



# Effects of sub-seabed CO<sub>2</sub> leakage: Short- and medium-term responses of benthic macrofaunal assemblages

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## ABSTRACT

The continued rise in atmospheric carbon dioxide (CO<sub>2</sub>) levels is driving climate change and temperature shifts at a global scale. CO<sub>2</sub> Capture and Storage (CCS) technologies have been suggested as a feasible option for reducing CO<sub>2</sub> emissions and mitigating their effects. However, before CCS can be employed at an industrial scale, any environmental risks associated with this activity should be identified and quantified. Significant leakage of CO<sub>2</sub> from CCS reservoirs and pipelines is considered to be unlikely, however direct and/or indirect effects of CO<sub>2</sub> leakage on marine life and ecosystem functioning must be assessed, with particular consideration given to spatial (e.g. distance from the source) and temporal (e.g. duration) scales at which leakage impacts could occur. In the current mesocosm experiment we tested the potential effects of CO<sub>2</sub> leakage on macrobenthic assemblages by exposing infaunal sediment communities to different levels of CO<sub>2</sub> concentration (400, 1000, 2000, 10,000 and 20,000 ppm CO<sub>2</sub>), simulating a gradient of distance from a hypothetical leakage, over short-term (a few weeks) and medium-term (several months). A significant impact on community structure, abundance and species richness of macrofauna was observed in the short-term exposure. Individual taxa showed idiosyncratic responses to acidification. We conclude that the main impact of CO<sub>2</sub> leakage on macrofaunal assemblages occurs almost exclusively at the higher CO<sub>2</sub> concentration and over short time periods, tending to fade and disappear at increasing distance and exposure time. Although under the cautious perspective required by the possible context-dependency of the present findings, this study contributes to the cost-benefit analysis (environmental risk versus the achievement of the intended objectives) of CCS strategies.

## 1. Introduction

The accelerating rise in atmospheric carbon dioxide (CO<sub>2</sub>) levels (IPCC, 2013) is causing ocean warming and acidification at unprecedented rates, posing critical threats to single species, habitats, oceanic regions and overall global ecosystem functioning (Caldeira and Wickett, 2003; Feely et al., 2004; Hale et al., 2011; Mora et al., 2013, Cerrano et al., 2013; Meadows et al., 2015; Gattuso et al., 2015). As a direct consequence, it is urgently needed to identify suitable options for reducing/mitigating CO<sub>2</sub> emissions (McCormack et al., 2016). One particularly promising technology involves capturing CO<sub>2</sub> from point source effluents (mostly, energy generation plants), then transporting it as a supercritical liquid to be stored in deep porous geological rock formations, such as saline aquifers or existing hydrocarbon reservoirs

(Gibbins et al. 2006; Holloway 2007). This process is defined as CO<sub>2</sub> Capture and Storage (CCS). In Europe and North America the technical feasibility of CCS approaches has been already demonstrated. For example, at the Sleipner West gas field in the Norwegian sector of the North Sea, CCS has been operational since 2000 with approximately 1 million tons of CO<sub>2</sub> pumped into the storage reservoir every year (Paulley et al., 2012, Jones et al., 2015). However, as with almost any other human activity, this technology is not risk-free in terms of posing potential environmental hazards (reviewed by Damen et al. 2006). Before industrial scale CCS activities become widely accepted and implemented these risks need to be identified and quantified. Perhaps the greatest environmental risk associated with CCS is that of CO<sub>2</sub> leaking into the marine environment either during transport, sequestration or from the geological storage reservoir itself. Whilst current evidence

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suggests that leakage from CCS reservoirs would be extremely unlikely it is not impossible (Blackford et al., 2009, 2014). Given that any major increase in seawater CO<sub>2</sub> concentrations, and the associated changes in carbonate chemistry, has the potential to considerably impact marine life and ecosystem functions, assessing the biological and ecological effects of CO<sub>2</sub> leakage is essential to support environmental risks assessments required by CCS operations (Widdicombe et al., 2013; Jones et al., 2015). This is especially relevant for benthic assemblages living in the immediate proximity of any potential leak, since they would be exposed to relatively large and rapid changes in carbonate chemistry, in both the sediment pore waters and the overlying seawater (Lichtschlag et al., 2014; Queiros et al., 2014).

The exposure to a range of CO<sub>2</sub> concentrations has been tested on a variety of marine organisms, as well as on some biogeochemical processes and ecosystem functions (Widdicombe et al., 2013, 2015; Laverock et al., 2013; Tait et al., 2014; Rastelli et al., 2015). It has also been demonstrated that the impact of elevated CO<sub>2</sub> on marine organisms depends on both the severity and the duration of the exposure (Blackford et al., 2013). In general, it is hypothesized that a CCS leakage is immediately associated with a localized acute exposure to harmful high CO<sub>2</sub> conditions whose effects are likely to get attenuated at increasing distance from the source. Moreover, more prolonged leakage or persisting influences of temporary seepage of any level could represent chronic stressed conditions to the surrounding abiotic and biological environment (Jones et al., 2015).

Whilst previous studies have started to provide a better understanding of the potential impacts of CCS leakage on specific benthic organisms (e.g. Widdicombe & Needham 2007), our knowledge of the possible effects at the community level remains limited (Widdicombe et al. 2015). In addition, the mechanisms underlying such changes are still largely unknown, as well as the difference between direct and indirect effects of increasing CO<sub>2</sub> leakages on the macrofaunal community. It has been reported, however, that low-pH levels predicted by realistic scenarios of CCS leakage might severely reduce the prokaryotic-mediated processes (Rastelli et al. 2015), while acidified conditions could favor blooms of benthic microbial primary producers including cyanobacteria and diatoms (Tait et al., 2015). Notably, the exposure to high CO<sub>2</sub> levels can alter microbial-mediated processes able to affect the quality and quantity of the sedimentary organic matter (OM) (Rastelli et al., 2015). Since the availability of OM is a key driver of the abundance, distribution and biodiversity of benthic fauna (Fabiano and Pusceddu, 1998; Pusceddu et al., 2009), the effects of changes in this variable due to CCS leakage might indirectly propagate to associated macrofaunal assemblages.

Full community level effects of CO<sub>2</sub> leakages can only be unequivocally assessed using simulated leakage experiments conducted in the field (e.g. Blackford et al. 2014) or from studying actual leakage events or accidents in areas where data on the response variables of interest are available before and after the event. Both options are normally unavailable either due to the lack of data or to logistic, financial and/or ethical constraints. Performing manipulative experiments in mesocosms can be a feasible alternative especially when the results are used to inform ecosystem level models. A strength of an experimental approach is that by exposing initially comparable assemblages to different levels of CO<sub>2</sub> concentration (such as ‘naturally’ occurring along a gradient of distance from a supposed leakage) under controlled conditions allows testing for their relative effects in an unconfounded way.

In this study, we performed a mesocosm experiment to test the potential impact of CO<sub>2</sub>-enriched (from 400 ppm to 20,000 ppm) seawater plumes on the abundance and diversity of soft-bottom macrofauna. Specifically, we tested the null hypotheses that (i) the whole structure (taxon composition and relative abundance), richness, total abundance of the macrofaunal assemblages and the abundance of individual taxa, did not differ depending on the CO<sub>2</sub> concentration; (ii) such a lack of differences was consistent between a few weeks (short-term) and some months (medium-term) of continued exposure.

## 2. Material and methods

### 2.1. Collection of sediment samples and associated fauna and mesocosm setup

Using a KC Denmark box corer, intact sediment samples containing natural infaunal assemblages were collected during the 3rd week of August 2012 from randomly selected points located some meters apart from each other at the outer Oslofjord (59°49.4788' N, 10°58.8595' E), Norway, at 100 m water depth. Each box corer was equipped with an inner liner, which allowed the sediments and the overlying water to be retrieved with minimal disturbance.

A total of 46 independent liners (0.09 m<sup>2</sup> each, with average sediment penetration of ~40 cm) were collected and transferred immediately to the benthic mesocosm systems at the Marine Research Station, Norwegian Institute of Water Research, Solbergstrand, Norway. During transportation, all liners were shaded and continuously covered with seawater to prevent desiccation and minimise temperature changes.

The experimental system was set up according to Widdicombe et al. (2009), as described in detail elsewhere (Queiros et al., 2015; Rastelli et al., 2015). Briefly, all liners were placed in an aquarium in a flow-through holding basin filled with seawater to a depth of 1 m (mesocosm) and supplied continuously with unfiltered natural seawater at a flow rate of 120 ml/min from a pipeline situated at 60 m depth in the adjacent fjord. All liners were maintained in these conditions for two weeks prior to the beginning of the experiment to allow the fauna, microbes and geochemical processes to acclimatize to mesocosm conditions.

### 2.2. Preliminary survey

To guarantee that the randomly assigned experimental levels of CO<sub>2</sub> were not confounded by initial differences between replicate cores in terms of hosted macrofaunal assemblages would have required us to compare macrofauna among all (allocated) treatments before manipulation. Unfortunately, the needed destructive sampling made such an option impossible. Alternatively, a total of 6 liners were chosen at random from the 46 liners initially collected and these 6 were randomly allocated to one of two groups of three. These were then compared (by means of one-way PERMANOVA, see Supplement S1) for the structure of macrofauna, under the hypothesis that the lack of significant difference between one group and the other could provide information (not exhaustive, but relevant) to assume that significant differences were not likely to exist also among the sets of replicate liners allocated at random to the experimental levels. In addition, data on the sediment grain size, estimated by laser analysis at the beginning of the experiment, were available for one liner per each of the total five experimental conditions (e.g. McCave, 2013).

For each liner, macrofaunal assemblages were sampled, after the acclimation period, by sieving all the sediment over a 500 µm mesh, with the residue from each sample being fixed in 10% buffered formalin until further processing. In the laboratory, the fauna was extracted from the residue under a binocular microscope and all specimens were sorted into major taxa and then identified to species level whenever possible. Species (or higher taxa) abundance was determined in each replicate and expressed as the total number of individuals per m<sup>2</sup> of sampled area.

### 2.3. Experimental setup and sampling

The 40 liners remaining after the preliminary survey were randomly allocated in equal numbers (Berge, 1990) to each of five CO<sub>2</sub> treatments: 400 (control), 1000, 2000, 5000, and 20,000 ppm, with two sampling times (2 weeks, 20 weeks). These levels were consistent with those specifically tested by Rastelli et al. (2015) and Queiros et al.

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