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Impact of intentionally injected carbon dioxide hydrate on deep-sea benthic foraminiferal survival

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Abstract

Sequestration of carbon dioxide (CO_2) in the ocean is being considered as a feasible mechanism to mitigate the alarming rate in its atmospheric rise. Little is known, however, about how the resulting hypercapnia and ocean acidification may affect marine fauna. In an effort to understand better the protistan reaction to such an environmental perturbation, the survivorship of benthic foraminifera, which is a prevalent group of protists, was studied in response to deep-sea CO_2 release. The survival response of calcareous, agglutinated, and thecate foraminifera was determined in two experiments at $\sim$ 3.1 and 3.3 km water depth in Monterey Bay (California, USA). Approximately 5 weeks after initial seafloor CO_2 release, *in situ* incubations of the live–dead indicator CellTracker Green were executed within seafloor-emplaced pushcores. Experimental treatments included direct exposure to CO_2 hydrate, two levels of lesser exposure adjacent to CO_2 hydrate, and controls, which were far removed from the CO_2 hydrate release. Results indicate that survivorship rates of agglutinated and thecate foraminifera were not significantly impacted by direct exposure but the survivorship of calcareous foraminifera was significantly lower in direct exposure treatments compared with controls. Observations suggest that, if large scale CO_2 sequestration is enacted on the deep-sea floor, survival of two major groups of this prevalent protistan taxon will likely not be severely impacted, while calcareous foraminifera will face considerable challenges to maintain their benthic populations in areas directly exposed to CO_2 hydrate.

Keywords: carbon dioxide sequestration, climate change, CO_2 injection, experiment, foraminifera, hypercapnia, meiofauna, Monterey Bay, ocean acidification, protist

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Introduction

In both the scientific and public arenas, much attention has recently focused on the issues of global warming and climate change. Although debate over the most appropriate mitigation pathway continues, it is certain that the atmospheric concentration of carbon dioxide (CO_2) has increased significantly in the recent past (e.g., Keeling *et al*., 1995; Keeling, 1998). One option to curtail the rapidly rising atmospheric CO_2 levels under consideration is to sequester waste CO_2 in the deep ocean (e.g., Caldeira *et al*. 2005). A number of scenarios have been proposed for such ocean carbon storage, including fertilization of the sea surface with iron to promote phytoplankton growth and accelerate the biological pump, thereby increasing dissolved inorganic carbon (DIC) export to the deep sea (e.g., Buesseler *et al*., 2004), CO_2 injection at mid-ocean water depths (e.g., Ozaki, 1997), and CO_2 injection onto the deep-sea floor (e.g., Brewer *et al*., 2000). Ongoing research is attempting to elucidate the benefits and drawbacks of each approach if implemented on the large scale. In particular, impacts on both ocean chemistry and inhabitants must be ascertained, especially given that CO_2 dissolution causes a concomitant decrease in pH (Brewer *et al*., 2000). Physiological responses to elevated CO_2, or hypercapnia, and ocean acidification are challenging to organisms in general (reviewed in, e.g., Seibel & Walsh, 2003; Pörtner *et al*., 2004); it is unclear which taxa, if any, will be unaffected by these environmental pressures.

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A series of *in situ* experiments have been conducted to assess the effects of direct injection of CO_2 on the seafloor, concentrating on the lower bathyal zone because it theoretically provides a longer period of sequestration compared with shallow water (Brewer *et al*., 1999, 2000; Barry *et al*., 2005). Although studies have begun to describe the effects of such CO_2 disposal on deep-sea fauna including fish (Tamburri *et al*., 2000), crustaceans (Barry *et al*., submitted), echinoderms (Barry *et al*., submitted), and metazoan meiofauna (e.g., Carman *et al*., 2004; Fleeger *et al*., 2006; Watanabe *et al*., 2006; Thistle *et al*., 2007), little is known about the effect of such activities on protista. Because protists comprise a substantial portion of the deep-sea benthos (e.g., Coull *et al*., 1977; Snider *et al*., 1984; Alongi & Pinchon, 1988; Gooday *et al*., 2000; Smith *et al*., 2002), it is important to establish the effects of bathyal CO_2 release on these single-celled eukaryotes. One study that surveyed the effects of CO_2 release on deep-sea meiobenthos observed that 4.5 weeks after seafloor CO_2 injection, many meiofaunal groups (i.e., nematode metazoans and flagellate and amoebae protists) experienced elevated mortality compared with sites removed from CO_2 manipulation (Barry *et al*., 2004). Robust conclusions about the response of some meiofaunal protists [i.e., allogromiid (thecate) foraminifera and ciliates] to that increased pCO_2 exposure could not be drawn, however, due to low population sizes (Barry *et al*., 2004) and because an accurate means to determine survival was not implemented for those taxa.

In a continuing effort to better ascertain the response of meiofaunal protists to elevated CO_2 exposure, a study was executed to determine *in situ* survival response of deep-sea benthic foraminifera to CO_2 release simulating seafloor disposal of this greenhouse gas. For the study, two experiments were conducted, each with a duration of 4.5 weeks in order to evaluate faunal responses to quasichronic changes in ocean chemistry, rather than ephemeral changes over short periods (i.e., days). Foraminifera were selected as the study taxon for two main reasons. First, they are a critical link in marine food webs (e.g., Legendre & Le Févre, 1995; van Oevelen *et al*., 2006; Rowe *et al*., in press). Second, while a large proportion of foraminiferal species secrete calcium carbonate shells called tests, the majority lack inorganic tests (so-called allogromiid or thecate forms, e.g., Gooday, 2002) or use detrital particles to construct agglutinated tests (Sen Gupta, 1999), allowing comparative experimentation between calcifying and noncalcifying species of the same taxonomic group of Rhizarian protists (Adl *et al*., 2005). This diversity in foraminiferal test composition is particularly useful when considering the biologic effects of ocean acidification. It may be hypothesized that the mortality of calcareous foraminiferal species will be higher in response to deep-sea CO_2 sequestration than the mortality of species of the two noncarbonate foraminiferan groups (i.e., thecate and agglutinated taxa).

Materials and methods

Two replicate experiments were conducted on the seafloor of Monterey Bay, of the California coast (USA). Using the surface support vessel RV *Western Flyer* and the ROV *Tiburon* for each experiment, a set of 40.6 cm diameter PVC cylinders were placed on the seafloor so that $\sim$ 15 cm extended above the sediment–water interface; 15 cylinders (Experiment 1) and 7 cylinders (Experiment 2) were configured in a circle with a diameter of $\sim$ 20 m. Then, over 2–3 days, liquid CO_2 was injected into each cylinder using methods described in, for example, Barry *et al*. (2004), Carman *et al*. (2004), and Fleeger *et al*. (2006). CO_2 hydrate formed immediately as a 'skin' on liquid CO_2 pools. The targeted pH decline within the $\sim$ 20 m diameter circle compared with *in situ* pH ($\sim$ 7.8; Thistle *et al*., 2007) was 0.2 pH units because data suggests that pH declines of that magnitude can be an important physiological threshold (e.g., Seibel & Walsh, 2003).

For both experiments, Conductivity–Temperature–Depth (CTD) instruments (SeaBird Model 19+ CTDs, SeaBird Electronics, Carlsbad, CA, USA) were deployed on the seabed at locations near the margin, $\sim$ 3–5 m from the margin, and in the center of the circle of CO_2 cylinders (Fig. 1). Each CTD was equipped with up to four Seabird Model SBE 18 pH sensors, positioned from 3 to 50 cm above the seabed. These instruments collected data at $\sim$ 2 min intervals throughout each experiment. Perturbations in the pH of surficial sediments were also measured during Experiment 2 within CO_2 cylinders and from 0.1 to 8 m from CO_2 pools, as reported in Barry *et al*. (submitted).

During the commencement of Experiment 1, a suite of control samples was obtained, as explained in the following text. The first experiment was initiated from 13 to 17 December 2004 at a water depth of 3088 m (36.6985°N, 123.0020°W) and terminated from 18 to 21 January 2005. Experiment 2 was initiated from 12 to 16 December 2005 at a water depth of 3266 m (35°48.6105′N, 122°34.0952′W) and terminated from 18 to 21 January 2006. The carbonate chemistry in the region of the experimental sites is typical of the deep Eastern Pacific (WOCE, www.nodc.noaa.gov/WOCE; Sector p17N, St. 10, 38.23733°N, 124.93833°W, 3087 m depth; DIC = 2352.7; TA = 2442.3; pH_{tot} = 7.7834; $Omega_{CA}$ = 0.93; $Omega_{AR}$ = 0.6).

As part of each experiment's termination, suites of samples were obtained from a site with no increase in CO_2 (i.e., $\sim$ 100 m from CO_2 release), from inside the

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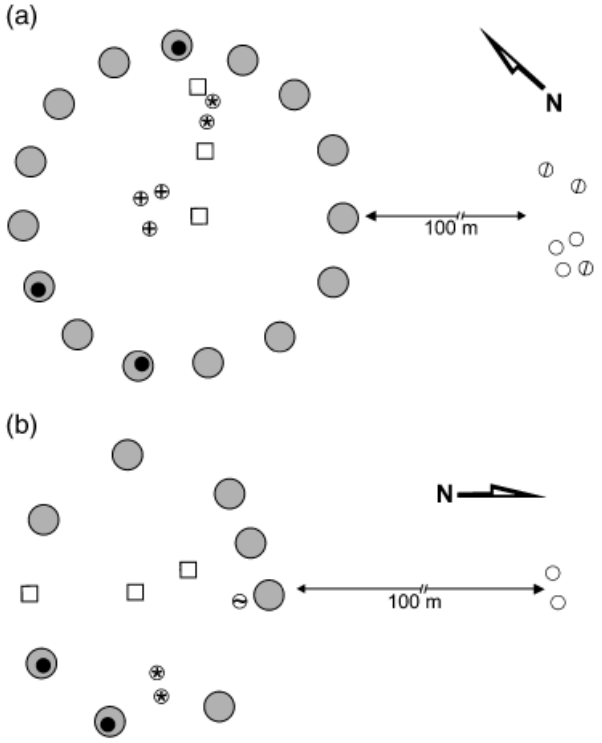


Fig. 1 Schematic representation of experimental configurations (a, Experiment 1; b, Experiment 2). The CO_2 cylinders are shown as gray circles, and Conductivity–Temperature–Depth instruments (CTDs) equipped with pH sensors are shown as squares. Pushcores analyzed for this study are shown as smaller circles; pushcore treatment categorizations are: open, Control; /, Initial Control; black, Direct Contact; +, Elevated Center; ∗, Elevated Edge; ∼, DMSO.

circle of PVC cylinders, and from inside individual PVC cylinders. These three treatments are hereby referred to as Control, Elevated, and Direct Contact, respectively. Elevated treatment samples were further distinguished depending on their distance from the PVC cylinders due to experimental logistics (see the following text): Elevated Center samples were collected from the approximate middle of the PVC-cylinder circle while Elevated Edge samples were obtained close to PVC cylinders (i.e., within ∼ 1–3 m; Fig. 1). The configuration of core locations was mandated by the fact that this experiment included sampling for multiple additional purposes and by the logistical considerations of working in the deep sea. For example, the ROV manipulator reach and seafloor disturbance in the form of sediment resuspension were critical factors for core placement. More details on the experimental design and concurrent science objectives are presented in Ricketts *et al*. (2005, submitted) and Thistle *et al*. (2007).

The fluorogenic probe CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate; Invitrogen, Carlsbad, CA, USA, hereafter referred to as CellTracker Green) was used to distinguish living from dead foraminifera (Bernhard *et al*., 2006) and, thus, establish survivorship. A fluorogenic probe is a nonfluorescent compound that yields a fluorescent product after structural modification; CellTracker Green accumulates intracellularly in live cells after enzymatic hydrolysis cleaves the molecule, thereby producing fluorescence (reviewed in Bernhard *et al*., 1995).

Quantitative sediment samples were obtained using pushcores outfitted with a device to allow injection of concentrated CellTracker Green after pushcore placement into the seafloor. This approach was adopted to allow *in situ* seafloor incubations with CellTracker Green because temperature and pressure changes during ascent could cause mortality due to sample recovery rather than due to *in situ* experimental conditions. CellTracker Green is soluble in dimethyl sulfoxide (DMSO); this stock solution was diluted with an equal volume of 0.2 μm-filtered seawater (FSW) to prevent freezing in the injector capillary tubing before dispensation.

Initially, the pushcores (7 cm inner diameter) were emplaced into sediments (Fig. 2a) so that the internal header space volume was within a targeted range. To do this, the pushcorer was positioned so that it penetrated the seafloor to a depth placing the sediment–water interface between two core-tube markings. The ROV manipulator arm was then used to carefully squeeze a large diameter (2 cm outer diameter) flexible tube filled with FSW, which flowed through a check valve to displace the concentrated CellTracker Green from the capillary tubing producing a final concentration of ∼ 1 μM CellTracker Green within each core's header space. These cores incubated *in situ* until the following day, when they were recovered by the ROV. A negative control core, which was injected with DMSO and FSW but not CellTracker Green, was similarly incubated and collected from an area with no increase in CO_2, during Experiment 2. To maintain the integrity of the sediment–water interface, the pushcores were not equipped with a stirring device.

After ROV recovery by the support ship, the incubated pushcores were taken within ∼ 10 min into an environmental room approximating *in situ* temperature (∼ 5 °C). Within an hour, the overlying water containing CellTracker Green was removed via siphon; the surface 1 cm of each core was sectioned from the underlying sediments, placed in a high-density polyethylene (HDPE) container, and fixed in ∼ 3.8% formalin buffered with sodium borate. All Direct Contact treatment cores became disturbed upon ascent due to degassing. In these instances, fine particles were suspended and the sediment–water interface was typically disturbed. Thus, for these cores, the overlying waters were re-

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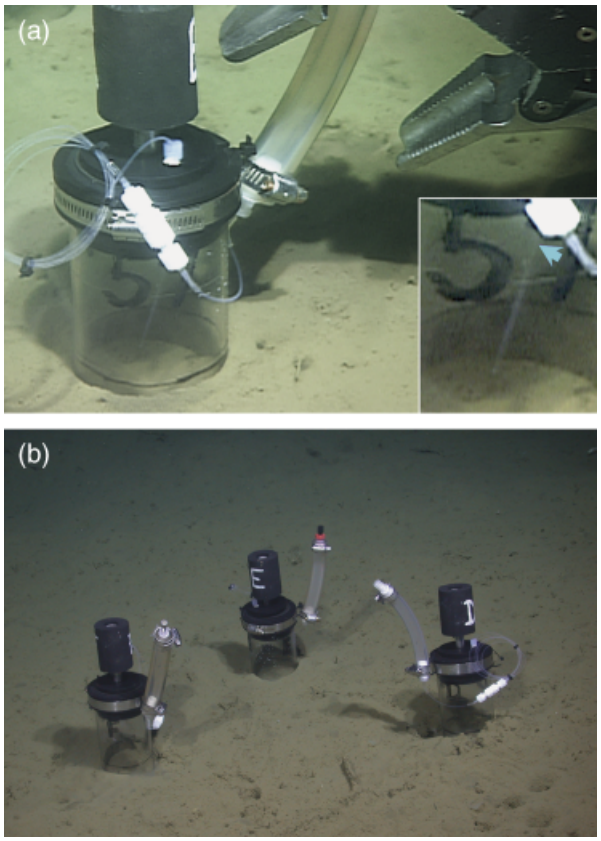


Fig. 2 Photographs of injector pushcores placed in the seafloor. (a) Representative pushcore from Elevated Center treatment immediately after the ROV manipulator released its grip on the thick injector tubing (upper left). *Inset*: Note the stream of whitish fluid (i.e., CellTracker Green and DMSO) entering the header space from the capillary tubing, which extends through the corer top, approximately to the arrow. (b) Suite of three Control pushcores incubating *in situ* during Experiment 1.

tained in addition to the surface sediment interval or allowed to settle for approximately an hour before sectioning.

In the shore-based laboratory, samples were sieved over a 63 μm screen with tap water, and the coarser fraction examined with a Leica MZ FLIII stereo dissecting microscope equipped with appropriate epifluorescence optics (excitation 480 ± 20 nm, emission ≥ 510 nm). Fluorescent foraminifera were isolated, segregated into brightly and dimly fluorescent groups, sorted by species within those groups, and enumerated. Thecate and 'soft-shelled' agglutinated taxa, which were not identified to species, were archived in buffered formalin while calcareous and robust agglutinated taxa were air dried and archived on micropaleontology slides. Because a single individual of observed unilocular cylindrical agglutinated foraminiferal taxa (i.e., *Bathysiphon*, *Rhabdammina*, *Rhizammina*) can easily break, those fragments were minimally enumerated to prevent over estimates of population density. Within a sample, it is typically clear which fragments likely arose from the same specimen due to test grain size, diameter, composition, texture, and color. During the picking process, the treatment of each sample (i.e., Control, Elevated, Direct Contact, negative control) was withheld from the microscopist. Species counts were normalized to *in situ* sample volume to provide abundance estimates.

Abundance data from each experiment were statistically analyzed separately for each taxonomic group. For thecate and agglutinated groups in Experiment 1, a one-way analysis of variance (ANOVA) was used to test for differences in density among the Control, Elevated Center, Elevated Edge, and Direct Contact treatments. For Experiment 2, a one-way ANOVA was conducted to test whether mean density of either group differed between the Control, Elevated Edge, and Direct Contact treatments. To homogenize variances, analysis was done on logged transformed thecate data and on square root agglutinated data.

To determine the effects of CO_2 injection on calcareous foraminiferal abundance, the mean abundance in the Control treatment was compared with the mean abundance in the Direct Contract treatment. For this comparison, the mean of the Control treatment was compared with a value of 0 under the null hypothesis with a one-sample *t*-test because calcareous foraminifera were absent in the Direct Contact treatment cores. Additionally, a one-way ANOVA was used to test whether mean densities of calcareous foraminifera differed between Control, Elevated Center, and Elevated Edge treatments. For Experiment 2, mean calcareous foraminiferal densities in the Elevated Edge and Controls were compared with a two-sample *t*-test and the mean density of the Control treatment was tested in a single sample *t*-test with a hypothesized mean of zero.

Results

Only cores that were visibly injected (Fig. 2a) were processed and analyzed. In one case, the capillary tubing parted from a coupling, thus CellTracker Green was not injected into that core so it was excluded from study. Replicate cores from Control and Elevated treatments of each experiment were typically taken within 1 m of each other (Fig. 2b); Direct Contact cores were obtained from different PVC cylinders. Careful manipulation by the ROV pilots resulted in absence of visible sediment–water interface disturbance within the corers, except for those collected from within the PVC cylinders (i.e., Direct Contact cores), which degassed during ascent as noted above.

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The negative control core (i.e., injected solely with DMSO, without CellTracker Green) lacked brightly fluorescent foraminifera, but had dimly fluorescent foraminifera. Thus, only brightly fluorescent foraminifera were considered to be living at the time of seafloor incubation with CellTracker Green. In CellTracker Green-incubated cores, the CellTracker Green diffused to at least 1 cm depth because some organisms in the 1–2 cm interval fluoresced brightly.

pH changes

The perturbations of pH measured in bottom waters during both experiments were variable but not large. In an area equivalent to the Elevated Edge treatment, the maximum pH reductions compared with ambient (pH reductions were calculated as perturbations from the background pH measured at the site) values were 0.15–0.25 pH units, with average changes <0.05 units. In the center of the circle (i.e., Elevated Center treatment), pH reductions sometimes approached 0.1–0.2 pH units, but typical pH perturbations were <0.05 units.

In areas corresponding to the Elevated Edge treatment, pore-waters of surface sediments at the beginning of Experiment 2 (i.e., 1–3 days after hydrate placement) were ~ 0.2 units lower than at control locations (~ 50 m away) but the pH of Elevated Edge pore-waters at experimental termination did not differ from the pore-water pH of control area sediments (Barry *et al*., submitted). Changes in the pH of surficial sediment pore waters (i.e., upper ~ 10 cm) were large within Direct Contact cylinders (~ -2.0 pH units compared with pore-water of control sites; Barry *et al*., submitted).

Species composition of living foraminiferal assemblages

The living calcareous foraminiferal assemblage, as determined by bright CellTracker Green labeling, in the Control and Elevated samples was dominated by rotaliid forms including *Uvigerina canariensis*, *Chilostomella oolina*, and *Globobulimina pacifica*; the most common miliolid form was *Quinqueloculina venusta* (Table 1; Fig. 3). The living noncalcareous foraminiferal assemblage in Control and Elevated samples was dominated by thecate and 'soft-shelled' agglutinated forms such as saccamminids, as well as *Reophax dentaliniformis*, *Reophax spiculifer*, *Hormosinella guttifera*, and *Paratrochammina challengeri* (Fig. 4). Agglutinated species such as *R. dentaliniformis* and *R. spiculifer* and thecate species dominated Direct Contact samples.

Abundances of live foraminifera

The foraminiferal abundance between cores within a treatment was highly variable for some treatments but not for others (Table 1). Abundances of all live foraminifera ranged from 4.4 to 5.7 specimens per 10 cm^3 in Initial Control cores of Experiment 1, and from 6.2 to 11.2 specimens per 10 cm^3 in Control samples collected at the end of either experiment (Table S1; Fig. 5). Thus, the average abundance of foraminifera in Control samples was 7.7 specimens per 10 cm^3 ($n = 8$, SD = 3.0). Foraminiferal abundances within the Elevated samples spanned a wider range (2.6–26.0 specimens per 10 cm^3; $\bar{x} = 12.1$, SD = 8.6) than those within Direct Contact samples (1.3–11.7 specimens per 10 cm^3; $\bar{x} = 5.4$, SD = 4.0). The average of total abundance of Elevated Edge samples ($\bar{x} = 14.9$, SD = 10.4) was higher than all other treatments (Fig. 5a and b).

Abundances of thecate foraminifera averaged 2.3 specimens per 10 cm^3 (SD = 2.3) in all Control cores and 3.3 specimens per 10 cm^3 both in Elevated samples and Direct Contact samples (SD = 3.8, 2.5, respectively). Abundances of agglutinated foraminifera averaged 2.1–2.2 specimens per 10 cm^3 in both the Control and Direct Contact samples, but were nearly twice as abundant in samples collected from within the circle (i.e.,

Table 1 Mean abundances of live foraminifera [(# per 10 cm^3); listed for all foraminifera and by foraminiferal group] for each treatment, presented by experiment

			Mean (SD)			
Treatment	Experiment	# Cores	Total	Thecate	Agglutinated	Calcareous
Initial Control	1	3	4.8 (0.8)	0.9 (0.5)	1.6 (0.8)	2.3 (0)
Control	1	3	9.5 (2.9)	3.3 (2.8)	3.0 (0.8)	3.2 (1.8)
	2	2	9.4 (1.8)	2.9 (3.3)	2.0 (0.9)	4.6 (0.5)
Elevated Center	1	3	8.3 (4.5)	2.4 (2.2)	3.0 (2.6)	2.9 (1.6)
Elevated Edge	1	2	14.3 (16.6)	6.5 (7.0)	6.1 (7.5)	1.7 (2.0)
	2	2	15.6 (6.9)	1.4 (0.6)	3.6 (1.5)	10.5 (5.0)
Direct Contact	1	3	4.7 (1.7)	3.0 (0.9)	1.7 (1.4)	0
	2	2	6.5 (7.4)	4.0 (4.6)	2.5 (2.8)	0

The number of replicates (# cores) is also listed. SD, standard deviation.

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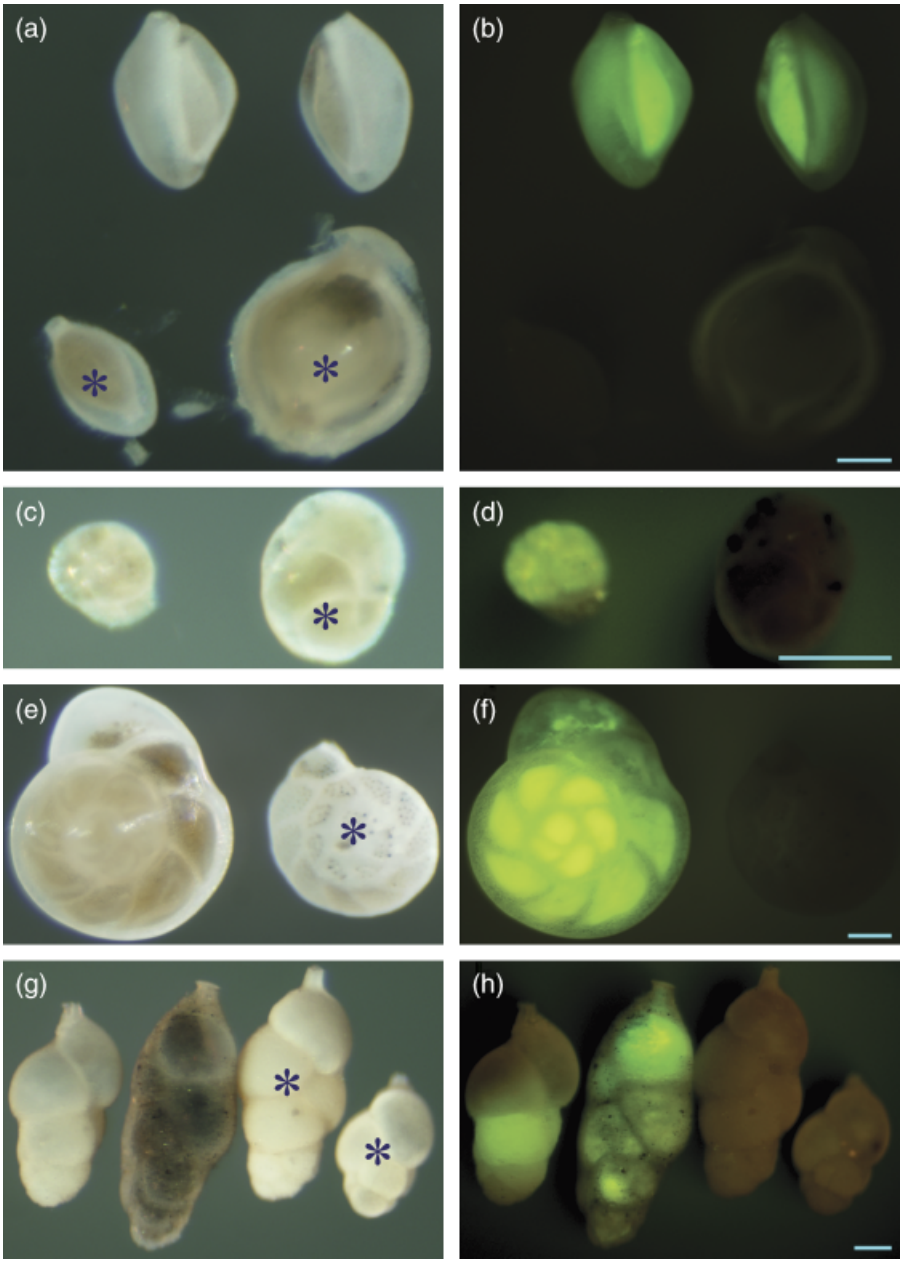


Fig. 3 Reflected light (a, c, e, g) and corresponding epifluorescence (b, d, f, h) micrographs of calcareous foraminifera collected at experimental termination. Those labeled with ∗ were from a Direct Contact treatment core (935-JB2); those without ∗ were from a Control treatment core (937-JB7). Note that none of the Direct Contact specimens fluoresce brightly. (a, b) Top row: *Quinqueloculina venusta*, bottom row: *Pyrgo murrhina*; (c, d) *Cassidulina* cf. *delicata*; (e, f) (left to right): *Hoeglundina elegans*, *Cibicidoides* sp.; (g, h) *Uvigerina canariensis*. Scale bars: 100 μm.

Elevated treatments combined; 4.1 specimens per 10 cm^3, SD = 3.8). The mean abundances of thecate and agglutinated groups were generally higher in Elevated Edge samples than in other treatments (Fig. 5c–f), the exception being thecate abundances for Experiment 2 (Fig. 5d). The mean density of thecate or agglutinated foraminifera did not differ significantly between the Control, Elevated Elevated Edge or Direct Contact treatments in Experiment 1 (one-way ANOVA, thecate, $F_{3,7} = 0.39$, $P = 0.762$; agglutinates $F_{3,7} = 0.49$, $P = 0.701$) or in Experiment 2 between Control, Elevated Edge, and Direct Contact (one-way ANOVA, thecate, $F_{2,3} = 0.09$, $P = 0.913$; agglutinates, $F_{2,3} = 0.40$, $P = 0.699$).

Calcareous foraminiferal abundances averaged 3.2 specimens per 10 cm^3 and 4.7 specimens per 10 cm^3 in Control and Elevated cores, respectively, but no calcar-

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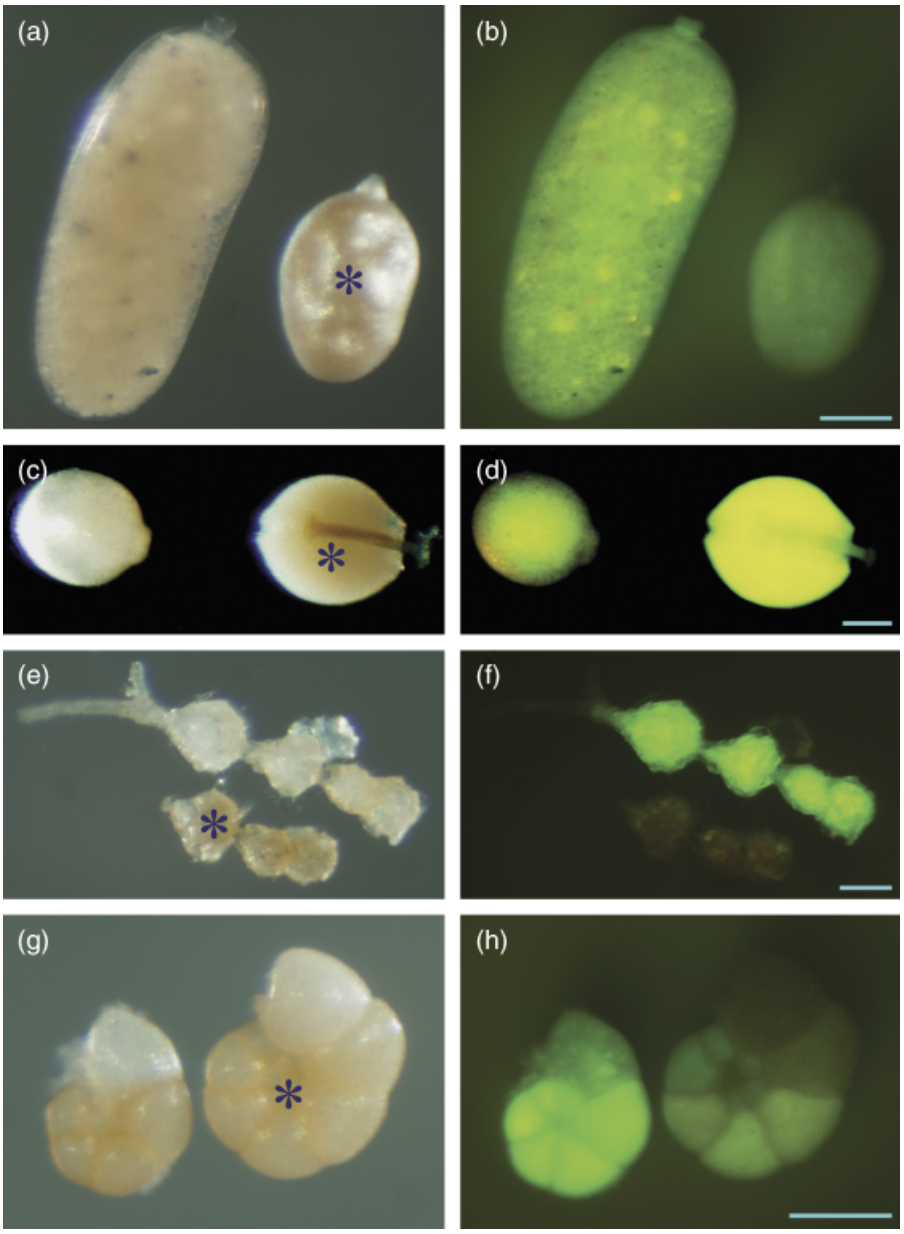


Fig. 4 Reflected light (a, c, e, g) and corresponding epifluorescence (b, d, f, h) micrographs of agglutinated and thecate foraminifera collected at experimental termination. Those labeled with ∗ were from Direct Contact treatment cores (935-JB2 or 935-JB3); those without ∗ were from Control treatment cores (937-JB6 or 937-JB7). Note that the Direct Contact specimen in c, d fluoresces brightly; other Direct Contract specimens were deemed dead. (a, b) (Left to right) unidentified allogromiid, saccamminid; (c, d) (left to right) *Pelosina* sp., unidentified allogromiid; (e, f) *Hormosinella guttifera*; (g, h) *Veleroninoides wiesneri*. Scale bars: 100 μm.

eous foraminifera were living in the Direct Contact cores (Table 1; Fig. 5g and h). For Experiment 1, the mean density of calcareous foraminifera did not differ significantly between the Control, Elevated Center or Elevated Edge treatments (one-way ANOVA, $F_{2,5} = 0.48$, $P = 0.645$) and, for Experiment 2, their mean density did not differ significantly between the Control and Elevated Edge treatments (two sample t-test, $-1/x$ transformed data, df = 2, $t = 2.88$, $P = 0.102$). In both experiments, the mean density of calcareous foraminifera in the Control samples was significantly different from a mean of zero (one sample t-test, Experiment 1: $t = 6.26$, df = 2, $P = 0.025$, Experiment 2: $t = 25.96$, df = 1, $P = 0.025$). Thus, there were significantly more calcareous foraminifera in the Control treatments than in the Direct Contract treatment, which lacked calcareous foraminifera in every core of both experiments.

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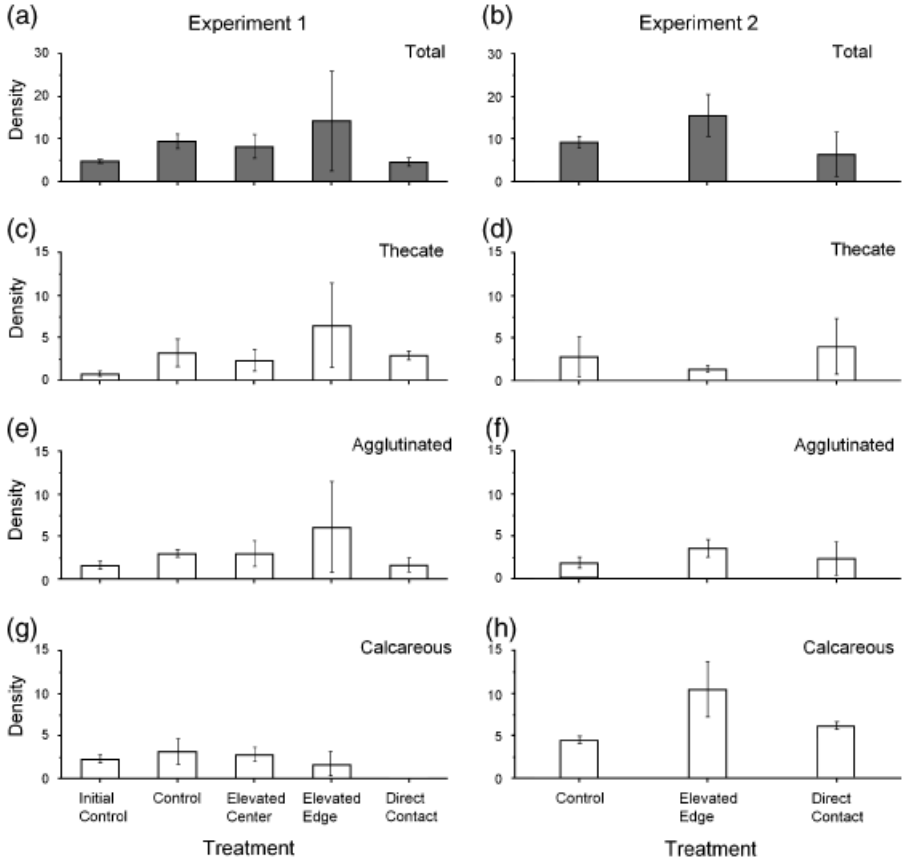


Fig. 5 Histograms of CellTracker Green labeled benthic foraminifera, presented as total density (specimens per 10 cm^3) and by group and treatment for each Experiment. Error bars reflect standard error.

Discussion

The hypothesis that higher mortality would occur in calcareous foraminifera compared with thecate and agglutinated foraminifera in response to deep-sea CO_2 sequestration is supported by our data. Survivorship of the thecate and agglutinated foraminiferal populations at our Monterey Bay sites were not significantly affected by direct exposure to CO_2 hydrate on the experimental time scale.

Although the water depth at our experimental sites likely exceeded the regional calcite saturation depth and the carbonate compensation depth (CCD), calcareous (calcitic) foraminifera were living in the area as evidenced by Control treatment results. The existence of calcitic foraminifera is not unexpected as most abyssal regions of today's oceans have live calcareous foraminifera (e.g., Bernhard, 1992; Linke & Lutze, 1993). In general, the observed abundances of foraminifera in control samples were comparable with abundances from similar water depths at sites $\sim$150 km to the south (Bernhard, 1992). Using a different viability method [i.e., adenosine triphosphate (ATP) assay], abundances of live foraminifera integrated over the surface 1 cm ranged from 0 to $\sim$12 specimens per 10 cm^3 in water depths from 3319 to 3728 m ($\bar{x}$ $\sim$6 specimens per 10 cm^3; $n = 4$; Bernhard, 1992). Deep-sea benthic foraminiferal distributions are known to be patchy on the scale of km to cm (e.g., Bernstein *et al.*, 1978; Bernstein & Meador, 1979), so it is not unusual to document considerable variations in foraminiferal abundance over short distances. Indeed, Bernhard (1992) also noted large variations in foraminiferal abundances between sites, although those cores were separated by kilometers, not meters, as in this study. The species compositions of agglutinated and calcareous assemblages encountered in our samples resemble those previously reported from the region at comparable water depths (e.g., Bernhard, 1992).

Even though some bathyal and abyssal foraminiferal species' abundances exhibit subsurface maxima (e.g., Corliss, 1985; Bernhard, 1992), a down-core analysis of our samples was not feasible. Because, in general, the majority of bathyal foraminifera live in the top cm (e.g., $\sim$52–71%, Gooday, 1986; $\sim$80%, Szarek *et al.*, 2007) and because we expect the maximum physical and

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chemical changes due to CO_2 hydrate release within the surface sediments, we feel our data reflects the typical survival response of bathyal benthic foraminifera to such environmental perturbations. It is possible that foraminiferans migrated in response to the changing milieu, as observed in other experimental studies (e.g., Alve & Bernhard, 1995; Moodley *et al*., 1998). In particular, if infaunal specimens migrated upward into the surface cm, the average abundances in treatments affected by CO_2 would have been higher than those of Controls. Indeed, the abundance data for agglutinated taxa were consistently higher, although not significantly different, in Elevated Edge samples compared with Controls (Fig. 5e and f). This trend was not evident for other groups (Fig. 5c, d, g and h). Why foraminifera may have migrated toward potentially stressful concentrations of CO_2 is unclear, except the option of migrating deeper into sediments would have been more stressful for aerobic migrants compared with facultative anaerobes, which are known for foraminifera (e.g., Bernhard & Alve, 1996; Moodley *et al*., 1998), or if the migrants were incapable of performing complete denitrification (Risgaard-Petersen *et al*., 2006; Høgslund *et al*., 2008). Recent findings suggest that elevated pCO_2 exposure is stressful to at least some meiofauna because higher numbers of harpacticoid copepods emerged from sediments in response to elevated pCO_2 compared with copepod emergence rates at control sites (Thistle *et al*., 2007).

Degassing of cores collected from PVC cylinders during ascent may have minimized the observed foraminiferal abundances in the Direct Contact cores. We discount this possibility because the average density of thecate and agglutinated morphotypes in Direct Contact samples was near or equal to their density in Control cores. If significant abundance dilution occurred due to degassing disturbance, we would expect all three groups of foraminifera to be similarly affected, which was not the case.

Observed patterns and magnitudes of pH change resemble those reported for similar experiments (Barry *et al*., 2005). Importantly, the observed bottom-water pH decreases in areas corresponding to Elevated Center treatments ($\sim$ 0.05 units for Experiment 1) were small compared with those declines corresponding to Elevated Edge treatments (initially 0.2 pH units, but $\sim$ 0.1 to 0.15 pH units at experiment end). Thus, it is not surprising that all three groups of benthic foraminifera (i.e., calcareous, agglutinated, thecate) tolerated the Elevated Center treatments because those sediments apparently did not experience extended large decreases in pH and, by inference, extended large increases in pCO_2. It is also noteworthy, however, that thecate and agglutinated foraminiferal survival was not negatively impacted by the substantial pH decrease ($>$1 pH unit) in the Direct Contact treatments.

Economically viable protocols of deep-sea CO_2 sequestration include formation of deep-sea CO_2 lakes and near-bottom injection of CO_2 (reviewed in Herzog *et al*., 2000; Caldeira *et al*. 2005). In all cases, a plume of CO_2-rich seawater will lead from liquid CO_2 or CO_2 hydrate drifting with currents and diffuse, eventually being diluted (Caldeira *et al*. 2005). Thus, CO_2 and pH gradients will range from substantial (e.g., the lowest pH measured is 4.5, a value measured within cm of CO_2 hydrate; Brewer *et al*., 2000) to mild (i.e., <-0.05 pH units) at some distance, depending on specific mixing conditions. Our Direct Contact treatment recreates the CO_2 lake approach, although on a much smaller scale. Benthos beneath any CO_2-hydrate pool will be directly exposed for long periods of time unless they can migrate from the hydrate pool (Tamburri *et al*., 2000). Deep-sea foraminifera move very slowly ($<25\,\mu m\,min^{-1}$; Gross, 2000), so active avoidance at these spatial scales is an implausible escape mechanism for them. The pH changes in our experiments are similar to those under any carbon sequestration option: conditions of our Elevated treatments are within the magnitude of pH change expected along the dilution gradient resultant from presently proposed mitigation scenarios.

The *in situ* response of foraminifera (and any other group of deep-sea organisms) to such CO_2-hydrate exposure is unknown over time periods longer than our experiments. Our results indicate that although the calcareous foraminiferal population tolerates short exposures to a 0.2 pH unit decrease, their survival response to direct CO_2-hydrate exposure results in a significant increase in mortality. If, however, plumes of dissolved CO_2 hydrate are diffused from the seafloor over a shorter time scale than our experiments, it is possible that calcareous foraminiferal survival will not be significantly impacted by large-scale CO_2 sequestration.

Although the effects of localized deep-sea CO_2 sequestration appear to negatively impact calcareous foraminifera (Ricketts *et al*., 2005, submitted; this study), two other protistan groups (i.e., flagellates and amoebae; Barry *et al*., 2004), and a number of metazoan meiofauna (Barry *et al*., 2004; Carman *et al*., 2004; Fleeger *et al*., 2006; Watanabe *et al*., 2006; Thistle *et al*., 2007), our findings suggest that survival of at least some protistan meiofauna (i.e., thecate and agglutinated foraminifera) are not similarly influenced by direct exposure to CO_2 hydrate. Because the abundance and diversity of thecate and agglutinated foraminifers are considerable in bathyal and abyssal sediments (e.g., Gooday *et al*., 1998, 2000; Smith *et al*., 2002) and their abundance can exceed that of other meiofauna (e.g.,

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Gooday *et al*., 2000), at least one major group of deep-sea meiofauna will likely not collapse if large-scale sequestration of CO_2 is implemented on the deep-ocean floor. Furthermore, our data showing that thecate and agglutinated foraminiferal abundances do not significantly decline in response to direct exposure to CO_2 hydrate at these spatial and temporal scales substantiate recent laboratory findings documenting a shallow-water thecate foraminiferan species survives $\sim$2-week exposure to extremely high pCO_2 (200 000 ppm) where some specimens even reproduced (Bernhard *et al*., in press).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Counts of CellTracker Green labeled (i.e., live) foraminifera presented by group and species, if available, for each core. Also presented are abundances normalized per core volume. First three digits of core designations refer to ROV *Tiburon* dive number. IC, Initial Control; EC, Elevated Center; EE, Elevated Edge; DC, Direct Contact.

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