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ECO2 Deliverable 4.3: Potential use of community and organism biomarkers for detecting and monitoring CO_2 leakage

by

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1 Introduction

Atmospheric levels of CO_2 have been increasing as a result of burning fossil fuels at a far higher rate than previously experienced in Earth's history. These anthropogenic emissions and the resulting rise in CO_2 levels is anticipated to be the primary factor for global warming and environmental effects associated with climate change (Hoegh-Guldberg and Bruno, 2010; Widdicombe and Needham 2007). There is however a growing acceptance that anthropogenic CO_2 emissions need to be significantly reduced in order to avoid or minimize further negative impacts on ecosystems.

A portfolio of mitigating actions and technologies are suggested to reduce or slow down the rise in atmospheric CO_2 levels. Technical CO_2 capture and storage (CCS) is one of the most promising measures for immediate regulation of CO_2 emissions (Holloway, 2005, IPCC, 2005). Such storage involves injection of CO_2 into geological formations. It has been suggested that the method potentially may reduce total emissions of CO_2 by 21-45% by 2050 (IPCC, 2050).

After injection, the carbon dioxide moves up through the geological formation at the storage site until it reaches an impermeable layer of rock. The existence of such an impermeable layer of rock is a prerequisite for being a suitable storage site. Suitable geological formations may be onshore or offshore. Most likely offshore sites with sediments at the bottom/seawater interface will predominate.

No matter of how comprehensive a potential site is investigated for its applicability for CO_2 storage there is always a certain probability for leakage, although the likelihood of leakage is extremely small (IEA 2008). It is nevertheless important to secure acceptance that the geological storage of CO_2 poses no significant risks to humans or the environment (Van Noorden, 2010; Monastersky, 2013). The necessary public support and permits for implementing CCS will not be given unless there is confidence that it can be executed safely. One should however bear in mind that the risk of environmental effects from a possible leakage from a storage site is orders of magnitude less than the risk of not implementing CCS altogether.

Since the possibility of a leak from storage site cannot be completely ruled out and causing local environmental consequences (Blackford et al. 2008), it is important to have methods for detecting leaks in order to carry out mitigating measures if a leakage should occur.

A leakage can result in physical, chemical and biological changes at and in the sea floor. Responses that are recognizable in the field and to some extent specific to CO_2 will be good candidates as part of a monitoring method for detecting a leakage.

In this document, we provide an evaluation of potential use of community and organism biomarkers for detecting and monitoring CO_2 leakage based on the available reports on experiments and investigations performed within the framework of ECO2 and does not anticipate to be a full review of the literature within the field, although some of the more recent literature has been included in the discussion.

2 Use of benthic community structure for detecting end monitoring CO_2 leakage

This chapter summarizes the report "Field observations at a natural CO_2 seep". Contribution to D4.1 from MPI, UniGent and OGS.

2.1 Observations at a natural CO_2 seep near the Panarea Island

The experiments were performed at a natural CO_2 seep at the Panarea site near the Stromboli Island with its active volcano. The effects of gas emissions on the microbial, meiofaunal and macrofaunal communities were investigated. Two CO_2-impacted and one non-gas-impacted background site where investigated: "HighCO2" (St. B1), "LowCO2" (St. B3) and "Ref" (St. B2).

The effect of CO_2 seabed emission was clearly visible on pore water chemistry as pH reduction, increase of DIC and alkalinity, and enhanced chemical weathering (high concentration of iron, manganese and silicate) along sediment profile of CO_2-impacted sites. Total organic carbon (TOC), total nitrogen content (TN) in the sediment and C:N ratio's did not differ between seep and control areas.

2.2 Macrofauna

Macrofauna densities at the "LowCO2" seep site were significantly lower than those at the other two sites ("HighCO2" and "Ref"). Although diversity did not differ, the macrofauna composition at both CO_2 seep sites differed from the "Ref" site based on the occurrence of more oligochaetes and amphipods and less polychaetes and gastropods at the seep sites. Grazing marks by the sea urchin *Paracentrotus lividus* were less abundant at the impacted sites as compared to reference site. Grazing marks by the fish *Sarpa salpa* were more abundant at the impacted sites

2.3 Meiofauna

Meiofauna densities were significantly higher in the control sediments at "Ref" site compared to the CO_2 seep, while in the seagrass shoots, the opposite was true. Where CO_2 seepage occurred, meiofauna densities where highest in the first two centimeters of the sediment and showed a steep decline with depth. At the control site, there was a more gradual decline in densities with depth.

Nematode species richness was significantly lower in the CO_2-impacted sites compared to the non-impacted sites. In the seagrass (leafs and shoots), no seepage-related significant differences were detected in meiofauna taxa, copepod species and nematode species assemblages.

2.4 Microphytobenthos/Bacteria

The highest microphytobenthic densities were consistently recorded at high CO_2 sites, about four times higher than the abundance observed at the reference site.

In the water column and on the seagrass leaves, the bacterial community structure did not show any significant differences between the sites investigated. Conversely, bacterial community analyses of recovered sediments showed difference between the CO_2-impacted sites and the "Ref" site without seepage. The results did also provide evidence of a reduction in the bacterial diversity in seep sites compared to the background site.

2.5 Planktonic communities

The results indicate that natural CO_2 emissions at the investigated sites do not seem to have any clear influence on the phytoplankton and microzooplankton community.

2.6 Conclusion

The natural CO_2 seeps at the Panarea Island have operated over a very long period, allowing for faunal responses that involve acclimation/adaption over a very long period. The use of the responses observed in the sediments as a method for the assessment of the impacts of short term CCS leakage is thus in principle questionable, as the biological responses observed (or not observed) at Panarea probably underestimate possible effects of an acute leak. Experiments on short term exposure of sediments to CO_2 do however show clear effects on biological processes such as increased nanobenthos density, methane production and sulphate reduction (Ishida et al 2013). Short term (37 days) and small (4.2 tons of CO_2) sub-seabed release of CO_2 did however also show that the bacterial community and macrobenthos is responsive to CO_2 but within a limited seafloor area (less than 25 meters from epicenter of exposure) and that recovery is fast (days to weeks) (Blackford et al. 2014).

3 Remote video and still images to monitor behavior and avoidance

> This chapter summarizes the report by Chris Hauton, Anthony Zardis and Elizabeth Morgan (University of Southampton). "Remote monitoring of organism avoidance behaviours as biomarker for CO_2 leakage, and the correlation of behaviour with physiological impact (WP4.3)"

3.1 Remote video and still images

Two different methods (continuous video recording from an underwater vehicle and colour still camera) for remote monitoring where tested. The work demonstrated that, in principle, color video and still imagery can be used to survey large areas of the sea bed in the context of CCS monitoring. Picture quality is however crucial and with the pixel resolution offered by currently deployed camera platforms it is a challenge to discriminate individual species. It was recommended that still images from stable platforms such as autonomous underwater vehicle (AUV) should be used over cabled platforms connected to a survey vessel in order to monitor possible changes in behavior of megafauna in response to CO_2 or brine leakage. The use of remote techniques do however represent methodological challenges related to picture quality and the development of methods for automatic image processing of a large number of pictures.

The use of still images in sediment profiling instruments are however, in general, a monitoring technique widely accepted by the scientific community and environmental impact assessment authorities for evaluating conditions in sediments (Rosenberg, R., Magnusson, M. and Nilsson, H.C., 2009, Rosenberg and Nilsson, 2005, Birchenough et al. 2013).

3.2 Use of responses of megafauna to detect CO_2 leakage

Species respond differently to an environmental perturbation. If changes in a behavior response should be used as a first and quick way of detecting a CO_2 leak it has to be observed with some sort of remote video or still technique (see chapter 3). This leaves megafauna (her defined as species possible to observe by remote techniques) as the most and probably also the only promising group of animals to be considered. A prerequisite for this is that the response can be seen on the sediment surface (for example animals coming to the surface of the sediments). An important question to be addressed is to what degree behavioral responses are consistent in relation to CO_2 exposure and thus can be used reliably for identifying CO_2 leakage. Picture quality render only changes in the behavior of large animals (megafuna) as a potential "instant" marker for a CO_2 leak. Echinoderms have been suggested as a vulnerable group to CO_2 due to the calcareous skeletons and typically poor acid base buffering capacity (Miles et al., 2007). There are however contrasting experience in how echinoderms (and other groups of species) respond to CO_2.

Two different echinoderm species where tested, *Paracentrotus lividus* and *Brissopsis lyrifera* (Hauton et al.). The investigation confirmed that whilst *P. lividus* lacks a significant medium term (65 days) buffer capacity in the absence of any detectable changes in HCO_3^-, it is still able to tolerate chronic hypercapnia (20,000 ppm) for up to two months. A longer exposure would probably lead to high rates of mortality. It was also observed that *Brissopsis lyrifera* can tolerate chronic hypercapnia (20,000 ppm) for 2 months. No obvious CO_2 related avoidance behavior was however identified for the two species. The results are not in accordance with the behavior of *Echnocardium cordatum* which has been shown to emerge from the sediment within hours of gas release (Pratt et al., in review). *E. cordatum* has also been found to emerge from the sediment under other kinds of unfavorable conditions like organic enrichment at the sediment surface and low oxygen conditions in bottomwater (Hollertz, 1998; Hollertz and Duchêne, 2001; Nilsson and Rosenberg 2000).

In the QICS project direct observations of the sea bed community identified the emergence of the urchin *Echinocardium cordatum* within hours after the gas release had started, but the sea star *Asterias rubens* did not to respond (Pratt et al., in review). In experiments with the urchins *Paracentrotus lividus* and *Brissopsis lyrifera* no obvious avoidance behavior was identified. It has also been documented that the links between CO_2 exposure, behavior pattern and organism physiology is not fully understood ().We conclude that behavioral responses among megafauna are not consistent in relation to CO_2 exposure and thus cannot be used alone as an unequivocally tool for identifying CO_2 leakage. At present the use of megafauna are however the only biological marker that has some relevance as a quick way of detecting a CO_2 leak under field conditions. The use of behavior changes in megafauna as a tool for identifying a CO_2 leak must however be used together with physical and/or chemical method in order to be reliable.

4 *The use of Brissopsis lyrifera*, *Nereis virens* and *Mytilus edulis* for detecting leakage of heavy metals following high CO_2 conditions

This chapter summarize the report by Eivind Farmen, Andrew K Sweetman, Dave Lowe, Elizabeth A Morgan, Steve Widdicombe "Effects of heavy metals under high CO_2 conditions".

Proposed mechanism: high CO_2 conditions modifies metal availability which can be detected as changes in organism metal content and/or effects on the test organisms in other ways that can be detected through changes in biomarker responses.

Five different CO_2 treatments were included in the laboratory (NIVA marine research station Solbergstrand, Oslofjord, Norway, Norway) experiment: ambient control (400 ppm), 1000, 2000, 5000 and 20000 ppm CO_2. Exposure to CO_2 led to increased mobilisation of a few metals and reduced mobilisation of others from the sediment. Some mortality was observed during the experiment with *B. lyrifera* as the most sensitive of the test organisms. The results show that after 8 weeks exposure to 20000 ppm CO_2, approximately 35 % of the *B. lyrifera* individuals had died. In the rest of the treatment groups, *B. lyrifera* mortality was less than 25 %, with a trend of higher mortality rate in test organisms exposed to sediments containing heavy metals. No consistent patterns in effects on mortality where observed for the two other species, *M. edulis* and *N. virens*.

Heavy metal bioaccumulation in the test organisms were not significantly altered by CO_2 treatment throughout the test period, although a trend of decreased levels of Al, Fe, Cr and V was observed in *N. virens* under high CO_2 conditions, similar to that seen for sediment. Changes in the extracellular pH in *B. lyrifera* did however confirm that CO_2 exposure affected the organisms.

The biomarker results suggested that cellular energy allocation, neutral retention and histology were not highly responsive to either contaminants in sediments or changes in CO_2 in this study.

The experiments indicate that changes in extracellular pH in *B. lyrifera* coinciding with increased mortality of *B. lyrifera* as a consequence of CO_2 exposure. In a field situation we expect that *B. lyrifera* under stress will move to the sediment surface. Such a response is however not exclusive for CO_2 but will probably also take place if the oxygen conditions of the bottom water becomes critical of other reasons as has been observed for the ophiuroid *Amphiura filifrmis* (Rosenberg et al. 1991).

Experiments with the brittlestar *Amphiura filiformis* has shown that rapid acidification events may not be lethal to benthic invertebrates, but may result in behavioural changes, like moving to shallower depths within the sediment changes (Murry et al, 2013). Such effects could have longer-term implications for species survival, ecosystem structure and functioning but is probably difficult to use as a method for detecting CO_2 leakage.

5 Discussion

Responses to CO_2 leakage can basically be broken down into those that act at the level of the individual and those that act at the level of the community. Individual biomarkers/bioindicators can be behavioral (e.g. animals coming to the surface) or physiological/biochemical. At the community level there are 3-6 main potential response candidates depending how different types of organisms are divided according to taxonomy and size: bacteria/Archaea, meiofauna/nanobenthos, and macrofauna/megafauna. Macrofaunal communities have long been used by industry and regulators as an effective environmental monitoring tool for a variety of potential stressors in the marine environment and is also an agreed tool within the Water Frame Directive. Macrofauna is an appropriate tool for detecting effects of stressors related to organic enrichment and reduced oxygen concentrations in bottom water, but this group does not seem to be optimal for detecting effects of industrial effluents like metal effluents (Oug, 2013).

Although it is has been shown that bacteria, meiofauna and macrofauna communities under certain experimental conditions can be affected by $CO_{2,}$ the response is not automatically practical for detecting and monitoring CO_2 leakage. There will also be a question of what is the aim of the monitoring. It is a significant difference in the methods that are suitable for detecting a leak in the first place and methods that are suitable for monitoring the effects of a leak in space and time after it has been detected or before a potential leak has taken place (baseline survey).

5.1 Baseline monitoring

Monitoring biological communities in the seafloor sediments overlaying a specific storage reservoir in order to collect reference information in case of a CO_2 leak is challenging. A fully comprehensive baseline of the biological system would require an initial mapping of mega-, macro- and potentially meio- and micro- biota within each habitat in the storage area, possibly also supported by other parameters (Blackford et al. in press). The basic challenge is to have the data needed in order to accurately discriminate the human impact (in this case CO_2 leakage) from natural short and long time environmental change (se Blackford et al. in press). The large areas involved and spatial temporal heterogeneity of the benthic fauna are also a challenge in terms of the extensive sampling needed in order to have sufficient background information for the different areas of the seafloor covering the reservoir. The objective of having a baseline program for collecting all the data needed in order to accurately discriminate the human impact from natural variability is considered unrealistically ambitious. It is however important that the baseline monitoring secures the data needed to make general predictions on possible effects if a leak should occur. It is therefore inevitable, at a certain stage, a decision has to be made on what sort of biological parameters should be incorporated in the baseline monitoring and which must be omitted.

Macrofaunal communities (here considered as makroscopic intvertebrate organisms larger than 1 mm, i.e. that are retained on a 1 mm sieve) have long been used by industry (oil and gas companies) and regulators as an effective environmental monitoring tool for a variety of potential stressors. Macrofauna are also responsive to CO_2 (Christen et al. 2012, Widdicombe et al 2009,). Although there are obviously also other communities that are responsive to CO_2, we feel that macrofauna at present is the primary choice for baseline monitoring. Our choice is based on the large amount of data already collected (in the North Sea) and the number of numerical ways macrofauna data can be treated in order to identify anthropogenic stress. Sampling of macrofauna may also contain some megafauna (not quantitatively). Information on the occurrence of megafauna on the seafloor overlaying the storage site is also important in order to be in a position to use emerging megafauna as a tool for detecting a leak (see below).

Meio- and micro- biota should not be omitted totally from the baseline monitoring and should be included based on the objective of collecting sufficient data to be able to design a comprehensive program for monitoring effects of an observed leak and recovery (see below).

The baseline monitoring should cover all the main types of sediment (sand, clay, etc.) areas overlaying the reservoir and should cover typical depth intervals. The orientation of the station network should be determined based on the form of the reservoir and possible week zones in the sedimentary overburden. It is anticipated that some sort of grid design of the stations is most relevant. The main objective is however that the stations should be positioned as optimally as possible in relation to the size and form of the reservoir and location of possible leaks. Expected dispersal patterns in case of a leak and benthic conditions should also be considered.

5.2 Detecting a new leak

Ideally, a suitable response for detecting a leak in the first place should be relatively easily and instantly recognizable in the field and, to some extent, specific to CO_2. No such absolute universal indicator of leakage or impact is however yet identified (Blackford et al, in press).

To be effective, it should be possible to apply the detection method for monitoring to large areas over a relative short time. The method should not require taking samples for later, delayed treatment. Detailed physical sampling for species identification will therefore in most cases not be a practical method for detecting a leakage, but can be a useful and relevant tool for a more detailed mapping of effects after a leakage has been identified by other methods. Modelling indicates that the spatial footprint of a CO_2 leak covers a small area. This does however also imply that localizing a leak is a challenge.

Methods for characterization biological communities are time consuming and expensive (especially macro- meio- and micro- biota) and the results are only available after some delay dependent on the sample treatment time. Behavioral responses of megafauna may however be more easily detected given that good automatic methods are available, which does not seem to be the case (see above). Physical and chemical characteristics, like acoustics and pH measurements, are instant measurements that are more specific for CO_2 and can be applied over relative large areas (surveying along parallel lines) with less effort than using most biological methods. The combination of surveying physical and chemical characteristics and biological responses of megafauna (remote methods) is suggested to be the best method for detecting a new leak. One should however bear in mind that the traces of emerging megafauna will tend to disappear over time due to degradation, at least in the cases where the endpoint of the behavioural response is mortality.

Short term exposure of CO_2 shows that benthic organisms such as meiobenthos, nanobenthos and bacteria and Archaea interact as a community (Ishida et al. 2013). Examples of such interactions are predator–prey relationships and competition. The first responses of the benthos after a CO_2 leakage are usually caused by the development of a more hostile environment caused by changes in the physical/chemical characteristics of the sediment pore-water and may result in increased mortality and alter the interactions among the different benthic organisms, for example by increasing the number of degrading bacteria. Benthic communities show significant changes in structure and reduced diversity in response to reduced pH after 30 days of exposure (Christen et al 2012).

The effects of these interactions will, however, change from the initial phase, where responses and counter-responses are anticipated to be fast, to a more steady state situation after long-time exposure. In theory, this means that biological methods used for detecting recent leaks could be different from those that are to be used for long-lasting leaks.

The primary area where the upward migrating CO_2 reaches the interstitial water of surface sediment is probably much smaller than the secondary area that may be influenced by CO_2 rich water that has escaped from the sediment as gas or in a dissolved state. Although larger, this secondary area will probably experience a much smaller and more variable influence of CO_2 than the primary area. It is therefore most important to find methods that can detect such primary areas.

5.3 Monitoring the effects of an observed leak and recovery

Monitoring the effects of an observed leak is a more straight-forward task than finding a leak, since the epicenter of the discharge in known. The challenge is however to design a monitoring program that both in space and time is sufficiently comprehensive to distinguish natural variability from changes caused by the leak.

It is unclear how large the affected seafloor area will be following a leakage. Several investigations and modeling studies do however indicate that the footprint of a leak will be localized to a relatively small area in the vicinity of the leak (Allendal et al. 2014, Blackford et al. 2014) and that only the most extreme scenarios are capable of producing perturbations that are likely to have environmental consequences beyond the locality of a leak event (Blackford et al, 2008).

Previous modelling estimates indicate that that large leaks may influence an area of a few km radius (Blackford et al. 2008), whereas experiments with sub-seafloor discharges of relatively small amount of CO_2 (4.2 tons over a period of 37 days) showed biological effects only within a few meters radius (Blackford et al 2014). Modelling performed within ECO2 indicates that the footprint of a leak will be localized to the vicinity of the leak (Allendal et al. 2014). Modelling seems to indicate that typology of the leak is a major dimensioning parameter for the footprint. Even large localized leaks leave footprints of a diameter less than 50 m on the seafloor, whereas leaks through fractures may leave larger footprints (Allendal et al. 2014).

The duration of the leakage depends on the cause and nature of the leak, local conditions and the action taken to stop the leak. It is probably possible to stop small uncomplicated leaks within some days, whereas larger complicated leaks will take much longer time, possibly tens of years (Allendal et al. 2014) if left untreated.

There are also time-related aspects involved as the community may endure high levels of CO_2 for a short time but not for longer periods. The leakage may first influence a relatively small area, which may increase until a steady state situation is reached, involving a larger area. Benthic communities are reported to undergo significant changes in terms of community structure and reduced diversity in response to reduced pH after 30 days of exposure (Christen et al 2012).

Monitoring programs in order to detect possible effects must be scaled in space and time according to the expected size of the footprint and the duration of the leakage. How far away from a leak it is expected to find detectable effects will influence the design of a monitoring program. In general, the sampling points should be arranged in a radial transect design where the stations are placed along two perpendicular axes where the epicenter of the leak is at the origin. The main axis should be in the prevailing direction of current flow. It is important that the design also covers several control stations as it otherwise can be difficult to distinguish between natural variability and effects of the leak . In case of more complicated leaks (fracture), the radial design may have to be adjusted in order to cover the potential affected area.

6 Recommendations

Based on the available information and the ECO2 results, our recommendations are as follows:

1. Biological baseline monitoring:

   Macro fauna is at present the first taxon choice for baseline monitoring. Meio- and micro- biota should however not be omitted totally but should be included based on the objective of collecting sufficient data to be able to design a comprehensive program for monitoring effects of an observed leak and recovery at a later stage (see below). The baseline monitoring should cover all the main types of sediment areas (sand, clay, etc.) overlaying the reservoir and should cover typical depth intervals. The orientation of the station network should be determined based on the form of the reservoir and possible week zones in the sedimentary overburden (some sort of grid is most relevant). Expected dispersal patterns in case of a leak and benthic conditions should also be considered.

2. Detecting a new leak

   As the primary method for detecting a possible leak(s), a survey on possible spatial related behavioral responses of benthic megafauna should be performed by remote methods in the area overlying the reservoir. This should be done in combinations with the use of physical and/or chemical characteristics of bottom water. If a leak is suspected, the area should be monitored by physical and/or chemical and biological (megafauna) methods in more detail, in order to localize the epicenter of the leak. When the epicenter is identified, a more detailed monitoring program in order to identify effects should be designed (se below).

3. Monitoring the effects of an observed leak and recovery

If biological effects are indicated based on megafauna observations and a leak is identified through use physical and/or chemical characteristics of bottom water, a more detailed biological study should be performed on community structure of macrofauna, meiofauna and microfauna, in order to map the range zone of the effect in more detail. Such an investigation should include monitoring stations along at least two perpendicular transects through the actual leakage area/point. If a leak is stopped, the recolonization of affected areas should be investigated by performing macrofauna/meiofauna/microfauna studies.

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