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Effects of Direct Ocean CO_2 Injection on Deep-Sea Meiofauna

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Purposeful deep-sea carbon dioxide sequestration by direct injection of liquid CO_2 into the deep waters of the ocean has the potential to mitigate the rapid rise in atmospheric levels of greenhouse gases. One issue of concern for this carbon sequestration option is the impact of changes in seawater chemistry caused by CO_2 injection on deep-sea ecosystems. The effects of deep-sea carbon dioxide injection on infaunal deep-sea organisms were evaluated during a field experiment in 3600 m depth off California, in which liquid CO_2 was released on the seafloor. Exposure to the dissolution plume emanating from the liquid CO_2 resulted in high rates of mortality for flagellates, amoebae, and nematodes inhabiting sediments in close proximity to sites of CO_2 release. Results from this study indicate that large changes in seawater chemistry (i.e. pH reductions of ~0.5–1.0 pH units) near CO_2 release sites will cause high mortality rates for nearby infaunal deep-sea communities.

Keywords:
· CO_2 sequestration,
· meiofauna,
· ecological impacts,
· deep-sea biology,
· hypercapnia.

1. Introduction

Warming of 0.75°C over the Earth during the last century (Mann *et al*., 1999) has been accompanied by broad changes in marine and terrestrial ecosystems (Parmesan and Yohe, 2003; Root *et al*., 2003), and is thought to be associated with the simultaneous rapid increase in atmospheric greenhouse gas concentrations, driven by emissions from the burning of fossil fuels. Earth's climate is expected to warm even more rapidly during this century, as global CO_2 emissions increase from present rates near 7 $GtCy^{-1}$, to 15 $GtCy^{-1}$ or more by 2050 (Marland *et al*., 2001). Estimates of warming expected through the 21st century vary among models, but all are responsive to levels of carbon dioxide in the atmosphere.

Direct injection of CO_2 into the ocean was suggested over 25 years ago (Marchetti, 1977) as one of several carbon sequestration alternatives to offset the accelerating rise in anthropogenic greenhouse gases (Reichle *et al*., 1999; Brewer *et al*., 1999). Sequestration would be accomplished by injecting carbon dioxide stripped from the flue gases of fuel-burning power plants into the bottom waters (~3000 m) of the deep-sea, where the circulation time of the world ocean would prevent CO_2 out-gassing to the atmosphere for centuries, thereby mitigating the peak atmospheric CO_2 levels expected during the next 200–300 years.

To be effective, an ocean CO_2 injection program would require that large quantities of CO_2 (i.e. billions of tons of carbon) be injected into the deep waters of the ocean. Dissolution of the liquid CO_2 into seawater will elevate CO_2 levels (hypercapnia) and, through a response of the carbonate buffering system of seawater, reduce its pH. Depending on the period and volume of injection, an ocean carbon sequestration program could eventually result in pH reduction of possibly up to tenths of pH units for the bottom waters of the entire world ocean. Acidification near sites of CO_2 injection would be far more severe, with pH values close to 4.0 at the seawater/CO_2 interface, and shifts of 1 unit or greater over meters to potentially 10s to 100s of kilometers.

Immersion in CO_2-laden, acidic seawater from CO_2 injection poses physiological challenges to marine animals that respond by tolerance, compensation, or death. Responses are based on physiological adaptation of species that have evolved to tolerate the range of natural environmental variability encountered. Animals that evolved in highly stable conditions typical of deep-ocean waters are, in general, more sensitive to a variety of environmental perturbations than shallow-water animals, including those associated with CO_2 injection (Seibel and Walsh, 2003). The main CO_2-related stresses can include acidosis of intra- and extra-cellular fluids, requiring pH com-

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pensation and inducing respiratory stress, and metabolic suppression, associated with hypercapnia (Pörtner and Reipschläger, 1996). Changes in ocean pH caused by direct sequestration or air/sea exchange that fall within the range of normal environmental variation are expected to be less stressful than more extreme perturbations. Over the world ocean, seawater pH varies today from ~7.3 to ~8.5, (www.nodc.noaa.gov), and differs among ocean basins. pH varies most in the upper ocean; the mean (SD) pH in depths <1000 m for the Atlantic and North Pacific Oceans are 8.2 (0.15) and 7.9 (0.22), respectively, representing variation of 0.6 and 0.9 pH units. Deep-sea environments are less variable; pH between 3000–4000 m for these areas is 8.0 (0.02) and 7.8 (0.05), with variation of 0.1 and 0.2 pH units. Individuals and populations are likely to experience even less natural pH variability.

Ecological impacts resulting from physiological stress during exposure to hypercapnic, low pH waters produced through CO_2 injection are an important concern regarding the role, if any, that ocean sequestration should play in a national or global carbon management strategy. Changes in pH caused by CO_2 injection are expected to exceed, in some cases by a large margin, the natural range of pH variability encountered by deep-sea organisms. In addition, the physiological literature on deep-sea animals suggests that they are much more sensitive to CO_2 related stresses than their shallow-water counterparts (Seibel and Walsh, 2003).

Nor are the ecological impacts of elevated oceanic CO_2 levels limited to those associated with a deep-sea CO_2 sequestration program. Air-sea gas exchange quickly equalizes CO_2 levels in the atmosphere and surface waters of the ocean, and has led to a rise in ocean CO_2 levels since the Industrial Revolution (Keeling and Whorf, 2002; Barnola *et al*., 2003), which will continue in the future (Marland *et al*., 2001). Roughly 1/3rd of current fossil fuel CO_2 emissions (~7 $GtCy^{-1}$) enter the sea surface through air-sea exchange (Houghton *et al*., 1990; McNeil *et al*., 2003), and have already acidified the upper ocean by –0.1 pH units (Sabine *et al*., 2002). Continued acidification of the surface ocean (–0.3 pH units by 2100; Haugan and Drange, 1992; Drange *et al*., 2001; Harvey, 2003) may place coral reefs and other shallow marine ecosystems in peril (Kleypas *et al*., 1999; Knowlton, 2001).

Together, the physiological challenges posed by hypercapnia and acidification of the surface ocean or in the deep-sea by direct CO_2 injection, elevate the importance of understanding the ecosystem consequences of these global perturbations of ocean chemistry. Ocean sequestration would reduce atmospheric emissions, but would add to the accumulating burden of fossil fuel CO_2 in the ocean. And while "dangerous anthropogenic interference" with the climate system has been debated widely, there has been little or no consideration of acceptable levels of anthropogenic change in ocean CO_2 levels. Thus, although direct deep-sea CO_2 injection is technically feasible (IPCC, 2001), the environmental consequences of large-scale CO_2 sequestration remain unknown and may be substantial (Seibel and Walsh, 2003). In view of the potential for significant changes in ocean pH, due either to continued invasion of anthropogenic CO_2 in the ocean surface (Caldeira *et al*., 2003), or to direct sequestration of CO_2 in the deep ocean, research concerning the biological and ecological impacts of elevated CO_2 on marine biota is a high priority. In addition, comparison of the sensitivities of deep and shallow-living marine biota to elevated CO_2 levels should receive considerable attention prior to initiating a direct ocean CO_2 injection as a carbon management strategy. Here we present the initial results of *in situ* deep-sea CO_2 release experiments designed to evaluate the sensitivity of sediment-dwelling deep-sea meiofauna to CO_2-rich, low pH seawater plumes emanating from deep-sea CO_2 pools.

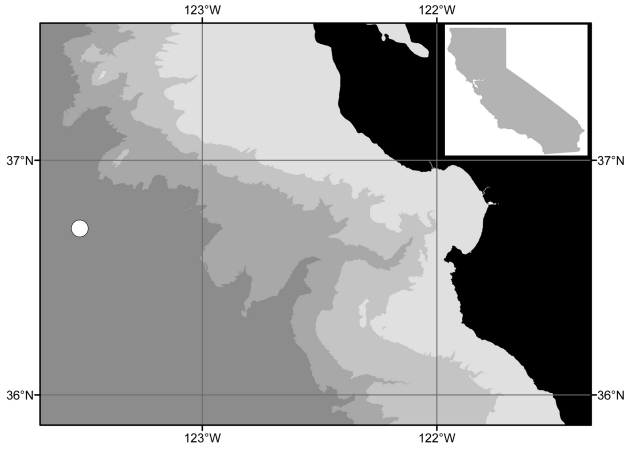

Fig. 1. Map of CO_2 Sequestration Study site, 85 nm west of Moss Landing, California. Water depth at study site (white circle) is 3600 m. Depth contours indicated in meters.

2. Methods

We measured the survival of deep-sea infaunal meiofauna to direct deep-sea CO_2 injection during an *in situ* experiment during June–July, 2001, in which organisms were exposed to the CO_2-rich dissolution plume from pools of liquid CO_2 released into PVC "corrals" on the seafloor. Our experiments were designed to investigate the potential effects of direct ocean CO_2 sequestration and develop deep-sea experimental techniques for controlled ecosystem CO_2 enrichment (e.g. DeLucia *et al*., 1999).

The study was performed at the base of the continental rise at 3600 m depth, 85 km west of Moss Landing, California (36°42′33.4″ N, 123°31′22.0″ W). This

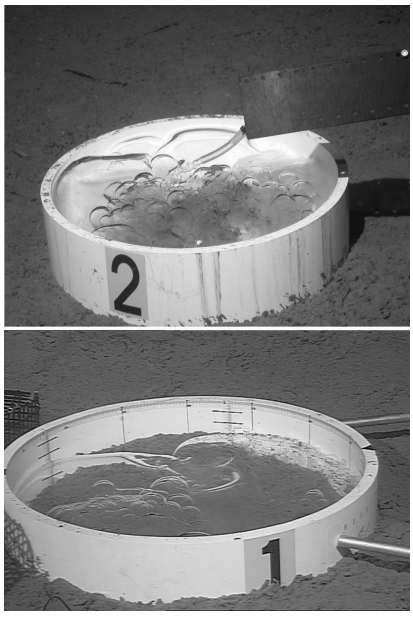

Fig. 2. Photograph of CO_2 corrals during filling operations. Liquid CO_2 is visible as a clear liquid partially (1) or fully (2) filling the corrals. Some sediment has been entrained in the CO_2 inside corral 2. CO_2 hydrate is also visible in corral 2. By the end of the experiment, much of the CO_2 had dissolved.

site is a flat, soft sediment environment with little relief at the base of the continental slope (Fig. 1). The local megafaunal assemblage is typical of abyssal deep-sea communities in the eastern Pacific, with moderate densities of macrourid (*Coryphaenoides armatus*) and zoarcid fishes (*Pachychara* sp.), octopus (*Benthoctopus* sp.), echinoderms (holothurians—*Peniogone* sp., *Abyssocucumis abyssorum*, *Scotoplanes globosa*), echinoids (*Cystechinus loveni*, *Aporocidaris milleri*), gastropod molluscs (*Mohina vernalis*), ophiuroids (unknown sp.), and a variety of less abundant species. The infaunal macrofaunal assemblage at this site is dominated by tube-dwelling ampeliscid amphipods (*Haploops lodo*), but also includes numerous other crustacean, polychaeta, mollusca, and cnidarians. Sediment-dwelling meiofauna are abundant, and dominated by nematodes, flagellates, and amoebae, with lesser densities of ciliates, foraminifera, and other groups.

2.1 Experimental CO_2 treatments

Three small (48 cm diameter × 15 cm high) PVC rings ("corrals") placed on the seafloor were filled with ~20 liters of liquid CO_2 using an ROV-mounted CO_2-release system (Brewer *et al.*, 1999) developed by the Monterey Bay Aquarium Research Institute (Fig. 2). Liquid CO_2 is slightly heavier than seawater at this depth, and dissolves slowly into the overlying seawater, producing a CO_2-rich, low-pH dissolution plume that is carried downstream in the prevailing current. Because it is rich in CO_2, the dissolution plume is also slightly heavier than seawater, and flows downstream across the seafloor as a dense plume, thereby exposing sediment-dwelling infauna (especially those inhabiting the surface) to seawater chemistry expected with a large scale CO_2 sequestration program. Release of liquid CO_2 into pools on the seafloor, selected here because it is experimentally tractable, is only one of many variants of proposed ocean CO_2 injection strategies (Haugan and Drange, 1992; Caldeira and Rau, 2000; Drange *et al.*, 2001).

The PVC corrals were filled with liquid CO_2 in late June, 2001, refilled 2 weeks later, and the experiment was terminated in late July, 2001. The liquid CO_2 persisted in liquid form, with a hydrate skin, throughout the study, and we did not observe large volume changes from massive hydrate formation (Brewer *et al.*, 1999).

Three additional PVC corrals were placed on the seafloor nearby (~30 to 50 m away) to serve as control corrals. Although these corrals were not filled with liquid CO_2, samples were collected near these corrals in the same manner used for experimental corrals.

2.2 Meiofaunal assemblage samples

Meiofaunal organisms were sampled using sediment cores, which were collected prior (June, 2001) to dispensing the CO_2 and after (July, 2001) 35d exposure, from the area immediately adjacent to both the CO_2 and control corrals. Three cores (7.5 cm diameter × 20 cm deep) were collected near (<1 m from the corral) each CO_2 corral and each control corral, from which subsamples were collected by extracting the top 1 cm of the sediment from a portion of the core using a 60 cc syringe with the Luer end removed. Subsamples were preserved immediately in a 2% glutaraldehyde solution in 0.1 M cacodylate buffered, filtered seawater. A Percoll density-gradient centrifugation technique was used to extract meiofauna from aliquots of the subsampled sediment. Details of this technique are discussed in Buck *et al.* (2000). Counts and biovolume measurements of meiofauna stained with the fluorochrome DAPI were made using epifluorescence microscopy. Estimates of abundance in each core represented the total biovolume estimated from the sample. Tissue condition (live/dead) was assessed for a subsample of nematode individuals collected. Individual nematodes stained with DAPI were inspected under epifluorescence microscopy for the presence (live) or absence (dead) of intact cell nuclei.

2.3 Physical measurements

Changes in seawater chemistry caused by the dissolution plume were measured using SeaBird pH sensors

attached to a SeaBird model 16 CTD. The pH probes were positioned 3–5 cm above the seafloor and located 1 m from CO_2 corrals. Seawater pH, as well as temperature, conductivity, and depth, was recorded at 10 minute intervals throughout the experiment to determine the intensity and duration of plume exposure near CO_2 corrals.

The direction and speed of near-bottom currents at the site were measured using an acoustic doppler current meter (ADCP) deployed 2 m above the bottom. This ADCP measured current speed at 10-minute intervals for bins 4 to 50 m above the seafloor. The current speed 10 m above the bottom was used to characterize the direction and speed of local currents.

Variation in pH and current speed were compared using spectral analyses to determine the dominant period of variation for the flow and pH changes near the corrals. Progressive vector diagrams were also plotted to determine the short (i.e. days) to medium (weeks) pattern of current flow.

2.4 Statistical analyses

The abundance of each meiofaunal taxon was compared between treatment (CO_2) and control groups by ANOVA at the beginning (prior to CO_2 release) and end of the experiment (after 1 month of exposure to CO_2). Because replicate cores used to estimate meiofaunal abundance were taken near each corral, these data were analyzed using a nested ANOVA design (corrals nested within treatments; Zar, 1999). Prior to analysis by ANOVA, variances of biovolume data for each meiofaunal taxon were compared between groups (treatment & control) using an F test (Zar, 1999). Square-root transformations were applied to biovolume data for taxa (Amoebae, Nematodes) with significantly ($p < 0.05$) different variances between treatments, after which, F-tests were non-significant.

The percentage of individuals that were dead for nematodes was compared between CO_2 and control treatments at the end of the experiment using a nested ANOVA, after applying an arcsine transformation to the percentage data. An F-test was used to evaluate heteroscedasticity among groups.

3. Results

Upon returning to the 3600 m CO_2 release experiment site with the ROV, our initial observations determined that much of the CO_2 released had dissolved, although a small amount of liquid CO_2 or CO_2 hydrate, or both, remained in some corrals. The sediment was disturbed in some corrals, apparently due to "frost heave" by the CO_2 hydrate, but the sediment surrounding the corrals, where samples were collected for meiofaunal analyses, was undisturbed.

The pH of seawater near corrals containing liquid CO_2 was highly variable during the experiment (Fig. 3), due to continued dissolution of the liquid CO_2 and shifting direction of bottom currents. This resulted in large peak pH perturbations (ΔpH ~ –1.5 units were observed within 1 m of the CO_2 corrals) during periods when currents were flowing over pH sensors, and little or no pH change when currents carried the CO_2 dissolution plume away from pH sensors. Excursions in pH greater than 1 unit were rare (<5% of the time) even near CO_2 pools, and reductions of ≥–0.2 units occurred only 25% of the time. Due to failure of a pH sensor placed near a control corral located 40 to 80 m from the CO_2 corrals, perturbations to seawater chemistry were not measured near these control corrals. However, it is expected that little to no variation in pH occurred near control corrals, based on subsequent measurements of pH versus distance that showed little to no pH perturbation at distances of ~50 m from CO_2 corrals.

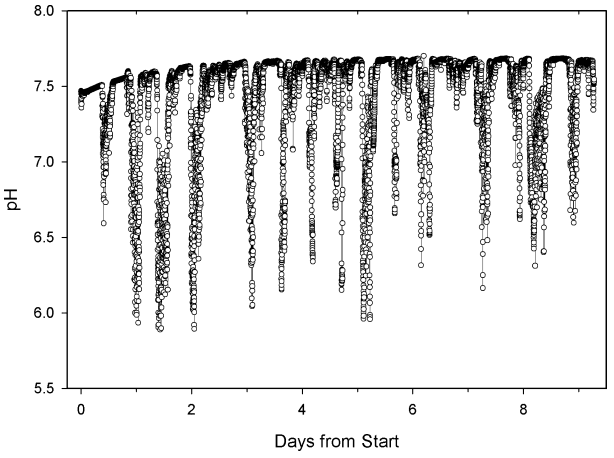

Fig. 3. pH perturbations near CO_2 corrals during CO_2 dissolution. Normal (background) pH is near 7.65 at this depth, and is reflected by the upper boundary of values recorded during the experiment. Note the large pH reductions approximately every 12 hours, which represent the advection of the dissolution plume over the pH sensor. Maximum pH shifts were near –1.7 pH units. The initially low background values (near 7.5) were due to the early equilibration of the instrument with the background deep-sea water pH, and are not due to the CO_2 dissolution plume.

Near-bottom currents were generally sluggish, but highly variable in direction (Fig. 4), as expected from the observed pH variability. Currents averaged 4.4 $cm \cdot s^{-1}$, with net transport to the SE at 1.7 $cm \cdot s^{-1}$, and rotated clockwise throughout the day over inertial periods, reversing direction approximately every 12 hours. Fourier analysis of currents and variation in pH values each indicated strong periodicity near 12.4 h, associated with the major semidiurnal lunar tidal constituent (M2). In effect,

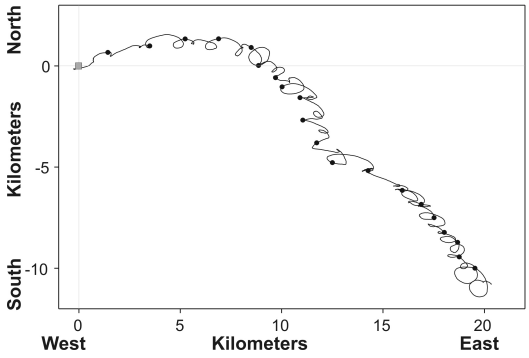

Fig. 4. Progressive vector diagram illustrating flow at 15 meters above bottom at 3600 m depth during the CO_2 experiment. The flow path of a parcel of water in the region is described by the advective transport from the starting point (gray box) to the end point (lower right corner). A black circle notes the start of each day. Note the rotary flow, which will disperse the CO_2 plume in several directions, even though the mean flow is to the SE. The major periodicity in both flow direction and speed is 12.5 hours, corresponding the semidiurnal lunar tidal constituent.

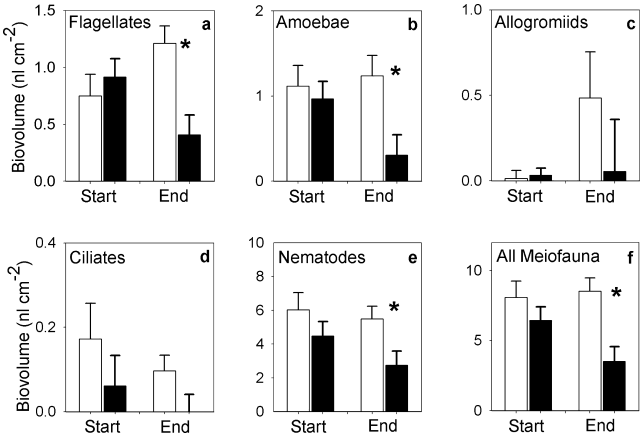

Fig. 5. Changes in biovolume of meiofaunal taxa after exposure to CO_2 dissolution plume. Open bars indicate core samples collected near control corrals. Dark bars are core samples collected near CO_2 corrals. Bars indicate mean (+SE) of treatments from nested ANOVA analyses. Start was at the beginning of the experiment prior to CO_2 injection into CO_2 corrals. End was after 4.5 weeks of exposure. Asterisk indicates significant (nested ANOVA, $p < 0.05$) different in abundance between control and CO_2 biovolumes.

the seafloor around each CO_2 corral (<2 m from the corral) was exposed to the CO_2-rich dissolution plume (i.e. ΔpH–1.0 or greater) for roughly 30 minutes twice per day, due to the inertial and tidal periodicity of bottom currents. The rotary character of currents then shifted the dissolution plume progressively clockwise, eventually bathing the entire seafloor around the corral with CO_2-rich seawater. The seafloor near the upstream side of each corral was exposed to normal seawater.

The population density or tissue condition of sediment-dwelling meiofauna declined after exposure to intense CO_2 stress, indicating that survival rates were low. Prior to CO_2 injection, no differences in the biovolume of any meiofauna taxon sampled (flagellates, amoebae, allogromiid foraminiferans, ciliates, and nematodes) were detected in samples collected near control corrals and CO_2 corrals ($p > 0.05$ for nested ANOVA tests). After one month intermittent exposure to the dissolution plume with pH perturbations up to –1.7 pH units, we detected significant differences in the biovolumes of the two dominant groups (Fig. 5). Flagellates increased slightly in biovolume near control corrals, but declined near CO_2 corrals, leading to a large difference in biovolume ($F = 12.0$, $p < 0.003$) by the end of the experiment. Amoebae exhibited a similar pattern of divergence in biovolume between control and CO_2 corrals. By the end of the experiment, control corral locations had a higher ($F = 6.7$, $p < 0.02$) biovolume of amoebae than CO_2 corral sites. Reduced densities of both groups probably reflect the death and nearly complete decay of individuals impacted by CO_2 exposure. Although decay rates are unknown, observations of degradation rates for much larger macrofaunal amphipods from this site indicated that significant tissue loss occurs over the month-long experiment. In view of their smaller size, we postulate that small meiofaunal taxa degrade completely during a similar period.

Unlike flagellates and amoebae, ciliates and allogromiid foraminifera did not decrease in biovolume during exposure to the CO_2 dissolution plume. Because these taxa are relatively low in abundance, however, our sampling design may be inadequate to detect small changes in abundance or biovolume.

Nematodes, the most prevalent meiofaunal taxon inhabiting the sediment, also declined significantly in biovolume near CO_2 pools during the experiment ($F = 7.9$, $p < 0.02$), and even higher rates of mortality were indicated from analyses of tissue condition. Detailed inspection of individuals stained with DAPI under epifluorescence microscopy (indicating the presence/absence of intact cell nuclei; Fig. 6), indicated that most (63.0%, SE = 12.1) individuals collected near CO_2 corrals had died prior to preservation. The percentage of decaying nematodes near control corrals (16.4%, SE = 5.8) was significantly ($F = 5.9$, $p < 0.02$) lower than near CO_2 corrals. These metazoans likely require longer decay times due to their chitinous cuticle.

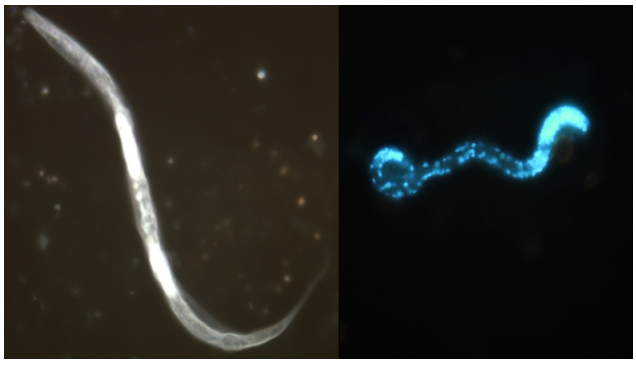

Fig. 6. Condition of nematodes exposed to CO_2 dissolution plume. A. Microscopic image of DAPI stained nematode with intact cell nuclei (light blue dots) indicating the individual was live immediately prior to preservation. B. Gray image of degrading nematode. This individual is also stained with DAPI, but exhibited no DAPI epifluorescence, and thus, no intact nuclei, indicating death prior to preservation. Individuals of this type were commonly observed near CO_2 corrals.

4. Discussion

These results provide a glimpse of the types of ecological impacts to deep-sea benthos that are likely to occur near sites of CO_2 injection during a direct ocean CO_2 injection program. In areas where CO_2 injection results in relatively large perturbations to seawater chemistry (i.e. ΔpH > –1.0 units), even for short periods as observed in these experiments, it is likely that meiofaunal taxa will exhibit high rates of mortality. All abundant meiofauna experienced high mortality near CO_2 pools, shown by reductions in abundance or biovolume, or higher percentages of dead individuals at the end of the experiment.

Although we were unable to perform any physiological measurements on these organisms, it is very likely that acidosis from immersion in hypercapnic waters exerted lethal physiological stresses. Meiofauna, including the amoebae, euglenoid flagellates, and ciliates studied here, as well as relatively primitive metazoans (nematodes), lack complex respiratory or circulatory systems that could be impaired by acidosis, but their ability to maintain normal physiological function is, nevertheless, linked to acid-base balance. CO_2-related acidosis, an imbalance in the optimal acid-base status of inter- and intracellular fluids, impairs physiological function in several ways, including changes in the activity of key enzymes and enzyme-protein interactions that affect even the most basic metabolic functions (Hochachka and Somero, 2002). Some pH compensation, via the buffering capacity of intracellular fluids, coupled with active proton-equivalent ion transport may restore normal internal pH for some degree of environmental pH perturbation (Seibel and Walsh, 2003). Failure to maintain optimal pH levels, however, will be lethal, or require a reduction in energy allocated to activity, growth, or reproduction. Physiological stresses caused by hypercapnia and acidosis often act together due to the bicarbonate buffering, and the separate effects of these remain somewhat obscure.

Our results support the expectation (Seibel and Walsh, 2003) that deep-sea species may be sensitive to pH stress that will accompany a direct CO_2 injection sequestration program. The perturbations to pH near our CO_2 corrals was large compared to that expected over large regions of the oceans during a CO_2 sequestration program, but also have relevance for smaller pH perturbations that will occur distant from CO_2 release sites. CO_2-related physiological stress, if not lethal as observed in this study, will convey higher "costs of living" through the energetic costs of acid/base balance, restricted aerobic capacity, and inhibition of protein synthesis. These costs are expected to be highest for deep-sea organisms, which typically have limited metabolic capacity, as well as narrow ranges of tolerance to environmental variation. Physiological responses of individuals to increased CO_2 levels may translate into changes in the survival, growth, and reproduction rates of populations, and shifts in the ecosystem dynamics of deep-sea communities.

The integrated impacts of a direct CO_2 sequestration program on deep-sea ecosystems will depend on the depths, locations, method of injection, and certainly the volume of CO_2 injected. If liquid CO_2 is released undiluted, it will produce a CO_2 dissolution plume near ~pH 4 in the boundary layer at the release site. The plume will disperse and mix downcurrent, finally approaching background levels at a distance determined by several factors, including current speed, injection method, total CO_2 injected, rate of injection, and other factors. Animals in close proximity to disposal sites are at risk of high mortality rates, as observed for benthic meiofauna in this study. Plume effects over larger scales may be estimated coarsely from expected pH fields. For example, if 0.25 to 4 $GtCy^{-1}$ as CO_2 is injected for 100 y beneath 3000 m and disperses worldwide (see methods), the pH of the deep-waters of the entire world ocean will shift by –0.02 to –0.3 units. Even larger pH perturbations will occur in mixing zones that may extend 10s to 100s of km around disposal sites (Haugan and Drange, 1992; Caldeira and Wickett, 2002).

Direct deep-sea CO_2 sequestration could mitigate the anthropogenic rise in atmospheric pCO_2 that will almost certainly accelerate through this century. Fossil fuel conservation and alternative energy sources should be primary carbon management strategies, and any decision to implement a direct ocean CO_2 sequestration program

should include careful consideration of the balance between the lesser of two evils—the unabated effects of climate warming or acidification, or both, on terrestrial and shallow marine ecosystems, or damage to deep-sea ecosystems by CO_2 sequestration. Ongoing research should provide guidance concerning the risks of direct CO_2 injection, and may mandate other methods or more environmentally benign CO_2 sequestration approaches (e.g. accelerated carbonate dissolution; Caldeira and Rau, 2000). Clearly, an ocean carbon sequestration program will be successful only if its intended benefits—a stabilization of atmospheric CO_2 and mitigation of climate warming consequences for terrestrial and shallow water ocean systems, outweigh its liabilities—energy expended on sequestration and damage to deep-sea ecosystems. Lacking presently is sufficient information on both sides of this balance.

Acknowledgements

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