ECO_2 project number: 265847

Deliverable Number D4.4: WP4 results summary report relevant for Environmental Best Practice; WP4; lead beneficiary: Plymouth Marine Laboratory

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WP4 result summary report relevant for "Environmental Best Practice"

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Abstract

This report presents a distillation of the main findings from ECO2 WP4, together with information available from other EU and Nationally funded projects, presented within and specifically for the context of Environmental Best Practice. The information and key messages contained within this deliverable (D4.4) will be directly applied to the project wide "Guidance on Environmental Best Practice" and will form the basis of Chapter 6 "Assessing biological impact of CO_2 leakage". There were 8 key findings that came from the ECO2 research conducted with WP4:

- Exposure to elevated levels of CO_2 has a negative impact on marine organisms
- There is a wide range of CO_2 sensitivities across different marine taxa and groups
- Care must be taken when predicting species specific response and sensitivity to CO_2 for Environmental Risk Assessments
- Exposure to elevated levels of CO_2 has a negative impact on marine communities, biodiversity and ecosystem processes / functions
- The leakage / release of formation water can have a negative impact on marine organisms
- Other environmental factors could exacerbate or ameliorate the impact of CCS leakage
- Some biological responses may be employed in a programme of Environmental Monitoring
- Collecting spatially and temporally referenced biological data is important for creating effective Baseline Surveys

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Introduction

This report will outline the current knowledge regarding the potential biological impact of leakage from CCS on marine organisms, communities and ecosystem processes or functions. Significant information has come from the experiments conducted in ECO2 but this chapter has also drawn information from other EC and national funded projects such as RISCS, QICS, and EPOCA. Carbon dioxide (CO_2) leakage from CCS will have two forms in the marine environment: in the active zone of leakage, CO_2 will pass up through the sediments initially impacting the sediment pore waters from below, whilst above and away from the leakage zone a plume of CO_2-enriched water will flow out from the centre of the leak active release zone impacting the water column and sediment surface. Due to the nature of the experiments conducted, this chapter primarily deals with the impacts of the CO_2 rich plume, although some of the sections are relevant to the former case as well. In addition, the focus of ECO2, as well as the majority of previous CCS studies, has been on assessing the potential impacts to the seabed ecosystem. It is assumed that the risk of potential significant impacts on planktonic organisms to be less than that for benthic organism. The biological studies in ECO2 used laboratory experiments, mesocosm studies, field observations in areas of naturally high CO_2 (primarily Panarea, Italy) and computer models.

Objectives

The objectives of this chapter are to:

- Describe the potential biological impacts of CCS leakage on marine ecosystems
- Give guidance for the collection of biological data to support an effective environmental baseline survey
- Assess the practicality of biological responses to monitor for CCS leakage
- Provide biological information to support Environmental Risk Assessment activities

Major Findings

• Exposure to elevated levels of CO_2 has a negative impact on marine organisms

As has been described in previous chapters, if leakage were to occur from sub-seabed storage sites, then the escaping CO_2 would react firstly with the sediment porewater and then, if the leakage was more severe, with the overlying seawater above the sediment surface. This reaction would change the carbonate chemistry of these fluids; a chemical effect known as seawater acidification (see previous chapters). This in turn would expose the flora and fauna living within, on or near to the seafloor to unnaturally high levels of CO_2 and low levels of pH and carbonate ions. The potential physiological impacts of this acidification on the health, behaviour, function and ultimate survival of marine species and communities have been intensively studied within several previous research projects (including ECO2) and have been detailed in a number of previous reviews (Seibel and Walsh 2001, 2003; Pörtner et al. 2004, 2005; Fabry et al. 2008; Widdicombe and Spicer 2008; Kroeker et al., 2013). 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 by two mechanisms. The concentration of extracellular bicarbonate can be 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 references therein). However, in other species from a variety of different taxa, such as mussels (Michaelidis et al. 2005), crabs (Wood and Cameron 1985; Pane and Barry 2007) and sea urchins (Miles et al. 2007) studies have reported only partial, or no, compensation in the extracellular acid-base balance. In these instances the uncompensated acidosis can lead to more or less severe metabolic depression in the affected organism (Pörtner, 2008) in turn having a negative impact on that individual's condition, and therefore its contribution to the ecosystem.

Perturbations in an organism's acid base physiology represent one potential impact of elevated CO_2 on marine benthic species. Species with calcified external structures are at risk of dissolution in response to seawater acidification. Seawater acidification increases the concentration of H^+ ions in solution, a process which reduces the pH of the external environment. Through a process of bicarbonate buffering these H^+ ions combine with carbonate ions in solution to form bicarbonate (HCO_3^- ions). This reaction limits the concentration of H^+ ions in solution and so buffers the reduction in system pH. The buffering capacity of carbonate sediments can be substantial, limiting the net change in ecosystem pH in response to limited CO_2 release (Lichtschlag et al., in press). This sediment pore water buffering may limit the magnitude of impacts to benthic infauna. However, in non-carbonate sediments or for large CO_2 releases the buffering capacity of the sediments might be exceeded. In these situations biogenic carbonate structures (bivalve shells and urchin tests) will undergo dissolution to liberate aqueous carbonate ions. The dissolution of biogenic calcified structures has been widely reported (Gazeau et al., 2007; Gazeau, 2008, Byrne et al., 2014) with effects generally more pronounced in juvenile and larval stages (Talmage et al., 2009; Sheppard Brennand et al., 2010, Stumpp et al., 2012, Long et al., 2013, Hu et al., 2014,). However, these impacts are not universal, and notable exceptions (normal calcification, hypercalcification) have been reported (e.g. Wood et al., 2008; Miller et al., 2009; Martin et al., 2011, Dorey et al., 2013), especially in situations in which the exposed shellfish are not resource limited (Thomsen et al., 2013). Ultimately, the variability in response of closely related species and individuals precludes the formation of general predictions of likely *in situ* impact. As such, it is currently necessary to adopt a precautionary approach to predicting the direction and magnitude of calcification responses to limited CO_2 release.

In extreme cases of CCS leakage, severe acidification will result in most organisms being killed. However, this will not be the case for every leak scenario as many marine species, even some heavily calcified taxa, can tolerate shorter periods of more moderate acidification. This is because that, unlike other potentially toxic substances, CO_2 is a naturally occurring and fluctuating compound in the marine environment. As a result of millions of years of exposure, marine creatures have incorporated this CO_2, along with other elements of carbonate chemistry, into many of their routine physiological processes. So whilst this means that large changes in seawater carbonate chemistry can potentially affect many aspects of an organism's physiology, there is also the potential for organisms to temporarily alter or adjust their physiology to cope with these chemical changes. So in addition to the process of extracellular buffering described previously, many species have been seen to change their respiration rates, their activity levels and their reproductive outputs when exposed to high CO_2 (Queirós et al. 2014). This response, known as physiological plasticity, affords some protection to organisms from rapid changes in their environment and can provide temporary protection against moderate acidification.

Plasticity, however, does not offer permanent protection for any organism against CCS leakage. This is because an organism's ability to express plastic responses is to a large extent governed by the energy it has available (Thomsen et al., 2013). To maintain calcification rates under low pH, low carbonate saturation state conditions, some organisms can temporarily reallocate more energy to this process and use less energy on other processes such as growth, locomotion or development of reproductive tissues. In the short term this can be an effective strategy to deal with an acidification shock. However, if leakage were to persist the increased energetic demand associated with living in a high CO_2 environment would inevitably lead to reduced growth, lower reproductive output and, eventually, death. The environmental consequences of CO_2 leakage therefore depend on both the severity and longevity of the leak. This means that even if a leak is fairly small, if it were to continue for many years it could ultimately cause some species to go locally extinct and change the structure and the function of the community living around the leak.

• There is a wide range of CO_2 sensitivities across different marine taxa and groups

As has been stated in the previous section, all aspects of the carbonate chemistry system [i.e. dissolved carbon dioxide levels (pCO_2), dissolved inorganic carbon (DIC), bicarbonate and carbonate concentrations, pH and carbonate (aragonite and calcite) saturation states] are intrinsically involved in a variety of physiological functions and therefore linked to the function and wellbeing of marine organisms. Consequently, the way in which organisms respond to elevated CO_2 levels, and to changes in the carbonate system, will vary greatly and will, to some extent, reflect the organisms underlying physiological mechanisms. Some progress has been made recently towards a generic response synthesis (Kroeker et al., 2010, 2013) and it is possible to identify classes of animals that are more vulnerable than others; in particular those that depend on carbonate based shells or have weak intercellular regulation are generally far more sensitive. So whilst the underlying sensitivity of marine organisms to CCS leakage might be set by their physiology, the precise nature and scale of this response can vary greatly even within species. In particular the following aspects need to be considered when assessing an organism's likely sensitivity.

1. **Resource availability or limitation**. A number of marine organisms have shown plasticity in their physiological, ecological and behavioural response to elevated CO_2. This plasticity comes at an energetic cost and can only be supported if resources are available. An example of this can be seen in Kiel fjord, Germany, where large densities of mussels appear not to be impacted by long periods of low pH primarily due to the high availability of organic material (Thomsen et al., 2013). A further complication arises as many high CO_2 exposure experiments have been conducted under conditions where food supply is not limited. This means that many species may be more sensitive than previous studies would suggest when CO_2 exposure actually occurs under more natural limited resource conditions.

2. **Life history stage**. Marine organisms go through a number of different developmental stages throughout their lives. Often, each of these life history stages displays very different physiological, ecological and behavioural traits. It is unsurprising therefore that numerous studies have shown large differences in CO_2 sensitivity between these stages often with larval or juvenile stages showing greater sensitivity than adults. So if CCS leakage were to occur the major impact may not be on adult populations but on juveniles and could have longer term effects on recruitment and future population success. In addition, even as adults the impacts of CO_2 exposure could be greater if it were to occur during periods of high energy demand (e.g. reproductive season or periods of intense growth such as moulting).

3. **Local adaptation in populations**. Recent studies have indicated that there is the potential for different populations of the same species to become more resilient to elevated CO_2 levels through adaption (or acclimation) to local conditions. For example, Parker et al (2011) showed that cultivated populations of oyster that had been selectively breed to increase energy efficiency and reduce food demands were better able to cope with high CO_2 conditions that the wild population. Such adaptation has also been observed in phytoplankton (Schluter et al., 2014)

4. **Variability between individuals within a population**. It has also been shown that even between individuals from the same population a high degree of variability can exist in CO_2 responses (Pistevos et al., 2011). This variability is often reflected in experiments by an increase in variance observed in data from high CO_2 treatments when compared to the controls.

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- **Care must be taken when predicting species specific response and sensitivity to CO_2 for Environmental Risk Assessments**

Given the high degree of natural variability which exists between different species, populations and even individuals of the same species (descried above), the most appropriate way to determine the underlying physiological sensitivity of an organism to the potential impacts of CCS leakage is to conduct controlled exposure experiments on the specific organism in question. Within these experiments every effort should be made to replicate, as closely as possible, the conditions under which the organisms' will be living when any such leak occurs. This is an important consideration given that environmental interactions, environmental stochasticity and animal behaviour can all impact upon how an organism's underlying physiological response is ultimately translated in terms of its health, function and survival. The experiments should also endeavour to encompass the range of exposure scenarios with respect to 1) the magnitude of the chemical perturbation away from normal values, 2) the duration of the exposure and 3) any variability in the exposure regime (e.g. is the exposure likely to be continuous or will there be temporal fluctuations in the severity of exposure due to local hydrodynamic processes such as tides or currents). A best practice guide (Riebesell et al., 2010) for conducting high CO_2 exposure experiments was published by the EU FP7 Integrated Project EPOCA (European Project on Ocean Acidification) and, although these guidelines were intended to inform Ocean Acidification experiments, they can still provide useful information for CCS related experiments. Of particular value are chapters on the artificial manipulation of the carbonate system for use in experiments and on the appropriate monitoring of carbonate chemistry.

In the absence of either the resources (time and/or money) or the capacity to conduct a fully comprehensive suite of exposure experiments prior to the start of a CCS project, an alternative approach would be to base the required Environmental Risk Assessment on existing, published species sensitivity information. Whilst this is a well-established and sensible practice for many traditional environmental stressors and toxicants there are a number of caveats which must be considered when doing so with respect to CCS leakage.

1. Although research efforts have increased enormously in recent years, the amount of published evidence currently available to anyone wishing to predict the sensitivity of specific species to potential CCS leakage is still extremely limited. In particular, the number of different species which have been subjected to high CO_2 exposure experiments is still low with many studies focusing on the same small group of species. Those species that have been extensively studied often carry little relevance to CCS activities (e.g. tropical corals).

2. Much of the recent research effort has focussed on the issue of Ocean Acidification and the experiments conducted are often restricted to CO_2 treatment levels that are too low to fully represent the likely pH and CO_2 chemistry changes associated with realistic CCS leakage scenarios.

3. Even if data are available for the specific species of interest, consideration should be given to the environmental context and conditions under which those data were collected. As discussed previously, a vast range of physiological, ecological and environmental processes and factors can affect an individual organism's sensitivity to elevated CO_2; e.g. temperature, food availability, habitat type, life-history stage, reproductive state, other stressors, predation and competition.

If a decision to use existing information is taken, every effort should be made to source high-quality, peer-reviewed published data. These data can either be sourced directly from the journals or the authors, or can increasingly be obtained from centralised data archive centres e.g. the British Oceanographic Data Centre (http://www.bodc.ac.uk/) which holds data from a large number of projects and programmes including the UK Ocean Acidification Research programme; the UK National Geoscience Data Centre (http://www.bgs.ac.uk/services/ngdc/) which host data from the QICS project; and Pangea (http://www.pangaea.de/about/) which holds data from many German national and EU funded projects including BIOACID, BIOACID II, EPOCA, RISCS and ECO2. Another potential source of CO_2 impact data is the database constructed and maintained by the Ocean Acidification – International Coordination Centre (IO-ICC) which is supported by the International Atomic Energy Agency (IAEA) and operated by its Environment Laboratories in Monaco. This database (http://www.iaea.org/ocean-acidification/page.php?page=2205) brings together published data from a large number of experiments which have looked at the impacts of elevated CO_2 on the marine environment.

If specific species have been identified as important to an area during the initial stages of an ERA and no CO_2-sensitivity information can be found on those species, either in the peer-reviewed literature, in publically accessible databases or in grey literature publications (e.g. non-peer reviewed reports), it has traditionally been acceptable to use any information that is available on closely related species. However, great caution should be exercised when using this approach for assessing the biological impacts of CO_2. Whilst it is probably safe to say that species within the same genus and originating from similar habitats are likely to respond in a generally similar manner to a stressor (c.f. Morley et al. 2009), the ability to assume a similar response declines rapidly as taxonomic relatedness grows more distant. In fact, there is rapidly growing evidence that an organism's sensitivity to CO_2 is governed by a wide range of physiological and environmental factors, not all of which are predictable by an organism's taxonomic classification. Consequently, it would be better to consider an organism's potential sensitivity to be more similar to other species with which they share many aspects of their CO_2 physiology, ecology and life history traits. However, even if this information is available for potentially similar species, further care should be taken to assess whether the information has been derived from comparable environmental conditions. In particular, it should be considered whether the information was gathered from an environment with comparable range of depth, pH, salinity, oxygen and temperature to the one being assessed.

A final approach would be to use some form of sensitivity index which could distinguish between potentially sensitive and tolerant species. Currently there is no such index specifically designed for assessing CO_2 sensitivity, however, there have been recent efforts to apply existing generic predictors of organism sensitivity to a range of environmental stressors. For example, the sensitivity scores of individuals used in the AZTI Marine Biotic Index (AMBI) have been applied to a range of different situations, including hypoxia, sand extraction, oil platform impacts, engineering works, dredging and fish aquaculture, with some degree of success (Muxika et al., 2005). In the absence of a specific CO_2 sensitivity index this may offer a workable solution, however, significant efforts to validate the index for CO_2 impacts before wide-scale application of this approach are required. In parallel, efforts to generate a CO_2-specific sensitivity index should remain a high priority.

• Exposure to elevated levels of CO_2 has a negative impact on marine communities, biodiversity and ecosystem processes / functions

As the previous sections have illustrated, in any marine community there will be some species which are physiologically better equipped to cope with elevated levels of CO_2 than others, so the potential for leakage to cause local species extinctions and biodiversity loss exists. So after exposure to extreme seawater acidification, we could predict that the resulting communities would be made up of species from a limited number of tolerant taxonomic groups. These more tolerant species may even increase in abundance due to a reduction in ecological pressures such as competition for food and predation. These alterations in community structure will certainly reduce both taxonomic richness and species diversity and could also lead to a reduction in some of the key ecosystem processes (e.g. bioturbation, mineralization) and functions performed by seabed ecosystems (e.g. nutrient cycling, production, remediation of waste).

The number of CCS leakage impact studies conducted on whole communities is still relatively low, compared with those studies conducted on individual species. However, a few laboratory-based experiments (Christen et al., 2013; Hale et al., 2011; Widdicombe et al., 2009; Dashfield et al., 2008) including one conducted within the ECO2 project (http://eprints.uni-kiel.de/26037/1/D4.1_AQ_final.pdf), have shown that exposure to low-pH / high-CO_2 seawater does cause significant changes to community structure, loss of biodiversity and reduced ecosystem function (e.g. bioturbation and community biomass) in benthic macrofauna and meiofauna.

These experimental results are supported by observations made at a naturally occurring CO_2 seep site in Ischia, Italy, where biodiversity was seen to decrease as you got closer to the centre of the leak site and the impact of the CO_2 on carbonate chemistry increased (Hall-Spencer et al., 2008). In the ECO2 project this observation was confirmed at another natural CO_2 seep in Panarea, also Italy, where both macrofaunal and meiofaunal abundance was lower and community structure different in areas where CO_2 was actively seeping out of the seabed, when compared with control areas where no CO_2 seepage was observed. Interestingly, there were no differences in the number of macrofauna species found at the seep sites and the number found at the control sites. However, the macrofauna community composition at CO_2 seep sites differed markedly from the control sites based on the occurrence of more oligochaetes and amphipods, and less polychaetes and gastropods at the seep sites. This illustrates again the potential for more CO_2-tollerant taxa to capitalise on the loss of more CO_2-sensitive competitors or predators and persist in areas of active CO_2 leakage.

One current concern is that the majority of evidence currently available to assess the likely impacts of CCS leakage on marine communities and biodiversity comes from approaches which, although they each have specific advantages, are still limited in some aspect of either their direct relevance to realistic CCS leakage scenarios. In the case of laboratory-based mesocosm studies, a major advantage is that a strong control over treatment levels can be maintained which makes it possible to run specific dose-response experiments on communities that are ecologically relevant to proposed storage sites. However, a limitation is that by removing the communities from the natural world many of the key ecological processes, such as immigration and recruitment, which can shape a communities response to disturbance and recovery are lacking. In addition, almost all of the laboratory based experiments conducted to date have looked at the impacts of a CO_2-enriched plume of seawater flowing over the seafloor rather than on the flow of CO_2 up through the sediment. In the case of natural CO_2 seeps, whilst the introduction of the CO_2 into the marine environment is more realistic than has been achieved in laboratory experiments, none of the seeps currently identified are found in areas or habitats that could potentially be at risk from CCS storage activities. For example, the long-term natural CO_2 seeps in the Mediterranean have very limited ecological relevance to potential CCS storage areas in the North Sea or in other temperate coastal environments. In addition, the natural seeps have been present for 10s or even 100s of years and therefore are of little use in determining the immediate impacts of leakage into an area which had previously never been impacted by high CO_2 levels before.

Recently, a new approach has been developed which looks to fill the gap between controlled manipulative experiments in the laboratory and the use of natural long-term seeps. The QICS project (Blackford et al., 2014; Taylor et al., 2014) conducted a controlled release of CO_2, 12m below the sediment surface in an area of ecologically relevant soft sediment. The CO_2 moved up through the sediment and bubbled out through the seafloor inducing large changes in the sediment chemistry at the centre of the leak. The biological response was measured and significant reductions in biodiversity and biomass, as well as large changes in community structure, were seen in both the macrofauna (Widdicombe et al., submitted) and the meiofuana (Ingels et al., in prep). To this end the initial impact results from the QICS project strongly support the conclusions that have emerged from the previous studies in the laboratory and from the natural CO_2 seeps. However, in this small release experiment the biological impact was limited to the area where CO_2 was actively leaking from the seabed, whilst in the control sites, the closest of which was only 25m away from the centre of the release, no CO_2 impact was detected. This would suggest that, in the case of small leaks and in dynamic areas of the seabed (e.g. bottom currents, tidal regimes), the impacts of CCS leakage on benthic communities could be extremely localised and only in the case of very large leaks, or in areas of restricted water flow, will the impacts of the CO_2-enriched plume have a significant impact. In addition, the QICS experiment actively studied the process of environmental recovery after a leak stops.

Understanding the potential for environmental recovery after leakage has a fundamental role to play in predicting the risks associated with CCS activities. Recent technological developments have provided potential mechanisms by which small leaks associated with bore holes or well heads may be stopped. Consequently, assessments of the potential risks posed to the surrounding area should consider both the severity of impact should leakage occur as well as the speed and completeness of any environmental post-leak recovery. The QICS experiment showed that not only was the impact of the manufactured leak relatively small in this system, but also that environmental recovery was rapid with normal chemical conditions within the sediment regained after a few days and biological recovery complete after a few weeks or months.

Clearly CCS leakage will have a significant impact on benthic biodiversity, community structure and ecosystem function. However, whilst generic understanding is rapidly improving, experimental and observational evidence from specific habitats and situations is still largely lacking making it extremely difficult to predict the precise nature or scale of impact that would be seen for any given leakage scenario at a specific storage site. This evidence will need to be collected to underpin effective risk assessment activities and to guide appropriate monitoring strategies.

- **The leakage / release of formation water can have a negative impact on marine organisms**

Formation water release may not occur in all CCS leak scenarios. However, the injection of gas into sub-seabed aquifers may lead to the displacement of fluids low in oxygen and highly enriched in ions, which, upon reaching the seabed, could come to represent a strong change in environmental conditions. For instance, based on seismic data, the Millennium Atlas (2003) indicates that the majority of aquifers in the North Sea may be filled by formation fluids of high salinities, in some cases in excess of 300 psu, a value similar to that of the Dead Sea (≈340 psu). Allowed to percolate to the surface of the seabed, such fluids could cause a ten- fold increase in local salinity, thus representing a potentially severe source of osmotic shock to organisms inhabiting the deep waters of the North Sea. The ability of organisms to cope with such disturbances, as with CO_2, will depend upon their tolerance windows corresponding to the imposed stressor combination (low oxygen and/or high salinity), and also on the magnitude and duration of the cause of stress. The width of that tolerance window will depend on the comparative range of each of the parameters typically experienced by the community in each area of the seabed. Environments of high salinity and low oxygen do exist naturally (Helly et al 2004, e.g. the Red Sea, some areas of the Arabian Sea). However, this combination of stressors as a transient disturbance, is not often observed in nature other than in estuarine environments and coastal lagoons, where strong variation to the flow of rivers is observed, seasonally (e.g. Newton and Mudge, 2003). In all cases, marine life inhabiting such extreme environments will have undergone a historical process of selection and adaptation, and may be well equipped to survive such harsh conditions, at least seasonally. However, benthic organisms inhabiting the comparably much more stable and hospitable seabed areas, in shelf or deeper ocean areas where CCS injection is likely to occur, will not have experienced such processes, and likely exhibit low tolerance to very marked environmental gradients. The results from the ECO2 formation water experiment strongly support this view (see ECO2 deliverable D4.1, http://eprints.uni-kiel.de/26037/1/D4.1_AQ_final.pdf).

There is currently limited data availability about the scale at which formation water release may occur in a commercial CCS operation injecting gas into an aquifer, its duration, volumes released, extent of impact areas, or dissolution rates. This gap needs to be quickly addressed by the geological modelling community and by industry. However, these parameters are likely to depend on the particular characteristics of the aquifer explored, the nature of the overburden, the rate of injection and local hydrodynamics. Thus, the ECO2 formation water experiment was carried out based on a relatively short exposure duration (i.e. two weeks) and a moderate change in seawater conditions in relation to those expected to characterise formation fluids (Millenium Atlas, 2003). Indeed, the level of hypoxia simulated (1.4 g oxygen/L c.f. expected seawater norm of approximately 8 g oxygen/L,) is milder than the anoxic conditions often observed in formation fluids. Equally, the increase in total salinity that was simulated (48 g NaCl/L) is also fairly conservative. Nevertheless, the results of the ECO2 experiments confirmed the expectation that this combination of stressors would severely impair benthic marine fauna (see ECO2 deliverable D4.1, http://eprints.uni-kiel.de/26037/1/D4.1_AQ_final.pdf, and results summarised below).

In the ECO2 formation water mesocosm experiment, marked changes were observed in most of the measured responses, for which data have been processed to date. Faunal abundances and community structure, behaviour and processes had changed markedly after two weeks, as had nutrient fluxes near the seabed, and within sediments. In some cases, sediment geochemistry was entirely altered (D4.1, sections 3.5.2 and 3.6.2, e.g. figure 36). This was particularly apparent in nitrogen cycling (D4.1, section 3.5.2, fig.27), a function which in marine ecosystems supports primary productivity, and hence the base of non- chemosynthetic marine food-webs. Comparatively, for many of these responses, these results far exceeded the impact of the two week and twenty week exposure to even the most severe CO_2 treatments observed in the project. These findings suggest that the release of formation water is a potential side effect of CCS activity that should not be overlooked in environmental risk assessments.

As shown in a recent CCS *in situ* controlled CO_2 release experiment (Blackford et al 2014), marine organisms and processes are strongly influenced by seasonal processes and hydrodynamics (Widdicombe et al, submitted) that can lead to variability of the same magnitude as those imposed by CCS related disturbances. For example, community bio-diffusive sediment transport associated with bioturbation, an important mediator of marine biogeochemistry, observed during the ECO2 formation water experiment (D4.1, section 3.6.2, figure 35a) decreased in experimental treatments in the range of 60-100% in relation to controls (D4.1, section 3.6.2, figure 35a). This decrease is comparable to the decrease in this parameter observed between late Spring and Winter in coastal communities (approx. 85%, Queirós et al. Submitted). As such, formation water release could result in changes in bioturbation and associated biogeochemical processes and community health (Aller 1982, Solan and Wigham 2005) that are comparable to those which communities experience as a result of seasonal fluctuations, albeit at a much faster pace. What remains unquantified, is how much change could be expected in the event of impacts associated with activity of industrial scale (i.e. what is the scale of a representative impact scenario), and what is the ability of bottom communities to recover, once the release of formation fluid ceases. In areas of the seabed where re-colonisation processes are limited and in vulnerable habitats (e.g. those inhabited by species with low mobility and or low recruitment potential), the impacts caused by formation water release are likely to be long lasting. It follows that baseline characterisation needs to take place well before injection is initiated as, based on the current project analyses (D4.1), formation fluid impacts are likely to be tangible, and larger than potential effects cause by the localised leak of CO_2 under moderate leakage scenarios.

Further investigation of this element of CCS using suitable scenario information and adequate experimental setups (i.e. including long term exposures, and simulations of seasonality and hydrodynamics as modulators, see D4.1) could strongly contribute to the definition of guidelines and monitoring strategies in support of safer CCS activity in the marine environment. CCS may prove to be a suitable strategy to a much needed curb in GHGE, but an adequate cost-benefit analysis still requires the clarification of some areas of uncertainty. A more complete assessment of risk associated with the release of formation water is one of them.

• Other environmental factors could exacerbate or ameliorate the impact of CCS leakage

When considering the likely impact of CCS leakage on marine organisms it should also be remembered that the CO_2 exposure that will occur from leakage will not impact upon organisms in isolation from other prevailing conditions and factors at the leak site. Consequently, a number of interacting factors will need to be considered.

1. **Sediment type.** Findings from a field based CCS release experiment (Blackford et al 2014) has shown that in some cases the mineralogy of the sediment can to some extent buffer the chemical changes within the seabed. This process would be particularly strong in sediments with high carbonate content. Specifically in the QICS experiment, increased concentrations of pore water alkalinity and Ca^{2+} indicated that the injected CO_2 promoted rapid dissolution of calcium carbonate ($CaCO_3$) naturally present in the sediment and that the rise in DIC was buffered by this carbonate dissolution (Blackford et al., 2014), at least in the short term.

2. **Presence of heavy metals and other pollutants.** Mobilization of metals bound within the sediment on exposure to CCS acidification have been demonstrated in laboratory investigations (Romanó de Orte et al., 2014). Metals including Al, Fe, Zn, Co, Pb and Cu have all been found to increase with acidification, compounded by increased time of exposure to CO_2 leak. Furthermore, acidification also influences the speciation of metals, transforming metals and metalloids, like As, into species much more toxic to biota. Additional investigations found an increase in the metals As, Cd, Ni, Pb and Zn leaching into the water column when seawater pH is reduced from pH 8 to pH 7.3, increasing As, Cd, and Zn concentrations by about 45% and at pH 6.8 by 66 - 82% (Payan et al., 2012). Evidence of metal accumulation and high mortality was observed in laboratory investigations simulating CCS leakage scenarios and using the polychate worm *H. diversicolor* as a model organism (Rodríguez-Romero et al., 2014). Mortality was significant at the lowest pH level in the sediment with highest metal concentrations. In general, metal concentrations in tissues of individuals exposed to the contaminated sediment were influenced by pH. These results indicate that acidification due to CCS leakages could provoke increased metal mobilization, causing adverse side effects in sediment toxicity. Metal mobilization therefore needs to be highlighted as a potential lethal secondary impact that may arise in the event of a CCS leakage scenario, further compounding the mortality observed and reduced the potential recovery/ tolerance limits of exposed organisms.

3. **Existing physical disturbance (e.g. trawling, aggregate extraction).** Marine ecosystems are under increasing pressure from a range of anthropogenic activities, the most widespread of which is benthic trawling. It is known that trawling disturbance has a substantial effect on the larger benthic fauna, with reductions in density and diversity, and changes in community structure, benthic biomass, production, and bioturbation and biogeochemical processes (Widdicombe et al. 2004, Queirós et al., 2006). In addition, nematode community structure changes in response to macrofauna presence and density, mainly as a result of the reduced abundance of a few dominant nematode species, and there may be a general indirect, macrofauna-mediated trawling impact on nematode communities. Removal or reduced densities of larger macrofauna species as a result of trawling disturbance may lead to increased nematode abundance and hints at the validity of interference competition between large macrofauna organisms and the smaller meiofauna, and the energy equivalence hypothesis, where a trade-off is observed between groups of organisms that are dependent on a common source of energy (Ingels et al., 2014). In addition, macrofauna such as the Norway Lobster *Nephrops norvegicus* subjected to trawling are much more stressed by trawling at high summer temperatures and have difficulty in recovering, with pronounced negative effects on their survival. Consequently, when baseline-monitoring studies are preformed the inclusion of past and present fishing and trawling activities should be determined. Fishing or trawling activities will have negativity impacted the local ecosystem and food webs. The bias targeting of economically important species, at specific life cycles will shift the environmental baseline for local marine communities.

4. **Existing environmental stress (Seasonal hypoxia, temperature).** Organisms are commonly confronted simultaneously with multiple environmental stresses (e.g. temperature, hypoxia, hypercapnia (i.e. high CO_2)) as well as range of direct human impacts (e.g. trawling, pollution, habitat destruction) compounding the impact of any single environmental stress (such as CO_2 exposure from CCS leakage) and severely inhibiting the scope for physiological adjustments to overcome that stress (Riedel et al., 2014). It is unlikely therefore that hypercapnia will be acting as an independent stressor, and caution is needed when calculating tolerances of species to environmental perturbations from studies what have only factored in one variable. Hypercapnia needs to be recognised at a duel stressor in conjunction to hypoxia, and the synergistic effects recognised. For example burrowing organisms inhabit hypoxia and hypercapnic environments, making them vulnerable to further increases in acidification that would compound hypoxia/hypercapnia. Species already existing at the limits of their physiological capacity should be classed as highly vulnerable. An example of the negative synergistic effects of hypoxia and hypercapnia, was found in the growth rates of clams, which was not detected when either pressure was investigated separately (Gobler et al., 2013). The role of temperature needs to be included when considering secondary impacts arising from a CCS leak, as it is the most pervasive environmental factor affecting all levels of biological organization (Portner 2008). With mean global temperatures increasing, this will have implications on the thermal tolerance limits of marine biota. Elevated temperature will compound the negative consequences of hypercapnic stress, by impeding respiratory capacity alongside increasing metabolic demands, pushing marine biota to the limits of their functional capacity. Therefore, a major factor determining the consequences of a CCS leak can be attributed to seasonal thermal conditions.

• Some biological responses may be employed in a programme of Environmental Monitoring

As previously discussed, CCS reservoirs will be extremely large and consequently, the area of seabed above them that could potentially be exposed to a leak is also very large. This vast spatial extent over which a leak could appear presents a serious challenge to using biological indicators to locate and identify CO_2 leakage from the seafloor. The best candidate indicators are therefore those that can be integrated into broad-scale visual mapping activities in which towed or autonomous vehicles fly close to the seabed and use high definition cameras to video large areas of seafloor.

A potential indicator that could be observed in this way is the unusual appearance of large numbers of animals on the sediment surface. Recent experiments have shown that exposure to high levels of CO_2 can elicit a surfacing response in echinoderms and in molluscs where by animals which normally burrow deep within the sediment (known as infauna) come up onto the sediment surface. This was also observed during the ECO2 high CO_2 experiments on natural communities (D4.1, section 3.6.1) at 20 but not 2 weeks of exposure. This is an extremely risky thing for infaunal species to do as it increases danger from predation and increases the chances of being relocated to less suitable habitats by strong tides, currents or storms. Whilst this surfacing behaviour is widely considered as classic stress-response and not necessary limited to high CO_2 levels, for example similar responses have been seen during times of hypoxia (also seen in the ECO2 formation water experiment, D4.1 figure 35b), it may still be a useful early indicator that something is having a negative impact on benthic fauna. As shown in the ECO2 formation water experiment, surfacing fauna may exhibit such poor condition (highlighted by low activity, D4.1 figure 35a) that they may be unlikely to survive regardless of added predation pressure. Nevertheless, at this point more targeted sampling can be used to determine the identity and source of the environmental impact. Care should be taken with this indicator as it will be a transient signal and requires a good understanding of the corresponding baseline. It will not take long for the dead or dying organisms at the sediment surface to be consumed by mobile predators and scavengers or to be decomposed by benthic microbes. If the surfacing organisms have shells or calcified skeletons these may remain on the sediment surface longer, but even these structures will eventually dissolve, particularly in a CO_2 enriched / low pH environment.

Another visual indication of CO_2 seepage could be the presence of microbial mats on the sediment surface. In the photic zone (typically water depths less than 50m) mats of benthic algae and photosynthetic bacteria form on sediment surfaces. Also known as microphytobenthos, these mats are coloured green, brown or even pink depending on the species present. During the QICS experiment, an increase in the activity of benthic algae was detected at the CO_2 release site when compared to the references sites (Tait et al. 2014), and a bloom of microphytobenthos was clearly visible during a mecososm experiment conducted for the RISCS project. The elevated levels of CO_2 likely enhanced the growth of these photosynthetic microbes. In areas receiving sufficient light input, monitoring for blooms of microphytobenthos may prove useful as an indicator of a CO_2 leak. However, this would require knowledge of the typical seasonal pattern of microphytobenthos activity within the area monitored. In deeper waters, where light levels are too low to support photosynthetic organisms, the presence of microbial mats can indicate the seabed leakage of substances other than CO_2. Most commonly these deeper mats are made up of methanogenic or sulphergenic microbes, i.e. those that use methane or sulphur as an energy source, with both of these compounds being a commonly formed by the breakdown of organic material in coastal sediments. It is conceivable that should CO_2 leak from a reservoir it could liberate methane or sulphur from the shallow sediment layers and that the presence of the associated microbial mats could indicate areas where future CO_2 leakage could be expected.

It should be remembered that although collecting video images of large areas of seabed can be done quickly, these images will still need to be analysed in order to identify possible anomalies. Currently this is done by a trained observer working through the images and is a time consuming activity. However, if more automated image recognition systems could be developed that could identify sections of footage which contained “unusual” frames, and only these needed to be checked by human eye, this could significantly speed up the process and reduce the costs.

Apart from the visible biological indicators of leakage described above, most other biological responses would be either too time consuming to measure or too spatially restricted to act as rapid indicators of leakage. To this end, biological responses are not best suited to locating potential leaks but are more ideally suited to monitoring the progress of leakage impact once a leak is detected, to assessing or quantifying the scale of the impact or to monitoring ecosystem recovery once a leak has stopped. For these purposes, biological responses offer an integrated measure of CO_2 exposure that actually relates CO_2 leakage to a biological consequence.

With that said it would be unwise to abandon biological monitoring altogether from any environmental monitoring plan for two main reasons. Firstly, the majority of public concern over offshore CCS activities is likely to focus on the potential for impacts on marine organisms and habitats. A robust and effective biological monitoring programme is a powerful tool in reassuring public concerns that any environmental impacts will be detected. Secondly, if circumstances were to arise in which chemical changes were restricted to sediment pore waters and for some reasons (e.g. water depth or hydrodynamic conditions) neither bubbles nor chemical changes were detectable in the water column, then biological monitoring may have a role.

Obviously it would be impractical to implement a full biological survey across the entire storage area, especially at the fine-scale spatial resolution needed to be sure of detecting all leaks. The most resource effective solution would be to concentrate seabed intensive survey activities around areas of perceived high leakage risk, such as existing or derelict wells or geological features such as chimneys. To this end, operators could employ a spatially nested approach to environmental monitoring whereby different monitoring activities were deployed across different spatial scales and with different spatial resolution. At the broadest scale visual surveys would be conducted across the whole storage area looking for the presence of unusual biological features such as microbial mats or the appearance of infauna at the sediment surface. These surveys could be conducted at the same time as the broad-scale chemical surveys using the same AUVs or ROVs. Around areas with potentially higher risk of leakage, comprehensive biological monitoring should be conducted. This monitoring would involve determining the identity, abundance, biomass and distribution of seabed fauna. In particular, observers should be looking for changes in the community structure and diversity of infaunal organisms that have been shown to be indicative of CO_2 impact (e.g. Widdicombe et al., submitted; Widdicombe et al., 2009). For CCS leakage, these changes could be a reduction in the presence of calcified taxa or a reduction in the biomass of calcium carbonate structures (e.g. shells and exoskeletons). As with all biological indicators, understanding the natural temporal variability in these data by the acquisition of a comprehensive biological baseline (see following section) will be needed so unnatural changes in benthic communities can be discriminated from those natural changes that occur between seasons and years. Not only is this important for identifying impact but also in guarding against the false attribution of natural changes to CCS activities. By adopting effective biological monitoring operators and regulators could address both of the issues of leak detection and public confidence.

- **Collecting spatially and temporally referenced biological data is important for creating effective Baseline Surveys**

As discussed, natural communities, and processes mediated by these, exhibit natural variability associated with environmental drivers. These can be driven by short term seasonal dynamics (Reiss and Kroncke 2005), spatial changes associated with gradients (e.g. sediment type, food availability, Dauwe et al., 1998), long-term change associated with environmental drivers (e.g. ocean warming, Hiddink and Hofstede 2008), and direct human induced change (e.g. fisheries, Queirós et al., 2006). For any given local system, it is difficult to define a specific, constant value that would identify a pristine, or undisturbed, condition when using ecological parameters such as diversity, temperature, biomass. Rather, in undisturbed conditions these parameters will fluctuate within a dynamic range, with a mean value that varies temporally and spatially, within a measurable interval. The definition of adequate baselines for areas of the seabed where CCS will take place thus requires an ability to capture these local natural dynamics. Without this, it is unlike that vulnerability can be suitably calculated, or that impact and recovery can be projected.

While it is unreasonable to suggest that baseline surveys should aim to quantify local long-term dynamics of the vast areas of the seabed above reservoirs, some understanding of seasonal dynamics, types of habitats covered and a reasonable mapping of ongoing parallel pressures (e.g. seasonal hypoxia, trawling grounds) is necessary. While some or all of these aspects are already regularly monitored by marine users and academia, it may be important to highlight the need for an understanding of local ranges of stressors which may be exacerbated by CCS impact scenarios. In particular, given the evidence described in this report and in the wider CCS and OA literature, determining vulnerability to risk factors associated with CCS will require some degree of understanding of what range for each of the parameters (pH, TA, DIC, O_2, Salinity) local natural communities have adapted to. This is because, as detailed in previous sections, there is wide evidence to support the perspective that local adaptation determines the response of individual species and populations to environmental change (e.g. Eliason et al., 2010; Morley et al., 2009). As such, baseline characterisation should aim also to cover such parameters.

In ECO2, significant efforts have been made to gather information on the state of the art methods for survey parameters associated with CCS leakage scenarios. Use of such methods to characterise natural ranges of variability in benthic and pelagic pH, TA, DIC, O_2 and salinity in CCS exploration areas should be prioritized within baseline surveys, as a means to establish natural ranges for each parameter for local biological communities. Such data would help to define local vulnerability thresholds (i.e. the limits of natural variability for a given parameter). Such data would also help to contextualize available literature about possible impacts of variations in such parameters associated with CCS leakage for local species, helping to define whether sufficient information exists in literature for a particular species (or for taxonomically close relative), from comparable habitats.

Deliverable Number D4.4: WP4 results summary report relevant for Environmental Best Practice; WP4; lead beneficiary: Plymouth Marine Laboratory

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