JRAP8: Monitoring of submarine CO_2 fluxes and ecological impact studies on natural analogues for CO_2 leakage

Deliverable JRAP-8/2:

Report on testing of automatic detection of offshore gas release, analysis of gas composition and quantifications of CO_2 concentration, water chemistry, and biological assessments of CO_2 tolerance

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ACRONYM : **CO_2GeoNet**

TITLE : **Network of Excellence on Geological Storage of CO_2**

PROJECT CO-ORDINATOR : **British Geological Survey – BGS (UK)**

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WPs involved:
IA : IA-1 (Research infrastructure), IA-2 (Communication & Information), IA-4 (Joint research development)
JRA: JR4-2 (Monitoring techniques - Geochemical); JR4-3 (Monitoring techniques Biological);
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SEA: SE-3 (Electronic)

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1. INTRODUCTION

It is the aim of this JRA to carry out a feasibility study for automatic sampling and detection of offshore gas release (CO_2 and CH_4). This work will deliver basic data and experience, and will assess knowledge and technical gaps in order to facilitate further developments of continuous geochemical monitoring devices as applied to open sea environments and lakes. First operational testing of basic gas monitoring installations is aimed to be conducted in the Gulf of Trieste and to be coupled to ecological investigations. Analysis of water chemistry shall be accompanied by a preliminary assessment (modelling) of CO_2 seabed behaviour, and exposure tests on mussels and other organisms exposed to likely CO_2 concentrations. It is the aim of the collaboration to establish the basic infrastructure within the team for coming gas and water monitoring (e.g. within the ´CO2REMOVE´ proposal) coupled to ecological impact investigation at a demonstrated CO_2 site. For this feasibility study, the Gulf of Trieste is chosen as an appropriate marine test site due to the available infrastructure.

This report is the second deliverable of JRAP8 (Monitoring of submarine CO_2 fluxes and ecological impact studies on natural analogues for CO_2 leakage). The first report was focussed on the technical prerequisites of offshore automatic detection of gases, data storage and data transfer. Topics of this report are (a) the testing of an automatically operating offshore monitoring buoy (quantitative detection of offshore gas both gaseous and dissolved with a focus on CO_2), analysis of water chemistry and (b) biological assessments of CO_2 tolerance.

2. RESULTS

2.1. MONITORING BUOY (BGR, OGS, URS)

2.1.1. BACKGROUND

During a kick-off meeting on Wednesday, 6th July 2005 at OGS in Trieste the technical prerequisites for the installation of the monitoring devices were discussed, integration of single devices due to power availability were proven and a work plan for the next six months was elaborated (all described in report JRAP 8/1).

The main topic of the meeting was the elaboration of a work flow and a concept for the technical development within the next six months (for concept see Fig. 1). Furthermore, the concept included a detailed plan for the partners´ activities and duties:

OGS: -provides the buoy as a platform for this monitoring JRA and power supply for the technical devices,
-initial plans were not to install more than three solar panels as for the original MAMBO buoys (this was changed during JRAP-8 lifetime)
-serves for connectors and fixation of the sampling funnel, and, if necessary, for diving activities,
-as a final task, OGS will test to establish an online-presentation of data on the CO_2GeoNet webpage.

BGR: -develops a gas monitoring device for CO_2, CH_4 and gas flow rates, including the preparation of tubing, connections and of a 3-way plastic sphere valve. This 3-way plastic sphere valve shall be placed close to the buoy to allow sampling from a boat.
-is responsible for data acquisition (for data acquired by BGR and URS) and transmission to GSM. Here, all partners may have access to the data.
-will provide sampling bottles for gas sampling by divers.

URS: -develops a monitoring device for dissolved CO_2 and CH_4 inclusive the relevant tubing and connections.

Before installation of the technical devices, samples of monitoring tubing had to be sent by BGR and URS to OGS in order to evalute the dimensions, the mechanical characteristics, and the weight in water to finally design the technical installation according requirements on the buoy by OGS.

All these prerequisites were fulfilled by the partners !

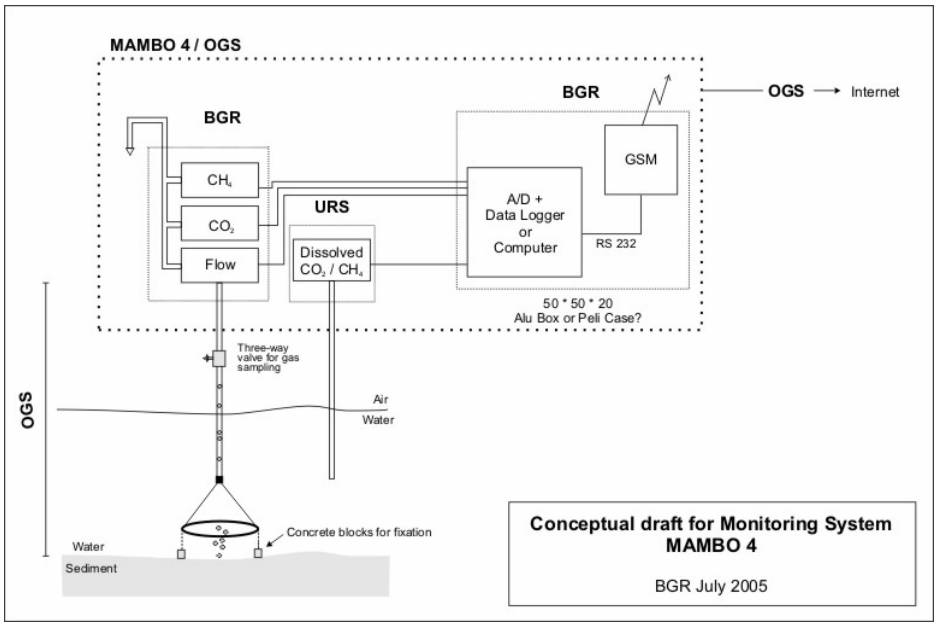

Fig. 1: Concept for the technical development of the monitoring buoy

2.1.2. DESIGN OF THE MONITORING BUOY

Technical work and final installation of all technical devices on the buoy were carried out during two meetings of all JRA partners in April (Fig. 2) and September 2006 in Trieste.

Fig. 2: Technical work during April 2006 at OGS

OGS has designed the monitoring buoy due to the required prerequisites (Fig. 3). Moreover, six solar panels instead of the originally planned four panels have been installed. Important to note that a plenty of construction details have been verified. Details on this and many additional specifications may be requested at OGS.

Fig. 3: Construction of the monitoring buoy at the OGS facilities

BGR has delivered the sampling funnel (Fig. 4a) which was incorporated in a frame (Fig. 4b). The frame was designed by OGS. The frame is (a) fixed by concrete blocks at the three tripods, and (b) larger than the present funnel to enable the installation of a larger sampling device if gas leakage at the sea floor is dispersed. Moreover, BGR designed a device for sampling gas from upwards migrating gas and analysis (Fig. 5). This was incorporated within the tube above sea level and allows gas migration upwards. All gas monitoring devices, data logger and GSM are installed within a pelicase (Fig. 6a, b). Details on this may be requested at BGR.

Fig. 4a, b: BGR sampling funnel for gas exhalations on the sea floor (left), and incorporated within the OGS frame (right)

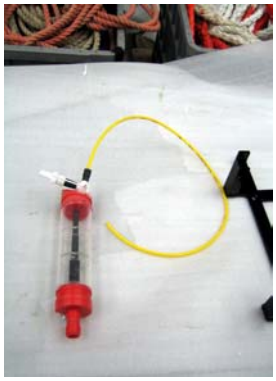

Fig. 5: Gas sampling device Inspection of buoy MAMBO 4

Fig. 6a, b: Pelicase with gas sensors, data logger, GSM, tubes and fixing frame

URS designed a system for the analysis of dissolved CO2 and CH4. The acquisition system is also incorporated within a closed box for the installation on the buoy (Fig. 7). Both methane sensor (Fig. 8a) and reinforced semi-permeable tube for dissolved CO2 analysis (Fig. 8b) are installed on the gas sampling funnel. Details regarding the scheme of the dissolved gases monitoring system and the sampling loop of dissolved gases are given in figs. 9 and 10. Details on this may be requested at URS.

Fig. 7: URS box with analytical devices for the detection of dissolved CO2 and CH4

Fig. 8a, b: Both methane sensor (left; see red arrow) and reinforced semi-permeable tube for dissolved CO2 analysis (right; see white arrow).

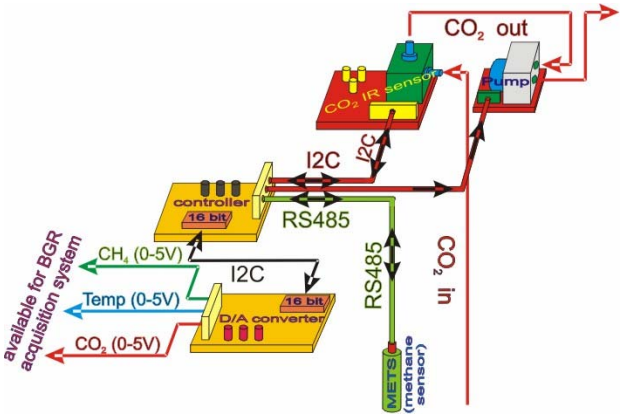

Fig. 9: Scheme of dissolved gases monitoring system

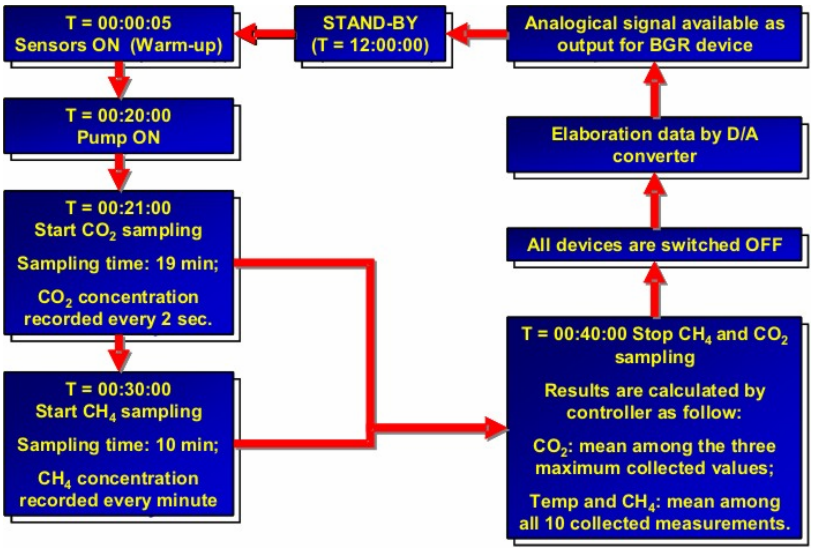

Fig. 10: Sampling loop of dissolved gases

The monitoring buoy with all technical devices was ready for deployment in September 2006 (Fig. 11a, b). OGS organized the deployment meeting which was attended by all partners (next chapter).

Fig. 11a, b: The monitoring buoy prior to deployment (left) and members of the JRA team (right).

2.1.3. TESTING AND FIRST OPERATION OF THE MONITORING BUOY

All partners met on the 20th September 2006 in Trieste for the final deployment of all monitoring devices. Prior to this meeting, OGS had already deployed the buoy in its offshore position including fixing the buoy and the monitoring funnel by divers.

The figures of this chapter including captions will give an impression of the buoy and its characteristics.

Fig. 12: Preparing the boat for the transport of technical devices to the buoy

Fig. 13a, b, c: Final work at the monitoring buoy. (a, left) Note the six solar panels and the meteorological station at the top, (b. middle) transition from pipes to sensors and data logger, (c, right) end of gas pipes is marked by balloons on the sea surface.



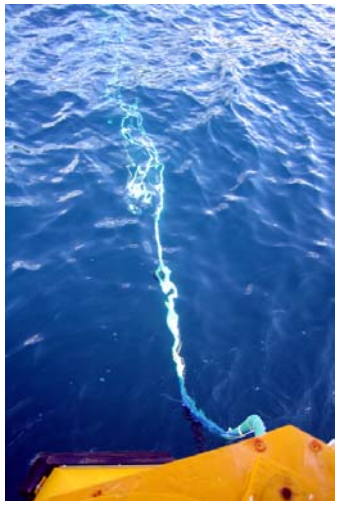

Fig. 14a, b, c: Final work at the monitoring buoy. (a, left) Note that the both upper solar panels can be moved for later workovers, (b. middle) gas pipe transition to the buoy with special safety fixation at the buoy, (c, right) gas pipe to depth.

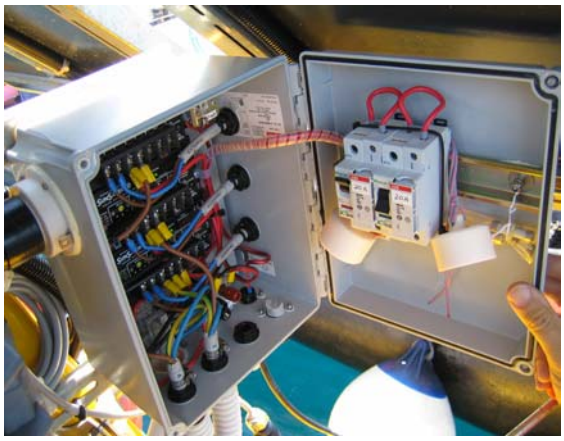
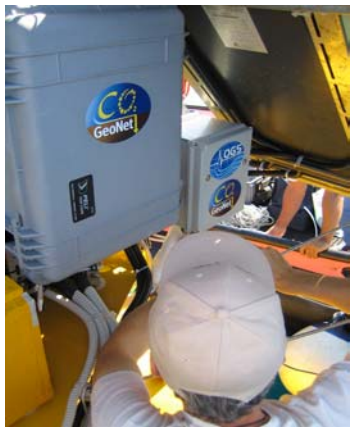

Fig. 15a, b: (a, left) open fuse box, (b, right) BGR pelicase and OGS fuse box.

Fig. 16: Ventilation of the BGR pelicase (works dependent on temperature; OGS construction).

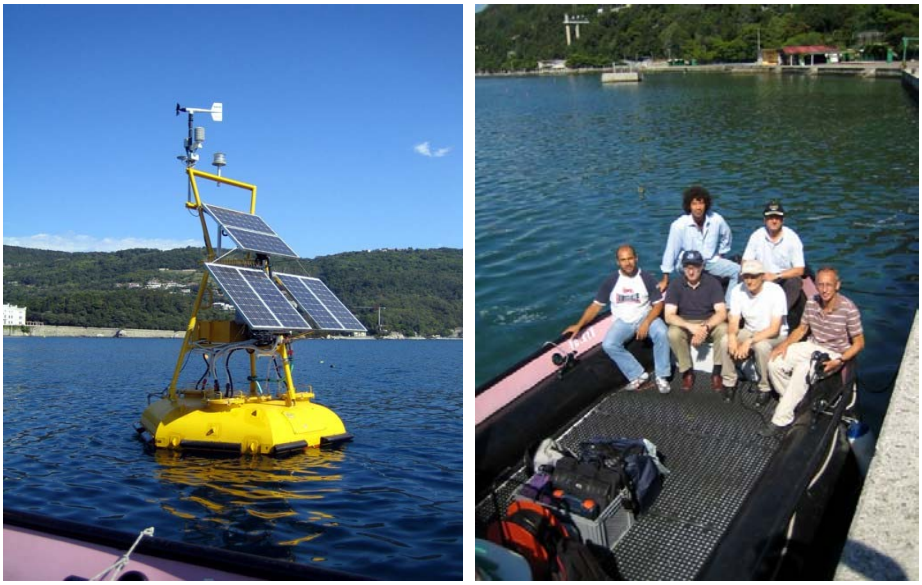

Fig. 17 (a, b): (b, left) the monitoring buoy after deployment and completion of all technical devices, (b, right) the monitoring team (front row from left to right: Aldo Annunziatellis, Guido Crispi, Jürgen Poggenburg, Dino Viezzoli; back row from left to right: Roberto Laterza, Hans-Martin Schulz; missing here: Stefano Graziani, Eckart Faber).

2.1.4. FIRST RESULTS OF THE MONITORING BUOY

After deployment of the buoy and final installation during September 2006 the monitoring buoy delivered first data for (a) gas flux and CO2 and CH4 concentrations (Fig. 18) and for dissolved CO2 and CH4 gas (Fig. 19).

The CO2 and CH4 gas concentrations are low. As gas fluxes are very low and occasionally tending to negative values (Fig. 18), variations may reflect wave motions which cause underpressure in the monitoring system. According to this, further testing is required. This can be conducted in the near future by gas injection into the subsea funnel by several options.

The concentrations of dissolved CO2 and CH4 are low, too (Fig. 19). General concentrations are about 20 nM/l CH4 and about 50 microM/L for CH4.

After a first testing period the URS-box has been switched off on Friday 29th September due to a disfunction of the pump and was re-started during the first October period. During the winter period the original SimCard (BGR) will be replaced by a new SimCard by OGS, additionally a new signal lamp will be installed.

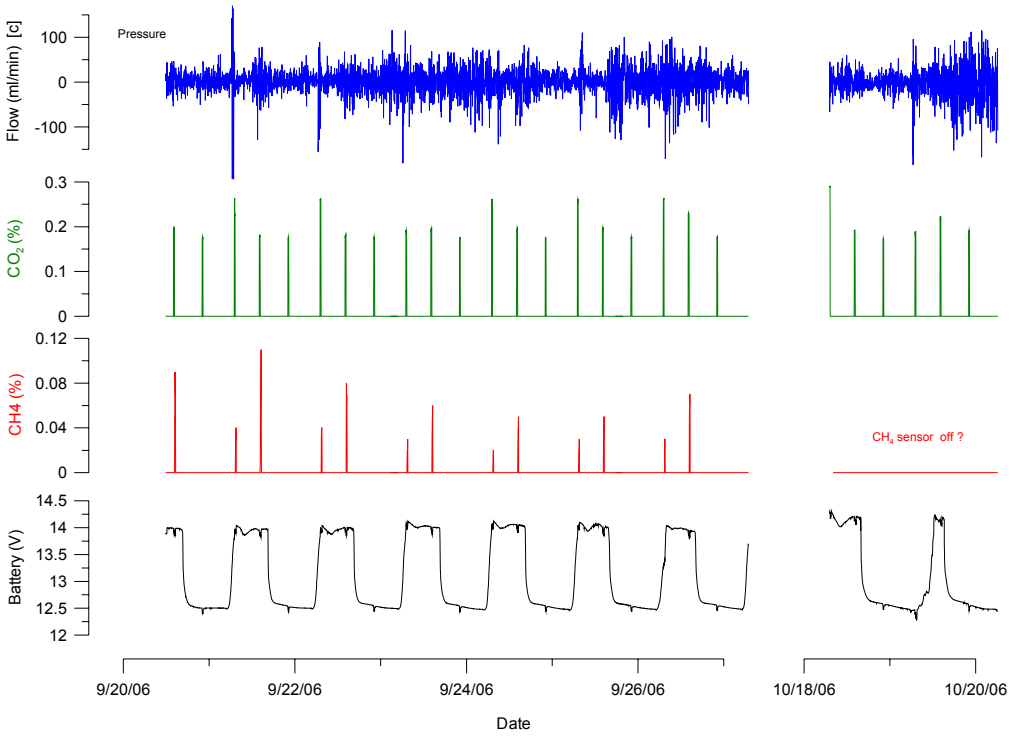

Fig. 18: Gas flow (ml/min), CO2 and CH4 concentrations (%), and battery status (V) for selected periods (here: September and October) of the monitoring buoy.

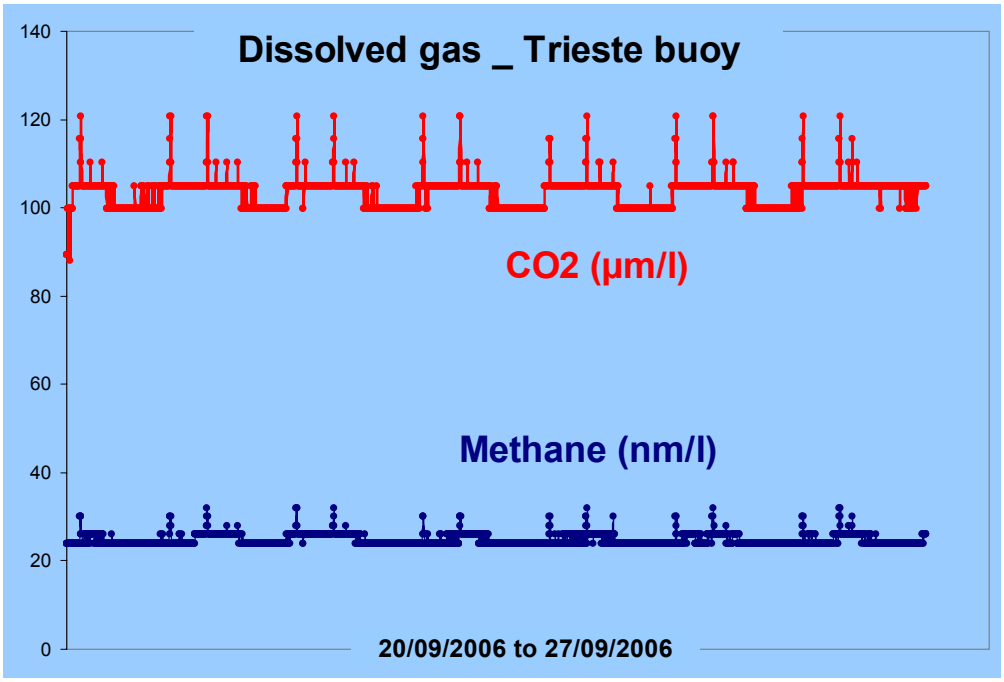

Fig. 18: Dissolved CO2 and CH4 concentrations (nm/l) for a selected period (here: September) of the monitoring buoy.

2.2. BIOLOGICAL INVESTIGATIONS (NIVA)

During July-September 2005, a unique CO_2 exposure experiment with a Japanese (RITE) Benthic chamber rig was performed at 400 m depth in Storfjorden in Møre og Romsdal, Norway (Fig. 18). Two consecutive 10-days exposures were performed: 20.000 ppm exposure in chamber I, 5.000 ppm exposure in chamber II and no extra CO_2 exposure in chamber III (control). The experiment was included as part of the JRAP8 (BGR), and also mentioned in JRAP4 (BGS) –but was basically a bilateral collaborative effort between NIVA and RITE/Japan.

Fig. 18: Map of the deep Storfjorden and adjacent coastal waters. The red dot marks the experiment site.

Before each deployment, rigid preparations and programming of sampling intervals etc took place. Several sensors monitored the water quality inside the chambers, and water samples were withdrawn approx. once per day. There are many pre-determined settings of timing, e.g. how long the chamber sits on the bottom before it starts penetrating the box chambers into the sediments and sealing off by the bottom lid (about six hours, to allow disturbances to settle in our case). Retrieval of the chamber was done right after the programmed end of measurements. The ballast weights were released by acoustic transponders, and the rig floated to the surface to be picked up from the boat. We rented the boat from the fishfarming company Pan Fish Norway, who has their HQ in the region of the site.

After each 10-days exposure in-situ, the rig was recovered and many sediment and water samples collected. The samples were divided between RITE/KANSO (Japan), NIVA, BGS, BGR and Aalesund College. Preliminary results on bacteria, meio/nanofauna, ATP and DGGE analyses etc have been provided from the partners.

Some results were presented at the network workshop in Venice, 25-28 April, 2006. A paper on some of the results was presented at the GHGT-8 conference in Trondheim, June 2006 (comparison between Storfjorden results and results from similar experiments at 2,000 m depth in the Pacific Ocean near Japan).

The experiment required a lot of logistics timing and support, something which was successfully achieved in a costwise manner at the Storfjorden site. The assembly of the chamber was done at the ODIM factory, which otherwise makes maritime equipment for offshore surveys.

Runde Environmental Centre (http://www.miljosenter.no) and Aalesund Univ. College provided good support to NIVA and the Japanese during the preparations and retrievals.

The total project period was from mid-July to beginning of October, 2005. The chamber and ascessories was shipped from and to Japan in a 20' container. Shipping time was about 6 weeks.

In connection with the experiments, a special workshop on CO2 storage was arranged in Aalesund on 1st September, 2005. It attracted CO2GEONET partners as well as several from Japan, plus Norway.

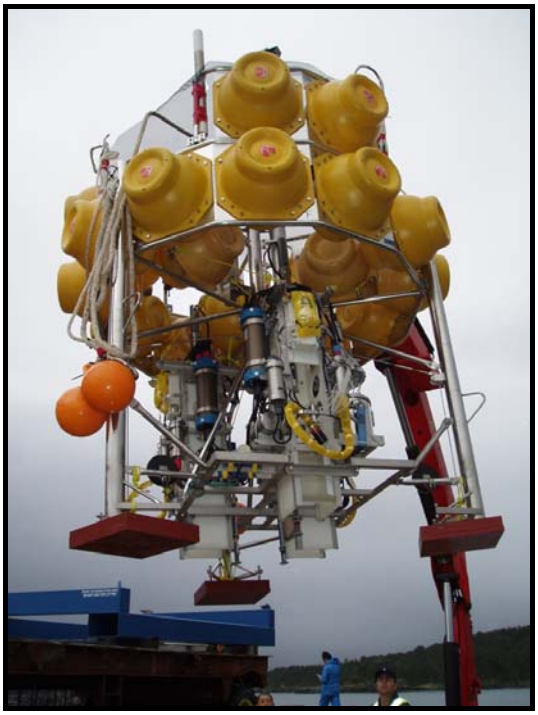

Fig. 19: The RITE accumulation chamber experiment prior to deployment

2.2.1. FIRST RESULTS OF MICROBIAL ACTIVITY AND CELL NUMBER MEASUREMENTS IN BENTHIC CHAMBER SAMPLES (M. Krüger, BGR)

2.2.1.1 Methods

In June 2005, sediment samples were collected during the NIVA benthic chamber experiment. Sediment was filled aseptically into sterile glass bottles (100 ml), which were sealed with butyl septa and screw caps. Further subsamples (2 ml) were taken for DNA extraction (stored at -20°C), and for microsocopy (fixed with 4 % p-formaldehyde (Eller et al. 2001).

Sediment samples were collected during two subsequent chamber experiments. According to the CO_2 concentrations applied in the chambers samples were either labelled "control" for background CO_2 concentrations, or "medium CO_2" for 5000 ppm_v in the gasphase, and "high CO_2" with 20.000 ppm_v. From the first set of experiments the living sample from the "high CO_2" treatment was lost during transport.

2.2.1.2 Microbial activity measurements

Sediments were converted into slurries by the addition of artifical seawater medium in a ratio of 1:1 (w/w). All manipulations were performed under an atmosphere of N_2/CO_2 (90/10 [v/v]) in an anoxic glove chamber (Mecaplex). Three replicates of 3 ml slurry from each sample were flushed with N_2 and incubated in Hungate tubes with a total volume of 5 ml to determine the initial potential methane production rates (MPR) (Krüger et al. 2001). All tubes were sealed with butyl stoppers and repeatedly flushed with N_2 to remove residual O_2. Headspace gas samples were taken daily after heavy shaking of the tubes by hand, and analyzed for methane on a gas chromatograph equipped with a flame ionization detector.

For the determination of sulphate reduction rates (SRR), incubations were set up as described above for MPR but this time additionally sulphate was added from an anoxic stock solution to a final concentration of 10 mM. Samples for chemical analysis were withdrawn with hypodermic needles and plastic syringes pre-flushed with N_2 through the butyl stoppers. Sulphide was determined photometrically using the formation of copper sulphide (Cord-Ruwisch, 1985).

Microbial activities were calculated by linear regression of the product increase with incubation time, and expressed in µmol g_{dw}^{-1} h^{-1} of soil.

DNA extraction and quantification of 16S rRNA genes

For quantitative PCR analysis (qPCR), high molecular weight DNA was extracted from the sediment samples stored at -20°C. The DNA extraction was based on cell lysis with 10% lauryl-sulphate solution and horizontal shaking for 45 s after addition of zirconium-silica beads, followed by DNA purification using NH_4-acetate and isopropanol precipitations as described in detail by Henckel et al. (1999).

Nucleic acids of *Eubacteria* and *Archaea* were quantified by real-time qPCR using an ABI Prism 7000 Sequence detection system (Applied Biosystems, Germany), following the protocol of Lüders et al. (2004) with the primer sets Ba519f / Ba907r and Ar109f/Ar912r, respectively. The gene copy numbers were converted into cell numbers using a conversion factor of 3.6 and 1 for *Eubacteria* and *Archaea*, respectively (Klappenbach et al., 2001).

2.2.1.3 Results & Discussion

Microbial activities and cell numbers of important groups

The determination of environmentally important microbial activities in the sediment samples showed significant differences between the CO_2 enriched "high CO_2" and "medium CO_2" sites and the "control" with background CO_2 concentrations. Methane production is an important anaerobic microbial process in organic matter degradation. Microbial methane formation was in the first experiment slightly stimulated by medium CO_2 addition (Figure 1), with rates increasing from 0.0097 ± 0.0001 to 0.018 ± 0.0007 µmol CH_4 produced g_{dw}^{-1} d^{-1}. In the second chamber epxeriment the increase of rates from the control to medium CO_2 was evene more pronounced, but then a decrease occurred at highest CO_2 concentrations. The stimulation of methane production rates is in good accordance with the increase in archaeal cell numbers detected by qPCR (Figure 3, see below). Maybe the methanogenic miroorganisms benefitted from dead other bacteria due to less competition for substrates or by eating the products of their degradation.

Sulfate is the most important electron acceptor and anaerobic microbial process in the degradation of organic matter in marine sediments. As was already observed for methane production, this process was stimulated by medium CO_2 addition in both sets of experiments (Figure 2). In the second chamber incubations sulftate reduction rates even increased again from medium to high CO_2 cocentrations. Presumably, sulfate reducing bacteria can tolerate changes in CO_2 concentrations and/or pH in their surroundings, and are also thriving on degradation products of other organisms which died as consequence of CO_2 addition.

In accordance with the microbial activities, also total numbers of microorganisms, determined via quantitative PCR (qPCR), showed significant differences between the control chamber and the sediments exposed to CO2 (Figure 3). Cell numbers of *Bacteria* generally were highest at the control site, and gradually lower after the addition of medium or high CO_2. Archaea, in contrast, seemed to be stimulated by medium CO_2 and only died off with high CO_2. The growing archaea might well be methanogens, since their activity is also stimulated, see above. Generally, cell numbers of *Bacteria* were higher than those of *Archaea*. In conclusion, for this marine site these still preliminary microbiological experiments show a beneficial effect of moderatelyelevated CO_2 concentrations on specific groups of the sediment microflora.

In summary, during the CO_2-exposure in the benthic chambers, significant effects of high CO_2 concentrations were observed on both, microbial activities and cell numbers. These preliminary results already indicate the stimulation or inhibition of distinct groups of microorganisms as a consequence of the long-term exposure of the sedimentary environment to elevated CO_2 concentrations.

Consequently, our future studies will focus on a more detailed analysis of presence and activities of key microbial groups and metabolic pathways. In addition to the already mentioned methane producing and sulphate reducing microorganisms this will include for example nitrogen or CO_2 fixing (autotrophic), ammonium oxidising, or other environmentally relevant microorganisms. At the end, this study should identify possible candidates in the microbial kingdoms, whose presence or absence provide easily detectable and accurate indicators for the leakage of CO_2 from deep reservoirs into near-surface marine ecosystems.

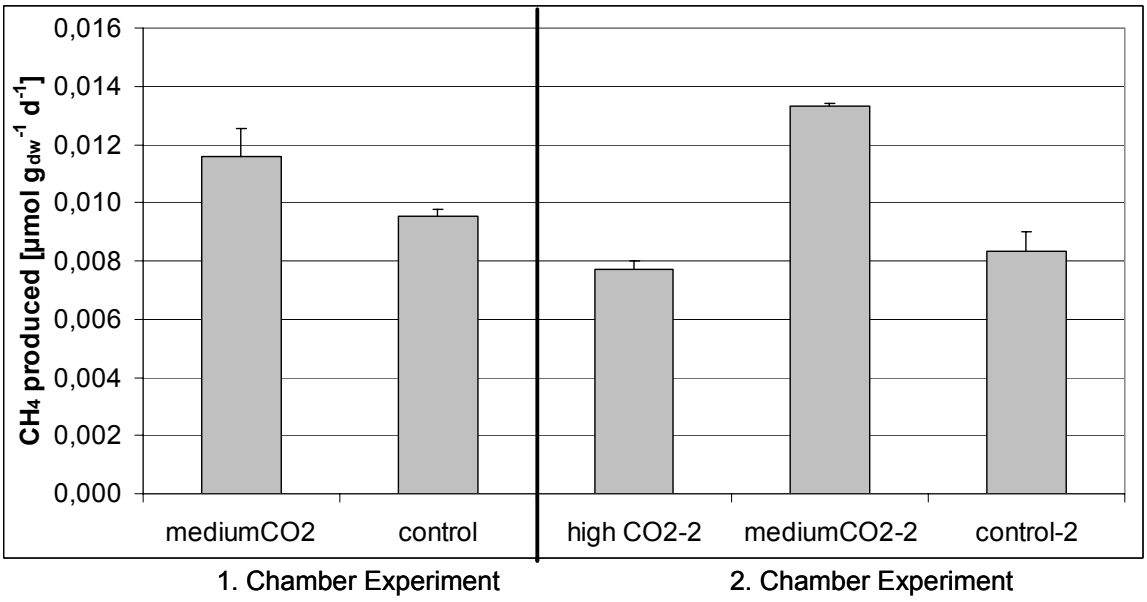

Figure 1: Potential rates of methane production in different sediment samples determined *in-vitro* (mean ± SD, n = 3-5).

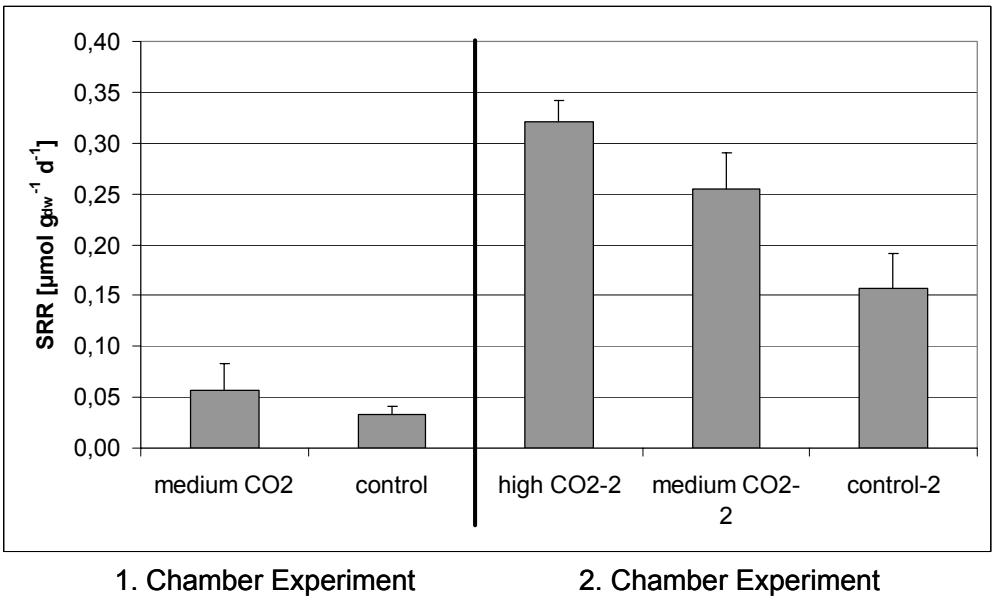

Figure 2: Potential rates of sulfate reduction in different samples determined *in-vitro* (mean ± SD, n = 3-5).

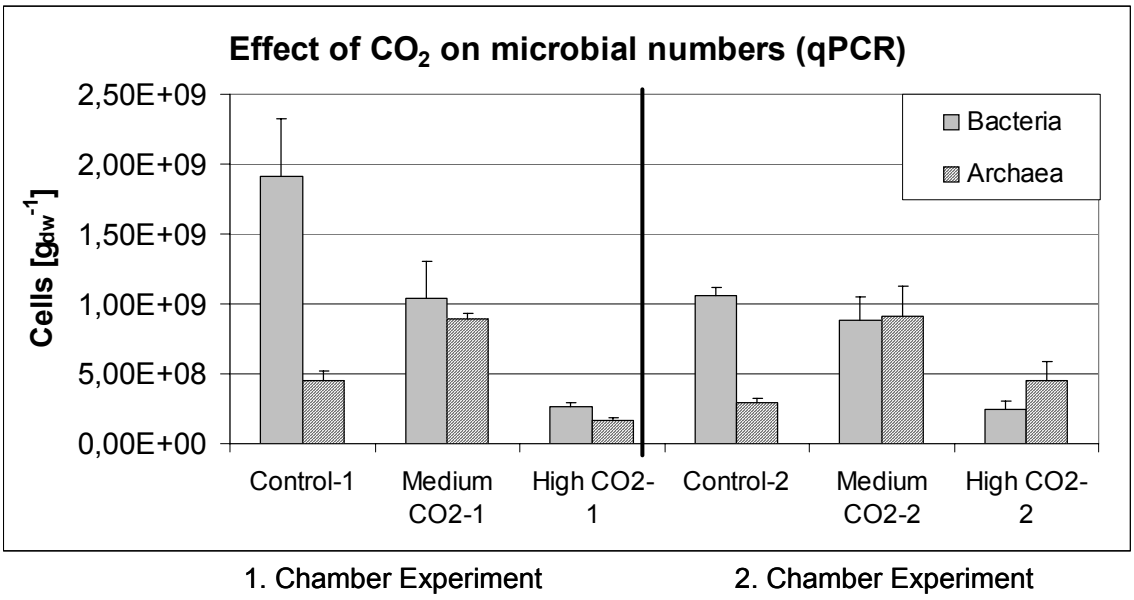

Figure 3: Cell numbers of archaea and bacteria determined via qPCR in different samples (mean ± SD, n = 8-12).

Literature

Cord-Ruwisch, R., 1985. A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria. J. Microbiol. Methods 4, 33-36.

Eller, G., Stubner, S. and Frenzel, P. (2001) Group-specific 16S rRNA targeted probes for the detection of type I and type II methanotrophs by fluorescence in situ hybridisation. FEMS Microbiol. Letters 198, 91-97.

Henckel, T., Friedrich, M. and Conrad, R. (1999) Molecular analyses of the methane-oxidizing microbial community in rice field soil by targeting the genes of the 16S rRNA, particulate methane monooxygenase, and methanol dehydrogenase. Appl. Environ. Microbiol. 65, 1980-1990.

Klappenbach, J.L., Saxman, P.R., Cole, J.R., and Schmidt, T.M. (2001) rrndb: the ribosomal RNA operon copy number database. Nucleic Acids Res 29:181–184.

Krüger, M., Frenzel, P., Conrad, R., 2001. Microbial processes influencing methane emission from rice fields. Glob. Change Biol. 7, 49-61.

Lüders, T., Manefield, M., and Friedrich, M.W. (2004) Enhanced sensitivity of DNA- and rRNA-based stable isotope probing by fractionation and quantitative analysis of isopycnic centrifugation gradients. Environ. Microbiol. 6:73–78.