

1 Title:

2 Sensitivity of deep-sea organisms to low pH waters associated with direct deep-sea carbon  
3 storage

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5 Running head:

6 Sensitivity of deep-sea organisms

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16

## 17 ABSTRACT

18           The effects of high ocean pCO<sub>2</sub> levels on deep-sea sea organisms caused by the direct  
19 injection of liquid CO<sub>2</sub> in the deep-sea as a method of ocean carbon storage are understood  
20 poorly. We examined the survival of common deep-sea, benthic organisms exposed to low pH  
21 waters emanating as a dissolution plume from pools of liquid carbon dioxide in a series of  
22 abyssal CO<sub>2</sub>-release experiments. Survival during month-long exposure to episodic, but severe  
23 environmental hypercapnia (pH reduced by as much as ca. 1.4 units) was low for urchins,  
24 holothurians, and the deep-sea amphipod, *Haploops lodo*. Microbial abundance did not change  
25 or increased under hypercapnia over two experiments. Survival under milder pH reductions in  
26 other experiments was higher for all taxa, including echinoderms. The gastropod *Mohnia*  
27 *vernalis* and octopus *Benthoctopus* sp. survived exposure to pH reductions that episodically  
28 reached ca. -0.3 pH units. The abyssal zoarcid *Pachycara bulbiceps* exhibited high survival  
29 (~90%) through month-long exposure to pH changes reaching ca. -0.3 pH units. Our results  
30 indicate that deep-sea microbial assemblages may be neutral or benefit, either directly or  
31 indirectly, from environmental hypercapnia, while many lower abyssal metazoan taxa are likely  
32 to suffer higher rates of mortality near CO<sub>2</sub> release sites, and perhaps over much larger areas  
33 under large deep-sea C storage programs. Higher organisms examined (one cephalopod,  
34 gastropod, and fish species) were more tolerant than most other taxa. Sublethal effects were  
35 not measured, but may include reduced aerobic capacity, as well as reduced growth and  
36 reproduction for many organisms. In addition, the impacts of ocean acidification on sensitive  
37 species are likely to have cascading indirect consequences throughout deep sea food webs.

38 Key words:

39 Ocean acidification, deep sea, carbon storage, environmental hypercapnia, high-CO<sub>2</sub> ocean

40

41 **Introduction**

42           Although it is widely accepted that reducing greenhouse gas emissions is a key to  
43 avoiding dangerous climate warming and associated consequences, international efforts to  
44 curtail emissions have been largely unsuccessful. Some progress has been made, with 19 of 37  
45 signatory nations to the Kyoto Protocol having met their emission reduction targets (IAEA,  
46 2011), but global emissions rose nonetheless from 21 to 29 Mt CO<sub>2</sub>y<sup>-1</sup> (+38%) between 1990  
47 and 2009. An effective emissions reduction program will require broad application of a portfolio  
48 of carbon-free energy alternatives, methods for increased energy efficiency, and carbon storage  
49 strategies (Pacala and Socolow 2004). Development of carbon capture and storage methods  
50 have focused on C storage in the biosphere and in suitable geologic strata such as deep  
51 aquifers, depleted oil and gas wells, or deep ocean sediments and porous subseabed  
52 formations (Anderson and Newell 2004, Yang et al. 2008, Herzog 2011). Carbon storage by  
53 direct injection of waste CO<sub>2</sub> into the deep ocean (e.g. Marchetti 1977) has been considered,  
54 but avoided owing to concern for environmental damage (Herzog 2001, Seibel & Walsh 2001).  
55 Deep sea C storage is also thought to be possible through iron fertilization of ocean surface  
56 waters (Buesseler et al. 2008; Vaughan and Lenton 2011), but has unknown efficiency and is  
57 also expected to alter environmental conditions and ecosystem function in the deep sea.

58           The urgency for climate stabilization is likely to increase as atmospheric CO<sub>2</sub> levels and  
59 related climate consequences rise through this century. If so, concern for the impacts of global  
60 warming on terrestrial and upper ocean systems may eventually outweigh consideration of the  
61 potential impacts of ocean C storage for deep-sea ecosystems. Society may then decide to ‘pull  
62 out all the stops’ to avoid runaway climate change, and expand the use of deep ocean carbon  
63 storage and other methods that are currently avoided due to cost or environmental concerns.

64           Biological communities in the deep-sea are threatened by elevated environmental CO<sub>2</sub>  
65 levels (environmental hypercapnia) caused by the direct injection of waste carbon dioxide, or  
66 through the leakage of CO<sub>2</sub> from subseabed C storage sites. Carbon dioxide released at or near  
67 the seabed reacts with seawater to form carbonic acid, and can produce large and highly  
68 variable changes in ocean pH and carbonate saturation, particularly near release sites. Small

69 scale experiments releasing liquid CO<sub>2</sub> in the deep sea have measured pH levels less than 4.0  
70 near pools of liquid CO<sub>2</sub> (Brewer et al. 2005). Models of boundary layer turbulence near CO<sub>2</sub>  
71 pools in the deep ocean indicate similar near-field and variable pH in the dissolution plume  
72 emanating from deep-sea lakes of sequestered CO<sub>2</sub> (Herzog et al. 2001; Fer and Haugan  
73 2003). The spatial extent and severity of pH perturbations near injection sites will depend upon  
74 the method of CO<sub>2</sub> injection, time-scale and rate of release, and local hydrography (Caldiera et  
75 al. 2005).

76 Deep-sea animals are expected to be highly sensitive to high-CO<sub>2</sub>, low-pH dissolution  
77 plumes near deep-sea CO<sub>2</sub> injection or storage sites. The ability of animals to tolerate  
78 environmental change are based on physiological repertoires that have evolved over thousands  
79 of generations, and those that evolved in the typically stable conditions in deep-ocean waters  
80 are generally more sensitive to environmental perturbations of any sort than related shallow-  
81 water taxa (Seibel and Walsh 2003). Most deep sea taxa have lower metabolic rates (largely  
82 due to reduced temperature) and reduced enzyme function – both key factors for coping with  
83 physiological stress - compared to their shallow water counterparts (Seibel and Walsh 2003).  
84 Energy limitation in the deep-sea may also constrain the ability of animals to increase energy  
85 allocation toward acid-base regulation and other physiological processes used to cope with  
86 physiological challenges associated with environmental hypercapnia.

87 Few studies have examined the sensitivity of deep sea animals to variable, low-pH  
88 conditions near deep-sea CO<sub>2</sub> storage sites. Low tolerance of key community taxa to high  
89 ocean pCO<sub>2</sub> levels caused by ocean carbon storage could disrupt the function of deep-sea food  
90 webs, leading to reduced biodiversity, shifts in community structure, and reduced community  
91 production. A series of experiments that released small pools of liquid CO<sub>2</sub> on the seabed off  
92 Central California was used to evaluate the potential impacts of a large scale deep sea carbon  
93 dioxide storage program on benthic deep sea communities (Barry et al. 2005). Reports from  
94 these experiments found that meiofauna, including harpacticoid copepods, euglenoids, and  
95 foraminifera experienced elevated mortality after exposure to episodic pH changes of ca. -0.2

96 units (Barry et al. 2004; Carmen et al. 2004; Thistle et al. 2005, 2006, 2007). In this paper, we  
97 report the response of various taxa to episodic exposure to low-pH dissolution plumes near  
98 pools of liquid CO<sub>2</sub> released on the seabed at abyssal depths, including changes in abundance  
99 and biodiversity.

100

## 101 **METHODS**

### 102 ***Study Area***

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

110 The sediment-dwelling macrofauna at Site A were dominated by a dense assemblage of  
111 *Haploops lodo*, a tube-dwelling ampeliscid amphipod, but also included numerous other  
112 Crustacea, Polychaeta, Mollusca, and Cnidaria. The macrofaunal assemblage at Site B was  
113 very similar to Site A, with much lower densities of *H. lodo*. Meiofauna were abundant at both  
114 sites, and dominated by nematodes, flagellates, and amoebae, with lesser densities of ciliates,  
115 foraminifera, and other taxa. The local abyssal megafaunal assemblage in the region is typical  
116 of the eastern N.E. Pacific, with moderate densities of macrourid (*Coryphaenoides* sp.), zoarcid  
117 (*Pachychara* sp.), and ophiidid (*Bassosetus nasus*, *Spectrunculus* sp.) fishes, octopods  
118 (*Benthoctopus* sp.), echinoderms (holothurians – *Amperima* sp., *Abyssocucumis abyssorum*,  
119 *Scotoplanes globosa*), echinoids (*Cystechinus loveni*, *Aporocidaris milleri*, *Echinocrepis* sp.),  
120 galethaid crabs (*Munidopsis* sp.), gastropod molluscs (*Mohnia vernalis*), and ophiuroids.

121

### 122 ***Experimental Methods***

123           We evaluated the response of deep-sea organisms to simulated deep-sea carbon  
124 storage in a series of *in situ* experiments by exposing deep-sea organisms to high CO<sub>2</sub>, low-pH  
125 dissolution plumes emanating from pools of liquid CO<sub>2</sub> released onto the abyssal seabed. All  
126 experiments were performed using the ROV *Tiburon* operated by the Monterey Bay Aquarium  
127 Research Institute (MBARI). Each ca. month-long experiment was initiated by releasing ~20-  
128 100 l of liquid CO<sub>2</sub> into small containers (sections of PVC pipe 48 – 100 cm in diameter x 15 –  
129 40 cm high) placed on the seabed (Fig. 2). Liquid carbon dioxide was transported to the seabed  
130 and injected into CO<sub>2</sub>-corrals using an ROV-mounted CO<sub>2</sub>-release system (Brewer et al. 1999).  
131 CO<sub>2</sub> in each corral dissolved slowly into bottom waters during each 2-6 week-long experiment,  
132 producing a CO<sub>2</sub>-rich, low-pH dissolution plume that is slightly heavier than ambient seawater.  
133 Advection and mixing of the plume in the oscillatory bottom currents led to episodic and variable  
134 exposure of organisms on the seabed and in surficial sediments to the CO<sub>2</sub> dissolution plume  
135 (Barry et al. 2005).

136           The spatial arrangement of CO<sub>2</sub> corrals and animal cages varied among experiments  
137 (Fig. 3). Details of the design of these experiments, including the configuration of CO<sub>2</sub> corrals,  
138 variation in current direction & speed, and patterns of pH variability are presented in Barry et al.  
139 (2005). Briefly, our initial design (E1) planned for an ANOVA comparison of survival by animals  
140 very near (<1m) and distant (>20 m) to CO<sub>2</sub> corrals. During experiment E2 & E3 we positioned  
141 animals at prescribed distances (1, 5, 10, 50 m) from centrally located CO<sub>2</sub> pools (~100 l total),  
142 attempting to capture the effects of a broader range of pH perturbations. A circular arrangement  
143 of CO<sub>2</sub> corrals with a diameter of ~15m was used in E5, attempting to produce relatively stable  
144 pH perturbation (-0.2 units) near the center of the circle where animal sampling was  
145 concentrated.

146           These experiments were performed at abyssal depths to assess the sensitivity of deep-  
147 sea animals potentially at risk from future ocean C storage efforts, but also because of the  
148 physical qualities of CO<sub>2</sub>. The pressure-temperature profile off California coastal waters defines  
149 a gas-liquid phase boundary for CO<sub>2</sub> near 350 m depth. Unlike water, liquid CO<sub>2</sub> is highly

150 compressible. From the depth of the phase boundary (350 m) to ca. 2600 m, liquid CO<sub>2</sub> has a  
151 specific gravity less than seawater, and will float toward the surface when released (Brewer et  
152 al. 2005). Our experiments were performed at 3300 - 3600 m, a depth where liquid CO<sub>2</sub> is  
153 denser than seawater (specific gravity ~1.07), and sinks into containers on the seafloor. CO<sub>2</sub>  
154 hydrate is also stable at depths greater than ~340 m and formed in various amounts in each  
155 CO<sub>2</sub> pool.

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

170 The tolerance of common abyssal megafauna (echinoderms – *Abyssocucumis*  
171 *abyssorum* (holothuroid), *Amperima* sp. (holothuroid), *Cystechinus loveni* (echinoid); Crustacea  
172 – *Munidopsis* sp. (galatheid); Mollusca – *Mohnia* sp. (gastropod), *Benthocopus* sp.  
173 (cephalopod); Vertebrata – *Pachycara bulbiceps* (zoarcid), *Coryphaenoides armatus*  
174 (macrourid)) exposed to CO<sub>2</sub> dissolution plumes was evaluated by positioning animals held in  
175 mesh cages near or distant from CO<sub>2</sub> pools during each experiment. We gently captured  
176 individuals of several benthic megafaunal species common at the study sites using an ROV-

177 operated suction device. Five to ten individuals of each species were captured and released into  
178 small mesh cages (46 x 46 x 20 cm), and placed at specified distances (<1 to 100 m) from CO<sub>2</sub>  
179 pools. Benthopelagic fishes (*Coryphaenoides armatus* and *Pachycara bulbiceps*) and the deep-  
180 sea octopod, *Benthoctopus* sp., were collected using baited traps prior to the start of some  
181 experiments (bait was removed after capture), and positioned near or distant from CO<sub>2</sub> pools.

182 The survival of megafauna was determined at the end of the experiment from visual  
183 observations of all individuals possible using the ROV's high resolution camera prior to  
184 collection from the cages. Movement of tentacles, spines, or feet, and active ventilation (e.g.  
185 fishes, octopus) were used to determine if animals were live. Animals were then recovered from  
186 cages and inspected in the laboratory for indications of tissue damage related to CO<sub>2</sub> exposure.

187 Because no metazoans collected at abyssal depths survived the ascent of the ROV to  
188 the surface (likely due to the barophilic nature of deep-sea animals (Somero, 1992)), the  
189 condition (live/dead) of individual animals in each experiment was determined from in situ  
190 observations (video) or examination of body condition upon collection. For larger macrofauna  
191 (e.g. amphipod, *Haploops lodo*) mortality caused by CO<sub>2</sub> exposure was distinguished from  
192 death during ROV ascent by evaluating the condition of body tissues. Condition was estimated  
193 subjectively on a scale of 1 (intact tissues, recent death - *live*) to 5 (degraded, little tissue  
194 remaining - *dead*) (Fig. 4).

195 Degraded individuals with considerable tissue loss were considered to have been dead  
196 for a period much longer than a few hours (i.e. did not die during the ascent of the ROV), based  
197 on comparisons with tissue degradation rates of amphipods measured in separate assays. For  
198 this purpose, samples of *Haploops lodo* were collected from Site A using sediment core  
199 samplers during the initiation of E3, brought to the surface with the ROV, and sieved from  
200 sediment samples. Groups of ~25 dead individuals of *H. lodo* were placed in nytex mesh bags,  
201 then redeployed (placed on the sediment surface) at the site for the duration (ca. 1 month) of  
202 the experiment. Mortality (% individuals dead) was calculated as the percentage of all  
203 individuals with tissue ratings of  $\geq 4$ .

204

## 205 ***Physical Measurements***

206           The direction and speed of near-bottom currents during most experiments (E1, E2, E5)  
207 were measured using an RDI Sentinel 300 mHz Acoustic Doppler Current Profiler. The  
208 instrument was deployed 2-4 m above the seabed in an ‘up-looking’ configuration, programmed  
209 to record profiles (1 m bins) of flow speeds in East, North, and vertical axes from 6-28 m above  
210 the seafloor at 5 minute intervals. Currents 8-15 m above the bottom were used to characterize  
211 the direction and speed of currents at the study sites.

212           The pH of the CO<sub>2</sub> dissolution plume was measured during each experiment using  
213 multiple pH sensors (SeaBird model 1278, logged on SeaBird Model 19+ CTDs) positioned 3-50  
214 cm above the seafloor at prescribed distances from CO<sub>2</sub> corrals. For example, time-series  
215 observations of pH (data intervals of 5 to 15 minutes) were obtained from 0.5 to 1 m away from  
216 one CO<sub>2</sub> and one control corral during E1, and at 1.5, 7.5, 25, 50, or 75 m from the central CO<sub>2</sub>  
217 corral during E2, E3, E5 (Barry et al. 2005).

218           In addition to the pH measurements described above, we measured the pH of pore fluids  
219 in the upper sediment column both in and at various distances (0.1, 1.0, 8, 50-100 m) from CO<sub>2</sub>  
220 pools during in two additional abyssal CO<sub>2</sub> release experiments (not reported here), to assess  
221 the penetration of the CO<sub>2</sub> signal in the surficial sediments inhabited by microbes and  
222 macrofauna. Profiles of pore-fluid pH were measured by inserting (by micromanipulator) an  
223 Orion pH microelectrode directly into sediment cores (chilled to in situ ambient temperature near  
224 1.5 °C, and under an N<sub>2</sub> atmosphere), or were measured in pore fluids extracted (squeezed)  
225 from 1 cm subsections of sediment cores. In all cases, pH profiles were obtained as soon as  
226 possible (~<2 h) after recovery of the ROV to the surface.

227

## 228 ***Data analyses***

229           For each experiment, we compared the condition or abundance of organisms collected  
230 before and after CO<sub>2</sub> release, using ANOVA or regression, depending on the experimental

231 design. ANOVA was performed using Systat (release 13) software, on raw or transformed data  
232 depending upon the normality and variance of samples. Non-parametric tests (Kruskal-Wallis)  
233 were used when transformations were ineffective in normalizing samples. Kolmogorov-Smirnov  
234 tests were used to compare frequencies of tissue condition indices of *H. lodo* between periods  
235 and treatments during E1. Regression analyses (% live vs. distance) were performed on arcsine  
236 transformed percentages, after inspection of the variance distribution among distances and  
237 application of a log-transformation, where appropriate. Comparisons of megafaunal survival  
238 were only performed for data collected at the end of experiments.

239

## 240 **RESULTS**

241

### 242 ***Variation in near-bottom pH and currents***

243 Most liquid CO<sub>2</sub> released into corrals dissolved during release experiments (Fig. 2),  
244 producing a low-pH dissolution plume that drifted down-current. Although several pH sensors  
245 failed during each experiment, deployment of duplicate sensors at most locations provided  
246 numerous continuous (5-15 minute intervals over periods of 9-31 days) pH records at various  
247 distances from CO<sub>2</sub> pools, documenting the frequency and intensity of CO<sub>2</sub> perturbations as the  
248 dissolution plume was advected across abyssal sediments.

249 pH in the vicinity of CO<sub>2</sub> corrals varied considerably during each experiment (Fig. 5). The  
250 largest pH shifts (ca 1.4 pH units) were measured very near (<0.25 m) CO<sub>2</sub> corrals during E1,  
251 and may have been due to small amounts of liquid CO<sub>2</sub> spilling out of CO<sub>2</sub> corrals to the seabed  
252 very close to the pH sensors. Excursions >1 pH unit during E1 occurred in <2.5% of  
253 observations over a 9 day pH record, with pH reductions of  $\geq 0.2$  units during 16% of the  
254 experiment. Reductions of pH associated with the dissolution plume were milder in subsequent  
255 experiments (E2, E3, E5) and diminished with distance from CO<sub>2</sub> pools (Fig. 6). At a distance of  
256 2-8 m from CO<sub>2</sub> pools, the maximum and mean pH decreases were ca. -0.2 and -0.005 units,  
257 respectively, with perturbations of -0.1 units occurring in less than 1% of measurements.

258 Records of pH during experiments E2, E3 and E5 were combined and analyzed in relation to  
259 distance from CO<sub>2</sub> pools using logarithmic regression ( $\Delta\text{pH}=\text{A}(\ln(\text{distance}))+\text{B}$ ) to characterize  
260 the general pattern of pH shifts with distance from CO<sub>2</sub> pools, indicating a rapid decline in the  
261 magnitude of pH perturbations with distance (Fig. 6). This regression model for maximum pH  
262 changes was used subsequently to estimate pH perturbations for regression analyses  
263 evaluating changes in abundance or survival of organisms measured in E2, E3, and E5.  
264 Maximum pH reductions were roughly 35 times greater than mean pH reductions measured at  
265 the same location ( $r^2=0.87$ ,  $p<0.01$ ,  $n=14$ ). Because of the variable current direction (see  
266 below), exposure to large pH reductions was typically limited to a short period each day when  
267 pH sensors (or animal cages) were down-current of CO<sub>2</sub> pools. pH perturbations decreased with  
268 distance from CO<sub>2</sub> pools and were small to undetectable beyond ca. 50 m from CO<sub>2</sub> sources.

269 Currents during each experiment were generally sluggish (mean speed  $\sim 3\text{-}6\text{ cm}\cdot\text{s}^{-1}$ ) and  
270 rotary in character, dominated by tidal oscillations (mainly a 12.4 h period, associated with the  
271 principal semidiurnal lunar tidal constituent M<sub>2</sub>) and inertial currents (20.02 h at this latitude;  
272 Barry et al. 2005). Owing to the variable direction and speed of bottom currents, exposure of  
273 surficial sediments and caged metazoans to the dissolution plume near CO<sub>2</sub> pools was highly  
274 variable (Fig. 5). These rotary currents with a dominant ca. 12 h period carried the dissolution  
275 plume toward any particular direction (i.e. megafaunal cages) approximately twice each day for  
276 a relatively short period, particularly for experiments with a single CO<sub>2</sub> pool (i.e. E2, E3).  
277 Additional details of pH variation are presented by Barry et al. (2005).

278 Profiles of pH in pore fluids showed similar patterns of pH change to measurements in  
279 bottom waters (Fig. 7). Interstitial pH in the upper 5 cm of the sediment within CO<sub>2</sub> corrals under  
280 liquid CO<sub>2</sub> at the start of the experiment 1-2 days after CO<sub>2</sub> release was 5.7-6.3, or 1.5-2 units  
281 lower than measured at control locations. Profiles 10 cm from CO<sub>2</sub> pools were  $\sim 0.2$  to 0.4 units  
282 below ambient. This pattern was also observed at the end of the experiment, after most or all of  
283 the liquid CO<sub>2</sub> in containers had dissolved. Interstitial pH 1 m or more from CO<sub>2</sub> pools was  
284 indistinguishable from control sites 100 m away.

285

## 286 ***Response of deep-sea organisms to low pH CO<sub>2</sub> dissolution plumes***

### 287 *Microbial Assemblage*

288 Prokaryotes (Bacteria and Archaea) were enumerated during 2 experiments (E1, E3)  
289 from sediment samples collected from very near (<1m) CO<sub>2</sub> pools to control sites ~40 m away,  
290 both before and after CO<sub>2</sub> exposure. Microbial abundance near (~0.25 m) CO<sub>2</sub> pools in E1  
291 decreased by 26% during the month-long experiment during exposure to pH perturbations as  
292 large as ca. -1.2 pH units. At control locations where pH changes were very small, microbial  
293 density dropped by only 11% (Fig. 8). The effect of distance from CO<sub>2</sub> pools was not significant  
294 (F=1.78, p>0.2) and the Distance X Period (before or after exposure) interaction term was also  
295 non-significant (F=0.01, p>.9). During experiment E3, microbial abundance was 3.8 to 17.4-  
296 fold higher than observed during E1. Abundance was relatively constant with distance from the  
297 central CO<sub>2</sub> pool before liquid CO<sub>2</sub> was released, but increased significantly (F=4.7, p<0.05)  
298 during the experiment, mainly near the CO<sub>2</sub> pool where maximum observed pH reductions were  
299 ~0.3 units. Microbial abundance increased by more than 8-fold at a distance of 5 m from the  
300 CO<sub>2</sub> pool.

301

### 302 *Macrofauna*

303 The abundance and species richness of sediment-dwelling macrofauna decreased near  
304 CO<sub>2</sub> pools after exposure to the dissolution plume. Macrofaunal abundance measured from  
305 sediment core samples during E2 did not vary significantly with distance from CO<sub>2</sub> corrals prior  
306 to filling them with liquid CO<sub>2</sub>. After ~40 days of episodic exposure to the CO<sub>2</sub> dissolution plume,  
307 macrofaunal density decreased significantly as the magnitude of pH perturbations nearer the  
308 CO<sub>2</sub> pool increased (Fig. 9). Polychaetes and nematodes captured on a 300 µm sieve each  
309 comprised roughly one third of the total macrofaunal abundance and 15 percent of the total  
310 biomass. While most major taxa showed a trend toward lower abundance or richness nearer

311 CO<sub>2</sub> pools, polychaetes were the only group whose abundance decreased significantly with  
312 declining pH (t=2.406, R=0.56, p<0.01).

313 Species richness also declined in relation to pH reductions near CO<sub>2</sub> pools by the end of  
314 E2, indicating that some taxa were sensitive to exposure to the environmental hypercapnia  
315 caused by CO<sub>2</sub> dissolution (Fig. 10). As observed for macrofaunal abundance, no pattern of  
316 species richness was detected before CO<sub>2</sub> release. The loss of species richness nearer CO<sub>2</sub>  
317 pools was explained mainly by the significant reduction in polychaete richness with larger  
318 maximum pH changes (t=2.65, R=0.59, p<0.01). A similar, but non-significant trend was  
319 detected for macrofaunal biomass (not shown).

320 The tube-building amphipod *Haploops lodo* occurred in dense beds at Site A and was  
321 the focal species for macrofaunal studies during E3 and E5. Largely intact specimens of *H. lodo*  
322 with a tissue condition index of <4 were assumed to have died recently. Decomposing  
323 specimens (condition index ≥4) were assumed to have died in response to environmental  
324 hypercapnia associated with CO<sub>2</sub> pools. Survival of *H. lodo* was lowest near CO<sub>2</sub> pools for both  
325 experiments (Fig. 11), where pH varied episodically by as much as -0.45 units. Regression of  
326 the proportion of live *H. lodo* (pooled samples from E3, E5) versus the maximum estimated pH  
327 changes (or distance from CO<sub>2</sub> pools) was non-significant prior to CO<sub>2</sub> release. By the end of E3  
328 and E5, survival of *H. lodo* decreased significantly with maximum pH change (post-E3, E5  
329 observations pooled; R=0.55, F=25.6, p<0.0001), indicating increased mortality following  
330 exposure to mild average pH changes coupled with episodic pH reductions of -0.3 or greater.  
331 Not surprisingly, nearly all *H. lodo* collected from sediment cores taken within CO<sub>2</sub> corrals (i.e.  
332 where liquid CO<sub>2</sub> had been present) were dead.

333

### 334 *Megafauna*

335 The response of benthic megafauna to month-long exposure to environmental  
336 hypercapnia caused by CO<sub>2</sub> dissolution varied considerably. A few taxa, notably echinoderms

337 were relatively intolerant and experienced high rates of mortality, while other groups (Mollusca,  
338 Chordata) were highly tolerant.

339

340 *Echinoderms.*

341 The echinoid *Cystechinus loveni* did not survive severe hypercapnic stress during E1. All  
342 *C. loveni* held in cages adjacent (<1m) to CO<sub>2</sub> pools during E1 (where maximum pH  
343 perturbations were ~-1.4 units) were dead within 2 weeks, and dissolution of skeletal elements  
344 was observed in several tests (Fig. 12, 13). Small amounts of liquid CO<sub>2</sub> that spilled from the  
345 corrals onto the seabed adjacent to megafaunal cages undoubtedly intensified the dissolution  
346 plume and probably contributed to the observed skeletal dissolution. In contrast, all urchins held  
347 in control cages ~30 m from CO<sub>2</sub> pools during E1 survived (U=0, p<0.025).

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

356 Holothurians (*Abyssocucumis abyssorum*, *Amperima robusta*, *Scotoplanes globosa*)  
357 exhibited variable survival near CO<sub>2</sub> pools. None of these species survived CO<sub>2</sub> exposure within  
358 1 m of CO<sub>2</sub> corrals, as was evident from the observations of dead, decaying individuals at the  
359 end of each experiment in cages near CO<sub>2</sub> pools. *A. abyssorum* did not survive in cages  
360 adjacent to CO<sub>2</sub>-filled corrals during E1, while survival was 100% in control cages 30 m away  
361 (U=0, p<0.025). Most *Abyssocucumis abyssorum* in cages more than 1.5 m from CO<sub>2</sub> corrals  
362 (i.e.  $\Delta \text{pH}_{\text{max}} \sim -0.2$  to  $-0.8$  units) had escaped by the end of E1. The holothurian *Amperima*  
363 *robusta* did not survive exposure to the dissolution plume within 5 m of CO<sub>2</sub> pools during E3

364 and E5 where maximum pH changes were -0.2 pH units or greater, even though mean pH  
365 changes were only -0.005 units. At greater distances, *A. robusta* exhibited 100 percent survival,  
366 based on a total of 17 individuals recovered from 7 cages positioned 1-50 m from CO<sub>2</sub> corrals  
367 (Fig. 13). *Scotoplanes globosa* was relatively rare at the study site and was used for limited  
368 observations. Three individuals were placed in direct contact with liquid CO<sub>2</sub> for short periods  
369 (~1 minute) to evaluate the effects of acute CO<sub>2</sub> exposure. Each of these individuals appeared  
370 to die within minutes. In addition, a single individual placed in a cage ~50 m from CO<sub>2</sub> pools  
371 (E3) survived the month-long exposure to the very weak dissolution plume at that distance.

372

### 373 *Mollusca.*

374 Molluscan survival was high during exposure to the CO<sub>2</sub> plume. Seven individuals of the  
375 abyssal gastropod *Mohnia vernalis* were recovered live from cages during E2 and E3,  
376 representing 17% of the number deployed in cages. The missing study animals could have  
377 escaped or were overlooked during inspection of the cage at the end of the experiments. The  
378 live individuals were recovered from cages 1 to 30 m from the centrally located CO<sub>2</sub> pools. In  
379 addition, many (10+ individuals) *M. vernalis* were observed crawling on the outside walls of the  
380 large CO<sub>2</sub> corral (91 cm diameter x 40 cm high) the end of E3, even though it was near (~85%)  
381 full of liquid CO<sub>2</sub> and the dissolution plume was presumably exposing these individuals to  
382 reduced pH waters.

383 Survival of the benthic octopod *Benthoctopus* sp. was high regardless of distance from  
384 CO<sub>2</sub> corrals in E3 and E5. Single individuals captured in traps with demersal fishes and placed 1  
385 and 25 m (E3) or 1.5, 4, and 100 m (E5) from CO<sub>2</sub> pools all survived exposure to pH  
386 perturbations of 0 to -0.3 units (maximum). Time-lapse observations of one of these  
387 *Benthoctopus* sp. are reported in Barry & Drazen (2007).

388

### 389 *Crustacea.*

390 Galatheid crabs (*Munidopsis albatrossae* and perhaps other *Munidopsis* sp.) were also  
391 relatively tolerant to pH stress near CO<sub>2</sub> pools, as all of 10 individuals assayed during E2  
392 survived. Single individuals of *M. albatrossae* held in each of 2 cages placed 1 m from the  
393 central CO<sub>2</sub> pool in E2 survived the 43 day experiment. Likewise, all individuals held at greater  
394 distances (5 individuals at 5 m distance, 3 individuals at 10 m) from the central CO<sub>2</sub> pool also  
395 survived.

396

397 *Pisces.*

398 The demersal zoarcid *Pachycara bulbiceps* exhibited high survival during exposure to  
399 elevated CO<sub>2</sub> levels. Of 26 individuals captured, 23 survived at distances of 0.5 to 100 m from  
400 CO<sub>2</sub> during E2, E3, and E5 where maximum and mean pH changes were as much as -0.4 and -  
401 0.01 units, respectively nearest CO<sub>2</sub> corrals. Those that died were 3 m (2 individuals) and 100 m  
402 (1 ind.) from the CO<sub>2</sub> pools in E5. In contrast, no individuals of the benthic-pelagic macrourid  
403 *Coryphaenoides armatus* (n=14) captured during E2, E3, and E5 survived inside traps,  
404 regardless of position with respect to CO<sub>2</sub>, suggesting that mortality was unrelated to CO<sub>2</sub>  
405 exposure. See Barry & Drazen (2007) for additional details.

406

407

## 408 **DISCUSSION**

409

### 410 ***Efficacy of CO<sub>2</sub> release experiments***

411 CO<sub>2</sub> release experiments reported here were designed to mimic changes in deep-ocean  
412 chemistry associated with a direct deep-sea carbon dioxide storage program. Although such an  
413 injection program would produce wide variation in ocean pH within mixing zones near injection  
414 sites, we had hoped that the pH perturbations produced would be relatively stable through the  
415 experiment, so that specific thresholds for tolerance could be identified for the taxa studied.  
416 Nevertheless, pH near CO<sub>2</sub> pools varied widely during each experiment, ranging as much as 0

417 to -1.7 units during E1 and roughly 0 to -0.3 units in other experiments. Large pH perturbations  
418 during each experiment were relatively rare (<1% of all observations) and mean pH shifts were  
419 well less than 0.1 units, except during E1. Although it is not possible to determine if the  
420 observed mortality was due mainly to the mean or maximum pH change, it is clear that variable  
421 pH fields near CO<sub>2</sub> disposal sites or released from subseabed storage locations will impact  
422 deep-sea benthos.

423

#### 424 ***pH sensitivity among taxa***

##### 425 *Microbial Community*

426 Lack of a change or an increase in the abundance of the microbial community in  
427 response to month-long exposure to simulated deep-sea CO<sub>2</sub> release (~-0.01 to -0.3 units)  
428 could be due to both direct and indirect effects of environmental hypercapnia. Tolerance or  
429 adaptation by the microbial assemblage could promote survival and persistence. Ishida et al.  
430 (2005) reported results similar to ours from *in situ*, deep-sea experiments, with an initial  
431 reduction in microbial respiration in response to very high pCO<sub>2</sub> levels (5000 – 20000 ppm),  
432 followed by an increase in their abundance and respiration, which they interpreted as an  
433 increase in the more tolerant taxa. In contrast, Coffin et al. (2004) observed a decrease in  
434 microbial growth over 96 hours, in high-pressure laboratory vessels with elevated pCO<sub>2</sub> levels  
435 (pH range = 5.6-7.6). Increased microbial abundance observed during E3 may have been due  
436 to a shift in the microbial assemblage to more tolerant taxa, but could also simply be an indirect  
437 consequence of the high mortality of meiofauna during the experiment (Barry et al. 2004; Thistle  
438 et al. 2005). The microbial assemblage presumably benefited from meiofaunal mortality through  
439 the simultaneous reduction in grazing/predation pressure, and increase in food availability vis-à-  
440 vis the moribund bodies of the meiofauna. Ishida et al. (2005) also documented a reduction in  
441 meiofaunal abundance. Together, these studies suggest that deep-sea microbial assemblages  
442 will be tolerant of projected changes in ocean chemistry, but this topic requires more careful

443 consideration, particularly considering a potential shift in microbial remineralization of organic  
444 debris under acidic conditions as suggested by Widdicombe and Needham (2007).

445

#### 446 *Meiofaunal Communities*

447 Although meiofaunal groups were not considered here, the response of various  
448 meiofaunal taxa to high CO<sub>2</sub> exposure during these experiments have been reported elsewhere  
449 and generally showed fairly high sensitivity to pH stress. Thistle et al. (2006, 2007) documented  
450 very low survival rates of harpacticoid copepods after month-long exposure to a CO<sub>2</sub> dissolution  
451 plume ( $\Delta$ pH  $\sim$ -0.7 units), with only 20 percent of all species surviving. Barry et al. (2004, 2005)  
452 reported similar rates of high mortality for several meiofaunal groups (flagellates, amoebae,  
453 allogromiid foraminifera, ciliates, nematodes) for E1 and E3 after exposure to maximum pH  
454 reductions of -1.7 and -0.33 units, and average pH changes near -0.14 and -0.008 units,  
455 respectively.

456

#### 457 *Macrofaunal Community*

458 The general reduction detected for macrofaunal density and species richness with  
459 increasing exposure to variable pH perturbations near sites of CO<sub>2</sub> released were driven  
460 principally by changes in the polychaete assemblage at the study sites. Vulnerability to pH  
461 stress is likely variable among taxa, with more active species expected to have greater  
462 physiological scope to compensate acid-base disruptions and other pH-related stress than less  
463 active taxa (Whiteley 2011). Widdicombe and Needham (2007) found little impact of reduced pH  
464 (-0.4 to -2.3 pH units) on the behaviour of an active infaunal polychaete (*Neries* sp.) from  
465 shallow subtidal habitats. In contrast, Batten and Bamber (1996) reported high mortality for a  
466 less active nereid polychaete under milder pH stress.

467 Sensitivity to pH stress is not limited to polychaetes, which were a numerically and  
468 taxonomically dominant group at our study sites. Crustacea varied in response to pH  
469 perturbations. Galatheid crabs appeared highly tolerant, while the ampeliscid amphipod

470 *Haploops lodo* experienced low rates of survival near CO<sub>2</sub> pools in all experiments. Survival of  
471 *H. lodo* was comparable to that reported for harpacticoid copepods under pH stress (Thistle et  
472 al. 2005). Exposure to environmental hypercapnia has also been studied in deep-sea  
473 lysianassid amphipods (*Eurythenes* cf. *obesus*), which under short term exposure (2-15  
474 minutes) became narcotized, but recovered upon immersion in normal pH waters (Vetter and  
475 Smith 2005). *H. lodo* may have experienced similar narcosis due to the CO<sub>2</sub> dissolution plume,  
476 which would have inhibited any potential escape behavior. Watanabe et al. (2006) report that  
477 pelagic copepods are sensitive to elevated CO<sub>2</sub> levels, and that deeper taxa were more tolerant  
478 than shallow species.

479

#### 480 *Megafaunal Community*

481 Survival of metazoans under environmental hypercapnia during these experiments  
482 varied among taxa in relation to phylogenetic differences in physiological complexity.  
483 Echinoderms, the group with the greatest observed sensitivity to pH stress, general have limited  
484 capacity to buffer internal pH due to their open water vascular system, weak ion exchange  
485 capacity, and largely cutaneous respiration system. Taxa with more developed  
486 adaptations for acid-base regulation, including complex respiration organs, strong ion-  
487 exchange capacities, and typically higher metabolic rates, such as fishes, cephalopod molluscs,  
488 and crustaceans, (including species studied here) are thought to be less sensitive to low pH  
489 stress (Widdicombe and Spicer 2008). All echinoderms studied exhibited a very low tolerance to  
490 changes in seawater chemistry caused by the CO<sub>2</sub> dissolution plume. Similar results have been  
491 observed for shallow water echinoids, which had little capacity for pH compensation (Miles et al.  
492 2007). The relative rarity of echinoderms in hydrothermal vent assemblages (Smirnov et al.  
493 2000) is also likely to be linked to their intolerance to elevated pCO<sub>2</sub>, since such vents are  
494 sources of high pCO<sub>2</sub> waters.

495 Megafaunal molluscs and crustaceans exposed to the same pH stress were highly  
496 tolerant, with no detectable mortality either near or distant from CO<sub>2</sub> pools. The gastropod

497 *Mohnia vernalis* and the deep-sea octopod *Benthoctopus* sp. have adaptations (e.g. thick  
498 epithelium and mantle, respiratory proteins, closed circulatory system (octopods)) that allow  
499 tighter control of internal acid/base balance than is possible for more primitive taxa (e.g.  
500 echinoderms) relying on diffusion-based respiration.. Their greater ability to defend internal pH  
501 through efficient gas exchange and internal ion regulation may promote their survival.

502 Fishes are expected to have even greater capacity for maintenance of internal acid/base  
503 balance (Ishimatsu et al. 2008; Pörtner et al. 2004), at least for short periods. Nevertheless, it  
504 was surprising to observe nearly 100 percent survival by *Pachycara bulbiceps*, even within 0.5  
505 m from pools of liquid CO<sub>2</sub>

506 The live / dead classification used for these experiments provides no metric for sublethal  
507 impacts of environmental hypercapnia, which may have been significant for these animals, even  
508 over the time scales of the experiments. For example, although *Benthoctopus* sp. survived  
509 these experiments, Seibel & Walsh (2003) showed that only a -0.3 unit reduction in internal pH  
510 can reduce by 40% the oxygen binding capacity of its hemocyanin. Even though it survived, its  
511 aerobic scope is likely to have been compromised during the experiments. Other taxa would  
512 have similar problems, with greater demand for both passive and active ion regulation to cope  
513 with internal hypercapnia that would result from immersion in high-CO<sub>2</sub> waters near CO<sub>2</sub> pools.

514

### 515 ***Implications for the future of deep-sea communities***

516 Mortality observed during the short-term (i.e. month-long) experiments reported here are  
517 only coarse indicators of the potential consequences of disturbance to deep-sea environments  
518 by deep-sea carbon storage, leakage of CO<sub>2</sub> from seabed storage sites, or eventually,  
519 ensuing passive ocean acidification. Taxa that exhibited high sensitivity to CO<sub>2</sub>-stress in our  
520 experiments will very likely experience significant stress in large changes in deep-sea pH,  
521 regardless of its source. Sublethal impacts to individuals and the potential sensitivity of early life  
522 history phases to environmental hypercapnia (Kurihara et al. 2004, Mayor et al. 2007, Dupont et  
523 al. 2010), neither which was addressed in these experiments, are likely to be more important

524 than direct mortality. Although sensitivity varies among taxa reports on shallow water taxa have  
525 documented a wide variety of effects from exposure to elevated pCO<sub>2</sub> levels, including reduced  
526 calcification (Kleypas et al. 1999, Riebesell et al. 2000, Orr et al. 2005, Orr et al. 2006, Gazeau  
527 et al. 2007, Kuffner et al. 2008), impaired early development (Kikkawa et al. 2003, Ishimatsu et  
528 al. 2004, Kikkawa et al. 2004, Kurihara et al. 2007), metabolic depression (Tamburri et al.  
529 2000), behavioural changes (Bibby et al. 2007, Widdicombe & Needham 2007), decreased  
530 survival and growth (Shirayama 1998, Shirayama & Thornton 2005, Hoegh-Guldberg et al.  
531 2007), and even neurotransmission in some fishes (Nilsson et al. 2012).

532 For deep-sea animals, the costs of CO<sub>2</sub> tolerance may be more significant than for  
533 shallow-living species. Seibel & Walsh (2003) compiled data from fishes, cephalopods, and  
534 crustaceans over a depth range from the surface to 1000 m, and found that the passive  
535 buffering capacity decreased logarithmically with depth of occurrence,, related presumably to  
536 the higher water content and lower tissue concentrations of weak acids and bases with  
537 increasing depth. Because compensation of acidosis by way of active ion exchange (e.g. H<sup>+</sup>-  
538 ATPase, Na<sup>+</sup>/K<sup>+</sup>-ATPase, or Na<sup>+</sup>/H<sup>+</sup> exchangers) is energetically expensive (Pörtner et al. 1998)  
539 and deep-sea animals live in a food-poor environment, their ability to up-regulate production of  
540 these key enzymes is likely to be highly limited, further impairing their ability to tolerate  
541 environmental hypercapnia.

542 The impacts of ocean carbon storage or leakage from seabed C storage locations are  
543 likely to be localized, but will have effects on ocean chemistry that accumulate over time and  
544 merge with passive ocean acidification, leading to significant changes in the chemistry of the  
545 deep ocean – a change of -0.4 pH units is eventually expected to occur due to ocean  
546 acidification. The consequences of such perturbations to deep ocean ecosystems cannot be  
547 predicted with confidence, however the fossil record supports the notion that rising ocean pCO<sub>2</sub>  
548 can have very significant impacts on ocean communities, as documented for a number of  
549 extinction events (Knoll et al. 1996, 2007; Veron 2011). Survival under a more acidic ocean can  
550 increase the costs of metabolic maintenance and performance for many animals. Allocation of

551 energy to pH compensation, increased ventilation, and other metabolic functions required for  
552 tolerance to elevated ocean carbon levels is highly likely to reduce energy available for growth  
553 and reproduction for individuals, leading to impacts on the demographic rates of populations.  
554 Reduced reproductive effort will likely constrain population growth rates, reduce resilience  
555 following physical and biological disturbances, and increase the likelihood of local extinctions.  
556 Consequences of these population-level changes for communities could include reduced  
557 biodiversity, disruption of energy flow through food webs, and reduced community resilience  
558 and stability.

559

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569

570

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702 FIGURE LEGENDS

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

705 Figure 2. Photographs of CO<sub>2</sub> release experiment E2. a. Overview of E2 showing fish traps with  
706 zoarcids (*Pachycara bulbiceps*) and octopus (*Benthoctopus* sp.), smaller megafaunal cages,  
707 and sensors. CO<sub>2</sub> containers (center) are mostly empty because the image was taken at end of  
708 experiment. b. Close-up of CO<sub>2</sub> containers at start of E2, showing liquid CO<sub>2</sub> (~100 liters) and a  
709 macrourid fish (*Coryphaenoides armatus*) swimming above the containers.

710 Figure 3. Design of CO<sub>2</sub> release experiments. Large solid circles represent CO<sub>2</sub> (filled) and  
711 control (open) containers. Sediment core samples indicated by small circles. Cages for  
712 fish/octopus, & megafauna indicated by rectangles and gray squares, respectively. Sensors  
713 (pH, T, S) shown by stars. Dashed circles indicate sampling sites near or distant from CO<sub>2</sub>  
714 pools. a. E1. b. E2 & E3. c. E5.

715 Figure 4. Tissue condition rating for *Haploops lodo*. Condition was assigned according to  
716 degree of degradation from 1-5, corresponding to images a-e, respectively. Tissue condition  
717 equal or greater than 4 were considered to be 'dead'.

718 Figure 5. Typical pH variation during CO<sub>2</sub> release experiments. a. Variation in pH at distances of  
719 >1m (E1), 5m & 50 m (E3) over 10 days. pH for each is offset to ease comparison among  
720 distances. Background pH for all experiments is ~7.78. Gray dots indicate raw data; black lines  
721 indicated smoothed data. Note the large perturbations near CO<sub>2</sub> pools in E1, and diminution of  
722 the pH signal with distance. b. Progressive vector diagram during E1 indicating flow direction  
723 during initial 23 days. Open circles indicate successive days. Note the tidal oscillations that  
724 result in a constantly changing flow direction for the dissolution plume, leading to variable pH  
725 perturbations, as observed in a. See Barry et al. (2005) for more information.

726 Figure 6. Distance versus mean and maximum pH perturbations from the combined pH  
727 observations during E,2,3,5. A logarithmic regression ( $\Delta\text{pH} = A*\ln(X) + B$ ) models the decrease  
728 in  $\Delta\text{pH}$  with distance (X)  $\text{CO}_2$  pools. The model is significant for maximum (F= 15.2,  $p<0.002$ ,  
729  $r^2= 0.52$ ; 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  
730 changes.

731 Figure 7. pH profiles of upper sediment column in, near, and distant from  $\text{CO}_2$  pools. Profiles are  
732 mean  $\pm$  SD (n=1-6) for cores collect inside  $\text{CO}_2$  pools (0 m, circles), and at distances of 0.1 m  
733 (squares), 1 m (up-triangles), 8 m (diamonds), and 50-100 m (control locations; black triangles).  
734 White-filled and gray-filled symbols indicate samples collected at the start and end of  
735 experiments, respectively. Note the depression of pH beneath  $\text{CO}_2$  corrals, particularly at the  
736 start of the experiments, and the close similarity of samples from 1-8 m with control samples.

737 Figure 8. Summary of microbial abundance measured during E1, E3. Values are billions of cells  
738 per g sediment  $\pm$  SE. Squares indicate cell density estimates based on phospholipid fatty acid  
739 analyses. Open symbols indicate values at start of experiments E1, E3, prior to  $\text{CO}_2$  release.  
740 Filled symbols for mean abundance at end of experiments. \* indicates a significant difference  
741 ( $p<0.05$ ) between samples collected before and after 1 mo. of  $\text{CO}_2$  exposure.

742 Figure 9. Abundance of macrofauna in surficial sediments before  $\text{CO}_2$  release and after 1 month  
743 of exposure to the  $\text{CO}_2$  dissolution plume during E2, in relation to expected maximum and mean  
744 pH perturbations. Regression for samples collected prior to  $\text{CO}_2$  release was not significant  
745 (linear; F=0.98,  $p>0.34$ ). Linear regression of abundance versus maximum pH perturbation for  
746 samples collected ca. 1 month after  $\text{CO}_2$  release was significant (F=15.5,  $p<0.002$ , R=0.74;  
747 black line, dashed line indicates 95% confidence band), with decreasing abundance of  
748 macrofauna nearer the  $\text{CO}_2$  pool where pH perturbations were largest. Regression for samples  
749 collected before  $\text{CO}_2$  release were not significant. Estimated distance (m) from  $\text{CO}_2$  pool  
750 indicated on upper x-axis.

751 Figure 10. Changes in macrofaunal species richness versus mean and maximum modeled pH  
752 perturbations during E2. Symbols as in Fig. 10. Regression prior to CO<sub>2</sub> release was N.S.  
753 (F=0.58, p>0.58). After ca. 1 month exposure to the dissolution plume species richness declined  
754 with higher plume exposure nearer the CO<sub>2</sub> pools (F=11.2, p<0.006, R=0.68).

755 Figure 11. Proportion of live *Haploops lodo*.(amphipod) in sediment cores versus ΔpH and  
756 distance from CO<sub>2</sub> corrals during E3 (circles) and E5 (triangles). “Before” samples (open  
757 symbols) indicate core samples collected prior to CO<sub>2</sub> release. “After” core samples (filled  
758 symbols) were collected ca. 1 month after CO<sub>2</sub> release. Maximum and mean pH changes (x-  
759 axes) were modeled from E2, E3, & E5 (lower X-axes). Regression of proportion live versus  
760 maximum pH changes was significant for “After” samples (combined for E3,E5; black curve,  
761 R=0.55, F=25.6, p<0.0001), but was non-significant for “Before” samples.

762 Figure 12. Photograph of *Cystechinus loveni* individuals from E1 showing normal skeletal  
763 condition (a) observed for animals at control locations and skeletal dissolution (b) observed near  
764 CO<sub>2</sub> pools.

765 Figure 13. Survival of megafaunal echinoderms (*Abyssocucumis abyssorum* – E1; *Cystechinus*  
766 *loveni* -E1(ΔpH>1.0),E3(ΔpH<0.4); *Amperima* sp. – E3) exposed to CO<sub>2</sub> dissolution plumes  
767 during E1 & E3. For each species, each point represents its percentage survival within a single  
768 megafaunal cage positioned from 0.5 to 100 m from CO<sub>2</sub> pools, scaled by the estimated  
769 maximum pH perturbation observed. The number of individuals in each cage varied from 1-10  
770 individuals. Data where pH changes exceeded -0.5 units are from E1. Observations with milder  
771 pH perturbations are from experiment E3. Data are altered slightly to avoid overlap. Mean pH  
772 changes are shown for E3 only.

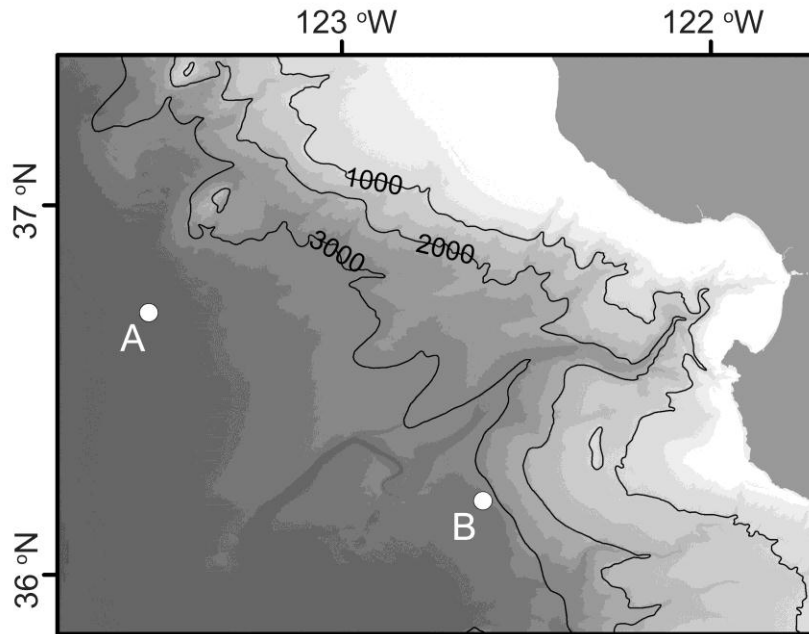
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777 Figure 1

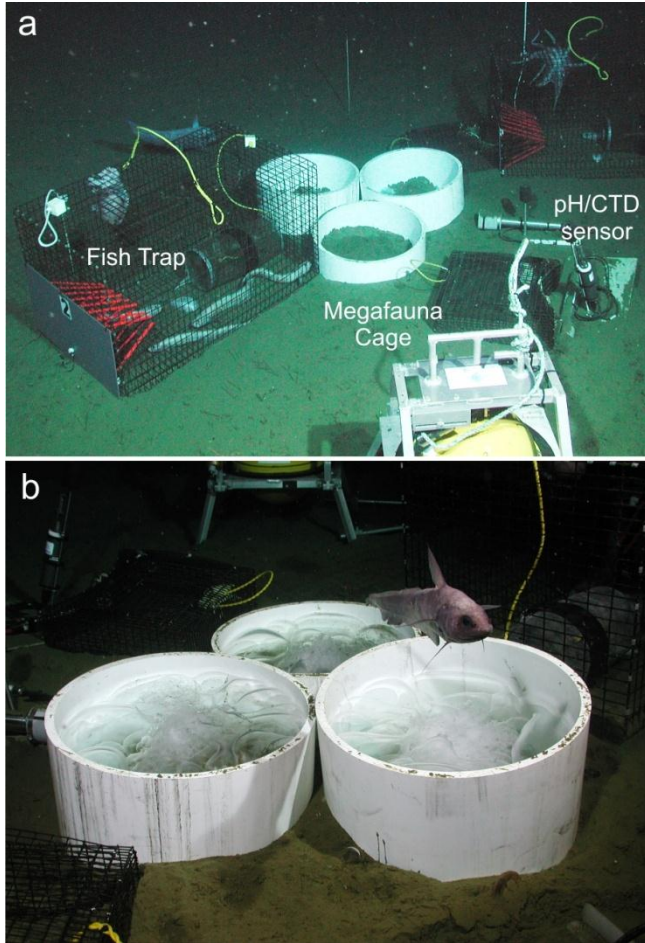


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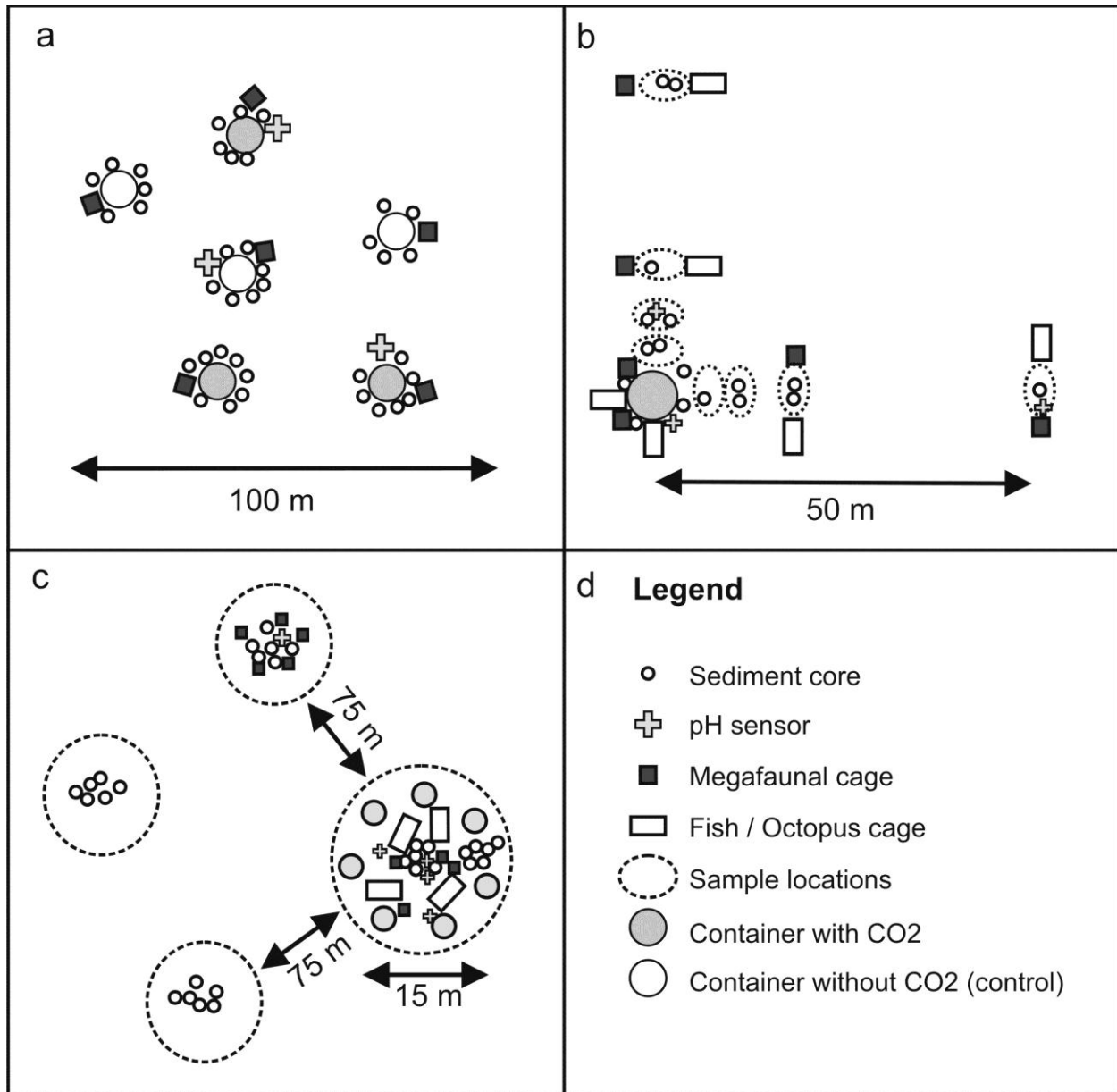
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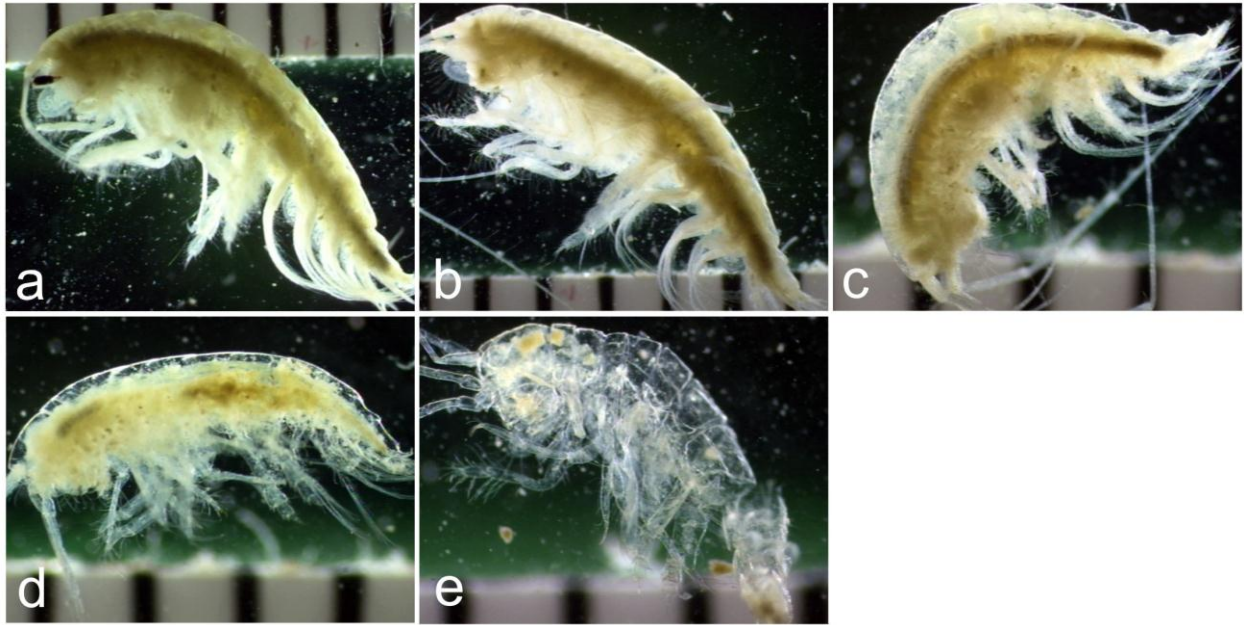
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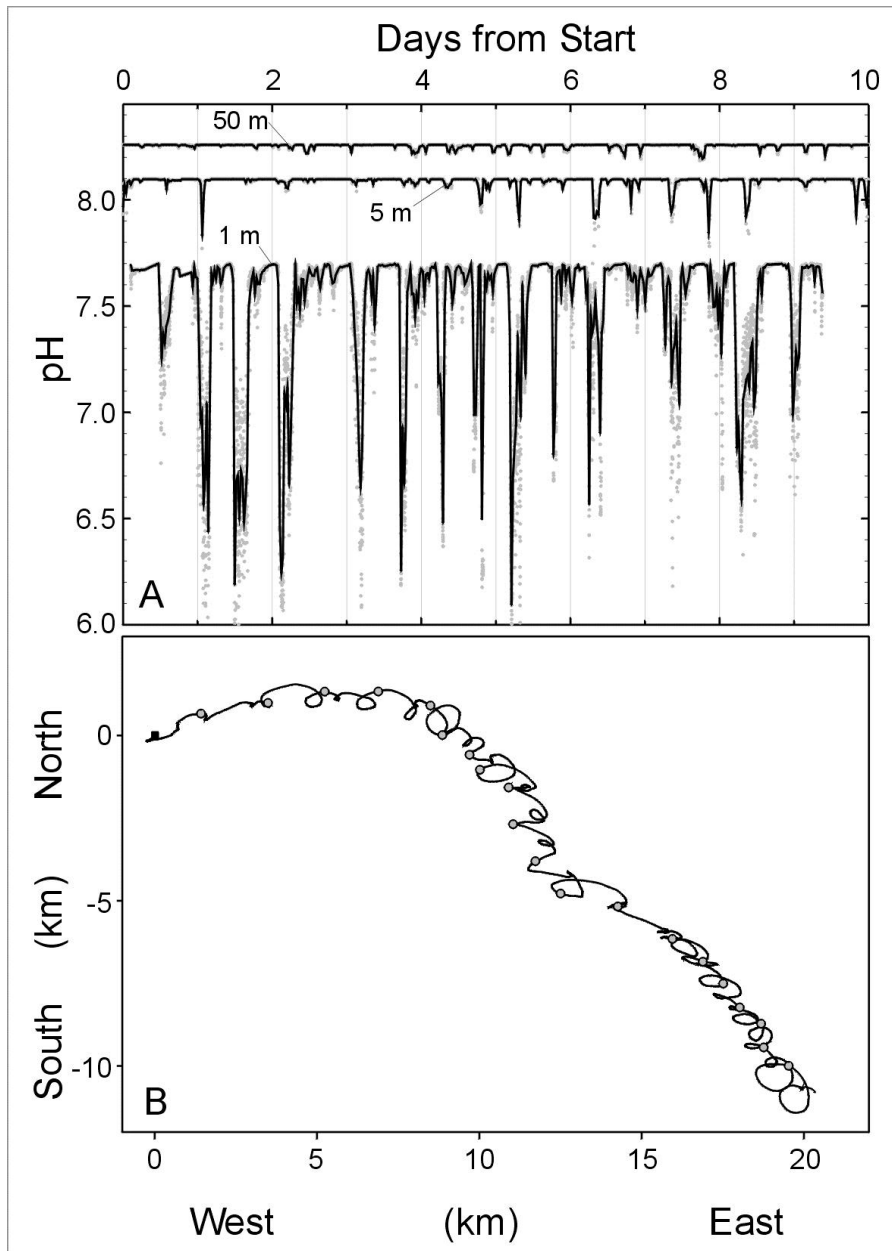


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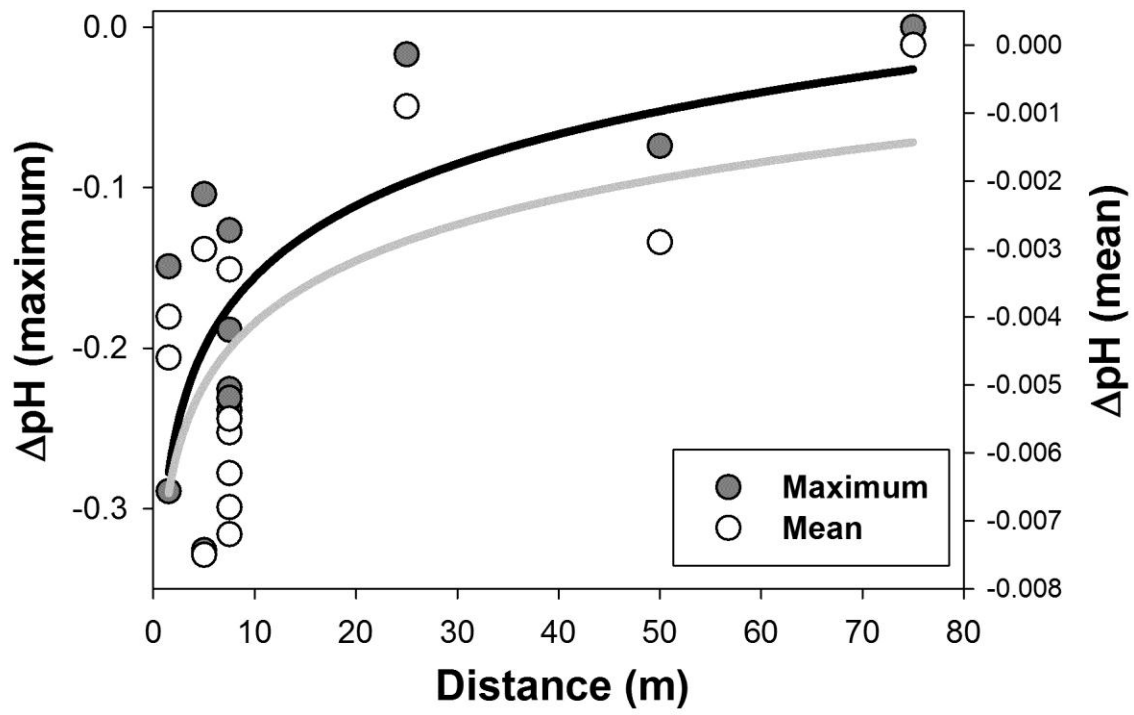


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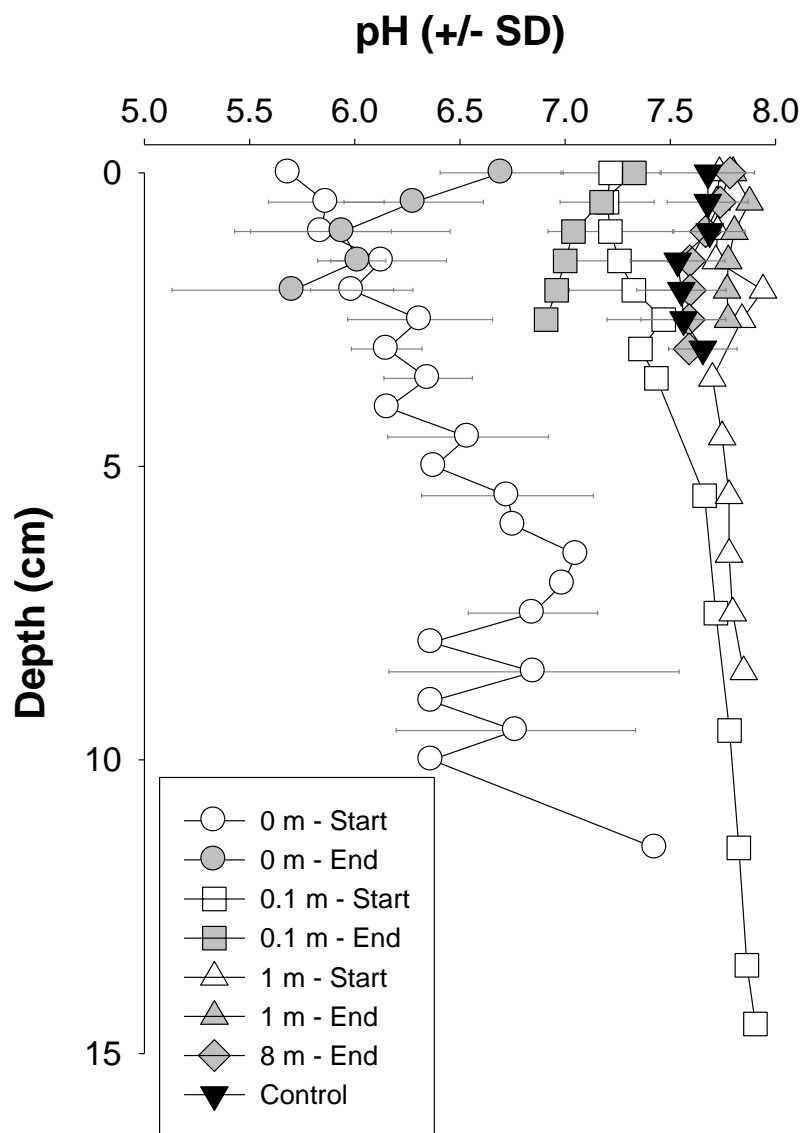
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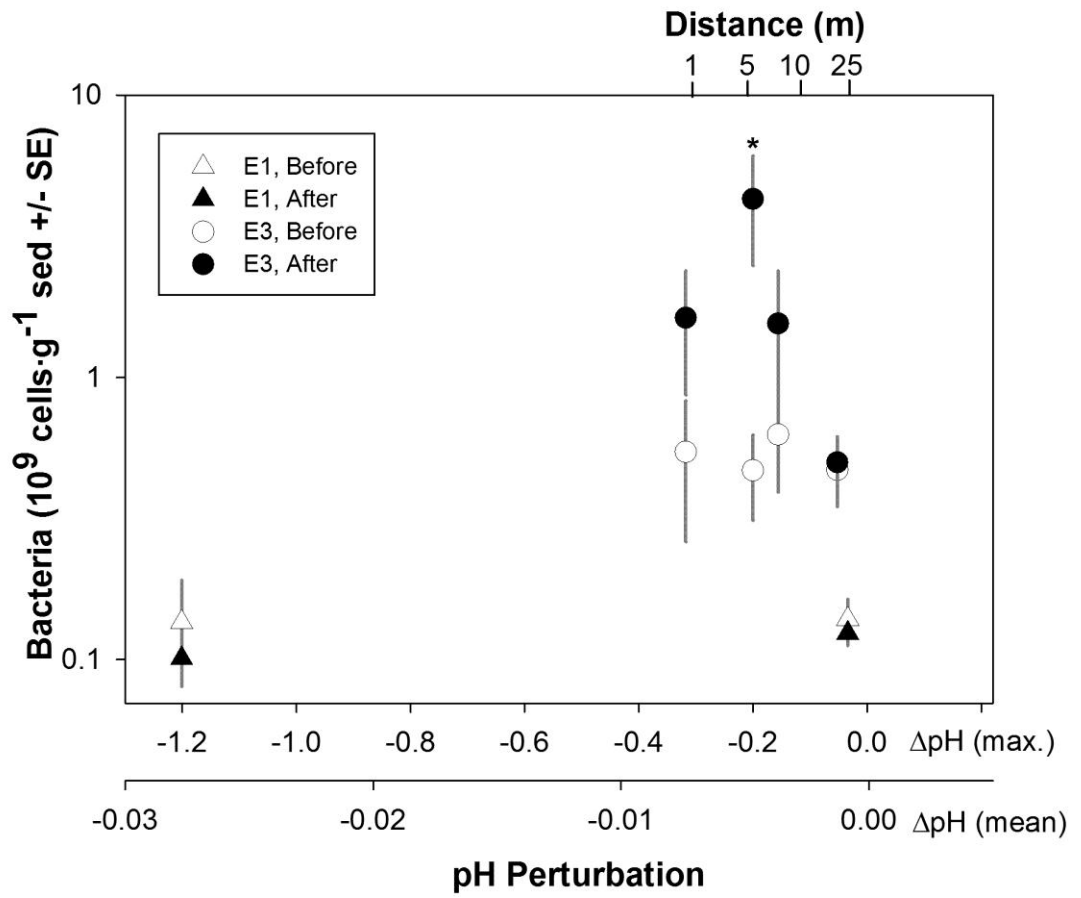


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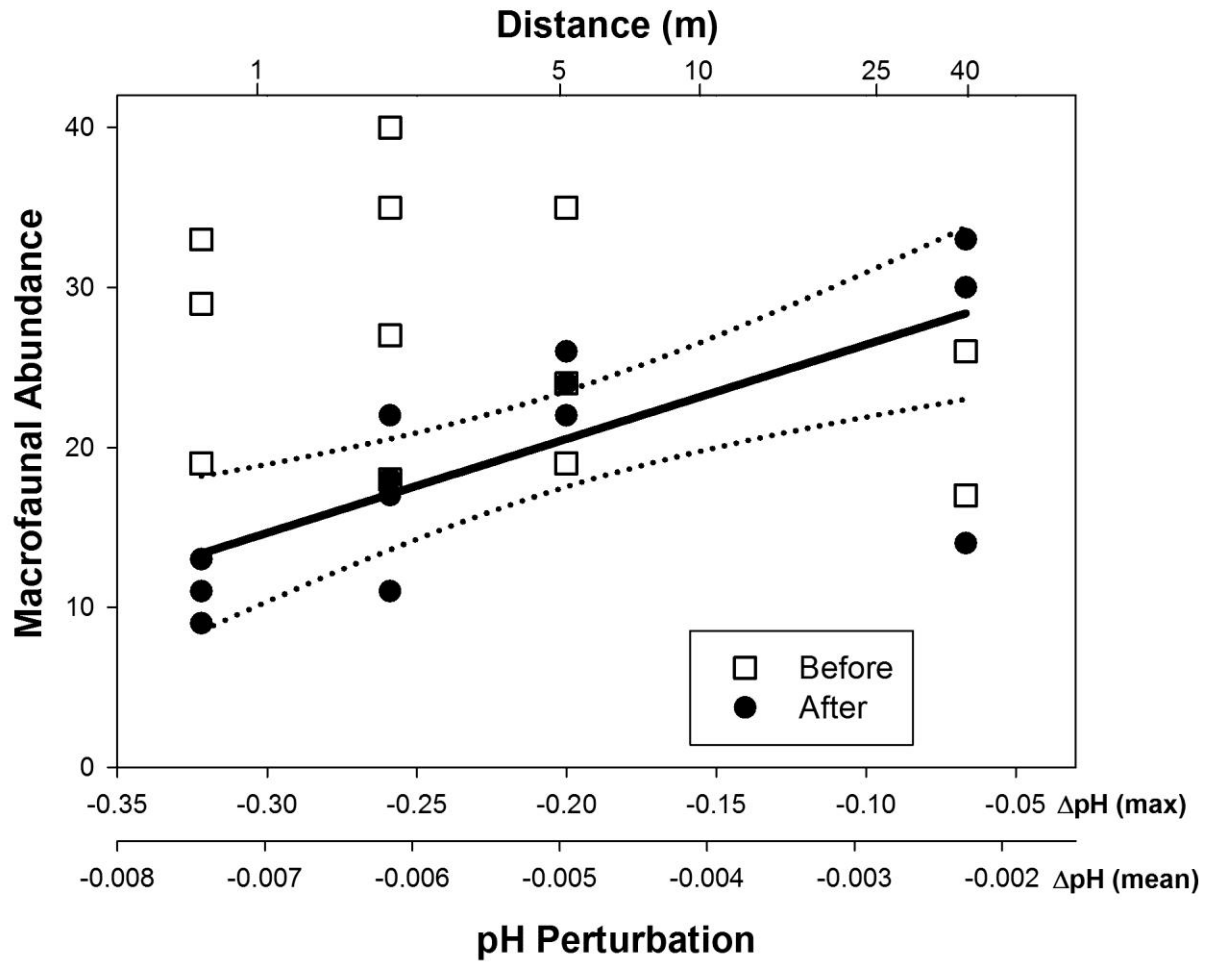
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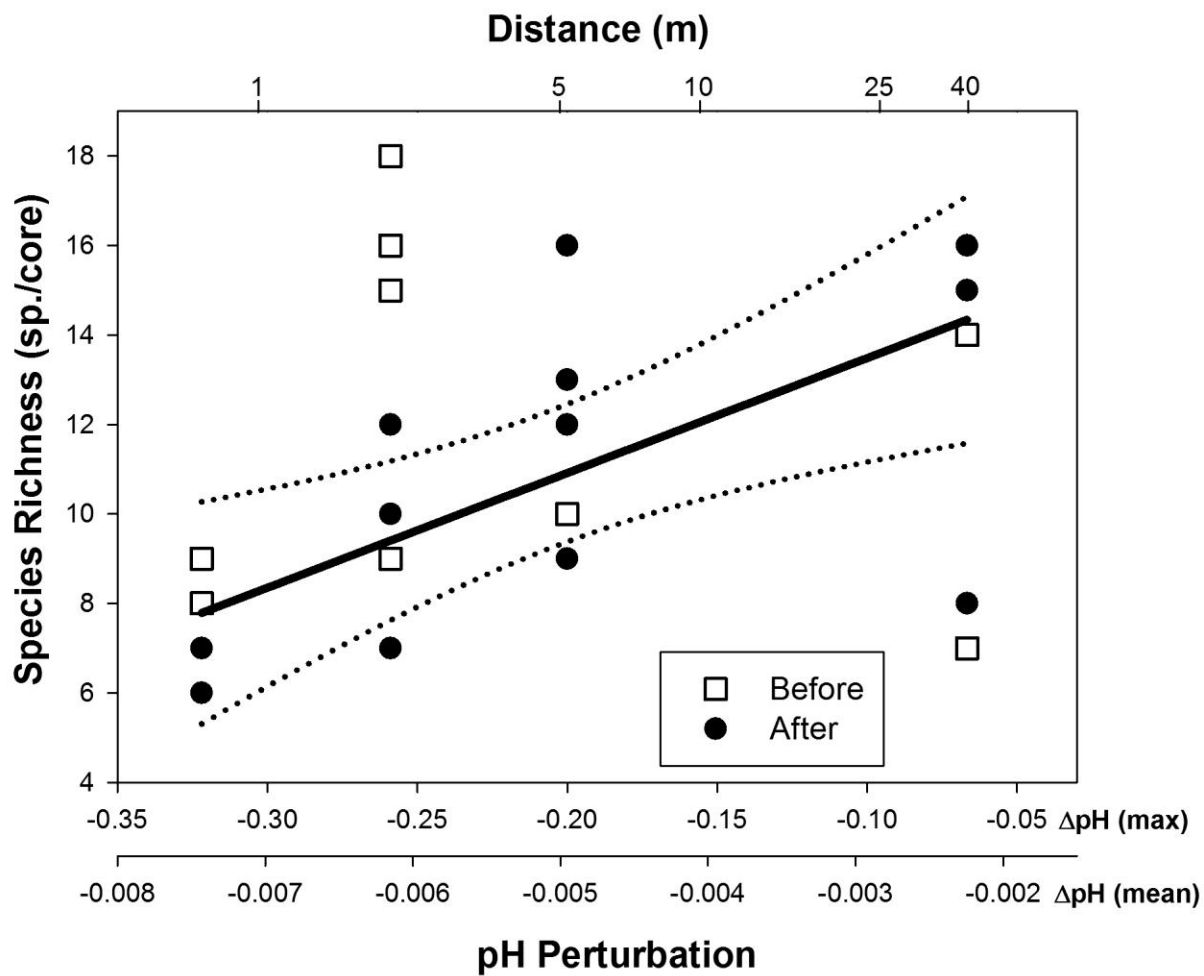
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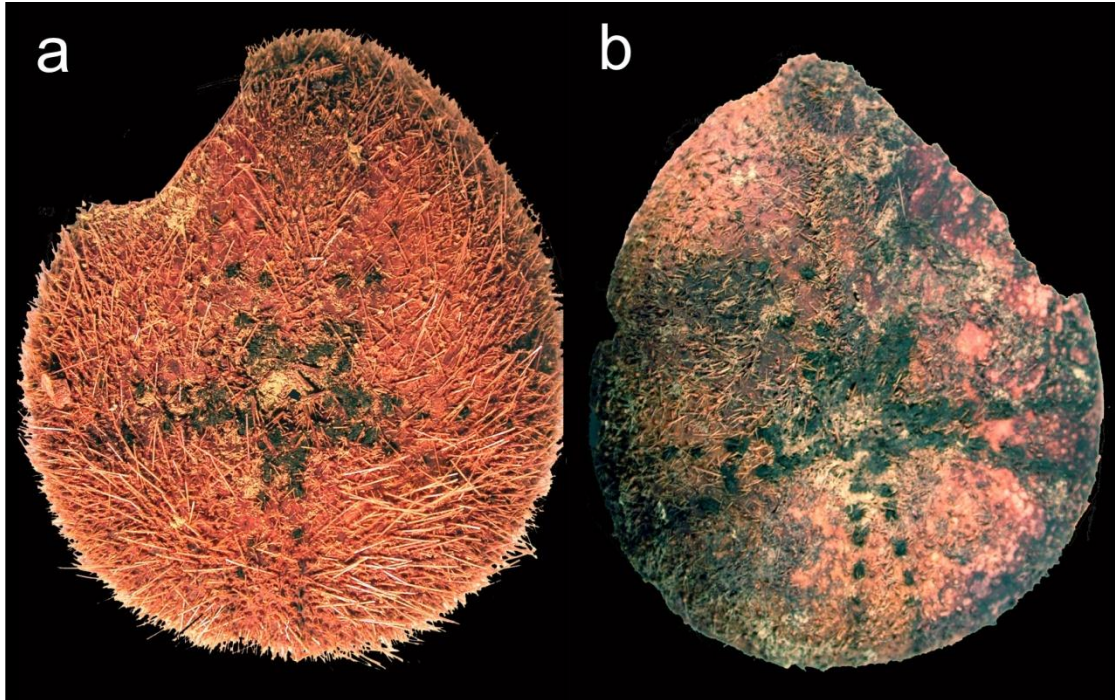
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827 Figure 12.

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831 Figure 13.

