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The response of abyssal organisms to low pH conditions during a series of CO_2-release experiments simulating deep-sea carbon sequestration

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ABSTRACT

The effects of low-pH, high-pCO_2 conditions on deep-sea organisms were examined during four deep-sea CO_2 release experiments simulating deep-ocean C sequestration by the direct injection of CO_2 into the deep sea. We examined the survival of common deep-sea, benthic organisms (microbes; macrofauna, dominated by Polychaeta, Nematoda, Crustacea, Mollusca; megafauna, Echinodermata, Mollusca, Pisces) exposed to low-pH waters emanating as a dissolution plume from pools of liquid carbon dioxide released on the seabed during four abyssal CO_2-release experiments. Microbial abundance in deep-sea sediments was unchanged in one experiment, but increased under environmental hypercapnia during another, where the microbial assemblage may have benefited indirectly from the negative impact of low-pH conditions on other taxa. Lower abyssal metazoans exhibited low survival rates near CO_2 pools. No urchins or holothurians survived during 30–42 days of exposure to episodic, but severe environmental hypercapnia during one experiment (E1; pH reduced by as much as ca. 1.4 units). These large pH reductions also caused 75% mortality for the deep-sea amphipod, *Haploops lodo*, near CO_2 pools. Survival under smaller pH reductions ($\Delta pH < 0.4$ units) in other experiments (E2, E3, E5) was higher for all taxa, including echinoderms. Gastropods, cephalopods, and fish were more tolerant than most other taxa. The gastropod *Retimohnia* sp. and octopus *Benthoctopus* sp. survived exposure to pH reductions that episodically reached −0.3 pH units. Ninety percent of abyssal zoarcids (*Pachycara bulbiceps*) survived exposure to pH changes reaching ca. −0.3 pH units during 30–42 day-long experiments.

1. Introduction

Although it is widely accepted that reducing greenhouse gas emissions is a key for avoiding dangerous climate warming and associated consequences, international efforts to curtail emissions have been largely unsuccessful. Some progress has been made, with 19 of 37 signatory nations to the Kyoto Protocol having met their emission reduction targets (IEA, 2011), but global emissions rose nonetheless from 21 to 29 Mt CO_2 y^{-1} (+38%) between 1990 and 2009. An effective emissions reduction program will require broad application of a portfolio of carbon-free energy alternatives, methods for increased energy efficiency, and carbon storage strategies (Pacala and Socolow, 2004). Development of carbon capture and storage methods have focused on C storage in the biosphere and in suitable geologic strata such as deep aquifers, depleted oil and gas wells, or deep ocean sediments and porous subseabed formations (Anderson and Newell, 2004; Yang et al., 2008; Herzog, 2011). Carbon storage by direct injection of waste CO_2 into the deep ocean (e.g. Marchetti, 1977) has been considered, but avoided owing to concern for environmental damage (Tamburri et al., 2000; Herzog, 2001; Seibel and Walsh, 2001). Deep-sea C storage is also thought to be possible through iron fertilization of ocean surface waters (Buesseler et al., 2008; Vaughan and Lenton, 2011), but has unknown efficiency and is also expected to alter environmental conditions and ecosystem function in the deep-sea.

The urgency for climate stabilization is likely to increase as atmospheric CO_2 levels and related climate consequences rise through this century. If so, concern for the impacts of global warming on terrestrial and upper ocean systems may eventually outweigh consideration of the potential impacts of ocean C storage for deep-sea ecosystems. Society may then decide to 'pull out all the stops' to avoid runaway climate change, and expand the use of deep ocean carbon storage and other methods that are currently avoided due to cost or environmental concerns.

Biological communities in the deep-sea are threatened by elevated environmental CO_2 levels (environmental hypercapnia)

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caused by the direct injection of waste carbon dioxide, or through the leakage of CO_2 from subseabed C storage sites. Carbon dioxide released at or near the seabed reacts with seawater to form carbonic acid, and can produce large and highly variable changes in ocean pH and carbonate saturation, particularly near release sites. Small scale experiments releasing liquid CO_2 in the deep-sea have measured pH levels less than 4.0 near pools of liquid CO_2 (Brewer et al., 2005). Models of boundary layer turbulence near CO_2 pools in the deep ocean indicate similar near-field and variable pH in the dissolution plume emanating from deep-sea lakes of sequestered CO_2 (Herzog et al., 2001; Fer and Haugan, 2003). The spatial extent and severity of pH perturbations near injection sites will depend upon the method of CO_2 injection, time-scale and rate of release, and local hydrography (Caldeira et al., 2005).

Deep-sea animals are expected to be highly sensitive to high-CO_2, low-pH dissolution plumes near deep-sea CO_2 injection or storage sites. The ability of animals to tolerate environmental change is based on physiological repertoires that have evolved over thousands of generations, and taxa inhabiting the typically stable conditions in deep-ocean waters are generally more sensitive to environmental perturbations of any sort than related shallow-water taxa (Seibel and Walsh, 2003). Most deep-sea taxa have lower metabolic rates (largely due to reduced temperature) and reduced enzyme function – both key factors for coping with physiological stress – compared to their shallow water counterparts (Seibel and Walsh, 2003). Energy limitation in the deep-sea may also constrain the ability of animals to increase energy allocation toward acid–base regulation and other physiological processes used to cope with physiological challenges associated with environmental hypercapnia.

Few studies have examined the sensitivity of deep-sea animals to variable, low-pH conditions near deep-sea CO_2 storage sites. Low tolerance of key community taxa to high ocean pCO_2 levels caused by ocean carbon storage could disrupt the function of deep-sea food webs, leading to reduced biodiversity, shifts in community structure, and reduced community production. A series of experiments used to evaluate the potential impacts of a large scale deep-sea carbon dioxide storage program on benthic deep-sea communities were performed by releasing small pools of liquid CO_2 on the seabed off Central California (Barry et al., 2005). Reports from these experiments found that meiofauna, including harpacticoid copepods, euglenoids, and foraminifera experienced elevated mortality after exposure to episodic pH changes of ca. –0.2 units (Barry et al., 2004; Carman et al., 2004; Thistle et al., 2005, 2006, 2007). In this paper, we report the response of various taxa to episodic exposure to low-pH dissolution plumes near pools of liquid CO_2 released on the seabed at abyssal depths, including changes in abundance and biodiversity.

2. Methods

2.1. Study area

Four carbon-dioxide release experiments (CO_2—1, 2, 3, 5; hereafter E1, E2, E3, E5) were performed at two abyssal sites near the base of the continental rise off the central California coast. Site A (3600 m, E1, E3, E5) and Site B (3320 m, E2) were both characterized by a flat, soft-sediment environment (Fig. 1). Bottom water temperatures were near 1.5 °C, with oxygen levels of ca. 125 μmol kg^{-1} and ambient pH of ~7.78 (SWS). Currents were generally sluggish (< 5 cm s^{-1}) and oscillated in direction over the dominant semidiurnal tidal period near 12 h (Barry et al., 2005).

The sediment-dwelling macrofauna at Site A were dominated by a dense assemblage of *Haploops lodo*, a tube-dwelling ampeliscid amphipod, but also included numerous other Crustacea, Polychaeta, Mollusca, and Cnidaria. The macrofaunal assemblage at Site B was very similar to Site A, with much lower densities of *H. lodo*. Meiofauna

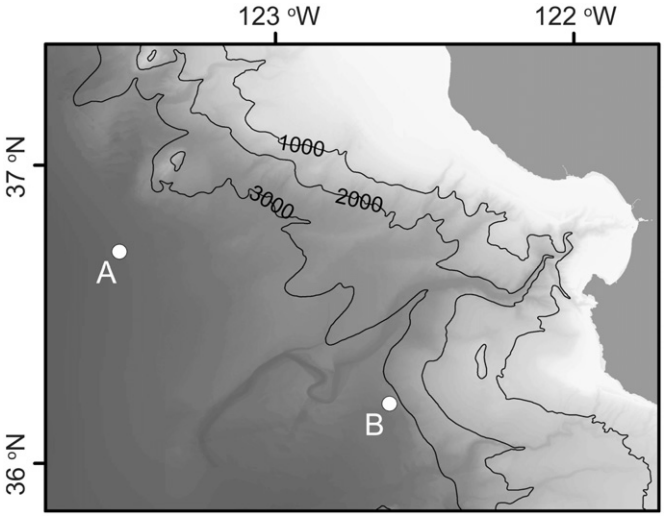

Fig. 1. Map of study sites on the continental slope off central California. Site A (3600 m) was used for E1, E2, and E5. Site B (3310 m) was used for E2. Depth contours in meters.

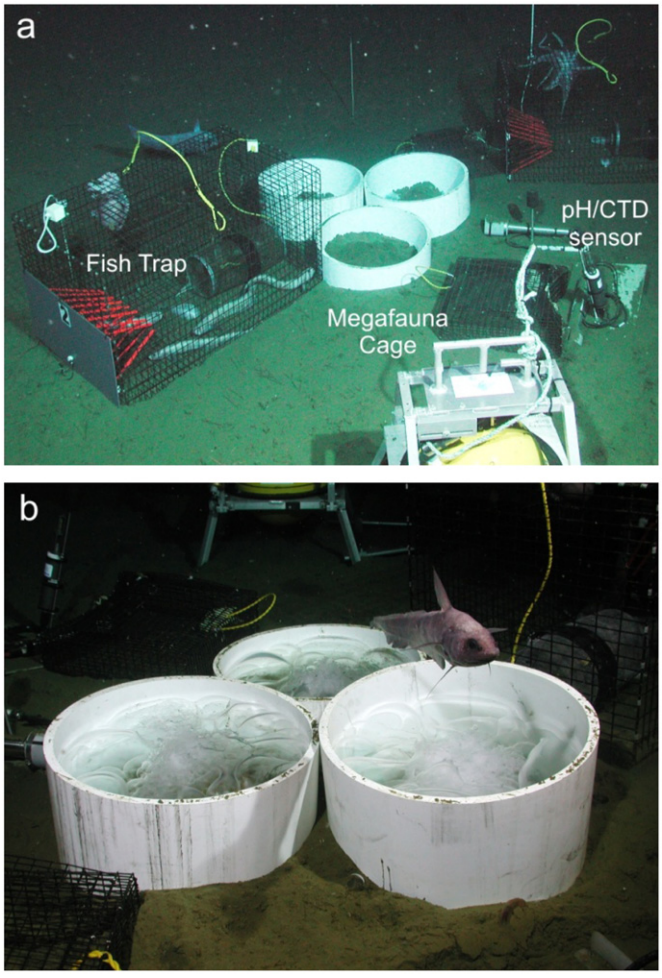

Fig. 2. Photographs of CO_2 release experiment E2. (a) Overview of E2 showing fish traps with zoarcids (*P. bulbiceps*) and octopus (*Benthoctopus* sp.), smaller megafaunal cages, and sensors. CO_2 containers (center) are mostly empty because the image was taken at end of experiment. (b) Close-up of CO_2 containers at start of E2, showing liquid CO_2 (~100 l) and a macrourid fish (*C. armatus*) swimming above the containers.

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were abundant at both sites, and dominated by nematodes, flagellates, and amoebae, with lesser densities of ciliates, foraminifera, and other taxa. The local abyssal megafaunal assemblage in the region is typical of the eastern N.E. Pacific, with moderate densities of macrourid (*Coryphaenoides armatus*), zoarcid (*Pachychara bulbiceps*), and ophidiid (*Bassozetus nasus, Spectrunculus* grandis) fishes, octopods (*Benthoctopus* sp.), echinoderms (holothurians – *Amperima robusta, Staurocucumis abyssorum, Scotoplanes globosa*; echinoids – *Cystechinus loveni, Aporocidaris milleri, Echinocrepis rostrata* and ophiuroids), galatheid crabs (*Munidopsis* spp.), gas tropod molluscs (*Retimohnia* sp.), and anthozoan cnidarians (Actiniaria, Pennatulacea).

3. Experimental methods

We evaluated the response of deep-sea organisms to simulated deep-sea carbon storage in a series of in situ experiments by exposing deep-sea organisms to high CO_2, low-pH dissolution plumes emanating from pools of liquid CO_2 released onto the abyssal seabed. All experiments were performed using the ROV *Tiburon* operated by the Monterey Bay Aquarium Research Institute (MBARI). Experiments varied in duration from 30 to 42 days (E1, 41 d; E2, 42 d; E3, 31d; E5, 31 d). Each experiment was initiated by releasing ~20–100 l of liquid CO_2 into small containers (sections of PVC pipe 48–100 cm in diameter $\times$ 15–40 cm high) placed on the seabed (Fig. 2). Liquid carbon dioxide was transported to the seabed and injected into CO_2-corrals using an ROV-mounted CO_2-release system (Brewer et al., 1999). CO_2 in each corral dissolved slowly into bottom waters during each experiment, producing a CO_2-rich, low-pH dissolution plume that is slightly heavier than ambient seawater. Advection and mixing of the plume in the oscillatory bottom currents led to episodic and variable exposure of organisms on the seabed and in surficial sediments to the CO_2 dissolution plume (Barry et al., 2005).

The spatial arrangement of CO_2 corrals and animal cages varied among experiments (Fig. 3). Details of the design of these experiments, including the configuration of CO_2 corrals, variation in current direction & speed, and patterns of pH variability are presented in Barry et al. (2005). Briefly, our initial design (E1) planned for an ANOVA comparison of survival by animals very near (<1 m) and distant (>20 m) to CO_2 corrals. During experiment E2 & E3 we positioned animals at prescribed distances (1, 5, 10, 50 m) from centrally located CO_2 pools (~100 l total), attempting to capture the effects of a broader range of pH perturbations. A circular arrangement of CO_2 corrals with a diameter of ~15 m was used in E5, attempting to produce relatively stable pH perturbation (−0.2 units) near the center of the circle where animal sampling was concentrated.

These experiments were performed at abyssal depths to assess the sensitivity of deep-sea animals potentially at risk from future ocean C storage efforts, but also because of the physical qualities of CO_2. The pressure–temperature profile off California coastal waters defines a gas–liquid phase boundary for CO_2 near 350 m depth. Unlike water, liquid CO_2 is highly compressible. From the depth of the phase boundary (350 m) to ca. 2600 m, liquid CO_2 has a specific gravity less than seawater, and will float toward the surface when released (Brewer et al., 2005). Our experiments were performed at 3300–3600 m, a depth where liquid CO_2 is denser than seawater (specific gravity ~1.07), and sinks into containers on the seafloor. CO_2 hydrate is also stable at depths greater than ~340 m and formed in various amounts in each CO_2 pool.

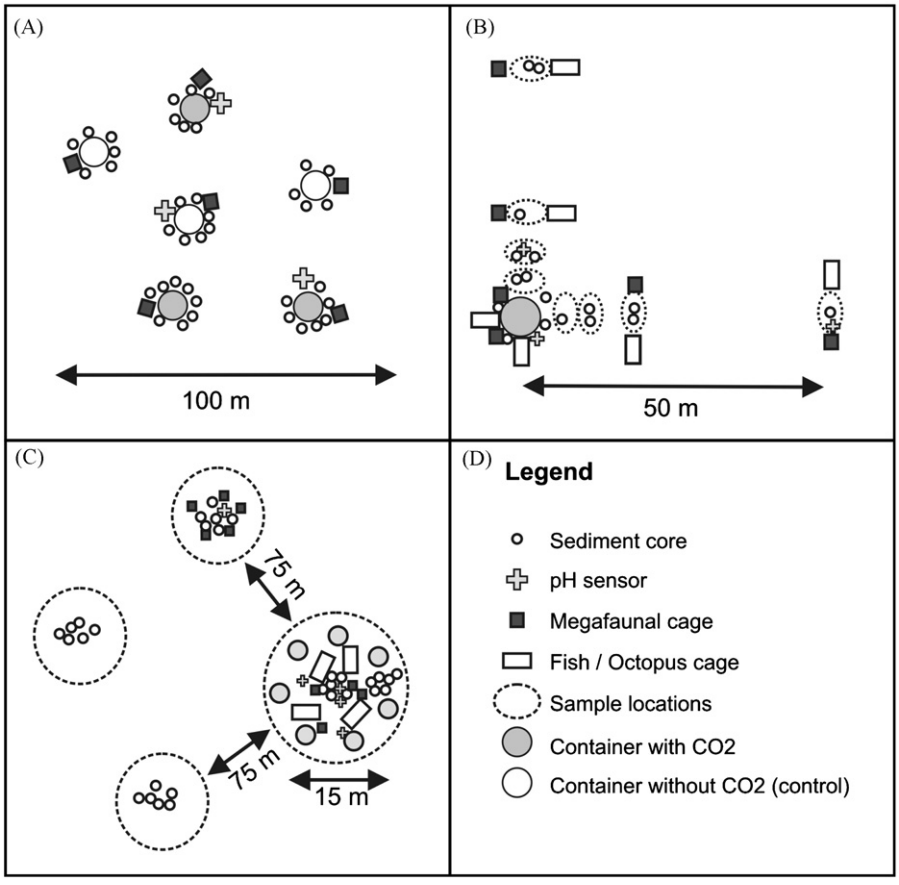

Fig. 3. Design of CO_2 release experiments. Large solid circles represent CO_2 (filled) and control (open) containers. Sediment core samples indicated by small circles. Cages for fish/octopus, & megafauna indicated by rectangles and gray squares, respectively. Sensors (pH, T, S) shown by stars. Dashed circles indicate sampling sites near or distant from CO_2 pools. (a) E1, (b) E2 & E3, and (c) E5.

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Tolerance of the abyssal microbial and macrofaunal assemblages to environmental hypercapnia caused by the dissolution plume was determined by comparing changes in their abundance (microbes) or percentage live/dead (macrofauna) at the start and end of each experiment. Samples for microbial and macrofaunal studies were obtained using replicate ROV-collected sediment cores (7.5 cm diameter × 20 cm deep) taken at specified distances (0–75 m) from CO_2 corrals both before CO_2 release and at the end of each experiment. Sediment cores were processed as soon as possible upon the recovery of the ROV to the surface. Microbial abundance was obtained from subcores (~1 cm^3) taken from the top 1 cm of replicate sediment cores. Samples for microbial abundance were preserved in 2% glutaraldehyde, and then counted using epifluorescence microscopy after dilution, vortexing, sonication to disassociate microbial cells from sediment grains, and staining with DAPI. For macrofaunal analyses, the top 0–5 cm section of sediment cores was sieved gently through 300 μm nytex mesh. Macrofaunal samples were preserved in a 10% formalin solution for 1–2 days, rinsed, and stored in 70% isopropyl alcohol until analysis.

The response of common abyssal megafauna [Echinodermata – (Echinoidea, *C. loveni*; Holothuroidea, *S. abyssorum*, *A. robusta*), Crustacea – (Galatheidae, *Munidopsis* spp.), Mollusca – (Gastropoda, *Retimohnia* sp.; Cephalopoda, *Benthoctopus* sp.), Chordata – (Zoarcidae, *P. bulbiceps*; Macrouridae, *C. armatus*)] to contact with CO_2 dissolution plumes was evaluated by positioning animals in mesh cages near or distant from CO_2 pools during each experiment. Several individuals of benthic megafaunal species common at the study sites were gently captured using an ROV-operated suction device. Five to ten individuals of each species captured were released into small mesh cages (46 × 46 × 20 cm), placed at specified distances (< 1 to 100 m) from CO_2 pools. Benthopelagic fishes (*C. armatus* and *P. bulbiceps*) and the deep-sea octopod, *Benthoctopus* sp., were collected prior to the start of some experiments using baited traps (bait was removed after capture), and positioned near or distant from CO_2 pools.

The nutritional status of each caged organism was unknown, and may have affected their ability to tolerate exposure to high-CO_2 waters. Macrofaunal organisms were unconfined and we expect that they would continue to feed normally unless there were important direct or indirect changes in food availability or quality caused by the dissolution plume. Cages confining smaller megafauna were open on the bottom to allow animals access to the sediment, and presumably allowing them to feed. Although caged fish and cephalopods were starved throughout the experiments, they (except for macrourid fish) exhibited the highest rates of survival among all organisms (see below).

The survival of megafauna was determined at the end of the experiment from visual observations of all individuals possible using the ROV's high resolution camera prior to collection from the cages. Movement of tentacles, spines, or feet, and active ventilation (e.g. fishes, octopus) were used to determine if animals were live. Animals were then recovered from cages and inspected in the laboratory for indications of tissue damage related to CO_2 exposure.

Because no metazoans collected at abyssal depths survived the ascent of the ROV to the surface, likely due to the barophilic nature of deep-sea animals (Somero, 1992), the condition (live/dead) of individual animals in each experiment was determined from in situ observations (video) or examination of body condition upon collection. For larger macrofauna (e.g. amphipods, *H. lodo)* mortality caused by CO_2 exposure was distinguished from death during ROV ascent by evaluating the condition of body tissues. Condition was estimated subjectively on a scale of 1 (intact tissues, recent death—*live*) to 5 (degraded, little tissue remaining—*dead*) (Fig. 4).

Degraded individuals with considerable tissue loss were considered to have been dead for a period much longer than a few hours (i.e. did not die during the ascent of the ROV), based on comparisons with tissue degradation rates of amphipods measured in separate assays. For this purpose, samples of *H. lodo* were collected from Site A using sediment core samplers during the initiation of E3, brought to the surface with the ROV, and sieved from sediment samples. Groups of ~25 dead individuals of *H. lodo* were placed in nytex mesh bags, then redeployed (placed on the sediment surface) at the site for the duration (31 days) of the experiment. Mortality (% individuals dead) was calculated as the percentage of all individuals with tissue ratings of ≥4.

3.1. Physical measurements

The direction and speed of near-bottom currents during most experiments (E1, E2, E5) were measured using an RDI Sentinel 300 MHz Acoustic Doppler Current Profiler. The instrument was deployed 2–4 m above the seabed in an 'up-looking' configuration, programmed to record profiles (1 m bins) of flow speeds in East, North, and vertical axes from 6–28 m above the seafloor at 5 min

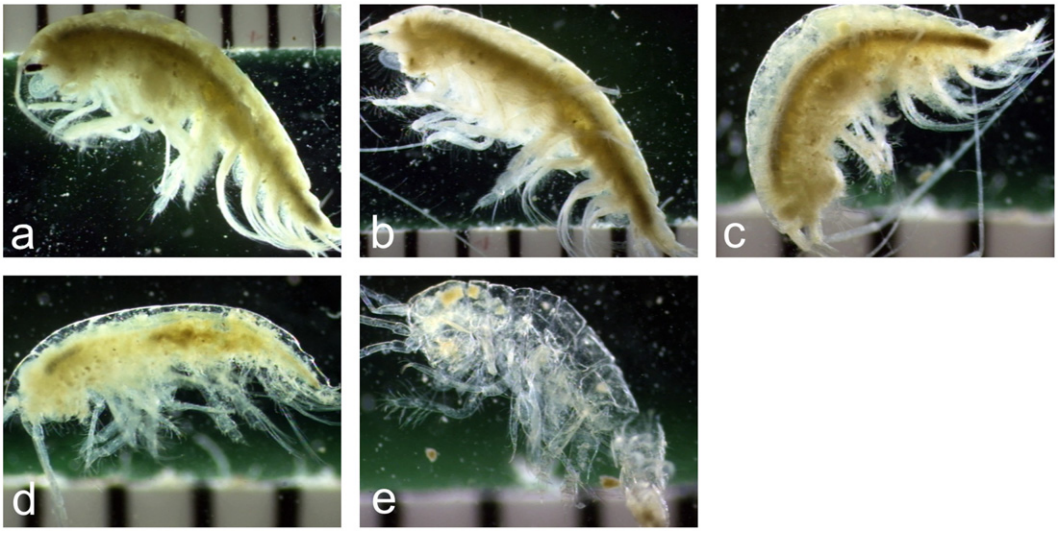

Fig. 4. Tissue condition rating for *H. lodo*. Condition was assigned according to degree of degradation from 1–5, corresponding to images a–e, respectively. Tissue condition equal or greater than 4 were considered to be 'dead'.

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intervals. Currents 8–15 m above the bottom were used to characterize the direction and speed of currents at the study sites.

The pH of the CO_2 dissolution plume was measured during each experiment using multiple pH sensors (SeaBird model 1278, logged on SeaBird Model 19+CTDs) positioned 3–50 cm above the seafloor at prescribed distances from CO_2 corrals. For example, time-series observations of pH (data intervals of 5–15 min) were obtained from 0.5 to 1 m away from one CO_2 and one control corral during E1, and at 1.5, 7.5, 25, 50, or 75 m from the central CO_2 corral during E2, E3, E5 (Barry et al., 2005). Temperature and salinity were recorded along with pH on each CTD.

pH sensors were calibrated using seawater standards (SWS) adjusted to pH 8.0 and 6.0 by titration with NaOH and HCl, respectively. SWS endpoints were monitored using an IQ Scientific Instruments pH probe calibrated against NBS buffers 6.00 and 9.18, and connected to Mettler Toledo Titration Controller. Slope and offsets were calculated prior to deployment for each sensor. In situ pH was calculated for the deployment depth based on water quality properties obtained from WOCE Station 17 (cdiac.ornl.gov/). pH data were adjusted using pre- and post-deployment calculations of slope to account for in situ, pressure-dependent intrusion of seawater to each pH sensor's electrolyte.

In addition to the pH measurements described above, we measured the pH of pore fluids in the upper sediment column both in and at various distances (0.1, 1.0, 8, 50–100 m) from CO_2 pools during in two additional abyssal CO_2 release experiments (not reported here), to assess the penetration of the CO_2 signal in the surficial sediments inhabited by microbes and macrofauna. Profiles of pore-fluid pH were measured by inserting (by micromanipulator) an Orion pH microelectrode directly into sediment cores (chilled to in situ ambient temperature near 1.5 °C, and under an N_2 atmosphere), or were measured in pore fluids extracted (squeezed) from 1 cm subsections of sediment cores. In all cases, pH profiles were obtained as soon as possible ($\sim < 2$ h) after recovery of the ROV to the surface.

3.2. Data analyses

For each experiment, we compared the condition or abundance of organisms collected before and after CO_2 exposure using ANOVA or regression methods, depending on the experimental design. ANOVA was performed using Systat (release 13) software, on raw or transformed data depending upon the normality and variance of samples. Non-parametric tests (Kruskal–Wallis) were used when transformations were ineffective in normalizing samples. Kolomogov–Smirnov tests were used to compare frequencies of tissue condition indices of *H. lodo* between periods and treatments during E1. Regression analyses (% live versus distance) were performed on arcsine transformed percentages, after inspection of the variance distribution among distances and application of a log-transformation, where appropriate. Effect sizes (Cohen's d) were calculated for 'before' versus 'after' comparisons for some macrofaunal analyses. The standard deviation of the 'before' samples pooled over all distances was used as the denominator (Cohen, 1992). Comparisons of megafaunal survival were only performed for data collected at the end of experiments.

4. Results

4.1. Variation in near-bottom pH and currents

Most liquid CO_2 released into corrals dissolved during release experiments (Fig. 2), producing a low-pH dissolution plume that drifted with near-bottom currents. Although several pH sensors failed during each experiment, deployment of duplicate sensors at most locations provided numerous continuous (5–15 min intervals over periods of 9–31 days) pH records at various distances from CO_2 pools, documenting the frequency and intensity of CO_2 perturbations as the dissolution plume was advected across abyssal sediments.

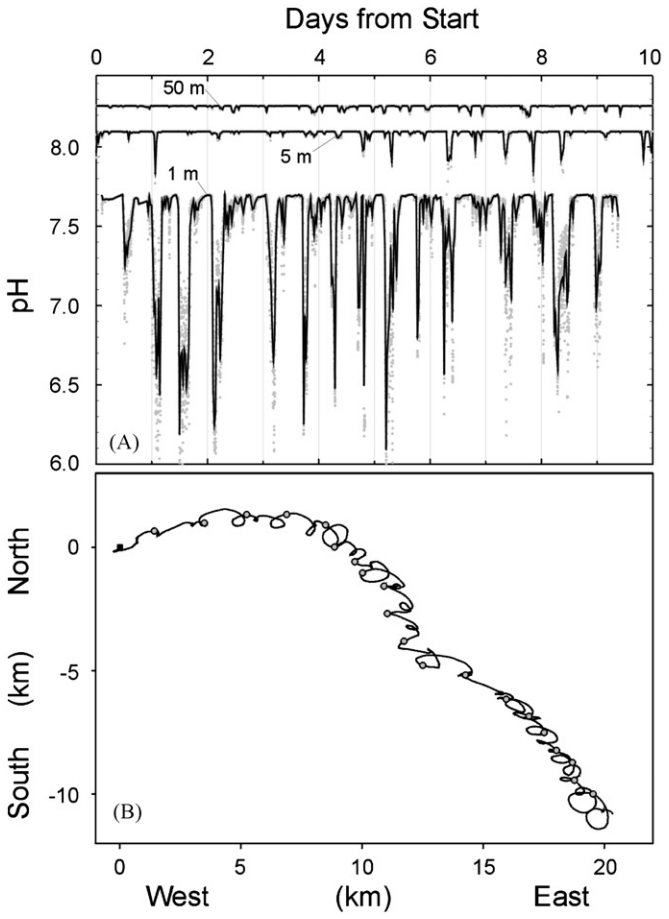

Fig. 5. Typical pH variation during CO_2 release experiments. (a) Variation in pH at distances of > 1 m (E1), 5 m & 50 m (E3) over 10 days. pH for each is offset to ease comparison among distances. Background pH for all experiments is ~7.78. Gray dots indicate raw data; black lines indicated smoothed data. Note the large perturbations near CO_2 pools in E1, and diminution of the pH signal with distance. (b) Progressive vector diagram during E1 indicating flow direction during initial 23 days. Open circles indicate successive days. Note the tidal oscillations that result in a constantly changing flow direction for the dissolution plume, leading to variable pH perturbations, as observed in (a). See Barry et al. (2005) for more information.

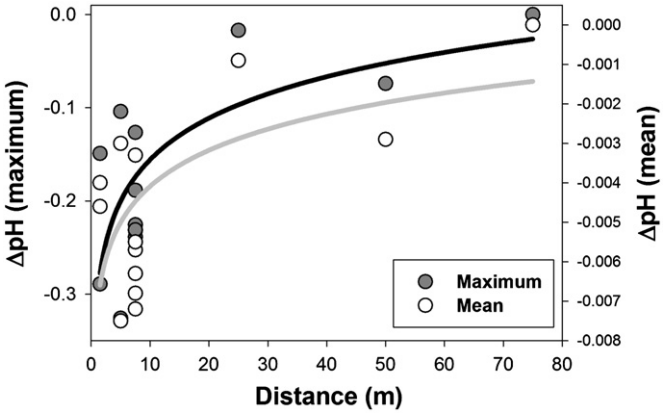

Fig. 6. Distance versus mean and maximum pH perturbations from the combined pH observations during E,2,3,5. Regression curves indicate maximum (black) and mean (gray) pH changes.

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pH in the vicinity of CO_2 corrals varied considerably during each experiment (Fig. 5). The largest pH shifts (ca. 1.4 pH units) were measured very near (<0.25 m) CO_2 corrals during E1, and may have been due to small amounts of liquid CO_2 spilling out of CO_2 corrals to the seabed very close to the pH sensors. Excursions >1 pH unit during E1 occurred in $<2.5\%$ of observations over a 9 day pH record, with pH reductions of ≥ 0.2 units during 16% of the experiment. Reductions of pH associated with the dissolution plume were smaller in subsequent experiments (E2, E3, E5) and diminished with distance from CO_2 pools (Fig. 6). At a distance of 2–8 m from CO_2 pools, the maximum and mean pH decreases were ca. −0.2 and −0.005 units, respectively, with perturbations of −0.1 units occurring in less than 1% of measurements. Records of pH during experiments E2, E3 and E5 were combined and analyzed in relation to distance from CO_2 pools using logarithmic regression ($\Delta pH = A(\ln(\text{distance}) + B)$ to characterize the general pattern of pH shifts with distance from CO_2 pools, indicating a rapid decline in the magnitude of pH perturbations with distance (Fig. 6). This regression model was significant for both maximum ($F=15.2$, $p<0.002$, $r^2=0.52$; $A=0.06$, $B=-0.30$) and mean ($F=8.45$, $p<0.015$, $r^2=0.34$; $A=0.001$, $B=-0.01$) pH changes. Maximum pH changes predicted by this model were used subsequently for regression analyses evaluating changes in abundance or survival of organisms measured in E2, E3, and E5. Overall, maximum pH reductions were roughly 35 times greater than mean pH reductions measured at the same location ($r^2=0.87$, $p<0.01$, $n=14$). Because of the variable current direction (see below), exposure to large pH reductions was typically limited to a short period each day when pH sensors (or animal cages) were located down-current from CO_2 pools. pH perturbations decreased with distance from CO_2 pools and were small to undetectable beyond ca. 50 m from CO_2 sources.

Currents during each experiment were generally sluggish (mean speed ~3–6 cm s^{-1}) and rotary in character, dominated by tidal oscillations (mainly a 12.4 h period, associated with the principal semidiurnal lunar tidal constituent M_2) and inertial currents (20.02 h at this latitude; Barry et al., 2005). Owing to the variable direction and speed of bottom currents, exposure of surficial sediments and caged metazoans to the dissolution plume near CO_2 pools was highly variable (Fig. 5). The dominant ~12 h period of these rotary currents carried the dissolution plume toward any particular direction (i.e. megafaunal cages) approximately twice each day for a relatively short period, particularly for experiments with a single CO_2 pool (i.e. E2, E3).

Profiles of pH in sediment pore fluids showed similar patterns of pH change to measurements in bottom waters (Fig. 7). Interstitial pH in the upper 5 cm of the sediment within CO_2 corrals under liquid CO_2 at the start of the experiment 1–2 days after CO_2 release was 5.7–6.3, or 1.5–2 units lower than measured at control locations. Profiles 10 cm from CO_2 pools were ~0.2 to 0.4 units below ambient. This pattern was also observed at the end of the experiment, after most or all of the liquid CO_2 in containers had dissolved. Interstitial pH 1 m or more from CO_2 pools was indistinguishable from control sites 100 m away.

4.2. Response of deep-sea organisms to low pH CO_2 dissolution plumes

4.2.1. Microbial assemblage

Prokaryotes (Bacteria and Archaea) were enumerated during two experiments (E1, E3) from sediment samples collected from very near (<1 m) CO_2 pools to control sites ~40 m away, both before and after CO_2 exposure. Microbial abundance near (~0.25 m) CO_2 pools in E1 decreased by 26% during the 41-day experiment during exposure to pH perturbations as large as −1.2 pH units. At control locations where pH changes were very small,

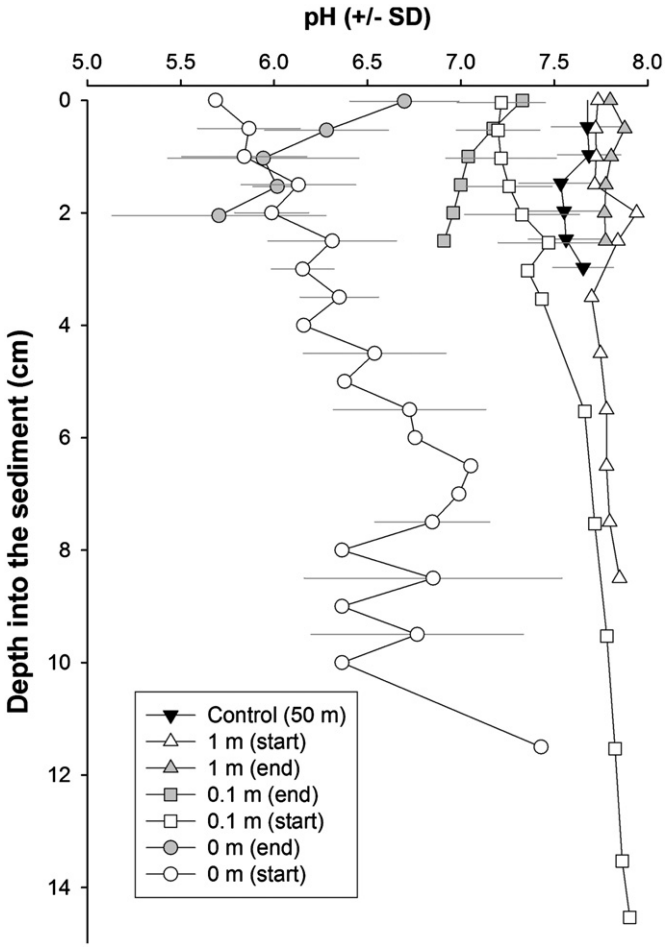

Fig. 7. pH profiles of upper sediment column in, near, and distant from CO_2 pools. Profiles are mean $\pm$SD ($n=1$–6) for cores collect inside CO_2 pools (0 m, circles), and at distances of 0.1 m (squares), 1 m (up-triangles), and 50–100 m (control locations; black triangles). White-filled and gray-filled symbols indicate samples collected at the start and end of experiments, respectively. Note the depression of pH beneath CO_2 corrals, particularly at the start of the experiments.

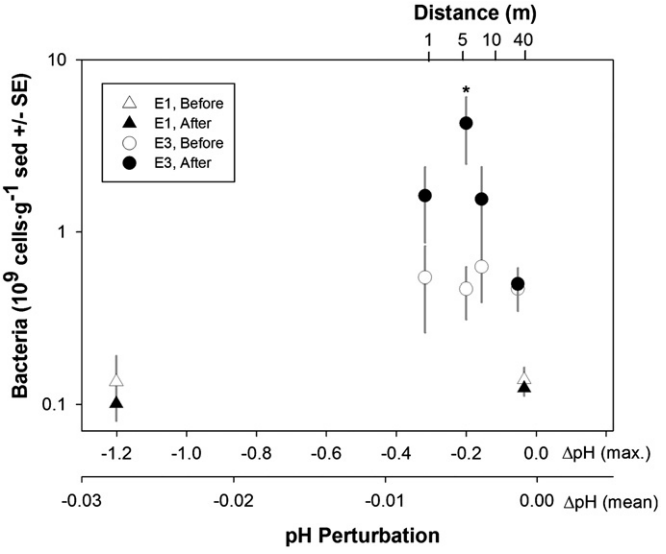

Fig. 8. Summary of microbial abundance measured during E1, E3. Symbols (circles, triangles) indicate billions of cells per g sediment. Open symbols indicate values at start of experiments E1, E3, prior to CO_2 release. Filled symbols represent mean abundance at the end of each experiment. * indicates a significant difference ($p<0.05$) between samples collected before and after CO_2 exposure during each experiment..

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microbial density dropped by only 11% (Fig. 8). The effect size (Cohen's d) was moderate or lower ($d < 0.4$), and ANOVA evaluating the influence of distance from CO_2 pools and time (before or after) was not significant ($F = 1.78$, $p > 0.2$). During experiment E3, microbial abundance was 3.8–17.4-fold higher than observed during E1. Abundance was relatively constant with distance from the central CO_2 pool before liquid CO_2 was released, but increased significantly ($F = 4.7$, $p < 0.05$) during the experiment, mainly near the CO_2 pool where maximum observed pH reductions were ~0.3 units. Microbial abundance increased by more than 8-fold at a distance of 5 m from the CO_2 pool, where the effect size was also largest ($d = 1.0$).

4.2.2. Macrofauna

The abundance and species richness of sediment-dwelling macrofauna decreased near CO_2 pools after exposure to the dissolution plume. Macrofaunal abundance measured from sediment core samples during E2 did not vary significantly ($F = 0.98$, $p = 0.34$, $r^2 = 0.09$) with distance from CO_2 corrals prior to filling them with liquid CO_2. After 42 days of episodic exposure to the CO_2 dissolution plume, macrofaunal density decreased significantly ($F = 15.49$, $p < 0.002$, $r^2 = 0.54$) as the magnitude of pH perturbations nearer the CO_2 pool increased (Fig. 9). The effect size between 'before' and 'after' samples declined with distance from CO_2 pools, from a value of 3.7 at 1 m, to 2.0 at 5 m, and less than 0.8 at greater distances. Polychaetes and nematodes captured on a 300 μm sieve each comprised roughly one third of the total macrofaunal abundance and 15% of the total biomass. While most major taxa showed a trend toward lower abundance or richness nearer CO_2 pools, polychaetes were the only group whose abundance decreased significantly with declining pH ($t = 2.406$, $r^2 = 0.31$, $p < 0.01$).

Species richness also declined in relation to pH reductions near CO_2 pools by the end of E2 ($F = 11.18$, $p = 0.009$, $r^2 = 0.46$), indicating that some taxa were sensitive to exposure to the environmental hypercapnia caused by CO_2 dissolution (Fig. 10). As observed for macrofaunal abundance, no pattern of species richness was detected before CO_2 release ($F = 0.01$, $p = 0.98$, $r^2 = 0.001$). The decline in species richness nearer CO_2 pools was explained mainly by a significant reduction in polychaete richness with larger maximum pH changes ($t = 2.65$, $r^2 = 0.35$, $p < 0.01$). Similarly, the size of the pH effect was greatest from 1–5 m from the CO_2 pools ($d = 5.0$, 1.8, respectively). A similar, but non-significant decrease in mean biomass under higher pH changes was observed (not shown).

The tube-building amphipod *H. lodo* occurred in dense beds at Site A and was the focal species for macrofaunal studies during E3 and E5. Decomposing specimens (condition index ≥4) were assumed to have died in response to environmental hypercapnia associated with CO_2 pools. Survival of *H. lodo* was lowest near CO_2 pools for both experiments (Fig. 11), where pH varied episodically by as much as −0.45 units. Regression of the proportion of live *H. lodo* (pooled samples from E3, E5) versus the maximum estimated pH changes (or distance from CO_2 pools) was non-significant ($F = 1.07$, $p = 0.31$, $r^2 = 0.02$) prior to CO_2 release. By the end of E3 and E5, survival of *H. lodo* decreased significantly with higher maximum pH changes (post-E3, E5 observations pooled; $F = 25.64$, $p < 0.0001$, $r^2 = 0.30$,), indicating increased mortality following exposure to mild average pH changes coupled with episodic pH reductions of −0.3 or greater. The effect size measured between 'before' and 'after' samples at each distance was greatest ($d = -2.9$) within CO_2 pools, large from 1 to 5 m (−1.4, −1.1, respectively), and low (< 0.3) from 10 to 100 m distant. Not surprisingly, nearly all *H. lodo* collected from sediment cores taken within CO_2 corrals (i.e. where liquid CO_2 had been present) were dead.

4.2.3. Megafauna

Benthic megafauna responded variably to environmental hypercapnia caused by CO_2 dissolution during 30–42 day-long experiments. A few taxa, notably echinoderms, were relatively intolerant and experienced high rates of mortality, while other groups (Mollusca, Chordata) were highly tolerant.

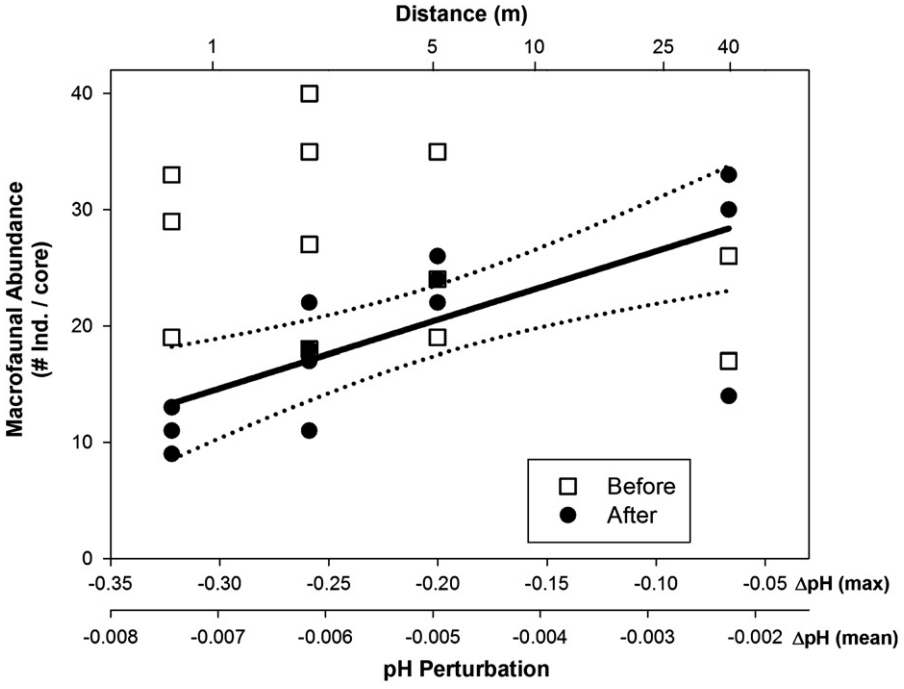

Fig. 9. Abundance of macrofauna in surficial sediments before CO_2 release and after 42 days of exposure to the CO_2 dissolution plume during E2, in relation to expected maximum and mean pH perturbations. Linear regression for samples collected 42 days after CO_2 release (black line, dashed line indicates 95% confidence band), with decreasing abundance of macrofauna nearer the CO_2 pool where pH perturbations were largest. Estimated distance (m) from CO_2 pool indicated on upper x-axis.

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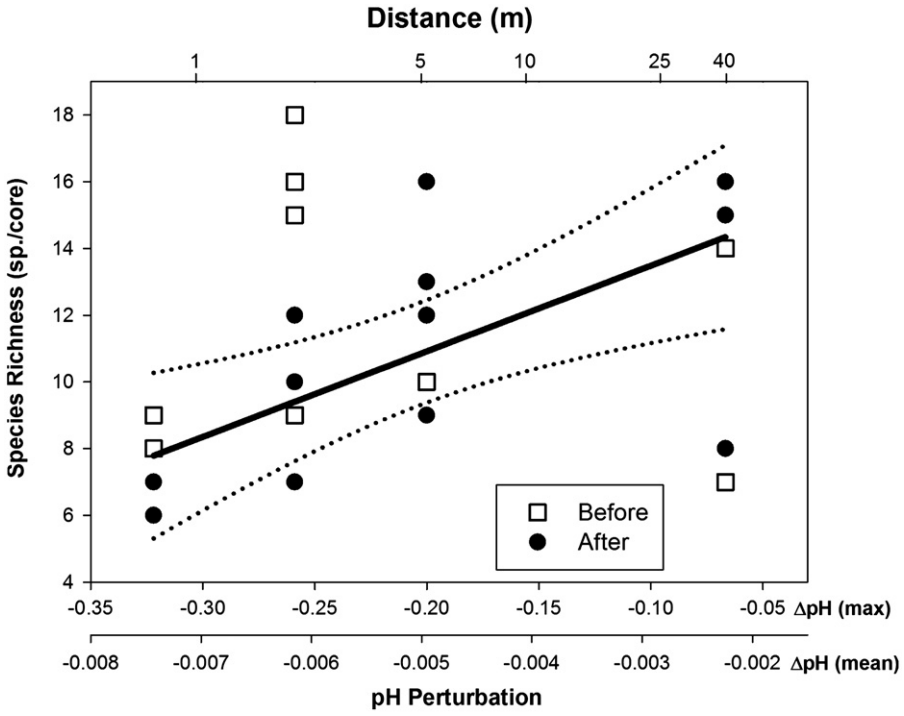

Fig. 10. Changes in macrofaunal species richness versus mean and maximum expected pH perturbations during E2. Symbols as in Fig. 9.

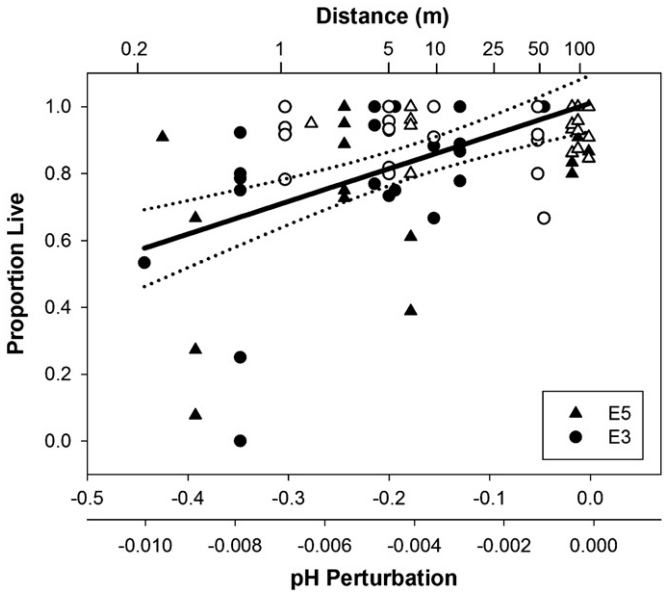

Fig. 11. Proportion of *H. lodo* (Amphipoda) individuals that were alive in sediment cores versus ΔpH and distance from CO_2 corrals during E3 (circles) and E5 (triangles). "Before" samples (open symbols) indicate core samples collected prior to CO_2 release. "After" core samples (filled symbols) were collected 31 days after CO_2 release. Maximum and mean pH changes (*x*-axes) were modeled from E2, E3, & E5 (lower *X*-axes). Black line represents the regression of proportion live versus maximum pH changes (combined for E3, E5).

4.2.4. Echinoderms

The echinoid *C. loveni* did not survive severe hypercapnic stress during E1. All *C. loveni* held in cages adjacent (< 1 m) to CO_2 pools during E1 (where maximum pH perturbations were ~−1.4 units) were dead within two weeks, and dissolution of skeletal elements was observed in several tests (Figs. 12 and 13). Small amounts of liquid CO_2 that spilled from the corrals onto the seabed adjacent to megafaunal cages undoubtedly intensified the dissolution plume and probably contributed to the observed skeletal dissolution. In contrast, all urchins held in control cages ~30 m from CO_2 pools during E1 survived ($U=0$, $p<0.025$).

A similar pattern of echinoid mortality was observed during experiment E3, even though pH perturbations associated with the CO_2-rich dissolution plume were likely milder (pH sensors within 1 m of CO_2 pools during E3 failed) than during E1. All urchins held in cages within 2 m of the central CO_2 pool died, but none showed obvious damage from skeletal dissolution. Survival was slightly higher 5 m from CO_2 pools after 31 days of exposure, where maximum pH reductions of 0.1–0.3 units were observed. All *C. loveni* held in distant cages (10, 50 m) where pH perturbations were mild and infrequent survived (KS=0.69, $p<0.05$ for survival among 1, 5, 10, 50 m),

Holothurians (*S. abyssorum*, *A. robusta, S. globosa*) exhibited variable survival near CO_2 pools. None of these species survived CO_2 exposure within 1 m of CO_2 corrals, as was evident from the observations of dead, decaying individuals at the end of each experiment in cages near CO_2 pools. *A. abyssorum* did not survive in cages adjacent to CO_2-filled corrals during E1, while survival was 100% in control cages 30 m away ($U=0$, $p<0.025$). Most *S. abyssorum* in cages more than 1.5 m from CO_2 corrals (i.e. ΔpH_{max}~−0.2 to −0.8 units) had escaped by the end of E1. The holothurian *A. robusta* did not survive exposure to the dissolution plume within 5 m of CO_2 pools during E3 and E5 where maximum pH changes were −0.2 pH units or greater, even though mean pH changes were only −0.005 units. At greater distances, *A. robusta* exhibited 100% survival, based on a total of 17 individuals recovered from 7 cages positioned 10–50 m from CO_2 corrals (Fig. 13). *S. globosa* was relatively rare at the study site and was used for limited observations. Three individuals were placed in direct contact with liquid CO_2 to evaluate the effects of acute CO_2 exposure appeared to die within minutes. In addition, a single individual placed in a cage ~50 m from CO_2 pools (E3) survived 31 days of exposure to the very weak dissolution plume at that distance.

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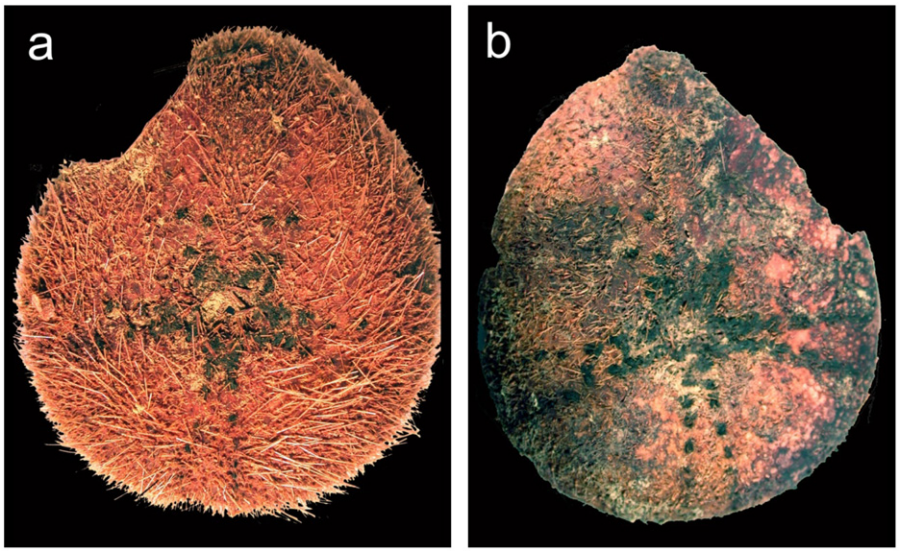

Fig. 12. Photographs of *C. loveni* from E1. (a) an individual collected far from a CO_2 pool showing normal skeletal condition, and (b) an individual within 10 cm of a CO_2 pool showing skeletal dissolution.

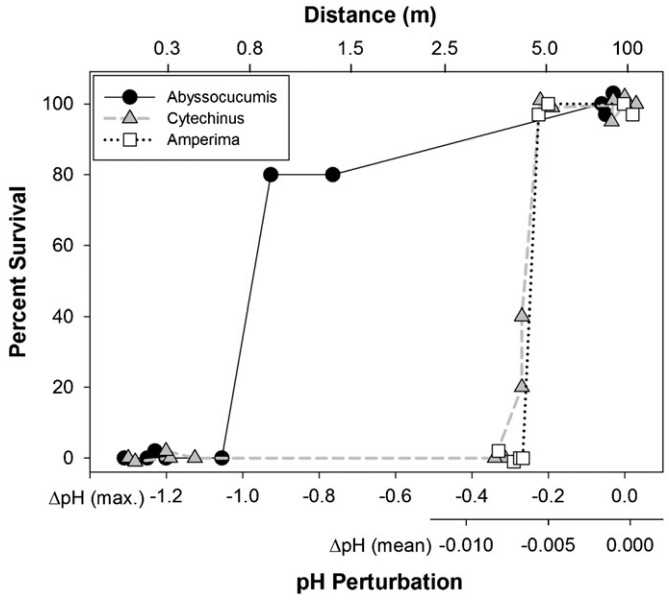

Fig. 13. Survival of megafaunal echinoderms exposed to CO_2 dissolution plumes during E1 & E3. For each species, each point represents its percentage survival within a single megafaunal cage positioned from 0.5 to 100 m from CO_2 pools, scaled by the estimated maximum pH perturbation observed. The number of individuals in each cage varied from 1 to 10 individuals. Data where pH changes exceeded −0.5 units are from E1. Observations with smaller pH perturbations are from experiment E3. Position of points were altered slightly to avoid overlap. Mean pH changes are shown for E3 only. Distance from CO_2 pools indicated in relation to pH changes.

4.2.5. Mollusca

Molluscan survival was high during exposure to the CO_2 plume. Seven individuals of the abyssal gastropod *Retimohnia* sp. were recovered live from cages during E2 and E3, representing 17% of the number deployed in cages. The missing study animals could have escaped or were overlooked during inspection of the cage at the end of the experiments. It is also possible that gastropods or other animals entered cages during each experiment, biasing results, but this seems unlikely, considering no species were discovered in cages that were not placed there initially. The surviving individuals were recovered from cages 1 to 30 m from the centrally located CO_2 pools. In addition, many (10+individuals) *Retimohnia* sp. were observed crawling on the outside walls of the large CO_2 corral (91 cm diameter × 40 cm high) the end of E3, even though it was nearly (~85%) full of liquid CO_2 and the dissolution plume was presumably exposing these individuals to reduced pH waters.

Survival of the benthic octopod *Benthoctopus* sp. was high regardless of distance from CO_2 corrals in E3 and E5. Single individuals captured in traps with demersal fishes and placed 1 and 25 m (E3) or 1.5, 4, and 100 m (E5) from CO_2 pools all survived exposure to pH perturbations of 0 to −0.3 units (maximum). Time-lapse observations of one of these *Benthoctopus* sp. are reported in Barry and Drazen (2007).

4.2.6. Crustacea

Galatheid crabs (*Munidopsis albatrossae* and perhaps other *Munidopsis* spp.) were also relatively tolerant to pH stress near CO_2 pools, as all of 10 individuals assayed during E2 survived. Single individuals of *M. albatrossae* held in each of 2 cages placed 1 m from the central CO_2 pool in E2 survived the 43 day experiment. Likewise, all individuals held at greater distances (5 individuals at 5 m distance, 3 individuals at 10 m) from the central CO_2 pool also survived.

4.2.7. Pisces

The demersal zoarcid *P. bulbiceps* exhibited high survival during exposure to elevated CO_2 levels. Of 26 individuals captured, 23 survived at distances of 0.5—100 m from CO_2 during E2, E3, and E5 where maximum and mean pH changes were as much as −0.4 and −0.01 units, respectively, nearest CO_2 corrals. Those that died were 3 m (2 individuals) and 100 m (1 individual) from the CO_2 pools in E5. In contrast, no individuals of the bentho-pelagic macrourid *C. armatus* ($n=14$) survived inside traps during E2, E3, and E5, regardless of position with respect to CO_2, suggesting that mortality was unrelated to CO_2 exposure. See Barry and Drazen (2007) for additional details.

5. Discussion

5.1. Efficacy of CO_2 release experiments

CO_2 release experiments reported here were designed to mimic changes in deep-ocean chemistry associated with a direct deep-sea

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carbon dioxide storage program. Although such an injection program would produce wide variation in ocean pH within mixing zones near injection sites, we had hoped that the pH perturbations produced would be relatively stable through the experiment, so that specific thresholds for tolerance could be identified for the taxa studied. Nevertheless, pH near CO_2 pools varied widely during each experiment, ranging as much as 0 to −1.7 units during E1 and roughly 0 to −0.3 units in other experiments. Large pH perturbations during each experiment were relatively rare ($<1\%$ of all observations) and mean pH shifts were well less than 0.1 units, except during E1. Although it is not possible to determine if the observed mortality was due mainly to the mean or maximum pH change, it is clear that variable pH fields near CO_2 disposal sites or released from subseabed storage locations will impact deep-sea benthos.

5.2. pH sensitivity among taxa

High CO_2 levels in the dissolution plume emanating from CO_2 pools alter the equilibrium concentrations of several seawater carbonate system parameters (e.g. pH) that can affect physiological processes in deep-sea taxa. Owing to the relatively constancy of abyssal environments, we assumed that the carbonate parameters of ambient waters at our experimental sites were very near those reported for WOCE station 17, near San Francisco (cdiac.ornl.gov/). At 3600 m depth, temperature (~1.52 °C), salinity (~34.68), and pH (7.78, SWS) are typically stable, with total DIC of 2348 μmol kg^{-1} and total alkalinity of 2445 μmol kg^{-1}. Ω_{ca} and Ω_{ar} are both undersaturated, near 0.88 and 0.58, respectively. The addition of CO_2 by the dissolution plume will alter these parameters. For example, a pH reduction of −0.5 units (pH_{SWS} 7.28) will drive Ω_{ca} to 0.29, and Ω_{ar} to 0.19. In addition to disruption of acid–base balance, maintenance of calcified structure is expected to be more difficult or energetically costly.

5.3. Microbial community

Lack of a change or an increase in the abundance of the microbial community in response to 31–41 days of exposure to simulated deep-sea CO_2 release (~−0.01 to −0.3 units) could be due to both direct and indirect effects of environmental hypercapnia. Tolerance or adaptation by the microbial assemblage could promote survival and persistence. Ishida et al. (2005) reported results similar to ours from in situ, deep-sea experiments, with an initial reduction in microbial respiration in response to very high pCO_2 levels (5000–20,000 ppm), followed by an increase in their abundance and respiration, which they interpreted as an increase in the more tolerant taxa. In contrast, Coffin et al. (2004) observed a decrease in microbial growth over 96 h, in high-pressure laboratory vessels with elevated pCO_2 levels (pH range = 5.6–7.6). Increased microbial abundance observed during E3 may have been due to a shift in the microbial assemblage to more tolerant taxa, but could also simply be an indirect consequence of the high mortality of meiofauna during the experiment (Barry et al., 2004; Thistle et al., 2005). The microbial assemblage may have benefited from meiofaunal mortality through the simultaneous reduction in grazing/predation pressure, and increase in food availability vis-à-vis the moribund bodies of the meiofauna. Ishida et al. (2005) also documented a reduction in meiofaunal abundance. Together, these studies suggest that deep-sea microbial assemblages will be tolerant of projected changes in ocean chemistry, but this topic requires more careful consideration, particularly considering a potential shift in microbial remineralization of organic debris under acidic conditions as suggested by Widdicombe and Needham (2007).

5.4. Meiofaunal communities

The response of various meiofaunal taxa to high CO_2 exposure during these experiments have been reported elsewhere and generally showed fairly high sensitivity to pH stress. Thistle et al. (2006, 2007) documented very low survival rates of harpacticoid copepods after ca. 1 month of exposure to a CO_2 dissolution plume (ΔpH ~−0.7 units), with only 20% of all species surviving. Barry et al. (2004, 2005) reported similar rates of high mortality for several meiofaunal groups (flagellates, amoebae, allogromiid foraminifera, ciliates, nematodes) for E1 and E3 after exposure to maximum pH reductions of −1.7 and −0.33 units, and average pH changes near −0.14 and −0.008 units, respectively.

5.5. Macrofaunal community

The general reduction of macrofaunal density and species richness under larger pH changes near liquid CO_2 pools were driven principally by changes in the polychaete assemblage, a numerically and taxonomically dominant group at the study sites. Vulnerability to pH stress is likely variable among taxa, with more active species expected to have greater physiological scope to compensate acid–base disruptions and other pH-related stress than less active taxa (Whiteley, 2011). Widdicombe and Needham (2007) found little impact of reduced pH (−0.4 to −2.3 pH units) on the behavior of an active infaunal polychaete (*Neries* sp.) from shallow subtidal habitats. In contrast, Batten and Bamber (1996) reported high mortality for a less active nereid polychaete under milder pH stress.

Crustacean taxa varied in response to pH perturbations. Galatheid crabs appeared highly tolerant, while the ampeliscid amphipod *H. lodo* experienced low rates of survival near CO_2 pools in all experiments. Survival of *H. lodo* was comparable to that reported for harpacticoid copepods under pH stress (Thistle et al., 2005). Exposure to environmental hypercapnia has also been studied in deep-sea lysianassid amphipods (*Eurythenes* cf. *obesus*), which under short term exposure (2–15 min) became narcotized, but recovered upon immersion in normal pH waters (Vetter and Smith, 2005). *H. lodo* may have experienced similar narcosis due to the CO_2 dissolution plume, which would have inhibited any potential escape behavior. Watanabe et al. (2006) report that pelagic copepods are sensitive to elevated CO_2 levels, and that deeper taxa were more tolerant than shallow species.

The observation of significant penetration of the hypercapnic signal into the sediment only within or very near the CO_2 pools may be related to the episodic character of dissolution plume dispersal by benthic currents. Penetration to below 5 cm, as measured near the CO_2 pools (Fig. 7), is expected to have strong impacts on the infaunal community, except perhaps for taxa capable of vigorous irrigation of sediment burrows. Our measurement of pH profiles may have been biased by the effect of handling on burrow structures. Vibration and other motion during collection, transport, and analysis very likely collapses small burrows, thereby inhibiting ventilation of the sediment and disrupting existing heterogeneity in pH due related to burrows. Thus, these profiles likely overestimate the magnitude of pH changes experienced by infaunal animals that benefit from active or passive irrigation of sediment burrows.

5.6. Megafaunal community

Survival of metazoans under environmental hypercapnia during these experiments varied among taxa in relation to phylogenetic differences in physiological complexity. Echinoderms, the group with the greatest observed sensitivity to pH stress, have limited capacity to buffer internal pH due to their open water

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vascular system, weak ion exchange capacity, and largely cutaneous respiration system lacking respiratory proteins. Taxa with more developed adaptations for acid–base regulation, including complex respiration organs, strong ion-exchange capacities, and typically higher metabolic rates, such as fishes, cephalopod molluscs, and crustaceans, (including species studied here) are thought to be less sensitive to low pH stress (Widdicombe and Spicer, 2008). All echinoderms studied exhibited a very low tolerance to changes in seawater chemistry caused by the CO_2 dissolution plume. This contrasts with mixed results reported from studies of shallow water echinoids. Miles et al. (2007) found that the urchin, *Psammechinus miliaris*, was severely impaired by pH changes of –0.4 units over 8 days. Longer-term experiments have shown that at least some echinoids can acclimate to chronic moderate pH stress. *Strongylocentrotus droebachiensis*, although sensitive to short term hypercapnic exposure (Widdicombe and Spicer, 2008), is less impaired after 4 months (Stump et al., 2012), and fully acclimated after 16 months, with few detectable negative impacts on physiology or reproduction (Dupont et al., 2012). *S. droebachiensis* may be preadapted to moderate ocean acidification through the exposure of some populations to seasonal hypoxic and hypercapnic events. Similar capacities may be found in echinoids on the continental slope, particularly in areas with strong oxygen minimum zones, low pH, and steep environmental gradients. In contrast, abyssal species seem more likely to be intolerant of hypercapnia, due to the absence of such conditions over long time periods. The relative rarity of echinoderms in hydrothermal vent assemblages (Smirnov et al., 2000) may also be coupled to physiological intolerance to elevated pCO_2, since such vents are sources of high pCO_2 waters. Paradoxically, weakly calcified echinoids are a dominant element of the abyssal megafauna, even though carbonate saturation levels are well below 1, the threshold for dissolution.

Megafaunal molluscs and crustaceans exposed low pH conditions in these experiments were highly tolerant, with no detectable mortality either near or distant from CO_2 pools. The gastropod *Retimohnia* sp. and the deep-sea octopod *Benthoctopus* sp. have adaptations (e.g. thick epithelium and mantle, respiratory proteins, closed circulatory system (octopods)) that allow tighter control of internal acid–base balance than possible for more primitive taxa (e.g. echinoderms) relying on diffusion-based respiration. Their greater ability to defend internal pH through efficient gas exchange and internal ion regulation may promote their survival.

Fishes are expected to have even greater capacity for maintenance of internal acid–base balance (Ishimatsu et al., 2004, 2008; Pörtner et al., 2004), at least for short periods. Nevertheless, it was surprising to observe nearly 100% survival by *P. bulbiceps*, even within 0.5 m from pools of liquid CO_2. *P. bulbiceps* doesn't have a swimbladder, but species that do could be more sensitive to low pH because they have strong root effects to facilitate gas secretion in the gas gland. Root effects are particularly strong in deep-sea species which must secrete oxygen against a strong gradient (Noble et al., 1986).

The live/dead classification used for these experiments provides no metric for sublethal impacts of environmental hypercapnia, which may have been significant for these animals, even over the time scales of the experiments. For example, although *Benthoctopus* sp. survived these experiments, Seibel and Walsh (2003) showed that only a –0.3 unit reduction in internal pH can reduce by 40% the oxygen binding capacity of its hemocyanin. Even though it survived, its aerobic scope is likely to have been compromised during the experiments. Other taxa would have similar problems, with greater demand for both passive and active ion regulation to cope with internal hypercapnia that would result from immersion in high-CO_2 waters near CO_2 pools.

5.7. Implications for the future of deep-sea communities

Measures of mortality observed during these short-term experiments are only coarse indicators of the potential consequences of disturbance to deep-sea benthic communities by deep-sea carbon storage, leakage of CO_2 from subseabed storage sites, or eventually, passive ocean acidification. Sublethal impacts that influence demographic rates of populations were not examined here and may be more important that direct mortality. Impaired reproductive success due to impacts on adult fecundity, larval development, or other life history processes, as well as reallocation of energy to cope with acid–base disturbance, calcification, or other key processes, thereby reducing energy for growth and reproduction, could also drive important changes in population and community dynamics.

Several life processes in shallow water taxa spanning most animal phyla have been shown to be affected by ocean acidification. Though there is considerable variation reported among taxa, even within major groups, effects are generally negative for survival, calcification, growth, development, and abundance (Kroeker et al., in press). Notably, the effect sizes detected for many processes examined in response to ocean acidification decreased as the length of studies increased. Could this result indicate that short-term vulnerability to hypercapnic stress may be resolved over longer periods for many species (e.g. Dupont et al., 2012)?

Few deep-sea species have yet been examined, but in general, they have narrow physiological scope for various key processes compared to upper ocean taxa. Deep-living taxa are typically poorly calcified, develop and grow more slowly, and are less capable of buffering acid–base disturbances than related taxa from shallower depths (Seibel and Walsh, 2003; Pane and Barry, 2007). Seibel and Walsh (2003) compiled data from fishes, cephalopods, and crustaceans over a depth range from the surface to 1000 m, and found that the passive buffering capacity decreased logarithmically with depth of occurrence, presumably related to the higher water content and lower tissue concentrations of weak acids and bases with increasing depth. Because compensation of acidosis by way of active ion exchange (e.g. H^+-ATPase, Na^+/K^+-ATPase, or Na^+/H^+ exchangers) is energetically expensive (Pörtner et al., 1998) and deep-sea animals live in a food-poor environment, their ability to up-regulate production of these key enzymes is likely to be highly limited, further impairing their ability to tolerate environmental hypercapnia. In summary, large changes in deep-sea pH and related carbonate system parameters, regardless of source, are expected to be stressful for many taxa, but it remains unclear if even the most sensitive species studied here are capable of acclimation and adaptation to chronic deep-sea hypercapnia.

The impacts of ocean carbon storage or leakage from seabed C storage locations are likely to be localized, but will have effects on ocean chemistry that accumulate over time and merge with passive ocean acidification, leading to significant changes in the chemistry of the deep ocean – a change of –0.4 pH units is eventually expected to occur due to ocean acidification. The consequences of such environmental perturbations to deep ocean ecosystems cannot be predicted with confidence; however, the fossil record supports the notion that rising ocean pCO_2 can have very significant impacts on ocean communities, as documented for a number of extinction events (Knoll et al., 1996, 2007; Veron, 2001; Payne and Clapham, 2012). Survival under a more acidic ocean can increase the costs of metabolic maintenance and performance for many animals. Allocation of energy to pH compensation, increased ventilation, and other metabolic functions required for tolerance to elevated ocean carbon levels is highly likely to reduce energy available for growth and reproduction for individuals, leading to impacts on the demographic rates of populations. Depending on the capacity and time scales required for acclimation, sensitive species may or may not persist. Reduced

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reproductive effort could constrain population growth rates, reduce resilience following physical and biological disturbances, and increase the likelihood of local extinctions. For example, will deep-sea urchins and other taxa shown to be intolerant of high pCO_2 in our experiments, survive long enough to acclimate to long-term pH changes, ultimately, reducing energy costs and returning to normal function? If so, impacts on the population dynamics for deep-sea species may be smaller than expected. If not, the consequences of these population-level changes for communities could include reduced biodiversity, disruption of energy flow through food webs, and reduced community resilience and stability.

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