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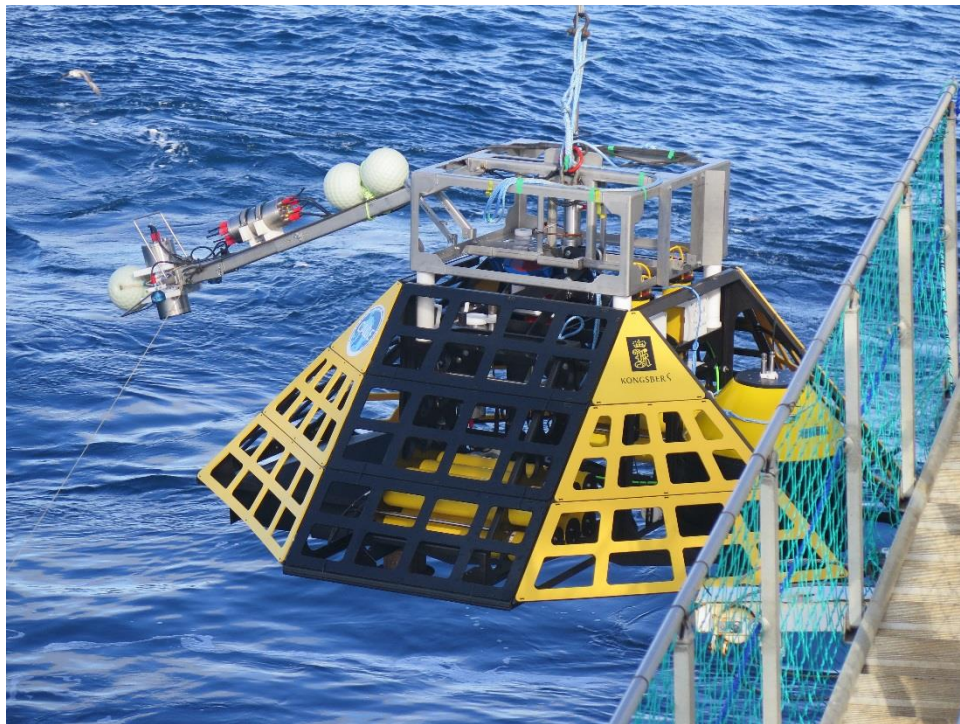
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CAGE Centre for Arctic Gas Hydrate, Environment and Climate, IG, UiT

Cruise report CAGE 15-3

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1. Objectives

The cruise had two main objectives:

- 1) Deployment of the two CAGE ocean floor observatories (OS1 and OS2) at shallow PKF site and deeper PKF site;

CAGE ocean floor observatories were designed and build as collaborative work of CAGE scientists with Kongsberg engineers. Observatories have identical set up except that only one of them have side looking multibeam.

The set up is:

Seabed Platform/seabed lander/mooring frame (x2) – OS1 has black mooring frame, OS2 has grey mooring frame, CTD (x2), Oxygen sensor (x2), CH₄ sensor (x2), CO₂ sensor (x2), pH sensor (x1), Fluorometer (x2), ADCP (x2), Current profiler (x1), Multibeam echosounder (x1) – grey lander, OS2, Broadband Hydrophone (x2), Flowmeter (x2)

For specific description of each lander, please refer to ‘taking over’ documents (WP4 team leader). Landers and sensors arrived to Tromsø with a track from Hamburg, A. Silyakova was a reference person to receive goods and shipping documents. Time period between 24 and 26 of June was the assemblage of observatories and tests of telemetry/communication/camera on a launcher. Pär Jeanson (PhD student WP4) and Reidar Kaasa (substitute engineer instead of Anoop in WP4) from CAGE were assigned to receive training on observatory assemblage/communication. 26 of June – taking over procedure. Persons present – B. Ferre, A. Silyakova., P. Jeanson, R. Kaasa from CAGE; M. Meyer, O. Rubinke, C. Frank, P. Linke from Kongsberg. After thoroughly going through the documents of acceptance, they were signed by M. Meyer and B. Ferre.

Sites for the deployment were discussed during preparatory phase. Water depth at the sites could not exceed 500 meters due to restrictions in relation to the recovery rope, which is only 500 meters long. Photographs from the tow cam used during CAGE 15-2 cruise (chief scientist on the cruise G. Panieri) revealed sites with bacterial mats on the ocean floor. Prior each deployment we did echosounder and multibeam survey to know where flares are highly concentrated. Information from the survey was mapped instantly. Target spots for both observatories were chosen based on all this combined information.

- 2) Oceanographic survey in the area of shallow PKF methane flares;

From the “Testing seep fertilization hypothesis” proposal:

‘During cruise CAGE 14-1 the USGS-GAS system detected elevated methane fluxes near the coast and over the shelf seep site in ca. 90m water depth. Methane fluxes above the 240 and 400m site are much less, although slightly elevated with respect to the open ocean (e.g. Vestnesa). Unexpectedly, high methane concentrations (up to 20nM) are often accompanied by low CO₂ concentrations. Initial estimates of the total CO₂ budget show that under those conditions seep areas are CO₂ sinks. What are the biological, geochemical and hydrographic conditions that made these seeps a CO₂ sink? Are the observations from CAGE 14-1 repeatable? And ultimately, what are the processes causing the strong CO₂ consumption.’



It was decided to test seep fertilization hypothesis during CAGE 15-3 cruise by conducting comprehensive water sampling for biogeochemical environment in the entire water column above the area of methane flares. At the same time, USGS-GAS system was onboard allowing to simultaneously measuring surface water/lower atmosphere gas concentrations. This potentially allows calculating vertical gas flux from one realm to another.

Depending on available time, collaborators equipment and human resources, water from 64 CTD stations was samples for following parameters: CH₄ concentration; discrete sampling to introduce into CDRS system – 13C CH₄, CO₂; pH; DIC and 13C DIC; DOC; MO_x; FISH; DNA; DMSP; CDOM; Nutrients (nitrate, silicate, phosphate).

2. Cruise participants

1. Anna Silyakova, chief scientist, CAGE, IG, UiT, anna.silyakova@uit.no
2. Steinar Iversen, CAGE, IG, UiT, Engineer, Instrument chef, steinar.iversen@uit.no
3. Reidar Kaasa, CAGE Engineer, substitute engineer
4. Pavel Serov, CAGE, IG, UiT, oceanographic survey, pavel.russerov@uit.no
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6. Friederike Grunder, CAGE, IG, UiT, oceanographic survey, friederike.gruendger@uit.no
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9. Erna Osk Arnadottir, CAGE, oceanographic survey, erna.o.arnadottir@uit.no
10. Peter Linke, GEOMAR, Kongsberg, Lander deployment, plinke@geomar.de
11. Carsten Frank, Kongsberg, Lander deployment, Carsten.Frank@km.kongsberg.com
12. Oliver Rubinke, Kongsberg, Lander deployment, Oliver.Rubinke@km.kongsberg.com
13. John Pohlman, USGS, CDRS and oceanographic survey, jpohlman@usgs.gov
14. Lee-Gray Boze, USGS, CDRS and oceanographic survey, lboze@usgs.gov
15. Cedric Magen, USGS, CRDS and oceanographic survey, cedric.magen@gmail.com
16. Fenix Garcia Tigreros, Uni of Rochester, Kessler lab, oceanographic survey, fenix.garcia.tigreros@gmail.com
17. Randall Hyman, Journalist, photographer, freelancer, randall@randallhyman.com

3. Participants, faces and tasks



Anna Silyakova, CAGE
Cruise leader



Steinar Iversen, CAGE
Engineer, Instrument chef



Reidar Kaasa, CAGE
Engineer



Pavel Serov, CAGE
Gas chromatography, GIS, multibeam



Pär Jeanson, CAGE
Sampling, ADCP, echosounder



Friederike Grunder, CAGE
Sampling, MO_x, FISH and DNA
filtration



Helge Nienman, University of Basel
Sampling, MO_x, FISH and DNA
filtration



Carolyn Graves, CAGE
Gas chromatography, sampling



Erna Osk Arnadottir, CAGE
Sampling, CH₄, CDOM, DMSP,
nutrients, DOC for John



Peter Linke, GEOMAR
Scientific adviser on deployment



Carsten Frank, Kongsberg
Lander deployment



Oliver Rubinke, Kongsberg
Lander deployment



John Pohlman, USGS
CRDS, sampling, DOC and discrete
13C isotopes of methane



Lee-Gray Boze, USGS
Sampling, discrete 13C isotopes of
methane and CO₂



Fenix Garcia Tigreros, Uni of Rochester
DIC, pH sampling and measurement, 13C
of CO₂



Cedric Magen, USGS
CRDS, discrete sampling of 13C for
methane and CO₂ to CRDS
(experimental sampling)



Randall Hyman
Journalist, photographer

4. Deployment procedures (by A. Silyakova)

Main objective of the cruise was to deploy two CAGE observatories. First deployment took place on 30.06.2015 in the area of deeper PKF shelf (240 meters). Second deployment took place on 02.07.2015 in the shallow PKF area (90 m). Both deployments started with the toolbox meeting on the bridge that involved everybody who was involved in the deployment – crew (captain or first mate, deck men), Carsten, Oliver, Peter, Anna and Steinar and Reidar. Prior the first deployment Carsten collected signatures from first mate, deck men, Steinar and Reidar to confirm that everybody is aware of the launching procedure and risks. Toolbox meeting followed by going through the checklist (done by Carsten and Reidar as a witness, takes about 1,5-2 hours). During that time, first mate reached the target spot and tested how the ship drifts without the propeller to find out how the ship should be pushed against wind and currents with the help of bow thrusters. Wind direction was from the North, so were the currents. It was rather easy situation, so we approached the target having lander in water pushing the ship from the south.

After checklist and telemetry preparations, lander was ready to be lifted. First lander was lifted from the side deck (multicore deck). After lifting, lander could not be placed on deck again due to system of splinters holding legs of the lander (which are supposed to break on the bottom). First lander was detached from the launcher, launcher was then turned 180 degrees for the procedure of unplugging and plugging telemetry again to set the connection (see “broken” telemetry part); after the communication with telemetry and the camera was in place, launcher was again connected to the top of the lander. Deck men lifted the lander with the crane, tested whether winch will hold the lander still keeping it on crane for the security. After it was clear that winch can hold lander, crane cable was removed, and lander was lowered down in the water (Steinar is on winch, Peter is on telemetry). Steinar and Peter came to the instrument room from the deck, Steinar controlled the winch remotely, and Peter was controlling the telemetry. Everybody in the room watched streaming from the camera; when the boat approached the target area for the first lander, we saw bubbles and bacterial mats on video, but also large flare on the echosounder; Anna made the decision to release the lander and pressed button “release”. First release was done from 2-3 meters height above the sea bottom, and as recommended height to release from is 1.5 meters, Anna signed compliance about releasing it from higher than recommended depth. Although it was higher than recommended, Peter said that it was ok to do it and since the structure of lander is very well tested, nothing could happen to the structure due to release from higher than recommended height. Weather during the first deployment was very good – calm, waves were less than a meter and very low wind speed. It took approximately 57 minutes from the time when lander touched the water until when it touched the sea floor.

Second lander was on the trawl deck, and during second deployment unplugging and plugging the telemetry process was easier and quicker because launcher didn't have to be detached. Lander was lifted from the trawl deck and directly transferred to water. Weather was a bit worse the second time, waves height was about 1,2-1,5 meters, and wind was picking up. However, we decided not to wait until the best weather 2 days in future, because it would be already too tight timing in proximity of the end of the cruise. Since we had more intense vertical motions of the ship, it was more work for Steinar to control height above the bottom with the winch, and it looked and sounded on the HD video that lander touched the bottom few times before we released it. The second release has happened from

recommended depth. Again, we saw bubbles on the video and massive field of flares on echosounder, so Anna decided to release the telemetry.

4.1. „Broken“ telemetry of the launcher (by Carsten Frank)

The telemetry of the launcher was tested on the launcher in the bunkerdepot one day before loading the launcher on to the Helmer Hanssen. A test on the Helmer Hanssen revealed that the telemetry – which also includes the essential video feed from the bottom tracking camera – did not work any more. We found that the plug of the camera at the housing of the camera was damaged between the last test in the bunkerdepot and the test on the Helmer Hanssen. The plugs on both sides were replaced while keeping the middle of the cable intact. Two standard subcon pigtails were used as a replacement. However, it was impossible to get the camera to work with this cable, therefore, the original shorter cable had to be used. The latter was still working if the telemetry as switched on prior to plugging in the camera.

As the camera should not change position on the launcher, the telemetry had to be moved in direction to the camera leading to an even more severe overweight of the camera arm compared to the rest of the system. However, after changing deployment procedures accordingly, both deployments could take place without problems.

4.2. Time needed between lander launches (by Carsten Frank)

The time needed between the release of the first lander to a possible release of the second lander is roughly 24h and consist of the following steps:

- 8h rest for KONGSBERG personnel to avoid errors due to tiredness
- 2-3h reloading the releaser
- 4-5h final test of the system including preparations such as programming the flasher and radio beacon, removing ‘packing’ ropes from the launchers rope etc.
- 2-3h for going through the checklist with a witness
- Between 1h and 2h to discuss weather situation and possible changes to deployment procedure, toolbox meeting(s)
- A time reserve to be sure not to work under too much pressure, which may lead to errors.

4.3. Sensor recalibration after lander recovery (by Carsten Frank)

KM Embient recommends to send all sensors back to factory recalibration after each recovery (at the very least on a yearly basis). Please also consider that biofouling may heavily impact the accuracy of at least some of the sensors. The recalibration intervals should be reduced accordingly.

5. Locations of the observatories after the deployment

5.1. Deployment CAGE OS1, black frame

78 39.2779N 9 25.9871E

30.06.2015 15:51 UTC time at bottom, 241.90 m

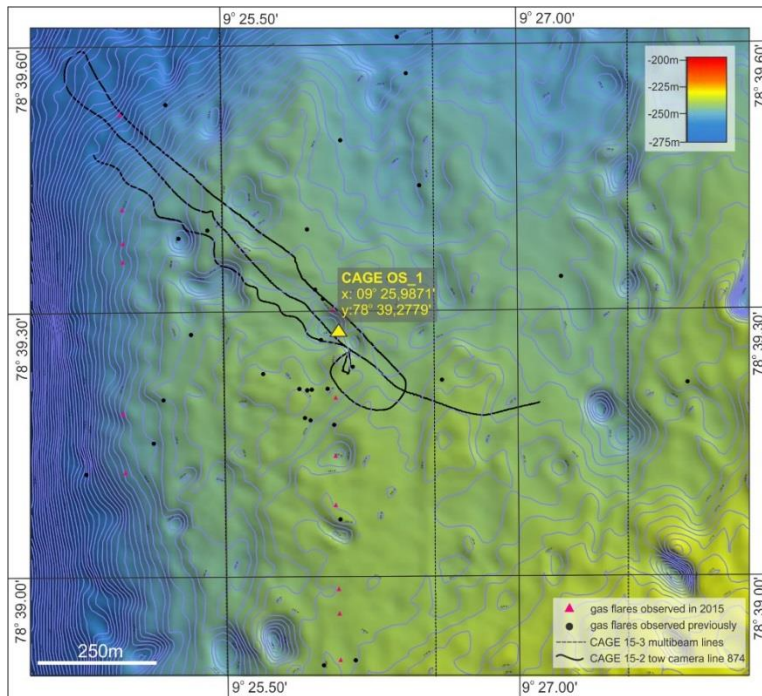


Figure 1. Location of the CAGE stationary observatory OS1

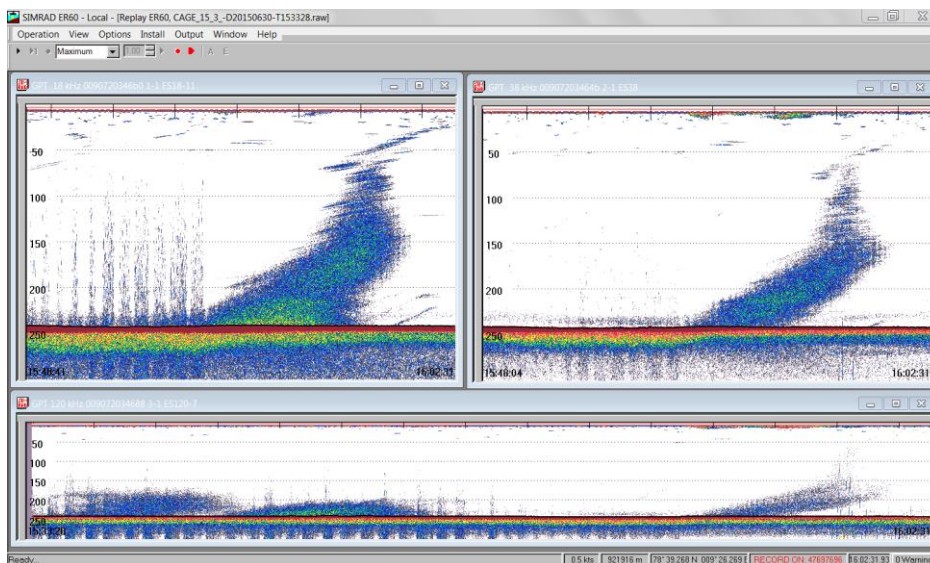


Figure 2 Echosounder screenshot from the time of the OS1 deployment.

Communication with cNode checked and is OK

5.2. Deployment CAGE OS2, grey frame

78 33.6765N 10 08.5356E, 02.07.2015, 14:18 UTC time, 90.56m

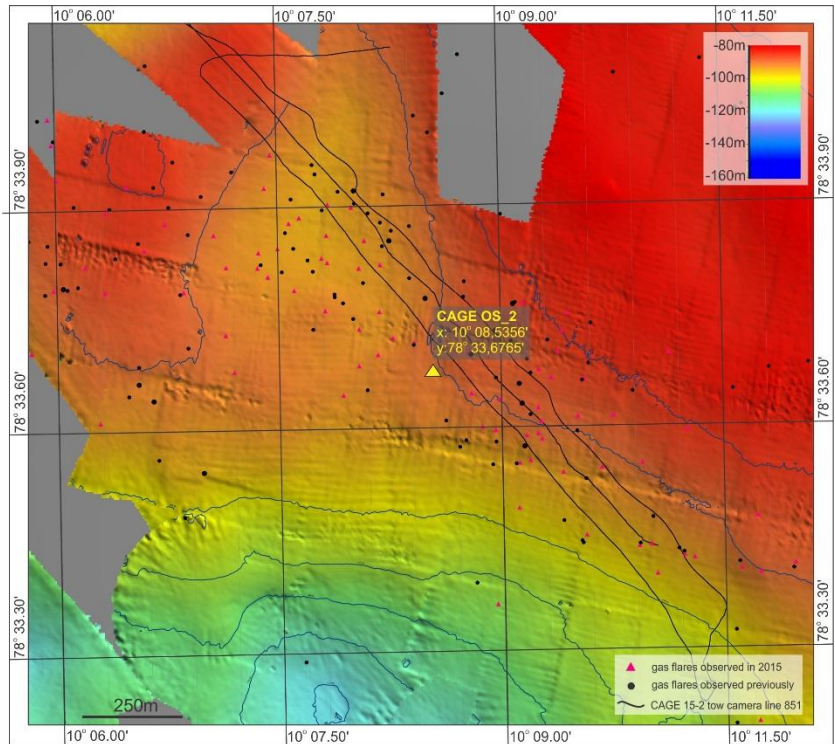


Figure 3. Location of the CAGE stationary observatory OS2

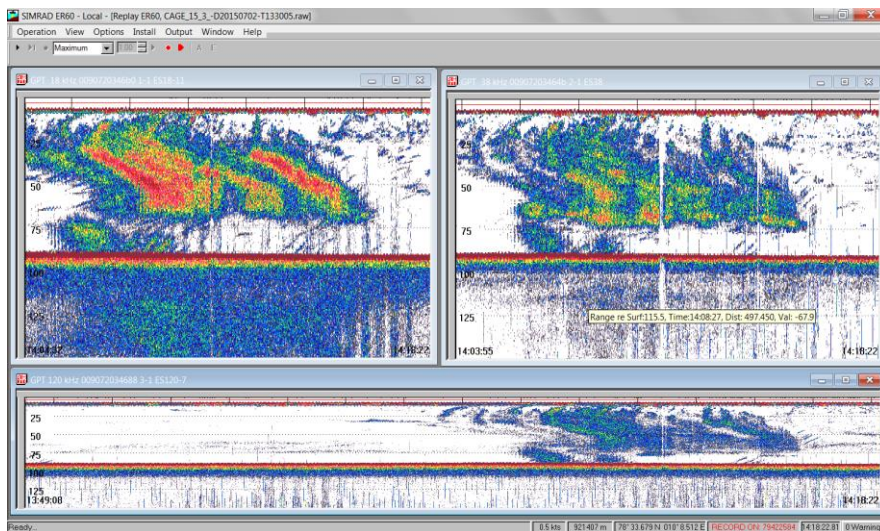


Figure 4 Echosounder while deploying OS2

Communication with cNode is checked and is ok

6. Oceanographic survey

6.1. Multibeam and echosounder

During cruise CAGE 15-3. The hull-mounted Kongsberg Simrad EM 300 multi-beam echosounder system was used for bathymetric mapping. The frequency of wavelengths is 30 kHz, which provides high resolution bathymetry information. Multi-beam echosounder was turned on during the entire cruise except the time when we did oceanographic survey. The reason for that is possible interference with looking down ADCP profiler. ADCP provided essential information for oceanographic study during the survey. The bathymetry information was partially used from 2014 cruise season and from the later survey made specifically for USGS gas program above the same area but in between lines of initial oceanographic survey.

Single beam echosounder data was recorded during the entire cruise, producing approximately 30 Gbytes of continuous data.

6.2. ADCP

We recorded ADCP (Acoustic Doppler Current Profiler) data during the shallow shelf survey while CTD casts were conducted. The Aqua Vision software “Visea DAS” recorded 30 files containing a total of 27 Mbytes of Doppler shifted backscatter data. This has to be post-processed after a compass calibration and corrections for transducer offset relative to GPS antenna. In order to achieve good data, we used a bin size of 16 meters, giving us 2 -5 depth cells with good velocity data.

6.3. Count Down Ring Spectroscopy (CDRS PICARRO) - USGS Gas Chemistry Program (by J. Pohlman)

Participating Scientists: John Pohlman, Cedric Magen, Lee-Gray Boze, Fenix Garcia-Tigeros

Methods

The USGS Gas Analysis System (USGS-GAS) was deployed to obtain real-time and continuous surface water concentrations of methane and CO₂ in conjunction with water chemistry and meteorological data to calculate the sea-air fluxes of methane and CO₂ (e.g., Wanninkhof, 1992). The analytical component of the USGS-GAS consists of two Picarro cavity ring-down spectrometers. A G2201i CRDS measures methane and CO₂ concentrations and stable isotopes from the headspace of a Weiss-type gas equilibrator supplied with water from an intake pump located 5 m below the surface of the water. An EXO water chemistry sonde measures the temperature and salinity of the incoming water stream. A G2301f CRDS measures methane and CO₂ concentrations from an air intake mounted on the bow 10 m above the surface of the water. An Airmar PB200 sonic anemometer installed near the air intake measures wind speed, which is utilized in the flux calculations. Gas from the equilibrator and the air intakes is delivered to the CRDS by USGS-built gas handling devices that regulate the gas flow and condition it for analysis.

Additionally, a discrete sample analysis system was tested and validated for analyzing methane and CO₂ concentrations and stable carbon isotope values from discrete samples collected from Niskin bottles attached to the CTD rosette. Headspace extractions were performed in 1000 ml Hamilton syringes by applying a 100 ml zero-air headspace and shaking vigorously for 2 minutes. A sample injection system delivered the extracted gas directly into the inlet of a Picarro G-2201i CRDS for direct measurement and isotopic analysis of the gas.



Figure 5. PICARRO CDRS system for surface waters and lower atmosphere

Concentration and isotope standards were measured on a regular basis to monitor instrument performance and determine offset calibration factors. Leak tests and equilibration rate tests were conducted before, during and at the conclusion of the campaign on USGS-GAS.

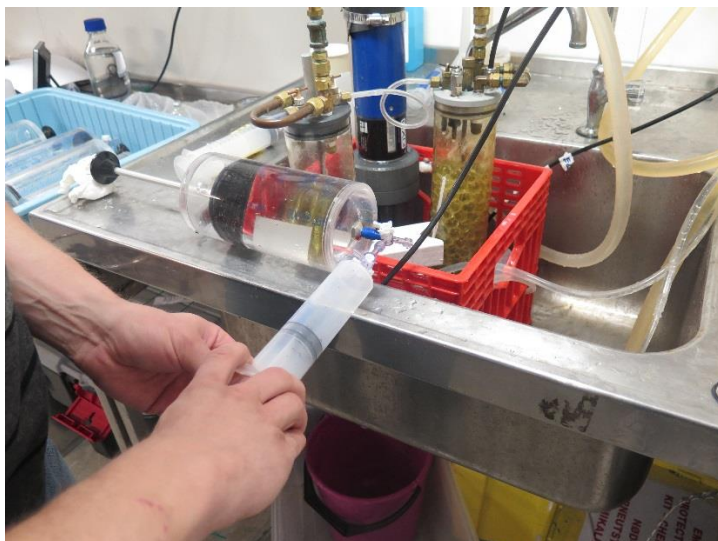


Figure 6. Injection of 100 ml zero air gas as a headspace to 1000ml syringe filled with water sample

6.4. CTD profiling

CTD (Conductivity, Temperature, Depth) sensors measure the physical properties of seawater. In addition to measuring the conductivity, temperature and pressure (from which depth is calculated), the CTD sensors can measure or calculate salinity of seawater, density, P-wave velocity, turbidity, fluorescence/chlorophyll, and oxygen content. Furthermore, it is possible to collect water samples from any depth of choice.

During our cruise, we used the sound velocity profiles from different CTD stations to calibrate depth calculations in the bathymetry data.

Oceanographic survey consisted of 64 CTD casts (Figure 7), at each . Coordinated for the stations sourced from CAGE 14-1 oceanographic survey. All stations were sampled for CH₄ concentration. For all other parameters, please refer to file ‘Sampling Protocol_aa.xls’.

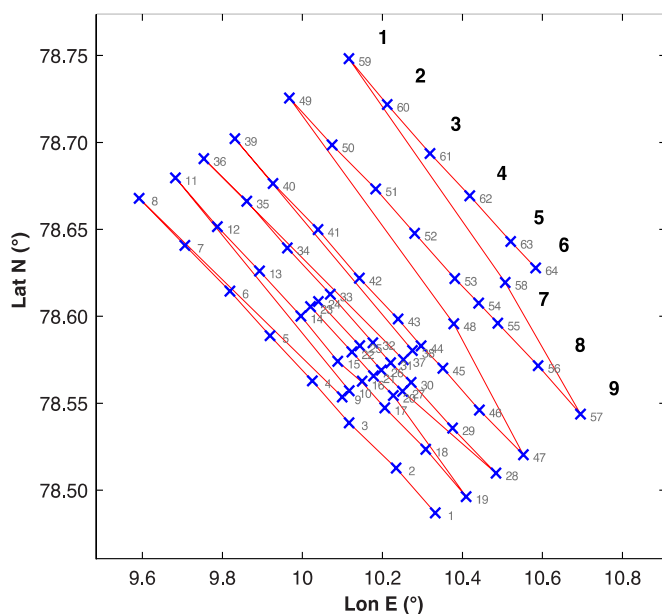


Figure 7. Scheme and internal numbering for CTD stations during oceanographic survey

6.5. Water sampling

6.5.1. Methane (by P. Serov)

To prepare water samples for measurements of methane concentrations we applied conventional headspace gas extraction technique. We injected 5 ml of nitrogen through the robber crimped septum to the water sample. By shaking the bottle for two minutes the headspace nitrogen equilibrated with the in situ water sample gas. Equilibrated headspace gas was injected to FID gas chromatograph Trace 1310 by 100 µl gastight syringe. Measured ppm methane concentrations were subsequently converted to nmol concentrations considering the sample temperature and the atmospheric pressure in the laboratory.

Set up for the gas chromatograph: injector T – 170, T detector – 190, T oven constant 40, column flow – 20 ml/min, Air 350, H₂ – 40, makeup – 35, Injections 100 ul, standards – 10ppm, 50 ppm, 100 ppm; FID acquisition rate – 60Hz.

6.5.2. Dissolved Inorganic Carbon (by F. Tigreros)

Objective: To collect Niskin bottle samples to measure DIC.

Procedure: Rinse sample bottles – Rinse bottle three times with sea water to remove any traces of previous sample. Fill sample bottle – Insert drawing tube till touching bottom of the glass bottle, fill the bottle smoothly from bottom to top using the tube. It is critical to **remove any bubbles** from the drawing tube before filling. Overflow the water by a full bottle volume or about 60 seconds.

Headspace – To determine headspace volume: close nisking bottle and pinch off the drawing tube before removing it from the sample bottle. The water level should be as a few mm from the bottle neck.

Adding Copper Sulfate – Once all samples have been collected and taken to inside the lab, poison water samples with 150 µl of a 2 M cupric sulfate solution.

Close and secure the stopcock – close bottles with rubber stopper and crimp bottles until analysis.

6.5.3. pH and UV-Vis (by F. Tigreros)

Objective: To measure pH in seawater samples. **UV-VIS:** A Cary 100 UV-Vis Spectrophotometer was used.

Reagents: A 2 mM L-3 dye solution of m-cresol purple is adjusted to match pH measurements from an oceanic profile using a 0.1N NaOH solution. This implies that for m-cresol purple A578/A434 = 1.6.

Indicator solution is susceptible to atmospheric contamination and should be stored minimizing contact with atmospheric CO₂ (collapsible container or syringe). **Sampling:** Flush cell for 15-20 seconds and seal with teflon cap ensuring there is no headspace. While awaiting analysis store sample in the dark at room temperature.

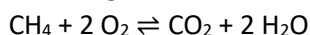
Procedure:

1. Warm sample cell to 25.0C (±0.1)
2. Measure absorbances for the cell + seawater
3. Inject 50 - 100 µL of dye. The amount of dye required is that which will produce absorbance values between 0.4 and 1 and each of the two absorbance peaks.
4. Measure absorbances of cell + seawater + dye

References: information was taken from the UV-Vis manual, SOP6b and Clayton and Byrne (1993).

6.5.4. Microbial activity and identity (by H. Nienman)

Aerobic methane oxidation (MOx) is final barrier for methane before its release to the atmosphere, where it acts as a potent greenhouse gas. MOx is mediated by bacteria and proceeds according to the following net reaction:



For analysis of MOx rates at discrete water depths, we sampled the water column with a 12 × 5-liter CTD/Rosette sampler and sub-samples were taken immediately upon recovery of the sampler. MOx rates were determined at sea from ex situ incubations with trace amounts of tritium labelled methane (C³H₄), allowing to trace the label transfer by measuring the activity of substrate (C³H₄) and product pools (C³H₂O)

after incubation (Berndt et al., 2014; Niemann et al., 2015, Steinle et al., 2015). For each sampling depth, six 20-ml crimp-top vials were filled and closed bubble-free with PTFE coated bromobutyl stoppers (Wheaton, USA). Subsequently, the each sample was amended with 5 μl gaseous $\text{C}^3\text{H}_4/\text{N}_2$ mixture (~ 5 kBq, <50 pmol CH_4 , American Radiolabeled Chemicals, USA) and incubated for 48 h at in situ temperature in the dark. The incubations were terminated by unsealing one triplicate and subsampling a 10 ml aliquot of the incubation medium. This was then amended with aqueous NaCl solution (1 ml, 20%, w/v) and purged for 30 min with air to strip out the remaining methane. The activity of the produced $^3\text{H}_2\text{O}$ will be determined in our home laboratories by liquid scintillation counting. The radioactivity of both, the remaining C^3H_4 and the produced $^3\text{H}_2\text{O}$ will be determined from the second triplicate (fixed with 0.5 ml HgCl solution after incubation) by liquid scintillation counting in our home laboratories. MOx rates will be corrected for (most probably insubstantial) tracer turnover in killed controls (fixed with HgCl solution just after tracer amendments). MOx rates will be calculated from the fractional turnover of labelled CH_4 and water column CH_4 concentration assuming first order kinetics (Reeburgh, 2007):

$$r\text{MOx} = k \times [\text{CH}_4]$$

where k is the first-order rate constant (determined from the fractional turnover of labelled CH_4 per unit time and corrected for tracer turnover in killed controls) and $[\text{CH}_4]$ is the concentration of CH_4 at the beginning of the incubation.

Additional samples were collected for determining the identity and abundance of key microbial communities through fluorescence in situ hybridisation (FISH) (Pernthaler and Pernthaler, 2007). For this, 200 ml of aqueous sample were fixed with 7 ml formaldehyde solution (30%) for 5 h at 4°C .

Subsequently, samples were filtered through polycarbonate filters (0.2 μm pore size) rinsed with deionised water and stored at -20°C until further analyses in our home laboratories.

Finally, we also collected particulate organic matter (POM) for microbial community analyses with DNA tools (next generation sequencing, Illumina). POM was collected from ~ 500 ml of sea water filtered through polycarbonate filters (0.2 μm pore size) and stored at -80°C until further analyses in our laboratories.

MOx rates will be determined from all collected samples (4 transects, ~ 300 distinct water depth amounting to ~ 1800 replicates). The resolution of microbial community

6.5.5. Nutrients (by A. Silyakova)

Water from Niskin bottle was subsampled into 20 ml scintillation vial and 200 μl of chlorophorm was added each sample immediately after sampling. Samples were stores in dark box in the fridge at temperature 2 centigrade.

6.5.6. DMSP

Water from Niskin bottle was subsampled into 60 ml falcon tube with 167 μmol H_2SO_4 added prior sampling. Samples were stored in dark and cold.



7. References

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