Australia's National
Science Agency

GISERA | Gas Industry Social and Environmental Research Alliance

Progress report

Environmental monitoring and microbial degradation of onshore shale gas activity chemicals and fluids

CSIRO | Australia Pacific LNG | QGC | origin | Santos | NSW Government | Australian Government Department of Industry, Science, Energy and Resources | Supported by Government of South Australia | Queensland Government | Northern Territory Government

Progress against project milestones

Progress against milestones/tasks are approved by the GISERA Director, acting with authority in accordance with the 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 is withheld.
  - 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 by GISERA Director.

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

TASK NUMBER	TASK DESCRIPTION	SCHEDULED START	SCHEDULED FINISH	COMMENT
1	Logistics, planning, bore and soil selection	May-19	Jul-19	
2	Sampling campaign	Jul-19	Jul-19	
3	Baselining microbial communities	Aug-19	Aug-20	
4	Microbial degradation and sole carbon growth trials	Aug-19	Aug-20	
5	Impact and indicator taxa	Nov-19	Nov-20	
6	Final Report	May-19	Nov-20	

Project schedule report

TASK 1: Logistics, planning, bore and soil selection

BACKGROUND

During Task 1, staff will work with team members of GISERA project W.16 to establish the sampling sites for large volume water samples required. This task will also identify the sampling sites for soil collection. Chemicals to be tested in subsequent tasks will be determined in Task 1 via consultation with industry and a regulator. Task 1 will interact with the parallel running project investigating stygofauna in the Beetaloo sub-Basin to ensure project compatibility. Task 1 will also include the safe and environmentally-sensitive planning, provisioning and logistics for the sampling campaign.

TASK OBJECTIVES

1. Establish water and sampling sites within the Beetaloo sub-Basin;
2. Liaise with project W.16 and stygofauna project teams for site selection and sampling campaign logistics.
3. Determination of chemicals of relevance for onshore gas production in the Northern Territory.
4. Identification of sites for soil collection for use in Tasks 2-5.
5. Preparation of sampling equipment/reagents.
6. Preparation for remote sampling fieldwork; and
7. Identification of any permits, permission or consultation required for sampling.

TASK OUTPUTS AND SPECIFIC DELIVERABLES:

A briefing document will be prepared for the sampling campaign describing the outcomes of task objectives 1-6.

PROGRESS REPORT

All seven of the objectives listed for Task 1 were achieved.

1. Project staff consulted team members of GISERA project W.16 to establish the sampling sites and acquire information useful for planning purposes. Electronic geoscience data, such as ESRI files for ArcGIS, other GIS data and borehole location data were purchased for a small fee from the relevant Northern Territory government department, acquired from CSIRO colleagues involved in W16 and provided by the supporting companies (Origin Energy and Santos). Maps were constructed for planning the sampling program and the preferred water sampling sites were selected. A detailed sampling schedule was also produced as a starting point in discussions with company representatives.
2. Following the first meeting project members of the W17 and W18 (the stygofauna project) liaised regularly to coordinate a joint sampling campaign for both projects as requested by company representatives. Coordinating a joint sampling campaign resulted in a slight delay and hence the field campaign was conducted between August 4th and August 20th.
3. A list of the relevant chemicals was confirmed by company representatives, with some additions by Santos.
4. Electronic data to produce soil maps of the study region were also obtained. These maps were used to select sites for soil collection for use in Tasks 2-5. The soil sampling activities were also included in the detailed sampling schedule.
5. An initial batch of the sampling equipment/reagents was prepared and shipped to Darwin using Toll Priority. The shipment was accidently sent by road freight by store personnel and would not have reached the destination in time to be collected by the field teams. Attempts were made to recover and divert the shipment but after Toll was unable to establish the location of the shipment while in transit, a new batch of the sampling equipment/reagents was prepared and transported via Qantas freight with the first field team. The initial batch of sampling equipment/reagents was eventually recovered. This incident added a small additional cost to the project, but the alternative arrangements ensured that sampling and other activities could take place as planned for a successful sampling campaign.
6. All internal safety documentation was completed including an Activity Risk Assessment and a Fieldwork Trip Plan. The team was also provided with Spot Trackers, EPIRBS and satellite phones, along with a daily call-in schedule. Spot Trackers allowed the base contacts to check the movements of field team members online. Field team members and base contacts also communicated regularly through a group chat on Facebook Messenger whenever the field teams had mobile phone reception.

7. Permission for sampling was first discussed with company representatives who then appointed their own field representatives to organise borehole access permission with individual landowners. Permission for sampling from NT government owned bores was organised with the relevant government department and keys to locked boreholes were provided to field teams by government representatives. In a few cases CSIRO was required to communicate with landowners directly for access permission and this was done predominantly via email and phone. In some cases planned boreholes were either closed in, unequipped with pumps or permission was not granted, such as in the case of Amungee Mungee. In these instances, company field representatives organised collection from alternative boreholes.

All arrangements for the joint sampling campaign were completed in time for the field trip to commence.

TASK 2: Sampling campaign

BACKGROUND

Task 2 will involve two staff travelling to the Beetaloo sub-Basin with the purposes of collecting: triplicate microbially preserved water samples from the 55 sites examined by project W.16 along with 50 soil samples (ten each of the five major soil types: tenosols, rudosols, kandosols, vertosols and chromosols of the Beetaloo sub-Basin). In addition, five large volume water samples will be collected anoxically for microbial degradation assays (identified in Task 1).

TASK OBJECTIVES

To collect oxic and anoxic samples:

1. Microbially preserved water samples will be collected from sites identified in Task 1/project W.16.
2. Five large volume anoxic water samples will be collected from sites identified in Task 1/project W.16.
3. Collection of 10 replicated soil samples from each of the five soil types completed: and
4. Five large volume soil samples will be collected from sites identified in Task 1.

TASK OUTPUTS AND SPECIFIC DELIVERABLES

Collection of preserved samples, oxic and anoxic samples to establish microcosms.

PROGRESS REPORT

All four of the objectives listed for Task 2 were achieved.

1. Thirty-eight microbially-preserved, replicated water samples from planned sites as per the sampling schedule (Task 1) and from alternative sites organised by company field representatives. Bores collected include (RN008481, RN029027, RN033608, RN033609, RN033670, RN033671, RN037665, RN038817, RN038818, RN040930, RN007658, RN025291, RN030325, RN037654, RN037655, RN037666, RN038179, RN038580, RN038581, RN005942, RN024616, RN028082, RN029012, RN031243, RN031382, RN031397, RN032961, RN033135, RN035130, RN035146, RN036654, RN036775,

RN038630, RN038811, Stuart Plains Homestead Bore, Hayfield Shenandoah Homestead Bore , Amungee NW1, Motel Bore)

2. Six large volume anoxic water samples were collected from selected sites as per the sampling schedule (Task 1)
3. Fifty replicate soil samples were collected from 10 locations (n=5), from each of the main five soil types in the region.
4. Five large volume soil sample were collected from five locations, one from each of the main five soil types in the region.

The joint field campaign was conducted successfully and there were no safety concerns. Six CSIRO staff members conducted the field campaign, four from W17 and two from W18, accompanied by some staff from Charles Darwin University. The hire vehicle of the north team had a flat tyre soon after reaching Daly Waters but team members and company field representatives were able to manage the situation efficiently and make adjustments to the sampling schedule as required, still ensuring the success of the campaign. To ensure that all samples arrived at North Ryde laboratories safely and in a timely manner, samples were packaged in Darwin on August 20th and transported by road with TSS Sensitive Freight. Samples and equipment/reagents arrived at North Ryde laboratories on August 28th and work on the following project tasks has commenced.

TASK 3: Baselining microbial communities

BACKGROUND

The 55 microbially preserved water samples and the 50 soil samples will be subject to DNA extraction along with 16S rDNA sequencing, and, for the soil samples ITS DNA sequencing.

TASK OBJECTIVES

The task will include the following objectives:

1. Complete DNA extractions from all samples.
2. DNA samples sent to external sequencing provider; and
3. Bioinformatics completed for microbial baselining of all samples.

TASK OUTPUTS AND SPECIFIC DELIVERABLES

Raw sequencing data from microbial community profiling available.

PROGRESS REPORT

This milestone is complete.

Task objectives 1 & 2) All soils, and aquifer baseline samples have been DNA extracted, subject to required PCR, and subsequently uniquely barcoded and purified. These purified PCR products were then pooled and sent to Macrogen Korea for sequencing.

Task objectives 3) Sequencing data was received back in Australia and has been subject to bioinformatic analyses. In brief, the amplicon library was demultiplexed (a process whereby DNA reads are assigned to bins based on their barcode), these bins were then subject to quality control (removing short, erroneous or chimeric reads). The bins are then combined to create a single pool

of reads from which singletons are discarded and a clustering process is undertaken to create OTUs (≈species). The original reads from the bins are then back-mapped onto the OTUs to create counts for each OTU in each bin.

TASK 4: Microbial degradation and sole carbon growth trials

BACKGROUND

Replicated microcosms containing either the soils or aquifer water will be established and used to determine the ability of microbes in these environments to degrade chemicals potentially used by industry. Chemical degradation will be determined either through direct measurement of the chemical in the soil or aquifer using analytical chemistry techniques or microbial growth assays

TASK OBJECTIVES

The task will include the following objectives:

1. Establish replicated microcosms.
2. Spike microcosms with target compounds at realistic concentrations.
3. Incubate at realistic conditions i.e., for soil microcosms, incubate at field relevant conditions (local temperatures and day/night cycle will be reproduced in the laboratory) for aquifer water microcosms relevant subsurface temperature will be used in the absence of light.
4. Harvest all soil treatments after two weeks and prepare samples for chemical analyses.
5. Harvest all water treatments after six weeks and prepare samples for chemical analyses.
6. Establish sole carbon source experiments.
7. Incubate at a relevant field conditions.
8. Inspect cultures for visual signs of growth and where possible (in the aquifer cultures) measure biomass; and
9. Statistical analyses of the resultant data.

TASK OUTPUTS AND SPECIFIC DELIVERABLES

Replicated Experimental data on the degradation of target compounds. Data prepared for analysis and final reporting.

PROGRESS REPORT

This milestone is complete.

Task objectives 1, 2& 3) Replicated microcosms were established for soils (oxic conditions) or aquifers (anoxic conditions) with and without chemical spikes.

Task objectives 4, 5, 6 and 7) All replicated microcosms were harvested and subject to chemical testing and DNA analyses (see: Task 5; Impact and indicator taxa). All sole carbon source experiments were established and incubated at relevant field conditions.

Task objective 8) Data on visual growth recorded at multiple time points and biomass estimated using DNA yields from Task 5.

TASK 5: Impact and indicator taxa

BACKGROUND

In the soils and the water microcosms, microbial community profiling will also be undertaken after exposure to individual chemicals will be carried out to ascertain impacts on microbial communities and to potentially identify putatively useful indicator taxa for monitoring environmental impacts.

TASK OBJECTIVES

The task will include the following objectives:

1. Extract DNA from soil and water from Task 4 to determine changes in microbial community profiles.
2. Statistical and bioinformatics analyses of the resultant data.

TASK OUTPUTS AND SPECIFIC DELIVERABLES

Experimental data on the microbial community changes in response to exposure to shale gas activity-related chemicals. Analyses complete and prepared for final report.

PROGRESS REPORT

This milestone is complete.

Task objectives 1) All soils, and aquifer spiked microcosm samples have been DNA extracted, subject to required PCR, and subsequently uniquely barcoded and purified. These purified PCR products were then pooled and sent to Macrogen Korea for sequencing along with the DNA from Task 3.

Task objective 2) Sequencing data was received back in Australia and has been subject to bioinformatic analyses. In brief, the amplicon library was demultiplexed (a process whereby DNA reads are assigned to bins based on their barcode), these bins were then subject to quality control (removing short, erroneous or chimeric reads). The bins are then combined to create a single pool of reads from which singletons are discarded and a clustering process is undertaken to create OTUs (≈species). The original reads from the bins are then back-mapped onto the OTUs to create counts for each OTU in each bin. In order to compare chemical data, statistical comparisons were undertaken, and p-values determined.

TASK 6: Project management, data analysis and reporting

BACKGROUND

The final report for this project will collate baseline data with microbial degradation, microbial community impact and useful indicator taxa for individual chemicals. These data will be combined with results from project W.16 to inform requirements for future toxicological studies and will provide information for a range of stakeholders.

Critical evaluation of the results is needed to understand the experimental outcomes of this study.

TASK OBJECTIVES

The task will include the following objectives:

1. Reporting results and analyses from Tasks 2-5.
2. Integration of this studies results with those of project W.16; and
3. Provide recommendations of chemicals with potential high residual risk requiring toxicological studies.

TASK OUTPUTS AND SPECIFIC DELIVERABLES

Final written report encompassing all the tasks outlined above and integration with the related project W.16.

PROGRESS REPORT

This milestone is underway. Visualisations of data from Task 3, 4 and 5 are on-going and will be complete shortly. The report will follow the completion of these visualisations.

Variations to Project Order

Changes to research Project Orders are approved by the GISERA Director, acting with authority, in accordance with the GISERA Alliance Agreement. Any variations above the GISERA Director's delegation require the approval of the relevant GISERA Research Advisory Committee.

The table below details variations to research Project Order.

Register of changes to Research Project Order

DATE	ISSUE	ACTION	AUTHORISATION
27 September 2019	Due to complexities discovered during logistics and planning, and loss of equipment in transit, an additional $24,000 ($18,000 GISERA funding & $6,000 CSIRO in-kind) is required for task 2 field campaign to sample soil and water collections.	An additional $24,000 ($18,000 GISERA funding & $6,000 CSIRO in-kind) is allocated for task 2 field campaign to sample soil and water collections.	
11 June 2020	Due to several significant, unplanned and unforeseen events, the research proponent has requested that the delivery dates for milestones 3-6 be moved. Additional funding has also been requested to cover the additional OPEX costs incurred.	There have been changes to the delivery dates of milestones 3-6 and an overall project budget increase of $20,000 ($15,000 GISERA funding and $5,000 CSIRO funding). This results in an overall total project budget of $291,964 and a project delivery date of 30 November 2020.	The NT RRAC approved the project variation including the additional $20,000 funding. Refer to Minutes.

As Australia's national science agency and innovation catalyst, CSIRO is solving the greatest challenges through innovative science and technology.

CSIRO. Unlocking a better future for everyone.

Contact us

1300 363 400
+61 3 9545 2176
csiro.au/contact
csiro.au

For further information

1300 363 400
gisera.csiro.au

GISERA is a collaboration between CSIRO, Commonwealth and state governments and industry established to undertake publicly-reported independent research. The purpose of GISERA is to provide quality assured scientific research and information to communities living in gas development regions focusing on social and environmental topics including: groundwater and surface water, greenhouse gas emissions, biodiversity, land management, the marine environment, and socio-economic impacts. The governance structure for GISERA is designed to provide for and protect research independence and transparency of research.