Australia's National
Science Agency

GISERA | Gas Industry Social and Environmental Research Alliance

Project Order

Short Project Title

Examination of stygofauna ecosystems of the Beetaloo Sub-basin

Long Project Title	In-depth examination of stygofauna ecosystems of the Beetaloo Sub-basin
GISERA Project Number	W.30
Start Date	01/07/2022
End Date	31/07/2024
Project Leader	Gavin Rees

GISERA State/Territory

- ☐ Queensland
- ☐ New South Wales
- ☒ Northern Territory
- ☐ South Australia
- ☐ Western Australia
- ☐ Victoria
- ☐ National scale project

Basin(s)

- ☐ Adavale
- ☐ Amadeus
- ☒ Beetaloo
- ☐ Canning
- ☐ Western Australia
- ☐ Carnarvon
- ☐ Clarence-Morton
- ☐ Cooper
- ☐ Eromanga
- ☐ Galilee
- ☐ Gippsland
- ☐ Gloucester
- ☐ Gunnedah
- ☐ Maryborough
- ☐ McArthur
- ☐ North Bowen
- ☐ Otway
- ☐ Perth
- ☐ South Nicholson
- ☐ Surat
- ☐ Other (please specify)

GISERA Research Program

- ☒ Water Research
- ☐ Health Research
- ☒ Biodiversity Research
- ☐ Social & Economic Research
- ☐ Greenhouse Gas Research
- ☐ Agricultural Land Management Research
- ☐ Other (please specify)

1. Project Summary

Stygofauna are valued as a biodiversity resource, as indicators of groundwater ecosystem health, and potential providers of ecosystem goods and services. A recent pilot-scale project showed that Northern Territory (NT) aquifers in the Beetaloo Sub-basin support a diverse range of stygofaunal species. However, there is a significant knowledge gap in the collective understanding of where stygofauna exist, the extent of their distribution across the Beetaloo Sub-basin and therefore, the extent to which these subsurface ecosystems could be vulnerable to any above ground activities.

This project will build on previous work by closely linking groundwater studies with biological sampling to target the key knowledge gaps. The project will involve collection and review of all relevant data on stygofauna in the NT, as well as detailed field programs that target bores across the Beetaloo Sub-basin. To this end, we will seek to understand the extent to which stygofauna present in bores reflect their presence more widely within aquifers, understand which physical and chemical drivers determine where stygofauna exist and the extent to which stygofauna communities may be connected, or whether there is genetic separation among communities (thus reflecting degrees of endemism at sites).

2. Project description

Introduction

A pilot-scale study recently demonstrated that aquifers in the Beetaloo Sub-basin of the Northern Territory (NT) support a diverse range of stygofaunal species. All stygofaunal communities were dominated by crustaceans, namely shrimps, amphipods, ostracods, copepods and syncarids. The fauna showed little affinity with the stygofauna recorded from the more extensively sampled aquifers in north-western Australia, with new genera and species very likely to be present in the Cambrian Limestone Aquifer (CLA). DNA analysis identified that the blind shrimp found across several bore samples as *Parisia unguis*. The presence of stygofauna at sites across ~500km of the CLA, particularly *P. unguis*, is consistent with some degree of connectivity within the aquifer. Whilst a wide diversity of stygofauna was found, their distribution was patchy, with 6 out of 26 monitoring bores sampled in a recent study in the CLA containing live stygofauna. DNA evidence indicated the presences of stygofauna in other bore water samples. This patchiness suggests a specific set of conditions is required for stygofauna community to persist locally, but what these conditions are is unclear at present for the CLA. The study raised the additional question as to whether sample bias existed due to the potential for clustering of organisms near bores if these bores represent a food source (energy or nutrients) due to their contact with the surface.

Maintaining the health of groundwater is especially important in Australia because over 70% of the continent is arid or semi-arid (annual rainfall is low, < 500mm) and surface waters are scarce. This is

particularly relevant to the Beetaloo region. In many remote areas, groundwater is the only water source for human infrastructure, agriculture, horticulture, mining and gas and oil extraction. Despite the importance of groundwater across inland Australia, the fact that it is stored underground means that it is often 'out of sight and out of mind' (Oberprieler et al 2021). Stygofauna are valued as a biodiversity resource, as indicators of groundwater ecosystem health, and potential providers of ecosystem goods and services. Such ecosystem goods and services may include nutrient cycling and storage (e.g. carbon, nitrogen, phosphorus), as well as organic matter cycling and redistribution (Glanville et al 2016; Smith et al 2016). Where groundwater has been either drawn down or contaminated, there have been serious and often irreversible impacts on stygofaunal biodiversity (Boulton 2020).

Understanding the risk of above-ground gas extraction activities upon the NT stygofauna requires good knowledge of the types of stygofauna present in the aquifers, a clearer picture of the distribution of stygofauna across the CLA and the extent to which bore samples truly reflect colonisation of the CLA. Understanding how animals are distributed across landscapes, and what factors affect those distributions, is critical for effective ecological management. Biogeography of species is driven by a number of factors including those that are biological (e.g. dispersal ability) and non-biological (e.g. barriers or landscape resistances to dispersal). Appropriate analyses can identify: 1) how connected populations are, 2) dispersal and connectivity pathways among populations, 3) landscape features that are likely to form barriers to dispersal, 4) where evolutionarily unique populations occur, and 5) the presence of species with natural high genetic variability.

The combined approaches here will give us the ability to predict presence of stygofauna based on aquifer depth and water chemistry, the extent to which different populations are connected, greatly informing potential risks of above ground activities.

Prior Research

Styogofaunal Research

In Australia there are three general types of aquifer in which stygofauna have been found—karstic, fractured rock and alluvial (Figure 1). Karstic systems are characterised by sink holes, caves and springs commonly developed in carbonate rocks such as limestone and dolomite. They are found across Australia, including on the Nullarbor Plain, in Cape Range National Park, and throughout northern Australia from the Kimberley to the Barkly Tableland (Tomlinson and Boulton 2008). Fractured rock aquifers occur when fissures or cracks develop in rocks of sedimentary, igneous or metamorphic origin. Groundwater flow follows the fractures but can also permeate the rock matrix, depending on the geology of the system (Tomlinson and Boulton 2008). Alluvial aquifers occur in unconsolidated sediments, often sands and gravels associated with river flood plains and deposits.

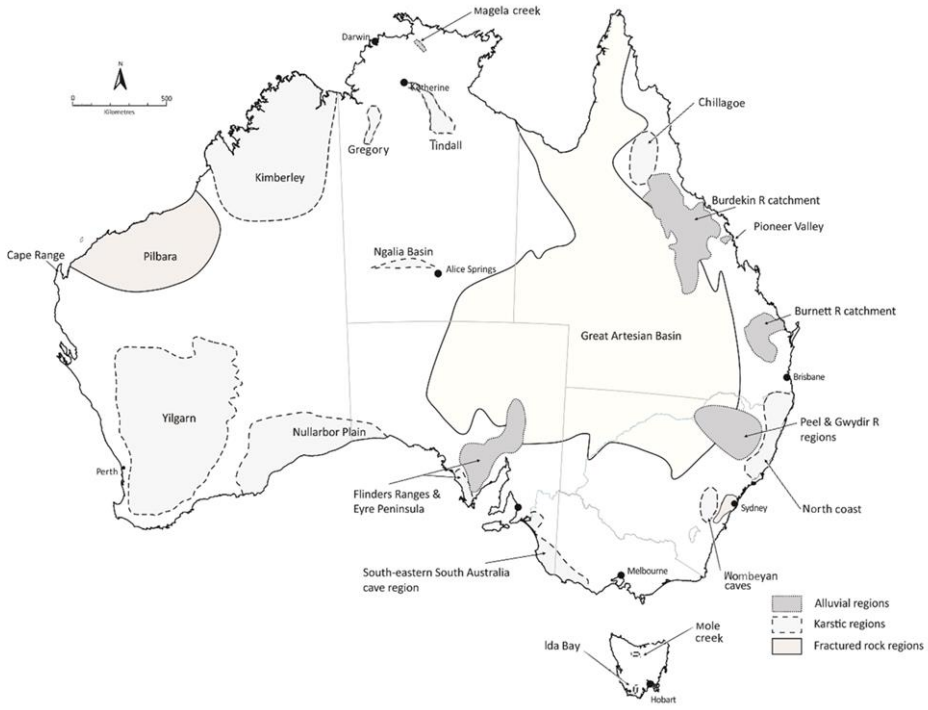


Figure 1. General aquifer types and regions where stygofauna have been found in Australia. Modified from Tomlinson and Boulton (2008) with additional information from Guzik *et al.* (2008), Hose *et al.* (2015a) and Chandler *et al*. (2017) and this study (the Tindall aquifer).

Although there are extensive karstic/carbonate systems across the Top End of the Northern Territory (Tickell 2005), there are few published records of stygofauna across the Northern Territory and these appear to be limited to sporadic surveys in five locations. In the arid Ngalia Basin calcrete aquifers, northwest of Alice Springs, taxa similar to those found in Western Australian calcretes have been reported (Balke and Ribera 2004, Balke *et al*. 2004, Watts and Humphreys 2006, Cho *et al.* 2006a, Leys and Watts 2008, S. Taiti, unpub. data in Hose *et al.* 2015a). Further north in the Cutta Cutta caves near Katherine, three species of blind atyid shrimps (Williams 1964, Bruce 1992) and a *Mesocyclops* copepod (Dumont and Maas 1983) are known. On the western border of the Northern Territory, in the Judburra/Gregory karst caves, two stygofaunal species were recorded alongside cave-dwelling taxa (Moulds and Bannink 2012). In the monsoonal north, the alluvial aquifers of Magela Creek are known to harbour undescribed hyporheic fauna (Dostine *et al.* 1997), and more recently also stygofauna have been described (Chandler *et al.* 2017). There have also been unpublished reports of stygofauna in the Gulf County and at Gregory National Park in the Victoria River District, and the Daly River catchment.

A pilot-scale survey by Rees et al (2020) has provided the greatest insight into the stygofauna present in the Beetaloo Sub-basin. Twenty six bores were sampled and live stygofauna were present in 6 of the bore water samples. All Beetaloo stygofaunal communities sampled were dominated by crustaceans, namely: shrimps, amphipods, ostracods, copepods and syncarids. This fauna showed

little affinity with the stygofauna recorded from more extensively sampled Western Australian aquifers, with new genera and species likely to be present in the Beetaloo Sub-basin. In addition to traditional netting techniques, examining the environmental DNA (eDNA) present in water samples indicated a wider presence of stygofauna across the Beetaloo sub-basin. These eDNA samples indicated the presence of stygofauna in 20 of the bores encompassing a broad range of taxonomic groups (bacteria, fungi, and invertebrates).

The Strategic Regional Environmental and Baseline Assessments (SREBA) is conducting a regional stygofauna assessment as part of aquatic ecosystem baseline studies required for the Beetaloo Sub-basin. This program aims to sample approximately 70 bores from across the region, with the design including sites both in the aquifers of the Gum Ridge, Anthony Lagoon Beds and Tindal Limestone formations, and basins that connect to the Daly and Roper River cantchments. In addition to general stygofauna sampling, using a standardised net haul approach, environmental DNA methods are being employed that are aimed at detection of *Parisia* sp. Basic ground water chemistry, including dissolved oxygen, electrical conductivity, temperature and pH are also being collected from the bores. This project will be finalised by the end of 2022.

Hydrogeology of the Beetaloo Sub-basin

The hydrogeology of the CLA in the Beetaloo Sub-basin has been reviewed at the regional scale as part of the Geological and Bioregional Assessment (GBA) of the Beetaloo GBA region (Evans et al 2020). There is a relatively good understanding of the regional groundwater flow systems for parts of the CLA but whole subregions (like the Wiso Basin) still have received limited attention, in part owing to the limited availability of infrastructure. There is a sizeable database available for some water quality parameters for CLA groundwater (such as major ion compositions) but less so for others (oxygen levels, dissolved organic matter, etc; GBA 2021b,c,d). Whilst there is widespread evidence for karst features in the CLA, its role in local and regional hydrogeological processes remains unclear. Recent environmental tracer surveys in CLA groundwater in the vicinity of Beetaloo Sub-basin showed a patchy distribution for tritium (an indicator of relatively ‘young’ groundwater; GBA 2021b,c,d). As much of the CLA is under cover in this area, this suggests localized point recharge via karst features is present (also consistent with observations by local landholders). Likewise, a recent monitoring program at three springs in Elsey National Park showed that one spring was partially sustained by water recharge by recent (weeks) rain event (GBA 2021a). This would be consistent with localised recharge and groundwater flow through karst at that spring but not at nearby ones.

Risk assessment

The GBA assessment found that pathways to impact unconfined aquifers through changes to water quality or ground water levels (characteristics of groundwater that impact groundwater dependent ecosystems, including stygofauna) were of low to very low concern throughout most of the region. However, the GBA impact assessment of unconventional gas development in the Beetaloo did not explicitly investigate impacts on stygofauna (Geological and Bioregional Assessment Program, 2021).

Project development

The previous stygofauna sampling program provided a snapshot of their presence in a small number of bores. However, whether the distribution is the result of specific environmental requirements, sampling artefacts, or some combination of the two is unclear. Owing to the relatively large size of some of the stygofauna, a link with karst features in the CLA is likely. This project will evaluate in more detail the links between stygofauna and the subsurface conditions that are likely drivers of the presence of stygofauna. The water chemistry, along with the physical conditions, (such as distribution of large pore spaces in the CLA), are likely key factors in determining the presence of stygofauna. This will be achieved in a first instance by examining in more detail the presence or absence of stygofauna (especially the larger organisms) from previous surveys relative to a range of local hydrogeological parameters (bore construction, evidence and location of karst or cavities from bore logs, etc). This will be used to formulate hypotheses about what may control stygofauna distribution in the CLA. These will be presented and debated at an expert workshop with the aim to test these hypotheses via a targeted field program. It is anticipated that this field program would seek to characterize in more detail the habitat where stygofauna are or are not found. In particular, it is anticipated that finer scale measurements in open boreholes will be used to describe the microhabitats where stygofauna tend to be found. This could include, for example, downhole profiling for various water quality parameters, the use of packer tests or similar approach to isolate and look at the specific properties of individual fractures or karst features, downhole cameras, etc. Also to be considered at the workshop is whether or not some monitoring infrastructure specific for stygofauna monitoring is required in order to address current perceived potential shortcomings of 'traditional' water quality monitoring bores. This could be as simple as using open (that is uncased) boreholes when these are feasible but other designs could be considered.

Combining groundwater information with ground truth sampling for stygofauna will increase predictive capacity in determining their distribution. A further detailed sampling design will also be able to unravel the key questions regarding whether animals present in bores accurately reflect those in aquifers, and whether subsurface biological communities are well connected, or endemic within small regions.

Need & Scope

The pilot-scale work of Rees et al (2020) and Oberprieler et al (2020) confirmed the direct presence of stygofauna in 6 of the 26 bores, with additional DNA evident of stygofauna in a total of 20 bores that were sampled in the Beetaloo Sub basin. This pilot-scale survey was designed so as to trial different approaches to collect water samples from different types of bores, thus trying to ensure the maximum likelihood of collecting animals, should they be present. The sampling design included bores with depth to water ranging from some 20m to up to ~100m, and approximately 300 km distance.

The outcomes of the pilot-scale project demonstrated the importance of carrying out a widespread systematic study in the Beetaloo Sub-basin, examining a diversity of bores and aquifers for the presence of stygofauna.

The project described within this proposal builds on the detailed work being undertaken as part of SREBA described above. The CSIRO project provides a highly complementary approach (and data) that will draw on the baseline assessment to address key knowledge gaps in our understanding of the controls on distribution of stygofauna across the Beetaloo Sub-basin.

Key questions required to understand the ecology of stygofauna at basin scale are:

- How widespread are stygofauna within the Beetaloo Sub-basin and are there clear relationships between presence of stygofauna and physical characteristics of bores (such as depth, temperature), and water chemistry?
- What degree of similarity is there between the communities that occur throughout the Beetaloo Sub-basin? Is there evidence of a high degree or low degree of connectivity between the bore water communities.
- Do bore water samples represent what may colonise the aquifers or does taking samples in the vicinity of bores bias the presence and frequency of species?
- Given the impracticalities of sampling all bores within the basin, is there potential to predict the presence of stygofauna such as, on aquifer/bore water physical and chemical properties, possible surface vegetation etc.
- What is the primary food resource for the stygofauna and is this potentially impacted by surface activities.
- What bore parameters can be used to predict the presence of stygofauna (i.e. depth at which the bore intersects the carbonate/limestone formation).

Answers to these questions form a core part in understanding any potential impacts that gas extraction activities may have on the subsurface fauna. The research activities in this proposal complement the SREBA stygofauna program, which is compiling baseline data on the distribution of fauna throughout the basin.

Key questions to understand how stygofauna may be impacted by gas extraction activities are:

- How may groundwater systems be impacted by gas development activities?
- Extent and duration of those impacts
- Consequences of those impacts on stygofauna at individual sites and across the basin
- Effectiveness of mitigation measures in preventing those impacts.
- Appropriate monitoring of the performance of mitigation measures

Objectives

The overall objectives for this project are:

- Defining the environmental conditions that drive the extent and distribution of stygofauna assemblages across the Beetaloo Sub-basin.
- Develop an understanding of the ecology of the environment that supports stygofauna in subterranean groundwater.
- Using the understanding of environmental conditions and ecology of stygofauna gained within the project to consider how they may be impacted by onshore petroleum activities and implications for future management and monitoring.

Methodology

Stage 1. Discovery phase.

The discovery phase will be used to ensure all the relevant information on stygofauna and groundwater systems in the Beetaloo region is captured prior to commencing work. The task involves a workshop with industry, stygofauna and groundwater experts to elicit what regulators and industry need to know to make decisions about managing potential impacts to stygofauna.

- Review available stygofauna literature (public and potentially grey literature, industry reports, where relevant), noting particular aspects particularly relevant to impacts from development activities.
- Review available data on stygofauna and groundwater systems in the Beetaloo region as input data into investigations of the environmental conditions that drive the extent and distribution of stygofauna assemblages, including:
    - Relevant literature on the groundwater systems (including water chemistry) in the Beetaloo to guide preliminary sampling campaign.
    - Baseline data collected by the SREBA stygofauna project.
- The data from this review use this to inform bore selection to be carried out in both the CSIRO sampling program and drilling of observation bores.
- Review stygofauna monitoring approaches to consider whether specific monitoring infrastructure is required in order to address perceived potential shortcomings of 'traditional' water quality monitoring bores.

The workshop will include an initial risk assessment (based on the Geological and Bioregional Assessments causal network for the Beetaloo region) to explore potential pathways for onshore gas activities to impact on stygofauna.

The outcome of the discovery phase will be used to refine the remainder of project scope.

Stage 2. Implementation phase – communities, environmental conditions and ecosystems

Stygofauna sampling

The implementation stage involves three field campaigns. While their timing is to be confirmed, it is likely that they would occur in the 2022, 2023 and 2024 dry seasons. The first sampling program is designed to target a small subset of bores, (potentially up to 6), where stygofauna are known to occur and where stygofauna are known to be absent. The second field trip is a major activity and will sample a more extensive range of bores, whose selection will be based on the outcomes of the Task 1, in particular, incorporating the findings of the SREBA stygofauna project. Up to 20 bores are planned to be sampled, with a further 10 included as backups if on site changes to the planning are required. Field trip three is to carry out an assessment on the new observation bores.

No one sampling method can used to sample the range of bore types in the Beetaloo sub-basin as bores vary in diameter and depth, and on a number of pastoral leases have fixed pumps on the bore head. As a consequence, water samples will be collected either directly from pumps, handheld nets, or via traps. Although the use of pumps allows the sampling of large quantities of water, the water comes from a single depth, unlike nets that can be drawn through the entire water column. A further disadvantage of pumping as it potentially leads to the destruction of stygofauna as they pass through the pump, making them difficult to identify.

As part of the detailed planning process, we will explore as many options for sampling that will address the issue of possible sample bias in bores. Part of this will also be to try and address the question relating to depth of bores, and where animals are present. One possible approach to resolve questions regarding the presence of stygofauna in bores vs aquifers is to carry out continued pumping on a given bore until up to 3x the volume of the bore has been removed, therefore sampling aquifer water. A further possibility is drilling of observation bores closely associated with bores to determine the physical properties closely associated with bore holes. Direct observation (see below) will part of these approaches.

For net sampling, a 50µm-sized mesh will be lowered to the bottom of the bore before being raised. Different diameter nets will be used in matching bores. The draw back with nets is that many of the older bores were corroding leading to blockages of nets while being raised.

Stygofaunal sample processing

All stygofaunal samples will be preserved in 70% ethanol and will be subsequently examined using stereo and dark-field enabled microscopy. Taxa will be identified, based on morphological characters, to the lowest taxonomic level possible. These identifications will be subsequently validated by experts: Dr Stuart Halse (Bennelongia Environmental Consultants) and Dr John Short (BioAccess Australia).

Environmental DNA

Analysing samples for environmental DNA (eDNA) is a very useful approach to examples where water sampling is very difficult (e.g. from pumps and taps). Approximately 300ml of bore water will be collected from each site and preserved in the field. DNA extraction, analysis for animals will be undertaken at the CSIRO laboratory in Thurgoona, NSW.

Direct observation of bore water

A video camera will be lowered into the bore to determine:

- The distribution of the stygofauna down the bore column, gaining an understanding of the habitat in which they survive.
- Video camera will also give some insight to the state of the bores.

Biogeography analysis.

We will carry out a DNA-based approach (Single Nucleotide Polymorphisms (SNPs), a method well recognized for investigation of species biogeography and population genetics (Zimmerman et al. 2020). We will aim to collect 10 individuals from different bores situated across the CLA. Data will be analysed using the DArTR (Gruber et al. 2018) R package and STRUCTURE (Pritchard Lab, Stanford University) to investigate genetic structure among populations. The analysis will provide a measure of how connected populations are (i.e. how much gene flow occurs among populations).

Laboratory analysis

Analysis of water quality samples and identification and enumeration of stygofauna samples will be carried out at CSIRO laboratories. Stygofauna will be identified to the lowest possible taxonomic resolution. Since stygofauna taxonomy is limited, 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 DNA analyses will include 16SrRNA (microbes) and 18S rRNA (animals) metabarcoding.

Groundwater variables

Water depth will be measured with a water level sounder. Electrical conductivity (EC), pH and water temperature (°C) will be measured on water samples. We would also measure dissolved oxygen concentration in bores (throughout the entire depth, to assist in understanding where stygofauna reside. To ensure no cross-site contamination, the pump heads, nets and catching jars will be thoroughly cleaned and disinfected between sites by washing in a bleach-solution.

Ecosystem analysis.

We will use a range of univariate and multivariate techniques to carry out exploratory analysis. Analysis will investigate the spatial distribution of stygofauna within the Beetaloo sub-region and explore how the spatial distribution is influenced by the physical and water quality properties of the

bores. Statistical analysis will be used to examine relationships between water physical conditions and chemistry.

Stage 2. Implementation phase –observation bores

The construction of several observation bores is proposed as part of the project's investigation of sampling and monitoring approaches for stygofauna. Purpose built bores will allow an investigation of sampling bias due to the age of a bore and the way the bore has been operated and constructed.

Planning

The planning stage for the observation bores will need to allow sufficient time for regulatory approvals. The planning steps will include the following:

- Refining objectives of the observation bores.
- Design of the observations bores.
- Determining suitable locations.
- Negotiating access with the relevant landholders.
- Obtaining relevant regulatory approvals.
- Apply for Authority Certificate(s) from the Aboriginal Areas Protection Authority.
- Obtaining quotations from suitability qualified licensed bore driller.

The project team will liaise with the NT Industry Capability Network (NTICN) and NT DEPWS to source a local drilling company. We anticipate drilling between 2-4 observation bores which will be sampled as part of a third field program. While the initial purpose of the observation bores is for stygofauna and groundwater chemistry sampling, multiple uses of the bores is possible and will be explored as part of the design process. Consideration of ongoing use of the bores post project completion will be part of the planning process.

Sampling

The observation bores will be sampled in the third field program. A wide range of sampling methods will be used for comparison with results obtained from existing bore data collected in the SREBA baseline assessment and in the other field campaigns in this project. Applying different sampling methods will also allow an assessment of the suitability of bore-based sampling for characterising stygofauna assemblages and optimization of these sampling methods. Additional consideration will be given to sampling methods that are efficient and effective for ongoing monitoring.

Stage gate / Decision point

There is a decision point during the implementation phase where the GISERA NT Research Advisory Committee will be presented for approval, the details on the drilling campaign and the wide-scale field campaign. *Figure 1* in section 6 Project Plan provides a schematic outlining the project stages and decision point.

Stage 3. Reporting phase

In the reporting phase the results of the reviews, field campaigns, laboratory work and analyses in stage 1 and stage 2 will be compiled and discussed in terms of the project's first two objectives. Final reporting will present what the project has found regarding:

- The environmental conditions that drive the extent and distribution of stygofauna assemblages across the Beetaloo Sub-basin.
- The ecology of the environment that supports stygofauna in subterranean groundwater.

The final report will also outline the observation bore drilling program and discuss the advantages and disadvantages of different approaches to sampling and monitoring of stygofauna.

Implications for management of potential impacts on stygofauna

The implications of the research's findings for ongoing management and monitoring of stygofauna will also be discussed to address the third objective of the project. The understanding of environmental conditions and ecology of stygofauna gained within the project will be used to consider whether and how stygofauna may be impacted by onshore petroleum activities. The project team will reconvene the industry, regulators and stygofauna and groundwater experts for a second workshop to discuss the results of the research and their implications. The workshop will revisit the initial risk assessment conducted at the first workshop to update it with the results of this project. The results of the project will be discussed in terms of their implications for:

- Approaches (including sampling methods) to characterise stygofauna at a specific site or within a geographic region.
- Assessment of potential impact pathways from onshore gas activities to stygofauna, including mitigation options.
- Development of environmental performance standards and measurement criteria for ongoing monitoring of stygofauna or the ecosystems they depend upon.

This work on stygofauna will benefit from other GISERA research on groundwater. The results of this project will provide knowledge that can inform site-specific or project-scale assessments. However, it is not intended to replace site or project specific assessments of environmental impacts that project proponents may be required to do through territory or Commonwealth regulations.

3. Project Inputs

Resources and collaborations

Researcher	Time Commitment (project as a whole)	Principle area of expertise	Years of experience	Organisation
Gavin Rees	96 days	Microbial ecology, with broad expertise in freshwater ecology and experience working with the petroleum industry. Led previous stygofauna project	>30 yrs	CSIRO
Paul McInerney	65 days	Ecosystem ecologist with a focus on freshwater ecology.	>20 yrs	CSIRO
Garth Watson	80 days	Molecular DNA techniques	>30 yrs	CSIRO
Cameron Huddlestone-Holmes	25 day	NT GISERA lead, geoscientist who has been working on gas development risk and the Beetaloo region for a few years now.	>20 yrs	CSIRO
Sebastien Lamontagne	72 days	Groundwater hydrology, lead researcher on groundwater quality in the GBA assessment of the Beetaloo.	>30 yrs	CSIRO
Maxine Piggott	38 days	Molecular Biologist with expertise in molecular tools including eDNA for biodiversity monitoring in freshwater and terrestrial ecosystems	>20 yrs	CSIRO
Research assistant	90 days	Field expertise in groundwater analysis	>10 yrs	CSIRO

Subcontractors (clause 9.5(a)(i))	Time Commitment (project as a whole)	Principle area of expertise	Years of experience	Organisation
Dr Michael Shackleton	30 days	Molecular DNA analysis and invertebrate taxonomy	>20 yrs	La Trobe University
Dr Daryl Nielsen	56 days	Freshwater ecologist, with expertise in invertebrate ecology. Key part of previous stygofauna project	>30 yrs	CSIRO Hon fellow
Chris Davey	50 days	Freshwater ecologist with experience in macroinvertebrate identification	>10 yrs	Information to be provided.
Drilling company, provider to be confirmed	N/A	Drilling contractor to build observation bores	>10 yrs	As per first column

Technical Reference Group

The project will establish a Technical Reference Group (TRG) aimed at seeking peer-to-peer technical advice on contextual matters and to discuss research needs as well as outputs as the project progresses. The TRG will include the core members of the project team and a group of different stakeholders that include, but not limited to:

- Stygofaunal expertise
- On-ground and industry expertise
- Government agency personnel including a representative from NT Department of Environment, Parks and Water Security (DEPWS)

Budget Summary

Source of Cash Contributions	2021/22	2022/23	2023/24	2024/25	2025/26	% of Contribution	Total
GISERA	$0	$495,858	$900,798	$14,517	$0	80%	$1,411,174
- Federal Government	$0	$384,290	$698,119	$11,251	$0	62%	$1,093,660
- NT Government	$0	$37,189	$67,560	$1,089	$0	6%	$105,838
- Santos	$0	$37,189	$67,560	$1,089	$0	6%	$105,838
- Origin	$0	$37,189	$67,560	$1,089	$0	6%	$105,838
Total Cash Contributions	**$0**	**$495,858**	**$900,798**	**$14,517**	**$0**	**80%**	**$1,411,174**

Source of In-Kind Contribution	2021/22	2022/23	2023/24	2024/25	2025/26	% of Contribution	Total
CSIRO	$0	$123,965	$225,200	$3,629	$0	20%	$352,793
Total In-Kind Contribution	**$0**	**$123,965**	**$225,200**	**$3,629**	**$0**	**20%**	**$352,793**

TOTAL PROJECT BUDGET	2021/22	2022/23	2023/24	2024/25	2025/26	-	TOTAL
All contributions	**$0**	**$619,823**	**$1,125,998**	**$18,146**	**$0**	**-**	**$1,763,967**
TOTAL PROJECT BUDGET	**$0**	**$619,823**	**$1,125,998**	**$18,146**	**$0**	**-**	**$1,763,967**

4. Communications Plan

Stakeholder	**Objective**	**Channel** (e.g. meetings/media/factsheets)	**Timeframe** (Before, during at completion)
Regional community / wider public	To communicate project objectives and key messages about the research	A fact sheet at commencement of the project which explains in plain English the objective of the project. A factsheet with general information on stygofauna to provide a resource for the community.	At commencement of the project.
Gas Industry	Industry adopts methods for improving biological assessment	Presentation of findings at joint Gas Industry/Government Knowledge Transfer Session	At Completion
Government	Advice provided to senior bureaucrats / ministers / policy makers	Presentation of findings at joint Gas Industry/Government Knowledge Transfer Session	At Completion
Community stakeholders, including land holders and traditional owners.	Presentation of research findings	A range of approaches will be explored to present findings to the community, with close alignment with GISERA communication's activities. This may include, but not necessarily be limited to, plain English documents (e.g., 'The Conversation' articles, fact sheets) and material shared by CSIRO on relevant social media forums. Opportunities for face-to-face forums with stakeholder groups (through NTCA, NLC, CLC for example) will be explored.	At Completion
Regional community/wider public, government, scientific community and industry	To report on key findings.	Public release of final report. Plain English factsheet summarising the outcomes of the research.	At project completion
Scientific Community	Provide scientific insight into the distribution of stygofauna in karst systems and data collected during the project.	Peer-reviewed scientific publication. Dataset(s) available through CSIRO's data repository.	After completion of project

In addition to project specific communications activities, CSIRO's GISERA has a broader communications strategy. This strategy incorporates activities such as webinars, roadshows, newsletters and development of other communications products.

5. Project Impact Pathway

Activities	Outputs	Short term Outcomes	Long term outcomes	Impact
Knowledge discovery and workshop	Scope of works and plans for sampling campaign finalized, based on knowledge of bore and most up to date available information from SREBA activities.	Provide information on the biodiversity and distribution on stygofauna in the Beetaloo sub-Basin. Field sampling will provide information on spatial variability across the project area and an understanding of the physical drivers that support stygofauna communities.	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. These data will provide scientific underpinning of the biodiversity values associated with the Beetaloo sub-Basin, that will lead to improved industry practices. Importantly, local communities will be informed of the ecological values associated with groundwater systems.	The main impact will be that industry operates in a financially effective manner, while ensuring biodiversity and values are maintained Policy makers will be informed of the subsurface biodiversity values, leading to better decision making and policy formation
Implementation of sampling program	Installation of dedicated infrastructure. Collection of samples. Databases of stygofauna. Database of relevant physical properties of the regions associated with the bores and groundwater chemistry.			
Analysis, reporting and communications	The final report will form the basis of all the final outputs, which will include a factsheet and a knowledge transfer session.			

6. Project Plan

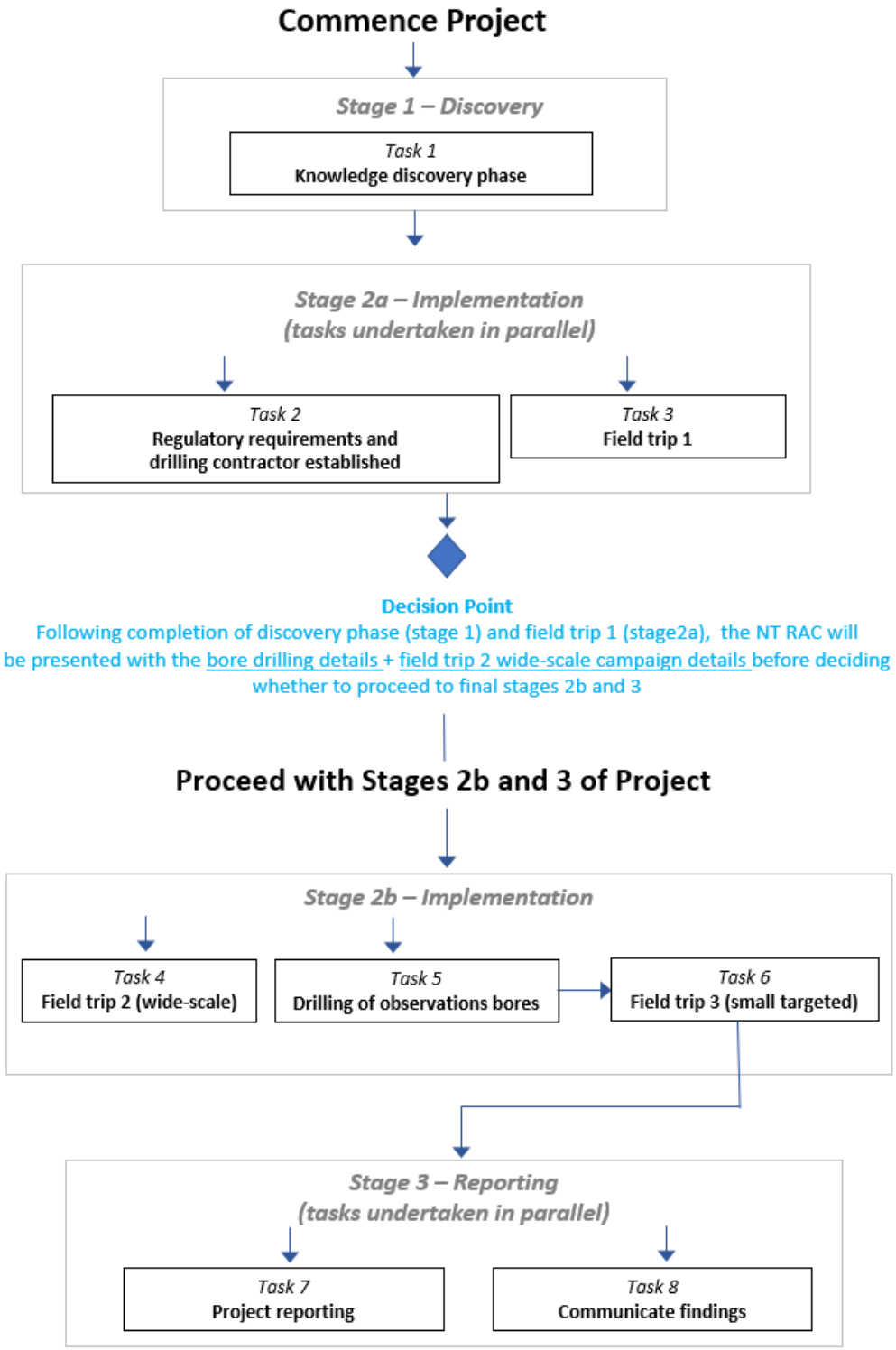


Figure 1. Pathway schematic outlining the project stages and decision point

Project Schedule

ID	Activities / Task Title (should match activities in impact pathway section)	Task Leader	Scheduled Start	Scheduled Finish	Predecessor
Task 1	Knowledge discovery phase	Gavin Rees	01 Jul 2022	30 September 2022	-
Task 2	Planning and approvals for drilling of observation bores + drilling contractor procurement	Gavin Rees	1 October 2022	30 September 2023	Task 1
Task 3	Field trip 1 – initial campaign	Gavin Rees	1 October 2022	29 February 2023	Task 1
Stage Gate / NT RAC Decision Point					
Task 4	Field trip 2 – wide-scale campaign	Gavin Rees	1 April 2023	29 February 2024	Tasks 1-3
Task 5	Construct observation bores for Stygofauna	Sebastian Lamontagne	1 July 2023	29 February 2024	Tasks 1-3
Task 6	Field trip 3 – small targeted campaign	Gavin Rees	1 March 2024	31 May 2024	Tasks 1-5
Task 7	Analysis and reporting	Gavin Rees	01 Nov 2022	31 July 2024	Task 1
Task 8	Communicate findings to stakeholders	Gavin Rees	Full duration of project		

Task description

DISCOVERY PHASE

Task 1: Knowledge discovery phase

OVERALL TIMEFRAME: 1 July 2022 – 30 September 2022

BACKGROUND: While some work has now been carried out on Stygofauna in the Beetaloo Sub-basin, along with a significant amount of work into the ground water system, it will be important to ensure all the relevant information is captured prior to commencing work. It is important that this knowledge base, combined with the latest findings from current projects in the NT inform the proposed activities and that they will address the important issues associated with industry. The task involves a workshop with industry, stygofauna and groundwater experts to elicit what regulators and industry need to know to make decisions.

TASK OBJECTIVES:

1) collate all relevant groundwater and stygofauna literature

2) carry our work shop that includes evaluation of the proposed scope of works, ensuring works address industry needs

TASK OUTPUTS AND SPECIFIC DELIVERABLES: A document addressing task objectives.

IMPLEMENTATION PHASE

Task 2: Planning and approvals for drilling of observation bores + drilling contractor procurement

OVERALL TIMEFRAME: 1 October 2022 – 30 September 2023

BACKGROUND: We intend drilling up to 4 observation bores (Task 5) to understand more on distribution of stygofauna, as well as develop an understanding of the potential bias in simple sampling bores directly. This task involves carrying out all the preliminary planning, procurement, administrative and regulatory requirements for implementation of drilling activities. The team will seek input from workshop participants (task 1) on the objectives for the observation bores.

The planning for the observation bores will consider :

1) Refining overall objectives and hypotheses to be tested.
2) Determine best type of bore to build and measurements to make to test the hypotheses.
3) Selection of suitable locations for the bores.

TASK OBJECTIVES: To develop a plan for the drilling of observation bores, including:

1) Plan bore drilling program and selection of bore locations
2) Negotiate access with land holder

3) Meet all relevant regulatory requirements for drilling the bores
4) Meet requirements from Aboriginal Areas Protection Authority (AAPA)
5) Work with NT Industry Capability Network (NTICN) to source local licensed water bore drilling company
6) Establish procurement plan for drilling contractor

TASK OUTPUTS AND SPECIFIC DELIVERABLES: A plan for the drilling of observation bores for RAC approval that includes:

1) Observation bore objectives, work program and bore locations.
2) Agreement(s) with land holders for access.
3) Relevant regulatory approvals/permits for drilling bores obtained.
4) AAPA Authority Certificate (either obtained or in progress).
5) Procurement plan for drilling progress and ready for execution.

Task 3: Field trip 1 (initial campaign)

OVERALL TIMEFRAME: 1 October 2022 – February 2023

BACKGROUND: The previous stygofauna sampling program provided a snapshot of their presence in a small number of bores. However, whether the distribution is the result of specific environmental requirements, sampling artefacts, or some combination of the two is unclear. This task sets out a structured process to examine the knowledge gaps associated with stygofauna and their distribution.

The task involves an initial field campaign, carrying out a very detailed examination of up to 6 bores known to harbour stygofauna and those where stygofauna are known to be absent. Outputs will used to inform a second field campaign (task 4). An internal workshop of technical experts will be engaged to provide commentary of the second field campaign (task 4).

TASK OBJECTIVES:

1) Carry out an initial field campaign that targets bores known to harbour stygofauna as well as an equal number of bores where stygofauna have not previously been detected.
2) Sample for stygofauna, while simultaneously carrying out detailed analysis of the physical and chemical aspects of the subsurface and ground water.

TASK OUTPUTS AND SPECIFIC DELIVERABLES:

1) Databases of stygofauna observations.
2) Database of relevant physical properties of the regions associated with the bores and groundwater chemistry.

3) Plan for second field campaign (task 4).

STAGE GATE / DECISION POINT:

Present to the NT RAC, the details on proposed drilling campaign and the wide-scale field campaign and seek approval to proceed with stage 2B (task 4, 5 and 6) and stage 3 (task 7 and 8) of project.

Task 4: Field trip 2 (wide-scale campaign)

OVERALL TIMEFRAME: 1 April 2023 – 29 February 2024

BACKGROUND: Field campaign 1 (Task 3) will provide detailed insight into properties of bores that support stygofauna. This field campaign seeks to broaden the number of sites (up to 20 bores, with a further 10 included as backups), thereby improving predictive capability to understand where stygofauna are found.

Connectivity of stygofaunal communities within the aquifers of the NT is unknown, yet key to understanding the ecology of stygofauna. This task will incorporate DNA-based biogeography analysis of *Parisia* sp. collected at different sites across the NT.

TASK OBJECTIVES:

1) Use outputs from field trip 1 (task 3) to inform this program.
2) Sample for stygofauna via a wide-scale sampling program.
3) Carry out analysis of date collected in the wide-scale field program.

TASK OUTPUTS AND SPECIFIC DELIVERABLES:

1) Databases of stygofauna.
2) Database of relevant physical properties of the regions associated with the bores and groundwater chemistry.

Task 5: Construct observation bores for Stygofauna

OVERALL TIMEFRAME: 1 July 2023 - 29 February 2024

BACKGROUND: This task is for the drilling of the observation bores according to the plan developed in Task 2.

TASK OBJECTIVES: To install dedicated infrastructure to monitor and collect information on stygofauna distribution.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Drilling and completion of 2-4 observation bores

Task 6: Field trip 3 (small targeted campaign)

OVERALL TIMEFRAME: 1 March 2024 – 31 May 2024

BACKGROUND: All stygofauna sampling carried out to date has been direct net capture or pumping water from bores. A key unknown is the extent to which bore samples represent ecosystems more broadly. This task involves carrying out a small, but targeted field campaign to measure ground water physical and chemical properties, and to collect samples for stygofauna analysis.

TASK OBJECTIVES: To take samples from drilled observation bores.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Information collected on stygofauna distribution and sampling methods.

REPORTING PHASE

Task 7: Project Reporting

OVERALL TIMEFRAME: 1 November 2022 – 31 July 2024

BACKGROUND: Information from this project is to be made publicly available after completion of standard CSIRO publication and review processes.

TASK OBJECTIVES: Prepare reports on all findings from the project.

TASK OUTPUTS AND SPECIFIC DELIVERABLES:

1) Deliver summary following first field campaign.
2) Preparation of a final report outlining the scope, methodology, scenarios, assumptions, findings and any suggestions/options for future research.

Task 8: Communicate project objectives, progress and findings to stakeholders

OVERALL TIMEFRAME: Full duration of project

BACKGROUND: Communications of GISERA research are an important component of outreach and dissemination of findings to diverse audiences.

TASK OBJECTIVES: Communicate project objectives, progress and findings to stakeholders through meetings, knowledge transfer session, factsheet and journal article, in collaboration with GISERA Communications officers.

TASK OUTPUTS AND SPECIFIC DELIVERABLES: Communicate project objectives, progress and results to GISERA stakeholders according to standard GISERA project procedures which may include, but not limited to:

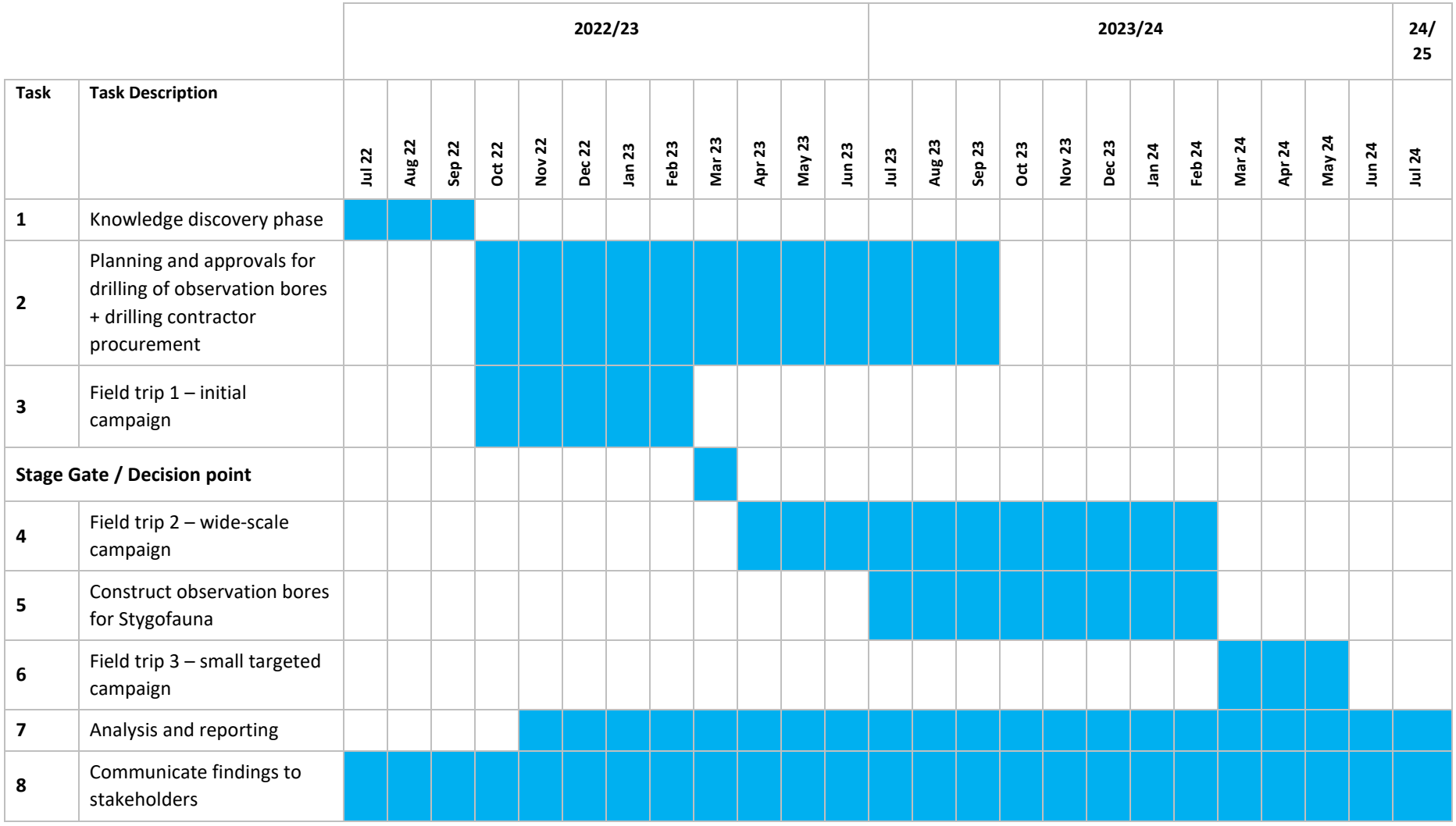
1) Knowledge Transfer session with Government/Gas Industry

2) Presentation of findings to Community members/groups. This may include, but not necessarily be limited to, plain English documents (e.g., 'The Conversation' articles, fact sheets), material shared by CSIRO on relevant social media forums and a face-to-face forum.

3) Preparation of article for GISERA newsletter.

4) Peer reviewed scientific manuscript ready for submission to relevant journal

Project Gantt Chart



7. Budget Summary

Expenditure	2021/22	2022/23	2023/24	2024/25	2025/26	Total
Labour	$0	$344,623	$366,598	$18,146	$0	$729,367
Operating	$0	$170,000	$147,000	$0	$0	$317,000
Subcontractors	$0	$105,200	$612,400	$0	$0	$717,600
Total Expenditure	**$0**	**$619,823**	**$1,125,998**	**$18,146**	**$0**	**$1,763,967**

Expenditure per task	2021/22	2022/23	2023/24	2024/25	2025/26	Total
Task 1	$0	$73,528	$0	$0	$0	$73,528
Task 2	$0	$54,556	$11,871	$0	$0	$66,427
Task 3	$0	$248,010	$0	$0	$0	$248,010
Task 4	$0	$132,680	$369,319	$0	$0	$501,999
Task 5	$0	$0	$516,226	$0	$0	$516,226
Task 6	$0	$0	$62,594	$0	$0	$62,594
Task 7	$0	$109,230	$156,633	$10,451	$0	$276,314
Task 8	$0	$1,819	$9,355	$7,695	$0	$18,869
Total Expenditure	**$0**	**$619,823**	**$1,125,998**	**$18,146**	**$0**	**$1,763,967**

Source of Cash Contributions	2021/22	2022/23	2023/24	2024/25	2025/26	Total
Federal Govt (62%)	$0	$384,290	$698,119	$11,251	$0	$1,093,660
NT Govt (6%)	$0	$37,189	$67,560	$1,089	$0	$105,838
Santos (6%)	$0	$37,189	$67,560	$1,089	$0	$105,838
Origin (6%)	$0	$37,189	$67,560	$1,089	$0	$105,838
Total Cash Contributions	**$0**	**$495,858**	**$900,798**	**$14,517**	**$0**	**$1,411,174**

In-Kind Contributions	2021/22	2022/23	2023/24	2024/25	2025/26	Total
CSIRO (20%)	$0	$123,965	$225,200	$3,629	$0	$352,793
Total In-Kind Contributions	**$0**	**$123,965**	**$225,200**	**$3,629**	**$0**	**$352,793**

	Total funding over all years	Percentage of Total Budget
Federal Government investment	$1,093,660	62%
NT Government investment	$105,838	6%
Santos investment	$105,838	6%
Origin investment	$105,838	6%
CSIRO investment	$352,793	20%
Total Expenditure	**$1,763,967**	**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	Knowledge discovery phase	GISERA	Jul-22	Sep-22	2022/23	$58,822
Task 2	2.1	Planning and approvals for drilling of observation bores + drilling contractor procurement	GISERA	Oct-22	Oct-23	2023/24	$53,142
Task 3	3.1	Field trip 1 – initial campaign	GISERA	Oct-22	Feb-24	2023/24	$198,408
Stage Gate / NT RAC Decision Point							
Task 4	4.1	Field trip 2 – wide-scale campaign	GISERA	Jul-23	Feb-24	2023/24	$401,599
Task 5	5.1	Construct observation bores for Stygofauna	GISERA	Jul-23	Feb-24	2023/24	$412,981
Task 6	6.1	Field trip 3 – small targeted campaign	GISERA	Mar-24	May-24	2023/24	$50,075
Task 7	7.1	Analysis and reporting	GISERA	Nov-22	Jul-24	2024/25	$221,051
Task 8	8.1	Communicate findings to stakeholders	GISERA	Jul-22	Jul-24	2024/25	$15,095

8. 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 13.1)	**Party**	**Commercialisation Interest**
	CSIRO	N/A
	Origin Energy	N/A
	Santos	N/A

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