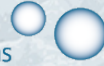


ECO₂

Sub-seabed CO₂ Storage:
Impact on Marine Ecosystems



ECO₂ Briefing Paper No. 2

*Potential impacts of CO₂ leakage from
sub-surface storage on seabed biology*

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Introduction

By Regina Klapper, German Marine Research Consortium

Anthropogenic carbon dioxide (CO₂) emissions have been continuously increasing since the advent of fossil fuel combustion during the industrial revolution, leading to significant changes in the Earth's climate. In order to limit

global warming to the by the global community agreed 2°C target, CO₂ emissions have to be significantly reduced. Carbon dioxide Capture and Storage (CCS), in which CO₂ from power plants or other industrial facilities is stored underground, onshore or offshore, is considered to be a key technology to reach this target. While the technology is seen to hold to great potential, CCS is still in its early stages. Several member states are beginning to employ this technology offshore. Accordingly, more research on the potential (environmental) risks and effects is needed. Financed under “The Ocean of Tomorrow call” of the EU’s 7th Framework Programme for Research, the ECO₂ project examines potential impacts on marine ecosystems from sub-seabed CO₂ storage. Based on a series of investigations of potential techniques, risks, and impacts of offshore CCS, guidelines for best environmental practices for the implementation and management of CCS are being developed in the project.

After the first Briefing Paper *The Scientific Basis of offshore CCS*, this second paper addresses the **potential impacts of CO₂ leakage from sub-surface storage on seabed biology**. The principal focus of this paper is to present the first results from the ongoing research of Work Package 4. Researchers within this Work Package seek to quantify the short- to long-term effects of CO₂ leakage on marine biota and ecosystems, assess the ability of organisms to adapt to CO₂ exposure over a prolonged period, and identify biological indicators which show a strong response to high CO₂ levels.



In the first section, results will be presented which arise from field investigations, mesocosm studies, and laboratory experiments. Within the ECO₂ project, experiments testing the effects of acidification from acute or chronic events are being conducted. In contrast to classic studies on ocean acidification, organisms in the CO₂ leakage studies are exposed to CO₂-levels far higher than the predicted future ocean scenarios. Low pH-levels can impact on organisms either directly through changes in the physiology or even cause the organism's death. The impacts may also come indirectly through changes to the food web and/or ecosystems. Because the survival of a benthic species is not only dependent on the well-being of adults, but on their whole life-cycle, experiments are also being carried out which focus on their larval stages. Tests are conducted on the influence of heavy metal toxicity due to changes in the reactivity of heavy metals under CO₂ leakage. The project further investigates how communities are affected and models are being applied in order to predict ecosystem functioning under different scenarios. The following section looks at case studies which are being performed on natural analogues, in order to assess whether marine organisms are able to adapt and how marine ecosystem functioning might be affected

by CO₂ leakages. Three case study examples are presented here: The first site is Panarea, Italy, in which submarine CO₂ seepage occurs due to the activity of the active volcano Stromboli. The second study location is situated at the Yonaguni Knoll IV, Japan, where liquid CO₂ seeps from active hydrothermal vents in a sedimentary valley. The QICS (Quantifying and Monitoring Potential Ecosystem Impacts of Geological Carbon Storage) experiment off the Scottish coast is also featured. QICS is a unique experiment simulating a leakage event, in order to understand initial ecosystem impacts and their recovery. Finally, the review articles in section three summarize the recent state of knowledge on how CO₂ exposure might impact on marine organisms by synthesising results from the ECO₂ project and ocean acidification studies.

The aim of this paper is to inform stakeholders about the progress in ECO₂. Its purpose is to provide a sound scientific background in order to inform and support the decision-making process for the future application of CCS. To help explain unfamiliar terms an ECO₂ Glossary 'The language of CCS – definitions and explanations' is available at

<http://oceanrep.geomar.de/20194>



Section 1: Impacts of CO₂

Potential impacts of CO₂ leakage from sub - seabed storage sites: Sensitive clams

By Hanna Schade and Frank Melzner, GEOMAR, Germany

In order to understand the sensitivity of benthic communities to CO₂ stress, we carried out a three-month laboratory experiment using sandy bottom communities from the Western Baltic Sea. Sediment samples containing natural meiofauna and microbiota were collected in shallow water (ca. 1-2 m) in Kiel Fjord with the vessel RV *Polarfuchs* (GEOMAR). These sandy sediments are characterized by very high densities of three bivalve (clam) species: *Cerastoderma edule*, *Mya arenaria*, *Macoma balthica* - species that are very common and ecologically important along the coasts of the Baltic and North Sea. Of these three species, *C. edule* (the common cockle) is the most abundant. We hypothesized that it would be particularly sensitive to seawater acidification, as its shell consists of aragonite and lacks a thick organic external cover (periostracum) that could prevent shell dissolution. Previous studies have shown that species with thick periostraca can withstand high levels of acidification.

We incubated the communities for three months in laboratory mesocosms in a climate chamber at a constant temperature in winter 2012 (Fig. 1). Mesocosms were continuously supplied with acidified seawater, thus simulating a plume of CO₂-enriched seawater that moves laterally over

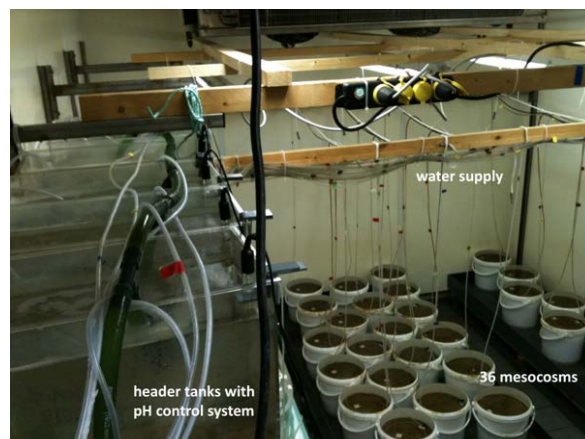


Figure 1: Experimental setup to study infaunal bivalve communities. A total of 36 mesocosms (diameter: 30 cm) supplied with six mixtures of acidified water from header tanks via gravity feed at a rate of 100 ml min⁻¹. Experimental units contained sandy sediment with three species of bivalves, meiofauna and microbiota. Cultured microalgae were supplied via the header tank in order to supply the bivalves with sufficient food. The experiment was run in a GEOMAR climate chamber under winter conditions for 3 months.

the sediment interface. The experiment consisted of six treatment levels ($p\text{CO}_2$: 900-24,000 μatm) that were replicated six times each (Fig. 1, 2D). Each mesocosm contained the same number of similarly sized clams. In order to supply the high metabolic demand of the filter feeding clams, food algae were cultured in parallel (*Rhodomonas* sp.) and continuously supplied to the mesocosms (see Fig. 1).

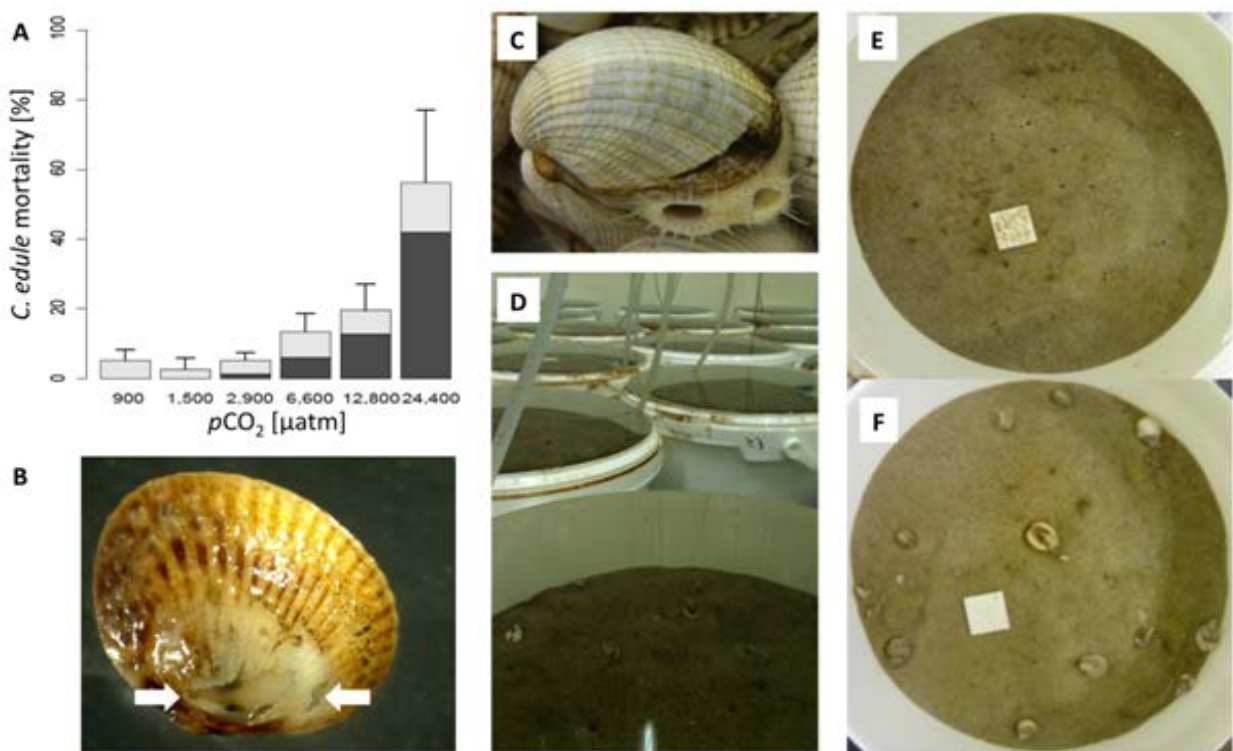


Figure 2: A: cumulative mortality of *C. edule* during the 3-month experimental incubation period, error bars = standard deviation. Dark grey patterning indicates proportion of animals with visible shell dissolution; B: Cockle (ca. 0.5 cm shell length) with severe shell corrosion that penetrates the entire shell (white arrows), 24,400 μatm treatment; C: control cockle (ca. 3 cm shell length) with intact shell, exhalant and inhalant siphos visible; D: mesocosms; E: control mesocosm after 6 weeks (900 μatm , water supply tube removed to enhance photo quality), all cockles buried in the sediment, exhalant and inhalant siphos visible; F: mesocosm after 6 weeks (24,400 μatm , water supply tube removed to enhance photo quality), moribund cockles accumulate on the sediment surface.

We observed a very high mortality rate of the cockle *C. edule*, while the other species survived all treatment levels. Cockle mortality significantly increased at a seawater $p\text{CO}_2$ of 6,600 μatm and beyond (Fig. 2A). At the same time, extensive shell dissolution could be documented (Fig. 2B). Slight external shell corrosion was apparent at 2,900 μatm already, while severe dissolution sites that penetrated the entire shell became apparent at 12,800 and 24,400 μatm (Fig. 2B). Tissue mass (normalized for size) was significantly smaller at the highest CO_2 treatment level, indicating a strongly negative energy balance in cockles exposed to this treatment level. Biochemical stress indices also indicated that *C. edule* is

relatively sensitive to elevated seawater $p\text{CO}_2$. The presence of moribund cockles can potentially be used in video observation techniques to monitor sites of strongly increased habitat $p\text{CO}_2$, which causes this species to leave the sediment and accumulate on the sediment surface (Fig. 2F). Currently, we are analysing microbial community structure and meiofauna composition in samples taken from this experiment in collaboration with colleagues from the MPI Bremen and the University of Ghent. We hypothesize that the strong changes in cockle densities should affect biogeochemical fluxes in the sediment and, in a secondary fashion, microbial community and meiofauna composition.

Potential impacts of CO₂ leakage from sub - seabed storage sites: Larval stages of benthic invertebrates

By Sam Dupont, University of Gothenburg, Sweden

Species sensitivity should be expressed as the success over the whole life-cycle when facing a stress such as CO₂ leakage from sub-seabed storage, including all life-cycle stages. Many benthic marine invertebrates develop by means of a dispersive planktonic larval stage and are characterized by external fertilization, early hatching and a succession of various small, free living larval stages within the water column. These larvae are morphologically and ecologically distinct from the adult until they reach the juvenile stage at metamorphosis. The duration of the planktonic period differs between species and is usually in synchrony with species-specific needs or tolerance (presence of food, optimal abiotic environment, etc.). About 55 to 85% of all benthic invertebrate species

produce long-lived planktotrophic larvae which spend weeks to months in the plankton, 5% produce short-lived planktotrophic larvae (spending hours to days in plankton), and about 10% produce non-feeding lecithotrophic larvae.

Each of these life stages differ in form and function with various degrees of autonomy and

thus have differing sensitivities to environmental stressors. A bottleneck or a dead-end in one life-cycle stage can drive the whole species response. It has been suggested that ion-regulatory ability defines tolerance to elevated CO₂ in marine animals (Melzner et al. 2009). The adults of some marine invertebrates have considerable acid-base regulation capacity and are able to survive in what would appear to be extreme CO₂ conditions, but there are few data on acid-base regulation for larval stages. As a consequence,

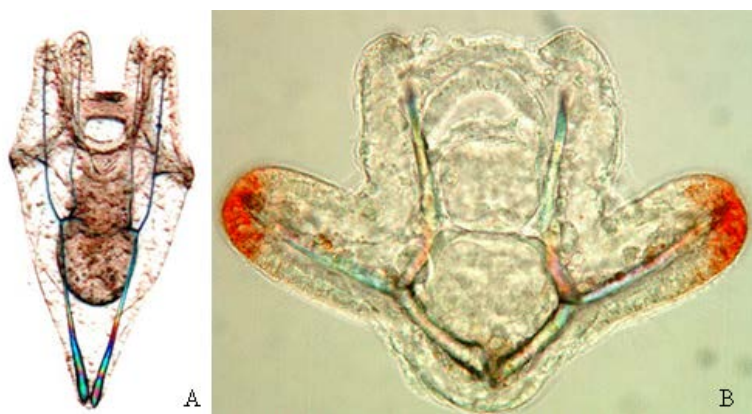


Figure 3: Pluteus larvae from two echinoderm species with different sensitivity to elevated CO₂: the green sea urchin *Strongylocentrotus droebachiensis* (A) being more tolerant than the brittlestar *Amphiura filiformis* (B).

larval stages are often considered as the true life-cycle bottleneck, even for the seemingly CO₂ tolerant organisms.

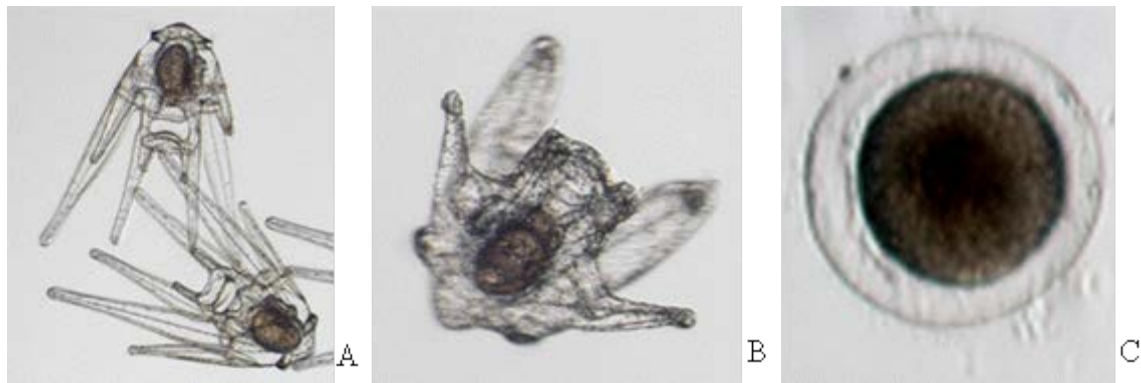


Figure 4: Sea urchin *Strongylocentrotus droebachiensis*: A, 14d normal pluteus larvae raised in $<2500 \mu\text{atm CO}_2$; B, 14d abnormal pluteus larvae raised in $>2500 \mu\text{atm CO}_2$; C, 7d fertilized eggs showing arrested development when kept in $20,000 \mu\text{atm}$.

There is now a large body of evidence documenting the impact of elevated CO_2 on marine invertebrate larvae in the context of ocean acidification. Chronic exposure to near-future CO_2 -driven acidification (e.g. pH between -0.1 and -0.5 units) can induce delays in development, increased mortality and abnormalities (including the potential for local extinction). We recently showed that some larvae are able to cope quickly with extracellular acidosis by full compensation at the intracellular level (pHi). The associated cost of pHi regulation leads to a shift in energy budget with less energy available for growth (Stumpp et al. 2012). However, some species appear to be more resilient than others and here larval development can remain unaffected or even positively affected when exposed to elevated CO_2 (Figure 3; Dupont et al. 2010). Despite some resilience to ocean acidification in adult and larval stages, strong negative carry-over effects between adult, larval and juvenile stages are likely to compromise the sustainability of some urchin populations. It was recently shown that pre-exposure of adults to

elevated CO_2 can translate into decreased fertility and larval/juvenile quality (Dupont et al. 2012).

Experiments on the impact of elevated CO_2 on invertebrate larval development have mostly been performed in the context of ocean acidification (chronic and slowly increasing exposure to elevated CO_2), and may be restrictively informative in the context of CO_2 leakages from sub-seabed storage. Magnitude and extent of such leakages can vary and lead to different CO_2 exposure scenarios (exposure time, intensity). For example, leakages may release liquid CO_2 slowly but continuously, resulting in a relatively constant increase in CO_2 , or may result in a sudden burst of the gas released from a compressed source. Organismal responses are dependent on the species' ability to cope with elevated CO_2 , but also on the intensity of the exposure and its duration. For example, larvae exposed to an acute CO_2 increase of $20000 \mu\text{atm}$ for 24h may respond very differently compared to larvae exposed to a chronic exposure of $1000 \mu\text{atm}$.

New experimental designs are needed if we want to understand the potential impact of CO₂ leakages from sub-seabed storage, including a broader range of tested CO₂ levels and different times of exposure. We investigated these aspects using sea urchin larvae, a classic model in developmental biology. First, we explored the impact of chronic exposure to CO₂ ranging between 400 and 20000 µatm. This

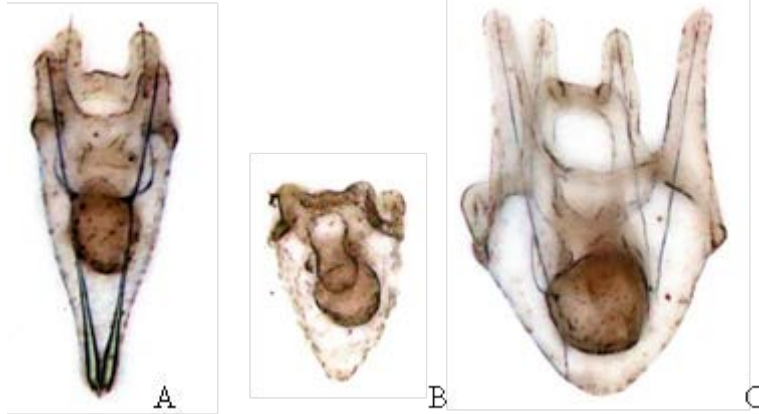


Figure 5: Sea urchin *Strongylocentrotus droebachiensis*: A, 7d sea urchin larvae raised in 400 µatm CO₂; B, same larvae (decalcified) after a 24h exposure to 20000 µatm CO₂; C, same larvae after 7d recovery in 400 µatm CO₂.

covered the range of current and near-future environmental variability (ocean acidification scenario), as well as levels of exposure expected under severe CO₂ leakage from sub-seabed storage. Only sub-lethal effects (e.g. delay in development) were observed within the range of today's CO₂ variability (400-2500 µatm). However, under more severe CO₂ exposures (>2500 µatm), lethal effects were observed (increased mortality and abnormality). Extreme CO₂ conditions (20000 µatm, relevant for extreme sub-seabed storage leakages) led to an arrested development (Figure 4; Dorey et al. in prep).

In another experiment, we investigated the impact of short term (1-7 days) exposure to extreme CO₂ conditions (20000 µatm). Here, even

a short one-day exposure had significant consequences for larvae, leading to a severe decalcification and developmental regression (Figure 5, Dupont et al. in prep). However, when transferred back to control conditions (400 µatm), these larvae were able to recover. This was not without a cost since this short term exposure to elevated CO₂ induced a 7-day delay in the developmental programme. Such a delay may translate into a 3-

times increased mortality due to predation in the plankton.

In conclusion, more experiments with new experimental designs are needed to fully capture the potential impact of different levels of CO₂ exposure from CCS leakage scenarios. Available evidence demonstrates that these leakages can have negative direct (increased mortality, delay in development) and indirect (decreased fecundity and larval quality from adult exposure) impacts on benthic invertebrates.

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Effects of heavy metals under high CO₂ conditions

By Eivind Farnen, Andrew K Sweetman, NIVA Norwegian Institute for Water Research Oslo; Elizabeth A Morgan, National Oceanography Centre Southampton; Dave Lowe, Steve Widdicombe, Plymouth Marine Laboratory

Local ocean acidification may be imposed from seeps from natural and anthropogenic CO₂ reservoirs in the seabed. Whereas such ocean acidification has been shown to affect various compartments of the marine ecosystem directly, it is not only the CO₂ itself that could be harmful to organisms, since acidification may also affect metal toxicity to benthic species. Typically, heavy metal bioavailability could be altered due to change in metal complexation equilibria and by change in concentration due to displacement of metal-particle reaction equilibria in the water column and sediments. Model predictions are difficult due to the high diversity of natural particles and insufficient knowledge of solid-solution interactions. Therefore, an 8-week experiment designed to study the metal uptake and toxicity under high CO₂ conditions has been carried out in the mesocosm at the NIVA marine research station Solbergstrand, Oslofjord, Norway. The aim was to experimentally identify biomarker responses on speed and severity of

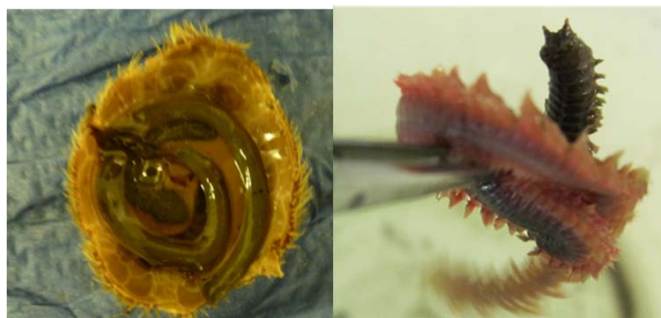


Figure 6: Test organisms. Left: Urchin *Brissopsis lyrifera*, right: ragworm *Nereis virens*.

biological response. Three test species were chosen to represent different classes of organisms associated with the sediments: heart urchin *Brissopsis lyrifera*, ragworm *Nereis virens* and blue mussel *Mytilus edulis* (Fig. 6). Two types of natural sediments were collected from the research vessel of University of Oslo (MF *Trygve Braarud*). Approximately 1000 L of reference sediment was collected in a clean part of the outer Oslofjord, and approx. 1000 L of sediment naturally contaminated by heavy metals was collected near Ulvøya in the inner Oslofjord. The five different CO₂ treatments included in the experiment were 400 µatm (ambient control),

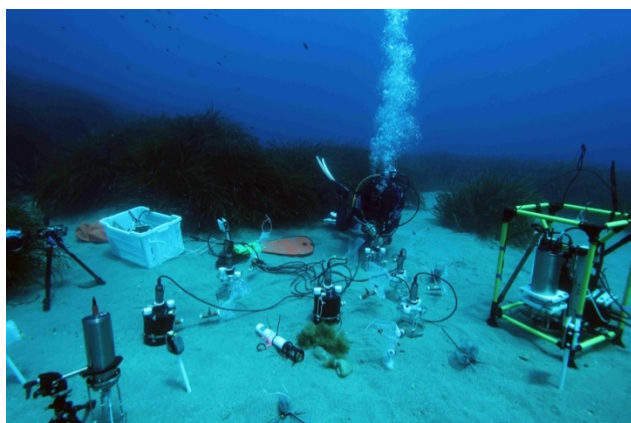
1000, 2000, 5000 and 20000 μatm CO_2 . Pre-exposure control samples (sediment, overlying water, biological tissues) were secured both from the day of organism addition, as well as following the four-week acclimation period. Following this, samples were taken after two and nine days, and further after eight weeks of CO_2 exposure. These sampling intervals were chosen to encompass rapid transcriptomic changes, long term bioaccumulation of metals, and chronic physiological effects measured by neutral red retention (mussel), respiration (urchin) cellular energy allocation (mussel, ragworm, urchin) and histology (mussel, ragworm, urchin).

Section 2: Case Studies

Panarea: A natural laboratory for studying the influence of high CO_2 on marine microorganisms

By Stefanie Meyer, Miriam Weber, Antje Boetius, Max Planck Institute for Marine Microbiology, Germany; Katja Guilini, University of Ghent

Panarea Island (Italy) is long known for the occurrence of submarine gas seeps due to the influence of the active volcano Stromboli (Aliani et al., 2010; Caramanna et al., 2011). The



Occasional mortality was observed during the experiment with *B. lyrifera* as the most sensitive of these test organisms. Preliminary results show that after eight weeks exposure to 20000 μatm 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 a higher mortality rate in test organisms exposed to the heavy metal containing sediment. Data from chemical analysis of the sediments and animal bioaccumulation is now in preparation and will be compiled with biomarker data to further study how increased heavy metal toxicity may impact on organismal health under high CO_2 conditions.

released gas is mainly composed of carbon dioxide. Due to the long-term seepage activity around the island, Panarea represents an excellent natural laboratory for investigating the effects of high CO_2 -low pH conditions on marine organisms and ecosystems (Caramanna et al.,

Figure 7: A comprehensive sampling and measuring campaign was conducted at natural CO_2 seepage sites and a non-gas-impacted background site off Panarea Island (Italy) in summer 2012, including sediment, seawater and seagrass sampling, transplantation experiments as well as deploying various *in situ* instruments. © Hydra

2011) and was chosen as key study site of the EU project ECO₂. Based on an initial sampling campaign in summer 2011, we found distinct differences in bacterial and meiofaunal community structures between CO₂ seepage sites and a non-gas impacted background site. Some organisms seem to avoid the high CO₂ areas, while others may be specifically adapted to the prevailing high CO₂-low pH conditions. This finding intrigued further studies on short- and mid-term acidification

effects on the structure of benthic communities, their functions and ecosystem services. During a recent field trip in summer 2012, the seepage and background sites were revisited for a comprehensive sampling and measuring campaign by several ECO₂ members (MPI, UGent, Hydra, Geomar, OGS, UniRoma1; Fig. 7). Future analyses of sediment samples, seagrass leaves, and biofilms will give insight into the diversity and functions of the residing microbes and meiofauna. Transplantation of natural sediment (Fig. 8a) between one of the seepage sites and the background site, as well as settlement experiments with terracotta tiles, glass slides (Fig. 8b), and seagrass mimics (Fig. 8c) will help to identify the effects of high CO₂-low pH conditions on the structuring and dispersal of bacteria and

meiofauna. *In situ* monitoring of e.g. pCO₂, pH, and O₂ as well as *ex situ* measurements of e.g. dissolved inorganic carbon, nutrients and extracellular enzymatic activities can help to

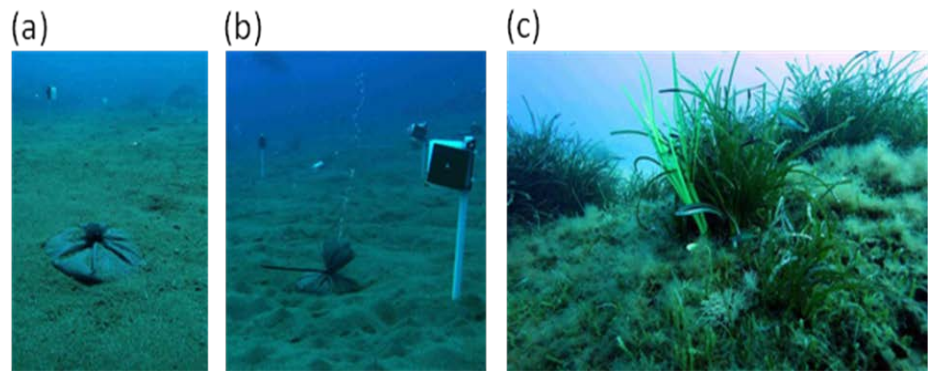


Figure 8: To infer on the short- and mid-term effects of high CO₂-low pH on the structuring and dispersal of bacteria and meiofauna, transplantation experiments were conducted with natural sediments (a), terracotta tiles and glass slides (b) as well as seagrass mimics (c). © Hydra

understand the geochemical dynamics and niche formation processes in the respective habitats. Overall, these results will contribute to a Best Environmental Practices Guide within the EU project ECO₂ by presenting knowledge on assessing the impact of high CO₂-low pH conditions on marine microbes and by offering improved monitoring strategies of affected ecosystems.

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Southern Okinawa Trough – CO₂ venting from hydrothermal sediments as a natural analogue

By Dirk de Beer, Judith Neumann and Antje Boetius, Max-Planck-Institute for Marine Microbiology, Bremen

Among the proposed measures against increasing CO₂ emissions to the atmosphere are mitigation strategies such as the Carbon Dioxide Capture and Storage (CCS) technique, including offshore submarine CO₂ injection into the seabed. A main question for CCS risk assessment concerns the effect of high CO₂ emissions on the functioning of marine ecosystems, for example from subsurface leakage pathways.

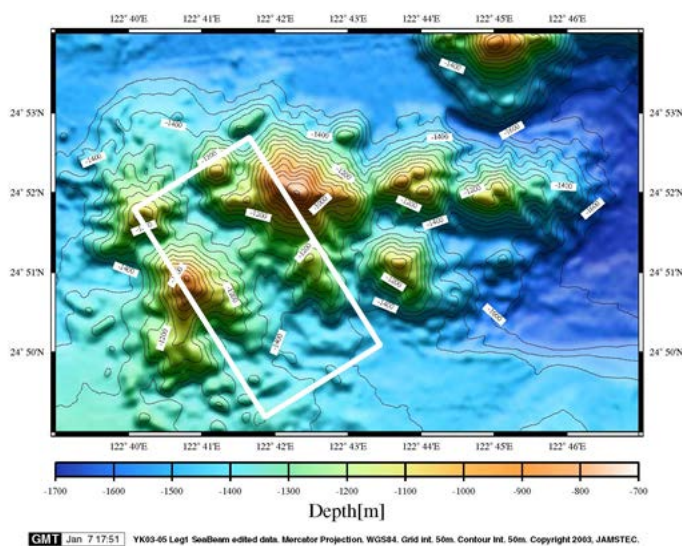


Figure 9: Bathymetric map of the Yonaguni Knoll >1300 m water depth. The CO₂ vented area is indicated with a white box. Yanagawa et al. 2012 ISMEJ doi:10.1038/ismej.2012.124

The Yonaguni Knoll IV (Fig. 9) comprises a sedimentary valley with a string of active hydrothermal vents, and is one of the few sites on Earth known where liquid CO₂ leaks through thick layers of sediments. The site is an ideal natural laboratory to study the effects of CO₂ leakage associated with CCS in the deep-sea

floor. We used samples and data from two previous cruises (2008, RV *Sonne* and 2010 RV *Natshushima*) to study the effects of extremely high CO₂ levels on the faunal and microbial diversity and ecosystem functioning.

A video transect from the reference site to the CO₂ impacted vent area showed that benthic fauna gradually disappeared, including ecologically relevant seafloor dwelling types such as sea cucumbers, polychaetes, burrowing sea anemones as well as starfish. The decline of faunal biomass and diversity was correlated to increasingly high CO₂ and low pH in the bottom water and surface sediments. Only on active chimneys were dense accumulations of chemosynthetic fauna observed, mainly *Bathymodiolus* mussels and large crabs (Fig. 10).

Further investigations showed that faunal diversity was affected mostly at the species composition level by low pH and high CO₂. In contrast, we had expected that microbial life would actually thrive on the reduced compounds in the vent fluids, and potentially show an increase in biomass and diversity towards the CO₂ point sources. It is well documented that prokaryotic organisms adapted to a wide range of extreme conditions, such as low and high pH (<1 and >12), and temperatures up to 121°C. Furthermore, the sediment influenced by CO₂ venting at Yonaguni Knoll showed high concentrations of reduced compounds (e.g. CH₄,

H₂S, H₂, Fe²⁺). These compounds can fuel a large diversity of autotrophic microbial communities, and may favour highly adapted microbial communities at CO₂ leakage sites. Hence, another main question of our studies addressed the combined effects of high CO₂ and potential co-leaking of subsurface substrates on microbial communities.

At Yonaguni Knoll, CO₂ is transported in its supercritical form from hot subsurface strata to the cooler surface seafloor where it liquefies and CO₂ hydrates may form. Accordingly, we observed the emission of liquid CO₂ from the sediments by penetrating the top 20 cm with the coring gear. Thus, liquid CO₂ was present in the sediments below 10 – 20 cm depth. Microprofiles showed that the pH decreased rapidly to 4.5 below 5 cm sediment depth. Indeed, only in the upper sediments high cell densities were observed, and a community indicative for bacterial types that can profit from the chemical energy in the vent fluids (Yanawaga et al. 2012).

But remarkably, microbial activities (AOM and SR) were restricted to the upper 7 cm, and were absent from deeper sediment strata above and

within the liquid CO₂ zone. Laboratory studies showed that the high temperature and low pH alone would not restrict microbial activity (Yanagawa et al., 2012). However, apparently the very high CO₂ concentrations reached at in situ pressure of > 100 bar induced a kinetic inhibition



Figure 10: Vent fauna adapted to high CO₂ leaking from chimneys: Mussels and crustaceans (Source: MARUM, SO196)

of the microorganisms, despite the availability of energy resources. We argue that the extremely high CO₂ concentration of 1.7 M in the deeper sediments uncouples the membrane potential and thus stops cellular energy generation. Life could not adapt to such a stress. Our findings have profound consequences for biogeochemical cycling at CO₂ impacted sites and thus for the ecosystem functioning at natural and man-made CO₂ leakage pathways.

A novel experimental release of CO₂ in the marine environment to aid monitoring and impact assessment: The “QICS” experiment

By Jeremy C Blackford, Plymouth Marine Laboratory, UK

European regulations state the need for a full characterisation of the storage domain, including the natural environment over the geological storage formation. Fulfilment of Article 13 of the EC directive (monitoring) requires the establishment of a framework for future “*detection of leakage*” and “*detection of significant adverse effects for the surrounding environment, human health, or users of the surrounding biosphere*”. Much storage potential for North Western Europe lies under the North Sea. Hence we are challenged to understand both the manifestation and impacts of released CO₂ in the marine environment.

(respectively) the complexity of the natural environment or the initial stages of leakage. Several studies have shown that observed impacts of CO₂ can depend on both direct effects and indirect effects such as release from predation or competition. The initial evolution of a complex community in response to high CO₂ is generally unknown. Detecting the early stages of leakage is also of importance for regulation and remediation. It is an open question as to how CO₂ would present in the biotic zone, whether dissolved in pore water or as gas bubbles and whether this would change as leakage physically modified the geological and sediment structures.

In response, the UK Research Councils have funded a project, QICS (Quantifying and Monitoring Potential Ecosystem Impacts of Geological Carbon Storage) that has developed a coupled experimental and modelling approach based around a unique real world release of CO₂ beneath the sea-floor (Fig. 11). The project aims to improve understanding of the environmental impact

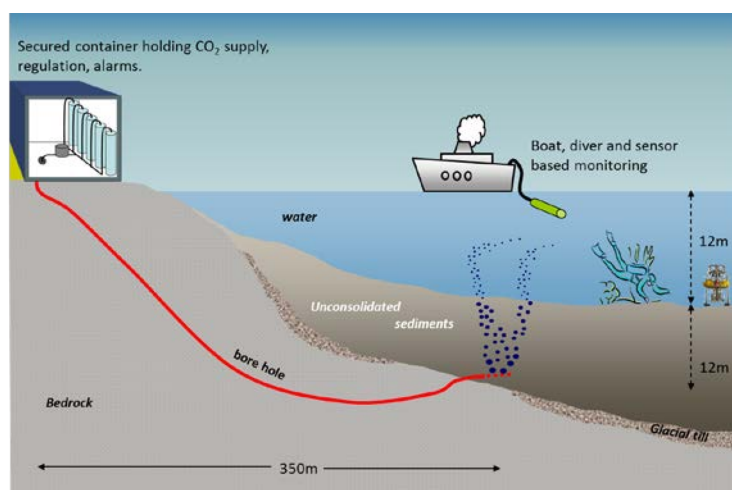


Figure 11: Schematic of the QICS experiment

Current research into possible environmental impacts and monitoring of the geological storage of CO₂ is largely based on laboratory and analogue site studies. Whilst these are of enormous use, they do not replicate

potential should a leak occur from a future carbon dioxide capture and storage (CCS) system. The plan was to mimic, on a very small scale, a leak event and monitor how the ecosystem is impacted and recovers. The project

involves a consortium of UK institutions working in partnership with a consortium from Japan.

The experimental plan involved drilling a narrow borehole from land approximately 350m in the horizontal and terminating the borehole in unconsolidated sediments 10 m below the sea floor which in turn is in 12-15 m of water in a semi-enclosed bay. Significant instrumentation was placed on the sea floor and a variety of sediment and water samples taken. The release was carried out in May-June 2012, continuing for 37 days with a cumulative release of 4.2 tons of CO₂ (Fig. 12). Monitoring continued for several months after the gas release to assess the rate of recovery.

The project has presented several engineering and regulatory challenges, which have required careful planning and communication strategies. Drilling boreholes using directional drilling is an established technology, however terminating the bore underground and designing the bore in such a way to minimise the risk of CO₂ escaping backwards or the drilling disturbing the overlying sediments was challenging. The risk in this respect was been minimised by extensive surveying of the geological strata, by lining the bore hole with welded stainless steel pipe and sealing the exterior with concrete. The end 5 m of the pipe was perforated to allow a flow of relatively fine bubbles over a wide area with the hope of

achieving an initially diffuse flow of CO₂ into the sediments. All but the last 10 m of the bore hole was drilled through solid rock to maximise stability and minimise disturbance.

Any experiment that seeks to deliberately 'pollute' even a minute part of the natural environment is inherently controversial, as is CCS itself in some circles. In order to avoid problems, the project has employed a highly structured communications strategy. Firstly, the project has underlined that it is neither an advocate nor critic of CCS, but has a specific role to generate knowledge that will be available to a wide range of stakeholders and facilitate the informed

discussion and regulation of CCS. To that end, the project has assembled a stakeholder group with representatives from industry, regulators, NGOs and the public.



Figure 12: Photograph of seafloor bubble streams and instrumentation (copyright Henrik Stahl, SAMS)

Secondly, the project has taken care to both

explain the experiment and obtain consent from a wide range of relevant parties, namely the owners of the land site and marine sediments, environmental protection agencies and local planning authorities. Thirdly, local populations who might interact with the experiment have been consulted (rather than told) about the experiment, and local users including both aquaculture and leisure have been involved to ensure minimal disruption to normal activities. In communicating with concerned parties it has

been useful to contrast the amount of CO₂ planned for release (80-800 kg per day) and the expected area of disruption with typical CO₂ production rates of for example households and the areal disturbance caused by typical trawling activities.

Results from the QICS experiment are beginning to emerge and show some significant and unforeseen outcomes. However, these are as yet preliminary and unpublished and will be reported in due course. The possibility of a repeat experiment is being discussed, with inputs from international research groups welcome.

Section 3: Lessons learnt

Impacts of elevated CO₂ on the physiology of marine organism and implications for biodiversity

By Steve Widdicombe, Plymouth Marine Laboratory, UK

If leakage were to occur from sub-seabed storage sites, then the escaping CO₂ would react with the seawater, exposing the flora and fauna living on or near to the seafloor to unnaturally low levels of pH (known as seawater acidification). The potential physiological impacts of this acidification on the health and function of marine species and communities have been intensively studied within ECO₂ (see accompanying sections by Morgan & Hauton; Schade & Melzner; Dupont) and have been detailed in a number of recent reviews (Seibel and Walsh 2001, 2003; Pörtner et al. 2004, 2005; Fabry et al. 2008; Widdicombe and Spicer 2008). In summary, when marine organisms are exposed to low pH seawater the primary physiological effect is a decrease in the pH or an “acidosis” of the extracellular body fluids such as blood, haemolymph, or coelomic fluid. In some species

this extracellular acidosis is fully compensated for as levels of extracellular bicarbonate are increased by either active ion transport processes in the gills or through passive dissolution of a calcium carbonate shell or carapace (see Widdicombe and Spicer 2008 and refs therein).

“So after exposure to extreme seawater acidification the remaining marine communities would be made up of species from a limited number of tolerant taxonomic groups...”

However, in other species from a variety of different taxa, such as the mussel *Mytilus edulis* (Michaelidis et al. 2005), the crabs *Callinectes sapidus* (Wood and Cameron 1985) and *Chionoecetes tanneri* (Pane and Barry 2007) and the sea urchin *Psammechinus miliaris* (Miles et al. 2007) studies have reported only partial, or no, compensation in the extracellular acid-base balance. Clearly, if some species are physiologically better equipped to cope with elevated levels of CO₂ than others, the potential for species extinctions and biodiversity loss exists. At the phylum level, early

evidence would suggest that echinoderms will be more vulnerable than molluscs, then crustaceans, with annelids showing the greatest tolerance to hypercapnia and acidification (Widdicombe et al. 2009, current experiments within WP4). This will certainly reduce both taxonomic richness and species diversity and could lead to a reduction in some of the key ecosystem functions performed by seabed ecosystems (e.g. nutrient cycling, production, remediation of waste). However, despite having identified these coarse descriptors of potential vulnerability based on an organism's taxonomy, even within the same taxonomic groups, variability in tolerance can exist between even closely related species with this variability seemingly linked to key elements of an organism's lifestyle. Organisms that already exist in habitats regularly exposed to highly variable levels of CO₂ may be more likely to possess the physiological mechanisms necessary to cope with rapid changes in environmental conditions than organisms from areas with more stable conditions. It seems clear that the likelihood that a species will be lost from an area as a result of CO₂ leakage will be determined by both its phylogeny and its ecology. Consequently, the potential exists for severe leakage to reduce the total number of marine species, the taxonomic richness of the communities which remain and the diversity of functions that these communities can perform.

"...If organisms have access to sufficient resources then they will to some extent be able to fund the physiological mechanisms needed to survive short-term exposure to high levels of CO₂...."

Dealing with environmental stress incurs an energetic cost

It was initially assumed that all organisms which rely heavily on calcification would be negatively affected by seawater acidification primarily due to the reduction in the saturation states of calcite or aragonite (Orr et al. 2005; Doney 2006; Fabry et al. 2008; Schiermeier 2011). However, recent research (including that within ECO₂) has shown that some animals can upregulate key physiological processes and thereby cope with exposure to high CO₂ in the short term (Fabry et al. 2008; Hendriks et al. 2010; Pistevos et al 2011). As an example, blue mussels (*Mytilus edulis*) from the North Sea showed a 25 % drop in calcification rates after exposure to high atmospheric CO₂, whereas a Baltic population of the same mussel did not experience the same problems (Thomsen et al. 2010; Schiermeier 2011). One emerging explanation is that the response of individuals to elevated CO₂ is governed by the energy they have available to fuel the physiological responses needed to maintain acid-base balance and physiological function. If you consider that organisms will need to actively elevate the pH (by removal of H⁺) around the sites at which biogenic calcification takes place, it is easy to see that, if surrounded by seawater of reduced pH, this process will become energetically more demanding. Consequently, to

maintain calcifying organisms will have to allocate more energy to this ion removal process. If energy (from food or from photosynthesis) is in short supply this will mean that organisms will need to make a physiological “choice”; do they maintain calcification at the expense of other important physiological processes (such as growth, reproduction, immune function) or do they respond with reduced calcification? Either way these organisms will be ecologically less fit than they would have been in a higher pH environment. This finding has significant implications for the survival of organisms during a CO₂ leakage event. If organisms have access to sufficient resources then they will to some extent be able to fund the physiological mechanisms needed to survive short-term exposure to high levels of CO₂ and reduced seawater pH. This means that organisms and communities could potentially be better able to survive short-term leaks than previously thought. However if leakage were to persist the increased energetic demand associated with living in a high CO₂ environment would inevitably lead to reduced growth, lower reproductive output and eventually death.

Physiological responses can underpin ecological change

In addition to the direct impacts of leakage on organism health and survival detailed above, long-term exposure to elevated levels of CO₂ due to a prolonged release (months to years) could also promote significant ecosystem changes and biodiversity loss through indirect impacts. In many marine systems the activities of individual

species can be instrumental in maintaining high levels of biodiversity in the associated fauna (see review by Widdicombe and Austen 2005). This biological control can be exerted through reducing competitive exclusion *via* density-dependent predation or consumption i.e. “keystone species” (Paine 1966), or through habitat modification and creation of environmental heterogeneity *via* processes such as reef building and bioturbation, i.e. “ecosystem engineering” (Jones et al. 1994; Lawton 1994). Biogenic habitats such as coral reefs, mearl beds and mussel banks are considered to be particularly important as “hot spots” for biodiversity. However, many of these important habitats rely heavily on the health and survival of heavily calcifying species which are considered to be at high risk from seawater acidification (Raven et al. 2005). It should also be considered that a reduction in the top-down biological control of biodiversity could come about not just through the complete loss of important species but also through changes in the rates of ecologically important activities, such as growth, feeding, bioturbation and burrow irrigation.

The recovery of biodiversity after CO₂ leakage

Evidence from the current ECO₂ research based at natural analogues (see section by Meyer et al.)

Areas with strong impact of leakage:

- Near to calcareous, biogenic habitats with long-lived, habitat-forming species
- Nursery ground with consequences for recruitment

and from a recently conducted controlled release experiment conducted by the QICS consortium in the UK (see section by Blackford) has suggested that, whilst the chemical and biological impacts of CO₂ leakage could be considerable within the immediate vicinity of the leak, these impacts will dissipate rapidly as you move away from the release point. In fact, for the majority of leakage scenarios it could be difficult to detect any impact of leakage on the seabed biota unless you are within a few 10s of metres of the leak itself. Consequently, once a leak has stopped and the sediment environment has returned to a natural state (a process that is likely to take just a few weeks) the recovery of the seabed communities could be fairly rapid. This recovery will initially be driven by lateral migration of individuals from the surrounding unaffected areas, ultimately assisted by seasonal inputs of larvae from the plankton. It is therefore expected that if the footprint of the leakage is less than 50 m across any significant impact of leakage of benthic community structure and diversity may well have disappeared within a year.

Whilst rapid recovery from CO₂ leakage may be the case for the majority of situations, it should be remembered that there are habitats and situations within which the impacts of leakage could be much longer lasting or more widely felt. Firstly, if leakage were to occur near to a calcareous, biogenic habitat, such as a mearl bed, a cold water coral reef or a bed of mussels, the loss of these long-lived, habitat-forming species would have a major impact on the biodiversity of

the area as a whole. In this case, it could take many years or even decades for these species to return and for the habitats to fully recover. Secondly, if leakage were to occur within an area that acted as an important nursery ground the consequences for recruitment and population health for key, sometimes commercially important species could be significant and wide reaching.

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Potential impacts of CO₂ leakage from sub - seabed storage sites: On the growth and health of commercial benthic species

By Elizabeth A Morgan and Chris Hauton, National Oceanography Centre Southampton University

Acute or chronic acidification events associated with leakage scenarios from carbon dioxide capture and storage (CCS) reservoirs can have direct or indirect impacts on the health and physiology of commercially important benthic species. Direct impacts to organisms occur through perturbations to physiology, including maintenance of acid base balance, and through the energetically demanding induction of homeostatic and adaptive mechanisms. Indirect effects of acidification events occur via impacts to the structure of benthic food webs and predator-prey relationships. In order to usefully predict outcomes from potential leakage scenarios on shelf-sea commercial fisheries, both indirect and direct impacts must be considered.

However, a significant challenge to making useful predictions is the degree of uncertainty in constraining the boundaries of any predicted

leakage scenario. The extent of local acidification resulting from a CO₂ leak - as low as < pH 4 has been predicted at CO₂ injection sites - (Barry *et al.*, 2004) and the likely duration of exposure that resident organisms experience remains uncertain. As a consequence, investigations have tended to include a range of pH extremes in addition to differing durations of exposure, rendering direct comparison between species in different experiments difficult. Furthermore, because of the current limited number of dedicated CO₂ leak experiments, current predictions of the potential impacts must be based on 'extreme' levels/treatments used by researchers investigating the impacts of atmospherically-derived ocean acidification (OA).

Direct impacts to commercial species vary as a function of the biological species in question and its life stage. Determining the full extent of



potential impacts on organism growth and health requires investigation of the physiological, immunological and behavioural scope for adaptation throughout the life cycle.

Mobile benthic species have conventionally been considered to be at reduced risk from the impacts of pollutant exposure as they can exhibit avoidance behaviours in response to challenging environments. However, two recent reports have suggested that even short-term

exposures of fish to high $p\text{CO}_2$, which are relevant to potential CO_2 leakage scenarios, might have a detrimental effect on health and growth. Baumann *et al.* (2012) demonstrated that

exposure of embryos of silversides *Menidia beryllina* to 1000 μatm CO_2 for a period of one week post-hatch reduced survival by 74% and caused an 18% reduction in the overall length attained by survivors. Frommel *et al.* (2012) reared Atlantic cod *Gadus morhua* larvae at $\sim 4200 \mu\text{atm}$ CO_2 for a period of two months and identified that, whilst the growth of fish appeared to increase as a function of lipid accumulation, development of organs was decoupled and lethal tissue damage occurred - particularly in liver, pancreas, kidney, eye and the gut. As both teams identified, the survival of larvae and juvenile stages is fundamental to recruitment success and persistence of future generations. The fact that early developmental stages have been shown to be susceptible to even short term exposures raises concern, especially for future stocks of

“...Direct behavioural impacts to commercially-important benthic crustaceans from exposure to elevated $p\text{CO}_2$ have also been reported...”

commercially important fish, such as plaice *Pleuronectes platessa* and turbot *Scophthalmus maximus* that are primarily benthic in habit.

Reports of direct impacts of CO_2 leaks to commercially important benthic crustaceans are also limited in the literature and, here again, inferences must be made from the impacts identified from experiments on extreme atmospherically-derived OA scenarios. As has

been reviewed by Whiteley (2011), the larval stages of many crustaceans demonstrate some resistance to high $p\text{CO}_2$ exposures. The planktonic zoeae of the European lobster *Homarus gammarus* are unmineralized and the benthic juveniles exhibit minimal calcification – this physiological adaptation potentially offers some protection from the impacts of elevated $p\text{CO}_2$ on these key stages that are fundamental to recruitment. Nevertheless, reports have been made of reduced growth rates in adult shrimp and prawns (*Palaemon pacificus*, *Penaeus occidentalis* and *Penaeus mondon*) when reared in the pH range of 7.6 or less (reviewed by Whiteley, 2011). In general terms it appears that decapods with high activity are better equipped to deal with perturbations in haemolymph acid base balance resulting from exposure to high environmental $p\text{CO}_2$. Shallow water and active species, e.g. the swimming crab *Necora puber*, have higher levels of haemocyanin and therefore have a higher oxygen carrying capacity and capacity for non-bicarbonate buffering than slow-



moving species such as the spider crab *Maja squinado* (Watt *et al.*, 1999). By contrast, decapods living in stable marine environments characterised by limited variation in pH, pO_2 and pCO_2 , typical of the deep sea, appear less tolerant of perturbations in acid base balance (Pane and Barry, 2007). For example, the deep-sea crab *Chionoecetes tanneri* is unable to compensate hypercapnic stress, unlike its shallow-water counterpart, the Dungeness crab *Cancer magister* (Pane and Barry, 2007). Briffa *et al.* (2012) have extensively reviewed the experimental evidence that elevated pCO_2 has impacts on the ability of crustaceans to respond to semiochemicals in the marine environment; to locate shelter and prey items and to avoid predators. It has been shown that the normal detection of food odours hermit crabs *Pagurus bernhardus* were impacted by acute exposure to high pCO_2 environments ($\sim 12,000 \mu\text{atm}$; $\text{pH}_{\text{NBS}} 6.80$) (de la Haye *et al.* 2012).

Species- and population-level variability in responses to elevated pCO_2 has been identified in bivalves. Whilst Gazeau *et al.* (2007) have recorded impacts in mussels *Mytilus edulis* and oysters *Crassostrea gigas* at pCO_2 levels above $1800 \mu\text{atm}$, Thomsen *et al.* (2010) have recorded recruiting populations of mussels in the Kiel Fjord where seawater pCO_2 peaks at $\sim 2300 \mu\text{atm}$ ($\text{pH}_{\text{NBS}} < 7.5$) in the summer and autumn. Thomsen *et al.* (2010) further reported that these mussels maintain somatic and shell growth to $\sim 1400 \mu\text{atm}$ when food is plentiful and that mussel recruitment takes place in waters of up to $\sim 1000 \mu\text{atm}$. However, adaptation to extreme

environment is achieved at some physiological costs. Metabolic and growth rates can be reduced to compensate for the hypercapnia, increasing the organism's vulnerability to predation (Tunnicliffe *et al.*, 2009). In the mussel *Mytilus galloprovincialis* exposed to seawater at pH 7.3; bicarbonate buffering occurred through shell dissolution, protecting the soft tissues. However, this resulted in reduced metabolic rates that lowered oxygen consumption and growth rates (Michaelidis *et al.*, 2005). Shell dissolution in the mussel *Mytilus edulis* has also been seen when exposed to sea water at pH 7.8, 7.6 and 6.5 in a 60-day exposure experiment. In contrast to the outcome in fish, tissue structures remained intact. Longer term exposures in *M. edulis* were found to reduce growth rate and immune performance of the commercially important species (Beesley *et al.*, 2008).

Deep sea mussels living in chronically acidified environments have been shown to exhibit long-term adaptation to such extremes using similar physiological mechanisms. A dense mussel bed (*Bathymodiolus brevior*) has been identified at the Eifuku volcano on the Mariana Arc, where liquid CO_2 creates acidified sea water between pH 5.36 – 7.29 (Tunnicliffe *et al.*, 2009). When exposed to hypercapnia, the mussels were shown to dissolve their shell to increase tissue bicarbonate, resulting in metabolic depression and decreased growth rates (Tunnicliffe *et al.*, 2009); observations that echo what has been reported for *Mytilus galloprovincialis*, at least (Kurihara *et al.*, 2009). Inhabiting such a corrosive



environment has clearly come at cost, as the mussels exhibited thinner shells as a result of reduced calcium carbonate precipitation leaving them vulnerable to predation. Survival in this instance was possible due to the absence of crabs that would normally prey on the mussels.

In contrast to crustaceans, the larvae of molluscs undergo biomineralization, depositing polymorphs of calcium carbonate from an early stage. Experiments on larval stages of bivalves have reported reduced shell synthesis in *M. galloprovincialis* in sea water at pH 7.4, indicating that acidification interferes with early development (Kurihara *et al.*, 2009) in this species and could potentially impact on future population recruitment.

As argued, the direct impacts of chronic or acute CO₂ leaks on commercially-important species cannot be considered in isolation. Indirect effects, occurring via impacts to benthic infaunal groups must also be taken into consideration

(Barry *et al.*, 2004). Whilst larger mobile benthic organisms may move away from an environmental stressor, less mobile and infaunal species cannot readily escape exposure. This will, in turn, cause impacts to the stability of benthic food webs that support commercially-important species (Hale *et al.*, 2011; Thistle *et al.*, 2007). Supporting this contention are the observations of Thomsen *et al.* (2010) on the Kiel Bight

„...many commercially-important benthic species and populations have some compensatory capacity that might furnish protection in high pCO₂ environments, these compensatory mechanisms usually come at some energetic cost to health and growth.”

mussels. In their work, these authors identify the importance of plentiful food supply to maintain somatic and shell growth in more extreme environments. The indirect effects of any leakage scenario on predator-prey dynamics will also have longer-term or far-field indirect impacts to commercially-important species.

The multiple effects of acidification from CO₂ leakage on the real ecological communities clearly requires further consideration (Tamburri *et al.*, 2000). As such, the role of surficial and burrowing echinoderms in bioturbation and nutrient cycling cannot be ignored; these species are pivotal ecosystem engineers which support ecologically diverse soft sediment communities that underpin the basis of a resilient food web. To date, few studies have investigated the impact of chronic acidification that would result from CO₂ leakage on echinoderms, and most of these studies have focused on the effect of atmospherically-driven ocean acidification

(Schram *et al.*, 2011; Stumpp *et al.*, 2011). Echinoderms, including the economically important *S. droebachiensis* that are cultivated for their roe, have high magnesium calcite tests making them particularly vulnerable to chronic acidification (Dupont and Thorndyke, 2012; Schram *et al.*, 2011). Similar to in mollusc shells, test dissolution has been reported to permit bicarbonate buffering of the coelomic fluid (Miles *et al.*, 2007). Short-term investigations with the urchin *Paracentrotus*



lividus have demonstrated that adults were tolerant of pH as low as 7.4, (Catarino *et al.*, 2012). At pH 6.63, significant mortality was observed in the intertidal purple tipped urchin *Psammechinus miliaris*, rising to 100% mortality at pH 6.16.

The compensatory capacity of epifaunal urchins to environmental acidification appears to be dependent upon the timescale of exposure. Time for acclimation to high $p\text{CO}_2$ has been found to range from 24 to 48 hours in urchins *Strongylocentrotus droebachiensis* (Dupont and Thorndyke, 2012). These data indicate that urchins are better adapted to compensating hypercapnia than seastars, another important group of ecosystem engineers in the marine environment. Indeed, acid base compensation was absent in common sea star *Asterias rubens* (Appelhans *et al.*, 2012; McElroy *et al.*, 2012) and immune suppression has been observed in this species when exposed to seawater at pH 7.70, compromising its resistance to pathogens (Hernroth *et al.*, 2011). Hypercapnia can exacerbate hypoxia, resulting in respiratory failures in sensitive organisms and this has been identified in the seastar *Parvulastra exigua*, resulting in metabolic depression. More widely, it has been predicted that reductions in sea water pH will have increasingly negative impacts on the immune response of echinoderms during chronic acidification events (Appelhans *et al.*, 2012).

In conclusion, predicting the biological outcome of CO_2 leakage scenarios is currently challenging. Difficulties stem from uncertainty in constraining

the physicochemical limits of any $p\text{CO}_2$ plume and the complex and differing biology of the species involved. Whilst it is clear that many commercially-important benthic species and populations have some compensatory capacity that might furnish protection in high $p\text{CO}_2$ environments, these compensatory mechanisms usually come at some energetic cost to health and growth. Whether these costs ultimately lead to mortality and population decline depend on the extent (in time and space) and $p\text{CO}_2$ saturation of the leakage plume. Time of year may create additional complexity in prediction, if the plume interacts with reproductively active adults or larval life stages. Finally, comprehensive and robust predictions of the outcomes of chronic long-duration leaks must take wider consideration of non-commercial species that have key roles in the maintenance of ecosystem diversity and stability; commercial species cannot be studied or protected in isolation.

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ECO₂ Notes ...*... upcoming research expeditions in 2013*

For the second year of the project (2013), 4 research cruises are scheduled and one is planned for summer. During 2012 we conducted the core part of the project's expeditions; in total 11 were conducted, from which the majority was targeting the area at and around the CO₂ storage site Sleipner (4 cruises) and the natural CO₂ seepage site Panarea (4 cruises). As shown in the table below these two sites will as well be the main focus of the 2013 cruises.

Time	Cruise Name	Study Site
22 March – 08 April 2013	RV Alkor AL412	Sleipner / North Sea
20 – 30 May 2013	Small Boat	Panarea
02 – 14 June 2013	Small Boat	Panarea
08 – 21 July 2013	RV Helmer Hanssen	Snøhvit
not yet scheduled	G.O. Sars	Sleipner / North Sea

*... upcoming project meetings in 2013***Post cruise meeting** "Sleipner cruises 2011/12", 06 – 07 March 2013 in Brussels, Belgium

During this meeting the results of the 2011 and 2012 research expeditions will be discussed and interpreted cross-disciplinary. Furthermore, during the meeting we will develop a set of monitoring advises based on the work carried out so far and work out some still open questions regarding the Sleipner area that should be solved during the upcoming cruises in 2013.

International Workshop on CO₂ leakage effects from natural analogues, 13 May in Bergen, Norway**2nd annual ECO₂ Meeting**, 13 – 16 May 2013 in Bergen, Norway

The meeting will bring all 27 project partners and the members of the external advisory boards (Scientific Advisory Board and Stakeholder Dialogue Board) together.

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