality.